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**APPLICATION OF STOCHASTIC NETWORK MODELS
FOR THE STUDY OF MOLECULAR TRANSPORT PROCESSES IN BONE**

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INTRODUCTION

Osteocytes are the most abundant cells in bone. They are entombed in lacunae within the bone matrix, but are interconnected via their processes that run within the canaliculi with other osteocytes, as well as with the osteoblasts and bone lining cells on the bone surfaces, and thus form a cellular syncytium. However, the osteocytes are not immediately connected with the vasculature of bone, which means that the transport of nutrients and hormones to the cells and the removal of waste products from the cells, as well as transport of signaling molecules between the cells, has to occur either via the pericellular fluid spaces in the lacunocanalicular network, via the matrix micropores between the collagen fibers and the apatite crystals, or via intracellular transport mechanisms. Only recently our laboratory and other research groups have started to examine the transport pathways of different molecular size substances within bone systematically, using experimental tracer methods (e.g. [1, 7]). These experiments have unveiled the molecular sieving characteristics of bone: While small tracers with molecular weights of 300 Daltons (Da, e.g. glucose and small amino acids) are found in abundance throughout the bone matrix and the lacunocanalicular network, larger molecules (e.g. cytokines and serum derived proteins) are only transported through the pericellular spaces of the lacunocanalicular network. Furthermore, the transport of these substances through the lacunocanalicular network can be enhanced by mechanical loading of bone [1]. These findings highlight the importance of the lacunocanalicular network for the survival of the osteocytes and thereby tissue health. However, the state of the osteocyte syncytium is affected by age and bone diseases. It has been shown that the number of

osteocytes in cortical bone decreases with age [6]. Furthermore, a histological study of cortical bone tissue samples from donors undergoing hip replacement surgery has shown that the morphology of the lacunocanalicular network is altered in diseased bone [2].

In order to simulate molecular transport along the different pathways in healthy and diseased bone, we are developing stochastic network models. This method has been previously used in chemical engineering for the simulation and optimization of column chromatography [3]. It is based on the percolation theory and is ideal for the computational simulation of flows and transport within hierarchical, porous networks and allows for taking different transport phenomena into account. In the case of bone, these may include diffusive or convective transport, sources and sinks, as well as cell initiated active transport mechanisms. We have used this method successfully to demonstrate the influence of age related osteocyte density decrease on the bone tissue permeability [4], as well as for a simulation of the molecular sieving characteristics of bone [5]. In the current study, we examined the influence of bone diseases, such as osteoporosis and osteomalacia, on the transport capacity of bone tissue. There is evidence that these diseases affect the state of the osteocyte syncytium, which can be described using parameters such as osteocyte density, connectivity and canalicular tortuosity. While the disease-related changes to the lacunocanalicular network have only been described semi-quantitatively (a quantitative study including age matched samples from a big number of donors is currently under way), the aim of this parametric study was to identify the parameters with the biggest influence on the transport of various molecules to and between the osteocytes.

Understanding of the disease related changes to the transport capacity of bone is essential for the development and planning of pharmacological therapies for the treatment of these diseases.

METHODS

A three-dimensional, cubic lattice network model with the dimensions $L \times L \times L$ ($L=15$) was constructed. The regular pores connecting the nodes simulate the matrix microporosity with two classes of randomly distributed pore diameters representing the pores between the collagen fibers and the apatite crystals, respectively. In addition, an overlying system of canaliculi connects the randomly distributed osteocytes across the network. Next, convective (driven by a pressure gradient) and diffusive (driven by a concentration gradient) transport of two classes of molecules was calculated across the network: A small molecule with a molecular weight of 300 Daltons, which is able to penetrate most matrix micropores, and a larger molecule (70,000 Daltons), which is more confined to the lacunocanalicular system. Convective flow was characterized by the calculated average flow velocity through the network, and diffusive transport by the calculated diffusivity of the system. In order to simulate different disease states, varying degrees of osteocyte connectivity and canaliculi tortuosity were used in the models: Osteocyte connectivity, the maximal number of canaliculi connecting two osteocytes, was changed between one and five, based on histological analysis. The canalicular tortuosity was accounted for with a tortuosity factor, the ratio between the direct connection of two osteocytes and the effective canalicular length, with values between one (direct line) and three (equivalent to a approximately the length of a circular arch between the two osteocytes). The calculations of the transport parameters were repeated twenty times for different networks, to reach statistical significance.

RESULTS

The convective flow calculations show for both sizes of molecules that the osteocyte connectivity seems to have a bigger influence on the average pore fluid velocities than the canalicular tortuosity.

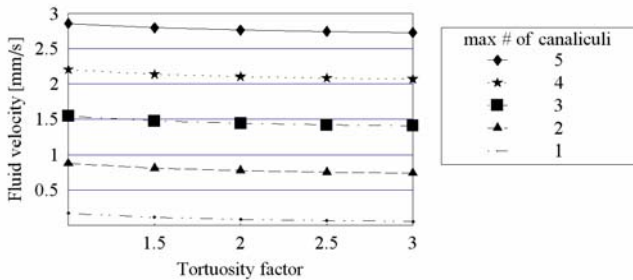


Figure 1: Decreasing average pore fluid velocity for a small molecule through networks with decreasing osteocyte connectivity and canalicular tortuosity.

The decrease in flow velocity with decreasing numbers of connecting canaliculi is almost linear, whereas there is only a small decrease for increasing tortuosity (Fig. 1).

For diffusive transport across the network, the tortuosity plays a bigger role (Fig. YY). The diffusivity decreases by approx. 50% if the tortuosity factor increases from one to three. For varying connectivities, the increase in diffusivity between one and maximal two connecting canaliculi is almost fourfold. For further increases in the number of connecting canaliculi the diffusivity increase is smaller.

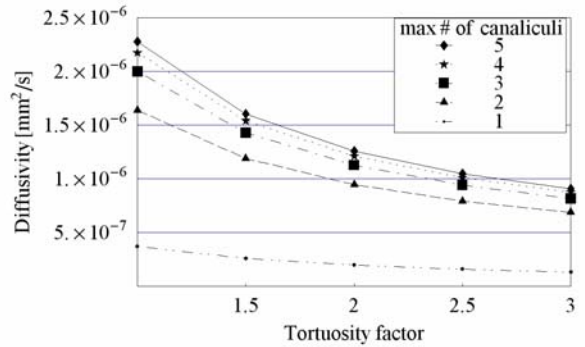


Figure 2: Decreasing diffusivity for a small molecule across networks with decreasing osteocyte connectivity and canalicular tortuosity.

DISCUSSION

Stochastic network simulations of convective and diffusive transport through bone revealed the interrelationships between disease related changes in the osteocyte syncytium and the transport capacity of bone tissue. The results of this study emphasize the early detection of changes to the structure of the cellular network. Decreased osteocyte connectivity and increased tortuosity influence both convective and diffusive transport of small and large molecules, which is likely to influence the osteocyte viability. This means that once the deterioration of the network has started, it becomes increasingly difficult for both small and bigger molecular weight substances to reach the osteocytes.

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