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Contribution of Fluid in Bone Extravascular Matrix to Strain-Rate Dependent Stiffening of Bone Tissue – A Poroelastic Study

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Contribution of Fluid in Bone Extravascular Matrix to Strain-rate Dependent Stiffening of Bone Tissue – A Poroelastic Study

Abstract

Osteoporotic fractures represent an increasing cost to society, and its diagnosis methods based on bone density still lack accuracy in identifying risk of fracture. This is why a better understanding of mechanical behavior of bone tissue is of importance, especially when it comes to relating experimental observations to realistic physiological fall loading conditions. This study aims at exploring the stiffening effect of pore fluid in bone extravascular matrix subject to high strain rate loading that is more realistic to simulate a physiological fall. A computational approach is used, where bone tissue microstructure extracted from micro-CT images is modeled using finite elements. The solid phase of bone tissue is modeled as a poroelastic material, a porous matrix filled with fluid. When the extravascular matrix experiences certain volumetric deformation, the fluid in pores presents load carrying capacity, which consequently varies the apparent stiffness of bone tissue. It is shown that effects of fluid stiffening in bone can be significant, depending on the chosen material properties, the amount of volumetric strain in tissue and the loading rate with respect to hydraulic conductivity and drainage conditions. It is also shown that such stiffening effect is influenced by bone microstructure, and is more significant in cortical bone than in trabecular bone.

Keywords: Bone mechanics; Poroelasticity; Hydraulic stiffening; Strain rate; Tissue microstructure.
1. Introduction

Bone is a porous medium filled with fluid, which can be found in pores at several length scales ranging from millimeters to approximately 1 micrometer. Trabecular bone, often found in the head of long bones and in vertebrae, is formed of a spongy structure. The solid matrix of the trabecular bone is formed by trabeculae that are spatially connected and its void is filled with bone marrow. The average distance between trabeculae is found to be of dimension of 0.5-1 mm (Ulrich et al., 1999). The vascular porosity of cortical bone, i.e. the denser bone forming the outer shell of long bones, is formed of microscopic canals of dimension in the order of 100 µm (Wang et al., 2003). The solid phase of these two types of bone tissue, often referred to as bone extravascular matrix, can also be seen as a porous medium, although at a lower length scale. Its porosity, often referred to as the lacunar-canalicular network, is formed by the lacunae containing osteocytes (of a dimension between 5-20 µm (Dong et al., 2014)) and spatially inter-connected by small channels, the canaliculi, of a diameter between 0.2-0.5 µm (Varga et al., 2015).

Osteoporosis, the loss of bone mass density with age, is a major health issue whose socio-economic cost increases with aging population. Current approaches against osteoporosis-related fractures include prevention of falls, exercise, nutrition and the administration of anabolic or antiresorptive drugs (Kanis et al., 2013). Clinical diagnosis relies on measurement of bone mineral density (BMD) using Dual-energy X-ray absorptiometry (DEXA) which can be further enhanced by patients risk factors, FRAX (Kanis et al., 2008), or the analysis of bone turnover biological markers (Kanis et al., 2013). Unfortunately these methods present certain limitations. BMD, for example, which is an index that takes into account bone size, porosity and mineral density, is somewhat incapable of accounting for the differences in bone loss pattern at the
microstructural scale (Seeman and Delmas, 2006). Quantitative computed tomography micro finite element (micro-FE) models have been proved to provide more accurate prediction of femoral strength than BMD by accounting for bone microstructure (Dall’Ara et al., 2013).

Fractures in osteoporotic bone usually occur after a fall at high loading rate. Although experimental measurement of strain in bone during fall conditions remains challenging (Grassi & Isaksson, 2015), recent development of drop tower tests, where a mass is dropped onto a femur from a certain height in order to reproduce a fall configuration (Gilchrist et al., 2013), has led to an estimate of strain rates above 20 s\(^{-1}\) (Ariza et al., 2015). Experiments on tissue samples of trabecular (Carter and Hayes, 1977) and cortical bones (Cloete et al., 2014; Hansen et al., 2008; McElhaney, 1966) have shown a notable strain rate dependency of bone elastic modulus. Although early studies determined a logarithmic relationship between the apparent Young’s modulus and strain rate (Carter and Hayes, 1977; McElhaney, 1966), some studies reported a drastic transition in strain rate sensitivity over a critical range of strain rate, over which the apparent stiffness of tissue increases significantly (Cloete et al., 2014; McElhaney, 1966). Nanoindentation of bone matrix has also shown such an increase of the derived Young’s modulus with respect to strain rate (Fan and Rho, 2003; Isaksson et al., 2010).

Incorporating such strain rate dependency into Finite Element models to assess bone quality can be done through the use of empirically derived viscoelastic models (Cloete et al., 2014; Johnson et al., 2010; Sasaki et al., 1993). The empirical nature of these models, where two or more time constants are determined to fit the experimental results, do not allow discriminating different physical relaxation mechanisms, which may include fluid motion in canals and viscoelasticity of collagen fibers (Lakes and Katz, 1979). It is worth noting that such physical mechanisms might be affected, to different extents, by tissue microstructure, loading and boundary conditions of the chosen sample. Although it has been
reported that collagen viscoelasticity could result in time dependent behavior in bone, the role of fluid phase in bone at high strain rate still remains unclear.

On one hand, there has been a long standing debate concerning whether or not bone, at organ level, could undergo hydraulic stiffening as a result of marrow pressurization (Bryant, 1995). Experiments where small holes were drilled in canine femoral heads in order to disrupt the fluid boundary conditions showed a significant reduction in bone stiffness due to altered fluid drainage (Ochoa et al. 1991). It has however been advanced that their loading scenario lacked relevance to physiological loading conditions (Bryant, 1995). Other experiments have shown a clear increase in stiffness and strength of trabecular bone samples at high strain rate, due to the presence of bone marrow, under undrained and confined compression tests (Carter and Hayes, 1977). But once again, the relevance to physiological loading conditions could be discussed. On the other hand, at the nanometer scale, bone mechanical properties clearly vary depending on its water content. Indeed, several nanoindentation studies (Guidoni et al., 2010; Wolfram et al., 2010) have reported higher elastic moduli for dehydrated compared to hydrated samples, which demonstrates an influence of water content in the mechanical properties of bone tissue. Based on the aforementioned experimental evidence from the literature, it is therefore important to investigate the role of fluid phase in the bone mechanical behavior, particularly at high strain rate, which is crucial for assessment of fracture risk in realistic fall conditions.

In this study, poroelasticity is employed, with the aim of understanding the physical mechanism of the stiffening effect in bone tissue subject to high strain rate, to explore how fluid in the bone extravascular matrix affects its time dependent behavior. Modeling of poroelasticity in human tissues has been extensively investigated for cartilage (Mow et al., 1984). Bone is a much stiffer material however, with a Young’s modulus
much higher than cartilage, and it is expected that pore fluid might have a different effect on its macroscopic mechanical behaviour. We use a micro-FE approach based on bone microstructures to study the interplay between the poroelastic behavior of bone extravascular matrix and bone microstructure at tissue scale. We then further investigate how fluid phase affects bone apparent stiffness at different strain rates. It is worth mentioning that although a poromechanics approach has already been used in literature to model bone tissue (Cowin, 1999; Hellmich and Ulm, 2005; Sandino et al., 2015) either from a theoretical or computational perspective, it is still unclear how various factors, such as the wide range of existing material properties, boundary conditions, loading rates, and tissue microstructures, affect the assessment of bone mechanical behavior, especially under high strain rates. Therefore, a key issue in this study is to determine the loading and boundary conditions relevant to physiological conditions that may better reflect the tissue behavior in vivo, for numerical investigations as well as experimental setups. Quantifying the stiffening effect of bone fluid in its apparent properties, depending on the aforementioned factors, could therefore lead to an improved assessment of bone fracture risk under realistic loading scenarios.

2. Materials and Methods

2.1. Poroelasticity formulation of the bone extravascular matrix

When applying mechanical loading on a porous microstructure filled with fluid, fluid sustains part of the load due to pressure increase induced by pore compression. Fluid within the bone extravascular matrix then flows driven by pressure gradient, following Darcy's law. The constitutive equation reads as (Cowin, 1999)
\[ \sigma_{ij} + \alpha p \delta_{ij} = K_d \varepsilon_{kk} \delta_{ij} + 2G_d \left( \varepsilon_{ij} - \frac{1}{3} \varepsilon_{kk} \delta_{ij} \right) \] (1)

where \( K_d \) and \( G_d \) are the drained bulk and shear moduli of the porous material, \( \alpha = 1 - \frac{K_d}{K_s} \) the Biot coefficient, \( K_s \) the bulk modulus of the solid phase, and \( p \) the pore pressure of fluid. Eq. (1) shows that fluid pressure affects the volumetric behavior of the material, through an additional term to the volumetric part of the apparent stress tensor. The shear behavior therefore remains unchanged subject to change of fluid pressure. In materials such as cartilage or soils, where poroelasticity is usually used, the porous matrix (i.e. sand) is highly compressible compared to its individual solid (i.e. grains) and fluid (i.e. water) components, therefore the components (i.e. grains and water) can then be assumed to be incompressible. In bone, however, the compressibility of the extravascular matrix is of the same order of magnitude as its individual constituents (i.e. fluid and solid phases), whose compressibility should therefore be accounted for.

The fluid content \( \zeta \) (variation of fluid volume per unit volume) is related to the change in stress and pore pressure, under drained conditions, through the following equation

\[ \zeta = \frac{\alpha}{3K_d} \left( \sigma_{kk} + \frac{3p}{B} \right) \] (2)

where \( B = \frac{\alpha K_f}{(\alpha - \phi(1-\alpha)) K_f + \phi K_d} \) is the Skempton pore pressure coefficient, where \( \phi \) is the porosity, and \( K_f \) the bulk modulus of the fluid.

Finally, the constitutive equations of poroelasticity are coupled with fluid flow through Darcy’s law, as
\[ q = -K_w \nabla p \quad (3) \]

where \( q \) is the flux (discharge per unit area) and \( \nabla p \) the pore pressure gradient. \( K_w = \frac{\kappa}{\mu} \), the hydraulic conductivity, is a function of the intrinsic permeability \( \kappa \) of the porous media as well as the fluid viscosity, \( \mu \).

### 2.2. Bone samples and imaging processing

In this study the data of bone tissue are from studies carried out by (Baruffaldi and Perilli, 2003; Perilli and Baruffaldi, 2003), where the bone specimen was taken from the femoral neck of a 61 years old male. The sample was scanned with a resolution of 19 \( \mu \)m. After applying a mean filter (1 pixel in radius), the images were segmented using a threshold of 75\% of the maximum grey value. Four cubic subregions with a side length of 1.3 mm were extracted from the cortical shell, and three with a side length of 3 mm were extracted from the trabecular core, as illustrated in Fig. 1. The samples are then meshed in ScanIP (Simpleware Ltd, Exeter, UK) using quadratic tetrahedral elements.

### 2.3. Micro-FE modeling

#### 2.3.1. Material properties

The micro-FE models were solved in ABAQUS (Dassault Systemes, Vlizy-Villacoublay, France) using the built-in coupled pore fluid flow and stress analysis, which is based on Eq. (2-4). Specific tetrahedral elements with additional degrees of freedom for pore pressure have been used (C3D10MP). Isolated collagen fibrils have been shown to exhibit a viscoelastic behavior which can be described by two time constants in the order of magnitude of 7 s and 100 s (Shen et al., 2011). Collagen viscoelasticity is therefore expected to contribute to the time dependent
behavior of bone tissue. However, the primary aim of this study is to explore the contribution of pore fluid to time dependent behavior of bone tissue, and the extravascular matrix is therefore assumed to be elastic and viscoelastic effect due to collagen is not modeled here. Poroelastic parameters were assigned to the extravascular matrix (as seen in Fig. 2), for which the range of values found in the literature is summarized in Table 1.

A wide range of values can be found in the literature for bone elastic modulus, which depends on the degree of mineralization, anatomical location, hydration state and direction of testing, as well as the measurement methods. Bending, buckling and tensile testing of single trabeculae often results in elastic modulus in the range of 1-15 GPa (Carretta et al., 2013). Such methods present the advantage of retrieving the average mechanical properties including bone packets and lacunar-canalicular porosities. It is however sensitive to trabecula geometry, which can lead to certain error if not properly accounted for in the theoretical/numerical model used (Lucchinetti et al., 2000). Moreover, moduli derived from loading experiments tend to underestimate the elastic modulus, as it includes inelastic effects (Luczynski et al., 2015). Elastic moduli reported from nanoindentation tests range from 10 to 25 GPa. This technique probes a very small volume within a single bone packet and is sensitive to the preparation protocol (Carretta et al., 2013). In this study, an intermediate value of 15 GPa for the Young’s modulus is adopted, except in the sensitivity analysis where a range of Young’s moduli from 5 to 20 GPa is used. Poisson’s ratio, on the other hand, has received less interest in literature up to now. A Poisson’s ratio of 0.3 is commonly assumed for the analysis of nanoindentation (Zysset, 2009), as well as in micro-FE models (van Rietbergen et al., 1995). Experimental measurement of Poisson’s ratio of the extravascular matrix is very challenging due to its small length scale. Theoretical approaches have been used to estimate the Poisson’s ratio (Blanchard et al., 2013; Cowin and Sadegh, 1991),
which lead to higher values in the range of 0.32-0.35. An indirect way to determine Poisson’s ratio experimentally is to analyze microindentation results using a poroelastic framework, which lead to lower Poisson’s ratio of 0.2 (Oyen et al., 2011).

It has been shown in the literature that bone extravascular matrix exhibits a certain degree of anisotropy in elasticity, with a transverse elastic modulus 30-40% lower than the axial one (Fritsch & Hellmich, 2007; Mirzaali et al., 2015), which is due to a preferred orientation of mineralized collagen fibrils in the extracellular matrix. Poroelastic properties exhibit anisotropy as well (Hellmich, Celundova, & Ulm, 2009; Hellmich & Ulm, 2005). Such anisotropy at the nano- and micro-scale is likely to affect the mechanical properties of bone tissue at the macroscale. However, it is challenging to incorporate such anisotropy in a numerical multiscale scheme, especially for trabecular bone samples. It is due to the fact that the anisotropy is likely to differ depending on the location in the bone tissue sample, and more importantly, the correlation of the orientation of the anisotropic stiffness tensor between the experimental set up and the numerical analysis is a difficult task. For the sake of simplicity, in this study, material properties are assumed to be isotropic.

Usual ways of measuring porous materials permeability, i.e. by measuring the fluid flow induced by a pore pressure gradient, cannot be effectively used to measure the permeability in the lacunar-canalicular network due to its small length scale. Permeability has therefore been derived from theoretical studies and from those involving both experimental measurements and analytical/numerical approaches. Resulting estimates span across several orders of magnitude \(10^{-25} \text{ to } 10^{-17} \text{ m}^2\), with higher permeability estimated by the theoretical approaches than combined experimental/theoretical approaches (Cardoso et al., 2013). The fluid in the lacunar-canalicular porosity is assumed to be equivalent to salt water, with a bulk modulus \(K_f=2.3 \text{ GPa}\) (Cowin, 1999).
2.3.2. Loading and boundary conditions

1% axial compressive strain is applied along the loading direction, which corresponds to the elastic range reported in the literature (Bayraktar et al., 2004). Eight different strain rates, ranging from $10^{-4}$ to $10^3$ s$^{-1}$, are used. The results are analyzed by computing the apparent stress along different directions. This apparent stress is calculated by summing the reaction forces at all FE nodes on the boundary, and divided by the total surface area (including solid and pores surfaces). For the purpose of comparison, an elastic analysis, using the drained elastic properties, i.e. $E_d$ and $v_d$, is carried out for each sample in order to compare the resulting apparent stresses with and without taking into account the stiffening effect of fluid.

From the constitutive equations, i.e. Eqs. (1-2), two extreme cases can be defined for fluid boundary conditions. When the material is loaded slowly compared to the time required for the fluid to drain from the sample and for the pore pressure to reach equilibrium, the pore pressure remains constant (i.e. drained conditions). Such behavior can be characterized by means of the drained elastic properties, $K_d$ and $G_d$ (or $E_d$ and $v_d$). When the boundaries of the material are ‘sealed’, or when the loading is applied so quickly that water does not have time to drain out (i.e. undrained conditions), the undrained bulk modulus ($K_u$) is defined as (Cowin, 1999)

$$K_u = K_d \left(1 + \frac{\alpha^2 K_f}{\phi K_d + (1 - \alpha)(\alpha - \phi)K_f}\right) \tag{4}$$

which shows that only the bulk modulus is affected in poroelasticity under such conditions. From Eq. (4), it can be seen that the parameters affecting undrained behavior include: the ratio between the drained bulk modulus and the bulk modulus of the solid constituent (through the Biot
coefficient $\alpha = 1 - \frac{K_d}{K_s}$, the porosity $\phi$, the drained bulk modulus $K_d$, and the bulk modulus of water $K_f$. Using the chosen values in Table 1, the drained and undrained elastic properties of bone extravascular matrix are, respectively, $K_d = 12.5$ GPa, $E_d = 15$ GPa and $\nu_d = 0.30$ and $K_u = 15.2$ GPa, $E_u = 15.36$ GPa and $\nu_u = 0.33$.

For the FE analysis, mechanical and fluid boundary conditions need to be applied on the sides of the volume samples $\partial \Omega$, as shown in Fig. 2. Mechanical boundary conditions, i.e. how the displacements are constrained at the boundary, are critical for assessment of bone behavior. Indeed, liquid phase affects mainly the volumetric behavior of bone extravascular matrix as seen in Eq. (1), and its effect is therefore related to the amount of volumetric deformation in the extravascular matrix induced by external loading. The amount of volumetric deformation induced is therefore crucial to fluid pressurization, and consequently to the apparent elastic properties. Two extreme cases are employed: the first one being a confined compression test where no lateral displacement is allowed, leading to higher volumetric deformations; and the second one being an unconfined compression test, where the sample is free to deform along the lateral directions, resulting in a relatively low volumetric deformation.

For the fluid boundary conditions, on the other hand, it is proposed not to explicitly model the fluid phase in the tissue microstructure, but to incorporate its effect through the application of boundary conditions at the interface ($\partial \Omega_f$, as illustrated in Fig. 2) between the extravascular matrix and the vasculature/marrow cavity. This is based on the assumption that the time scale required to reach pressure equilibrium in the vasculature is much lower than the one for the extravascular matrix (Cowin and Cardoso, 2015). The fluid pressure state at tissue level depends on deformation and fluid flow induced by loading conditions at higher length scale, and could be obtained from a poroelastic analysis of bone at
its organ level. It is yet another complex problem, and no consensus has been reached concerning whether whole bone should be considered drained (Cowin and Cardoso, 2015) or undrained (Hellmich and Ulm, 2005) under physiological loading. We therefore considered both extreme cases to provide possible lower and upper bounds for the bone behavior. The first type of fluid boundary conditions assumes a completely drained case, with the pore pressure remaining zero in the marrow cavity/vasculature, and is modeled by applying a zero pore pressure condition at the interface (\(\partial \Omega\), Fig. 2). In contrast, the second type of fluid boundary conditions assumes a completely undrained case, where the fluid cannot flow out of the marrow cavity. It is therefore also a ‘pressurized’ case and is thus modeled by applying a non-flux condition at the interface. It is worth noting that the ‘drained’ and ‘undrained’ conditions described in this paragraph and the following are related to the hydraulic boundary conditions used in the Finite Element model.

All aforementioned boundary conditions (including for both fluid and solid) are then combined in order to reproduce 3 representative experimental set-ups often used in experimental studies at bone tissue scale reported in the literature, which are summarized in Fig. 3. Firstly, a confined compression test with drained fluid conditions corresponds to experiments carried out by (Carter and Hayes, 1977) on samples from which bone marrow has been removed. Secondly, an unconfined compression test with drained fluid conditions corresponds to boundary conditions in simple compression and Hopkinson bar testing (Cloete et al., 2014; McElhaney, 1966). Finally, a confined compression test with undrained fluid boundary conditions corresponds to experiments carried out by (Carter and Hayes, 1977) on samples with bone marrow at high strain rate. Studying these various combinations of fluid and mechanical boundary conditions enables critical investigation of the effect of various strain states and drainage conditions, which are not well known under physiological conditions \textit{in vivo}. It is of importance to study the
effect of these conditions for both fluid and solid phases, in order to help design meaningful numerical models and experimental set-ups which better represent *in vivo* physiological conditions of the chosen bone samples.

3. Results and discussion

In this section, it is first analyzed how pore fluid induces changes in apparent stiffness in bone subject to various strain rates. A sensitivity analysis to the poroelastic parameters of bone extravascular matrix is carried out. Similarly, results derived from using three sets of boundary conditions are compared, and the effect of poroelasticity in different bone microstructures is investigated.

3.1. Strain rate dependency

Fig. 4 shows the evolution of three stress components with respect to various strain rates, for four cortical bone samples undergoing a confined drained compression test. Fig. 4a shows the evolution of the axial ($\sigma_{zz}$) and lateral ($\sigma_{xx}$ and $\sigma_{yy}$) stresses with varying strain rates. The apparent values of the three stress components rise when the strain rate increases. Most of the change appears to occur within a certain range of strain rates (between $10^{-2}$ and $10^{0} \text{ s}^{-1}$ in the scenario using chosen parameters), below and above which the apparent stress remains constant. A similar trend has been reported in experimental work on bovine and human cortical bone, where a drastic change in apparent stress over a relatively small range of strain rate was observed (Cloete et al., 2014; McElhaney, 1966). However, results from these two studies also showed an increase of the stress at lower strain rates, although much smaller, which could not be captured by our model. This could be due to a secondary time-
dependent mechanism related to collagen viscoelasticity, which has not been accounted for in the proposed model, since the aim of this study is to quantify the contribution of poroelastic stiffening to the time dependent behavior of bone tissue.

Fig. 4b shows the apparent stress components normalized by the ones obtained from the elastic analysis (without taking into account fluid stiffening effect). This allows for a comparison of poroelastic effects for different microstructures, parameters and loading conditions in a unified framework. Although in Fig. 4a the fluid at high strain rate results in an increase in apparent stress similar in all directions, the lateral directions (i.e. x and y), for which the absolute value of the apparent stress is lower, are proportionally more affected, as seen in Fig. 4b.

Similar results are obtained from trabecular bone samples, as shown in Fig. 5. Poroelastic effects on bone apparent stress under higher strain rate are lower than those in cortical samples, and the strain rate required to stiffen the bone is higher ($10^1$ to $10^2$ s$^{-1}$) than for cortical bone ($10^2$ to $10^0$ s$^{-1}$). Results on trabecular samples also appear to show a higher degree of anisotropy, presenting notable difference between the two lateral apparent stress in Fig. 5a, as well as different stress increase at higher strain rate in Fig. 5b.

As aforementioned in Table 1, a wide range of values for the poroelastic properties can be found in the literature. It is therefore of particular interest to investigate the sensitivity of poroelastic stiffening to the parameters which exhibit high variability, i.e. the elastic properties of the extravascular matrix (i.e. $E_d$ and $v_d$), and hydraulic conductivity $K_w$. The different cases are compared using the apparent stress along the loading direction (i.e. z). Figs. 6a and b. illustrate the sensitivity to elastic properties of the porous matrix, i.e. Young’s modulus and Poisson’s ratio, respectively, using one of the cortical samples (C1) as an exemplar. It has been shown in Eq. (4) that the fluid phase mainly affects the bulk modulus, related to the compressibility of the extravascular matrix. Parameters reported in the literature are, however, usually the Young’s
modulus and Poisson’s ratio, which are functions of both the bulk and shear moduli. The volumetric change of the extravascular matrix is contributed by both solid and pore phases, depending on the relative compressibility between the extravascular matrix and its solid phase. When the extravascular matrix is relatively compressible compared to its solid phase, which corresponds to low values of Young’s modulus and Poisson’s ratio, the solid phase volume remains constant (i.e. incompressible) and volume changes mainly happens in pore phase, which increases the influence of fluid in the apparent stress at high strain rate. In contrary, when the compressibility of the porous matrix is of the same order of magnitude as the one of the solid phase (i.e. when the Young’s modulus and Poisson’s ratio of the extravascular matrix is higher), such effect becomes negligible. This is due to the fact that the solid phase of the porous matrix experiences some volumetric reduction, therefore the volumetric changes of pore phase becomes less, reducing the level of pore pressure increase and subsequently the load bearing contribution of the fluid phase. Fig. 6c shows the sensitivity to hydraulic conductivity, which has little effect on the undrained apparent properties at high strain rate. It however changes the strain rate at which stiffening effect starts to become significant.

This sensitivity to poroelastic parameters has to be kept in mind when using parameters obtained from experiments in micro-FE models, and one should determine to which poroelastic parameter the measured apparent properties correspond. Apparent elastic properties measured experimentally depend on the loading rate, and can correspond either to drained or undrained conditions. It is therefore important to assess whether the experiment corresponds to a drained or undrained scenario, with respect to loading rate and hydraulic conductivity as well as experimental set-ups. It has also been shown that poroelastic parameters obtained from indentation data differ between nano- and micro-indentation (Oyen et al., 2011), and for modelling purpose one should therefore adopt experimental data measured at the correct scale. Results
presented in Fig. 6 highlight the fact that accurate estimation of bone poroelastic parameters is crucial for assessing fluid stiffening effects on bone mechanical behavior, since elastic properties appear to be critical in determining the amount of stiffness increase at high strain rate due to poroelastic stiffening effect.

3.2. Effect of bone microstructure

Fig. 7 shows the evolution of the axial stress along the loading direction in different samples with respect to increasing strain rate. The normalization using the stress from the elastic case allows a comparison of poroelastic effects, for samples having different elastic apparent stiffness due to their different microstructures. It can be seen that cortical and trabecular samples exhibit very distinct behaviors. The range of strain rates over which apparent stiffness starts increasing due to poroelastic effect is significantly different between cortical and trabecular samples. Trabecular samples require higher strain rates to trigger poroelastic stiffening effects, as shown in Fig. 7, which is thought to be due to a shorter distance to the drainage interface, thus leading to a shorter drainage time. This characteristic range of strain rate is expected to be correlated to various microstructural indices such as trabecular width. Furthermore, the stress increase at high strain rate is much higher in cortical samples than in trabecular ones. The denser cortical samples also clearly exhibit higher hydraulic stiffening effects compared to lower density cortical samples. The difference in total increase at high strain rates is thought to be due to different volumetric deformation in the extravascular matrix. The denser the sample, the more volumetric compression the extravascular matrix will experience leading to higher pore pressure increase.
Fig. 8 shows on a single graph the maximum stress increase at high strain rate, as a function of the volumetric deformation of the extravascular matrix, under various sets of boundary conditions. It is shown that different microstructures lead to different volume change in the extravascular matrix, under the same uniaxial loading condition. Cortical bone samples experience higher volumetric change compared to trabecular ones, resulting in higher effect of hydraulic stiffening. These results are consistent with the findings in (Gilchrist et al., 2014), where whole proximal femurs were tested at quasi-static constant displacement rate, as well as using an impact fall simulator. In their experiments carried out at the organ level, it was reported that high density bones present more significant strain-rate stiffening than low density ones. Specimens that showed an increased stiffness under impact conditions have a higher bone mineral density compared to samples that appeared stiffer in quasi-static conditions. From their study, it was proposed that such behaviors are related to hydraulic stiffening due to the presence of bone marrow. Although their experiment was carried out at a higher length scale (i.e. whole bone), the observation of the relationship between hydraulic stiffening and bone density provides a relevant, although indirect, evidence of a possible analogy to the poroelastic stiffening effect examined in this study.

3.3. Effect of boundary conditions

Fig. 9 shows the increase of axial stress normalized by the stress in the elastic case, for cortical bone samples subject to three different prescribed sets of boundary conditions that have been previously discussed in Fig. 3. The undrained confined test (in red), at low strain rate, presents apparent stress higher than the one obtained for the elastic analysis, which is due to the fluid being trapped in the extravascular matrix, resulting in an increase of the pore pressure and subsequently increasing load bearing capacity of the fluid phase. It however does not exhibit a significant
sensitivity to strain rate. The drained confined compression test (in black), on the other hand, has the same mechanical boundary conditions as the previous one, however with permitted fluid transfer between the extravascular matrix and the pore channels. It presents a transition from a behavior where fluid plays little role at low strain rate, to one similar to the undrained case at high strain rate. This is due to fluid transfer, despite being permitted, having insufficient time to exhibit its effect at high strain rate, leading to an undrained apparent behavior. Finally, the unconfined drained compression test (in blue) also exhibits increase in stress as strain rate increases, although much lower than in the confined tests (in black and red). The difference between the results of the confined and unconfined compression tests is due to the amount of volumetric change in the extravascular matrix induced by a specific loading case, as seen in Fig. 8, where it is shown that volumetric change in the extravascular matrix induced by unconfined tests is much lower than in confined tests. This results in less fluid stiffening, since pore fluid mainly affects the volumetric behavior, as seen in Eq. (1).

Fig. 10 shows the increase of axial stress normalized by the stress in the elastic case, similarly to Fig. 9, for trabecular bone samples subject to three different prescribed sets of boundary conditions. The influence of boundary conditions presents similar trends compared to the cortical samples shown in Fig. 9, undrained fluid conditions leading to higher stress at low strain rate, and drained samples exhibiting an increase in stress when strain rate increases. It can also be observed that in the confined cases the stress increase is higher compared to unconfined ones. However, the apparent stress in the undrained confined compression test (in red) is influenced by strain rate, which is not the case in cortical bone in Fig. 9. It is hypothesized to be due to the trabecular microstructure that is indeed much more complex than the cortical one. In trabecular samples, because of the complex microstructure and trabeculae connectivity, local volumetric strain distributes more heterogeneously in the
microstructure, which induces pressure gradient resulting in fluid transfer inside the trabeculae, even under the undrained condition where fluid transfer is prohibited at the interface between solid and marrow cavity. However such fluid transfer in the extravascular matrix would lead to distinct behavior subject to different strain rates – under the high strain rate, such fluid transfer within the extravascular matrix does not have time to respond therefore the pore pressure remains high in the areas of high volumetric deformation thus contributing to the apparent loading bearing capacity; whereas under the low strain rate, such fluid transfer would make pore pressure distribution more uniform so that the loading bearing capacity caused by the high local pore pressure is reduced. Furthermore, it is worth noting that the difference between confined and unconfined conditions (between black and blue curves) in trabecular samples is also less than in cortical bone. Indeed, Fig. 8 shows that the difference in volumetric strain induced between confined and unconfined conditions is less in trabecular samples compared to cortical ones, which results in less difference in the apparent hydraulic stiffening effect.

Results shown in both Figs. 9 and 10 illustrate that the boundary conditions of the micro-FE models have a strong influence in the resulting apparent stiffness of bone tissue, and more importantly, in its strain-rate dependent stiffening effects. It is of particular importance to highlight here that those boundary conditions used in the micro-FE models to evaluate the bone behavior in vivo, as illustrated in Fig. 3, need to be carefully chosen. The boundary conditions that truly represent the in vivo case, are expected to lie in between these extreme cases. For low strain rates, fluid boundary conditions appear to be decisive, and a realistic case should lie in between the drained and undrained cases. For high strain rates, mechanical boundary condition plays a major role in determining the apparent behavior, since the drained and undrained conditions
become equivalent. Therefore at high strain rate the apparent behavior should lie in between the ones observed under confined and unconfined conditions.

In terms of experimental set-up, these results show that different mechanical and fluid boundary conditions could lead to different apparent behavior of bone tissue. Experimental testing set-ups should be carefully designed if one wants to assess poroelastic effects, or the fluid stiffening effect has to be taken into account under certain loading conditions. It is worth noting that the work conducted by (Sandino et al., 2015), who concluded that the relaxation behavior of bone tissue could not be explained exclusively by poroelasticity, was based on unconfined compression tests. Such boundary condition has been shown in our study to be less sensitive to fluid stiffening effect compared to confined ones, which in fact is in line with their findings and once again suggests that importance of the boundary conditions (in both experimental and micro-FE studies) to the mechanical characterization of bone tissue.

4. Conclusion

The aim of this study was to assess the influence of pore fluid in bone extravascular matrix in its mechanical behavior at high strain rate, which is of critical importance to prediction of osteoporotic fractures that usually happen after a fall. However, bone mechanical behavior is often assessed by means of quasi-static experiments, and it remains unclear how the quasi-static response can be extrapolated to be adapted in physiological loading scenarios, and more importantly, what the physical mechanism is that leads to the stiffening behavior at high strain rate except for the intrinsic viscoelasticity of bone tissue. In this study micro-FE modeling on cortical and trabecular bone samples with a poroelastic capability has been carried out in the attempt to model the fluid stiffening effect under high strain rate that has already been observed in
experiments. It is worth highlighting here that the contribution of viscoelasticity of collagen fibers to such strain-rate dependency is acknowledged in this study, however not modeled, in order to investigate the physical mechanism that drives the poroelastic stiffening effect in bone extravascular matrix. The evolution of the apparent stress with various strain rates was studied subject to different loading configurations, sets of material properties and bone microstructures.

Illustrated results have shown that poroelasticity effects can become from negligible to significant in the apparent stiffness of bone tissue, depending on the bone density and the volumetric strain in the extravascular matrix. Poroelasticity of extravascular bone material is likely to have less effect on the mechanical properties of trabecular bone, which confirms previous findings (Sandino et al., 2015). However, its effect on behavior of cortical bone is much higher, especially under boundary configurations that induce volumetric strains. Considering the extravascular matrix as a poroelastic material allows to reproduce time-dependent bone behavior that has been already observed in experiment, such as the increase of apparent stiffness over a certain range of strain rates (Cloete et al., 2014). It is also interesting to note that the poroelasticity effects at high strain rate differs depending on the microstructure, which is in line with what has been observed experimentally (Gilchrist et al., 2014). Such strain-rate dependency in bone apparent stiffness are related to bone density, and more importantly, the distance between pores, which varies the time scale at which hydraulic stiffening can be observed. It also depends on the pore distribution and solid phase connectivity, which may affect local volumetric/deviatoric stress, and consequently the apparent stiffening effect at high strain rates.

The results presented in this study also suggest that the boundary and loading conditions as well as the mechanical properties of bone tissue, need to be carefully chosen when assessing the mechanical behavior of bone tissue, which may significantly affect the apparent stiffening effect
particularly under high strain rates. It is shown that the poroelastic stiffening under high strain rate is significantly affected by the boundary conditions used in micro-FE analysis. Such boundary conditions need to be carefully chosen for the purpose of predicting bone mechanical behavior \textit{in vivo} using micro-FE approach, as well as in the case of designing \textit{ex vivo} experiment for bone tissue characterization. The parameters used to describe the poroelastic stiffening effect in bone extravascular matrix are found to have significant influence in its apparent strain-rate dependent behavior. It is thus critical to assess the applicability of the poroelastic parameters, due to the considerable variation in their values reported in literature. Experimental configuration (e.g. mechanical and drainage boundary conditions as well as the loading rate) and the length scale being measured should be accounted for when investigating the fluid-induced poroelastic stiffening behavior in bone tissue.

This study presents certain limitations. Isotropic poroelastic properties for bone extravascular matrix are used. Bone material exhibits a certain degree of anisotropy and it is expected to result in different poroelastic effects depending on the loading direction, especially in cortical bone, where the direction of anisotropy is expected to be much more homogeneous within a given region of interest, compared to trabecular bone. In addition, only compressive loading condition is considered in this study. Although the rate-dependent trends are expected to be similar in tension, in elasticity, it would be interesting to extend this study to incorporate tensile loading case, which may be relevant to assessment of bone fracture especially in bending. Moreover, the difference between compression and tension loading is expected to be of greater importance to strength analysis. Furthermore, only stiffness is discussed here. Effect of pore fluid on bone strength is a much more complex problem, since it depends on local fluid effects. Further work is therefore required to determine whether bone pore fluid affects bone strength similarly to stiffness.
To conclude, the proposed micro-FE poroelastic framework showed a significant strain-rate dependent poroelastic hydraulic stiffening of bone tissue due to the fluid in bone extravascular matrix, which is influenced by the microstructure of bone tissue, the mechanical properties of the extravascular matrix and the loading scenarios including boundary conditions of both solid and fluid phases. Such strain-rate dependency may become significant under certain physiological conditions, especially for assessment of fracture risk under fall conditions for osteoporotic patients, therefore needs to be taken into account, where relevant, in the mechanical characterization of bone tissue.

Acknowledgements

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References


Figure and Table Caption

Table 1. Range of poroelastic parameters of bone tissue found in the literature and values chosen for this study.

Figure 1. Left: 2D slice of the bone specimen and chosen subregions. Right: four cortical and three trabecular samples are investigated in this study. BV/TV denotes the bone volume fraction (bone volume / total volume).

Figure 2. Poroelastic representation of the bone extravascular matrix and illustration of the fluid and mechanical boundary conditions in micro-FE analysis.

Figure 3. Summary of different mechanical and fluid boundary conditions used in this study.

Figure 4. Evolution of stress components with respect to various strain rates (a), and stress components normalized by the stress obtained from the elastic case (b), for four cortical bone samples (BV/TV=0.97, 0.95, 0.89 and 0.92, respectively).

Figure 5. Evolution of stress components with respect to various strain rates (a), and stress components normalized by the stress obtained from the elastic case (b), for three trabecular bone samples (BV/TV=0.17, 0.20 and 0.16, respectively).

Figure 6. Sensitivity to poroelastic parameters, (a) Young's modulus; (b) Poisson's ratio and (c) Hydraulic conductivity, where cortical bone sample C1 is used here as an exemplar.

Figure 7. Evolution of axial stress with respect to increasing strain rates for different bone microstructures (i.e. four cortical and three trabecular bone samples)
**Figure 8.** Maximum stress increase at high strain rate (100 s⁻¹) as a function of the volumetric deformation in the extravascular matrix.

**Figure 9.** Evolution of axial stress of cortical bone samples with respect to increasing strain rate subject to three different sets of boundary conditions.

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Confined Drained compression test

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Unconfined Drained compression test

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Confined Undrained compression test

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<th>Range</th>
<th>Chosen value</th>
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<tr>
<td>Porosity of the Lacunar-Canalicul networks, $\phi_{LC}$</td>
<td>(Benalla et al., 2014)</td>
<td>$4.51 \pm 2.01%$</td>
<td>4%</td>
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<td>Bulk modulus of water, $K_f$</td>
<td>(Smit et al., 2002)</td>
<td>2.3 GPa</td>
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<td>Bulk modulus of the solid phase, $K_s$</td>
<td>(Smit et al., 2002)</td>
<td>17.66 GPa</td>
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<td>Extravascular matrix drained Young’s modulus, $E_d$</td>
<td>(Carretta, Lorenzetti, &amp; Müller, 2013)</td>
<td>1 – 25 GPa</td>
<td>15 GPa</td>
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<tr>
<td>Extravascular matrix drained Poisson’s ratio, $\nu_d$</td>
<td>(Smit et al., 2002)</td>
<td>0.325</td>
<td>0.3</td>
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<tr>
<td></td>
<td>(Blanchard, Dejaco, Bongaers, &amp; Hellmich, 2013)</td>
<td>0.346</td>
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<td></td>
<td>(Oyen, Shean, Strange, &amp; Galli, 2011)</td>
<td>0.2</td>
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<td>Intrinsic permeability, $\kappa$</td>
<td>(Cardoso, Fritton, Gailani, Benalla, &amp; Cowin, 2013)</td>
<td>10-25 - 10-17 m²</td>
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<td>Dynamic Viscosity of water, $\mu$</td>
<td>(Nauman et al., 1999)</td>
<td>0.001 Pa.s</td>
<td>/</td>
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<tr>
<td></td>
<td>(Abdalrahman et al., 2014)</td>
<td>0.007 Pa.s</td>
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<td>Hydraulic conductivity, $K_w = \frac{\kappa \rho g}{\mu}$</td>
<td>Calculated ($\rho$=1000 kg/m³, $g$=10 m/s², $\mu$=0.001 Pa.s)</td>
<td>10-18 - 10-10 m/s</td>
<td>10-13 m/s</td>
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Highlights of the submitted work include:

(1) Examined rate-dependent role of fluid in bone using a poroelastic micro-FE approach;

(2) Hydraulic stiffening depends highly on bone microstructure;

(3) Poroelastic rate-dependency in bone is more significant in cortical samples;

(4) Hydraulic stiffening in bone depends highly on loading and boundary conditions.

(5) Discussed suitable boundary conditions for in vivo mechanical characterization.