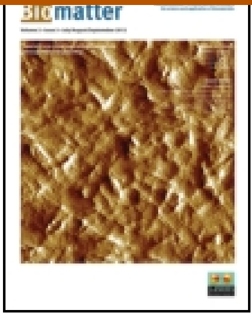

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Water uptake and swelling in single trabeculæ from human femur head

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Keywords: bone nano structure, diffusion, swelling, collagen, hydroxyapatite

The swelling of air-dried single trabeculae from human femur heads was obtained by complete immersion in water and the dimensional changes of the samples were measured over time. The experimental results were analyzed under the viewpoint of the diffusion through a porous material. The dimensional changes of the single trabeculae were 0.26 ± 0.15 percent (length), 0.45 ± 0.25 percent (width) and 1.86 ± 0.97 percent (thickness). The diffusion coefficients were then calculated from the swelling recorded over time and a value of (4.12 ± 0.8) x 10^{-10}(m^2s^{-1}) (mean ± standard deviation) was found.

Since the dimensional variations of the specimens is due to the swelling of the collagen bone matrix, this technique could offer new insights for (1) a selective characterization of bone microstructure at the collagen matrix level and (2) the dynamics of diffusion through bone tissue.

Introduction

Understanding the behavior of fluid flow and diffusion through bone is a concern to assess many physical and physiological properties of this tissue. Strictly speaking, bone is a composite material whose mechanical properties are determined by the intimate relationships among the constituent phases, that is the collagen fibers and the apatite nanocrystals.[1,2] Given the macro and microstructure of these phases and their specific physical-chemical properties, the bone results a porous and hygroscopic medium. Its porosity is evident on many distinct scales, namely those of intertrabecular, vascular, lacuno-canalicolar, collagen-apatite and bone collagen matrix, with characteristic dimensions spanning from millimeters down to few nanometres. Among them, the latter is determined by the arrangement of collagen molecules to form a regular pattern. The characteristic length of this porosity is imposed by the intermolecular collagen bonds (cross-links), the water content and by the degree of mineralization.[3-6] The investigation of the bone tissue down to this scale may lead to a better comprehension of mineralization of collagen.

The water in the collagen structure was classified is in 5 regimes[7] characterized by increasing water concentration from 0–0.010 g/g (regime I), to >0.5 g/g (regime V). Regime I water can only removed at 100 °C under vacuum while regime II water is removed at room temperature under vacuum. Regimes I and II were identified as hydrogen bonded water forming bridges inside the triple helix of collagen. The room-temperature drying removes water of regimes III and IV. Regime V is characterized by free water between microfibrils. In bone collagen, water of regime V is replaced by mineral.[3]

For the purposes of the present study it is important to point out that re-hydrating the specimens from regime III causes a consistent large increase of the lateral spacing of the collagen molecules and thus produces a measurable swelling.[7,8]

Shrinkage of bovine cortical bone were provided and discussed by.[5,9] Volumetric shrinkage of cancellous bone was reported by.[10] Transmission Electron Microscope measurements were performed by[11] on dimensional changes of equine osteonal bone after dehydration.

On the other hand, few studies are reported about the water dynamics in bone matrix. Transport of water into the mineralized matrix of human dentine was investigated by.[12,13] Diffusion coefficient[14] and water distribution[15] were measured by NMR respectively on rabbit and human cortical bone.

Despite the importance of these issues, to our knowledge there are no available data about dimensional changes and diffusion coefficient of single human trabeculae.[10]

Given a substantial lack of experimental data about this particular topic, the main goal of this work is to contribute to the study of the water transport through the collagen apatite porosity and thus a better comprehension of the mineralization process and the nutrient exchanges between bone matrix and osteocytes. In this paper we first illustrate the measurement of the swelling of single trabeculae from human femur heads during water imbibition. Since the swelling is caused by water while diffusing

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Table 1. Mean percent dimensional changes

			Length	Width	Thickness
Present study	Human, single trabeculae	Air-dried (27 ± 1)°C, (41 ± 3)%RH, then rehydrated in water	0.26	0.45	1.86
			Axial	Tangential	Radial
Lees et al., (1981)[5]	Bovine, cortical	From fully hydrated to vacuum dried at room temperature	0.6	2.4	3.5
Finlay ey al, (1994)[9]	Bovine, cortical	From fully hydrated to vacuum dried	0.92	2.7	4.1
Finlay ey al, (1994)[9]	Bovine, cortical	From fully hydrated to oven-dried, 40 °C	~0.7	~2	~3
Uktu et al., 2008[11]	Equine, osteonal	Dehydrated from 100%RH to 42%RH at 5 °C.	-0.12	1.41	1.39

from external surfaces to the core of the sample, by measuring the sample swelling over time, we obtained direct information about the transport of water into the collagen matrix.

Results

Swelling

The measured dimensional changes of single trabeculae are listed in **Table 1**, together with those reported by other authors. Percent mean and standard deviation of measured values averaged over all the specimens were 0.26 ± 0.15 (length), 0.45 ± 0.25 (width) and 1.86 ± 0.97 (thickness).

Discussion

The amounts of dimensional changes were appreciably lower than those reported for bovine cortical bone reported by,[5,9] but in good agreement with those at single osteon level[11] for equine bone (**Table 1**). The analysis of these discrepancies are beyond the scope of this work and should be investigated considering the different arrangement of the lamellae in trabecular or in osteonal cortical bone.[16,17] Also different initial and final water content are likely to affect the measured swelling.

The average value ± standard deviation for the diffusion coefficient was (4.12 ± 0.8) × 10^{-10}(m^2s^{-1}), roughly one order of magnitude higher than (3.56 ± 0.78) × 10^{-11} (m^2s^{-1}) measured using NMR for four cortical bone specimens from rabbit tibia by.[14]

This probably arises from the different lamellar structure and organization of the cortical bone with respect to the trabecular one. However our result is much more similar to that found by[18] about the solute transport in the bone lacunar-canalicular system. In this work the diffusion coefficient of fluorescein measured in mice intact bone was (3.3 ± 0.42) × 10^{-10} (m^2s^{-1}). This evidence is somewhat confirmed by the diffusion coefficient measured for the intertubular dentine[12] (1.74 ± 0.42) × 10^{-10}(m^2s^{-1})

Analogously to the findings of,[12,13] our results suggest that the water uptake could be described by a combination of the (relatively fast) capillary suction of water through the lacunar-canalicular network and the subsequent (relatively slow) diffusion of water into the trabecular tissue matrix.

This means that is reasonable that our measurements are not affected by the "fast" diffusion of water through canalicular network. Conversely, our method based on the hygroexpansion of the mineralized matrix appeared sensitive essentially on the water transport at collagen matrix-apatite scale.

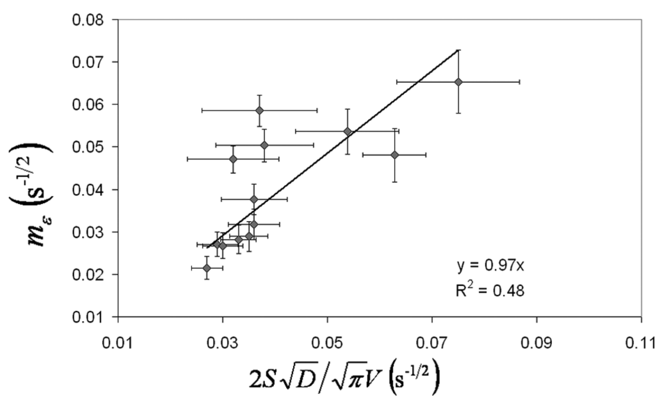

Figure 1. Slope of the normalized hygroscopic strain plotted against the surface to volume ratio of the tested specimens, according to Equation 6.

As a concluding remark, one important issue for estimating *D* with the present method is the quite cumbersome measurement of the dimensions of the specimens since single trabeculae are often irregularly shaped yielding relatively dispersed results (**Fig. 1**). However, these preliminary results are encouraging and suggest that further analysis could give a fundamental insight onto the microstructure of bone tissue, depending on anatomical sites as well as normal or pathologic conditions.

Materials and Methods

Specimen preparation

Thirteen specimens of cancellous bone were extracted from human femur heads withdrawn from five donors (female: age 67, 62, and 61; male: age 65, 60) suffering by moderate coxo-arthritis (CA). Their capita were substituted by hip arthroplasty surgery. From preliminary Dual-energy X-ray Absorptiometry (DXA) a slight degree of osteopaenia was found for three of them. For the other two donors DXA assessed normal values of Bone Mineral Density. We do not used cadaveric femoral heads as controls vs. surgical samples. In fact it seemed important to evaluate the specimens without possible post-mortem changes. Trabeculae

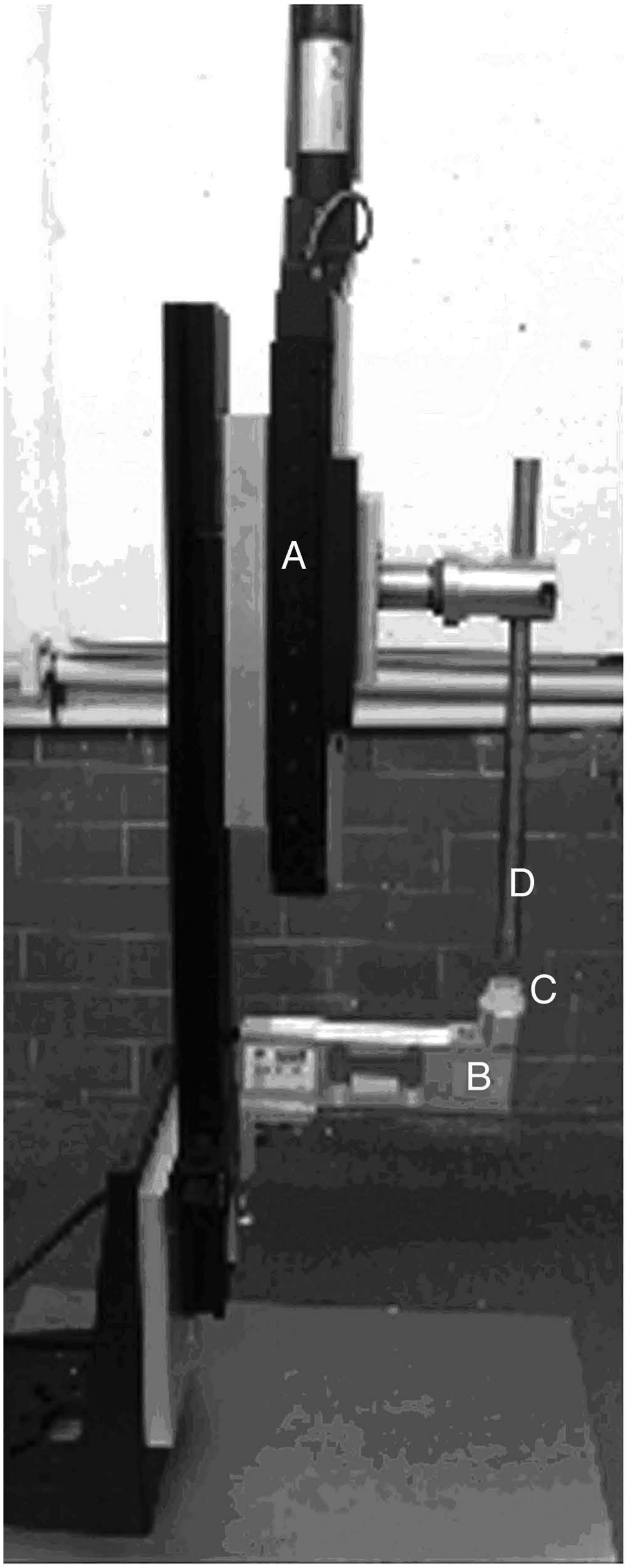

Figure 2. The apparatus for the measurement of swelling of a single trabecula. Micro translation stage (**A**) and tip (**D**); load cell (**B**); water filled reservoir (**C**) for the imbibition of the specimen.

were dissected from slices of about 10 mm corresponding to the frontal plane in the middle of the femur capita.[19] To our experience, the trabecular patterns of the withdrawal site appeared not substantially altered.

Measurement of swelling

Referring to **Figure 2**, to measure the free expansion of the tissue during the imbibition, we improved a previously released MicroTensile Device (MTD)[20] designing an apparatus (APP) composed by a microtranslator (**Fig. 2A**) (Physik Instrumente M-410 DG, 0.1µm repeatability) and a strain gage load cell (**Fig. 2B**) (Vishay M1042, 50N full scale). The apparatus was mounted in vertical position in order to accommodate a cylindrical reservoir (**Fig. 2C**) of about 10 ml onto the free end of the load cell. An average value ± standard deviation of (0.1 ± 0.01) N of preload was imposed to assure proper but gentle fixation of the specimen between the load cell and the tip (**Fig. 2D**) of the microtranslator.

The specimen was then rapidly and totally submerged in distilled water. As soon as the specimen begins to swell the microtranslator is moved upward by a feedback loop which actively maintains the applied preload. The swelling over time is measured as the travel of the microtranslator required to maintain the preload at its constant value. In this way, the APP operates as a high sensitivity dilatometer. For each specimen, we measured the swelling along three natural axis (length L, width W and thickness T) of the trabecula.

The experiments were conducted at room temperature and relative humidity (41 ± 3%RH and 27 ± 1 °C). Dimensions of the dissected trabeculae were measured with a vernier caliper (resolution 0.05mm) prior to each test.

Theoretical framework

Diffusion

The moisture concentration over time within a porous sample of arbitrary shape can be expressed after[21] by Equation 1, for solids of arbitrary shape in the neighborhood of time zero.

$$\frac{c-c_0}{c_s-c_0}=\frac{2}{\sqrt{\pi}}\frac{S}{V}\sqrt{Dt}-K\left(\frac{S}{V}\right)^2 Dt \qquad (1)$$

where S/V is the surface to volume ratio of the sample, c is the moisture concentration, $c_s=c|_{t\to\infty}=c_\infty$ is the surface moisture concentration, $c_0=c|_{t=0}$ is the initial moisture concentration, D is the diffusion coefficient and K is a sample-dependent constant. Equation 1 can be rewritten to point out the mass uptake of water by the sample,[22] neglecting higher order terms:

$$\frac{M_t}{M_\infty}=\frac{2}{\sqrt{\pi}}\frac{S}{V}\sqrt{Dt} \qquad (2)$$

M_t and M_∞ are the mass uptake at time t and for $t\to\infty$.

Swelling

The amount of hygroscopic strain ε_h due to swelling is normally assumed to be linearly proportional to the moisture content $c(g/m^3)$ by the coefficient of expansion[23-27] $\beta(m^3/g)$

$\varepsilon_h = \beta c$ (3)

The linear relationship between swelling and mass uptake is even confirmed by the model proposed by[28] that was even utilized for the diffusion and swelling of chitosan acetate tablets.[29] For $t \rightarrow \infty$, i.e., when the sample is fully saturated, one must have

$\varepsilon_\infty = \beta c_\infty$ (4)

Equations 1–4 yield

$$\frac{(c-c_0)}{(c_\infty - c_0)} = \frac{M_t}{M_\infty} = \frac{(\varepsilon_h - \varepsilon_{h0})}{(\varepsilon_\infty - \varepsilon_{h0})} = \frac{\Delta\varepsilon_h}{\Delta\varepsilon_\infty} \quad (5)$$

so we can express the relationships between the first tract of hygroscopic strain of the trabeculae and the diffusion coefficient over time:

$$\frac{\Delta\varepsilon_h}{\Delta\varepsilon_\infty} = \frac{2}{\sqrt{\pi}}\frac{S}{V}\sqrt{Dt} = m_\varepsilon\sqrt{t} \quad (6)$$

in which m_ε is the slope of the normalized swelling vs. $\sqrt{t}$ (**Fig. 3**). The validity of the approach is further confirmed by a comprehensive review[30] and a recent measurement of diffusion coefficient of collagen-like materials by measuring their swelling rate.[31]

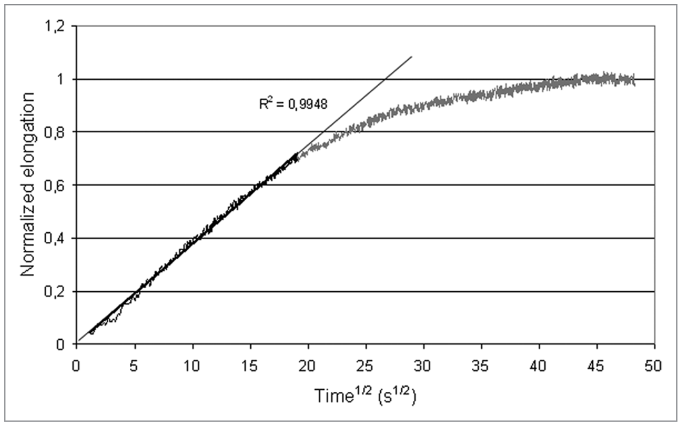

Figure 3. Typical diagram showing the elongation of a trabecula over time$^{1/2}$. Superimposed to the experimental trace (gray) was placed the linear fit of the initial tract, showing a linear dependence (black).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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