# THE EFFECTS OF CIRCUMFERENTIAL WIRING ON CANCELLOUS BONE HEALING AFTER OSTEOTOMY IN OVARIECTOMIZED RATS

Hiroyuki Tsuchie, Naohisa Miyakoshi, Yuji Kasukawa, Hiroshi Aonuma and Yoichi Shimada

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*Department of Orthopedic Surgery, Akita University Graduate School of Medicine, 1-1-1 Hondoh, Akita 010-8543, Japan*

**Abstract**

**Background :** Osteoporotic fractures often occur at sites rich in cancellous bone. However, most animal models of fracture healing have been established using cortical bones, and the fracture healing process in cancellous bone has not been reported.

**Methods :** Seven-month-old female Sprague-Dawley rats underwent ovariectomy (OVX). All rats underwent complete mid-sagittal osteotomy of the proximal tibia and were divided into a wiring group and a non-wiring group ($n=14$ each). Rats were sacrificed at 2 and 4 weeks after osteotomy, and bone histomorphometry was performed to evaluate the percentage of cancellous bone union and fibrous union.

**Results :** The percentage of bone union was significantly higher in the wiring group than in the non-wiring group ($p=0.004$) at 2 weeks after osteotomy. There was also no significant difference between the wiring and non-wiring groups at 2 and 4 weeks after osteotomy regarding the percentage of fibrous union (2w : $P=0.062$, 4w : $P=0.063$).

**Conclusions :** The circumferential wiring technique improved cancellous bone union at the osteotomy site of the proximal tibia in the ovariectomized rats. The wiring may improve cancellous bone union by improving contact at the bone-to-bone interface and reducing the interposition of fibrous tissue.

**Key words :** Cancellous bone, Osteotomy, Osteoporosis, Wiring

## Introduction

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone structure, resulting in bone fragility. Fall-related osteoporotic fracture is a serious medical problem in a society characterized by an increasing proportion of elderly people. Osteoporotic fractures are often observed in cancellous bones, such as the femoral neck, distal radius, and vertebrae[1], and surgical treatment of these fractures typically involves the use of screws or plates to stabilize the fracture site.

The literature contains various observations of the fracture healing process in cortical bones such as the shaft of the femur or tibia after surgical intervention in animal models of osteoporosis[2-4]. However, no such studies have addressed fracture healing of cancellous bones after injury or surgical intervention. We therefore created a cancellous bone fracture model in which osteotomy of the proximal tibia was performed, and we evaluated the effects of intermittent administration of parathyroid hormone (PTH) on fracture healing in this model in a previous study[5]. In that study, two different interventions, administration of PTH and circumferential

Correspondence : Hiroyuki Tsuchie, M.D.
Department of Orthopedic Surgery, Akita University Graduate School of Medicine, 1-1-1 Hondoh, Akita 010-8543, Japan
Tel : 81-18-884-6148
Fax : 81-18-836-2617
E-mail : tsuchie@doc.med.okita-u.ac.jp

wiring, were performed to evaluate the combined effects on fracture healing at the cancellous bone. However, the effect of the surgical intervention, circumferential wiring, on fracture healing after cancellous bone osteotomy remains unclear. The healing process of cancellous bone fracture after osteotomy is also unknown. In order to evaluate the effects of any medical intervention on fracture healing after cancellous bone osteotomy in this model, we need to determine the effect of circumferential wiring.

The aim of this study was to evaluate the effects of an operative treatment, circumferential wiring, on the bone healing process and bone union of cancellous bone fracture created by osteotomy in ovariectomized (OVX) rats.

## Materials and methods

### Animals

Seven-month-old female Sprague Dawley rats (Charles River Laboratory Inc., Kanagawa, Japan) were housed in a controlled environment at 22°C with a 12-h light/dark cycle. The rats were pair fed and allowed free access to water and standard food (CE-2, Clea Japan Inc., Tokyo, Japan) containing 1.14% calcium, 1.06% phosphorus, and 250 IU vitamin D3 per 100 g of food, as described previously[6,7]. Osteopenia was induced by bilateral ovariectomy, as described previously[8], the response to which was confirmed by uterine atrophy when the animals were sacrificed[9].

### Experimental design

Animals were divided into the following 2 groups ($n$=14 in each group): (1) non-wiring group; and (2) wiring group. General anesthesia was induced with an intraabdominal injection of pentobarbital (30 mg/kg) (Nembutal, Abbott Laboratories, Chicago, IL, USA). In all animals, cancellous bone osteotomy at the right proximal tibia was performed as previously described, 4 weeks after bilateral OVX, timing that was considered to ensure sufficient osteopenia[5,10].

A median parapatellar incision from the knee joint through the proximal half of the tibia was made at the right hind limb, and complete mid-sagittal osteotomy from the joint surface to the outer tibial diaphysis was performed using an electric bone saw (Fig. 1A, B). Half of the animals were assigned to the wiring group, and in this group the osteotomized proximal tibia was then fixed with circumferential wire (0.4 mm diameter) (Fig. 1C). The other animals were assigned to the non-wiring group and underwent osteotomy alone with no wiring applied for fixation. After the surgery, the animals were allowed to move freely. No animal was observed to have abnormal gait or impaired locomotion postoperatively. Because it allows us to evaluate cancellous bone which is the main site of fracture in patients with osteoporosis, this model was appropriate to evaluate fracture healing in OVX rats. Moreover, this wiring technique was sufficient to ensure fixation of the fracture site[5].

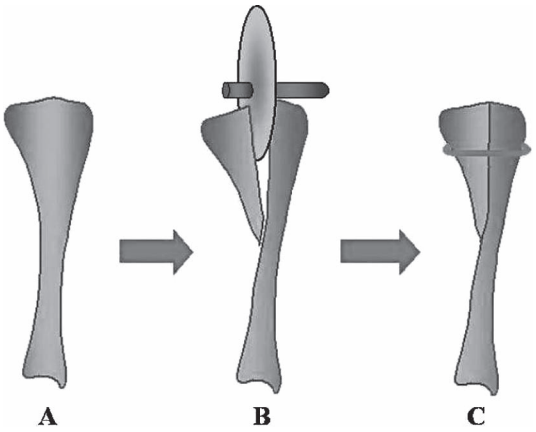


Fig. 1. Schema of the surgical procedure of cancellous bone osteotomy. Complete mid-sagittal osteotomy from the knee joint surface to the outer tibial diaphysis was performed using an electric bone saw (A, B). The osteotomized proximal tibia was then fixed with circumferential wire (0.4 mm diameter) in the half the animals (C).

The animals were euthanized under anesthesia with pentobarbital (30 mg/kg), 2 or 4 weeks after osteotomy. The right tibia was harvested and fixed in neutral-buffered formalin until preparation for histological examination. Our animal experimental protocols were approved by the Animal Committee of Akita University Graduate School of Medicine, and all animal experiments conformed to the "Guidelines for Animal Experimentation" of Akita University.

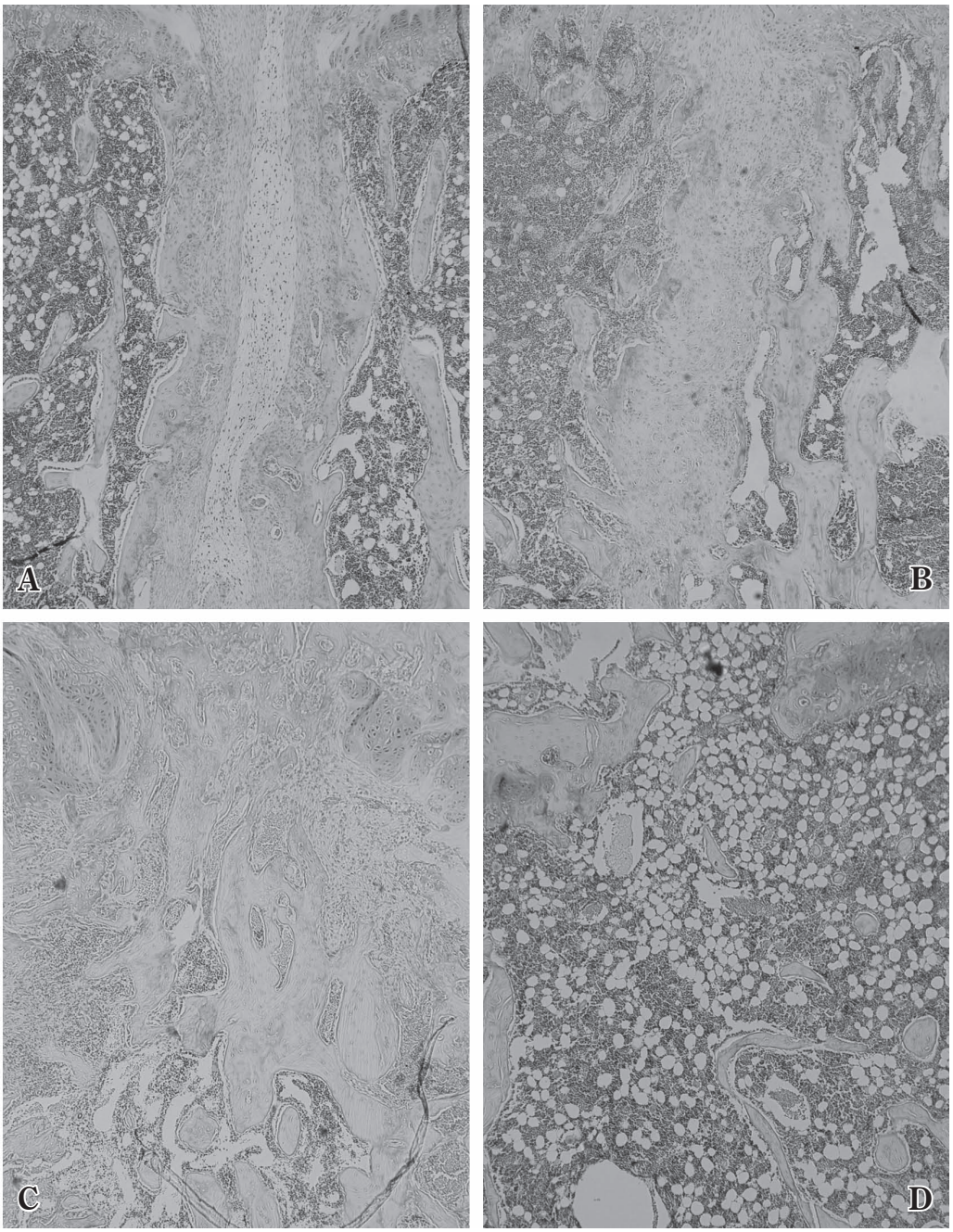


Fig. 2. Histological sections stained with hematoxylin and eosin at the osteotomy site with a magnification of 40×. Stage I : fibrous tissues were present at the osteotomy site (A). Stage II : cartilage cells and woven bone occupied most of the osteotomy site (B). Stage III : lamellar bone occupied most of the osteotomy site (C). Stage IV : lamellar bone had been absorbed (D).

### Sample preparation

The right proximal half of the tibia, including the osteotomy site, was decalcified with neutral 10% ethylene diamine tetra-acetic acid (EDTA) for 3 weeks and embedded in paraffin. Then, 3-μm-thick mid-frontal sections were prepared for hematoxylin and eosin (H & E) staining in preparation for cancellous bone histomorphometry.

### Evaluation of bone union after osteotomy

H & E stained sections were used to evaluate cancellous bone union. The state of bone union at the osteotomy site was classified into the following 4 stages : Stage I, fibrous tissues present at the osteotomy site (Fig. 2A) ; Stage II, cartilage cells and woven bone observed at the osteotomy site (Fig. 2B) ; Stage III, lamellar bone occupying most of the osteotomy site (Fig. 2C) ; and Stage IV, lamellar bone absorbed (Fig. 2D). The ratio of Stage I to the other stages was then calculated. The percentage of bone union (rate of the length of bone union to the total length of osteotomy) was calculated with a semiautomatic graphic system (Histometry RT Camera, System Supply Co., Nagano, Japan) at a magnification of 40×. Complete bony union (bone-to-bone binding at the site of osteotomy) was defined as bone union, whereas fibrous union (fibrous binding) was defined as non-union. In addition, the percentage of fibrous union (ratio of the length of fibrous union to the total length of osteotomy) was also calculated with a semiautomatic graphic system.

### Statistical analysis

All values are expressed as means±standard deviations (SD). A Student *t*-test and Wilcoxon rank sum test were used for comparison between the non-wiring and wiring groups at 2 and 4 weeks. Chi-square test was used for comparison of the ratio of stage I to other stages. Probability values less than 0.05 were considered statistically significant.

## Results

### Stage of bone union (Table 1)

All seven animals in the wiring group were classified as Stage II, and demonstrated cartilage cells and woven bone at the osteotomy site 2 weeks after wiring. Three of seven animals in the non-wiring group were classified as Stage I, with fibrous tissue visible at the osteotomy site, and one of these three showed complete fibrous binding. Four weeks after osteotomy, although only one of seven animals in the wiring group showed Stage I union, four of seven in the non-wiring group had this level of union, and one of these four exhibited complete fibrous binding. When the proportion of stage I union was calculated, there was no significant difference between the wiring and non-wiring groups at 2 or 4 weeks after osteotomy (2 weeks : $P=0.193$, 4 weeks : $P=0.165$).

### Percentage of bone union (Table 2)

At 2 weeks after osteotomy, the percentage of bone

Table 1. Number of Rats with Each Bone Union Stage at The Osteotomy Site

| Stage | I | II | III | IV |
|---|---|---|---|---|
| Non-wiring 2 w | 3 | 2 | 2 | 0 |
| Wiring 2 w | 0 | 7 | 0 | 0 |
| Non-wiring 4 w | 4 | 0 | 1 | 2 |
| Wiring 4 w | 1 | 0 | 1 | 5 |

w : weeks

Table 2. Percentage of Bone or Fibrous Union

| | Non-wiring 2 w | Wiring 2 w | Non-wiring 4 w | Wiring 4 w |
|---|---|---|---|---|
| Bone union rate (%) | 27.5 ± 22.2 | 68.9 ± 16.6* | 34.3 ± 28.9 | 28.4 ± 12.9 |
| Fibrous union rate (%) | 31.4 ± 41.9 | 0 | 34.8 ± 34.7 | 5.2 ± 11.9 |

w : week
All values are means ± standard deviations
*$p=0.004$ vs. non-wiring group at 2 weeks after osteotomy.

union in the wiring group was significantly greater than that in the non-wiring group ($P$=0.004). However, at 4 weeks after osteotomy, there was no significant difference in percentage of bone union between the two groups ($P$=0.749). There was also no significant difference between the wiring and non-wiring groups at 2 and 4 weeks after osteotomy regarding the percentage of fibrous union (2w : $P$=0.062, 4w : $P$=0.063), and when we compared the percentage of fibrous union between 2 and 4 weeks after osteotomy in the non-wiring group, no significant difference was evident ($P$=0.875).

## Discussion

Bone union is affected by various factors, such as blood flow and mechanical stress[11,12]. Gaps between bone fragments and damage to the periosteum also delay bone union and promote fibrous union or pseudarthrosis[13,14]. In this study, wiring after cancellous bone osteotomy advanced bone union significantly in the 2 weeks after the operation. Moreover, although there was no significant difference between the wiring and non-wiring groups regarding the percentage of fibrous union, the percentage of fibrous union tended to be higher in the non-wiring group at 2 and 4 weeks. Although there was no significant difference in bone union at 4 weeks after operation, most of the osteotomy sites were classified as Stage IV in both the wiring and non-wiring groups. Four weeks after wiring equated to 8 weeks after ovariectomy, thus bone turnover was increased and trabecular bone volume was decreased[15]. This means that the measurement method of bone union in the present study could not evaluate bone union correctly at 4 weeks after wiring. Based on these findings, the wiring significantly promoted the bone healing and tended to reduce intervention of fibrous tissue at the osteotomy site of the proximal tibia.

In this study, fibrous tissue intervention in the fracture gap existed in half the specimens in the non-wiring group. Lamellar bone at the osteotomy site in the non-wiring group was absorbed almost as well as in the wiring group in areas without this fibrous tissue. We also found no significant difference in the percentage of fibrous union between 2 and 4 weeks in the non-wiring group after osteotomy. This finding indicates that once fibrous tissue has intervened in the fracture gap in osteoporotic patients, it may remain unabsorbed and become remodeled into trabecular bone. As a result, the strength of the bone union site may decline and the risk of re-fracture may increase. Thus, firm fixation provided as soon as possible, such a hard brace for vertebral fractures or operative treatment using screw and plate implantation for femoral neck fractures, may be necessary in osteoporotic patients.

Cortical bone union after fracture of the femoral shaft is thought to require 8 weeks (2). In this study, cancellous bone union at the osteotomy site was almost complete in both groups at 4 weeks after osteotomy. Based on this finding, cancellous bone union may be completed earlier than cortical bone union. Although the literature contains several reports of ovariectomy delaying bone union in rats[2-4], bone union speed did not differ significantly between the wiring and non-wiring groups in the OVX rats in the present study. The bone union speed was similar at the osteotomy site of the cancellous bone in OVX rats, whether or not fixation of the bone fragments was performed.

In conclusion, the present study is the first to our knowledge to evaluate bone union of cancellous bone fractures and the effect of operative treatment in this osteoporotic model. The present wiring technique at the osteotomy site effectively promotes bone union and prevents fibrous union. Although bone union occurred without wiring in several OVX rats in this study, this may have happened incidentally where there was no interposition of fibrous tissue. Henceforth, when we evaluate the effects of any medical intervention on cancellous bone healing after osteotomy, wiring should be applied to maintain the osteotomy site in the same condition.

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