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Viscoelastic Characterization of Bovine Trabecular Bone Samples

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***Abstract*—**Knowledge of bone mechanical properties is important for bone substitutes design and fabrication, and more efficient prostheses development. The aim of this study is to characterize the viscoelastic behavior of bone specimens, through stress relaxation and fatigue tests performed to trabecular bone samples from bovine femoral heads. Relaxation tests consisted on preloading the samples at five different magnitudes and evaluate them for 1020 seconds, adjusting the results to a KWW mathematical model. Fatigue tests consisted of 700 load cycles and analyze their status at the end of the tests. As a conclusion we have that between relaxation stress and each preload there is linear relation and for samples with initial Young´s modulus greater than 1.5 GPa showed no effects due fatigue test loading cycles.

***Keywords*—**Bone viscoelasticity, fatigue test, stress relaxation test, trabecular bone properties.

I. Introduction

VISCOELASTICITY is a characteristic polymers behavior; many biological materials are mostly made of polymers and therefore present this kind of behavior. Both trabecular and cortical bone exhibit viscoelastic behavior, but it is more obvious in the trabecular one due to its content of bone marrow in its cavities. Trabecular bone is usually modeled as cellular solid, which structural walls are called trabeculae, inside the structure is filled with bone marrow and a liquid phase.

Assign viscoelastic properties to trabecular bone, helps to explain its ability to dissipate energy during deformation below its yield limit, stress relaxation and the creep.

In the last 40 years, several experiments have been conducted to analyze behavior and viscoelastic properties of trabecular bone, for example: creep tests [1], [2] stress relaxation tests [3], [4] and dynamic analysis [5]-[8], that have been carried out on bovine, porcine and human bone samples by tensile, compression, bending and torsion tests.

The bone viscoelastic properties have already been explained as a result of different phenomena like thermo-elasticity, piezoelectricity, movement of biological fluid through channels, as well as intrinsic viscoelasticity of collagen fibers [6].

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Iyo [4] developed stress relaxation tests to evaluate cortical bone elastic modulus of a 36 months bovine. The experiment consisted on obtaining small femur bars, the bars longitudinal direction was both perpendicular (5 pieces) and parallel (7 pieces) to the main femur axis, evaluating them through a stress relaxation tests, adjusting the results to an empirically created mathematical model. Equation (1) shows this mathematical model KWW, which is also the model that was used in this paper:

$$E(t) = E_0 \left\{ A_1 \exp[-(t/\tau_1)^{\beta}] + (1 - A_1) \exp[-(t/\tau_2)^{\gamma})] \right\} \quad (1)$$

where; E_0 – initial Young's modulus; τ_1 – relaxation time for the quick region; τ_2 – relaxation time for the slow region; β and γ – geometric parameters; A_1 – fractional contribution of the rapid relaxation region with respect to total relaxation.

Quaglini [9] performed stress relaxation tests on bovine femoral heads, they worked with 55 cylindrical specimens of 10 mm diameter and 20 mm height whose main axis was parallel to principal femur axis. The magnitudes of the preloads used during the test were 168, 320, 445, 577 and 727 N, strain rate was 0.015 s^{-1} and the load decreases was evaluated for 600 s. The resultant curves was normalized by dividing them between its initial preload, they concluded that the applied preload determines their behavior during relaxation test: the higher the preload, slower stress relaxation rate and higher stress magnitude to reach steady state.

Rapillard [10] developed fatigue tests to cylindrical specimens of human trabecular bone from vertebrae. The tests were carried out under the stress controlled regime, load was a sine wave with a 2 Hz frequency, the minimum load was 16% of the material yield stress (around 0.5 MPa) and for the maximum load they considered 6 different levels, between 55 and 90% of the yield stress. They observed a lower slope in the elastic region at greater cycles, which shows an elastic module decrease, as well as an increasing of hysteresis area in the last load cycles.

Topolinski [11], performed compressive fatigue tests to trabecular bone specimens from human femoral heads. The main characteristic of these tests is that load was increasing every certain number of cycles: at 1 Hz frequency, applying a cyclic sine load wave to the samples for 500 cycles and then increased in 10N the maximum load value, repeating the process until the specimen failure. They concluded that the specimens last between 3750 and 50,200 cycles and as loading cycles increase, the failure probability will also do so, but this increasing was linear ($R^2 = 0.9564$).

II. Experimental Methodology

Electromechanical universal testing machine Shimadzu ™ was used for tests with a 100 KN load cell. Both fatigue tests and stress relaxation were developed with trabecular bone specimens from bovine femoral heads. The relation h/d = 2 was used for cylindrical samples, this value generates more homogeneous results and presents less friction between the plates and samples during the test [12]. The specimens diameter was 10 mm, therefore the height was 20 mm, with these dimensions it was satisfied continuum theory and also the edge effects on the specimens were reduced at the time of its machining to less than 10% over the final results [13], [14].

For samples preservation, they were kept at room temperature submerged in a solution of 50% saline substance and 50% ethanol, this is the suggested method to preserve samples for short periods of time (less than 3 months), under these conditions the impact on the mechanical properties will be minimal [15], [16]. The samples were stored for a 4 days maximum period.

We used 5 different preloads: 250, 500, 750, 1000, and 1250 N for stress relaxation tests at a constant strain rate $\dot{\varepsilon} = 0.0075\ s^{-1}$. The best five successful results were selected for the subsequent analysis. The relaxation time was 1020 s, because we observed in unofficial tests that at this point were obtained load variations less than 0.5%.

Fatigue tests were conducted at constant strain rate ($\dot{\varepsilon} = 0.0075\ s^{-1}$), specimens was cyclically loaded for 700 cycles to 50-950 N. Fig. 1 shows the way that test were done; the load application speed was variable because the objective was a steady strain rate.

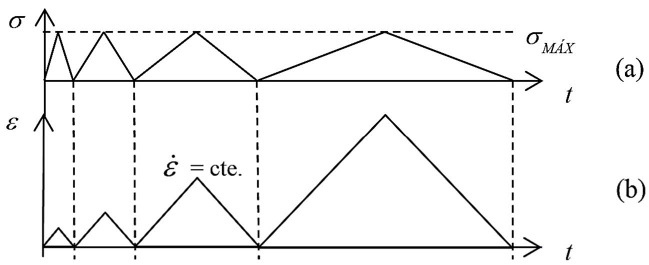

Fig. 1 (a) Load application versus time, (b) Strain variation along time

III. Results Analysis

A. Stress Relaxation

There were evaluated 34 specimens, of which 25 of them were approved, 5 for each preload value, 9 were rejected due to the results moved away from the average obtained for each preload (7% maximum error), or because they fail when they were pre-loaded.

Fig. 2 shows the 25 curves approved stress versus time, we can see five different groups of curves that correspond to each preload, between the curves of each group are not appreciated considerable differences at low preloads but as these increase, the results present higher dispersion.

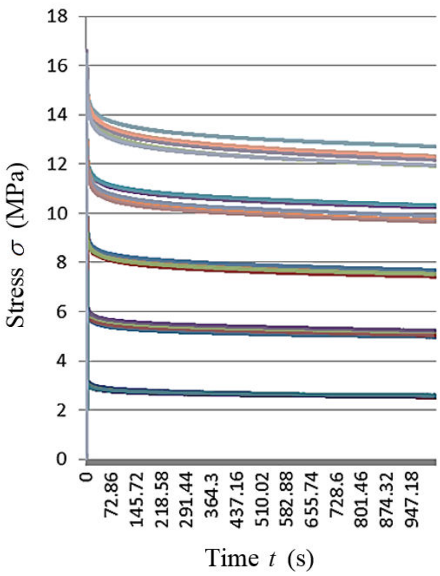

Fig. 2 Stress vs time of 25 selected specimens at five different preload values: 250, 500, 750, 1000, and 1250 N

To define better the data dispersion that was just mentioned, it was calculated the average σ_{RELAX} of the 5 data corresponding to each preload. Table I shows the results obtained for this and the standard deviation between the σ_{RELAX} values. It is seen that the deviation increases as the preload magnitude is increased, this explains the fact that at low loads, as in the case of 250 N, is not difficult for samples recover, but in the case of 1250 N, factors as the amount of bone marrow contained in specimens as well as structural variations from one specimen to another, become important.

TABLE I
Average σ_{RELAX} for Each Preload

Pre load response MPa	σ_{RELAX} avg MPa	Std Dev
3.183	2.540	0.030
6.366	5.093	0.095
9.549	7.559	0.105
12.732	9.967	0.268
15.915	12.215	0.284

Subsequently, it was calculated the stress reduction percentage for the evaluated specimens. We selected 5 results that showed less variation with respect to the average for each preload, so these were representative of the other 4. The results are presented in Table II, the reduction percentages are between 22 and 26%.

As it was shown in Table II, the stress percentage of reduction was similar for all cases, so it is concluded that regardless of the preload value, the percentage of reduction will be the same. This statement contradicts to [9], which concludes that stress reduction is inversely proportional to preload magnitude, however, [6] and [14] also found that the percentage of reduction is independent of the reached maximum stress.

TABLE II
REDUCTION PERCENTAGES IN THE VALUE OF σ_0 FOR EACH PRELOAD

σ_0 MPa	Reduction percentage %
3.18	23.57
6.37	22.25
9.55	23.04
12.73	23.72
15.92	26.30

Then curves normalization for each preload was obtained dividing the stress variation σ(t) between σ_{RELAX}. In Fig. 3, it can be seen standard curves coinciding with each other, which validates that the reduction percentage is independent of the preload magnitude along all evaluation time. From approximately 280 seconds the difference between curves is negligible, around 22% of the evaluation time, relaxation curves are homogenized.

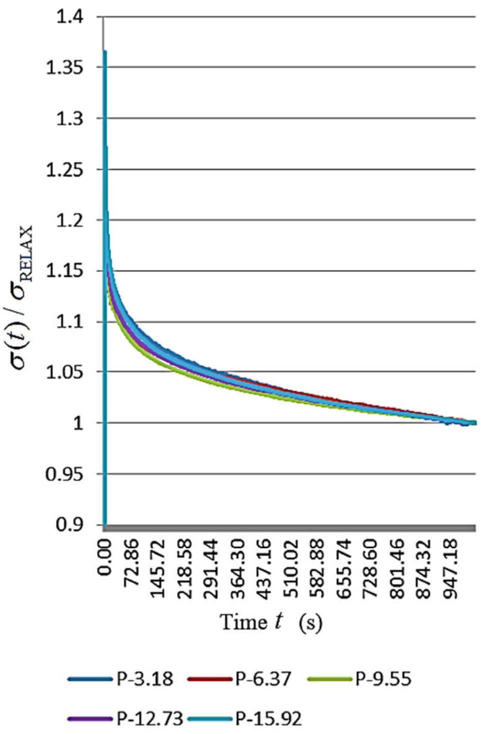

Fig. 3 Stress normalized curves respect of relaxation stress (σ_{RELAX})

Searching for a complete analysis, the results were fitted to a variation of the KWW model presented in (1). The modification consists in describing the stress variation as a function of time, obtaining 5 specific constants for the material regardless the preload value. For this, the model was normalized regarding σ_0, so we worked with the equation that is shown below:

$$\sigma(t)/\sigma_0 = A_1 \exp[-(t/\tau_1)^{\beta}] + (1-A_1)\exp[-(t/\tau_2)^{\gamma}] \quad [0 < A_1,\ \beta,\ \gamma < 1] \tag{2}$$

Through a mathematical adjustment $A_1, \beta, \gamma, \tau_1$ and τ_2 were optimized so that they describe in the best possible way the relation $\sigma(t)/\sigma_0$. As the five curves were very similar among themselves, only over one average curve the adjustment was made, obtaining the values presented in Table III.

TABLE III
OBTAINED VALUES FOR THE EQUATION 2 MATHEMATICAL FIT

A_1	β	γ	τ_1 s	$\tau_2 \times 10^6$ s
0.12	0.18	0.23	65.68	4.56

Fig. 4 shows five normalized stress curves versus time for the 5 preloads applied, as well as the curve obtained from (2). As it can be seen, the model fits to the experimental values so this part of the analysis is considered successful, likewise, numerical analysis reveals that it has a coefficient $R^2 = 0.98$, that is why KWW model is considered valid for this study.

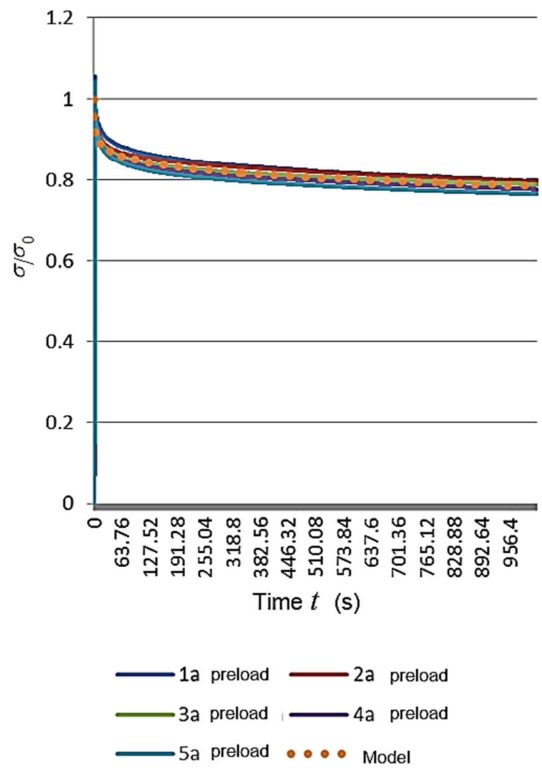

Fig. 4 Graph of normalized stress (σ/σ_0) depending on the time for the five different preloads, also the curve obtained by (2)

Then, it was verified if there were linearity in the results obtained between each preload and average σ_{RELAX} values, then a mathematical fit was made to a linear model. Fig. 5 shows that it was an equation that models the behavior of these values and it was obtained a correlation coefficient $R^2 = 0.97$.

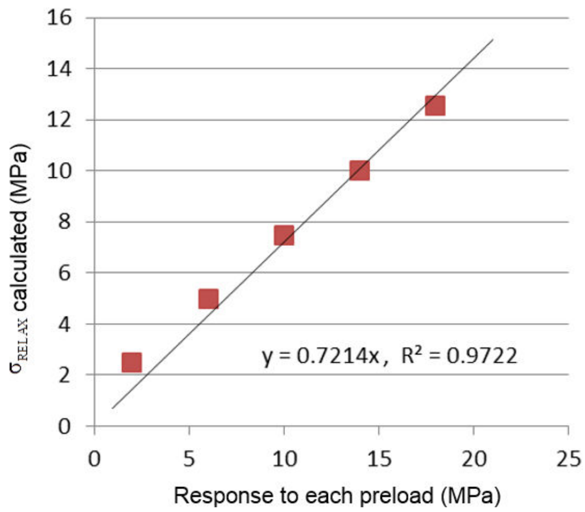

Fig. 5 Average σ_{RELAX} values calculated for each preload

B. Fatigue Tests

For this part of the research were evaluated 8 specimens at 700 cycles in a load range of 50 to 950 N with a constant strain rate $\dot{\varepsilon} = 0.0075\ s^{-1}$. Three of them show a decrease in its elastic module (samples 1, 4 and 5), three of them showed an apparent increase in stiffness (2, 3, 6 specimens) and 2 of them could not be evaluated because they collapsed before the test finishes.

To determine if the specimens were fatigued or not after completing the planned load cycles, its elastic module was calculated at different points in its evaluation. The calculation was made based on the data obtained at the beginning and at the end of a particular load cycle; this was carried out using the expression:

$$E = \frac{\sigma}{\varepsilon} = \frac{\sigma_{final\ load} - \sigma_{initial\ load}}{\varepsilon_{final\ load} - \varepsilon_{initial\ load}} \quad (3)$$

Table IV shows the specimens numerical results that resisted the 700 load cycles. It presents the total time that test lasted for each case, the specimens initial and final Young's modulus, (E_0 and E_F), the variation percentage at the end of the test in relation to E_0, maximum strain (ε_{MAX}) over 700 cycles and residual strain (ε_{RES}) immediately after loading cycles.

The values presented in Table IV show that the specimens that were fatigued (samples 1, 4 and 5) presented initial Young's modulus minor to 1.5 GPa, while the other 3, which showed no fatigue (specimens 2, 3 and 6), presented higher initial modulus.

In the case of the specimens that fatigued, their Young's modulus were reduced, this indicates that there was a structural damage, a large number of fractured trabeculae that weaken the structure throughout the cycles of load. For specimens whose stiffness not decreased, its Young's modulus apparently increased between 9 and 10%, may be due statistical variation or for these number of cycles the samples structures are not affected as the same as the load magnitude applied on these tests.

TABLE IV
RESULTS FOR 6 SPECIMENS AFTER 700 LOADING CYCLES

	1	4	5	2	3	6
Time s	1795.01	1054.52	2124.41	1332.73	1683.21	1800.18
E_0 MPa	1103.23	1226.2	1201.75	1800.51	1564.31	1929.25
E_F MPa	427.57	1154.8	541.44	2015.1	1709.91	2106.36
E_0 var %	-61.24	-5.82	-54.94	11.91	9.3	9.18
ε_{MAX} mm/mm	0.256	0.365	0.511	0.013	0.021	0.009
ε_{RES} mm/mm	0.23	0.355	0.492	0.007	0.015	0.004

The previous data table does not explain well everything of the specimen behavior during 700 loading cycles. Looking for a better visualization of Young's modulus variation along the time, it was decided to evaluate the elastic modulus at the first full cycle of loading and unloading, in the last full cycle, as well as 3 intermediate cycles 25%, 50% and 75% of the total evaluation time for each sample.

Fig. 6 shows Young's modulus variation for each sample. For specimens 2, 3 and 6, the initial module suffered an initial increase, but at around 30% of the time, this value became constant. So we considered that initial stage only as a stabilization zone.

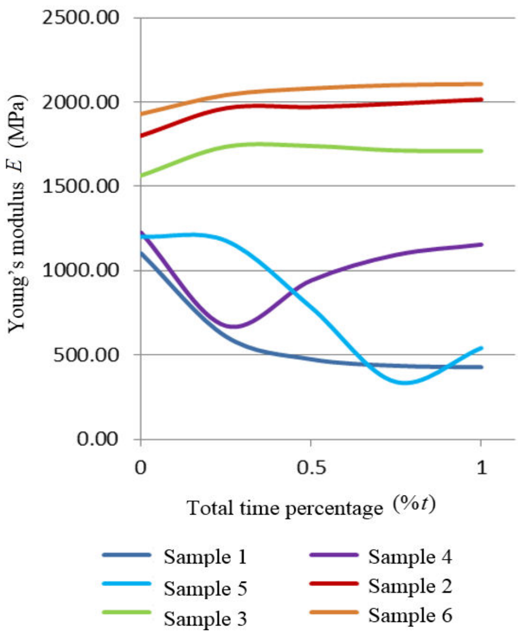

Fig. 6 Young's modulus of 6 specimens at 700 loading cycles

On the other hand, specimens that fatigued since the beginning showed elastic module decrease, it indicates that all the time presented trabecular structure failures.

Now are presented two stress-strain curves that completed the 700 loading cycles. To be representative of all the results obtained, one corresponds to a specimen that presented fatigue and the other one whose structure was not affected during the evaluation. The curves were fragmented looking for a better visualization; it was graphed only 5 loading and unloading cycles, which correspond to the first full cycle, as well as three intermediate cycles corresponding to 25, 50 and 75% of the total time and the last full cycle too.

Fig. 7 shows the stress-strain segmented curves for specimen 1 which is an example of what happened with the fatigued ones. It can be seen that cycle's distribution is not uniform over time; the displacement on the horizontal axis shows the strain accumulation.

The first cycle was a leaning line because loading and unloading follow the same trajectory, this fact suggests that hysteresis of the process is negligible, the reason for this is that the specimen absorbed energy during loading, but returned to regain its natural state once downloaded. Therefore does not present viscous response.

On the other hand, as loading cycles increase, hysteresis rings formation can be seen in cycles, the energy absorbed by the specimen during loading cycle is not immediately returned.

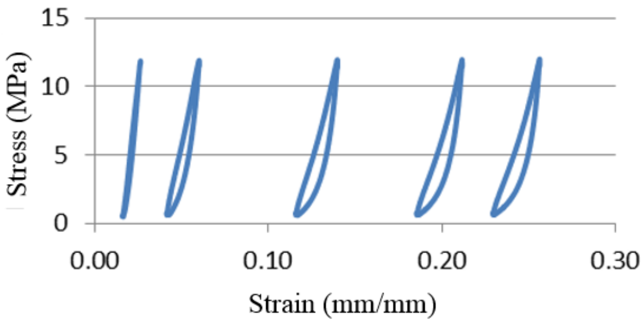

Fig. 7 Loading and unloading cycles for specimen 1

Fig. 8 represents specimens which presented no damage after 700 testing cycles and shows loading and unloading cycles for specimen 2. In this case, cycles are very near one to another, since the specimen strain was mostly elastic, deformation remnants effect did not influence in this case.

IV. Conclusions

For stress relaxation tests, it is concluded that as the applied preload value increases, σ_{RELAX} results will present a higher standard deviation.

It is concluded that decreasing percentage at the end of the test is independent of the preloading magnitude. In 5 selected results of the total number of specimens analyzed, reduction percentages between 22 and 26% were obtained without showing a relationship with the applied preload.

The relaxation stress presented a linear relation with the corresponding preload in the femoral heads evaluated specimens.

The results allowed a mathematical model KWW fit for stress relaxation, getting the specific constants for femoral trabecular bone.

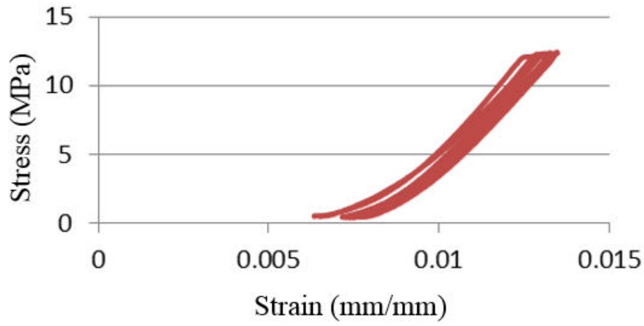

Fig. 8 Loading and unloading cycles for specimen 2

From fatigue tests, it was concluded that if the initial elastic modulus is less than 1.5 GPa, 700 loading cycles will be enough to damage their structures and cause significant stiffness decreases. They showed hysteresis rings so we can conclude that specimens absorbed energy was not being returned, and then their behavior is not perfectly elastic.

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