Chapter 1

Aim and Scope of this Thesis

Aim and Scope of this Thesis

Bone has the unique property of complete regeneration after disruption of its architecture. The mechanisms behind this phenomenon are of great interest not only to understand the repair and maintenance processes of bone tissue, but also considering its implications for the regeneration of non-skeletal tissues. Growth factors are now generally accepted to play a crucial role in regulating bone formation and bone resorption. The maintenance of bone depends on the delicate equilibrium between formation and resorption. After disruption of the architecture of bone as occurs in skeletal fractures, new bone formation is critical to regain its supportive function. The general response of bone to fracture is formation of excessive fibrocartilage, which is mineralized and eventually forms a bony callus reuniting the fracture segments. Distraction osteogenesis, in which bone formation occurs under gradual distraction of two bone surfaces, relies on this ability of bone to repair itself. This technique allows for lengthening procedures of long bones and reconstruction of bone deficits by the induction of new bone. Distraction osteogenesis proved being an effective treatment option both in humans and in canine patients. In addition, distraction osteogenesis is a valuable asset in experimentally studying the intricate role of growth factors during bone formation.

The aim of this thesis was threefold. The first goal was to evaluate the clinical use of distraction osteogenesis in treating antebrachial growth deformities in the dog. The second aim was to investigate experimentally the role of bone growth factors during distraction-induced bone formation. The third goal was to study the effect of growth hormone treatment on bone regeneration in a critical sized bone defect model.

In the general introduction of this thesis (**Chapter 2**) an overview is given of bone histology and histiogenesis, growth plate injuries, healing of bone fractures, and distraction osteogenesis. Attention is then focused on circular external skeletal fixation systems, which are used in the clinical and experimental studies in this thesis. The hormonal regulation of bone is the next item in the general introduction. An extensive review is presented concerning the role of skeletal growth factors in bone formation and resorption. This overview is completed with a description of the most important markers of bone metabolism.

The following chapter of the thesis focuses on the clinical use of distraction osteogenesis in dogs. The outcome and prognostic factors are presented when treating antebrachial growth deformities with a lengthening procedure in canine patients. Emphasis is put on the role of incongruity of the elbow joint and antebrachiocarpal subluxation concomitant with antebrachial growth deformities (**Chapter 3**).

The second part of the thesis is concerned with growth factors which play a role during distraction osteogenesis. A cascade of growth factors is essential for bone formation both during distraction-induced bone regeneration and bone healing. These growth factors are expressed locally, but are also known to affect circulating levels of these factors. We hypothesized that the local expression of growth factors and systemic levels of factors associated with bone regeneration differ between distraction osteogenesis and osteotomy bone healing. A canine crural lengthening model was used to explore the local expression of insulin-like growth factor-I (IGF-I), insulin-like growth factor II (IGF-II), growth hormone (GH), growth hormone receptor (GHR), and bone morphogenetic protein-2 (BMP-2) in combination with the circulating levels of GH, IGF-I, IGF-II, insulin-like growth factor binding protein-4 (IGFBP-4), and insulin-like growth factor binding protein-6 (IGFBP-6) (**Chapter 4**).

In the past, monitoring of progression of osteogenesis during active distraction and subsequently in the maturation and consolidation phase of bone regenerate has depended mainly on repetitive radiographic examinations. Radiography evaluates the amount of mineralization in the newly formed callus. During active lengthening the bone regenerate mainly consists of fibrous tissue and blood vessels. Mineralization becomes evident in the consolidation phase of the bone regenerate. We hypothesized that markers of bone formation and bone resorption in plasma could effectively monitor osteogenesis comparing distraction osteogenesis with osteotomy bone healing. The bone markers under scrutiny were osteocalcin (OC) as a proposed marker of bone formation and carboxyterminal cross-linked telopeptide of type I collagen (ICTP) as a marker of bone resorption (**Chapter 5**).

By analogy with the use of bone markers, we evaluated delayed image bone scintigraphy to assess distraction-induced bone formation. Bone scintigraphy is a non-invasive, quantitative method to evaluate changes in the activity of bone metabolism. In contrast to radiography, which addresses the amount of mineralization, delayed image bone scintigraphy evaluates the uptake of Technetium-99m tracer by immature bone regenerate at places of increased bone

turnover and thus precedes the actual accretion of bone. We hypothesized that delayed image bone scintigraphy could effectively distinguish between the amounts of distraction-induced bone and bone formed during osteotomy healing (**Chapter 6**).

Although bone has the capability to completely regenerate under optimal circumstances, delayed or absent bone healing is a major problem in orthopedic patients. Growth hormone and growth factors, including IGF-I, BMP, and transforming growth factor-β (TGF-β) have been used with varying success to stimulate bone healing in fracture and osteotomy gap models. Very little information is available to date concerning their ability to stimulate bone regeneration in a critical-sized bone defect, i.e., a segmental defect, which will not heal spontaneously. We hypothesized that continuous infusion with GH could effectively induce bone formation and bone healing in a critical-sized bone defect model. In addition, we speculated that local GH application had the largest stimulatory effect on bone regeneration within the defect (**Chapter 7**).

The findings in this thesis are summarized and discussed in **Chapter 8**. The thesis is concluded with a summary in English (**Chapter 9**) and a summary in Dutch (**Chapter 10**).