Bone healing is a physiological process that includes the following steps: induction of acute inflammation, regeneration of parenchymal cells, migration and proliferation of parenchymal and connective tissue cells, synthesis of protein and extracellular matrix, remodelling of connective tissue and parenchymal component, and, as the last step, collagen formation. Tooth extraction and removal of chronic periapical lesions sometimes results in bone defects in the jaw. Similar to the general pattern, bone socket healing after tooth extraction takes place in the following stages: formation of a blood clot filling the socket, organization of the clot, epithelialization of the surface of the wound, formation of woven bone in the connective tissue filling the socket, and the replacement of woven bone (characterized by lacunae not arranged in parallel rows) by trabecular bone (the mature form of bone in which the bundles of collagen fibers in the bone matrix
are organized into successive sheets or lamellae, in which all of the bundles are oriented in the same direction) remodelling the socket.³

Undisturbed healing of bone defects and filling of the wound with bone tissue is the main prerequisite for normal bone function. A period of approximately 6 months is necessary for the formation of lamellar bone after a bone defect in humans has been created as a result of tooth extraction or periapical surgery, and even more time is needed for a defect to completely fill with mature (trabecular) bone tissue. Prolonged (delayed) healing time, or departure from regular procedure, can cause a pathological outcome.

To this day, an ideal technique of wound healing stimulation, inducing more rapid bone regeneration, has not been presented. The regenerative procedure has not been accelerated, even with new bone substitute materials.⁴ Numerous data from the literature show the stimulating effect of soft (low-power) lasers on the process of wound healing.⁵,⁶ This effect is characterized by the proliferation of fibroblasts, faster collagen production, and enhanced enzyme activity. However, there are no precise data concerning the influence of low-power lasers on the speed and degree of bone regeneration. The aim of this study was to histologically evaluate the possible enhancing effect of low-power laser on the rate of bone healing of experimentally made bone defects in rats.

**MATERIALS AND METHODS**

The study was carried out on 8 albino Wistar rats, weighing 440 ± 31 g. Procedures were performed under intra-peritoneal anesthesia with Nembutal (0.1 ml/100 g BW). The hair was shaved off the right (experimental side) and left (control side) femur region. Following this, the skin was cut, and the femur exposed by blunt preparation. The periosteum was cut, and round defects (3 mm wide, 2 mm deep) were made in the bone on both sides using a round steel bur (Fig 1). Efforts were made not to open the bone marrow space. Thereafter, the periosteum, soft tissue, and skin were sutured with catgut (4-0).

Immediately after the operation, defects on the experimental (right) sides were treated daily with a 637-nm GaAlAs low-power laser, at a power output of 50 mW, using 4 J/cm² per defect. The treatment lasted 7 days. The treated area included skin around the defect and the defect itself (Fig 2). Bone defects from the control (left) sides were allowed to heal spontaneously.

Half of the experimental animals (4 animals) were sacrificed 2 weeks after surgery and the others (4 animals) 3 weeks postoperatively. The femurs were completely extracted and fixed in 10% formaldehyde, then left in 20% ethylene diamine tetraacetate (EDTA) for a period of 2 weeks. Decalcified material was embedded in paraffin blocks. The samples were stained with hematoxylin-eosin and analyzed under an optical microscope. Five sections per defect were evaluated: one from the central area of the defect, two from the periphery, and two from the areas in between.

**RESULTS**

Microscopic findings showed that all the laser-treated defects exhibited signs of bone repair. Prominent inflammatory infiltrates and fibrous alteration were not observed.
Histological analysis showed osteoblastic activity 2 weeks after surgery in all the investigated areas of bone defects of the experimental sides. Defects in femurs treated with low-power laser were filled to a great extent by mature lamellar bone of trabecular nature (Fig 3). In the defects of the control group 2 weeks after surgery, new bone formation activity was also noticed. Newly formed bone sprouts were found at the periphery of bone defects, and fibroblastic tissue with no signs of new bone formation was observed in the central area of the bone defects (Fig 4).

Three weeks after surgery, experimental bone defects were completely filled with cancellous, lamellar bone with sparse areas of bone marrow (Fig 5). Results of microscopic analysis of non-laser-treated bone defects revealed mature lamellar bone in the periphery areas, and still-immature bone, characterized by thin bony trabeculae, in the central areas (Fig 6).

**DISCUSSION**

Healing and regeneration of bone defects is still a debated phenomenon of special interest to surgeons. Bone response to trauma generally follows basic wound healing steps. Injury results in a well-defined
cascade of tissue reaction designed to produce a new matrix to restore bone continuity and architecture. The sequence and timetable of these events are bone-specific, depending on functional differences between various bones.7

Experimental studies on the effect of a surgical intervention per se (ie, creation of bone defects in the cortical bone plate) on the healing process have been carried out on different experimental models, mainly on long bones. The general impression is that the diameter of the defect greatly influences the rate and speed of healing.8,9 It is estimated that in animal models, defects greater than 3 mm in diameter heal more slowly than smaller defects. Thus, the defects created in this study could be considered relatively large.

There is an understandable desire in clinical oral surgery practice to accelerate the bone healing process. After some surgical procedures which create bone defects in the maxilla or the mandible, the coagulum retraction inside the defect might cause the appearance of “dead spaces”, and proliferation of connective tissue into the defect. This can retard formation of new bone tissue and cause anatomic aberrations and functional disturbances.10

The stimulating effect of low-power laser on connective tissue and bone regeneration has been established both clinically5,6,11,12 and experimentally.12-14 Nagasawa12 used low-power laser (GaAlAs) for radiation of bone defects on rat femurs and found active formation of cancellous bone with trabeculae. In the group without laser treatment, he found only a few osteoclasts and some cancellous bone and trabeculae. Jovanovic et al,14 studying the effect of the same low-power laser on wound healing after tooth extraction in dogs, found an obvious favorable effect of laser irradiation, reflected in more rapid epithelium regeneration, creation of granular tissue, and fibroplasia. However, it is generally held that experimental animal studies cannot be simply transferred to the human situation.15 Furthermore, the specific types of low-power lasers, wavelengths, and dosage protocols need to be clarified and established.

The results of the present study in rats show that the use of a 637-nm GaAlAs low-power laser, under the conditions used in this experiment (power output of 50 mW, 4 J/cm² per defect), had an obvious influence on the speed of healing bone defects. The histopathological investigations carried out in rat femurs pointed to the fact that bone regeneration was obviously faster under the influence of laser, especially in early stages of wound healing (2 to 3 weeks after the creation of bone defects), accelerating the process of bone healing by ca 1 week under the conditions of the experiment. Thus, the strong biostimulating effect of low-power laser on the process of healing bone defects noticed previously2 has been confirmed by this experimental model, indicating the importance of the conditions of laser irradiation for the bone healing effect.

REFERENCES

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