CHAPTER 8

GENERAL ABSTRACT
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Bone is a living tissue and is able to alter its mass and structure to its mechanical environment. Increased mechanical loading results in a gain of bone mass and mineral density in vivo, while unloading of bones is known to reduce bone formation, mineral content, and bone matrix protein production. This process is known as functional adaptation of bone, and it serves to obtain bones that combine a proper resistance against mechanical failure with a minimum use of material. Adaptation of bone to changing environmental demands is obtained during the complicated process of bone remodeling. It is currently believed that the process of bone adaptation is governed by the osteocytes, which respond to the loading-induced flow of interstitial fluid through the lacuno-canalicular network by producing signaling molecules that can regulate the activity of the effector cells, the osteoclasts and the osteoblasts, which subsequently leads to adequate bone mass and structure.

This thesis examined the role of mechanical loading in various aspects of bone adaptation such as occurs during an orthodontic tooth movement, at the cellular level. Osteocyte apoptosis likely regulates bone remodeling by attracting osteoclasts. Nitric oxide (NO) is an important signaling molecule in response to mechanical loading, and is produced by osteocytes through the activity of constitutive endothelial nitric oxide synthase (eNOS) and/or inducible nitric oxide synthase (iNOS). We hypothesized that the osteocytes play a key role in the adaptation of bone to mechanical loading, and that this bone adaptation process is regulated by NO. To test this hypothesis, we addressed the following scientific questions:

1. Is there a relationship between osteocyte density, bone remodeling parameters, gender, and osteoporosis?
2. Does mechanical stimulation by fluid flow inhibit tumor necrosis factor-α-induced apoptosis of osteocytes, and is the inhibition of osteocyte apoptosis mediated by loading-induced NO production?
3. Which apoptosis-related genes alter their expression in response to
To find answers to these questions we investigated the relationship between osteocyte density, bone remodeling parameters, gender, and osteoporosis. We showed that the number of osteocytes embedded in the bone matrix depends on gender, and differs between healthy and osteoporotic subjects (Chapter 2). Osteoporotic patients showed reduced bone turnover and changed bone architecture, which is characteristic for osteoporosis, and the results are consistent with impaired osteoblast function in osteoporotic patients. The reduced osteocyte numbers in female osteoporotic patients might relate to imperfect bone remodeling leading to reduced bone mass and strength (Chapter 2).

Osteocyte apoptosis likely is the signal for osteoclast recruitment. Osteoclastic attack is directed towards apoptotic osteocytes, suggesting a key regulatory role for osteocyte apoptosis in bone remodeling, such as occurs after orthodontic load application. We studied osteocyte apoptosis induced by tumor necrosis factor-α (TNF-α) after pulsating fluid flow (PFF) application (Chapter 3). To validate that NO may modulate apoptosis, the release of NO was inhibited by N\textsuperscript{6}-Nitro-L-Arginine Methyl Ester (L-NAME). We found that fluid shear stress inhibits TNF-α-induced apoptosis specifically in osteocytes, but not in osteoblasts or periosteal fibroblasts. This inhibitory effect was, at least in part, mediated by NO. This suggests a regulatory role for osteocyte apoptosis in osteoclastic bone resorption during bone remodeling such as occurs after application of an orthodontic load (Chapter 3).

Which apoptosis-related genes alter their expression in response to a physiological mechanical load is unknown. We studied apoptosis-
related gene expression in response to PFF in osteocytes, osteoblasts, and fibroblasts (Chapter 4). To validate that NO may modulate apoptosis-related gene expression in osteocytes, we inhibited the release of NO by L-NAME. We measured gene expression of Bcl-2, caspase-3, p53, and c-Jun, because these molecules are key regulating molecules of cell apoptosis. We found that PFF stimulates Bcl-2 gene expression and inhibits caspase-3 gene expression, but did not alter p53 and c-Jun gene expression in osteocytes. Inhibition of NO synthesis by L-NAME prevented the PFF-mediated changes in Bcl-2 and caspase-3 gene expression in osteocytes. This suggests that NO is, at least in part, responsible for the loading-induced inhibition of osteocyte apoptosis via alterations in Bcl-2 and caspase-3 gene expression (Chapter 4).

Pericellular fluid may be critical in transmitting soluble mediators from osteocytes to other bone cells to produce a desired response to mechanical stimulation. In depth examination of the effects of conditioned medium from mechanically-stimulated osteocytes on osteoclast formation and activity can help to expand our understanding of the soluble factors released by osteocytes into the pericellular fluid. We investigated if mechanically-stimulated osteocytes are capable to modulate osteoclast formation and bone resorption via soluble factors (Chapter 5). To validate that NO may modulate osteoclast formation and bone resorption, the release of NO was inhibited by L-NAME. We found that osteocytes subjected to PFF inhibit osteoclast formation and resorption via soluble factors, and the release of these factors was at least partially dependent on activation of an NO pathway in osteocytes in response to PFF. It appeared that the osteocyte is more responsive to PFF than the osteoblast or periosteal fibroblast regarding to the production of soluble factors affecting osteoclast formation and bone resorption (Chapter 5).

Orthodontic tooth movement is characterized by sequential reactions of the periodontal tissues to biomechanical forces. We hypothesized that eNOS and iNOS regulate the tissue response to orthodontic force, and therefore we investigated eNOS and iNOS expression in osteocytes during orthodontic force application in a rat model (Chapter 6). Immunohistochemical staining revealed that in the
tension area, eNOS-positive osteocytes increased from 24 hrs on, while iNOS-positive osteocytes remained largely constant. In the compression area, iNOS-positive osteocytes increased already after 6 hrs, while eNOS-positive osteocytes increased after 24 hrs. This suggests that eNOS mediates bone formation in the tension area, while iNOS mediates inflammation-induced bone resorption in the compression area. Both eNOS and iNOS seem to regulate bone remodeling during orthodontic force application (Chapter 6).