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Experimental Study on the Effect of Mechanical Stimulation on the Early Stage of Fracture Healing

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Abstract

In an attempt to ascertain the effects of mechanical stimulation on callus in the early stage of bone fracture healing, a tibial fracture was induced in rats and mechanical stimulation applied to the fractures. The callus was then measured quantitatively, while the fractures were analyzed both radiographically and histologically.

Following the induction of a closed transverse fracture in the tibia, external anchors were applied and the rats raised by suspending the fractured leg. The rats were divided into two main groups: a Stimulation Group (S Group) and a Control Group (C Group) without the application of any mechanical stimulation. The S Group was further divided into the following three subgroups: an axial compression group (Sc Group) receiving stimulation in the positive direction; an axial distraction group (Sd Group) receiving stimulation in the negative direction; and an axial dynamization group (Sdy Group) receiving stimulation in both directions alternately. For mechanical stimulation, 1.4-N sine waves were applied continuously for 30 minutes a day, three times a week, starting 2 days after fracture-inducing surgery. At 3, 7, and 14 days after surgery, transverse sections of each fractured bone sample were prepared. At 14 days after surgery, each transverse section was divided into two peripheral and central regions to permit calculation of the area ratio of callus.

Radiographically, no marked differences were observed among the groups; histologically, differences were seen 7 days after surgery, suggesting that mechanical stimulation facilitated bone healing soon after surgery. At 14 days after surgery, the amount of callus for the C Group was less than that for all three stimulation groups. In the C Group, the amount of callus in the peripheral region was greater than in the central region, and in the Sc Group, the results were the same: callus in the peripheral region was greater than in the central region. In the Sd Group, callus was greater in the central region than in peripheral regions. In the Sdy Group, favorable callus was observed in both the central and peripheral regions. These findings suggest that axial compression facilitates callus primarily in the peripheral region, while axial distraction facilitates callus primarily in the central region. When axial compression and distraction were alternated (dynamization), callus was significantly facilitated in both the central and peripheral regions. Of the three axial stimulation techniques, dynamization was the most effective in facilitating callus in the early stage of bone fracture healing.

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Key words: mechanical stimulation, dynamization, fracture healing, NIH image

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Introduction

One objective in treating bone fractures is to achieve bone union as early as possible, and various methods of doing so have been developed. The effects of mechanical stimulation on bone fracture healing have been documented clinically over many years, and it has been known for some time that appropriate mechanical stimulation facilitates bone fracture healing[1,2]. However, several studies have reported that certain types of stimulation can prevent bone union[3]. Although many experiments have been conducted to determine the effects of mechanical stimulation on bone formation and resorption, no conclusive findings have been made on the relationship between stimulation type and bone formation.

In clinical settings, when interlocking nailing is performed on fractures of long tubular bones, dynamization, in which the direction of axial stimulation is changed by removing interlocking screws during bone fracture healing, is often employed. It is generally accepted that this technique promotes bone union. Although many experiments have been conducted on the effects of mechanical stimulation such as dynamization, the majority of these studies have discussed their results using hypotheses based on past clinical experience[4-6]. In the past, many studies categorized bone fracture healing as either static (without mechanical stimulation) or dynamic (with mechanical stimulation)[7-9]. However, the conditions under which mechanical stimulation was applied were not properly defined, and reproducibility was poor in some studies. In addition, no detailed study investigating the relationship between stimulation types and callus formation has been conducted.

In the present study, we prepared a reproducible animal model with which defined mechanical stimulation could be applied, then investigated the relationship between stimulation type and callus formation to identify a more effective mechanical stimulation technique. In other words, during the early stage of bone fracture healing, three types of axial stimulation (dynamic healing) were applied, and callus formation was compared between dynamic and static healing. Furthermore, the location and quantity of callus formation were compared and analyzed.

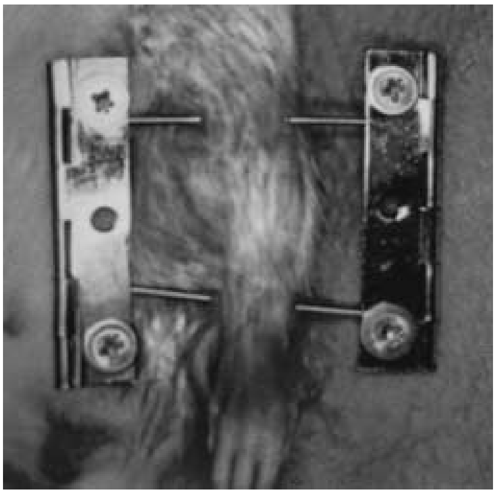


Fig. 1 Post operative fixation
The Kirschner wires were sandwiched using aluminum boards as external anchors.

Materials and Methods

(1) Materials

The left tibias of 102 male Sprague-Dawley rats, weighing 200～250 g, were used. First, in order to prevent displacement during fracture-inducing surgery, in accordance with the method of Otto et al.[10], a small skin incision was made above the left patellar tendon following intraperitoneal injection of Nembutal (pentobarbital sodium) to induce anesthesia, and 0.5-mm-diameter piano wire sterilized using hibitane was inserted into the medullary cavity. Next, a closed transverse fracture was prepared 10 mm from the proximal end of the tibia, and Kirschner wire with a diameter of 1.0 mm was inserted into both ends of the fractured tibia. Aluminum boards were used for external skeletal fixation (**Fig. 1**). In order to prepare a closed transverse fracture in a stable manner, Bonnarens and Einhorn's three-point bending method[11,12] was employed. Radiographs were taken following fracture-ind ucing surgery, and rats with a fracture line of more than 30 degrees in relation to the line perpendicular to the bone axis were excluded.

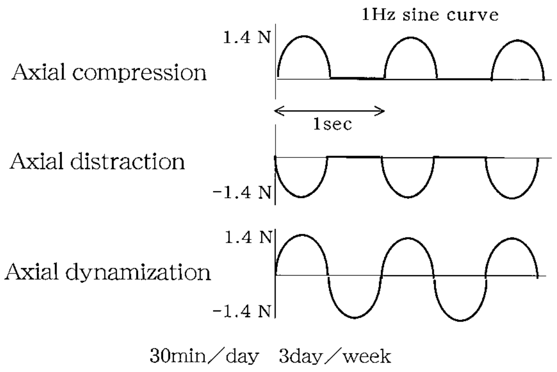


Fig. 2 Mechanical stimulation method
Sine waves (1.4 N, 1 Hz) were applied continuously for 30 minutes a day, three times a week, starting 2 days after fracture-inducing surgery.

Postoperatively, each rat was placed in a body cast. The ventral side was attached to a removable plastic table, while the dorsal side was immobilized using an acrylic bar. The rats were kept in cages with their limbs hanging in the air.

The rats were divided into two main groups: the stimulation group (S Group) and the control group (C Group) without the application of any mechanical stimulation postoperatively, the control rats were placed in cages with their limbs hanging in the air. The S Group was further divided into three subgroups with respect to stimulation types. As for the amount of stimulation, rats were made to walk on their hind limbs on a scale prior to the present study, and the results showed that the average amount of stress placed on the hind limbs by walking was 1.4 Newtons (N). As a result, 1.4-N 1-Hz sine waves were applied 30 minutes per day starting two days after fracture-inducing surgery three times per week in one of the following three ways: axial compression (Sc Group), in which stimulation was applied only in the positive direction; axial distraction (Sd Group), in which stimulation was applied only in the negative direction; and axial dynamization (Sdy Group), in which stimulation was applied in both directions alternately (**Fig. 2**). Data obtained 3 days, 7 days, and 14 days after fracture-inducing surgery was compared (**Table 1**).

Table 1 Distribution of materials and post operative periods

Groups \ Postoperative periods	3 days	7 days	14 days	Total
Axial compression	4	3	19	26
Axial distraction	3	4	19	26
Axial dynamization	4	3	19	26
Control	3	3	18	24
Total	14	13	75	102

(2) Experimental Apparatus

Prior to the study, we developed a mechanical stimulation device (Japan Servo co. Kanda Tokyo) with which defined mechanical stimulation could be applied in a constant manner. This device housed a stepping motor (Japan Servo co. Kanda Tokyo) with which the direction, angle, and frequency of rotation could be adjusted using a computer. Rotational movements of the motor were converted into linear movements via a lead screw. Based on the relationship between the migration length and spring coefficient, a certain amount of power was transmitted to the movable arm (**Fig. 3-a**). The angular velocity and rotational direction of the motor were set from a programming console (KEYENCE KZ-P 3).

(3) Experimental Methods

For each rat, after the skeletal fixation (aluminum boards) were removed, the plastic table was attached to the handle of the stimulation device. After the proximal and distal ends of the Kirschner wire were attached to the immobilization arm, mechanical stimulation was applied (**Fig. 3-b**).

The rats were euthanized through the administration of Nembutal intraperitoneally 3 days: stimulation were applied (30 minutes per day) a time, 7 days: stimulation were applied three times, and 14 days: stimulation were applied six times after fracture-inducing surgery. After some radiographs were taken, transverse sections of the fractured tibia were stained using HE for histological analyses (**Fig. 4-a**). In this manner, radiographic and histologic findings were compared.

At 14 days after fracture-inducing surgery, callus formation on each transverse section of each rat was quantified in two regions: the peripheral (A) and

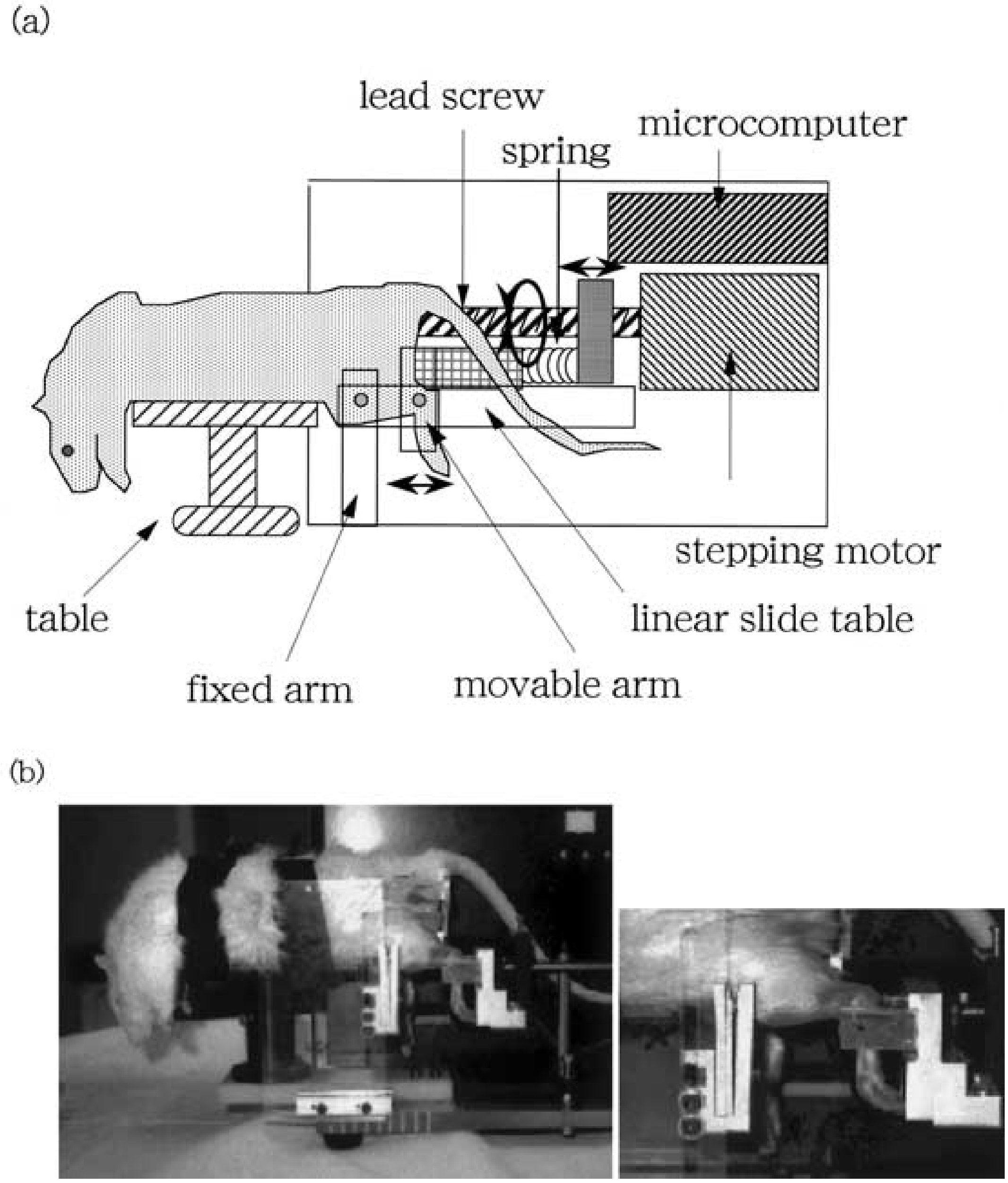


Fig. 3 Mechanical stimulation apparatus and the way of setting a rat to the apparatus
(a) Revolving movements of the stepping motor were converted into linear movements via the lead screw. Based on the relationship between migration length and spring coefficient, power of a certain magnitude was transferred to the movable arm.
(b) Following removal of the external anchors (aluminum boards), a plastic rat immobilization devise was placed and secured to the stimulation apparatus table. Both ends of the proximal Kirschner wires were then attached to the fixed arm, while both ends of the distal Kirschner wire were attached to the movable arm.

central (B) regions. In both the peripheral and central regions, two 1 mm × 1 mm squares were randomly selected, and the callus area ratio was calculated and averaged (**Fig. 4-b**). With NIH Image, the area corresponding to the callus formation in each square was traced on a paper in handwriting and then half-fixed quantified (**Fig. 5**). With non-paired t-test, a significant difference with a significance level of $<1\%$ was analyzed statistically between the control group and stimulation groups.

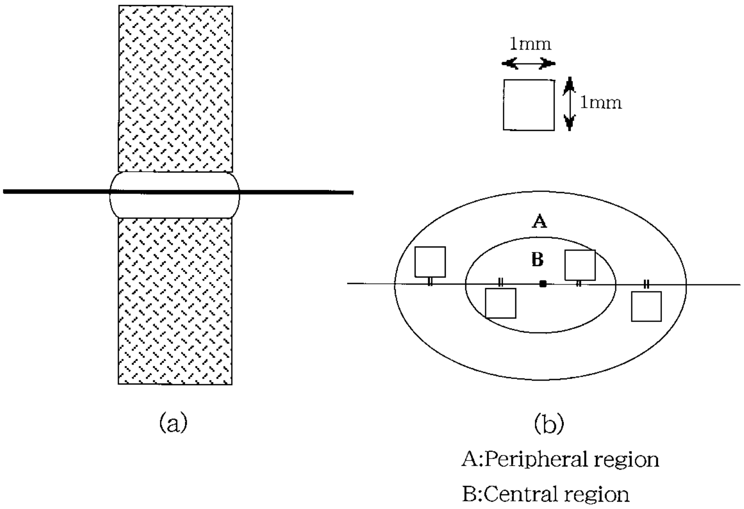


Fig. 4 Transverse sections of fracture for histological analyses
(a) Schema
(b) Each transverse section was divided into peripheral (A) and central (B) regions. In each region, two 1-mm × 1-mm areas were randomly selected to measure the area ratio of callus.

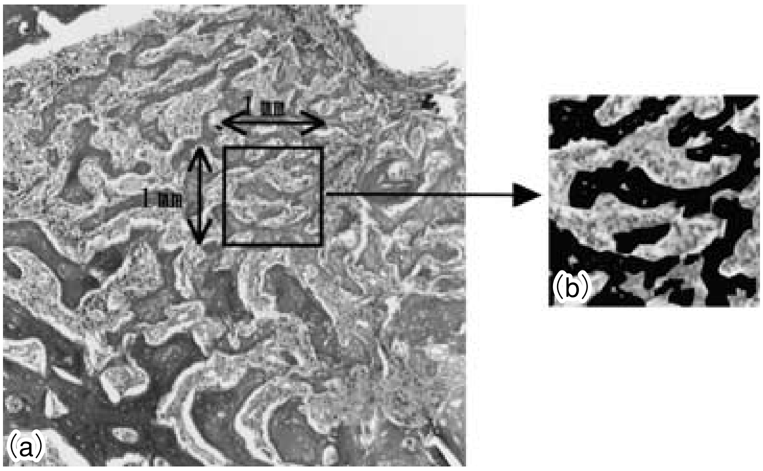


Fig. 5 Method of analysis
(a) The 1-mm × 1-mm area was selected randomly.
(b) The area corresponding to the callus formation in each square was traced on a paper in handwriting and then half-fixed quantified with NIH image.

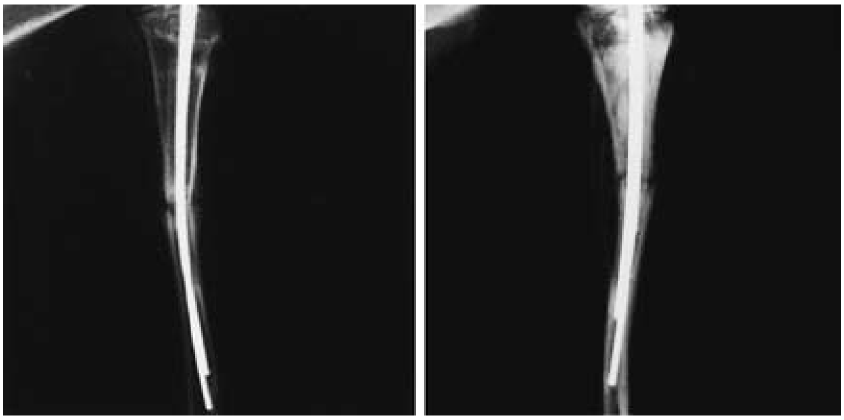
Results

(1) Radiographic Findings

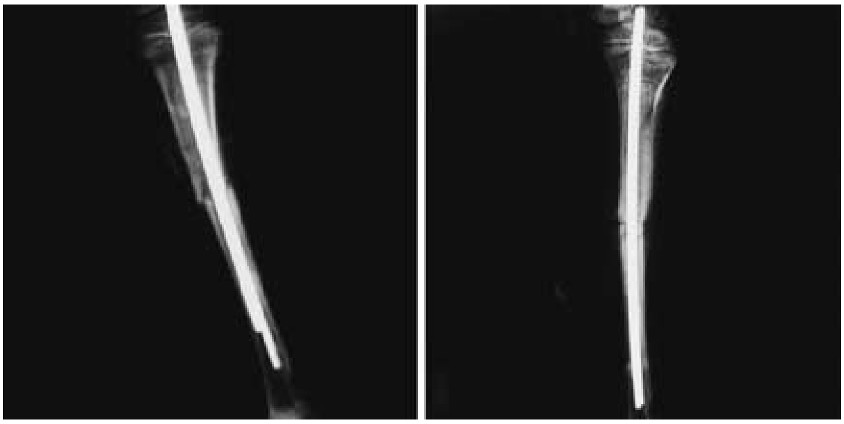
The radiographic findings were compared for each postoperative period. At 3 days and 7 days after fracture-inducing surgery, X-rays revealed no callus formation for all cases of the three S Groups or the C Group. But at 14 days after fracture, callus formation was confirmed in all cases of all groups (**Fig. 6**). However, there were no marked differences in the location or quantity of callus formation among the four groups.

(2) Histologic Findings

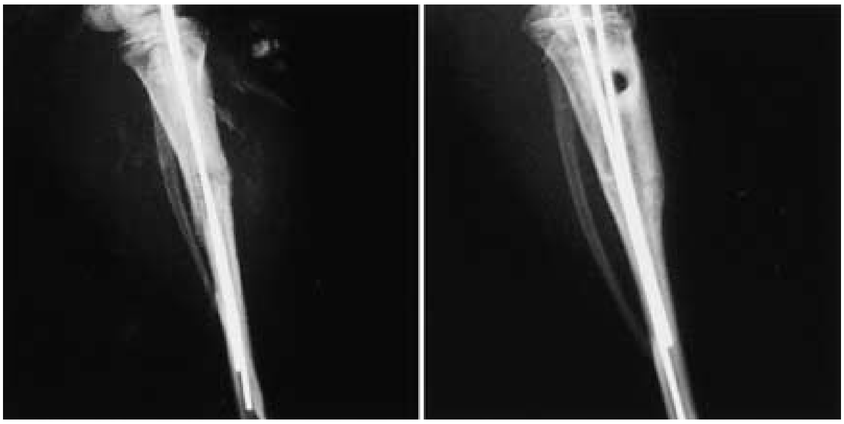
At 3 days after the fracture, hematoma and marked inflammatory cell accumulation at the fracture gap were observed in both the control and stimulation groups. At 7 days after the fracture, these findings were more marked in the stimulation groups. At 14 days after the fracture, no clear



(a) 3days control group stimulation group (Sdy group)



(b) 7days control group stimulation group (Sdy group)



(c) 14days control group stimulation group (Sdy group)

Fig. 6 Radiographic examination
(a) and (b): At 3 and 7 days after surgery, no callus was observed in any of the four groups.
(c): At 14 days after surgery, callus was seen in all groups.

differences could be seen among the four groups at the ends of the fractured tibia. Callus formation were confirmed in both the control and stimulation groups (**Fig. 7**). However, it seemed that the level of callus formation for the three stimulation groups was higher than for the control group. For the three stimulation groups, callus formation tended to occur in certain regions. In order to clarify this point, callus formation was quantified 14 days after fracture.

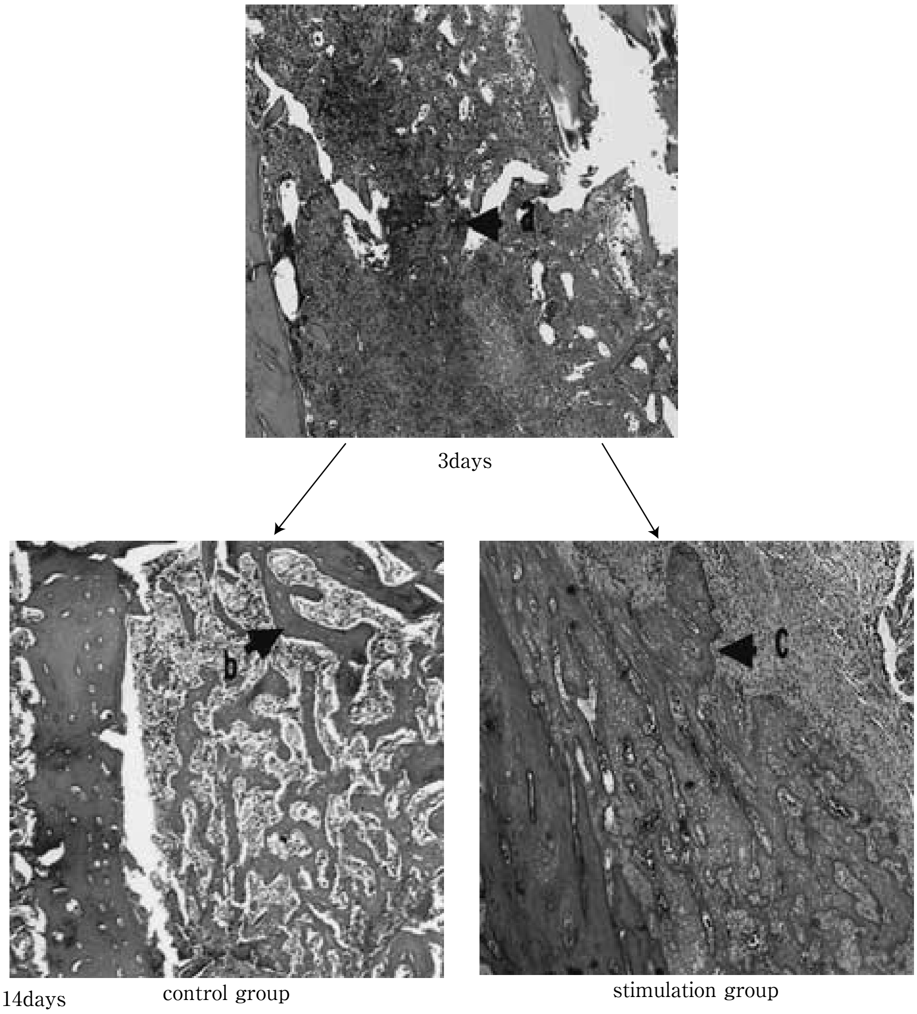


Fig. 7 Histologic examination (Hematoxylin and eosin stain × 40, sagittal plane)
a. hematoma and marked inflammatory cell
b. **c**. callus formation
At 3 days after the fracture, hematoma and marked inflammatory cell accumulation at the fracture gap were observed in both the control and stimulation groups. At 14 days after the fracture, no clear differences could be seen among the four groups at the ends of the fractured tibia. Callus formation were confirmed in both the control and stimulation groups.

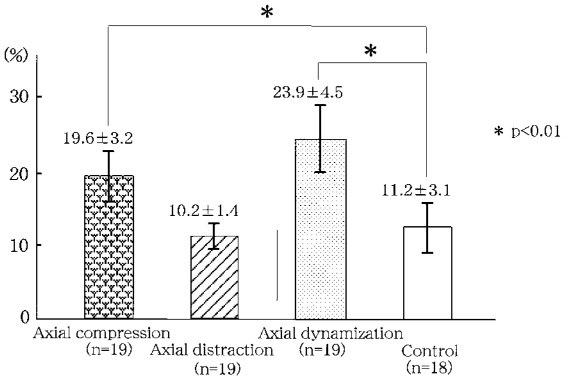
(3) Callus Area Ratios 14 Days after Fracture

In both the peripheral and central regions, two 1 mm × 1 mm squares were randomly selected, and the callus area ratio was calculated. With NIH Image, the area corresponding to the callus formation in each square was traced and then quantified.

In the C Group, the average callus area ratio in the central region was 2.6 ± 1.1%, while that in the peripheral region was higher, at 11.2 ± 3.1%. Additionally, in the Sc Group, the average callus area ratio in the central region was 2.7 ± 1.1%, while that in the peripheral region was higher, at 19.6 ± 3.2%. On the other hand, in the Sd Group, the average callus area ratio in the central region was 11.3 ± 3.4%, while that in the peripheral region was lower, at 10.2 ± 1.4%. In the Sdy Group, the average callus area ratio in the central region was 11.9 ± 4.8%, while that in the peripheral region was also favorable, at 23.9 ± 4.5%.

Compared to the C Group, there was a significant difference ($p < 0.01$) in peripheral callus formation with the Sc and Sdy Groups, clarifying that axial compression and dynamization facilitate peripheral

(a) Peripheral region



(b) Central region

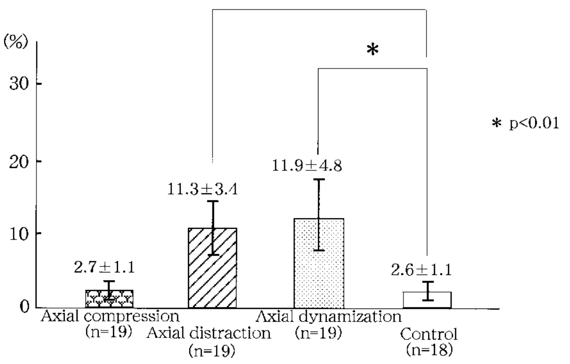


Fig. 8 Area ratio of callus

(a) A significant difference with a significance level of <1% was observed between the control group and Sc/Sdy groups.

(b) A significant difference with a significance level of <1% was observed between the control group and Sd/Sdy groups.

callus formation (**Fig. 8-a**).

In addition, compared to the C Group, there was a significant difference ($p<0.01$) in central callus formation with the Sd and Sdy Groups, thus clarifying that while dynamization facilitates central callus formation, axial compression does not (**Fig. 8-b**).

Discussion

Ideally, bone-fracture therapy should achieve bone union at as early a stage as possible. Various hormones, such as calcitonin and chondroitin sulfate, were once studied as compounds that facilitate bone union. In recent years, cytokines such as BMP have been examined[13-15]. However, except for calcitonin, these compounds have not been used clinically. Physical stimuli such as electric and mechanical stimulation have been studied for some time. Yasuda[16] and Fukada[17] reported that when bones were physically stimulated, vibration of the bones and collagen generated piezoelectricity. They hypothesized that dynamic and electric energies were involved in callus formation. Furthermore, Bassett et al.[18-21] and Brighton et al.[22] investigated the effects of electric stimulation on bone fracture healing, demonstrating the efficacy of pulsing electromagnetic fields (PEMFs). Consequently, electric stimulation is being utilized in clinical settings. Although many studies have been conducted to determine the effects of mechanical stimulation such as dynamization on callus formation, no standard method has been established. In recent years, research on mechanical stimulation has been actively conducted[1,2,7,8].

The relationship between mechanical stimulation and bone fracture healing has been investigated over many years, and the results of animal studies have shown that greater stimulation correlates with greater callus formation, while less stimulation correlates with less callus formation[23,24]. While many studies showed that mechanical stimulation was effective in the treatment of bone fractures, stimulation was applied under different conditions. Although different types of stress, such as compression, tensile, bending, and vibration, could have been present, the problems associated with the qualitative and quantitative properties of stimulation were not addressed.

In 1990, using the tibias of adult dogs, Hannu et al. histologically examined fractures in the periosteal, intracortical, and endosteal regions under rigid external skeletal fixation or dynamic compression, but found no clear differences[25]. However, they did not specify the direction, intensity, or frequency of stimulation in detail, thus limiting the application of the resulting histological findings in different regions. In this study, we induced a fracture and applied one of the three types of mechanical stimulation as consistently as possible. Prior to the study, we developed a device with which the intensity, direction, and frequency of stimulation could be freely set. The use of this device made it possible to apply mechanical stimulation with a higher degree of accuracy, and we were able to analyze the relationship between stimulation type and the location and quantity of callus formation. There are no standards for the start or frequency of stimulation. Since callus formation can be detected radiographically only two weeks after fracture-inducing surgery, we investigated the effects of mechanical stimulation during the early stages of bone fracture healing by applying three types of mechanical stimulation.

The results of histological analyses showed hematoma and marked inflammatory cell accumulation at the fracture gap 3 days after fracture-inducing surgery in the three stimulation groups, and these findings were more marked in the stimulation groups 7 days after the fracture. The results suggest that a fracture damages or destroys the periosteum, the cortical bone, the bone marrow, and the surrounding muscles and soft tissues to form a hematoma, and that mechanical stimulation during the early stages of bone fracture healing facilitates cellular necrosis and inflammatory reactions. Repeated stimulation delays inflammatory reactions, activates cells such as osteoblasts, and facilitates callus formation. In addition, at 14 days after fracture-inducing surgery, peripheral callus formation was greater than central callus formation in the axial compression and control groups. As there are more osteoblasts in the peripheral region than in the central region, peripheral callus formation is naturally more active, but axial compression appeared to have further elevated peripheral callus formation. On the other hand, axial distraction facilitated central callus formation, suggesting that central callus formation does not depend on osteoblasts. The results of the present study suggest that while axial compression facilitates peripheral callus formation, axial distraction facilitates central callus formation. Furthermore, dynamization, in which axial compression and distraction were performed alternately, facilitated both central and peripheral callus formation.

As far as callus formation is concerned, contradictory theories have been proposed regarding bone fracture healing without physical stimulation. At present, no conclusive evidence has been obtained regarding the callus-formation facilitating action of mechanical stimulation. However, it appears reasonable to assume that regional changes in physical environments due to mechanical stimulation facilitate callus formation. In recent years, three independent concepts have been proposed by researchers who investigated bone fracture healing without physical stimulation: 1) the hematoma concept states that cellular infiltration turns a hematoma, which forms at a fracture site, into stromal tissue; through granulation, the hematoma is replaced by osseous and cartilaginous tissues[26]; 2) the proliferation concept states that hematoma does not play a role in bone fracture healing[27], and that bone fracture healing occurs based solely on cellular proliferation from the periosteum and endosteum[28,29]; and 3) the multi-origin concept states that bone fracture healing is attributable to proliferating periosteal and endosteal cells and inducer cells originating from the surrounding soft tissue[30–32].

In this study, transverse sections of the fractured tibia were divided into the peripheral and central regions. Initially, we assumed that the peripheral region would reflect intramembranous calcification, while the central region would reflect endochondral calcification. If the proliferation concept is true, peripheral callus formation occurred due to intramembranous calcification, and axial compression facilitated intramembranous calcification. However, if the hematoma concept is true, central callus formation occurred due to endochondral calcification, and axial distraction facilitated endochondral calcification.

Wolff's Law[33] states that bone grows in response to mechanical stress to produce an anatomical structure best able to resist the applied stress. Frost et al. have explained the effects of regional strain as follows: the balance between bone resorption and bone formation is adjusted in response to strain caused by mechanical stimulation, and in an environment in which strain is regionally above the threshold, callus formation becomes pronounced. Due to the subsequent increase in bone strength, the strain is below the threshold, even when the level of stress remains unchanged (mechanostat theory)[34]. The results of the present study also suggest that mechanical stimulation applied soon after fracture-inducing surgery regionally caused strain above the threshold to increase callus formation.

The results of the present study shed some light on the mechanism through which mechanical stimulation applied soon after fracture-inducing surgery facilitates callus formation. By improving the accuracy of mechanical stimulation, we were able to clarify that axial compression and distraction facilitated peripheral and central callus formation, respectively, and that the direction of stimulation affected callus formation differently. Furthermore, dynamization, in which axial compression and distraction were applied alternately, was shown to be the most effective stimulation method.

Conclusions

1. The results suggest that mechanical stimulation applied during the early stages of bone fracture healing facilitates inflammatory reactions and activates osteoblasts.
2. The results of half-fixed quantitative analyses showed that callus formation varied, depending on the stimulation type: while axial compression primarily facilitated peripheral callus formation, axial distraction facilitated central callus formation.
3. Dynamization, in which axial compression and distraction were applied alternately, facilitated both peripheral and central callus formation under the conditions established for the present study. Of the three axial stimulation methods, dynamization was most effective in facilitating callus formation during the early stages of bone fracture healing.

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