LACUNOCANALICULAR PERMEABILITY MEASUREMENTS IN HEALTHY & OSTEOPOROTIC PATIENTS: AN EXPERIMENTAL FLUID MECHANICS APPROACH USING SCALED PHYSICAL MODELS

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INTRODUCTION: Osteocytes, the putative mechanosensors in bone, are organized in a functional syncytium that provides a biological network for transport and communication across bone tissue. In healthy bone, the cellular syncytium connects cells deep within bone tissue to cells on bone surfaces and near the blood supply. This so-called lacunocanalicular network allows for transmission of chemical, electrical and mechanical signals between cells that have the "machinery" to remodel tissue (osteoclasts, osteoblasts) and those that have the capacity to affect the population of bone remodeling cells [1]. With the onset and progression of osteoporosis, cellular connectivity decreases and efficiency of signal transmission is expected to decrease as well [2]. Interstitial fluid flow within this lacunocanalicular network is postulated to play a key role in mechanotransduction through modulation of mechanical and chemical signals at the cellular level. However, flow regimes within this space are poorly understood due to their remote location and nano-microscale dimensions. One fluid mechanics approach to understanding flow regimes in very large or small scale systems is to scale down (*e.g.* in the case of a power station pump) or scale up actual flow geometries; experiments are then run on the scaled model to measure flow parameters, which in turn are scaled up or down to determine relevant parameters for the actual scale system. The goal of this study was to create scaled-up physical models from actual cellular syncytia and to measure permeability through the lacunocanalicular network, for specimens obtained from healthy, early osteoporotic and late osteoporotic patients. It was hypothesized that, with the progression of osteoporosis, permeability through the cellular network decreases compared to normal bone.
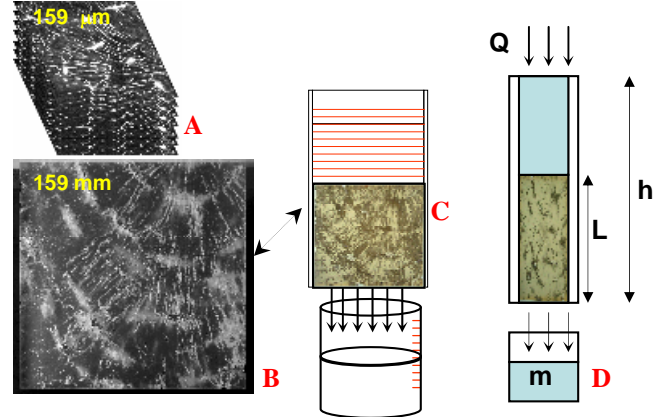
METHODS: *Creation of scaled-up, physical models:* Experimental tissue permeability measurements were carried out on scaled up (1000x), anatomically accurate physical models of osteocyte syncytia diagnosed by a clinical pathologist as normal or osteoporotic. Specimens were obtained from the cortical sheath of the femoral neck from human patients undergoing orthopaedic surgery (IRB approved, exemption #4). The tissue was bulk-stained with basic fuchsin to mark the lacunocanalicular network and processed for hard tissue histological sectioning. An image stack, comprising fifty-four transverse plane images (159 x 159 x 0.53 µm) was obtained for each specimen (Fig. A) using the laser scanning confocal microscope (SP2 AOBS, Leica Microsystems, Mannheim). Each image stack was reconstructed into a digital volume of interest containing the cellular network (Mimics, Materialise Inc., Ann Arbor, MI). Dimensions were then scaled up by 1000 times to yield a magnified version of the volume of interest. A physical model of the volume of interest was created by converting the digital model into a solid resin block with interconnected cellular channels (159 x 159 x 28.5 mm, Fig. B) using a stereolithography rapid prototyping machine (SLA-250, 3D Systems, Valencia, CA).

Permeability measurements: Permeability of the scaled up physical models was tested for each flow direction. The remaining five sides of each model were sealed to eliminate exiting flow; four acrylic sections enclosed these surfaces, with a silicon rubber gasket inserted between the acrylic and bone section to prevent leakage. For the first set of experiments, water was used as the perfusate medium. In a second set of experiments, silicone oil with 1000x viscosity of water (1000 cSt) was used to incorporate the 1000x scaling into permeability measurements. By incorporating this scaling coefficient for all variables in the experiment, appropriate permeability estimates can be made of the osteocyte syncytium by scaling back down to cellular length scales. For each specimen, the mass of fluid that traveled through the section in a given time interval was recorded along with section dimensions, fluid properties, and total pressure head for twenty samples (Fig. C, D). Permeability was then calculated using the constant head method, a form of Darcy's Law for this range of permeability according to the formula

$$k = \frac{m\Delta L\mu}{Ah\Delta t\rho^2 g} \quad (1)$$

where k is permeability, m is mass of fluid allowed to permeate in the specific time interval t, L is section length, A is cross section of the flow surface, h is total pressure head, μ is viscosity, $?$ is density of fluid, and g is the gravitational constant. Finally, an uncertainty analysis was carried out using the Uncertainty General Solution,

$$\left(\frac{U_k}{k}\right)^2 = \left(\frac{X_1}{k}\frac{\partial k}{\partial X_1}\right)^2\left(\frac{U_{X1}}{X_1}\right)^2 + \left(\frac{X_2}{k}\frac{\partial k}{\partial X_2}\right)^2\left(\frac{U_{X2}}{X_2}\right)^2 + ...+ \left(\frac{X_n}{k}\frac{\partial k}{\partial X_n}\right)^2\left(\frac{U_{Xn}}{X_n}\right)^2 \quad (2)$$

RESULTS: Based on preliminary data using water as a perfusate in the 1000x scaled up physical models, transverse permeability of the lacunocanalicular network from a specimen diagnosed as "healthy" or normal was 5.67×10^{-11} m^2 with 1.8% uncertainty; for a constant head of 0.239 m, the mean time, t, for a mean mass of fluid, m, to pass through the model volume was 65.5 s for 252 g of water. A parallel approach was applied to study transverse permeability in a specimen diagnosed as "late osteoporotic"; none of the fluid permeated the scaled up model in this case due to lack of connectivity in the lacunocanalicular network. Ongoing parallel studies in further healthy and osteoporotic specimens will provide an indication of population variance in lacunocanalicular permeability. Furthermore, implementation of a perfusate with proportionally scaled up viscosity (silicone oil has a viscosity 1000x that of water) will allow for correlation of scaled-up permeabilities to actual bone permeabilities through application of the scaling formula.

DISCUSSION: Scaled up fluid mechanics models offer a means to understand fluid flow through the nano/microscale pericellular spaces in bone for which technology is not currently available to observe and measure in situ; to our knowledge this is the first such application of the method to understand changes in lacunocanalicular permeability resulting from osteoporosis. Based on preliminary studies, permeability of this network is decreased dramatically with the progression of osteoporosis. This is expected to have a profound effect on transmission of extra-cellular and intracellular molecular signals through osteoporotic tissue.

The calculated permeability for water in the scaled up, healthy bone section is substantially higher than that reported in the literature for bone tissue [3]; this is due in part to differences between cellular level and tissue level permeability as well as to the scaling up of the model. Hence, it is expected that fluid flow and molecular transport through the lacunocanalicular system will be significantly diminished in osteoporotic bone. This is expected to have profound effects on mechanotransduction at a cellular level, where forces imparted by the fluid to the cells and transport of molecular signals between osteocytes would drop off at an alarming rate with the advance of osteoporosis.

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