

Biorheology, 40 (2003) 1-3 pp 347-353

IOS Press

**COMPARATIVE ANALYSIS OF DIFFUSIVE AND STRESS INDUCED NUTRIENT
TRANSPORT EFFICIENCY IN THE LACUNAR-CANALICULAR SYSTEM OF
OSTEONS**

Nikola Petrov and Solomon R. Pollack*

Institute of Mechanics and Biomechanics, 1113 Sofia, Bulgaria, petrov333@gmail.com

**Department of Bioengineering, University of Pennsylvania, PA19104-6392, USA,
spollack@seas.upenn.edu*

Abstract. Marker migration experiments suggest that cyclic mechanical loading of cortical bone in vivo increases marker penetration into bone. Is this a result of stress induced fluid flow or of stress stimulation of active transport processes? Active lacunar-canalicular transport of nutrients was suggested by Ham in 1979 on the basis of the presence of actin filaments in osteocyte processes and their suspected role in cell motility. In addition, Tanaka in 1984 observed active transport of microperoxidase in bone and Tanaka-Kamioka et al., in 1998 observed experimentally that osteocyte processes are able to actively change their form.

In this study we performed parametric and comparative analyses of the transport efficiencies of diffusion and stress generated fluid flow of (glucose) nutrients in lacunar-canalicular systems in cortical bone. The result obtained is that neither diffusion nor stress induced fluid flow is capable of sustaining osteocyte viability. It is possible that cyclic stress stimulates an active nutrient transport mechanism to supplement stress flows.

1. Introduction

Osteocytes in cortical bone are located within lacunar-canalicular spaces. These spaces interconnect permitting gap junction communication between neighboring osteocytes. In

addition, the lacunar-canalicular volume is larger than the osteocyte so that a porous fluid filled region surrounds the cells and their processes. It is important to note, however, that the volume of the fluid surrounding the osteocyte is smaller than (or equal to) the volume of the osteocyte itself. This small fluid volume can not sustain the osteocytes for indefinite periods without transport of essential media ingredients. There are four possible mechanisms by which osteocytes in cortical bone can receive nutrients and rid themselves of waste products. These include passive diffusion between the lacuna and the Haversian canal, arterial to venous flow from capillaries in the Haversian canal through the lacunar-canalicular system, stress induced flow caused by external and muscle forces, and active transport mechanisms as yet not defined. The presence of actin filaments in osteocyte processes [3] and the fact that cytoplasmic processes of osteocytes are connected to one another by gap junctions suggests a possible role for active transport processes [5]. However there is little experimental data [13] supporting the argument for the existence of active transport mechanisms within the lacunar- canalicular- cell processes system related to either nutrient or metabolic waste transport.

The object of the present analysis is to estimate the efficiency of stress induced fluid flow and diffusion to provide glucose levels sufficient to sustain osteocyte viability. This work will be expanded to determine if active mechanisms are required.

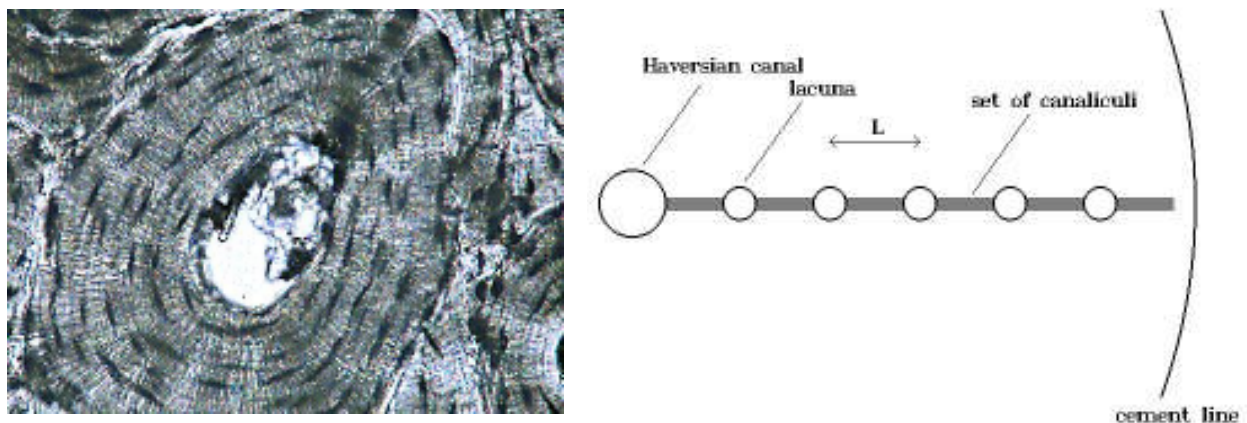


Fig. 1. Piekarski and Munro model of Haversian Canal- lacunae- canaliculi system. The same number of canaliculi connected each lacuna and the same distances between the lacunae are assumed.

2. Physical and mathematical model

Because of the complex microanatomic structure of the osteon, it is reasonable to use simplifying assumptions, as in papers of Pollack et al. [11] and Kufahl and Saha [8]. The assumptions we use are: bone matrix is isotropic and elastic; pores are spherical fluid filled inclusions; dilatation of the pores is computed as a function of the uniaxial stress; the cement line of osteons are impenetrable for the diffusing particles; the lacunar-canalicular configuration is represented by the model system of Piekarski and Munro [10], as shown on Fig. 1.

The data in Table 1 are used in all illustrative computations.

Table 1. Anatomical data and physical moduli

Anatomical data	
$23 \times 12 \times 6 \mu m$	- mean value of dimensions of lacunae; [1]
$R_{cnl} = 0.1 \mu m$	- mean value of canalicular radius; [1]
$R_{proc} = 0.05 \mu m$	- accepted by authors as mean value of osteocyte process radius; [1]
$L = 40 \mu m$	- mean canalicular length; [1]
$n = 32$	- mean number of canaliculi radiating from one side of the lacuna; [1]
$M = 5$	- accepted by the authors as the typical number of lacunae in one lacunar -canalicular chain;
$C_0 = 4.0 \times 10^{-4} g.cm^{-3}$	-glucose blood level;
Physical moduli	
$\mu_1 = 5.3 GN m^{-2}$	- shear modulus of bone matrix; [12]
$K_1 = 35 GN m^{-2}$	- bulk modulus of bone matrix; [12]
$S^{\max} = 5.88 MN m^{-2}$	- physiologic stress;
$D = 6.7 \times 10^{-10} m^2 .s^{-1}$	- diffusion coefficient for glucose in water [16].

2.1. Condition for existence of osteocyte homeostatic metabolic equilibrium if nutrients are supplied by diffusion

Using the Piekarski and Munro model, we can formulate the homeostatic diffusion problem within the lacunar-canalicular system with the equations:

$$(1) \quad \begin{aligned} D n \sigma \frac{d^2}{du^2} C &= \hat{\rho}(u) \\ C_0 = C(0) \quad , \quad \frac{d}{du} C(u^c) &= 0 \quad , \\ u^c &= 5.5L \end{aligned}$$

where C is the glucose concentration within the lacunar-canalicular system, C_0 is blood level glucose concentration within the Haversian canal, D is the glucose diffusion constant, $\hat{\rho}$ is the density of the glucose consumption sources, u is the coordinate measured from the Haversian canal wall, u^c is the distance from the Haversian canal wall to the cement line, L is the center to center distance between two neighboring lacunae, n is the number of canaliculi connecting two neighboring lacunae and σ is the portion of the canalicular cross section open for fluid flow and diffusion. The anatomical model number of 5 lacunae in series is typical and corresponds to electron microscopic observation data. The boundary conditions in the boundary problem (1) represent the blood level glucose concentration within the Haversian canal and zero diffusion flux on the osteon cement line.

The general solution of the boundary problem (1) would be obtained by double integration of diffusion Eq. (1)₁, taking into account boundary conditions (1)_{1,2}, e.g.

$$(2) \quad C(u) = C_0 - \frac{1}{D n \sigma} \int_0^u \int_u^{u^c} \hat{\rho}(u'') du'' du'$$

A problem appears with the fact that we don't know the real distribution of the source function corresponding to the osteocyte nutrient consumption. Accordingly, we consider the limiting cases, namely a uniform source distribution and a singular source distribution. In the limit of uniform source distribution along the whole lacunar-canalicular length

$$(3) \quad \hat{\rho} = \frac{5\hat{R}}{5.5L} \quad ,$$

where \hat{R} is the single osteocyte glucose consumption rate. By substitution of $\hat{\rho}$ from (3) in (2) we obtain for the glucose concentration distribution

$$(4) \quad C(u) = C_0 + \frac{\hat{R}}{D n \sigma L} \left(\frac{u^2}{2} - u^c u \right) \quad , \quad u \in [0, u^c]$$

The limit of the singular source distribution is described by a set of source singularities, each one positioned within a lacuna, in which case;

$$(5) \quad \hat{\rho} = \sum_{k=1}^5 \delta(u - kL) \hat{R} \quad ,$$

where $\delta(u)$ is the Dirac delta function. By substitution of $\hat{\rho}$ from (5) in Eq. (2) we obtain for the glucose concentration the function defined at the lacunar sites with linear segments between the sites as given by;

$$(6) \quad C(kL) = C_0 + \frac{\hat{R}L}{D n \sigma} \left(\frac{k^2}{2} - 5.5k \right) \quad , \quad k = 0, 1, 2, \dots, 5 \quad .$$

The glucose concentrations on the cement line predicted by the both limit cases are the same:

$$(7) \quad C(u^c) = C_0 - 15 \frac{\hat{R}L}{D n \sigma} \quad - \text{ for uniform source distribution}$$

$$(8) \quad C(u^c) = C_0 - 15 \frac{\hat{R}L}{D n \sigma} \quad - \text{ for singular source distribution} \quad .$$

One can see that both cases lead to the same computational result. It follows that the precise accounting of the real distribution of glucose consumption is not of importance for this determination.

To be in homeostatic metabolic equilibrium by diffusion alone, the quantity of glucose delivered by diffusion should be in balance with the quantity consumed. As the concentrations on the cement line $C(u^c)$ should be nonnegative, we obtain from (7) the following upper limit restriction for the osteocyte consumption

$$(9) \quad \hat{R} \leq D \frac{n \sigma C_0}{15 L} \quad ,$$

which represents a necessary condition for the existence of homeostatic metabolic equilibrium for this source of glucose.

Substituting in (4) the data for the parameters taken from Table 1 we obtain:

$$(10) \quad \hat{R} \leq 3.3 \times 10^{-16} \text{ g} \cdot \text{sec}^{-1} .$$

2.2. Condition for existence of osteocyte homeostatic metabolic equilibrium if nutrients are supplied by stress induced fluid flow

The delivered portion of glucose, ΔM , for one loading-reloading cycle within a single lacuna is

$$(11) \quad \Delta M = \Delta V^{exch} C^{loc} ,$$

where ΔV^{exch} is the lacuna dilatation during the loading-reloading cycle and C^{loc} is the local glucose concentration in the canaliculi radiating from the lacuna. As the fluid pressure resists the macroscopic stress, the dilatation of an empty lacuna should be an upper limit for the dilatation ΔV^{exch} of one fluid filled lacuna. Exploring the Hashin [4] theory for the dilatation of a single pore embedded in a matrix subjected to uniaxial loading, we obtain:

$$(12) \quad \Delta V^{exch} < \frac{\bar{V}_0}{3K_1} \frac{4\mu_1 + 3K_1}{4\mu_1} \Delta S ,$$

where ΔS is the uniaxial stress increase during the loading-reloading cycle, \bar{V}_0 is the reference lacunar volume, μ_1 and K_1 are the shear and bulk moduli of bone matrix. In addition, because of consumption, the blood concentration, C_0 , is the upper limit for the local canalicular concentration C^{loc}

$$(13) \quad C^{loc} < C_0$$

Consequently, ΔM , the glucose delivered to a lacuna by dilatation caused by one cycle of loading is limited by:

$$(14) \quad \Delta M \leq \Delta V^{exch} C_0 < \frac{\bar{V}_0}{3K_1} \frac{4\mu_1 + 3K_1}{4\mu_1} \Delta S C_0 .$$

For normal human locomotion the loading-reloading period is about one second and the minimal stress is zero. Taking into account that, for homeostasis, the amount of glucose consumed by an osteocyte can not be greater than the delivered amount, we obtain the following upper limit restriction for the osteocyte consumption rate:

$$(15) \quad \hat{R} < \frac{\bar{V}_0}{3K_1} \frac{4\mu_1 + 3K_1}{4\mu_1} S^{\max} C_0 .$$

Equation (15) should be considered as a condition for the existence of homeostatic metabolic equilibrium.

Substituting in (15) the data for the parameters in (15) taken from Table 1 we obtain:

$$(16) \quad \hat{R} < 1.7 \times 10^{-17} \text{ g.s}^{-1} .$$

3. Discussion and conclusions

By mathematical analysis of the diffusion and stress induced fluid flow in the Munro-Piekarski model of the canalicular-lacunar system, we obtained limiting restrictions for osteocyte consumption rate representing the conditions for the existence of homeostatic metabolic equilibrium. Comparing the inequalities (10) and (16) we found the stress induced fluid flow is a more strongly restricted transport process than diffusion and consequently less effective. The last conclusion suggests the need for a new interpretation of experimental results where it was found [6] that cyclic loading increases marker penetration within lacunar-canalicular systems. There are some possible ways to resolve this problem. One way is that cyclic stress stimulates an active transport mechanism. Another way is that the Munro-Piekarski model, as also used by Kufahl and Saha [8] Weinbaum et. al., [17] must be altered to permit greater flow to sustain osteocytes.

It is of importance to compare these upper limits for the rate of osteocyte glucose consumption with experimentally measured values. McCarthy and Yang [9] argued that because of morphological and functional similarity, cortical bone has a rate of consumption and cell density similar to articular cartilage. The value of glucose consumption in articular cartilage was measured by Bywater [2] and is $0.2 \text{ mg.ml}^{-1} \cdot \text{h}^{-1}$. If we relate this value to a single chondrocyte, assuming that the mean distance between the chondrocytes is similar to the distance between osteocytes, we obtain for the chondrocyte glucose consumption rate

$$(17) \quad \hat{R} \sim 10^{-15} \text{ g.s}^{-1} .$$

Komarova et al., [7] measured glucose consumption rates in fetal rat calvarial cells in culture and found for these active primary osteoblast like cells a value for glucose consumption of

$$(18) \quad \hat{R} = 1.6 \times 10^{-14} \text{ g.s}^{-1} ,$$

with a range from 1.0 to $2.4 \times 10^{-14} \text{ g.s}^{-1}$. This represents an upper limit to the glucose consumption of osteocytes, which are less active. We conclude that the range of 10^{-14} to $10^{-15} \text{ g.s}^{-1}$ is reasonable for the glucose consumption of osteocytes. This value is one to two orders of magnitude greater than the upper limit for osteocyte consumption obtained by diffusion analysis and two to three orders greater than the upper limit obtained by stress induced fluid flow

analysis. The result suggests that both diffusion and stress induced fluid flow using a Munro-Piekarski model would be ineffective as nutrient transport mechanisms. To accept or reject this supposition, however, it is necessary to use data directly measured for the osteocyte rate of glucose consumption.

Acknowledgment

The authors thank Professor Ian McCarthy for the detailed and very useful discussion in regard to the acceptance of reasonable scale for the osteocyte rate of glucose consumption.

References

- [1] A. Boyde, Scanning Electron Microscope Studies of Bone, in: *The Biochemistry and Physiology of Bone*, G. H. Bourne, ed., vol. 1, Academic press, New York, (1972), pp. 259-310.
- [2] E. G. Bywater, The metabolism of joint tissues, *J. Pathol. Bacteriol.* **44** (1937), pp. 245-253.
- [3] A. W. Ham and D. H. Cormack, Bone and bones, in: *Histology*, chap. 15, J. B. Lippincott Company, Philadelphia and Toronto, 1979, p. 403.
- [4] Z. Hashin, The inelastic inclusion problem, *Int. J. Engng Sci.* **7** (1969), pp. 11-36.
- [5] M. E. Holtrop and J. M. Weinger, Ultrastructural evidence for a transport system in bone, in: *Calcium, Parathyroid Hormone and the Calcitonins*, R. V. Talmage and P. L. Munson, ed., Excerpta Medica, Amsterdam, 1970, p. 365.
- [6] M. L. Knothe Tate, P. Niederer and U. Knothe, In vivo tracer transport through the lacunocanalicular system of rat bone in an environment devoid of mechanical loading, *Bone* **22** (1998), pp. 107-117.
- [7] S.V.Komarova, F.I. Ataulakhanov and R.K.Globus, Bioenergetics and mitochondrial transmembrane potential during differentiation of cultured osteoblasts, *Am J. Physiol Cell Physiol.* **279** (2000), pp. C1220-C1229.
- [8] R. H. Kufahl and S. Saha, A theoretical model for stress - generated fluid flow in the canaliculi-lacunae network in bone tissue, *J. Biomechanics* **23** (1990), pp. 171-180.
- [9] I. D. McCarthy and L. Yang, A distributed model of exchange processes within the osteon, *J. Biomechanics* **25** (1992), pp. 441-450.
- [10] K. Piekarski and M. Munro, Transport mechanism operating between blood supply and osteocytes in long bones, *Nature* **269** (1977), 5623, pp. 80-82.
- [11] S. R. Pollack, N. Petrov, R. A. Salzstein, G. Brankov and R. Blagoeva, An anatomical model for streaming potentials in osteons, *J. Biomechanics* **17** (1984), pp. 627-636.

- [12] R. A. Salzstein and S. R. Pollack, Electromechanical potentials in cortical bone-II. Experimental analysis, *J. Biomechanics* **20** (1987), pp. 271-280.
- [13] T. Tanaka and A. Sakano, Differences in permeability of microperoxidase and horseradish peroxidase into the alveolar bone of developing rats, *J. Dent Res.* **64** (1985), pp.870-876.
- [14] T. Tanaka, Transport pathway and uptake of microperoxidase in the junctional epithelium of healthy rat gingiva, *J. Periodont Res.* **19** (1984), pp. 26-39.
- [15] K. Tanaka-Kamioka, H. Kamioka, H. Ris, S. Lim, Osteocyte shape is dependent on actin filaments and osteocyte processes are unique actin-rich projections, *J. Bone Miner. Res.* **13** (1998), pp. 1555-1568.
- [16] J. C. Weaver, K. T. Powel, R. A. Mintzer, S. R. Sloan, H. Ling, The diffusion permeability of bilayer membranes, *Bioelectrochemistry and Bioenergetics* **12** (1984), pp. 405-412.
- [17] S. Weinbaum, S. C. Cowin and Y. Zeng, A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses, *J. Biomechanics* **27** (1994), pp. 339-360.