Journal of Biomechanics 48 (2015) 3066–3071

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Water in hydroxyapatite nanopores: Possible implications for interstitial bone fluid flow

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ARTICLE INFO

Article history:
Accepted 23 July 2015

Keywords:
Bone water
Molecular dynamics
Hydroxyapatite
Nanofluidics
Water self diffusion
Slip length

ABSTRACT

The role of bone water in the activity of this organ is essential in structuring apatite crystals, providing pathways for nutrients and waste involved in the metabolism of bone cells and participating in bone remodelling mechanotransduction. It is commonly accepted that bone presents three levels of porosity, namely the vasculature, the lacuno-canalicular system and the voids of the collagen-apatite matrix. Due to the observation of bound state of water at the latter level, the interstitial nanoscopic flow that may exist within these pores is classically neglected. The aim of this paper is to investigate the possibility to obtain a fluid flow at the nanoscale. That is why a molecular dynamics based analysis of a water-hydroxyapatite system is proposed to analyze the effect of water confinement on transport properties. The main result here is that free water can be observed inside hydroxyapatite pores of a few nanometers. This result would have strong implications in the multiscale treatment of the poromechanical behaviour of bone tissue. In particular, the mechanical properties of the bone matrix may be highly controlled by nanoscopic water diffusion and the classical idea that osteocytic activity is only regulated by bone fluid flow within the lacuno-canalicular system may be discussed again.

1. Introduction

Bone is a dense tissue that is composed of a porous matrix saturated by fluid. Its solid phase is composed of a mineral phase (70%), organic matter (20%) and water (10%) (Freemont, 1998). The main component of the mineral part of bone is hydroxyapatite (HAP), whose unit cell is given by the formula ($Ca_{10}(PO_4)_6(OH)_2$), whereas the organic phase is mainly type I collagen.

As the third major component of calcified tissues, the role of water has been studied extensively (Robinson and Elliott, 1957; Timmins and Wall, 1977). We can distinguish two main types of bone water according to its free or bound states. Structural bone water can be a constituent of the mineral phase, occupying the calcium ion coordination sites in the apatite-like crystals (about 35 mg of water per gramme mineral) (Neuman and Neuman, 1958), or can occur as water molecules bound to the organic phase. Free bone water, or bulk water, occupies the different porosity levels of the bone tissue. The movement of this bulk water is known to contribute to bone activity through the transmission of remodelling signals for instance (Fritton and Weinbaum, 2009; Lemaire et al., 2011). Obtaining insight into bone's ability to transport fluid by quantifying the bone permeability is a challenging topic of contemporary bone biomechanics (Oyen, 2008; Gardinier et al., 2010; Lemaire et al., 2012; Cardoso et al., 2013; Pereira and Shefelbine, 2014). It is commonly accepted that bone presents three levels of porosity (Knothe Tate, 2003), which are nested hierarchically one inside another as a set of Russian dolls in microcirculatory pathways (Cowin et al., 2009). The macroscopic porous network corresponds to the vascular (or Haversian) porosity (VP), which consists of the Havers and Volkmann canals (typical diameter of 50 μm). The lacunar-canalicular porosity (LCP) made of the osteocytic lacunae and canaliculi channels contains the osteocytes' stellar network of bone (typical pore size of 100 nm), whereas the smallest porosity level in bone corresponds to the spaces inside the collagen-apatite structure (CAP, typical pore size of 5 nm).

Since it is thought that at this lowest level most of the water is bound to ionic crystals (Wehrli and Fernandez-Seara, 2005; Cardoso et al., 2013) and plays a key role in structuring the apatite mineral (Wilson et al., 2006; Wang et al., 2013), the water flow at

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http://dx.doi.org/10.1016/j.jbiomech.2015.07.025
0021-9290/

this nanometric scale is often simply omitted in bone fluid flow representations, resulting in a two-fold porous treatment of bone poromechanics (Cowin et al., 2009; Rohan et al., 2012). In this paper, we aim to explore the validity of this assumption, *i.e.* whether it is appropriate to neglect a possible interstitial flow within the CAP network. Indeed, if there is mobile water inside bone nanopores, it could modify classical approaches of bone poromechanics and thus change our perception of bone activity. In particular, as discussed in this paper, the water diffusion at the nanoscale engenders a supplementary dissipation when submitting bone tissue to mechanical stimulation, and thus may modify the physical vicinity of the osteocytes.

To support our suggestion that CAP water cannot be ignored, we note that: (i) the typical size of CAP nanopores measured by Holmes et al. (1964) between 5 nm and 12.5 nm, or by Cardoso et al. (2007) between 10 nm and 20 nm, is roughly of the same order of magnitude as the fiber-to-fiber distance of the pericellular matrix that occupies the canalicular pores, being around 35 nm (You et al., 2004); and (ii) for water under normal conditions, the Navier–Stokes equation may remain valid in nano-channels down to typically 1–2 nanometers (Bocquet and Charlaix, 2010). As to the first point, as the osteocyte pericellular matrix is mainly composed of charged glycosaminoglycans molecules, it has been suggested that its electrostatic interactions with water molecules may be important and strongly affect canalicular fluid flow (Lemaire et al., 2008). If so, similar phenomena clearly could be relevant in CAP nanopores as well. As to the second statement, this indicates that, contrary to the classical assertion that in CAP bone water is mainly bound water, water can be theoretically both bound and free at this scale. This paper therefore aims to investigate whether any fluid flow is possible inside the HAP matrix, that is to say at the bone nanoscale. For the purpose of simplification, our analysis is only focussed on nanopores between HAP platelets, and other voids such as those which may exist between the collagen molecules and the HAP crystals are not investigated at this stage. Furthermore, there is currently no clear evidence that this nanoscopic porous network inside the collagen-apatite matrix is fully interconnected. Notwithstanding these restrictions, we propose to develop a numerical investigation to provide information on the state of water inside the bone matrix. We therefore calculate the self-diffusion of water confined between two HAP platelets and estimate if the confinement effect is strong enough to avoid its mobility. Since the relevant pore sizes are nanometric, continuum mechanics theory is not suitable for this purpose and we have thus chosen atomic-level molecular dynamics simulations (MD) to perform this study.

First, we briefly present our strategy based on molecular dynamics. This numerical modelling of nanoscopic HAP-water systems is part of an extensive and general study aimed at quantifying several physical properties of confined water, presented in a companion paper (Pham et al., 2015). Second, the water self-diffusivity is computed for pore sizes in the nanoscopic range of the CAP void sizes. Then a new perspective on bone interstitial water transport by computing a Poiseuille flow in such a nanoporous system is presented. We finally discuss the consequences of these simulations on the role of water in bone properties.

2. Application of molecular dynamics simulations to model a HAP-water layered structure

One of the principal tools in the theoretical study of nanoscopic systems is the method of molecular dynamics simulations. This computational method calculates successive configurations of a molecular system by integrating Newton laws of motion. The first MD simulation of a condensed phase system was performed by

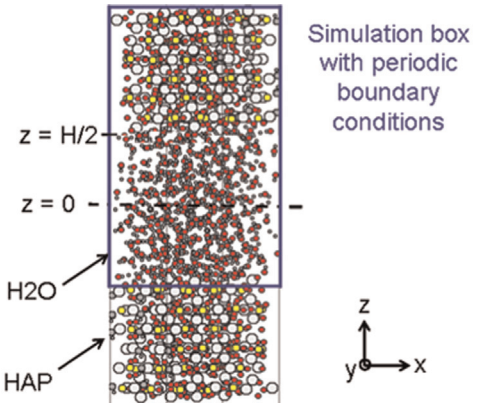

Fig. 1. View of the water-HAP simulation box (Ca=gray, P=yellow, $O_{phosphate}$ and O_{water} = red, O_{OH} = dark gray, H=white). The number of initial water molecules will a posteriori determinate the inter-platelets distance. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

Alder and Wainwright in the late fifties introducing a hard-sphere model (Alder and Wainwright, 1957), which has since led to increasing sophistication in both the interatomic potentials employed and the complexity of the modelled systems.

2.1. Molecular system in the simulation box

Here, we propose to mimic a nanoscopic water-saturated pore of HAP. As shown in Fig. 1, this nanopore is represented by a face-to-face configuration of parallel HAP platelets. The normal direction of these platelets coincides with the third axis of the orthogonal base (**x**, **y**, **z**). The pore size, which corresponds to the inter-platelet distance H, typically varies from 2 to 8 nanometers.These values correspond to the narrowest pore diameters measured by Holmes et al. (1964), that is to say, to the most confined situation for bone matrix water. This geometrical configuration is motivated by the fact that, in bone tissue, hydroxyapatite is present in the form of thin micro-plates with dimensions ($L \times l \times e$), where $L = 250 - 500$ Å, $l = 150$–250 Å and $e \sim 25$ Å (Weiner and Traub, 1986).

The chemical composition of the hydroxyapatite unit cell is given by the formula ($Ca_{10}(PO_4)_6(OH)_2$). Its crystal structure is described as an approximate hexagonal close packed set of spheres, where each sphere represents a tetrahedral PO_4^{3-}ion (Pham et al., 2015). The simulation box consists of a parallelepiped cell with $2 \times 2 \times 3$ slab of HAP crystal and a water layer whose thickness may change. When compared to the two other typical sizes of the hydroxyapatite platelet, the typical thickness of a water-hydroxyapatite layered structure remains small. As a result, the system can be assumed to be infinite mineral and water layers stacked in the z-direction and periodic conditions can be considered at the box edges.

The initial density of water is set at its bulk value under classical room temperature and pressure conditions: $1000\ kg\ m^{-3}$. Cell parameters and crystallographic data of Sudarsanan and Young (1969) are used for the initial configuration of the HAP structure ($a = b = 9.424$ Å, $c = 6.879$ Å, space group $P6_3/m$ symmetry). The inter-platelet distance, *i.e.* the thickness of the water layer, is controlled by the number of water molecules that are put into the initial simulation box. For instance, the initial box presented in Fig. 1 contains 310 water molecules, which corresponds to an inter-platelet distance of approximatively 25 Å after convergence.