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PREDICTIVE MODELS FOR AGE-RELATED BONE TIME-DEPENDENT MECHANICAL PROPERTIES AT THE MICRO SCALE

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Abstract. Quantification of the mechanical properties of cortical bone through life span models is an invaluable source of data for bone modeling and numerical simulations. This data allows the validation of different theories of bone remodeling and corroborates the interactions between different microstructural components. However, human life span models are difficult to obtain due to ethical and manipulation samples issues. In this study, the Wistar rat life span model was used to quantify the evolution of the mechanical properties with ageing. The life span covers from growth until senescence with samples of 1, 4, 9, 12, 18 and 24 months old. The surfaces of the samples were ground and polished in order to expose all their microstructural features. The experimental data was obtained from nanoindentation tests by using a specific indentation protocol allowing the quantification of several mechanical properties from a single test [1,2]. The mechanical properties include elasticity, viscoelasticity, plasticity and viscoplasticity. From the experimental data, predictive models were computed to estimate the values of the mechanical properties at different ages. Two types of predictive equations for fitting the experimental data are proposed in this work. The first type is based on a growth model inspired in the Gompertz curve [3]. That growth model describes the evolution of the results as a function of the age. It is composed of and exponential function integrated by an asymptotic parameter, the growth rate and an adjusted factor. The second type of equations was calculated using multivariable linear regression. For that purpose, previous physical-chemical properties measured in a similar set of bone samples [4] were correlated to the mechanical properties. Then, the best-correlated parameters were used to perform multivariable linear regressions. Results show that both predictions equations are useful to describe the mechanical behavior of bone. However, supported on the determination coefficient, the equations computed from physical-chemical properties using multivariable linear regressions are more accurate that those obtained from Gompertz model.

1 INTRODUCTION

Ageing is a natural process inducing variation of bone properties and is widely investigated in literature. However, assessments on human bone models are rarely reported. Therefore, animals' models such as rabbit or rat models are commonly used to investigate bone structural, mechanical and physico-chemical variation due to diseases, dietary conditions or single physical activities [5–7]. Rat models are useful in such investigations due to availability of young specimens and because the availability to include the complete life span from growth to senescence.

At the micro scale, time-dependent mechanical properties are commonly assessed using nanoindentation and different mechanical models. Mazeran et al. [1] proposed and new four stages protocol (load-hold-unload-hold) combined with a time-dependent mechanical model allowing the calculation of the elastic, viscoelastic, plastic and viscoplastic properties from a single nanoindentation test. This method has been successfully tested on polymers [1] and bones [2,8].

In order to have a better understanding of the variation of the morphological and structural variation through ageing, it is necessary to perform measurements i.e. tissue microporosity, mineral, phosphates, carbonate and collagen content over a longer period (from very young to old specimens). In this order, a previous study performed in our laboratory by Vanleene et al. [4] provides the data of the micro structural and physico-chemical properties.

In this work, the Gompertz growth model and the multivariable linear regressions using the physico-chemical properties are used to developed predictive models of the evolution of the time-dependent mechanical properties due to bone ageing from growth to senescence.

2 MATERIALS AND METHODS

2.1 Bone samples

Femoral cortical bones of male rats RJHan:WI Wistar (ages 1, 4, 9, 12, 18, 24 months old) were used. Five samples per age coming from five different specimens were cut transversely at the proximal and distal end of the femoral diaphysis (Figure 1).

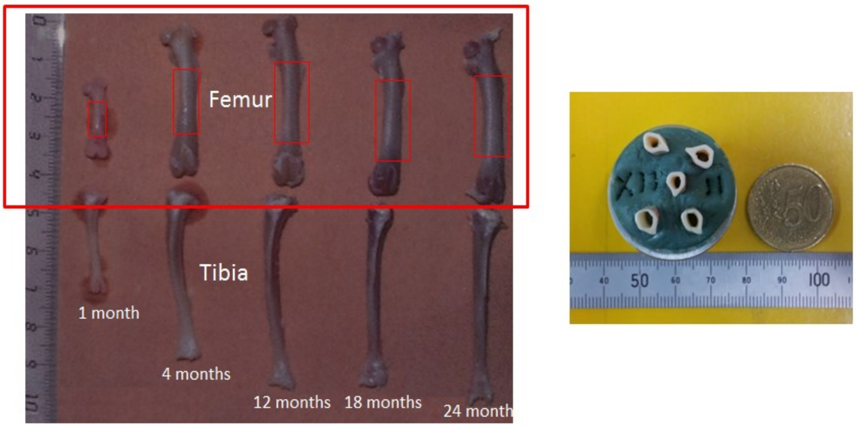

Figure 1: Femurs and tibias from Wistar rat classed according to their age: 1, 4, 12, 18, 24 months old [9].

2.2 Nanoindentation tests

Nanoindentation is a sophisticate technique allowing the calculation of the mechanical properties of classical and biological materials at the micro and nano scale. Nanoindentation tests were conducted with a Nano Indenter G200 (Agilent Technologies) using a Berkovich tip (Micro Star Technologies) and the measurement of the contact stiffness via the Continuous Stiffness Measurement (CSM) method. The nanoindenter tip was calibrated using a fused silica sample. For this study, which comprises 50 indents per age including ten indentations tests per sample performed in the longitudinal direction of the femoral section. All specimens were dry during nanoindentation assessments.

The indentation protocol is composed of four stages (loading, hold load plateau, unloading, and hold load plateau). These four stages are necessaries to differentiate the elastic, viscoelastic, plastic and viscoplastic behaviors. Indeed, the loading and unloading stages exhibit the elastic-plastic and purely elastic behavior respectively. The hold load plateaus allow exhibiting the reversible and irreversible viscous behaviors and thus viscoelasticity and viscoplasticity. In details, The four stages are 1) a loading stage at constant load rate/load ratio until an indentation depth of 3000 nm; 2) a hold time of 300 s; 3) an unloading stage at constant unload rate /load ratio until 50% of the maximal load value and 4) a second hold time of 300 s. This protocol has been used in previous studies to determine the time-dependent mechanical properties of polymers [1] and bones tissues [2,8].

2.3 Calculation of the mechanical properties

The mechanical model used to describe the material is based on a combination of one elastic modulus (Eelast), two-viscoelastic modulus (Eve and η_{ve}), a hardness (H) and one viscoplasticity η_{vp} in series. Two viscosities are enough to describe the viscoelastic behavior of the sample. To compute the mechanical properties a nanoindentation mechanical model composed of different mechanical elements (spring, Kelvin-Voigt elements, slider and dashpot Figure 2.) is used to fit the experimental indentation depth vs time curves. These elements have a quadratic response (square root of the load proportional to displacement and/or displacement velocity). After the experimental curves have been correctly fitted the nanoindentation mechanical model, the time-dependent mechanical properties can be computed.

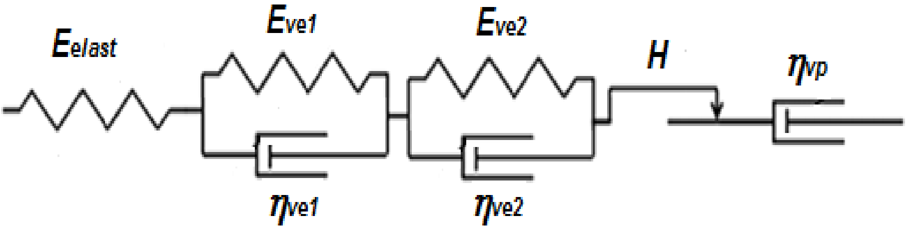

Figure 2. Material mechanical model used to assess the time-dependent mechanical properties. This model is composed of a pure elastic, two viscoelasticity with different time-constants, a plastic and a viscoplastic component

2.4 Physico-chemical properties

In this study, the values of the micro structural and physico-chemical properties obtained from a same set of samples by Vanleene et al. [9,4] were used. Those properties were obtained with different characterization methods such as ESEM images analyses, Fourier Transformed Infra-Red spectroscopy (FTIR) and X-ray diffraction.

2.5 Predictive models

- Gompertz growth model

An exponential growth model (Eq. 1) is used to predict the evolution of the mechanical properties related to ageing.

$$X = k_0 * \left(1 - exp^{(-k_1 - k_2.Age)}\right) \tag{1}$$

Where, the term X corresponds to each mechanical property of the model, k_0 is the estimated asymptote value when age approaches infinity. The term k_1 is the coefficient to adjust the equation for the initial conditions and the term k_2 is the growth rate. The asymptote value k_0 represents the end of growth. These prediction equations allow one to identify a possible maturation age for each mechanical property. This maturation age is considered as the age when the mechanical property reaches the 95% of its maximum value.

- Multiple regression analyses

The correlation coefficient and the determination coefficient were computed for all mechanical and physico-chemical properties using the statistical analysis and graphics software SYSTAT version 2012 (SYSTAT Software Inc.). Multivariable regressions were computed in the non-normalized experimental data. These regressions were carried out using the physicochemical properties that are in a good correlation and could fit better the mechanical response. In this work, all physico-chemical variables were considered as independent even if they are strongly correlated. They are different only when multicollinearity is detected.

These regressions were performed to obtain the best fit of the experimental data and to assess the relevance of each physico-chemical property to increase or decrease the mean value of the mechanical response. The Eq. 2 represents the model used for these regressions.

$$M_{property} = Constant + A * X_{Property} + B * Y_{property} \dots .. \tag{2}$$

Where, M is the mechanical property and X, Y are the higher correlated physico-chemical properties and the sign positive or negative indicate their effect in the mechanical response.

3 RESULTS

3.1 Time-dependent mechanical properties

The mean values ± standard deviation of the mechanical properties computed from the nanoindentation data are summarized in Table 1.

Table 1. Values of the mechanical properties computed from the nanoindentation experiments in the longitudinal direction of the rat femoral cortical bone.

Age (Months)	**E_{elast} (GPa)**	**E_{ve1} (GPa)**	**$\eta_{ve1}\times10^2$ (GPa.s)**	**E_{ve2} (GPa)**	**$\eta_{ve2}\times10^3$ (GPa.s)**	**H (GPa)**	**η_{vp} (GPa.s)**
1	26.4 ± 3.4	43.2 ± 6.1	17.6 ± 3.3	79.0 ± 12.3	48.0 ± 9.9	0.70 ± 0.09	250.9±28.8
4	40.7 ± 6.7	57.6 ± 16.9	19.3 ± 4.6	114.8 ± 15.8	64.0 ± 13.6	0.93 ± 0.06	334.6±27.9
9	35.9 ± 3.8	75.6 ± 17.7	28.3 ± 8.2	140.1 ± 23.0	73.6 ± 12.1	0.97 ± 0.10	364.3±43.0
12	39.8 ± 6.3	78.2 ± 18.4	23.6 ± 9.6	150.1 ± 25.9	57.4 ± 13.5	1.04 ± 0.12	357.6±45.5
18	38.4 ± 6.8	75.1 ± 19.1	28.2 ± 8.4	150.4 ± 25.9	71,5 ± 15.1	1.06 ± 0.10	381.6±35.8
24	34.6 ± 4.6	71.4 ± 15.4	22.6 ± 9.6	146.0 ± 18.6	68.6 ± 18.2	1.13 ± 0.09	408.5±43.1

Mean ± standard deviation

3.2 Physical-chemical properties

The values of the micro structural and physico-chemical properties obtained from a same set of samples by Vanleene et al. [4,9] are summarized in Table 2.

Table 2: Variation of the physico-chemical properties of Wistar rat femoral cortical bone with age

Age (Months)	**Porosity%**	**CO_3W%**	**PO_4%**	**Ca%**	**N%**	**Collagen%**
1	8.1	4.1	20.7	42.2	4	21.4
4	3.1	4.9	18.2	39.2	3.6	19
9	3.3	6.1	17.9	39.4	3.3	17.6
12	2.6	6.1	18	39.7	3.3	18.3
18	3.6	6	18	39.3	3.3	17.8
24	4	6	18	39.3	3.3	17.4

3.3 Correlations between the mechanical and physical-chemical properties

Mechanical properties obtained by nanoindentation were correlated with micro structural and physico-chemical properties. The correlation coefficients are reported in Table 3.

Table 3: Simple correlation coefficient R for the mechanical and physicochemical properties of rat bone

Mechanical properties	**Porosity%**	**CO_3W%**	**PO_4%**	**Ca%**	**N%**	**Collagen%**
E_{elast}	-0.949	0.608	-0.867	-0.878	-0.703	-0.673
E_{ve1}	-0.828	0.992	-0.885	-0.780	-0.981	-0.919
η_{ve1}	-0.576	0.842	-0.682	-0.597	-0.810	-0.778
E_{ve2}	-0.842	0.981	-0.914	-0.837	-0.991	-0.948
η_{ve2}	-0.679	0.744	-0.822	-0.844	-0.794	-0.884
H	-0.799	0.900	-0.899	-0.864	-0.937	-0.940
η_{vp}	-0.781	0.903	-0.908	-0.886	-0.942	-0.974

3.4 Predictive models

Results of the predictive models computed by the two methods are reported in Table 4.

Table 4: Predictive models of the evolution of the mechanical properties using the Gompertz model

Mechanical properties	Gompertz Model	Coefficient R^2
E_{elast}	$37.9 * (1 - exp^{(-0.00712-1.190*Age)})$	0.79
E_{ve1}	$75.8 * (1 - exp^{(-0.533-0.279*Age)})$	0.94
η_{ve1}	$2611 * (1 - exp^{(-0.816-0.234*Age)})$	0.61
E_{ve2}	$150 * (1 - exp^{(-0.486-0.252*Age)})$	0.99
η_{ve2}	$83165.5 * (1 - exp^{(-0.630-0.250*Age)})$	0.60
H	$1.10 * (1 - exp^{(-0.868-0.188*Age)})$	0.94
η_{vp}	$391 * (1 - exp^{(-0.857-0.210*Age)})$	0.93

Then the results of the prediction model are used to predict a maturation age for each mechanical property and to identify the growth rate (Table 5).

Table 5: Maturation age and growth rate of the different mechanical properties for male rats RJHan:WI Wistar. Maturation age was computed at the 95% of the maximal values of the function**.**

Mechanical properties	Maturation Age (Months)	Growth rate
E_{elast}	2.7	1.19
E_{ve1}	8.7	0.28
η_{ve1}	9.2	0.23
E_{ve2}	9.8	0.25
η_{ve2}	9.3	0.25
H	10.9	0.19
η_{vp}	10.1	0.21

The results obtained using multivariable regression analyses are reported in Table 6.

Table 6: Predictive models of the evolution of the mechanical properties using the multivariable regression

Mechanical properties	Multivariable regression	Coefficient R^2
E_{elast}	$73.7 + 8.03 * PO_4\% - 4.42 * Porosity - 4.21 * Ca\%$	0.97
E_{ve1}	$110 - 55.0 * N\% + 7.27 * CO_3W\% + 5.76 * Collagen\%$	0.99
η_{ve1}	$-10236 + 2733 * N\% + 1097 * CO_3W\% - 160 * Collagen\%$	0.74
E_{ve2}	$1278 - 396 * N\% - 60.2 * CO_3W\% + 18.9 * PO_4\,\% + 11.2 * Collagen\%$	0.99
η_{ve2}	$420 + 17.3 * \mathrm{P}O_4\% - 12.9 * Ca\% - 8.60 * Collagen\%$	0.87
H	$18.3 - 6.16 * N\% - 1.65 * CO_3W\% + 0.368 * Ca\% - 0.0780 * Collagen\%$	0.99
η_{vp}	$4810 - 1457 * N\% - 418 * CO_3W\% + 97.8 * Ca\% - 53.5 * Collagen\%$	0.99

4 DISCUSSION

The Gompertz growth model used in this work is frequently used to describe monotone mechanical behaviors but sometimes this situation do not corresponds to the experimental results. In that case, it must be informed that others growth models could be used to fit mathematically the experimental data but most of the time they do not have any physiological sense. According to our results, the best-fitted responses were the elastic components of viscoelasticity, hardness and viscoplasticity. Maturation ages were found to be different for the purely elastic response but within the same range of age for the others properties. This fact could be noted from the values of the mechanical properties. Elastic response was found to increase considerably between 1 to 4 months old meanwhile the others properties increase even until 12 months old.

Using the multivariable regression analyses, the predicted variables, which affect the viscoelastic behavior of bone, take part in bone capacity to accumulate and to release kinetic energy during and after a mechanical stress. One should note that R^2 of the viscous components are lower than the elastic components of viscoelasticity. This fact denotes that other parameters are necessary to better fitting the viscous elements of viscoelasticity. Others factors could be the hydration state of the sample. It is known that viscoelastic response could be also affected by layered particles of water and other fluids [10]. These particles may play an important role as damping elements of the viscoelastic response.

The prediction variables were selected because they provide a good R^2 coefficient. This does not mean that others physico-chemical variables cannot affect the mechanical properties of bone. In fact, even if the selection of the variables was extensive, there is always the probability of new variables that have not been considered or even defined yet be critical to the outcome. Nevertheless, this information could be useful to understand how and which mechanical properties of bone are affected by the variation of some physico-chemical properties..

5 CONCLUSIONS

- Life span models of rat cortical bone are useful to quantify the evolution of the mechanical properties mainly for new properties such as the time dependency or to test the influence of dietary or metabolic factors.
- Using the Gompertz growth model is possible to predict the evolution of the mechanical properties with age. Particularly, the elastic response was found to have a maturation age faster (2.7 months) and growth rate higher (1.19) than the others mechanical properties i.e. viscoelasticity, hardness and viscoplasticity have growth rates between 0.19 and 0.28.
- Mechanical properties could be predicted as a function of different physico-chemical properties. Porosity was found to be strongly linked to the elastic response meanwhile nitrogen content affects all the others mechanical properties.
- Predictive models could be included in bone modeling and others numerical simulation to increase the accuracy of the results or to predict the variation of the mechanical response due to changes in structural, metabolic and physico-chemical factors.

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