GENERAL SUMMARY

Bone is a biological system that demonstrates adaptive response to mechanical loading both at the tissue and cellular levels. It is currently thought that bone cells do not directly sense the loading of the bone matrix, but rather respond to the strain-induced flow of interstitial fluid. In response to flow, bone cells produce signaling molecules like nitric oxide (NO) or prostaglandin E_2 (PGE_2) that drive the adaptive response of bone to mechanical loading. Several studies have shown that the rate of the mechanical load or stress rather than its magnitude relates to bone formation. Thus, the rate of loading seems to be a decisive factor in bone formation and maintenance. However, the underlying physical picture for understanding how the rate of stress might relate to a meaningful physiological response remains poorly understood.

In this thesis we found that bone cells release increased amounts of NO in relation to the rate of fluid shear stress. This supports the notion that bone formation *in vivo* is stimulated by dynamic rather than static loads. This rate-dependent response was found to occur, provided that the cells initially experience a stress-kick (*i.e.*, a sudden increase of stress). Both these findings provide an explanation for sustained bone formation despite the sporadic occurrence of high amplitude strains in normal physiological loading conditions. High rates of stress and stress-kicks are expected in mechanical loading experienced during high-impact activities, as may occur in sports or exercise. Thus, although high-impact activities might be infrequent, the resulting stresses could stimulate bone cells to trigger the adaptive responses of bone.

We have shown the property that the bone cell response to stress is enhanced by the addition of noise. The nitric oxide released by bone cells reached a maximum at the application of an optimum noise-level by fluid shear stress. Our study used noise by fluid shear stress stimulation to find differences in the response of MLO-Y4 and MC3T3-E1 cells, used as models for osteocytes and osteoblasts, respectively. By using a theoretical model, relating

stress information to the molecular response of bone cells, under the influence of noise, this study suggested that there are differences in threshold-activation for NO and PGE_2 production for both osteocytes and osteoblasts. Thus, at conditions of high stress with noise, MLO-Y4 cells could have a peak NO response, while MC3T3-E1 cells, a PGE_2 response. Since NO is known to drive away osteoclasts, and PGE_2 might promote the recruitment of osteoclasts, it is possible that *in vivo*, osteoclasts are driven to be active close to osteocytic regions at low stress conditions with noise. On the other hand, osteoclasts are driven to be active near osteoblastic regions at high stress conditions with noise. Clearly, low stress might promote bone loss; however, high stress seems to promote the activity of osteoclasts near osteoblasts. Furthermore, the possible role of noisy stress in the mechanical adaptation of bone provides a partial explanation for the positive effects of indirect stress applications to stimulate bone formation. The threshold-activation property of bone cells indicates a capacity for enhanced response at stress conditions obscured even by minute stress sources, as from musculatory vibration, ultrasound, or vibratory motion, typified by low-magnitude high frequency loading.

Using a two-particle *in vitro* assay for characterizing the viscoelasiticity of cells, we probed the mechano-activity and mechanosensitivity of various bone cell types and fibroblasts. Mechano-activity is characterized by the induction of force traction on attachment sites by cells, and mechanosensitivity is the ability of cells to sense forces. We found that osteocytic cell types (primary osteocytes from chicken and MLO-Y4 cells) induce a relatively higher traction force on attached particles than osteoblastic cells (primary osteoblasts from chicken and MC3T3-E1 cells). Fibroblastic cells (CCL-224) are even more mechano-active compared to MLO-Y4 cells, which explains the propensity of fibroblasts for motility *in vitro*. In our two-particle *in vitro* assay, MLO-Y4 cells release nitric oxide simultaneous with increasing force application. It is clear that force traction, morphology change, and possibly the release of signaling molecules are all related in similar pathways, in response to environmental stress conditions.

We have outlined above two discernible properties of bone cell response to fluid shear stress, which are, rate-dependence and threshold-activation. The rate-dependent response was interestingly demonstrated by bone cells in a broad range of frequencies induced by vibration stress. The final local effects on bone, under conditions of loading are further understood by having a quantifiable interplay between cell types, due to the resulting release of signaling molecules.

We have shown that bone cells are responsive to dynamic stress. This supports findings that the rate rather than the magnitude of loading is the important parameter for osteogenic properties of mechanical loading to bone. This insight has relevant implications on the local activity of osteocytes for directing the mechanical adaptation of bone. We have also shown that the released signaling molecules of bone cells are related to the frequency of stress in an empirical relation. Since the activation of bone cells is highly dependent on the frequency of experienced stress, bone cells might be capable of responding to very meager amounts of strain, after overcoming a stress threshold. Thus, under extreme conditions of unloading (*e.g.*, microgravity environment) it might be possible to counteract the onslaught of bone loss by sporadic bouts of high-impact loading. We have shown here relations between what we consider relevant parameters of mechanotransduction in bone, which are the applied stress, the amount of signaling molecules released by osteocytes, and their possible roles for directing the local activity of osteoclasts and osteoblasts. Bone loss can be understood as resulting from a disturbance of the homeostasis of these parameters. Since these parameters are closely related as we have shown, it might be possible to restore their homeostasis despite extreme conditions of unloading.

However, the question remains as to how cells might detect microgravity directly. We probed the activity of bone cells by measuring the forces induced by cells on attached fibronectin-coated beads. We showed that the force fluctuation of bone cells had a characteristic frequency spectrum signature. This force fluctuation is indicative of intra-cellular processes. The

characteristic signature was shown to be an indication for the diffusive properties of intra-cellular particles or organelles. Diffusive processes govern the relation between microtubule re-organization, and possibly intra-cellular transport, which might be affected by gravity. Thus, intra-cellular transport might be crucial for mechanosensing. Bone cells might be able to detect changes in the gravitational field directly by the gain or loss of gravitational forces influencing the re-structuring of self-organizing polymers inside cells, thereby influencing intra-cellular transport. Thus, it is possible that the signature for force fluctuations inside cells might change under microgravity influencing bone cell mechanosensitivity as indicated by a changed release of signaling molecules.