Abstract. This study evaluated bone distracted in rabbit mandibles at different intervals and with different daily rates of distraction histologically with the goal of attaining a universally accepted distraction protocol. Osteogenesis was investigated in mandibles distracted at different rates in 24 New Zealand rabbits using a custom-made submerged distractor. Distraction was started on the third postoperative day for a total of 10 mm. The animals were divided into four groups each containing six rabbits. Group 1 was distracted 0.5 mm twice a day; Group 2 was distracted 1.0 mm once a day; Group 3 was distracted 1.0 mm twice a day and Group 4 was distracted 2.0 mm once a day. All the animals were sacrificed 6 weeks after completion of distraction. Half of the distracted mandibles were decalcified for H&E staining and polarized light microscopy studies. Sections of the undecalcified half of the samples were stained with Goldner’s stain. The results indicate that a distraction rate of 1.0 mm per day produced the best osteogenesis among the tested rates. There was no great difference in osteogenesis between 1.0 mm once a day and 0.5 mm twice a day. However, 0.5-mm distraction may result in immature bone healing. Distraction of 1.0 mm twice a day resulted in incomplete osteogenesis, while distraction of 2.0 mm once a day resulted in fibrous union. It is clear from these results that a shorter period of device fixation should be achieved by methods other than rapid distraction.

Key words: mandibular reconstruction; distraction osteogenesis; distraction rate.

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Different clinicians apply distraction at different intervals and rates per day.
This study histologically evaluated bone distracted in rabbit mandibles at different intervals and rates per day, in order to assist in developing a universally accepted distraction protocol.

Material and methods
Twenty-four New Zealand rabbits each weighing 4.0 kg (± 0.25 kg) were used in this experiment. The animals were anaesthetized preoperatively with an intramuscular injection of ketamine 10 mg/kg (TEKAM®; Al Hikama Pharmaceuticals, Amman-Jordan) and xylazine 0.15 mg/kg (Seton® 2%; Laboratorios Calier, S.A., Barcelona, Spain). In addition, 1.0 ml of local anaesthetic (2% lidocaine/epinephrine 1:80 000; Astra, USA) was injected at the surgical site just before the start of the operation. Postoperatively, all animals were given an i.m. injection of long-acting antibiotics (Duphapan® Strep B.P., 0.2 ml/kg, 12 500 000 IU benzyl penicillin benzathine and 5 g streptomycin per 100 ml; Solvay, Italy) and an analgesic dose of Analgin (Pharmalgin®; 0.5 ml Arab Drug Co., Cairo, Egypt).

The animals were fed and maintained in separate cages in the animal house facility at King Saud University.

The author designed a distractor suitable for rabbit mandibles. The device is designed to be fixed subcutaneously and allows simultaneous bilateral distraction of the body of the mandible without the need for two separate devices (Fig. 1). The distractor is activated from the tip of the distraction bar with a screwdriver. Two complete counterclockwise turns of the distraction bar equal 1 mm of distraction.

Each rabbit was operated on in the supine position. The submandibular and cheek hairs were shaved and the area scrubbed with iodine solution before the skin was incised longitudinally in the midline in the submandibular area. Skin and periosteal flaps were raised to expose the mandibular bone between the premolars and the apices of the incisors. Anterior to the premolars, the mental neurovascular bundle on each side of the mandible was identified and preserved to maintain blood supply to the distracted bone. Corticotomy of the mandibular cortex was performed instead of osteotomy to avoid damaging the apices of the incisors. The bone cortex was cut across both sides of the mandible just anterior to the mental foramina with a micro-reciprocating saw with copious irrigation using sterile saline. The two mandibular segments were separated completely at the corticotomy line with a small chisel. Details of the surgical procedure and distractor fixation were described previously.

For the initial feeding of rabbits in the recovery stage, 50 ml of 20% dextrose solution were injected s.c. in the abdominal area. Glucose water was given to the animals for the first postoperative day and the animals were fed normally thereafter.

In each animal, the wound site was debrided daily and cleaned with chlorhexidine wash. A local antibiotic ointment (Baneocin®; Bacitracin Zin and Neomycin, Biochemie GmbH, Vienna, Austria) was applied daily for the first week.

The distractor was first used on the third postoperative day, and the total distance distracted was 10 mm (Figs 1 and 2). The animals were divided into four groups each containing six rabbits. The distraction rate protocol applied in each group was as follows: Group 1: 0.5 mm twice daily; Group 2: 1.0 mm once daily; Group 3: 1.0 mm twice daily; Group 4: 2.0 mm once daily.

All animals were sacrificed 6 weeks after the completion of distraction.

Histological processing
The distracted bones were cut out en bloc. The block extended at least 0.5 cm into the adjacent original bone on each side of the distraction zone (Fig. 1). Samples were prepared, labelled, and placed in 10% formalin solution for histological examination. The specimens were divided in two. One half of the specimens were decalcified and the other used for undecalcified sections.

The sections to be decalcified were fixed in formalin solution for 24 h and then placed in Cal-Ex solution (Fisher Diagnostics, NJ, USA) until they were fully decalcified. Then, they were rinsed...
in running tap water for 5 h to remove excess acid from the tissue before they were dehydrated in an ethanol series (70–90%) in an automated processor. The specimens were cleared with chloroform for 1 h, impregnated with melted paraffin wax, embedded in paraffin wax, and sectioned in a rotary microtome at 5 μm. Sections of the distraction gap and part of the adjacent original bone were taken from the buccal side of the bone blocks (Fig. 1), mounted on Plexiglas slides using photo-polymerizing glue, and stained with haematoxylin–eosin (H&E). The stained sections were washed with water and dried. A precision adhesive press affixed the stained sections to parallel Plexiglas slides with photo-polymerizing glue.

Similar sections were studied histologically. The area of examination included the distracted bone on one side with part of the original bone adjacent to the distraction zone from the same side, to compare bone quality between the newly formed and original bone.

Haematoxylin–eosin stain was used to examine details of the healing process. In the same sections, polarized light microscopy was used to compare the density of the distracted bone and the adjacent original bone.

Similar undecalcified sections were prepared. Bone blocks were fixed in 10% formalin solution for one month and dehydrated through a graded series of ethanol (70–90%). The infiltrating and embedding resin used for the undecalcified specimens was from a JB-4 Plus® embedding kit (Polysciences, Inc., Washington, PA, USA). The infiltration resin was prepared by mixing 1 g of JB-4 Plus® Catalyst powder with every 100 ml of JB-4 Plus® Solution A. The bone samples were immersed in the infiltration solution for 10 days at 40°C in a dark bottle.

Embedding and polymerization were accomplished by mixing 15 ml of fresh infiltration solution with 1 ml of JB-4® solution B for each sample. To retard premature polymerization while embedding, the solution was stirred well and placed in an ice bath. Polymerization was achieved at room temperature within 8 h. Anaerobic conditions were maintained by covering the specimens tightly during this stage.

The bone was sectioned to a thickness of 50 μm with an electric diamond cutting wheel (Leitz, Germany). A vacuum adhesive system was used to mount the polymerized tissue sections on Plexiglas slides using photo-polymerizing glue. The surface of the final specimen was treated with 30% hydrogen peroxide for 1 min and then stained with Goldner’s trichrome stain. The stained sections were washed with alcohol, then with water, and finally dried. A precision adhesive press affixed a parallel Plexiglas slide to the stained sections with photo-polymerizing glue.

Goldner’s stain was selected to identify the quantity and maturity of new bone deposited in the distraction zone.

**Results**

All animals recovered well from the surgery. Four rabbits died before the end of
the observation period due to aspiration pneumonia or gastrointestinal obstruction. Two of these rabbits were in Group 1, one was in Group 3 and one was in Group 4. Distraction was well tolerated in Groups 1 and 2. The animals in Groups 3 and 4 appeared to be in pain when the distractors were activated. Therefore, Pharmalgin® 0.5 ml was injected i.m. 30 min before the time of distraction each day for all animal groups.

Clinical views of the distracted mandibles from the different groups are shown in Fig. 2. The distracted bone in Groups 1 and 2 was clinically sound and barely distinguishable from the adjacent original bone, while the distraction zones in Groups 3 and 4 were softer and easily differentiated from the adjacent original bone. The soft tissue gap in the distraction zones was larger in Group 4 than in Group 3.

H&E-stained sections of the distraction zone in Group 1 showed vascular-rich compact bone. Prominent reversal lines of bone healing indicated rapid bone formation (Fig. 3A). Polarized light microscopy also showed reversal lines in the distraction gap, and showed that the formed bone was mature and similar to the adjacent original bone (Fig. 3B). In this group, the undecalcified Goldner’s stained sections showed mature bone in the distraction gap. This bone was reconstructed predominantly in a lamellar fashion similar to the original adjacent bone. Moderate marrow spaces formed throughout the distraction gap (Fig. 3C).

The predominant histological feature of the decalcified sections in Group 2 was a rich fibrillar matrix, which tended to align itself in a parallel manner in the direction of distraction. Lamellar-like tissue compactly filled the distraction zone and some of these lamellae, especially near the bony cut line, were arranged in concentric circles to form

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Fig. 3. Prepared specimens from Group 1. (A) H&E stain: vascular-rich compact lamellar bone (☆). Note the reversal lines of bone healing (♩). (B) Polarized light microscopy of the same section showing the compact nature of the distracted bone (★), which is similar to the original bone on the left (△). Also, note the reversal line showing bone healing (↔). (C) Goldner’s stain: the lamellar bone is greenish-blue, while osteoid, the bone marrow, and blood vessels are red. The field of the distraction zone (left side, ☆) is predominantly filled with lamellar bone.
osteons. Micro-vascularization (angiogenesis) was predominant throughout the formed matrix, providing a healthy environment for osteogenesis and haversian system maturation (Fig. 4A). Polarized light examination reflected the same features seen in the H&E-stained sections. The light contrast was similar in the original bone margins and the distraction zone (Fig. 4B). Sections of undecalciﬁed specimens from this group showed deposition of mature bone throughout the distraction zone. Prominent layers of active osteoblasts started to form osteons, indicating that active bone remodelling took place in the distraction area (Fig. 4C).

Decalciﬁed sections from Group 3 revealed increase areas of marrow and ﬁbro-fatty tissue, with less lamellar bone (Fig. 5A). Cross-polar light microscopy also indicated a mixture of woven and lamellar bone, with increased marrow spaces (Fig. 5B). In this group, Goldner’s stained sections showed reduced amounts of mature bone deposited in the distraction zone. A few ﬁne bone bundles joined the two margins of the gap (Fig. 5C).

H&E-stained sections of Group 4 revealed a preponderance of bone marrow spaces within the distraction zone and less active ﬁbrous connective tissue stroma than in any of the other groups (Fig. 6A). Polarized light microscopy also showed that minimal bone remodelling took place within the distraction zone and that this area was less like the original bone (Fig. 6B). In addition, the undecalciﬁed Goldner’s stained sections showed a central area of non-union in the distraction zone with a very few small, scattered, spots of woven bone (Fig. 6C).

Discussion

The distractor used in this study was a submerged device custom designed to fit

Fig. 4. Prepared specimens from Group 2. (A) H&E stain: vascular-rich lamellar bone (♂) is aligned in a longitudinal manner in the direction of distraction. Osteons of distracted bone (●) are seen in some areas near the osteotomy line (○). (B) Polarized light microscopy of the same section: the density of lamellar bone in the gap (left side, ♂) is similar to that of the original bone seen on the right (△). (C) Goldner’s stain: mature bone is deposited throughout the gap (♀) in a parallel arrangement of lamellar bone following the direction of distraction. Prominent layers of active osteoblasts laid down in layers are starting to form osteons (○).
rabbit mandibles. It is different from the extra-oral distractors used in rabbits by others. The submerged distractor has the advantage of being buried subcutaneously, which reduces the incidence of mobilization caused by movement of the animal’s head against the cage bars. The position and size of the screws in the device avoid trauma to the adjacent teeth roots. The size of the distractor and method of activating the device enable distraction of both sides of the rabbit mandible simultaneously, eliminating the need for separate distractors on each side of the mandible. The weight of the device (2 g) makes it more convenient for adaptation to the rabbit compared to other distractors that weigh over 15 g.

The corticotomy line was planned in such a way as to avoid injury to the mandibular neurovascular bundle, thus maintaining good blood supply to the distracted bone.

After almost 10 years of experience in facial bone distraction, there is not yet a universally accepted rate of distraction among surgeons. Clinicians and researchers have shown that the latency period does not influence the physical and technical properties of the distracted bone, as was previously thought.

The potential and rate of bone healing vary with the size and age of the experimental animals. In this study, the author observed variable bone repair for each of the tested distraction rates using the same animal species.

Among the investigated distraction rates, Groups 1 (0.5 mm twice a day) and 2 (1.0 mm once a day) had equal maximum bone osteogenesis. These findings were confirmed in the undecalcified Goldner’s stained specimens as well as in the H&E and polarized light sections. Other researchers investigating

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Fig. 5. Prepared specimens from Group 3. (A) H&E stain: increased marrow spaces (†) throughout the distraction zone and a vascular-rich matrix (‖) with moderate longitudinal bone deposition are clearly distinguished from the original bone (△). Note bone joint (○) between original and new bone. (B) Polarized light microscopy of the same section: the distraction bone consists of a mixture of woven (less contrast, ‡) and lamellar (more contrast, △) bone with increased bone marrow spaces (top left, ★). (C) Goldner’s stain: the distraction zone contains fibrous connective tissue predominantly, with a reduced amount of new bone (‡). A few bundles of new bone ingrowth are seen at the margins of the gap (○).
distraction osteogenesis in rabbit mandibles have also demonstrated complete bone healing using distraction rates between 0.36 and 1.0 mm per day for distraction distances up to 20 mm.

The prominent reversal lines noticed in mandibles distracted by 0.5 mm twice a day indicate rapid bone healing, which may result in premature bone union, as observed by ILIZAROV.

Healing in Group 3 (1.0 mm twice a day) resulted in incomplete bone repair after 6 weeks of distraction. Healing was poorer in Group 4 (2.0 mm once a day), in which only fibrous union occurred. STEWART et al. examined two rates of distraction in rabbit mandible: 0.5 and 1.5 mm twice a day. Twenty-eight days after distraction, they noted that the more rapid distraction (3.0 mm per day) resulted in fibrous union, whereas bony union occurred in mandibles distracted by 0.5 mm twice daily (1.0 mm per day).

CHIN & TOTH reported clinical cases with distraction rates of 0.25 mm four times a day, 0.5 mm two to four times a day, and 1.0 mm once a day. They noted that relapse of the distracted facial bone occurred when distraction was rapid. Relapse was obvious with 0.5-mm distraction four times daily (2 mm per day).

From his earlier animal studies of long bone distraction, ILIZAROV concluded that 1.0 mm distraction per day gave the best results. He found that 0.5 mm once daily resulted in premature union, while 2.0 mm daily resulted in a greater incidence of non-union.

In addition to the poor quality of bone healing with rapid distraction, this author noted that rapid distraction caused pain in the rabbits in Groups 3 and 4 for up to 24 h.

In conclusion, distraction by 1.0 mm, once daily is the most acceptable rate. Distraction by 0.5 mm once a day may result in premature union, while 0.5 mm twice a day does not improve the process.

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Fig. 6. Prepared specimens from Group 4. (A) H&E stain: predominant marrow spaces within the gap (▲) are mixed with fibrous connective tissue (◇). (B) Polarized light microscopy of the same section: the matrix in the distraction zone is composed mainly of fibrous connective tissue (◇) with increased amounts of fibro-fatty tissue and marrow spaces (▲). Note the contrast between the original bone (right side, ▲) and the distraction field (left side, △). (C) Goldner’s stain: there is central fibrous union (◆) in the distraction zone. A few small, scattered, spots of new bone are seen in the distraction zone (greenish blue, ▲).
of bone healing markedly. It is more convenient to the patient and the clinician to activate the distractor once a day. This study also clearly showed that a shorter period of fixation of a distractor should be achieved by methods other than rapid distraction.

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References


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