Journal of Biomechanics 44 (2011) 304–312

Contents lists available at ScienceDirect

Journal of Biomechanics

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

# Top down and bottom up engineering of bone

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ARTICLE INFO

*Article history:*
Accepted 13 October 2010

*Keywords:*
Solid–fluid interactions
Bone mechanics
Multiscale
Computational modeling
Mechanophysiology
Tissue engineering

ABSTRACT

The goal of this retrospective article is to place the body of my lab′s multiscale mechanobiology work in context of top-down and bottom-up engineering of bone. We have used biosystems engineering, computational modeling and novel experimental approaches to understand bone physiology, in health and disease, and across time (*in utero*, postnatal growth, maturity, aging and death, as well as evolution) and length scales (a single bone like a femur, m; a sample of bone tissue, mm–cm; a cell and its local environment, μm; down to the length scale of the cell's own skeleton, the cytoskeleton, nm). First we introduce the concept of flow in bone and the three calibers of porosity through which fluid flows. Then we describe, in the context of organ–tissue, tissue–cell and cell–molecule length scales, both multiscale computational models and experimental methods to predict flow in bone and to understand the flow of fluid as a means to deliver chemical and mechanical cues in bone. Addressing a number of studies in the context of multiple length and time scales, the importance of appropriate boundary conditions, site specific material parameters, permeability measures and even micro-nanoanatomically correct geometries are discussed in context of model predictions and their value for understanding multiscale mechanobiology of bone. Insights from these multiscale computational modeling and experimental methods are providing us with a means to predict, engineer and manufacture bone tissue in the laboratory and in the human body.



## 1. Background

### 1.1. 25 years—paradigm shift in putative mechanism of mechanoadaptation

Paradigms for understanding bone mechanoadaptation have shifted over the past twenty-five years. Past paradigms emphasized the concept of preprogrammed cells adapting to perturbations about specific strain or stress thresholds, much like a furnace adjusting to a thermostat. This was referred to as the preprogrammed cell stress theory (Rubin and Lanyon, 1984) or the mechanostat hypothesis (Frost, 1987). Current paradigms treat cells as smart micromachines that not only have the capacity to adapt to their spatiotemporally dynamic habitat, but also to modulate the mechanical milieu of their own habitat through production of extracellular matrix (ECM) (Knothe Tate and Niederer, 1998; Knothe Tate, 2001b). In addition to modulating resident cell (osteochondroprogenitors, osteoblasts, osteocytes and osteoclasts) adaptation, mechanochemical signals also influence proliferation, migration and differentiation (as reviewed recently in Knothe Tate et al., 2008, 2010a,c).

Whereas one emphasis of engineering bone is to create microenvironments or habitats for cells (Sorkin et al., 2004; Anderson and Knothe Tate, 2007a,b), recent work emphasizes de novo manufacture of tissue by cells, such as during in utero development and generation of bone in critical sized defects (as reviewed in Knothe Tate et al., 2007, 2008, 2010a,b; Knothe Tate, in press; Knothe et al., 2010). An understanding of bone's remarkable adaptive properties may provide new strategies for the development of smart implants and/or materials (Knothe Tate et al., 2009, 2010a-c, Knothe Tate, in press).

The flow channels in bone provide conduits for fluid flow (Fig. 1), enhancing molecular and cellular transport and inducing shear stresses via fluid drag at cell surfaces (Bassett, 1966; Piekarski and Munro, 1977; Knothe Tate et al., 1998a,b). These flow channels, comprising the nano-micro scale porosity of the cross linked ECM, the pericellular spaces that are imbibed with interstitial fluid, and vascular channels, result directly and indirectly from mechanoadaptation and are essential for cell survival (Fig. 1) (Knothe Tate and Niederer, 1998; Reilly et al., 2001; Knapp et al., 2002; Stegemann et al., 2005; Orr et al., 2006; Humphrey, 2008; Davies, 2009).

### 1.2. The concept of mechanical load-induced fluid flow through bone

Already several decades ago, bone was proposed to exhibit poroelastic properties like a stiff, fluid-filled sponge subjected to physiologic loads (Bassett, 1966; Piekarski and Munro, 1977). Under compression, Piekarski and Munro (1977) hypothesized that pressure gradients force fluid from the small peri-osteocytic spaces of the lacunocanalicular system to the larger spaces of the Haversian Canal

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0021-9290/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jbiomech.2010.10.019

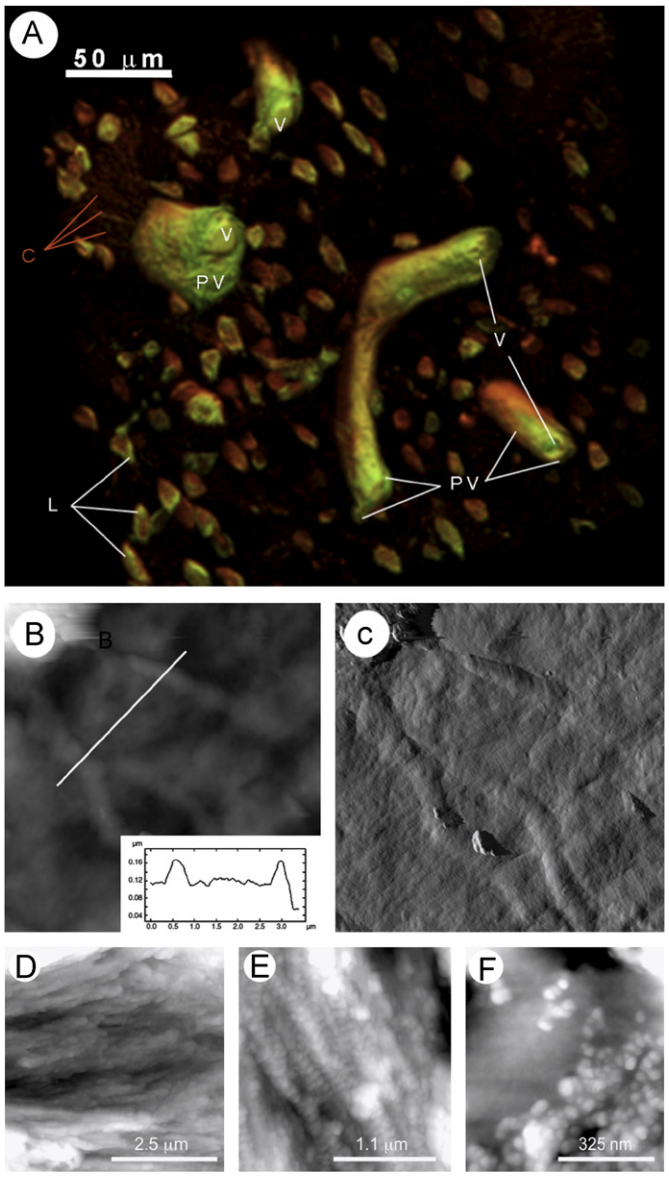


**Fig. 1.** High resolution images of fluid spaces in bone, including the (peri)vascular, (peri)cellular and matrix microporosities. (A) Confocal images of 3000 Da anionic (red) and neutral (green) fluorescent dextran tracer distribution in rat ulna. Different caliber spaces are identified as perivascular (PV), vascular (V), extravascular lacunar (L) and canalicular (C) spaces. (B,C) Images taken with an atomic force microscope in tapping mode, showing canaliculi in relief after etching with hydrochloric acid. The topography (B) and amplitude (C) signal are measured along the white line, producing the relief profile map (inset). (D–F) Atomic force microscope images, taken in tapping mode, of fractured rat bone embedded in polymethylmethacrylate (PMMA), for (D) native bone in PMMA, (E) after treatment with EDTA (an inorganic etching agent) and (F) after treatment with papain (an organic etching agent). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
(A) after Tami et al., 2003a,b; (B–F) after Reilly et al. (2001) and Knapp et al. (2002), used with permission.

where blood vessels reside (Piekarski and Munro, 1977). Conversely, upon relaxation of loading, flow reverses direction. These studies provided the inspiration for much of the work related to the interplay of mechanical loading, mass transport and cellular mechanotransduction described in this manuscript. At the onset of our studies, a body of published work focused on elucidation of streaming potential or electromechanical phenomena in bone (described in more detail in an early survey of fluid flow in bone published in this journal, i.e. see Knothe Tate, 2003; Johnson et al., 1982; Johnson, 1984; Pollack et al., 1984; Salzstein and Pollack, 1987; Petrov et al., 1989; Chakkalakal, 1989; Kufahl and Saha, 1990; Dillaman et al., 1991; Cowin et al., 1991), emphasizing electromechanical phenomena in bone. These early computational and experimental studies provided a foundation for the nascent field of bone fluid flow.

Subsequent theoretical and experimental studies on fluid flow could be shown in bone demonstrated nonlinear effects of loading, where convective fluid flow could result in augmentation of transport through bone or differential transport to cells far from the blood supply (Knothe Tate and Niederer, 1998; Knothe Tate et al., 1998b). Parallel studies explored mechano- and mechanoelectrical excitation of osteocytes by fluid flow in bone (Cowin et al., 1994; Weinbaum et al., 1994). The feasibility of load-induced fluid flow and its role in transport to and from osteocytes could be shown using an analytical approach (Knothe Tate et al., 1995; Knothe Tate and Niederer, 1998), demonstrating the inefficiency of diffusive transport alone. However, the dearth of experimentally measured transport and flow parameters provided impetus to develop methods for not only experimental validation, but also for quantification of fluid flow through bone (Knothe Tate et al., 1995).

### *1.3. Experimental tracer approaches to track molecular transport through bone*

In early studies, enhancement of transport by convection was demonstrated using fluorescent tracers administered intravenously to anaesthetized rats (Knothe Tate et al., 1998a,b). Studies in tibiae subjected to four-point-bending loads (Knothe Tate et al., 2000) and in ulnae exposed to compression (Tami et al., 2003a) showed that mechanical loading enhances tracer transport through the bone (Fig. 2).

### *1.4. Trimodal porosity distribution in bone: matrix, pericellular and vascular porosities*

Seminal tracer studies in the past ten years have shown that the different caliber flow channels in native bone serve as a molecular sieve with mechanoactive filtration properties (Fig. 2) (Knothe Tate, 2001a,b; Tami et al., 2003a; Steck and Knothe Tate 2005). Whereas molecules in the size range of hundreds of daltons readily permeate different caliber bone pores without load-induced fluid flow, larger molecules such as those in the range of 10,000 Da show increased transport when load-induced fluid flow is present.

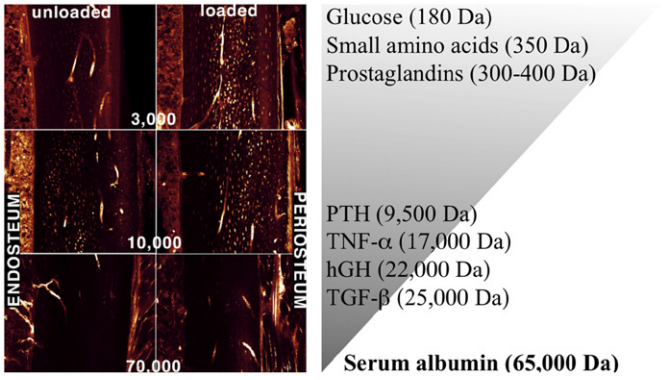


**Fig. 2.** The porosities of cortical bone act as a molecular sieve and convective transport via load induced fluid flow augments transport of molecular tracers through bone. Fluorescent tagged dextran tracers, of molecular weight mimicking those of biological analogs including small molecular weight entities (on right: glucose, amino acids and prostaglandins) and larger weight molecular agents including hormones and growth factors (on right: PTH, parathyroid hormone, etc.), show increased presence in loaded bone. In some cases convective transport by load-induced fluid flow appears necessary for transport to occur at all, e.g. in the case the most prevalent transporter protein in blood and plasma, albumin.
After Tami et al. (2003a), used with permission.