

# Effect of bone marrow and low power lasers on fracture healing with destruction of both periosteum and endosteum in rabbits

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## Abstract

Ten mature rabbits of local breed were used in this study; weighing between 1.5 to 1.75 kg and aged about 1–2 years. These animals were divided into two equal groups; in group A destruction of both periosteum and endosteum was done one centimeter from each side of mid-shaft femoral bone fracture, then sufficient amount of autogenously bone marrow was injected directly at the fracture site after immobilization by intramedullary pin. In group B a similar procedure was achieved as in group A, but in addition to that He-Ne infrared laser therapy was used for several sessions. The result of radiological findings indicated that, the fracture healing occurred within group B at fifteen weeks, whereas in group A the healing occurred at eighteen weeks after operation. The implantation of autologous bone marrow enhanced the fracture healing, whereas using of combinations of autologous bone marrow and He-Ne infrared laser therapy hastened the healing.

**Keywords:** Fracture, Bone marrow, Laser, Rabbit.

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## تأثير نخاع العظم والليزرات واطئه الطاقة على التئام الكسور بعد تحطيم السمحاق الخارجي والداخلي في الأرانب

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## الخلاصة

استخدم في هذه الدراسة عشرة أرانب ناضجة، تراوحت أوزانها ما بين 1,5-1,75 كغم وأعمارها ما بين 1-2 سنة. قُسمت هذه الحيوانات عشوائياً إلى مجموعتين متساويتين، في المجموعة الأولى تم تدمير السمحاق الخارجي والداخلي لمسافة تقارب الـ 1 سم على جانبي خط الكسر والمحدث في منتصف عمدة عظم الفخذ، تم حقن كميته كافيه من نخاع العظم والمأخوذ من نفس الحيوان في منطقه الكسر بعد تثبيت طرفي العظم المكسور بوساطة السفود داخل النخاع. أما المجموعة الثانية فقد طبقت فيها نفس الخطوات المتبعة في المجموعة الأولى فضلاً عن ذلك فان حيوانات هذه المجموعة تم تعريضها للاشعة الليزرية المكونة من هليوم نيون وتحت الحمراء وبعدها جلسات. أشارت الفحوصات الشعاعية إلى أن الإسراع في التئام الكسر حدث في المجموعة الثانية في الأسبوع الخامس عشر في حين ان المجموعة الأولى كان التئام الكسر فيها في الاسبوع الثامن عشر.

## Introduction

Studies suggested that, the periosteum was more essential than endosteum in callus formation. If there is destruction of both periosteum and endosteum the results will be absence of bone formation around the destructed area (1,2). Bone marrow stem cells residing in the bone

marrow are the progenitors for osteoblasts (3-5). Autologous bone marrow is a safe, simple and reliable method for treating delayed and nonunion (6-8). Low energy laser irradiation has positive effects on bone fracture healing. The mechanisms by which low-energy laser irradiation affect on bone healing still not clear (9). He-Ne low-level energy treatment accelerated the deposition of

bone matrix and increases vascularization after seven days of irradiation (10). Direct irradiation of the whole injury with He-Ne laser on days five, six post-injury altered the osteoblast and osteoclast cell population (11,12). Studies on animals were performed on the effect of low-level laser of fracture healing indicated that, the laser enhanced healing (13-15). The aim of this study was to investigate the effect of bone marrow and low-level laser energy on healing of femoral fracture after destruction of both periosteum and endosteum in rabbits.

## **Materials and methods**

Ten mature rabbits of both sex of local breed aged 1-2 years and weighing between 1.5-1.75 kg. All rabbits tolerated on the same manner of condition and housing along the periods of study. The experimental design divided into two equal series; Group A: five animals underwent transverse fracture at the mid-shaft of femur bone then about one centimeter of both periosteum and endosteum has been destroyed and treated with sufficient amount of bone marrow, Group B: five animals underwent the same conditions of group A in addition to that irradiated with He-Ne infrared laser for several sessions.

Surgical procedure was performed under general anesthesia by using protocol of anesthesia include atropine sulphate 1mg/kg b.w. intramuscularly as a premedication after fifteen minutes later a mixture of Xylazine and Ketamine hydrochloride given intramuscularly at a dose of 5 and 20 mg/kg b.w., respectively. The surgical site was prepared under aseptic technique. Skin incised directly at the mid-shaft of femoral bone to expose the bone and osteotomy was performed, about one centimeter of periosteum and endosteum were destroyed from both sides of fractured end with bistoury scalpel. The fractured ends fixed by stainless steel 2.4 $\phi$ ×120 mm intramedullary pinning. Muscles sutured by simple continuous using catgut NO. 2/0, then sufficient amount of bone marrow which aspirated from femoral head of other side (using needle gauge 18) was injected at the site of fracture in-between the stitches, after that, suture the fascia and skin. While in group B in addition to that, animals were exposed to radiation with He-Ne infrared lasers (the He-Ne wave length: 632.8 nanometers, frequency: 50-60 Hz were the wave length and frequency of infrared are 904 nanometers, 700-1200 Hz respectively) applied at the fractured site, in a series of eight sessions (1-3, 5, 7, 9, 11 and 13 days) after operation, the total dose of He-Ne infrared lasers for each animal was 3.6 joule/cm<sup>2</sup>.

Post-operative care by using penicillin-streptomycin at a dose 10000 I.U, 10 mg/kg b.w. injected intramuscularly for four days. Clinical examination and weekly radiological studies were performed for the fracture site to determine the stage of fracture healing.

## **Results**

The results of this study showed that mild inflammation at the site of operation during the first few days after operation, these signs represented by; pain, red, hot, swelling, but subsided quickly during fifth days in group A and fourth days in group B. The callus formation was tested clinically by stress of the fracture site by finger palpation, at the end of 2<sup>nd</sup> week in group A and at the end of 3<sup>rd</sup> week on B. It has noticed that the callus formation around the fractured area in group A larger than in group B. The callus disappeared clinically at eight and six weeks in group A and B respectively. The radiographical finding revealed that, the healing occurred at the eighteenth and fifteenth week in group A and B respectively, which characterized by the invisibility of fracture line and the bone taken about the normal shape. The periods of fracture healing are summarized in (Table 1).

## **Discussion**

The periosteum, endosteum and bone marrow provided cells that proliferate and differentiate into osteoblasts, chondroblasts and fibroblasts, which contribute to new bone formation (2,3). It was demonstrated that autologous bone marrow contains mesenchymal stem cells that are able to form bone, cartilage and enhancement of the osteogenesis in fracture healing (5,6).

In our study the destruction of both periosteum and endosteum at the fracture site and injection of sufficient amount of autologous bone marrow led to healing at about eighteenth week. This observation was confirmed by other authors (5,7). Who said that, the autologous bone marrow enhanced of bone healing. On the other hand this result agreed with other authors (1,2). Who recorded that, the periosteum and endosteum are important in fracture healing, which when both removed at the fracture area resulting in absence of callus formation.

Low level laser therapy can accelerate the bone regeneration. This was appeared by altered osteoblast activity at the fracture site as reflected by alkaline phosphatase activity (11). Laser irradiation also caused a significant increase in calcium accumulation at the fracture site (13). In this study, the results confirm these facts, which revealed that, the laser therapy enhanced the fracture healing in-group B when compared with group A. The destruction of both periosteum and endosteum at the fracture site then treated with a combination of laser and autologous bone marrow as in group B, which exhibited better results than using autologous bone marrow alone by other authors (6-8). We believed that this combination of autologous bone marrow and He-Ne infrared laser therapy may be not mentioned in literature.

Table 1: Radiographic findings of group A and group B.

Week	Group A	Group B
1	Slight proliferation of periosteum at a distance from fracture site. Clear fracture line.	Slight proliferation of periosteum at a distance from fracture site. Clear fracture line.
2	External callus become active and migrated in to the fracture site. Clear fracture line.	Slight increase of external callus around the fracture site. Clear fracture line.
3	Increase internal callus and partially bridged fracture site. Clear fracture line.	External callus around the fracture site. Visible fracture line.
5	Thick external callus bridged the fracture site. Still clear fracture line (fig. 1).	Thin external callus bridged fracture site. Still visible fracture line.
8	External callus began to absorb. Still visible fracture line (fig. 2).	Good alignment of the cortex at the fracture site. Bone almost taken about the normal shape. Disappearance of fracture line (fig. 3).
10	Thin external callus around the fracture site. Invisible fracture line.	Thin external callus around the fracture site. The bone taken about the normal shape (fig. 4).
12	The bone began to taken about the normal shape. Thin external callus still around the fracture site (fig. 5).	The bone taken the normal shape. Still appear very thin external callus around the fracture site (fig. 6).
15	Still visible external callus. Bone almost taken the normal shape.	The bone is normal in shape. Still very thin external callus around the fractured area (fig. 7).
18	Slight invisible callus. Bone may be taken about the normal shape.	

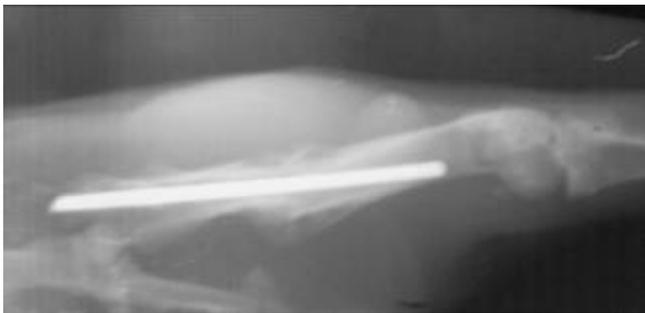


Fig. 1: Radiographic picture shows thick external callus bridged the fracture line. Still clear fracture line, five weeks after operation in group A.



Fig. 3: Radiographic picture shows bone almost taken the normal shape. Invisible fracture line, eight weeks after operation in group B.

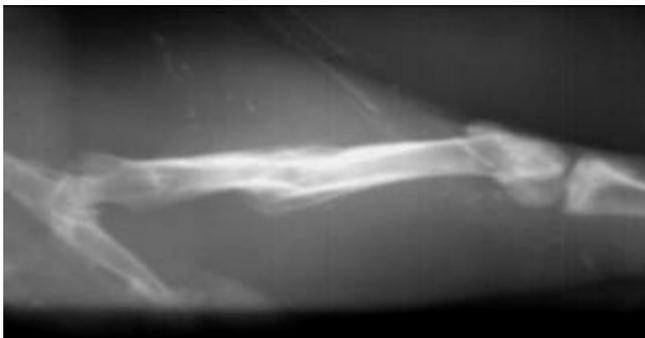


Fig. 2: Radiographic picture shows external callus began to absorb. Still visible fracture line, eight weeks after operation in group A.



Fig. 4: Radiographic picture shows thin external callus around the fracture site. The bone taken about normal shape, ten weeks after operation in group B.



Fig. 5: Radiographic picture shows the bone began to taken about the normal shape. Thin external callus around the fracture site, twelve weeks after operation in group A.



Fig. 6: Radiographic picture shows the bone taken the normal shape. Still appear very thin external callus around the fracture site, twelve weeks after operation in group B.



Fig. 7: Radiographic picture shows normal shape of bone. Very thin external callus around the fracture area, fifteen weeks after operation in group B.

The radiographic findings revealed that, the healing of the fractured bone is noticed in eighteenth, fifteenth week in group A and B respectively. These findings may be due to the He-Ne infrared laser therapy enhancement of osteogenesis and hasten the healing. This result was agreed with other authors (11,13). Who said that, the low level laser therapy can accelerate the osteogenesis. The external callus was larger in animals of group A than in group B,

this may be due to the He-Ne infrared lasers causes high activity of the source of osteoblasts and mesenchymal stem cells of autologous bone marrow for direct formation of bony material to fill the gap of fracture line instead of cartilage or fibrocartilage tissue formation as seen in common stages of fracture healing. The bone return to the normal feature approximately in eighteenth week in group A and fifteenth week in group B, this variation of period between these both groups may be due to the action of combination of autologous bone marrow and He-Ne infrared laser which accelerate the osteoblasts and osteoclasts activity to deposition and resorption of bone matrix.

In conclusion of this study which revealed that, the using of autologous bone marrow was enhanced the fracture healing. While using of combinations of autologous bone marrow and He-Ne infrared laser therapy caused more hastened healing.

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