INTRODUCTION

Until recently, an osteocyte was defined as an “osteoblast that is embedded in bony tissue and which is relatively inactive” [1]. Recent studies implicate an active role of this cell in mechanotransduction and functional adaptation of bone tissue [2,3]. The osteocyte lies within a space referred to as a lacuna and its cell processes are contained within long cavities called canaliculi. These processes interconnect osteocytes to each other as well as to vascular channels, the medullary cavity and the periosteal surface of bone. Hence, osteocytes form a syncytium that links cells deep within bone tissue to the marrow, from which the vascularization of bone derives, and the surface of the bone, to which mechanical loads are imparted via tendons and ligaments [4]. The lacunocanalicular system provides a conduit for “metabolic traffic and exchange” and is essential for maintenance of osteocyte viability [3,5].

Given the important role of the osteocyte syncytium as well as fluid flow through the lacunocanalicular system in mechano-sensation and transduction phenomena in bone, we hypothesize that the state of the syncytium reveals the state of the tissue. Hence, the purpose of this study was to investigate the osteocyte syncytium as well as the pericellular space defined by the lacunocanalicular system.

MATERIALS AND METHODS

Cortical bone was examined from human femora diagnosed by a pathologist as healthy or as overtly osteoporotic, osteoarthritic and osteomalacic. Samples were stained with basic fuchsin stain. Image stacks were obtained using the laser scanning confocal microscope with excitation/emission spectra comprising 568 nm/580-624 nm. Each z-stack consisted of 64 x-y images. They were rendered in three dimensions using Velocity® and OpenLab® software on a Macintosh G4 computer. In order to maintain real dimensions, interpolation parameters for the third direction (z axis) were calculated to account for the magnification used during sampling. To enhance visualization, movie sequences were produced with the stacks rotating along different axes.

RESULTS

In the healthy human femur (Fig.A), a myriad of osteocytes and their processes are visible across the section. The interconnectedness of the syncytium decreases across the cement line but some processes do cross the cement line and interconnect with interstitial bone as well as with neighboring osteons.

The interconnectedness of the syncytium is particularly striking when observed in three dimensions. In contrast, at early stages of osteoporosis (Fig.B), the interconnectedness of the syncytium is decreased. This effect is exacerbated in advanced stages of the disease, where not only the interconnectedness of the syncytium is decreased, but the osteocytes appear plumper. This is due to the fact that osteocyte osteolysis has occurred, widening the lacunae. The orientation of the lacunocanalicular system has decreased as well. These effects are more extreme in advanced stages of osteoporosis, where individual osteocytes appear isolated. Their processes are not interconnected and appear tortuous. In osteoarthritic bone (Fig.C), the orientation of the lacunocanalicular system appears intact, but the degree of interconnectedness between cells has decreased, as has the viability of the cells. The less viable cells have pyknotic nuclei. In osteomalacia specimens (Fig.D), the interconnectedness of the cells appears increased, but the lacunocanalicular system appears disoriented. The lack of mineral in these bones is apparent when one considers the space occupied by the many processes and lacunae.

DISCUSSION

In summary, comparing healthy and osteoporotic bone, healthy bone is exemplified by its high degree of interconnectedness as well as by the degree of orientation of the syncytium. This would be expected to result in a decrease in resistance to flow and mass transport and optimization of mechanochemical transduction through the tissue. In contrast, osteoporotic bone shows a marked decrease in interconnectedness and orientation, resulting in an increased resistance to fluid flow and mass transport. This is expected to impair mechanochemical transduction through bone. Comparing osteoporotic, osteoarthritic and osteomalacic bone, the distribution of stain in osteoarthritic bone looks much like that in bone after fatigue loading. Although overt microdamage is not visible in this micrograph, there is evidence of a decrease in cell viability, manifested through morphology of the cells as well as a decrease in interconnectedness. Orientation has not been affected by the disease, but due to the decrease in interconnectedness, it is expected that resistance to flow and transport would increase, making mechanochemical transduction less efficient. Interestingly, in the bone from a patient with osteomalacia, interconnectedness increased dramatically. Whereas one would expect a decrease in resistance to flow and transport to result, it is expected that mechanotransduction would be impaired by lack of orientation and increased tortuosity of the network.

In conclusion we were able to show that the state of the syncytium reflects the state of the tissue and vice versa. These, in turn, reflect the state of osteocytes, which are believed to sense mechanical loads at a local level. The interconnectedness and orientation of the lacunocanalicular system are both important predictors of fluid flow and mass transport through the tissue. Hence, we expect them to have a profound effect on mechanotransduction in bone tissue, modulating the state of the tissue both in health and disease.

REFERENCES


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**Institute of Biomedical Engineering, ETH, Zurich, Switzerland.

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