Chapter 6

Summary & General Discussion
**General discussion**

Distraction osteogenesis (DO) is a technique, originally developed in orthopedic surgery for bone lengthening, later also used in maxillofacial surgery to regenerate or augment oro-craniofacial bone. Basically, DO involves an osteotomy of bone creating a fracture area in which callus tissue will form. Tension is applied on this callus tissue by gradually distending the bone segments. The tension induces the formation of hard tissue in the gap area, and simultaneously extends muscles, nerves and soft connective tissues. This technique that uses mechanical loading is a powerful and rapid way to augment bone and can be used alternatively to other bone augmentation methods, such as grafting of autologous, allogenic or synthetic bone.

Animal studies have provided useful data to optimize treatment modalities of DO and to obtain insight in the cellular and molecular changes that are associated with bone formation during DO (Sato et al. 1998; Eingartner et al. 1999; Liu et al. 1999; Mehrara et al. 1999; Steinbrech et al. 2000; Bouletreau et al. 2002; Carvalho et al. 2004). So far few detailed reports have been published on cellular and molecular mechanisms that operate during bone healing in patients undergoing DO. For clinical use the conditions for distraction have largely been extrapolated from experimental studies in animals. Clinical results have mostly been evaluated retrospectively. In addition, the clinical healing process of DO has almost exclusively been examined by non-invasive methods. The placement of dental implants in bone regenerate after distraction osteogenesis in jaw bone provided us a possibility to obtain bone biopsies for detailed histological analysis of bone regeneration in humans without additional discomfort. This model was used in the present thesis and enabled us to test various aspects of bone regeneration in edentulous mandibles of older patients undergoing DO prospectively (chapters 2-4). In chapter 5 another experimental model was used: the rabbit long bone (Li et al. 2005), in order to test a new synthetic peptide (TP508) as a potential candidate to accelerate bone formation during DO.

In chapter 1 a literature review is presented in which various aspects of distraction osteogenesis and its technique are discussed and the gaps in
information on technical aspects of DO for clinical use in craniofacial surgery identified.

Chapter 2 describes a prospective study with 16 elderly patients who underwent alveolar bone augmentation of the mandible by vertical DO. The purpose was to assess at which of two (commonly used) distraction rates the highest amount of bone was formed in the distraction gap if the same healing time was provided. Another aim was to find out how much time was required to close the gap after distraction had stopped, and with the distractor in a rigid (neuatrofixation) position to avoid too much movement at the healing site. Bones were distracted at 0.5 or 1 mm/day for a total lengthening of 10 mm and left in neuatrofixation for 7-20 weeks. A problem was that almost half of the recovered biopsies were incomplete as they broke off in the area of the distraction gap during removal. Other biopsies recovered with a completely intact distraction gap were shorter than expected, as calculated from distension of the distractor. All biopsies were processed undemineralized for histology and histomorphometric analyses. In addition a number of the intact biopsies was also scanned by for microcomputerized tomography (μCT) prior to sectioning to analyze the closure of the gap independently from histology. Histology showed that some biopsies were clearly compressed or that the soft tissue in the gap was somewhat skewed, as a result of which a full 10 mm wide gap was not obtained. However, the (intact) upper bone segments containing trabeculae and some soft connective tissue from the distraction gap were suitable for quantitative bone morphometry to assess the amount of bone formation.

Histomorphometric analysis of bone biopsies from these patients revealed that more bone was formed in the group of patients with a distraction rate of 0.5 mm/day. This group had longer bone trabeculae with somewhat higher bone volume. Moreover, bridging the distraction gap with bone seemed to occur earlier in the 0.5 mm/day group, although the number of intact biopsies was very small.

Our study showed that in spite of the relatively high age of the patients and the atrophic nature of the bone, distraction of the fracture was still capable of
inducing a significant response of the cells in making new bone. The 0.5 mm/day distraction rate that gave better results in edentulous mandible of elderly patients was lower than the rate found to be optimal in mandible as well as in long bones of rabbits, porcine and sheep models (Li et al. 1997; Farhadieh et al. 2000; Troulis et al. 2000; al Ruhaimi 2001). Also the time needed to bridge the gap with bone differed from that found in animal studies: it took much more time in patients than reported in animals (Rowe et al. 1998; Li et al. 1999; Cope & Samchukov 2000; Richards et al. 2000). These differences may be accounted for by differences in bone metabolism (low in man), age (high in the present patient group), tissue vascularisation (variable in elderly patients) and vitality of the bone (atrophic with some areas with empty osteocyte lacunae in our patient group).

Mechanical loading enhanced not only normal fracture healing and induced formation of new bone in the soft connective tissue of the gap; it had also effect on the old bone. Loading increased bone forming activity in the upper bone segment that was moved by distraction compared to undistracted bone as shown by a 4-fold increase in osteoid volume. Thus, moderate distraction induces new bone formation and enhances bone formation in the old bone to enhance its load-bearing capacity. This is in agreement with Wolff’s law that bone is capable of adapting its structure to its mechanical demands.

In short, the data described in chapter 2 strongly suggest that optimal treatment modalities for DO found in animal experiments cannot simply be extrapolated to patients but have to be used with caution as they may be different.

Blood vessel formation proceeds bone formation and we noticed in a few patients that bone had not, or only scarcely, formed when blood vessels were lacking. Various animal studies indeed reported the importance of blood vessel formation for bone formation (Kitano et al. 1998; Ferguson et al. 1999; Li et al. 1999). In chapter 3 we tested the hypothesis that bone formation was correlated with the blood supply in the gap. We evaluated the effect of the two distraction rates on blood supply by histomorphometry of blood vessel volume on the same biopsies as used for bone volume morphometry. A selection of two groups
of 3 patients was made that had similar average age, good quality biopsies to locate vessels and only differed in distraction rate. A higher blood vessel volume density was detected in the group of patients that had undergone distraction at the lower rate of 0.5 mm/day. This was only found in the area that contained young, rapidly forming bone trabeculae near the center of the gap, not in the area with older, earlier formed bone trabeculae near the osteotomy line. This increase in blood vessel volume corresponded with an increase in blood vessel number. A positive correlation was observed between blood vessel volume and bone volume, only in the area with young rapidly forming bone. This correlation suggested that indeed vessel formation is needed for bone formation. That a lower distraction rate improves vessel volume can be explained in several ways: (1) in elderly patients, it may be that the standard distraction rate of 1 mm/day disrupts the capillaries that are forming in the soft connective tissue of the gap, but not at half that rate; (2) a slow distraction rate may directly stimulate angiogenesis in the soft connective tissue of the gap more than the standard distraction rate.

The results of chapter 3 imply that the slower distraction in elderly patients favors vessel formation and consequently bone formation which in its turn depends on vascular supply. The clinical relevance of this finding is that in elderly patients, the total healing time needed to bridge the gap may be shortened by decreasing the standard distraction rate of 1 mm/day down to 0.5 mm/day.

Based on animal studies it is assumed that the cellular and molecular mechanisms of bone formation during DO are basically similar to those effective during normal fracture healing (without distraction) (Sato et al. 1998; Liu et al. 1999; Weiss et al. 2005). This has not been verified in human distracted bone before, probably because of the difficulty of obtaining biopsy samples of the regenerating areas.

In chapter 4, we examined the cellular activity of bone cells in histological sections of distracted regenerating bone of the human mandible by immunohistochemistry. We used antibodies to RUNX2, a very early marker of osteoblast lineage, before cells become osteoblasts. RUNX2 is a transcription
factor, a master-switch that controls transcription of the major extracellular matrix proteins made by bone cells (Komori et al. 1997; Otto et al. 1997; Karsenty et al. 1999; Feng et al. 2003). Without RUNX2 protein no bone can be formed. The presence of RUNX2 in undifferentiated connective tissue cells indicates that the cells are potentially osteogenic. If the fibroblastic cells in the soft connective tissue of the distraction gap do not express RUNX2 protein, they likely originate from another (non-osteogenic) lineage, possibly involved in a fibrous non-union of the osteotomized bone ends. We also used antibodies to late markers of the osteoblast-lineage to identify (pre)osteoblast and osteocytes: osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein-1 (DMP1) and matrix extracellular phosphoglycoprotein (MEPE), all non-collagenous proteins that belong to SIBLING protein family. The SIBLING proteins are extracellular matrix (phosphoglyco) proteins that contain RGD sequences capable of binding cells. The RUNX2 protein controls transcription of most of these SIBLING proteins.

The distribution of these proteins in distracted human tissues was similar to that reported for normally healing fractures of animal tissues or for distracted animal bone. Many fibroblastic cells in the soft tissue of the gap stained for RUNX2 proteins, thus identifying them as potentially osteogenic. As these cells differentiated into osteoblasts and osteocytes they also stained for OPN and BSP. DMP1 and MEPE staining was seen almost exclusively in osteocytes. Comparison between predistracted and distracted bone showed that distracted tissues contained more cells stained for RUNX2 and matrix proteins than undistracted tissues. The intensity of staining was also higher in distracted tissues than in undistracted tissues. These phenomena were seen both in the new bone trabeculae in the gap and in the old bone already present when distraction was started. The latter finding is in line with data presented in chapter 2, confirming that indeed more bone cells in distracted bone are active and deposit more osteoid.

The distribution of the osteocyte markers DMP1 and MEPE in old bone had also changed after distraction. Both proteins were also seen in few mature
(flattened) osteocytes of lamellar bone after distraction; a phenomenon not seen before distraction. It has been reported for mouse bone that mechanical loading can activate loading-sensitive regulatory elements located in the promoter region of the DMP1 gene which enhances transcription of DMP1 in osteocytes (Yang et al. 2005). Osteocytes are bone cells that are sensitive to mechanical loading of bone and react by sending signals to bone surface cells in order to adapt the bone structure to loading. Based on this concept we hypothesized that the increase in DMP1 staining (and possibly also MEPE) in osteocytes seen in distracted bone is the response of osteocytes to distraction in order to adapt the bone structure to mechanical demands.

Several drawbacks and complications during distraction osteogenesis have been reported (Swennen et al. 2002). A lengthy neutrofixation time during which the distractor must be kept in place often leads to an infection around the device. Stabilization of the healing area is essential to ensure adequate new bone formation. Healing time in the mandibular jaw with the distractor in place is relatively long, at least 10-12 weeks after ending a 10 mm distraction. Occasionally, this period has to be extended in the case of a slow bone formation such as in aged patients or patients with a poor vascularisation. A way to accelerate the bone formation process in the gap may therefore be advantageous to the patients. Injection of bone cell-stimulating agents into the gap such as bone morphogenetic proteins (BMPs) or transforming growth factor β’s (TGFβs), application of ultrasound or acceleration of vessel formation by factors as vascular endothelial growth factor (VEGF) are options that are tested (Li et al. 2002; Schortinghuis et al. 2005), though with variable results (Eckhardt et al. 2003). A recently developed factor to enhance healing is thrombin peptide TP508, a synthetic peptide consisting of 23 amino acids. TP508 mimics the natural amino acid sequence of the receptor-binding domain of human thrombin, increases soft tissue healing and is capable of enhancing bone formation in animals during fracture healing (Li et al. 2005; Ryaby et al. 2000; Sheller et al. 2004; Wang et al. 2005). In chapter 5 we report the effect of TP508 on ECM protein expression during distraction osteogenesis in rabbit long bone.
Semi-quantitative immunohistochemistry revealed the presence of more RUNX2- and OPN-protein expressing cells in the distraction area of bones injected with high doses (300 μg) TP508 than in bones injected with saline or with low doses (30 μg) TP508. This suggests that TP508 might increase the number and/or activity of osteogenic cells in the gap by directly enhancing RUNX2 production in undifferentiated fibroblastic cells with either no or very low Runx2 gene expression. To investigate this hypothesis we exposed the (mouse) osteoblast-cell line MC3T3-E1 in vitro to TP508 and measured the mRNA levels of Runx2 by quantitative PCR. In contrast to what was expected TP508 failed to significantly increase Runx2 mRNA levels in MC3T3-E1 cells in vitro. This result suggests an indirect effect of TP508 on the differentiation of osteogenic cells in the gap area, likely by stimulating vessel growth which in turn enhances bone formation.

Thus, high levels of TP508 injected in the distraction gap stimulate closure of the gap, not by increasing the number of osteogenic cells and osteoblasts but presumably by increasing vascularization.

In summary, many of the molecular, cellular and structural aspects of bone formation during distraction of the atrophic human mandible are similar to those reported in animal studies. Yet, our data seem to indicate that the rate of distraction should be lower in the human jaw than in animal tissues. Vascularization appears to be crucial for bone formation in the gap. Improving vascularization in the gap area is an attractive option to improve treatment.
References


Chapter 6


