Ultrasound stimulation of mandibular bone defect healing
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Chapter 5

Ultrasound to stimulate early bone formation in a
distraction gap: a double blind randomised clinical trial
in the edentulous mandible

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Submitted
Abstract
In a double blind randomised clinical trial, it was investigated whether low intensity pulsed ultrasound stimulates early bone formation in a distraction gap created in a severely resorbed mandible. Eight patients underwent a mandibular vertical distraction over an average distance of 6.6 ± 1.1 mm. Ultrasound self-therapy or placebo therapy was started at the first day of distraction and continued daily until the implants were inserted. After 31 ± 3.8 days of consolidation, the distraction device was removed, a transmandibular biopsy was taken, and two endosseous implants were inserted. All patients complied well with ultrasound therapy. During an average of 18.1 ± 4.1 months follow-up, no complications did occur. Microradiographic examination of the biopsies revealed a comparable mean area of mineralised tissue in the distraction gap of 1.9 ± 1.7 mm² in the ultrasound treatment group and 1.9 ± 1.3 mm² in the placebo treatment group. Histological examination indicated that active woven bone was present within the distraction gap just adjacent to the osteotomy plane, with no apparent differences between the treatment groups. The lamellar bone formation outside the distraction gap appeared to have started as well. The results of this trial indicate that ultrasound treatment does not significantly promote early bone formation in the distraction gap during a 31-day consolidation period. It appeared that bone formation within and outside the distraction gap had just started, so that a possible beneficial effect of ultrasound therapy on bone formation can not be excluded. Therefore, a longer consolidation period has to be studied.
Introduction
Due to the continuing bone resorption of the mandible after loss of teeth, a limit is sometimes reached, making wearing of a lower denture too heavy a burden for the patient. Patients with a severely resorbed mandible frequently suffer from pain and difficulties with eating and speech. The key to a solution is to provide sufficient stability, especially for the lower denture. Nowadays, this is accomplished by using dental implants to support the denture. However, in the severely atrophic mandible (i.e., bone height less than 8 mm), there is insufficient bone to reliably insert implants, which might result in increased loss of implants in the long run. In these cases, bone augmentation procedures have to be performed before implants can be inserted. A pre-implant procedure that is increasingly applied to enable placement of implants in the severely resorbed mandible is the vertical distraction osteogenesis. After distraction, a bone healing time of 8 - 12 weeks is generally taken into account before implant insertion. After implant insertion, another 12 weeks should be allowed to ensure full osseointegration. During this period, the patient cannot wear a denture. Because the bone-healing period is a major factor that determines the total treatment time, ways of shortening the bone healing process may be of substantial benefit to the patient.

Ultrasound is a relatively unknown therapy that can stimulate the bone healing process. This has been investigated in the case of fresh tibial and radial fractures and in various delayed and non-unions. In the case of osteodistraction, there are indications that ultrasound therapy may stimulate bone formation within the distraction gap (the regenerate- or callusmaturation). These studies indicate that ultrasound therapy may accelerate the mineralisation of the tissue within the distraction gap (the consolidation). In case of osteodistraction, it seems that the primary effect of ultrasound occurs early in the treatment process and that the overall bone healing process occurs, therefore, relatively early. Although the potential of ultrasound to stimulate maxillofacial bone healing has been investigated before, no experiments have been published about ultrasound therapy and human mandibular distraction. To investigate this further, it was decided to evaluate whether therapy with low intensity pulsed ultrasound can stimulate early bone formation in the vertical distracted mandible in humans.

Materials and methods
Patient selection. Patients referred to the Department of Oral and Maxillofacial Surgery of the University Hospital Groningen between May 1 until November 30, 2001, were selected to participate in the study when they met the following criteria:
- having complaints related to insufficient retention and stability of their dentures,
- the presence of a severely resorbed edentulous mandible with a mandibular height at the canine region of less than 8 mm,
- unsatisfactory relief of complaints with conventional dentures.

Patients who smoked or used medications were not excluded to participate. All patients approved of the study and signed a written informed consent statement to participate. The study protocol was approved by the Medical Ethics Committee, University Hospital Groningen, The Netherlands (METc 2001/046).

Operative procedure. The severely resorbed mandible underwent a vertical distraction using the Groningen Distraction Device (GDD, Martin Medizin Technik, Tuttlingen, Germany) according to the procedure described by Raghoebard et al. After a latency period of 5 days after insertion of the distraction device, active vertical distraction was started at a rate of 1 x 1.0 mm/day until the appropriate height was obtained to insert two implants with an implant length of 12.0 mm. At the first day of active distraction, ultrasound treatment was started (Figure 1).

Figure 1. The sequence of the study protocol depicted in a time schedule (d = day, wk = weeks).
**Ultrasound treatment.** In this study, placebo and active Sonic Accelerated Fracture Healing System devices were used for ultrasound treatment (SAFHS model 2000®, Smith & Nephew, Memphis, TN, USA). This battery operated device consists of a main operating unit to which the ultrasound transducer is attached. The active devices emitted an ultrasound signal consisting of a 1.5 MHz pressure wave that is emitted in pulses of 200 µs. Between pulses a 800 µs pause was present (on:off period = 1:4). The average intensity over space and time was 30 mW cm⁻². The placebo-devices did not emit an ultrasound pulse. Prior to the commencement of the study, the placebo and active devices were identified and blinded by coding the devices by subsequent numbering using randomisation software (B.S). The placebo and active devices were indistinguishable from each other by appearance and during function. Next, the devices were allocated to the patients in subsequent order (J.S).

![Figure 2. Ultrasound self treatment. The transducer is placed on the chin ventral of the distraction gap (a). A lateral skull radiograph was taken to assure this proper positioning of the transducer (b) so that ultrasound is directed towards the distraction gap.](image)

The ultrasound self-treatment involved a daily treatment with ultrasound for 20 minutes on the skin of the chin covering the distraction gap (Figure 2a). During treatment, the patients were instructed not to move the transducer but keep the transducer stationary on the skin with light pressure. A lateral Tele-X radiograph with the transducer on the chin was made to verify proper positioning of the transducer in such a way that the ultrasound is directed towards the distraction gap (Figure 2b). To monitor patient compliance, the devices have an internal memory chip which records the treatment day and time, and possible errors of the device such as low battery, disconnected cables, and improper coupling between transducer and skin. The coupling alarm was only active in the active devices. The patients were instructed to keep a logbook to record the treatments.
and possible problems as well. Comparing the memory chip readouts with the self-kept logbooks assessed patient compliance.

Assessment of bone formation. Four weeks post distraction, the distraction device was removed, two ITI Bonefit® implants (Straumann AG, Waldenburg, Switzerland) with an implant length of 12.0 mm were inserted, and a transmandibular bone biopsy was obtained using a trephine burr (2.0 mm inner diameter). The biopsy was fixed in buffered formaline for 24 hours and used for microradiographic and histological analysis. To assess lamellar bone formation within time, patients were asked to take a two-day course of tetracycline (250 mg, four times daily, for two days) after the last day of distraction and again after two weeks. The total treatment protocol is presented in figure 1.

Figure 3. Microradiograph (a) and histological image (b) of a transmandibular bone biopsy, taken 4 weeks after the active distraction. As can be observed on the microradiograph, the native lamellar (cortical) bone (LB) is clearly visible, and only partial bridging of the distraction gap by mineralised tissue. Histologically, the gap is bridged by collagenous fibres (CN) arranged in the distraction direction. Woven bone (WB) is beginning to appear inside the distraction gap. Magnification x 100.

Microradiographical analysis of bone biopsies. Subsequently, a standardised, high-resolution microradiograph of the fixed biopsy was taken by a method described previously and digitised14 (Figure 3). Inside the distraction gap, the following variables were scored by the principal investigator using the digitised microradiographs and image analysis software (Scion, Frederik, USA): the gap
fill area, defined as the area in square millimetres of the microradiograph that encloses the calcified tissue within the distraction gap, next to the osteotomy plane; and the gap grey percentage, defined as the mean grey value of the gap fill area (0% = absolute black; 100% = absolute white).

**Histological analysis of bone biopsies.** After the microradiograph was taken, the biopsy was dehydrated in series of ethanol and subsequently embedded in methylmethacrylate under negative pressure without decalcification. Sections of 4 µm thickness were cut parallel to the long axes of the biopsy using a microtome (Jung-K, Heidelberg, Germany). The sections were stained according to the Goldner trichrome method (Figure 3), and some sections were left unstained for fluorescent microscopy. Histological assessment took place on both sides next to one osteotomy plane (Figure 4):

Within the distraction gap, because early bone formation, if present, can be detected here; and outside the distraction gap in the remaining mandibular bone, where lamellar bone formation was expected to occur. Within and outside the distraction gap, assessment was both quantitative and qualitative.

Within the distraction gap, the gap fill length, defined as the maximum distance between the osteotomy plane and bone formation front at right angles to the osteotomy plane, was measured quantitatively using digitised images at 200 x magnification of the histological sections (Leica DM RA microscope, Leica DC 200 digital camera, Leica QWin® Software, Leica, Germany).

Qualitatively, histological scoring was performed next to the osteotomy plane inside the distraction gap to identify:

Whether new bone formation was present or not as indicated by the presence of osteoid (score 0 = no bone formation).

If present, whether the appearance of the newly formed bone itself was that of woven (score = 1), or lamellar bone (score = 2).

Also the type of bone formation was assessed (i.e., endochondral versus intramembranous).

Outside the distraction gap (i.e., within the remaining mandibular bone), the mineral apposition rate of the lamellar bone was measured quantitatively using the unstained histological sections excited by light of 354 to 425 nm wavelength. In this way, two fluorescent tetracycline bands appeared in the sections. The average distance between the two labels was calculated by measuring the total area between two bands and dividing it by their average length. A minimum of eight consecutive labels per biopsy was measured and averaged (Leica QWin® Software, Leica, Germany).
Qualitatively, histological assessment of the remaining mandibular bone outside the distraction gap was performed to determine possible newly formed bone as assessed by the presence of osteoid.

After all measurements were completed, the code was broken and the quantitative variables were averaged for the ultrasound treatment group and the placebo treatment group.

![Histological image of woven bone next to the osteotomy plane (* in Figure 3) (Goldner Trichrome stain). The dotted line represents the osteotomy plane. At 4 weeks, calcified woven bone (WB) is beginning to appear within the distraction gap next to the lamellar bone (LB). Osteoid (OI), the non-mineralised bone matrix, is formed next to the woven bone indicating active bone formation. Inside the calcified woven bone, osteocytes (OC) are present indicating that the bone is vital. The space between the woven bone is filled with bone marrow (BM). Magnification x 200.](image)

**Figure 4.** Histological image of woven bone next to the osteotomy plane (* in Figure 3) (Goldner Trichrome stain). The dotted line represents the osteotomy plane. At 4 weeks, calcified woven bone (WB) is beginning to appear within the distraction gap next to the lamellar bone (LB). Osteoid (OI), the non-mineralised bone matrix, is formed next to the woven bone indicating active bone formation. Inside the calcified woven bone, osteocytes (OC) are present indicating that the bone is vital. The space between the woven bone is filled with bone marrow (BM). Magnification x 200.

*Follow-up.* After implantation, the patients were requested to complete a short questionnaire on their experiences with the ultrasound treatment. The two most important questions were whether the handling of the device was easy or difficult, and whether 20 minutes continuous treatment was easy or difficult to maintain. Also, the following clinical complications were scored during treatment and follow-up: inflammation around the screws, loss of distraction screws, loss of implants, mandibular fracture, wound infection, wound dehiscence, instability of the cranial bone fragment and sensory disturbances of lip and chin.
Results
Eight patients (2 males, 6 females, mean age 65 ± 8.8 years, range 50 - 79 years) were selected to participate in the study. All patients completed the study protocol. The vertical distractions and implantations were uneventful. In total, sixteen implants were inserted. The overall average height of the mandible in the canine region prior to surgery was 7.1 ± 1.1 mm, and the amount of distraction averaged 6.6 ± 1.1 mm (Table 1). The average latency time was 5.5 ± 0.8 days and the average consolidation time was 31.1 ± 3.8 days. The patients were exposed to either ultrasound or placebo treatment for an average of 12.4 ± 1.2 hours (Table 1).

Table 1. Differences between the ultrasound treatment group and the placebo group (average ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Ultrasound (n=4)</th>
<th>Placebo (n=4)</th>
<th>Overall (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>61.5 ± 8.3</td>
<td>69 ± 8.5</td>
<td>65 ± 8.8</td>
</tr>
<tr>
<td><strong>Initial mandibular canine height (mm)</strong></td>
<td>7.3 ± 1.5</td>
<td>7.0 ± 0.8</td>
<td>7.1 ± 1.1</td>
</tr>
<tr>
<td><strong>Latency time (dy)</strong></td>
<td>5.3 ± 0.5</td>
<td>5.8 ± 1.0</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td><strong>Distraction distance (mm)</strong></td>
<td>6.8 ± 1.0</td>
<td>6.3 ± 1.5</td>
<td>6.6 ± 1.1</td>
</tr>
<tr>
<td><strong>Consolidation time (dy)</strong></td>
<td>32.8 ± 4.3</td>
<td>29.5 ± 2.9</td>
<td>31.1 ± 3.8</td>
</tr>
<tr>
<td><strong>Total time ultrasound/placebo exposure (hr)</strong></td>
<td>13.0 ± 1.5</td>
<td>11.9 ± 1.7</td>
<td>12.4 ± 1.2</td>
</tr>
<tr>
<td><strong>Microradiography gap fill area (mm²)</strong></td>
<td>1.9 ± 1.7</td>
<td>1.9 ± 1.3</td>
<td>1.9 ± 1.3</td>
</tr>
<tr>
<td><strong>Gap grey percentage (%)</strong></td>
<td>29.7 ± 31.7</td>
<td>40.8 ± 13.4</td>
<td>36.0 ± 21.4</td>
</tr>
<tr>
<td><strong>Histology gap fill length (mm)</strong></td>
<td>0.9 ± 1.1</td>
<td>1.4 ± 0.8</td>
<td>1.1 ± 0.9</td>
</tr>
<tr>
<td><strong>Histological score</strong></td>
<td>0.75 ± 0.5</td>
<td>1.0 ± 0.0</td>
<td>0.88 ± 0.35</td>
</tr>
</tbody>
</table>

Latency time = number of days from operation until active distraction
Consolidation time = number of days from end of distraction until insertion of implants/bone biopsy
Microradiography gap fill area (mm²): area enclosing calcified (radiopaque) tissue in distraction gap, measured only from osteotomy side with maximum bone formation.
Gap grey percentage: average grey percentage of calcified tissue area (0% = black, indicating no tissue calcification; 100% = white, indicating high degree of tissue calcification)
Histological gap fill length (mm): maximum distance between osteotomy plane and bone formation front (measured at right angles to the osteotomy plane).
Histological score: 0 = no bone formation next to osteotomy plane within gap
1 = woven bone formation next to osteotomy plane within gap
2 = lamellar bone formation next to osteotomy plane within gap
Four weeks after distraction, it was difficult to obtain an intact transmandibular biopsy. Only three biopsies remained intact, and of these, the gap area was compressed in two (Table 2). All other biopsies broke, or only half or less of them could be recovered.

Microradiographical analysis of the biopsies revealed no difference in the area of mineralised tissue inside the distraction gap or next to the osteotomy plane. The gap fill area in the ultrasound treatment group measured $1.9 \pm 1.7 \text{ mm}^2$, and in the placebo ultrasound group measured $1.9 \pm 1.3 \text{ mm}^2$. Also, there were no significant differences or a trend seen in the gap grey percentage.

Histologically, within the distraction gap there was no significant difference in gap fill length. Qualitatively, all but one biopsy showed new bone formation towards the middle of the distraction gap next to the osteotomy plane (Figure 3). In the intact biopsy, the new bone was located only at one osteotomy plane, but not at the other (Figure 3). This newly formed woven bone appeared to be formed by intramembranous ossification. There were no apparent differences between the ultrasound and the placebo group. There were no signs of endochondral ossification.

Outside the distraction gap in the remaining mandibular bone, only one biopsy had a clear distinct tetracycline double label. The lamellar bone formation (mineral apposition rate) in this biopsy measured $2.63 \text{ µm/day}$. All other tetracycline labels were diffuse, and could not be measured. Histologically, locations of osteoid formation could be distinguished in the remaining mandibular bone. There were no apparent differences between the ultrasound and the placebo group.

The readouts of the SAFHS memory chips matched the logbooks of the patients. All patients had been treating themselves on a daily basis. According to the memory chip readouts, a total of 351 treatments had been administered; every one of which corresponded to the logbook administration. The treatment was interrupted 39 times (11%) due to disconnected cables, an improper contact between transducer and skin, or a low battery. In these cases, treatment could be resumed after correcting the error. Only one time a patient forgot a treatment.

All questionnaires were completed. All 8 patients judged the handling of the device as being easy, and the 20 minutes treatment as being convenient.

The clinical follow-up after successful insertion of the implants was $18.1 \pm 4.1$ months (as on July 1, 2003). During this period no complications occurred. No inflammation around the screws, no loss of distraction screws, no loss of implants, no mandibular fracture, no wound infection, no wound dehiscence, no instability of the cranial bone fragment and no sensory disturbances of lip and chin were encountered.
Table 2. Gross, microradiographic and histological analysis of the distraction biopsies.

<table>
<thead>
<tr>
<th>No.</th>
<th>Biopsy</th>
<th>Distraction distance (mm)</th>
<th>Treatment</th>
<th>Microradiography gap fill area (mm²)</th>
<th>Gap grey percentage (%)</th>
<th>Histology gap fill length (mm)</th>
<th>Tetracycline bands</th>
<th>Mineral apposition rate (µm/day)</th>
<th>Histological score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Broken, two parts</td>
<td>4.5</td>
<td>Placebo</td>
<td>0.95</td>
<td>24</td>
<td>-</td>
<td>Diffuse</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Intact, gap compressed</td>
<td>8.0</td>
<td>Placebo</td>
<td>3.06</td>
<td>51</td>
<td>1.38</td>
<td>Clear double</td>
<td>2.63</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Broken, one part</td>
<td>6.0</td>
<td>Ultrasound</td>
<td>2.74</td>
<td>26</td>
<td>0.83</td>
<td>Diffuse</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Broken, two parts</td>
<td>8.0</td>
<td>Ultrasound</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Diffuse</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Intact, gap compressed</td>
<td>5.5</td>
<td>Ultrasound</td>
<td>2.99</td>
<td>63</td>
<td>2.50</td>
<td>Diffuse</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Broken, two parts</td>
<td>5.5</td>
<td>Placebo</td>
<td>0.56</td>
<td>52</td>
<td>0.61</td>
<td>Diffuse</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Broken, two parts</td>
<td>7.0</td>
<td>Ultrasound</td>
<td>-</td>
<td>-</td>
<td>0.34</td>
<td>Diffuse</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Intact, gap intact</td>
<td>6.0</td>
<td>Placebo</td>
<td>2.90</td>
<td>36</td>
<td>2.20</td>
<td>Diffuse</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

*: unable to measure

Distraction distance: amount of bone lengthening according to screw torques.
Microradiography gap fill area (mm²): area enclosing calcified (radiopaque) tissue in distraction gap, measured only from osteotomy side with maximum bone formation.
Gap grey percentage: average grey percentage of calcified tissue area (0% = black, indicating no tissue calcification; 100% = white, indicating high degree of tissue calcification)
Histological gap fill length (mm): maximum distance between osteotomy plane and bone formation front (measured at right angles to the osteotomy plane).
Histological score: 0 = no bone formation next to osteotomy plane within gap
1 = woven bone formation next to osteotomy plane within gap
2 = lamellar bone formation next to osteotomy plane within gap
Discussion
This study suggests that ultrasound treatment does not stimulate early bone formation in the severely resorbed mandible during and after a vertical distraction procedure. This was not expected because others did find a stimulating effect of the same daily 20 minutes ultrasound treatment on bone healing within a distraction gap. This was found in femur bones of rats,\textsuperscript{7} tibial bones of rabbits,\textsuperscript{12,15} metatarsal bones of sheep,\textsuperscript{9,10} and in the human leg.\textsuperscript{11} Moreover, a positive effect of the same ultrasound treatment as used in our study was found on bone formation within a mandibular distraction gap in rabbits.\textsuperscript{8} On the cellular level, other ultrasound fields have been capable of stimulating human mandibular osteoblasts to proliferate\textsuperscript{16} and produce angiogenesis-related cytokines, such as interleukine-8, basic fibroblast growth factor, and vascular endothelial growth factor in vitro.\textsuperscript{17} Ultrasound can cause a reversible increase in the intracellular level of second messenger calcium in chondrocytes,\textsuperscript{18,19} and an increase in calcium incorporation in differentiating cartilage and bone cell cultures\textsuperscript{20} as well, which could stimulate the regenerate maturation after distraction.

Although only a limited number of patients was included in our study, making strong statements impossible, our results do suggest that ultrasound did not seem to have an effect on the amount of calcified tissue formation during the consolidation period of 31 days. The two groups studied are comparable to each other (Table 1) limiting bias of age, initial mandibular height, distraction distance, and consolidation time. Furthermore, the ultrasound treatment was administered with a high compliance rate as indicated by the internal memory chip readouts and logbooks, so that the distraction gap was exposed to the ultrasound pressure waves.

An explanation that no effect was seen may be related to the differences between the study model used (severely resorbed mandible versus ‘healthy’ mandibular bone and long bones), to the timing of the biopsy (the period of consolidation) or to the medications used by the patients.

First, there are perhaps differences between regenerate maturation of the long bones and the severely resorbed mandible that may serve as an explanation. In previous studies concerning osteodistraction and ultrasound, rabbit tibia,\textsuperscript{12,15,21} rat femora,\textsuperscript{7} sheep metatarsus\textsuperscript{9,10} and sheep mandibles,\textsuperscript{8} have been used as study model. Here, the stimulating effect of ultrasound on regenerate maturation has been described. In these studies, healthy animals were used with no known pathology that could influence bone healing. By contrast, the severely resorbed mandible is characterised by dense cortical bone, with a poor vascularisation, and almost no bone marrow space. However, it must be noted that a positive
effect of ultrasound on bone healing in poor vascular conditions has been observed in cases of scaphoid fractures\textsuperscript{22} and mandibular osteoradionecrosis.\textsuperscript{23} Second, a reason why no effect was seen may be related to length of the consolidation period. Unfortunately, it was not possible to determine accurately how much volume of bone had grown within the complete distraction gap, because it was difficult to obtain an intact transmandibular biopsy. Most biopsies were very fragile and tended to break in half. Therefore, the microradiographic and histological measurements were performed only on the part of the biopsy that showed the most bone growth within the distraction gap. Perhaps a more accurate measurement would alter the results of this study. The microradiograph and the histological section of the intact non-compressed biopsy shows that, at 4 weeks post distraction, the distraction gap is not yet bridged by bone (Figure 3). The other biopsies do not indicate complete bridging of the distraction gap either. Comparing the intact 4-week biopsy with a previously published 8-week transmandibular biopsy,\textsuperscript{24} the mineralisation zones in the intact 4-week biopsy are considerably smaller. Histologically, at 4 weeks, woven bone can be distinguished next to the osteotomy plane, but not yet the more mature lamellar bone (Figure 4). The soft tissue within the gap consists of collagen fibres, arranged according to the distraction direction. Because collagen is much weaker than bone, this may be the reason of breakage of the biopsies. Thus, the consolidation period may have been too short to evaluate the effect of ultrasound therapy on mandibular regenerate maturation. The finding that 7 out of 8 tetracycline labels were diffuse, and not clear, suggests this as well. As can be observed in table 2, only one tetracycline double label could be measured, the other labels were diffuse. It may be that the tetracycline tablets were not taken according to the protocol leading to improper labels. However, the patients were highly compliant with ultrasound treatment, making non-compliance for the tetracycline labelling in 7 out of 8 patients unlikely. A more reasonable explanation may be that the timing of bone labelling was inappropriate. The labelling was done directly after the active distraction at approximately postoperative day 11 - 12 and again at day 25 - 26. It seems that the lamellar bone formation in the mandibular bone is not yet fully active at this time because no clear labels were seen in most specimens. This may also account for the fact that no difference could be found in the amount of calcified tissue inside the distraction gap after 31 days between the ultrasound and placebo group. Thus, during the first 31 days of regenerate maturation, the bone formation within the distraction gap appears to be just beginning and it seems that this period is too short to state whether or not ultrasound may be of value in accelerating mandibular regenerate maturation.
A third reason why no effect was seen may be related to the medications of the patients. Because most elderly patients have co-morbidity that requires medical treatment, it was decided not to exclude patients that use medications. Illnesses that may influence bone healing are alcohol/drugs abuse, diabetes, osteoporosis, cancer, renal insufficiency and vascular disease. Drugs that may influence bone healing are calcium channel blockers, non-steroid anti-inflammatory drugs (NSAID’s), anticoagulants, and systemic steroids. The smoking status may also influence bone healing. In the placebo group, one patient used calcium channel blockers (no. 1 in Table 2), one patient used an anti-osteoporosis drug that prevents bone resorption by osteoclasts (no. 2), the other used a low dose NSAID for cardiac reasons (no. 6) and number 8 used inhalator steroids. In the ultrasound group, the drugs used were a low dose NSAID for cardiac reasons (no. 3), and inhalator steroids (no. 4). Patient number 5 used no medications; patient number 7 smoked six cigarettes a day.

Although ultrasound can compensate for the negative effect smoking has on fracture healing, and ultrasound can achieve healing of delayed unions and non-unions in healing disorders related to disease or medications, the results in our study may be biased by the drugs used.

Although the finding that the distraction gap is not yet bridged by bone may suggest that the consolidation period before implant insertion was too short, no complications did occur during or after the insertion of the implants at 4 weeks. No implant failure was observed, and no complications were encountered during further treatment. After implant insertion, an osseointegration period of 12 weeks is considered before the dentures can be made. During this time, it is presumed that sufficient bone will be formed to ensure further implant stability. The finding that successful implantation at 4 weeks post distraction can be accomplished, even without bony bridging of the distraction gap between the fragments, raises the question whether the bone healing process is the limiting factor in determining the time before implant surgery in these patients. On a theoretical basis, one could speculate that the time in which the soft tissue can be stretched to cover the distracted bone seems to be the limiting factor that determines the time to implantation in these patients, but only on the condition that sufficient stability of the cranial fragment is maintained during implant surgery. This was accomplished by removing only one screw of the distraction device and insertion of a threaded implant at the time. The cranial fragment maintained stability at least by one distraction screw and the guide screw.
Based on the findings of this study, the following can be summarised:

1. During a 31-day consolidation period, ultrasound does not appear to stimulate bone formation in the severely resorbed vertical distracted mandible and it seems that this period is too short to evaluate properly if there is an effect.

2. It is possible to insert implants after 31 days of consolidation with clinical success, although the distraction gap does not appear to be bridged by bone at that time.

We feel that the trial should be continued with a longer consolidation period. It is hoped that more transmandibular biopsies will remain intact, the tetracycline labelling will be better, and that a firmer statement can be given whether or not ultrasound therapy may stimulate regenerate maturation in the severely resorbed mandible.

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