Chapter 2

Vertical Distraction Osteogenesis in the Human Mandible: a Prospective Morphometric Study

L. R. Amir¹, A. G. Becking², A. Jovanovic³, F.B.T. Perdijk⁴, V. Everts¹, A.L.J.J Bronckers¹


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¹Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), Universiteit van Amsterdam and Vrije Universiteit, Amsterdam, The Netherlands
²Department of Oral & Maxillofacial Surgery Oral Pathology, Vrije Universiteit Medical Center, ACTA, Amsterdam, The Netherlands
³Department of Oral & Maxillofacial Surgery, Alkmaar Medical Center, The Netherlands
⁴Department of Oral & Maxillofacial Surgery, Gelderse Vallei Hospital, Ede, The Netherlands
Abstract
Vertical distraction osteogenesis receives considerable interest as a way to augment bone prior to implants placement. However, very little is known of the appropriate distraction protocols in the human mandible. In this study we evaluate the effect of the distraction rate and the duration of neutrofixation on bone formation and closure of the gap in the human mandible.

Vertical distraction was performed in the atrophic mandible of sixteen edentulous patients, aged 62 ± 6 years. The bone was distracted for approximately 10 mm at a rate of either 0.5 or 1 mm/day. Bone biopsies were taken after seven to twenty weeks of neutrofixation.

Histological analysis demonstrated newly formed bone in the distraction gap in all biopsies. The bone was predominantly of the woven type. After 10 weeks of neutrofixation, the gap was bridged by new bone in two out of three intact samples in the 0.5 mm/day group, but not in two intact samples of the 1 mm/day group. Histomorphometry revealed longer bone trabeculae (p=0.02) and a somewhat increased bone volume in the area where new bone formation started (p=0.07) in the group of patients with 0.5 mm/day of distraction rate than in the 1 mm/day group.

We conclude that in elderly patients a distraction rate of 0.5 mm/day results in a faster osteogenesis in the distraction gap than a rate of 1 mm/day. A minimum of 10 weeks of neutrofixation seems to be needed to close a 10 mm gap after cessation of distraction.

Introduction
Distraction osteogenesis (DO) is a process of bone regeneration induced by gradual traction on the osteotomized bone segments and was initially used for elongation of long bones (Ilizarov 1989a,b). The biological phenomena of the early phase of DO share many features with fracture healing. Following the osteotomy, a haematoma is formed that encircles the osteotomized bone segments. Granulation tissue that consists of soft-connective tissue cells, neutrophils and invading capillaries replaces the haematoma after several days
and this tissue subsequently converts to soft callus. As tension forces are applied to the tissue of the gap, the collagen meshwork becomes oriented in the direction of tension and the formation of woven bone follows this collagen scaffold (Ilizarov 1989b; Aronson et al. 1990). The critical mechanical and biological factors regulating bone formation in the distraction gap involve: (1) preservation of periosteum; (2) a delay or latency period after osteotomy prior to distraction; (3) a stable fixation to eliminate undesirable micromotion; (4) distraction at a slow rate with small but frequent steps; and (5) a neutral fixation period after completion of distraction (Ilizarov 1989a,b).

Recently DO has been applied as an alternative technique for alveolar bone augmentation of the human mandible. This approach is considered to be more rapid and to generate a more predictable amount of alveolar bone than other augmentation techniques and simultaneously increases the surrounding soft tissue volume (Chin & Toth 1996; Chin 1999; McAllister 2001; Perdijk & van Strijen 2001a,b; Rachmiel et al. 2001; Jensen et al. 2002; Raghoebar et al. 2000, 2002; Chiapasco et al. 2004a,b). The first animal and clinical reports indicate the feasibility of distraction in augmenting alveolar jaw bone (Chin & Toth 1996; Block et al. 1996, 1998). A human case study reported the increase of bone mass of the anterior alveolar mandible by vertical DO enabling the placement of endosseous implants (Chin & Toth 1996; Chiapasco et al. 2004a,b).

One of the factors for successful DO is the daily rate with which the bone segments are distracted. Animal studies showed that a distraction rate of 1 mm/day is optimal in terms of the amount of new bone formed, mineralization, and mechanical strength (Choi et al. 1997; Farhadieh et al. 2000; al Ruhaimi 2001; King et al. 2003). A lower rate (0.5 mm/day) very often led to premature closure of the distraction gap and a higher rate (2 mm/day) resulted in a fibrous union (Ilizarov 1989b; al Ruhaimi 2001). These results were extrapolated for clinical application in man and were the basis for the assumption that in the human jaw a distraction rate of 1 mm/day is optimal (McAllister 2001; Rachmiel et al. 2001; Raghoebar et al. 2000, 2002).
Despite of the clinical success of DO and extensive experimental studies, information on DO in the human jaw is still limited. The few reports evaluated the effects of DO clinically or by non-invasive techniques, such as X-rays which have low resolution and do not give detailed information on type and amount of bone formed, degree of mineralization or the histological aspects of osteogenesis (Chin & Toth 1996; Chin 1999; Perdijk & van Strijen 2001a,b; McAllister 2001; Rachmiel et al. 2001; Raghoebhar et al. 2002). In addition, animal studies and clinical applications differ in a number of aspects. Mandibular bone augmentation in animals has been carried out mainly in a horizontal direction and has used fast growing young or adult animals with their dentition still in place. However, in a clinical situation vertical DO is performed primarily in elderly patients with severe atrophic edentulous jaws. Since it is known that there is a reduced capacity of bone regeneration in elderly people (Shirota 1993; Aronson et al. 2001; Aronson 2004), the optimal rate of distraction in a clinical situation may be different from that assessed in animals. Hence, the data from animal experiments are not necessarily applicable to patients. Accordingly, there is a definite need for quantified information on the effect of DO in the human jaw, preferably at the histological level. No quantified histomorphometric data have been reported for DO in the human jaw.

This study examines two aspects of vertical DO in the human mandible: the rate of distraction on osteogenesis in the distraction gap and the time required to close the distraction gap during neutrofixation. Two questions are raised: (1). Is there a difference in the amount of bone formed between distraction rates of 0.5 and 1 mm/day, given the same healing time? (2). How long does it take to close the gap with new bone after cessation of distraction?

**Materials & Methods**

*Patients*

Sixteen edentulous patients, 14 female and 2 male, aged 47-73 years (mean ± SD: 62 ± 6) were included in this study (Table 1). They suffered from severe mandibular bone atrophy (mandibular height approximately 8 mm, Cawood
Bone formation during vertical distraction osteogenesis

classification V-VI; Cawood & Howel 1988) with concomitant prosthetic complaints. They all underwent ridge augmentation using distraction osteogenesis. The selection criteria for the patients were as follows: a good general physical and mental health, no drug and/or alcohol abuse, no systemic diseases that could interfere with normal wound healing, and a good oral health. All patients gave their informed written consent prior to surgery. Recruitment of patients and surgery were performed at two locations, each by an experienced surgeon. No randomization was made; the treatment protocols were exactly the same in both centers except for the distraction rate. In the first center a rate of 0.5 mm/day was used and in the other 1 mm/day. Patients gave informed consent and approval of the Medical Ethical Committee of North Holland was obtained (registration number M03-010).

Surgical procedures

Operations were performed under general anesthesia with penicillin prophylaxis (1 g intravenously). An intraosseous type of distractor (Groningen distractor, KLS Martin) was used in one patient and two extraosseous types of distractors were used in the other fifteen patients (KLS Martin and Mondeal Medical System, Tuttlingen, Germany) (Table 1). Initially, the operation site was exposed by a 2-4 cm horizontal vestibular incision on the labial surface, followed by a careful elevation of mucoperiosteum flap to avoid injury to the mental nerve. This incision is believed to hold the advantage over a mid-crestal incision to preserve blood supply for the crestal aspect of the transport bone segment, thereby maintaining its vitality. The lingual mucosa remained attached to the bone, also to provide a blood supply via the periosteum. Before performing osteotomies, the distraction device was temporarily placed to create a mark for the screws in order to have the correct position. The vertical line of osteotomy was marked halfway the ridge height (approximately 4 mm, the height of the bone transport segment). Then the planned outline of horizontal and vertical osteotomy was drilled. A saw was used to complete the osteotomy. The vertical osteotomy was done convergently in the apical direction to avoid any undercut that prevents the transport bone segment
from moving superiorly. Next, the distraction device was fixed in place with screws and finally the surgical wound was closed with the activation screw exposed to the oral cavity. All patients received antibiotic medication consisting of amoxycillin 3x500 mg per day for 7 days post-operatively.

**Table 1. Overview of patient's data**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Age (year)</th>
<th>Distraction type</th>
<th>Rate*m/mm/day</th>
<th>Healing time** (days)</th>
<th>Distraction gap length (mm)</th>
<th>mm length of trabeculae</th>
<th>μCT</th>
<th>Baseline</th>
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<td>1</td>
<td>F</td>
<td>63</td>
<td>Extra-osseous</td>
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<td>69</td>
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<td>3.2</td>
<td>2.8</td>
<td>√</td>
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<td>5.1</td>
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<td>1.0***</td>
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<td>7</td>
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<td>0.2***</td>
<td>1.2</td>
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<td>-</td>
<td>√</td>
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<td>2.0</td>
<td>-</td>
<td>√</td>
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<tr>
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<td>154</td>
<td>6.1</td>
<td>3.5</td>
<td>0.6</td>
<td></td>
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*Rate: Distraction rate; ** Healing time is distraction plus neurofixation time; ***: Compressed biopsy. - : absent in the biopsy. √: scanned by μCT or biopsy taken also before distraction.

From four randomly chosen patients of one group (1 mm/day of distraction rate), bone biopsies were taken also prior to the osteotomy. The biopsies were harvested from the alveolar bone in the area just next to the transport bone with a cylindrical hollow trephine burr. The size of the bone biopsies was approximately 1.5 mm in diameter and 3 mm in length. These biopsies served as baseline bone samples and will be termed as pre-distraction bone.
**Distraction protocol**

After a 5-7 days latency period to allow the formation of a reparative callus, the patients themselves activated the distractor by turning the screw with a rate of either 0.5 or 1 mm/day (2 times per day 0.5 mm). The total lengthening was approximately 10 mm (distraction period: 10 days for a rate of 1 mm/day in the first group and 20 days for a rate of 0.5 mm/day in the second group). After the desired lengthening was achieved, the activation was discontinued and the transport bone segment was maintained in neutral position for 7 to 20 weeks, prior to removal of the distraction device. Following removal of the distractor, the bone was prepared for implant insertion that provided the opportunity to obtain biopsies. Vertical biopsies were taken with a cylindrical hollow trephine burr with a 3.5 mm outer diameter (kindly donated by Straumann, Waldenburg, Switzerland) drilling through the upper (transport/incisal) segment, the distraction gap and into part of the lower (apical) bone. In order to avoid compression and distortion of the delicate regenerate, the material was not pushed out from the trephine bur but carefully removed after opening the trephine along its long axis with a rotary diamond disk.

Since patients with a 0.5 mm/day of distraction rate underwent 10 more days of distraction than patients with a distraction rate of 1 mm/day, for the purpose of statistical analysis, the neutrofixation time of the 1 mm/day group was extended with 10 days, making the healing time (i.e., distraction time plus neutrofixation time) of both groups equal.

**Histological procedures**

The biopsy specimens were fixed in 4% formaldehyde solution in 0.1 M phosphate buffer pH 7.3 at 4°C for 24-48 h and rinsed in 0.1 M phosphate buffer pH 7.3, 2 times 1 h at room temperature. Specimens were dehydrated through ethanol series at 4°C and embedded in low temperature polymerising methylmetacrylate (MMA) without decalcification. Longitudinal sections of 5 μm thick were cut on a Jung heavy-duty microtome. For histomorphometry
five groups of five consecutive sections were collected at different depths of the biopsy, with intervals of 125 μm between the groups. The sections were mounted on glass slides and stained with Goldner’s trichrome. This procedure results in the following staining: green for mineralized tissue, red for osteoid, blue or black for cell nuclei and light orange or red for cytoplasm. To detect cartilage, sections were stained by safranin-O. To visualize osteoclasts, tartrate resistant acid phosphatase staining (TRACP) was used (Tadjoedin et al. 2000; Zerbo et al. 2003). All sections were analysed by light microscopy.

_Histomorphometric procedures_

Quantitative histology measurements were carried out using the Leica Qwin image analysis system (Leica Imaging System, Ltd, Cambridge, England) as previously reported (Tadjoedin et al. 2000; Zerbo et al. 2003). Three Goldner’s trichrome-stained sections from various depth of each biopsy were measured and values were averaged.

Bone formation in the distraction gap was measured in two ways: (1) Assessment of the length of the bone trabeculae. Trabecular length (in mm) was defined as the shortest distance from the osteotomy line to the growth tip of the longest bone trabecula. (2) Assessment of the bone volume in the distraction gap in the 1st and the 2nd one-mm area distant from the osteotomy line over the full width of the biopsy (Fig. 1). Bone volume was defined as mineralized bone tissue as percentage of total tissue volume (BV/TVx100%). The osteoid volume was defined as osteoid as percentage of the bone volume (OV/BVx100%). All measurements were performed at x100 magnification. The values of the different sections collected from each biopsy were averaged.

_Micro-CT scanning_

For additional information on the three-dimensional structure of the new bone, seven bone biopsies embedded in MMA were scanned with a micro-computer tomograph (μCT40, Scanco Medical AG, Bassersdorf, Switzerland). Analysis
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consisted of a scout view, selection of the examination volume, automatic positioning, measurement, off-line reconstruction and evaluation. For each biopsy, approximately 200 micro-tomographic slices were obtained with a resolution of 10 μm.

Fig. 1. Schematic drawing of the areas of measurement in the distraction gap

Statistical analysis
Values obtained from histomorphometry were calculated using Graphpad InStat 3 for Windows and are presented as means and standard deviations. The mean values (and standard deviation) for trabecular length and bone volume of the 1 mm/day and 0.5 mm/day groups were tested for significant differences, using the nonparametric Mann-Whitney test (two-tailed). Two biopsies that were overtly compressed (patients 6&7) as well as a sample from a patient with an exceptionally long healing time (patient 16) were not included in the calculations. The mean values of bone volume within the first mm of the upper (transport/incisal) bone and lower (apical) bone of the same biopsies were tested for differences by the paired t-test. The significance was accepted when p<0.05 (two-tailed).
Results

Theoretically, the distraction process should create an alveolar ridge augmentation of approximately 10 mm based on the extension of the distraction screw. This was not confirmed in the biopsies for the following reasons. (i) The biopsies were often broken in the area of the distraction gap. The lower bone portion was missing in seven biopsies while intact biopsies containing upper and lower bone segments with a complete distraction gap were found in nine patients. (ii) As the distracted bone was still young and not fully mineralized, compression of the biopsy could also happen at the time of pushing the rotating trephine into the bone. (iii) The angle under which the biopsies were taken was not always completely perpendicular to the osteotomy site but slight variations were unavoidable. (iv) Upon removal of the distractor it was noted that distraction of the bone segments was not always completely vertical but somewhat skewed. As a result, the true length of bone augmentation that was found in the biopsies did not correspond to the theoretical length and varied from 2.0 – 9.3 mm (Table 1). The analysis of pre-distraction bone was performed in biopsies of four patients (patients no. 12-15) while the analysis of post-distraction bone was done in biopsies of all patients.

Pre-distraction bone

The biopsies taken from the four patients prior to distraction served as baseline values for atrophic mandibular bone. From these patients, biopsies were also BV: Bone Volume as percentage of tissue volume, OV: Osteoid Volume in old bone as percentage Bone Volume. Data were collected from 4 patients (#12-15) and presented as percentage. The mean bone and osteoid volume were analyzed with T-test. Osteoid volume was significantly higher in post-distraction old bone compared to pre-distraction bone. *p=0.02.

Table 2. Histomorphometric results of the upper old bone before and after distraction

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Pre-distraction</th>
<th>Post-distraction</th>
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<tbody>
<tr>
<td></td>
<td>BV (%)</td>
<td>OV (%)</td>
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<tr>
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<td>59.8</td>
<td>1.5</td>
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<tr>
<td>13</td>
<td>58.2</td>
<td>0.6</td>
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<tr>
<td>14</td>
<td>66.3</td>
<td>0.2</td>
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<td>15</td>
<td>50.3</td>
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<tr>
<td>Mean±SD</td>
<td>58.7±6.6</td>
<td>0.9±0.6</td>
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</table>
taken after distraction, thus enabling us to compare the reaction of the existing bone on distraction (Table 2).

Before distraction the bone consisted of normal cortical bone of the lamellar type arranged concentrically around vascular channels. The presence of very few osteoid seams and osteoblasts indicated a low bone-forming activity (Fig. 2A). The bone volume varied from 50% - 66% (Table 2).

**Post-distraction bone**

**Old bone**

After ten-twelve weeks of neutrofixation, bone present prior to the distraction (‘old bone’) demonstrated active bone formation. At this time point, the osteoid volume was on average more than 4-fold higher (p=0.02) (Table 2), particularly in the area close to the osteotomy line and in the most incisal part of the upper bone segment that was covered by oral mucosa (Fig. 2B). In line with this, higher numbers of osteoblasts along the mineralized tissue were found. The bone volume was not different from that in the predistraction bone. However, considerable areas of non-vital bone, as indicated by the presence of fields of empty osteocyte lacunae, were found in the zones adjacent to the osteotomy line (Fig. 3A). TRACP staining showed the presence of (only) a few osteoclasts (not shown). In most biopsies the bone density of the old bone near the osteotomy line (area at 1 mm distance) was higher in the upper bone segment than in the lower segment. The upper bone segment also contained more empty osteocyte lacunae.

**New bone in the distraction area**

Seven to twenty weeks after cessation of the distraction the osteotomy lines were still apparent but at some locations they could not be seen any more, probably due to bone remodelling (Fig. 3A). The newly formed bone in the distraction gap was predominantly of the woven type (approximately 80%) and contained a high-density of vital osteocytes. Some lamellar bone was evident only in the area adjacent to the osteotomy line (Fig. 3B). In none of the biopsies
cartilage was found. This was confirmed with safranin-O staining: in the sections no positive staining was noted.

Bone trabeculae, blood vessels and collagen fibres were oriented in an apico-incisal direction. The soft connective tissue between the trabeculae was highly

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**Fig. 2.** Light micrographs of pre- and post-distraction old bone of the same patient (# 14).
(A) Predistraction bone. Normal cortical bone (B) with very few discernible osteoid layers (arrows, O). BM=bone marrow. Goldner’s trichrome staining. x50.
(B) Post-distraction old bone. Note abundance of osteoid layers (red staining, arrows) along the mineralized tissue. B=Bone. BM=bone marrow. x50.

**Fig. 3.** Histological details of bone in the distraction gap.
(A) Osteotomy line (OL). Note in some areas (*) the osteotomy line has disappeared. The incisal old bone (OB) consists of lamellar bone with some empty osteocyte lacunae (within dotted circle) adjacent to the osteotomy line. New bone (NB) is of the woven type. Note the staining of the recently deposited bone is lighter green due to low mineralization compared to mature old bone. x200.
(B) Lamellar bone (LB) is deposited against woven bone (WB) in the distraction area adjacent to the osteotomy line. x200.
vascularised. In many areas, but predominantly near the osteotomy lines, osteoid was seen to connect separate bone trabeculae (Fig. 3C). Inflammatory cells that are usually present in the early phase of wound healing were still present in close vicinity to blood vessels. A few osteoclasts were seen particularly in the area adjacent to the osteotomy line.

(C) Osteoid formation. Osteoid (O) was produced in between the bone spicules, possibly to make a cross connection. Note abundance of osteoblasts (Ob) that generate this unmineralized bone matrix. x200.

(D) After 7 weeks of neutrofixation (patient # 1). The central area of the distraction gap was still filled with fibrous tissue (FT). Note that the orientation of the collagen fibres was rather skewed, probably due to compression during removal of the biopsy. x25.

(E) After 11 weeks of neutrofixation (patient # 10). The central area of the distraction gap was filled by new bone. x25.

(F) Micro CT-scan of biopsy obtained after 10 weeks of neutrofixation period (patient # 6). Bar = 1.0 mm. The distraction gap is completely filled by new trabecular bone. Incisal and apical old bone (cortical bone) were found sandwiching the distraction gap.

(continued)
The closure of the distraction gap could be evaluated in only nine intact biopsies (six in the 0.5 mm/day group, three in the 1 mm/day group); the other seven biopsies were incomplete. After 7-9 weeks of neutrofixation (about 10-12 weeks of healing time), fibrous tissue was still present in the central area of the gap in all three intact biopsies of the 0.5 mm/day distraction group (Table 3). After 10-11 weeks of neutrofixation (healing time about 13-14 weeks) fibrous tissue was found in one out of three intact biopsies of the 0.5 mm/day distraction group.

Table 3. Histomorphometric results of new bone formed in the distraction gap

<table>
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<tr>
<th>Patient no.</th>
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<td>2nd one-mm incisal area</td>
<td>1st one-mm apical area</td>
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<thead>
<tr>
<th>Patient no.</th>
<th>Healing time (days)</th>
<th>Bone Volume (%)</th>
<th>Osteoid Volume (%)</th>
<th>Closure of the gap</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1st one-mm incisal area</td>
<td>2nd one-mm incisal area</td>
<td>1st one-mm apical area</td>
<td>2nd one-mm incisal area</td>
</tr>
<tr>
<td>11</td>
<td>65</td>
<td>18.8</td>
<td>15.0</td>
<td>19.0</td>
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<td>12</td>
<td>85</td>
<td>25.2</td>
<td>17.1</td>
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<td>14</td>
<td>89</td>
<td>23.5</td>
<td>24.2</td>
<td>-</td>
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<tr>
<td>15</td>
<td>93</td>
<td>36.2</td>
<td>24.9</td>
<td>-</td>
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<td>16^</td>
<td>154</td>
<td>14.1^b</td>
<td>NA</td>
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<tr>
<td>Mean ± SD</td>
<td></td>
<td>29.5±10.3</td>
<td>18.8±5.4*</td>
<td>22.9±5.4</td>
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</table>

a=Healing time comprises distraction and neutrofixation period. b=Absent in the biopsies. NA: Not applicable due to compression of biopsy. FT: fibrous tissue. The closure of the gap was determined only in the intact biopsies. ^b=Biopsies from patients with lack of vascularisation in the incisal old bone. c=Mean without patient no. 16 who had an exceptionally long healing time. * p=0.07 for 0.5 mm/day vs. 1 mm/day (Mann–Whitney)
mm/day group (Fig. 3D) and closure in the two other biopsies (Fig. 3E). Closure in the 0.5 mm/day group was confirmed by micro-CT analysis (Fig. 3F). However, in the 1 mm/day group closure was seen neither in the one intact biopsy taken after 8 weeks of neutrofixation (about 9 weeks healing time) nor in the two intact biopsies taken after 11 weeks of neutrofixation or longer (Table 3). In two biopsies we found insufficient vascularisation of the upper (old) bone segment.

Quantitative analyses showed that the amount of bone formed in the investigated areas of distraction gap ranged from 10%-46% (Table 3). Since all sixteen biopsies contained new bone growing away from the surface of the upper bone segment we selected the upper segment to measure the amount of bone formation in the distraction gap. We focused on two separate areas: the incisal 1st one-mm area from the osteotomy line (Fig. 1) representing the ‘oldest’ new bone, and the (more apical) 2nd one-mm area from the osteotomy line, which is the bone formed later. In the 1st one-mm area there was no difference in bone volume between the 0.5 mm/day group and 1 mm/day group (Table 3). In the 2nd one-mm area there was more bone in the 0.5 mm/day group than in the 1 mm/day group, although the difference was not statistically significant (p=0.07) (Table 3; Fig. 4). Also the new bone trabeculae growing into the soft connective tissue in the centre area of the distraction gap were significantly longer in the 0.5 mm/day group than in the 1 mm/day group (p= 0.02, Fig. 5).

In biopsies that contained both bone ends (upper/incisal and lower/apical segments), bone was formed at each end at a comparable rate except in patients number 7 and 16 (Fig. 6). In those two patients less new bone (about one third) was formed at the upper bone end than at the lower end. Close inspection of the upper bone ends of these patients showed that the old bone next to this poorly-grown new bone in the gap contained more empty osteocyte lacunae than its counterpart in the lower segment. In addition the upper bone segment was very poorly vascularised.
Fig. 4. Scatter plot of the effect of distraction rate on bone volume. Bone volume was measured in the 2nd one-mm area from the osteotomy line in the upper bone segment. Filled ovals: distraction rate 0.5 mm/day, triangles: distraction rate 1 mm/day. Means and SD: 27.1% ± 6.8% for 0.5 mm/day and 18.8% ± 5.4% for 1 mm/day. Difference between treatment: not significant (p=0.07, Mann-Whitney).

Fig. 5. Scatter plot of the effect of distraction rate on trabecular length. Trabecular length from the upper bone segment was measured. Filled ovals: distraction rate 0.5 mm/day, triangles: distraction rate 1 mm/day. Means and SD: 3.8 mm ± 1.1 mm for 0.5 mm/day and 2.2 mm ± 0.3 mm for 1 mm/day. Difference between treatments, *p=0.02 (Mann-Whitney).
In the present study we examined and quantified bone formation in the distraction gap during vertical distraction osteogenesis in the human mandible. All biopsies showed the deposition of new bone within the distraction gap. The overall orientation of the bone appeared to be in the direction of distraction force. The major part of the new bone formed in the distraction gap was of the woven type suggesting that this bone was still young and probably in an active state of remodelling. Lamellar bone was found only in the distraction gap area close to the osteotomy line (‘the oldest new bone’). Also the density of bone in the distraction gap suggested this bone was immature as it had attained approximately half the value seen in the adjacent pre-distraction bone. The direction of trabecular growth was initially dictated by the direction of distraction force, i.e. in similar direction and deposited along the fibres of dense fibrous tissue in the gap area. In later stages cross connections of osteoid were seen to link separate bone trabeculae. This might further stabilize the bone tissue formed
between the distracted ends. We did not find any signs of cartilage in the
distraction gap. The absence of cartilage appears to divert from studies in animals
where this type of tissue was found at early stages of regeneration during
mandibular distraction (Karp et al. 1992; Komuro et al. 1994; Rowe et al. 1998;
Li et al. 1999; Cope & Samchukov 2000; Richards et al. 2000). Although it can
be argued that cartilage may have been present transiently in early stages of
distraction before biopsies were taken, we think it is unlikely to have missed
cartilage, since its remnants persist relatively long during endochondral
ossification and are only slowly replaced by bone. Moreover, deposition of
osteoid in the fibrous tissue in the central area of the gap in our biopsies was a
clear indication of intramembraneous ossification. Overall, our histological
findings demonstrate that bone regeneration in the human mandible shares some
similarity to that reported in animal models.

We report here that vertical distraction at a low rate enhances bone formation
in the human mandible more than distraction at a higher rate. This difference
was found by measuring the length of the bone trabeculae and by assessing the
volume of the bone formed. In the bone density measurements the difference
between both groups was not found in the first one mm area from the osteotomy
line, but it was apparent for the second one mm area. This suggests that
particularly the initiation and early stages of bone formation is improved by a
lower rate of distraction. Later, this difference could disappear suggesting the
increase in bone formation at low distraction rate is temporary. A possible
explanation for a faster bone formation at the lower distraction rate is that the
preceding growth of blood vessels is better. In rabbit tibia the distraction rate
was shown to strongly influence angiogenesis in the central fibrous zone and
was optimally stimulated at 0.7 mm/day and 1.3 mm/day but much less at
lower or higher rates (Li et al. 1999). Optimal stimulation of cell proliferation
in osteogenic areas was reported at 0.7 mm/day whereas higher distraction
rates induced necrosis, formation of cysts and also cartilage (Li et al. 1997).
During fracture healing an inhibition of angiogenesis resulted in a complete
lack of bone formation (Hausman et al. 2001; Carano & Filvaroff 2003).
Moreover, gene targeting of endothelin-1 (which is secreted by vascular endothelial cells and stimulates osteogenic cells) caused severe hypoplasia of facial bones in mice, suggesting that blood vessels have a stimulating function on osteogenic cells during embryonic craniofacial development (Kitano et al. 1998). The present study supports the relevance of angiogenesis for bone formation in the distraction gap as illustrated in the two patients with poor bone regeneration which appeared to be associated with insufficient vascularisation of the upper segment.

The appropriate time to remove the distractor is not exactly known yet. It is a crucial time, since the new bone in the distraction gap will receive an implant. Experimental studies show a wide variation in the time needed for closure of the distraction gap. This time ranges from 2-12 weeks and appears to depend on the species used, location of the distracted bone tissue and age of the animals (Rowe et al. 1998; Li et al. 1999; Cope & Samchukov 2000; Richards et al. 2000). We were not able to find any published report on the closure time of the distraction gap in human mandibles. Our histological and microCT results show that after 10 mm of distraction, the earliest time point that the fibrous tissue in the centre of the gap had disappeared was 10 weeks after cessation of distraction (0.5 mm/day). Given the necessity not to compress the newly formed bone during the placement of the implants, thus compromising the stability of the implants, we consider it important that the gap is completely bridged by bone prior to implant placement. Our present data suggest that after 10 mm of distraction with a rate of 0.5 mm/day, 10 weeks is a minimal time for healing before removal of the distractor and placement of the implants. Based on the relatively low bone density (about half of pre-distraction value) and immaturity of the bone (predominantly woven bone) at that time, our data also suggest that dental implants should not be loaded for some time after their placement.

For practical reasons each of the two groups examined in this study had been assigned to one of both surgeons. Since no randomisation of the two groups was carried out, one could argue that the positive effect on healing
found in this study reflects differences in treatment or operative skill of the
surgeon. Though we cannot completely exclude this possibility we have no
reason to assume this was the case. Both surgeons are well experienced, and
used the same procedures, settings and selection criteria. Extension of the
screws was carried out by the patients and is likely to vary equally in both
groups. There was also no clinical or histological evidence indicating
significant differences in the healing process between both patient groups.
Accordingly the effects found are likely induced by extension rate, not by
other factors.

In conclusion, this study strongly suggests that in elderly patients
distraction at 0.5 mm/day gives better results in stimulating bone formation
in the distraction gap than at 1 mm/day. We suggest that after distraction of
10 mm a minimal neutrofixation period of 10 weeks is needed to bridge the
distraction gap by new bone before the distractor is removed and implants
are placed.

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References


distraction osteogenesis of rat’s tibia. Immunostaining study of the proliferating cell nuclear antigen, osteocalcin, and transglutaminase C. Bulletin for Hospital and Joint Disease 56: 34-40.


