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Anatomic Variations of the Lacunar-Canalicular System Influence Solute Transport in Bone

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Abstract

Solute transport in the lacunar-canalicular system (LCS) is essential for bone metabolism and mechanotransduction. Using the technique of fluorescence recovery after photobleaching (FRAP) we have been quantifying solute transport in the LCS of murine long bone as a function of loading parameters and molecular size. However, the influence of LCS anatomy, which varies among animal species, bone type and location, age and health condition, is not well understood. In this study, we developed a mathematical model to simulate solute convection in the LCS during a FRAP experiment under a physiological cyclic flow. We found that the transport rate (the reciprocal time constant for refilling the photobleached lacuna) increased linearly with canalicular number and decreased with canalicular length for both diffusion and convection. As a result, the transport enhancement of convection over diffusion was much less sensitive to the variations associated with chick, mouse, rabbit, bovine, dog, horse, and human LCS anatomy, when compared with the rates of diffusion or convection alone. Canalicular density did not affect transport enhancement, while solute size and the lacunar density had more complicated, nonlinear effects. This parametric study suggests that solute transport could be altered by varying LCS parameters, and that the anatomical details of the LCS need systemic examination to further understand the etiology of aged and osteoporotic bones.

Keywords

osteocytes; bone fluid flow; lacunar density; canalicular number; transport enhancement

Introduction

It has become well accepted that osteocytes play an important role in bone maintenance and adaptation in response to mechanical stimuli [1,2]. Osteocytes can detect mechanical loads by sensing fluid flow through the lacunar-canalicular network (LCS), an interconnected pore system surrounding osteocytes and their processes [2]. In response to the mechanical loads osteocytes can alter the expression of signaling molecules such as nitric oxide (NO), prostaglandins E₂ (PGE₂), osteoprotegerin (OPG), receptor activator of nuclear factor- κ B ligand (RANKL), and sclerostin, which may regulate other bone cells during bone modeling and remodeling [1-6]. For example, PGE₂ was found to increase bone mass by stimulating

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osteoblast recruitment and NO may inhibit proliferation of osteoblast precursors and accelerate their differentiation into osteoblasts [5]. Osteocyte-derived sclerostin negatively regulates osteoblastic bone formation through the Wnt signaling pathway [3,4]. OPG binds to RANKL and prevents RANKL from binding and activating receptor activator of nuclear factor- κ B (RANK) on osteoclasts and osteoclast precursors [6]. Transport of these osteocyte-derived signaling molecules in bone is believed to occur through the lacunar-canalicular system because the mineralized matrix was found to be impermeable as demonstrated using exogenously injected tracers [7,8]. Moreover, osteocytes obtain nutrients and dispose of metabolic wastes through the same transport pathways [2,9].

LCS anatomic parameters vary to different degrees depending on animal species, bone type, location, age, and health condition. The LCS is mainly composed of two types of pores: the larger lacunae (order 10 microns) that house the osteocyte bodies, and the smaller tubular canaliculi (order 0.5 microns) that connect neighboring lacunae and contain osteocyte processes. The shape of lacunae is roughly a tri-axial ellipsoid in parallel-fibered and lamellar bones, and globular in woven bone, and its density is higher in woven-fibered bone than in parallel-fibered bone and lowest in lamellar bone [10,11]. Based on density data for lacunae and canaliculi, the canalicular length has been estimated as 23 to 50 micron among seven species (Chick, mouse, rabbit, bovine, horse, dog, human), with approximately 41 to 115 canaliculi originating from each lacuna [12,13]. Lacunar size among the seven species has not been shown to vary significantly [11]. Aging tends to reduce the lacunar density in adult cortical bones. In contrast, osteoporosis generally increases the lacunar density [14,15] but changes in the lacunar size have been contradictory in literature [14-16]. Gender and race effects on the lacunar density have also been found in recent studies [17,18]. At the canalicular level, there are no detailed data on variations in canalicular dimensions among different species, except for a study reporting average diameters of canaliculi and cell process in mouse diaphysis (259 ± 129 nm and 104 ± 69 nm, respectively) [19]. Within the canalicular channels, a gel-like fiber matrix with a fiber spacing of approximately 7 nm, similar to that of the glycocalyx on endothelial cells, has been proposed to fill the pericellular space in LCS [20]. This hypothesis is partially supported by the tethering fibers observed in the pericellular space [19]. Results from perfusion of various solutes in bone suggest that the pericellular matrix serves as a molecular sieve with an effective pore size between 6-12 nm in rat [8]. However, the exact composition and ultrastructure of the pericellular matrix and any variation among different species, bone type, and location are not known.

Because the LCS is the transport pathway for osteocytes to communicate with each other and with cells outside the bone matrix, the influence of LCS anatomic variation on solute transport and molecular signaling warrants closer examination. Results of our recent mathematical modeling of fluorescent recovery after photobleaching (FRAP) showed that solute diffusion in the LCS is proportional to solute diffusivity, the volume ratio between canaliculi and lacuna, as well as lacunar density [13]. This simple relationship is valid only for the case of diffusion in the LCS. Because bone experiences mechanical loading in vivo and load-induced fluid flow greatly enhances solute transport via convection [9,21], it is important to understand how the anatomical parameters of LCS affect convective transport in bone. Previous models that treated bone as a homogenous porous media without considering the LCS microstructure and variation are unlikely to answer this question [9,22-24]. Our recent studies of FRAP in loaded bone found that the transport rate between the photobleached lacuna and its neighbors is a power function of the dimensionless Strouhal number [25]. This Strouhal number depends not only on bone fluid velocity, loading frequency, solute diffusivity, but also on canalicular length, suggesting that solute convection is sensitive to LCS anatomy [25]. The objective of this study was to elucidate how LCS anatomical variability influences solute diffusive and convective transport within the bone LCS.

Materials and methods

A hypothetical FRAP experiment

The model developed in this study aims to quantify solute transport (diffusion and convection) under fluid flow with varying lacunar and canalicular number densities. A hypothetical FRAP experiment is modeled here, where a fluorescent solute with a diffusivity D remains in equilibrium in the entire LCS when a brief photobleaching of one lacuna is introduced using a high intensity laser, resulting in a rapid drop of its fluorescent solute concentration. The processes of unbleached solute refilling of the disturbed lacuna through diffusion and convection are studied here. A hypothetical FRAP experiment is modeled because 1) the FRAP process is an analogue of the spreading process of molecules among osteocytes, which is vital for bone's mechanotransduction, and 2) the model predictions can be tested experimentally using direct FRAP studies in the future. The model considers solute transport under loaded and non-loaded conditions with either sinusoidal flow or no flow present in the LCS for a series of configurations with varying canalicular length (inversely related to lacunar density) and canalicular number. The ranges of flow and LCS parameters are given in the following sections based on the previously reported values in the literature.

LCS transport model with cyclic fluid flow

Similar to our previous model [25], we focus on solute transport that occurs $\sim 30 \mu\text{m}$ below the anterior-medial periosteal surface of the mid-shaft of a murine tibia subjected to cyclic compression applied through its two ends. The mouse tibia model was chosen because (i) the mouse has a small body size that makes imaging on the microscope stage easier; (ii) external mechanical loading to the mouse tibia is well documented and the tibia is easily manipulated, and (iii) the tibial anterior-medial surface is devoid of muscle attachment, readily accessed under skin, and relatively flat for imaging [25]. Under mechanical load the local circumferential flow at this specified location was found to dominate over the radial flow component [25]. Thus, transport was simplified to a one-dimensional problem. The fluid velocity waveform was also found to resemble a sinusoidal curve with the same frequency as the applied load. The peak value of the induced fluid velocity for a mouse tibia subjected to a physiological loading magnitude of 3-N (corresponding to a surface strain of 800 micro-strain) was $80.6 \mu\text{m/s}$ [25]. Although changes in LCS anatomy could lead to varied LCS permeability and fluid velocities [26], we simplified the study by imposing the same sinusoidal fluid flow to all LCS networks considered here, assuming they are under similar physiological strains.

The current transport model, similar to our previous one [25], consists of a central photobleached lacuna (L) connected with neighboring lacunae (modeled as two reservoirs S_1 and S_2 on either side) through two sets of $n/2$ canaliculi (C_1 and C_2) (Fig. 1). The ellipsoidal central lacuna is modeled as a cylinder with a length of $2*d_s$ ($16 \mu\text{m}$) and a cross-sectional area of A_f ($14.6 \mu\text{m}^2$). The junction between the lacunar space and the canalicular space is tapered to make a smooth transition ($d_c = 0.5 \mu\text{m}$). Fluid velocity varies along the entire pathway due to spatial changes in the fluid cross-sectional area and fluid continuity requirements. To simulate the anatomical variations seen in vivo, the length of canaliculi d is varied from 20, 30, 40, 60, to $80 \mu\text{m}$ to reflect the variations in lacunar density found among different species. Previously, we found that 22% of the total number of canaliculi emanating from the photobleached lacuna contributed to tracer refilling, whereas the rest were located in the photobleaching laser path and thus did not contribute for the FRAP [13]. Based on the estimated range of canalicular number originating from one lacuna (about 40 to 120) [11,12], the contributing canalicular number (n) is set to be 8, 12, or 16. Since the results from these three cases convincingly demonstrate the linear relationship between solute transport and canalicular number (detailed in the Results section), the analysis with higher n values are not presented here. The total fluid cross-sectional area ($n/2*A_c$) for the canalicular set on each side of the

photobleached lacuna is the sum of individual fluid space (A_c), defined as the area of the annular space between the canalicular wall (diameter 259 nm) and cell process (diameter 104 nm). Transport of three representative tracers (small, medium, large) with varied diffusion coefficients ($D = 3, 30, 300 \mu\text{m}^2/\text{sec}$) is studied.

In the current transport model we defined the x -axis along the center line of the flow pathway with the origin located at the center of the photobleached lacuna. The cross-section area of the flow pathway is a function of x . Because the longitudinal length scale of the flow pathway is much larger than the transverse length scale, the molecule concentration is considered to be one-dimensional as a function of time t and location x . Therefore, the concentration function satisfies the one-dimensional diffusion-convection equation:

$$\frac{\partial C}{\partial t} + (1 - \sigma_f)u \frac{\partial C}{\partial x} = \frac{D}{A} \frac{\partial}{\partial x} \left(A \frac{\partial C}{\partial x} \right) \quad (1)$$

where D is the diffusion coefficient in the LCS and is chosen to be 3, 30, or $300 \mu\text{m}^2/\text{sec}$ for large, medium, and small molecules, respectively; A is the cross-section area of the flow pathway varying with x ; u is the fluid velocity ($u = 80.6 \sin(2\pi t/T) \mu\text{m}/\text{sec}$); T is the time period of the oscillating flow ($T = 2 \text{ sec}$); and σ_f is the reflection coefficient of the tracer in the LCS. The reflection coefficient σ_f represents the sieving effect of the pericellular fibers on the tracer movement through porous media. The solute flux and velocity is reduced by a factor of $(1 - \sigma_f)$ relative to the fluid velocity due to the hindering effects from the pericellular matrix. The σ_f varies from 0.015, 0.36 to 0.84 for large, medium, and small tracers, respectively [27].

To obtain the spatiotemporal evolution of the solute concentration within the system, the following initial and boundary conditions are used. Since the total volume of neighbor lacunae is much larger than the photobleached lacuna and canaliculi, we assume the compartments S_1 and S_2 to be reservoirs with constant concentration C_0 . Right after photobleaching, the concentration in the central lacuna L is reduced to the half of its initial value ($C_b = 0.5 * C_0$) and a linear distribution of concentration is achieved in canaliculi due to the finite time of the photobleaching period [13]. A finite difference scheme (Modified Total Variation Diminishing Lax-Friedrichs Method) [28] is implemented in Matlab (Mathworks, MA) to solve the differential equation. The LCS transport model (Fig. 1) is discretized using a non-uniform mesh, with a more refined mesh placed on the tapered sections where the velocity gradients are greater. Although the geometry is symmetric, the entire model is meshed and used in the calculation due to the anti-symmetric nature of the oscillating flow in the two canalicular sets (C_1 and C_2).

Model outputs

The time course of tracer concentration recovery within the photobleached lacuna is calculated for the three representative tracers in LCS networks with varying canalicular number n and length d . As our previous study demonstrated, the concentration of the photobleached lacuna at the end of each flow cycle increases exponentially with time and reaches a plateau at steady state $[(C - C_0)/(C_b - C_0) = \exp(-kt)]$ [25]. Therefore, the coefficient k is used as a characteristic transport rate. The transport rate under pure diffusion condition k_0 is also obtained when fluid velocity is set to be zero. With these diffusive and convective transport rates, we calculate the transport enhancement k/k_0 for all cases. We also calculate the Strouhal number ($St = d/d_{ss}$), which represents the ratio of the characteristic transport length (the canalicular length d) over the maximal displacement that the solute front travels during oscillation (termed as the solute stroke displacement, $d_{ss} = 80.6 * (1 - \sigma_f) * T/\pi \mu\text{m}$).

Results

For all the results presented below, the oscillating flow velocity in the canaliculi is set as $u = 80.6 \sin(2\pi t/T)$ $\mu\text{m}/\text{sec}$ and a cycle period of $T = 2$ sec if not otherwise stated.

Transport Rate Under Pure Diffusion Condition (k_0)

The transport rate k_0 under the pure diffusion condition is proportional to the canalicular number and the diffusion coefficient of the molecule, and is inversely proportional to the canalicular length. The calculations can be fit with the following equation very well ($R^2 = 0.998$), which agrees with our previous theoretical model [13]:

$$k_0 = 0.84 \frac{nD}{d}, \quad (2)$$

where n is the contributing canalicular number, D is the diffusion coefficient, and d is the canalicular length.

Effect of Canalicular Number n on Transport Rate k under Oscillating Flow

Transport rate k under convection increases with canalicular number n for all the three tracers and five different canalicular lengths (Fig. 2). The lines fitting data points of the same D and d almost pass through the origin, suggesting that the transport rate is proportional to the canalicular number. The slopes of these lines increase with increasing diffusion coefficient D and decreasing canalicular length d .

Effect of Canalicular Length d on Transport Rate k under Oscillating Flow

Transport rate k under convection decreases with canalicular length with a trend toward more rapid decreases for medium sized tracers (Fig. 3). For the larger molecule ($D = 3 \mu\text{m}^2/\text{sec}$), the transport rate is approximately inversely proportional to the canalicular length. For the medium molecule ($D = 30 \mu\text{m}^2/\text{sec}$), the transport rate is much more sensitive to the canalicular length from 20 to 40 μm and is not simply proportional to d^{-1} . For the small molecule ($D = 300 \mu\text{m}^2/\text{sec}$), the curves appear much straighter. For the flow condition considered here, the critical canalicular lengths d that equals the solute stroke displacement ($St = 1$) are 8.2, 32.8, and 50.5 μm for large, medium and small molecules, respectively. The critical canalicular length d differs for the three tracers due to their different reflection coefficients and the corresponding solute flow velocities. It appears that the transition of $St < 1$ to $St > 1$ is associated with the transition from rapid to slower decline of k as a function of canalicular length d .

Effect of Canalicular Length d and Number n on Transport Enhancement k/k_0 under Oscillating Flow

The transport enhancement k/k_0 is less sensitive to the LCS anatomical variations than the convection and diffusion rates themselves (Fig. 4). The transport enhancement varies within a relatively small range for the examined canalicular lengths d and diffusion coefficients D , and it does not vary with the canalicular number n (Fig. 4). The k/k_0 curves for different canalicular numbers are almost identical. The effect of canalicular number is canceled out because transport rate is proportional to canalicular number in both convection and diffusion. The relationship between transport enhancement and canalicular length is much more complicated. For the large molecule, the enhancement remains almost constant (2.6~2.7). Transport enhancement of the medium-sized molecule varies from 7 to 3.5 and monotonically decreases for increasing canalicular length. For the small molecule, transport enhancement varies across a smaller range (1.8~2.5) in a biphasic manner (peaking at $d = 40 \mu\text{m}$). Under the physiological flow condition considered here, the medium-sized molecules show a higher enhancement

(3.5~7) than those of small and large molecules (1.8~2.5 and 2.6~2.7, respectively). This finding is consistent with our previous findings [25].

Discussion

The most important finding from the present study is that variations in the anatomical parameters of the LCS greatly affect convective and diffusive transport, but they have less influence on the transport enhancement ratio (k/k_0) of convection over diffusion. In our previous study, we found that the rate of transport due to pure diffusion k_0 was proportional to the canalicular density and inversely proportional to canalicular length [13]. In this study, we focused on how the two anatomical parameters affected solute transport under a representative flow condition. The results showed that the convective transport rate k was also proportional to the canalicular density and inversely related to canalicular length (Figs. 2 and 3). However, the LCS variations examined in this study had a much-reduced influence on transport enhancement (k/k_0). Canalicular number density did not influence transport enhancement at all, and the transport enhancement varied only slightly within a narrow range (1.8-7) for all cases of canalicular length (20-80 μm) and solute sizes ($D = 3, 30, 300 \mu\text{m}^2/\text{sec}$) (Fig. 4).

The dimensionless number St (d/d_{ss}) seemed to be the major factor controlling the level of transport enhancement, similar to our previous finding [25]. When $St > 1$ (the solute stroke displacement d_{ss} during one half cycle of the sinusoidal flow of solute is shorter than the canalicular length d), the bulk solute movement was confined in the canaliculi channels and the solute flow did not reach the central lacuna. In this case, although the convection helped solute mix within the canaliculi, enhancement of transport in the central lacuna was relatively small and it always decreased with increasing canalicular length (Fig. 4). When $St < 1$, the flow carried the solute into the central lacuna and the degree of the transport enhancement was dependent on the solute diffusivity: it was maximized for the medium molecule, while decreased for small and large molecules (Fig. 4). This complex behavior arose from two competing processes occurring within the lacuna, these were i) the convective fluxes into and out of the lacuna and ii) solute mixing in the finite volume. As elucidated in the tracer profiles during cyclic loading in our previous study [25], the amount of tracer retained in the lacuna during a cycle of cyclic flow represented the net difference between in-flux and out-flux, which were dependent on flow velocity and solute diffusivity. For large molecules, lacunar mixing was slow and thus the tracers carried into the lacuna during in-flux were pushed out when the flow reversed in direction during the next half cycle. For small molecules, lacunar mixing was fast; the tracers carried into the lacuna from one side of canaliculi exited the lacuna in the same half cycle, reducing the net amount of tracer retained in the lacuna. Only for the medium sized molecules was the retained tracer concentration in the central lacuna maximized.

As an application of the model, we examined the effects of the inter-species LCS variability and found significant variations in baseline diffusion and load-induced convection, but very limited variation in transport enhancement (Table 1). The anatomical parameters for the LCS of seven species, including chick, mouse, rabbit, bovine, horse, dog, and humans, were assembled from the literature (please see references cited in [11-13]). The diffusive transport rate k_0 , convective transport rate k , and their ratio (transport enhancement k/k_0) were estimated by linear interpolation of our model results. Large coefficients of variations (percentage of standard deviation over mean values, 40%~52%) were observed among the diffusion and convection rates for the seven species (Table 1). In contrast, the coefficient of variations for transport enhancement were much smaller; 1~2% for small and large molecules and 19% for medium ones. It is noted that baseline diffusion varied significantly among the species with the different LCS anatomy, while the loading-induced flow enhanced transport relatively uniformly regardless of the LCS anatomical variations.

Overall, the present study suggests the important role that LCS anatomy plays in osteocyte metabolism and bone's response to mechanical loading and its removal. If a LCS configuration ensures relatively slow baseline diffusion among osteocytes, a relatively slow convective transport rate would also be expected under loading conditions. This can help us to understand bone metabolism and adaptation in aged and diseased bones. It was reported that the lacunar density of 70~80 years old subjects is only 60%~70% of 20~30 years old subjects [15]. Therefore, the average canalicular length in aged bones is about 1.2~1.3 times greater than in young bones, which would significantly reduce transport rate under diffusion and oscillatory flow conditions. This reduced transport of signaling molecules during mechanical loading may be partially responsible for the reduced mechanosensitivity seen in aged bones, because the cells harvested from elderly donors did not show altered sensitivity to in vitro fluid flow [29]. In addition, disuse osteoporosis has been found to be site-specific across the skeleton and to correlate with baseline morphology and cellular activity [30,31]. It will be interesting to examine whether the bone sites susceptible to disuse contain LCS networks with higher baseline transport. As suggested by our model, these locations would be expected to experience a greater reduction in overall transport during disuse.

There are several assumptions and limitations in the present study. First, we imposed an identical flow profile with a peak velocity of 80.6 $\mu\text{m}/\text{sec}$, which was found in murine long bone under a physiological strain (~ 800 microstrains) [25], to the various LCS networks examined in this study. In fact, a specific mechanical load could induce different flows in these LCS networks due to their varying hydraulic permeability [26]. The relationship between the LCS permeability and its anatomy has been established through a series of models by Weinbaum and colleagues [20,26]. In this paper, we simplified our investigation into the influence of LCS anatomy on solute transport by imposing the same flow condition to the various LCS network. For other flow profiles with different peak values, the numerical values for transport rate and enhancement will differ from the results presented in this study. However, the general behaviors of transport rates and enhancement are expected to maintain the same dependence on the dimensionless number St . Secondly, we examined the effects of two anatomical parameters of LCS, i.e. canalicular length and canalicular number, on solute transport because these two parameters are relatively well documented in different species, bone type, and locations. Other anatomical parameters including lacunar shape and size, the diameters of the canalicular wall and osteocyte process, as well as pericellular matrix structure are also important factors for fluid and solute transport in LCS. The values of these parameters were fixed in the current study based on the data from mouse tibiae [13]. We did not consider variations in these parameters because no significant difference in lacunar size has been found in different species [11] or simply because there are no data available on variation in the ultrastructure of the canaliculi and the pericellular matrix. Further investigations are needed to obtain such data so that they can be incorporated into future studies investigating their influence on solute transport in bone.

Despite these limitations, the model developed in this study demonstrates the significant influence of LCS anatomy on solute transport, and its potential implications on osteocyte metabolism and cell-cell signaling. The most important result presented herein is that LCS anatomy affects both baseline and convective solute transport in a similar manner, i.e., transport rate increases with canalicular and lacunar density. As a result, transport enhancement (i.e. the ratio between convection and diffusion) for different sized solutes is much less sensitive to the LCS anatomy than the rates of diffusion or convection themselves. Further investigations into LCS anatomy and ultrastructure in different species, bone site, and type are needed to fully understand the possible roles of osteocytes in bone mechanotransduction in health and diseased conditions.

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Fig. 1.

A three-compartment model describing the flow-enhanced solute transport in the LCS during a representative FRAP experiment. The central photobleached lacuna (major radius of $8\ \mu\text{m}$) is connected with its neighboring lacunae, which serve as two reservoir compartments (S_1 and S_2 , with constant tracer concentration C_0) at each side, through two sets of contributing canaliculi channels (C_1 and C_2) with a number of $n/2$ channels and a channel length of d . To mimic the LCS variation seen in vivo, the canaliculi number n is varied from 8, 12, to 16 and d is varied from 20, 30, 40, 60, to $80\ \mu\text{m}$. A sinusoidal flow velocity with a magnitude of $80.6\ \mu\text{m}/\text{sec}$ and a frequency of $0.5\ \text{Hz}$, corresponding to a representative flow condition under approximately ~ 800 microstrain at the murine tibial surface, is imposed from the reservoir towards the central lacuna. The flow pattern is simplified to one dimensional flow in the circumferential direction at the typical FRAP site ($30\ \mu\text{m}$ below the surface). The initial conditions used are representative of a typical FRAP experiment where the tracer concentration in the central lacuna is lowered to half its initial value ($0.5C_0$) with the tracer distributed linearly along the canaliculi at the beginning of the FRAP experiment. Refilling of the central lacuna is numerically simulated using a diffusion-convection equation (Eq. 1 in the text). The reciprocal time constant of the exponential refilling process, defined as the transport rate k , is analyzed for all the LCS network configurations and for three typical solutes with a diffusivity of 3, 30, and $300\ \mu\text{m}^2/\text{sec}$, and a reflection coefficient of 0.015, 0.36, and 0.84, respectively.

Fig. 2.

The transport rate k of various-sized molecules under convection in bone LCS increases linearly with increasing canalicular number. (A) Large molecules ($D = 3 \mu\text{m}^2/\text{sec}$); (B) Medium molecules ($D = 30 \mu\text{m}^2/\text{sec}$); (C) Small molecules ($D = 300 \mu\text{m}^2/\text{sec}$).

Fig. 3.

The Transport rate k of various-sized molecules under convection in bone LCS decreases with increasing canalicular length. (A) Large molecules ($D = 3 \mu\text{m}^2/\text{sec}$); (B) Medium molecules ($D = 30 \mu\text{m}^2/\text{sec}$); (C) Small molecules ($D = 300 \mu\text{m}^2/\text{sec}$). Note: the data shown here are the same as in Fig. 2, but organized differently to illustrate the influence of canalicular length on the transport rate.

Fig. 4.

Transport enhancement k/k_0 is less sensitive to the LCS variations than the baseline diffusion and convection rates. The transport enhancement varies slightly with solute size and canalicular length, but it does not vary with canaliculi number as shown in the nearly identical curves for the different canaliculi numbers. (A) Large molecules ($D = 3 \mu\text{m}^2/\text{s}$): transport enhancement remains constant for the canalicular length varying from 20-80. (B) Moderate molecules ($D = 30 \mu\text{m}^2/\text{s}$): transport enhancement decreases monotonically with increasing canalicular length. (C) Small molecules ($D = 300 \mu\text{m}^2/\text{s}$): transport enhancement shows a biphasic patterns, peaking at the canalicular length of $40 \mu\text{m}$. Notes: These comparisons are made using the same sinusoidal flow with a magnitude of $80.6 \mu\text{m}/\text{sec}$. Panels (A-C) are plotted in different scales. To compare with other flow magnitudes and frequencies, the Strouhal number is given for each case of canalicular length and solute size.

Inter-species LCS variations, diffusive and convective transport rates (k_0 , k), and transport enhancement (k/k_0) under a physiological oscillating flow for various-sized molecules.

Table 1

	Chick	Mouse	Rabbit	Bovine	Horse	Dog	Human	Mean $\pm$ STD	CO (STD/Mean)
Canalicular length d (μm)	23	26	33	37	31	50	43	36 ± 9.5	0.26
Total # of canaliculi emanating from a lacuna (N)	54	54	60	85	115	81	41	70 ± 25	0.36
# of canaliculi contributing to the transport ($n = 22\%N$)	12	12	14	18	26	18	10	16 ± 5	0.35
Diffusive transport rate k_0 (sec^{-1})	Large solutes	2.7E-4	2.2E-4	2.6E-4	4.5E-4	2.0E-4	1.2E-4	$2.5\text{E-}4 \pm 1.0\text{E-}4$	0.40
	Medium solutes	2.6E-3	2.3E-3	2.1E-3	2.5E-3	1.9E-3	1.2E-3	$2.4\text{E-}3 \pm 9.4\text{E-}4$	0.39
	Small solutes	2.6E-2	2.3E-2	2.1E-2	2.5E-2	1.9E-2	1.2E-2	$2.4\text{E-}2 \pm 9.4\text{E-}3$	0.39
Convective transport rate k (sec^{-1})	Large solutes	7.2E-4	6.4E-4	5.8E-4	6.7E-4	1.1E-3	3.2E-4	$6.6\text{E-}4 \pm 2.5\text{E-}4$	0.38
	Medium solutes	1.7E-2	1.4E-2	1.1E-2	1.2E-2	2.4E-2	4.9E-3	$1.3\text{E-}2 \pm 6.2\text{E-}3$	0.48
	Small solutes	5.0E-2	4.7E-2	4.8E-2	5.9E-2	9.6E-2	2.8E-2	$5.3\text{E-}2 \pm 2.1\text{E-}2$	0.40
Transport enhancement k/k_0	Large solutes	2.7	2.6	2.6	2.6	2.6	2.6	2.6 ± 0.04	0.01
	Medium solutes	6.6	6.3	5.4	4.7	5.6	4.2	5.2 ± 1.0	0.19
	Small solutes	1.9	2.1	2.3	2.4	2.3	2.4	2.3 ± 0.18	0.08

Notes: 1) The LCS anatomy data for the seven species are from references (Beno et al., 2006, Wang et al., 2005, and the references therein); 2) the diffusive rates under no flow and the convective transport rates under a sinusoidal flow with a magnitude of $80.6 \mu\text{m/sec}$ and a frequency of 0.5 Hz , as well as the transport enhancement are all estimated by linear interpolation of our model results.

3) A much reduced coefficient of variation (CV) was found for the transport enhancement for all the species compared with those of diffusive and convective transport rates themselves.