NOVEL *IN SILICO* VIRTUAL & SCALED UP PHYSICAL MODEL PLATFORM TO BRIDGE GAPS IN UNDERSTANDING *IN SITU* FLOW REGIMES AT MULTIPLE LENGTH SCALES IN BONE

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INTRODUCTION

Fluid flow through the pericellular network plays a key role in molecular transport through the dense tissue of bone. Fluid flow also serves as the dominant mechanism by which exogeneous mechanical stimuli are translated to cells and trigger cellular remodeling activity as well as tissue level adaptation. Osteocytes, the putative mechanosensors in bone, are organized in a functional syncytium that provides a biological network for transport and communication within and across bone tissue. Although osteocyte mechanobiology has thrived as a research arena in recent years, we still do not understand the prevailing mechanobiological environment of osteocytes *in situ* or how extrinsic stimuli are transduced at a cellular level. Here we implement a novel approach to bridge gaps in understanding between cell and tissue mechanobiology in bone using *in silico* virtual as well as scaled up physical models; these experimentally validated models give us a novel platform to extrapolate prevailing *in situ* flow regimes around single cells and networks of cells (which, together with their extracellular matrix comprise bone tissue) that are currently impossible to measure or observe directly.

METHODS

In Silico Models:

To explore fluid flow at the level of the cell and within the pericellular space of the lacunocanalicular network, an idealized osteocyte was modeled with multiple connecting canaliculi using a computational fluid dynamics package (CFD-ACE). Fluid flow was induced by a pressure gradient of 300 Pa over the length of the osteocyte with a perfusate medium similar to water (ρ=997 kg/m^3, μ=0.000855 kg/ms). Simulations of steady flow (governed by Navier-Stokes equations) provided velocity and pressure profiles within the fluid space, where shear stress at the surface of the cell and processes was calculated from wall strain rate and laminar viscosity [*after* 1]. In order to address lower length scales, we studied parametrically the effect of subcellular structures on prevailing flow regimes and network permeability. This was accomplished by calculating velocity and pressure profiles as a function of i) the presence and state of the macromolecular network in the pericellular space as well as ii) differences in relative gap size for flow between the osteocyte body–lacuna and the osteocyte process–canaliculus.

Scaled-Up Physical Models:

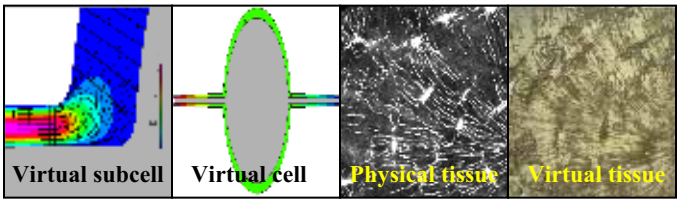
To study fluid flow at the tissue level (flow around networks of cells), experimental tissue permeability measurements were carried out on scaled-up (1000x), anatomically accurate (hollow) physical models of the lacunocanalicular network. Specimens were obtained from the cortical sheath of the femoral neck from human patients undergoing orthopaedic surgery (IRB approved). Hard-tissue histological sectioning and the creation of a three-dimensional image stack using confocal microscopy (SP2 AOBS, Leica) was used as the template for the rapid prototyping of a physical scaled-up (1000x) model. Permeability was tested in the transverse plane of the specimen for both water and viscous silicone oil (1000x viscosity of water, to counter effect of scaling) as the perfusate medium. By incorporating this scaling coefficient, appropriate permeability estimates can be made of the osteocyte syncytium by scaling back down to cellular length scales. Darcy's Law was used to calculate permeability for a specific mass flow rate and pressure gradient [*after* 2].

RESULTS AND DISCUSSION

Cell level *in silico* models show that fluid flow resulting from mechanical loads subjects the virtual osteocyte surface to hydrodynamic pressure of nearly constant magnitude within the lacuna, and high gradients of shear stress along the processes within the canaliculi. Incorporation of subcellular structures into this virtual model reveals a dominant effect of gap size ratio (between fluid gap size within the lacuna and within canaliculi) as compared to the presence of a fluid saturated macromolecular mesh within the pericellular space. Tissue level physical models allow for actual measurements of permeability based on scaled-up, anatomic cell network dimensions. In the scaled-up physical model, permeability is calculated to be $2.78\text{x}10^{-10}$ m^2 with 1.8% uncertainty in the transverse direction using silicone oil. Using the scale-factor relationship, specimen permeability at the cellular length scale is $2.78\text{x}10^{-16}$ m^2 for hollow geometry. Hence, actual permeability can be calculated from model-based experimentally determined permeability using,

$$k_{1x} = \frac{k_{1000x}}{scalefacto\ r^2}$$

The differently scaled models are then bridged by applying the relationship acquired in the sub-/cellular *in silico* models to the experimentally measured data of the hollow physical network model to calculate permeability for a partially (cell and process) filled virtual network model (Figure).



CONCLUSIONS

These studies provide, for first time to our knowledge, a novel platform to elucidate fluid flow within the lacunocanalicular network of bone across length scales, from the subcellular to the tissue level. An accurate description of flow across length scales paves the way for understanding the prevailing mechanical environment of bone cells, which will yield unique insight regarding translation of extrinsic signals to the cellular, tissue and organ level and its role in bone (patho)physiology.

REFERENCES [1] Anderson EJ, *et al*. *Ann Biomed Eng* 33, 52-62, 2005. [2] Anderson EJ, *et al*. Trans *BMES* 2004, 1215.