A Multi-level Finite Element Analysis of Fluid/Solute Flow in Mechanically Loaded Bone

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Introduction: Transport of nutrients, signaling molecules and fluid in the bone lacunar-canalicular system (LCS) is critical for osteocyte survival and function. Using the fluorescence recovery after photobleaching (FRAP) approach in combination of mathematical modeling, we have quantified fluid flow velocity and solute transport rates in the mouse tibial LCS \cite{1}. However, due to technical difficulties, the measurements were limited only to cortical regions 30-50 µm underneath periosteum. Transport measurements on deeper cortex and trabecular bone areas are lacking. The goal of this project was to employ a finite element (FE) approach to map the macroscopic tissue strain and the microscopic fluid and solute fluxes at the LCS level in a mechanically loaded bone. The material properties assumed in the FE models were validated against experimentally measured strain and FRAP data. Our preliminary results demonstrated the feasibility of this computational approach in estimating fluid flux in the LCS and the cellular stimulation forces (shear and drag forces) for osteocytes in not only cortical but also trabecular bones.

Methods: (i) Whole Bone Model: An intact mouse tibia from an adult C57BL/6J male mouse was imaged using a Scanco µCT35 scanner at a voxel size of 20 µm. The reconstructed 998 slices were exported in DICOM format to ScanIP\textsuperscript{®} (Simpleware), which meshed the entire tibia including cortical and trabecular bone into 5,112,690 tetra elements (Fig.1A). In Hypermesh\textsuperscript{®} (Hyperwork, Altair), fixed constraints were imposed at the proximal tibial plateau. Similar to our experimental setup \cite{1}, a 3N cyclic compressive load was applied to the distal end of the tibia (Fig.1B). Assuming elastic material in the model, strain field was obtained using OptiStruct\textsuperscript{®}, a FEA linear solver in HyperWork. The predicted strains were compared with experimental measurements to ensure that the material properties (20GPa Young’s modulus and 0.33 Poisson’s ratio) assigned to the FEA model were reasonable. (ii) Transport Model: For analysis of fluid and solute transport in LCS, a 3mm segment of tibial metaphysis consisting of ~ 700,000 tetra elements was cut from the whole bone FE model (20-40% distal of the proximal end, Fig.1A). The segment model was then imported to FEBio (http://www.febio.org) and the elements were assumed to be biphasic-solute material. FRAP experiment \cite{1} was simulated by setting the initial solute concentration to 0.2 at the elements around the selected photobleached element ~30µm below the anterior-medial periosteum, which was assumed to be photobleached, while the other elements were assigned 1.0. First, the FRAP transport rates (K\textsubscript{diff}) were obtained by simulating non-loaded conditions with varied solute diffusion coefficients (D) in this 3D transport model. Based on the experimental results of K\textsubscript{diff} for sodium fluorescein \cite{1} a best-fit D was determined. Secondly, the loading FRAP experiment was simulated by applying the dynamic displacements, which were obtained from the whole bone model under 3N peak load, to the two cross-sectional surfaces of the transport FE model (Fig.1A) with a 0.5Hz sinusoidal waveform followed by 2 sec resting periods \cite{1}. The boundary conditions for fluid and solute transport were listed in Fig.2A. To capture the active mixing mechanisms of canalicular flow influx into the photobleached element from surrounding elements, the increase of local solute concentration was simulated separately for the flow and reverse flow phases within one loading cycle,
which both were found to enhance solute transport previously [2]. The local solute concentration during a total of 8 loading cycles (32 sec) was simulated, from which the transport enhancement ($K_{load}/K_{diff}$) was obtained for sodium fluorescein. The model was validated by comparing against experimentally observed transport enhancements [1]. (iii) Outcomes: The strain and pore pressure of the region of interest, solute concentration of the photobleached element, as well as fluid and solute fluxes. Tissue level fluxes were scaled to the LCS levels based on porosity, assumed to be 10% [3]. The cellular stimulation forces such as fluid shearing force and drag force on transverse pericellular fibers were calculated using the Brinkman equation describing the canalicular flow [4] and measured pericellular matrix fiber spacing [5] as performed in our previous study [6]. The parameters used in calculations were 76nm cell process radius and 160nm canalicular wall radius, measured from adult B6 mice [7], and PCM edge-to-edge spacing of 10.3nm and a fiber radius of 2 µm [5].

**Results:** (i) The non-loaded FRAP FEA simulation showed that the solute diffusion coefficient $D$ was linearly correlated with the transport rate $K_{diff}$. The diffusion coefficient of sodium fluorescein (376 Da) was found to be 31.8µm²/s for experimentally observed $K_{diff}=0.01709$sec⁻¹ in the 3D porous model (Fig.2B), which was consistent with our previous estimations after factored with 10% LCS porosity [1]. (ii) The loaded FRAP FEA simulation showed a transport enhancement of 1.24, which fell in the range of experimentally data ($K_{load}/K_{diff}=1.31+0.24$) [1]. These two results validated the FE model, which was further used to predict cellular stimulation forces at various locations. (iii) The fluid flux in the model showed heterogeneous distribution, with relatively higher flux values around endosteal surface (Fig.3A). For six selected locations, the flux varied from 0.6 to 3µm/s (Fig.3B), which resulted in much higher drag forces than shearing forces for all the locations (Fig.3C).

**Discussion:** Using the image-based finite element analysis approach, we aimed to simulate the FRAP experiments and expand our understanding of load-induced fluid and solute transport in a large volume of bone tissue consisting both trabecular and cortical bone. In combination with our previous pericellular ultrastructural model [5], we were able to address this important issue from multiple levels, spanning from the entire bone level, LCS pore level, to the pericellular fiber level. Our results showed that this approach was feasible to estimate fluid flow and cellular stimulation forces on any locations, allowing further studies of how osteocytes’ activation correlates with functional bone formation in vivo. There are several limitations in this study. The predicted canalicular flow velocity, which is sensitive to porosity, is about one order of magnitude lower than our previous estimations [1]. The porosity will be better optimized in future simulations. Sealed boundary was assumed in our model for better convergence in solute concentration simulations. However, permeability of the periosteum can introduce solute and fluid flow across the boundary and will be considered in future studies.

**Significance:** Using this new tool, fluid flow and cellular stimulations can be quantified in mechanically loaded bone, enhancing our understanding of bone adaptation.
Fig. 1. (A) Whole bone FEA model of a murine tibia with the proximal end being fixed in position and load applied at distal end; A 3 mm segment was used for detailed transport modeling. (B) Loading profile consisted sinusoidal compressive load at 0.5 Hz followed by a 2 sec of resting period, similar to the one used in previous FRAP studies [1].

Fig. 2. (A) Boundary conditions in the transport model. (B) Simulated solute recovery was shown under non-loaded or loaded conditions at the FRAP site. An effective D was found to agree with experimentally observed $K_{\text{diff}}$ and the $K_{\text{load}}$ was predicted by the model.
Fig. 3. (A) Fluid flux field in the 3D model (FRAP site: location A); (B) Canalicular fluid velocity profile at different locations; (C) Predicted cellular stimulation forces at the corresponding locations.