X-ray observation on tibial fracture healing due to mechanical stress stimulation

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Abstract: The effects of mechanical stress 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. However, several studies have reported that certain types of stimulation can prevent bone union. Although many experiments have been conducted to determine the effects of mechanical stress stimulation on bone fracture healing, no conclusive findings have been made on the relationship between stimulation type and bone fracture healing.

In this paper, the optimal mechanical stress stimulation for bone fracture healing was investigated. A total of 108 healthy rabbits were used to establish the V-shape tibial fracture models and determine the fracture healing effects at six(6) mechanical stress levels ($\sigma = 0, 1.13, 2.90, 3.97, 4.73, 6.02$ kgf/ cm^2) and four(4) fracture healing time points (t = 1, 3, 5, 8 weeks). The fracture healing was monitored by X-ray radiography.

The radiographic findings were compared for each postoperative period. The experimental results were as follows:

- At 1 or 3 weeks after operations, no obvious healing effects could be found.
- At 5 weeks after operations, there existed a "⌒-shape" relationship between healing score and mechanical stress of bone fracture. The optimal stress stimulation levels ranged from 2.90 to 4.73 kgf/cm^2. These were the following fracture healing effects. When σ= 2.90, 3.97, 4.73 kgf/cm^2, the bone fracture line became indistinct or almost disappeared, and a great amount of callus had been able to joint two fracture ends. When σ = 6.02 kgf/ cm^2, bone fracture line was still clearly or partly visible, although a great amount of internal callus had been able to joint the related bone fracture ends. When σ = 0, 1.13 kgf/cm^2， bone fracture lines were very clearly visible and only little callus between two fracture ends was seen.
- At 8 weeks after operation, there also existed a "⌒-shape" relationship between healing score and mechanical stress of bone fracture. It was similar to the healing effects at 8 weeks after operation. However, when σ= 2.90, 3.97, 4.73 kgf/cm^2, the bone fracture healing effect was better at 8 weeks than at 5 weeks after operation.

In conclusion, the authors had described an open tibial fracture model of the midshaft tibia that showed distinctive patterns of bone fracture healing. Furthermore, it was implied from the stated x-ray observation results that the potential optimal mechanical stress stimulation and optimal fracture healing time were available. In detail, the mechanical stress level of 2.90-4.73 kgf/cm2 and fracture healing time of more than five(5) weeks comprised the optimal mechanical stress stimulation conditions to enhance tibial fracture healing.

Keywords: Tibia; Fracture healing; mechanical stress stimulation; X-ray

1 Introduction

The effects of mechanical stress 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. However, several studies have reported that certain types of stimulation can prevent bone union. Although many experiments have been conducted to determine the effects of mechanical stress stimulation on bone fracture healing, no conclusive findings have been made on the relationship between stimulation type and bone fracture healing.

In this paper, the optimal mechanical stress stimulation to induce tibial fracture healing was investigated. A total of 108 healthy rabbits were used to establish the V-shaped tibia fracture models. A half circular external fixture was specially designed to simulate the mechanical stress stimulation of fracture ends. Using a X-ray machine, fracture healing was monitored by radiography at different test time points. Furthermore, the location and quantity of tibial fracture healing were compared and analyzed.

2 Materials and Methods

2.1 Animals

This study, including care of the animals involved, was conducted according to the official edict presented by the China Ministry of Agriculture and the recommendations of the Declaration of Helsinki. Therefore, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher. These experiments were approved by our institutional animal care and use committee. Adult healthy rabbits were used in all experiments. The animals were housed individually in isolated cages and were fed and watered *ad libitum*. Animals were on a 12-h light-dark cycle.

2.2 Surgery

All rabbits were anesthetized with 30 g/L Pentobarbital Sodium delivered via ear-border vein. The surgical preparation included: to fix the animal and its right hind-limb and to remove hair from the projected wound region in a conventional approach.
These are the following surgical steps:

- To make a longitudinal incision about 15 mm on the outside of the lower middle right hindlimb under sterile conditions.
- To expose the animal tibia through this longitudinal incision.
- To cut circumferentially the periosteum and extend this circumferential periosteum incision to be about 1 mm wide.
- To approximately transversely divide the tibia by means of a dental drill with a diameter of 0.5 mm.
- To produce a V-shaped tibia fracture.
- To apply a specially designed half circular external fixator (Shown in Figure 1) to fix the broken tibia.

Figure 1 Half circular external fixator of rabbit fracture tibia

The wound was closed using nylon sutures. Surgical procedures were performed in a sterile fashion by an experienced veterinary or orthopedic surgeon. Three days after operations, all animals were injected with panicillin via muscle at the rate of 200 thousand units a time and two times a day.

2.3 Experimental Groups

A total of 108 rabbits 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 S Group was further divided into 20 subgroups (S11 to S54) with respect to five(5) mechanical stress levels and four(4) experimental endpoints. The detailed experimental groups were shown in Table 1.

Table 1 Experimental Groups

		Experimental endpoints			
		1 week	3 weeks	5 weeks	8 weeks
Mechanical stress level (kgf/cm^2)	0	C01	C02	C03	C04
	1.128±0. 171	S11	S12	S13	S14
	2. 898±0. 391	S21	S22	S23	S24
	3.965±0. 257	S31	S32	S33	S34
	4. 727±0. 209	S41	S42	S43	S44
	6. 023±0. 283	S51	S52	S53	S54

At the experimental endpoints of 21 days, 35 days, or 56 days, animals were euthanased using carbon dioxide and specimens collected postmortem for mechanical testing and histology, whose experimental data will be reported in the other paper.

2.4 X-ray Observation

Using a X-ray machine, fracture healing was monitored by radiography at the experimental time points of 7 days, 21 days, 35 days, or 56 days and in the events where external fixation had failed (due to pin slippage, bending or breakage) the affected rabbit was culled and excluded from subsequent analysis.

2.5 Bone Fracture Effect Scale

A bone fracture healing scale (0-6, see also Table 2) established by our laboratory was used to quantify the tibial fracture healing, where approximately 4 or more than 4 represents good or excellent fracture healing.

2.6 Statistical Analysis

The data were presented as mean±SD, statistical significance was determined by Student's t-test and coherent analysis with one-way and stepwise regression.

Table 2 Bone fracture healing score standard

Bone fracture healing effect	Reference score
The bone fracture line is clearly visible, and no calluses are growing.	0
The bone fracture line is clearly visible; A small amount of callus are growing and these callus haven't been able to joint two fracture ends.	1
The case is in accordance with one of the following things: (1) The bone fracture line is clearly visible; A small amount of callus are growing and these callus have been able to joint two fracture ends. (2) A great amount of callus are growing and a small amount of internal callus have been able to joint two fracture ends. (3) The bone fracture line is clearly visible. A great amount of callus are growing. These callus haven't been able to joint two fracture ends, but the width of bone fracture line has decreased by more than 50%.	2
The bone fracture line is clearly or partially visible; A great amount of callus are growing and a great amount of internal callus have been able to joint two fracture ends.	3
The bone fracture line is indistinct; A great amount of callus are growing and these callus have been able to joint two fracture ends.	4
The bone fracture line is basically disappeared; A great amount of callus are growing and these callus have been able to joint or enclose two fracture ends.	5
The bone fracture line has been disappeared formed at the original fracture ends. Besides, the connected medullary cavity was seen again.	6

3 Results

The radiographic findings were compared for each postoperative period. Shown in Figure 1 and Table 3, the experimental results were as follows:

- At 1 or 3 weeks after operations, no obvious healing effects could be found.
- At 5 weeks after operations, there existed a "⌒-shape" relationship between healing score and mechanical stress of bone fracture. The optimal stress stimulation levels ranged from 2.90 to 4.73 kgf/cm^2. These were the following fracture healing effects. When σ= 2.90, 3.97, 4.73 kgf/cm^2, the bone fracture line became indistinct or almost disappeared, and a great amount of callus had been able to joint two fracture ends. When σ = 6.02 kgf/ cm^2, bone fracture line was still clearly or partly visible, although a great amount of internal callus had been able to joint the related bone fracture ends. When σ = 0, 1.13 kgf/cm^2, bone fracture lines were very clearly visible and only little callus between two fracture ends was seen.
- At 8 weeks after operation, there also existed a "⌒-shape" relationship between healing score and mechanical stress of bone fracture. It was similar to the healing effects at 8 weeks after operation. However, when σ= 2.90, 3.97, 4.73 kgf/cm^2, the bone fracture healing effect was better at 8 weeks than at 5 weeks after operation.

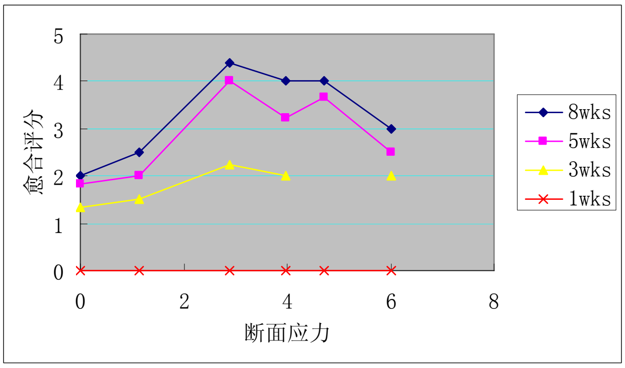

Figure 2 Relationship between tibial fracture healing scores and mechanical stress

Table 3 Bone fracture effect scores

		Experimental endpoints			
		1 week	3 weeks	5 weeks	8 weeks
Average stress level (kgf/cm^2)	0	0±0*	1. 33±0. 816*	1. 83±0. 410*	2. 00±0. 632
	1.128±0. 171	0±0*	1. 50±0. 577	2. 00±0. 000	2. 50±0. 577
	2. 898±0. 391	0±0*	2. 25±0. 500	4. 00±1. 220	4. 40±0. 894
	3.965±0. 257	0±0*	2. 00±0. 000	3. 22±1. 090	4. 00±1. 225
	4. 727±0. 209	0±0*	2. 00±0. 000	3. 67±1. 580	4. 00±0. 000
	6. 023±0. 283	0±0*	2. 00±0. 000	2. 50±0. 550	3. 00±0. 545

*: Animals weren't euthanased.

4 Discussion

4.1 Experimental fracture

Fracture healing has been studied with several animals and with different types of fracture in different bones, depending on the aims of the studies. The marked variation of methods used in fracture healing studies shows that it is rather difficult to standardize experimental fractures (Arnoczky & Wilson 1986b). The major diaphyseal fracture models are mouse, rat, rabbit, dog, sheep, goat, cat and calf, but the ones most commonly used are rat, rabbit, dog and sheep. For a review, see (Arnoczky & Wilson 1986b, An *et al.* 1999). Although the bones of some animals (e.g. rat and sheep) differ physiologically from human bone (i.e. they do not undergo normal haversian remodellation), they have been widely used in orthopaedic research (Miller *et al.* 1995, Sietsema 1995, An & Friedman 1999). Results obtained with animals cannot be extrapolated directly to humans, and studies on higher animals, like dogs and monkeys, are still needed (Arnoczky & Wilson 1986a). The challenges in experimental fracture models pertain to the stability of fixation and reproducibility. There have been four major fracture-producing methods for diaphyseal fracture models, including manual fracture (Penttinen 1972), three-point bending methods (Greiff 1978), a guillotine-like fracture apparatus (Bonnarens & Einhorn 1984) and osteotomy (Nyman *et al.* 1996). Immobilisation or fixation of the fracture site determines the amount of cartilaginous callus formation and the mode of healing. A fracture may heal without any fixation, but diaphyseal fractures are usually fixed with internal fixation, such as intramedullary rods or pins (Tarvainen *et al.* 1994c), plates and screws (Nyman *et al.* 1995) and threads or wires used alone or in combination (Arnoczky & Wilson 1986b, An *et al.* 1999). In a detailed presentation about animal models of fracture healing by An and Friedman (An *et al.* 1999), the authors preferred the pre-nailed rat tibial fracture made by a guillotine-like apparatus, which is a method modified from Bonnarens and Einhorn (Bonnarens & Einhorn 1984), as the most applicable method in fracture healing studies.

4.2 Evaluation of experimental fracture healing

Radiography is a basic method for evaluating fracture healing both in clinical use and in animal studies: radiographs are able to visualise callus formation after mineralization (Aronson & Shen 1994, Kato *et al.* 1998, Reichel *et al.* 1998). Radiographs are usually taken immediately after surgery to examine the location of the fracture and the quality of fixation. After the sacrifice of animals, high-resolution x-ray images are usually taken, which can be used for a variety measurements, such as bone density or bone dimensions. For long bone fractures, the healing parameters, such as periosteal reaction (callus 20 formation), quality of union and bone remodelling, can be quantitated on radiographs by different scoring systems (Bos *et al.* 1983, Lane & Sandhu 1987, Tarvainen *et al.* 1994c).

Histology is another basic method for evaluating fracture healing. Longitudinal sections through the fracture callus and the surrounding area are usually cut and stained. Common histological parameters, including callus formation, bone union, marrow changes and cortex remodelling, can be established (Eriksen *et al.* 1994a, An *et al.* 1999).

Mechanical testing of healing fractures is a useful tool in evaluating fracture healing. Bending, tension and torsional tests are mostly used (An *et al.* 1999, Keller & Liebschner 2000, Lopez & Markel 2000, Furman & Saha 2000), and all of them have their own particular applications (Ekeland *et al.* 1981). Bending has proved sensitive in measurements of the mechanical properties of healing callus in several animal models, such as mouse (Hiltunen *et al.* 1993), rat (Mølster *et al.* 1983), rabbit (Aalto *et al.* 1987), dog (Lenehan *et al.* 1985) and sheep (Augat *et al.* 1997). The torsional test is more functional, as it evaluates the mechanical properties of the entire healing bone, indicating the weakest section (Burstein & Frankel 1971, Ekeland *et al.* 1981). It is used for rat (Bonnarens & Einhorn 1984), rabbit (Paavolainen *et al.* 1979) and dog (An *et al.* 1999). Tension has been used mostly for rat (Nyman *et al.* 1993), and it has proved to be most useful during the initial phases of fracture healing (Walsh *et al.* 1997, Ekeland *et al.* 1981). Basic biomechanical parameters, such as maximum strength, stiffness and energy absorption, can be calculated from the results of mechanical testing.

Densitometry gives important information about the healing of bone. Densitometry has proved to correlate with the biomechanical status of healing callus at least in the early phase of fracture healing (Sano *et al.* 1999). Densitometry of healing callus has been performed traditionally by inspecting radiographs; the optical density of a radiograph is theoretically an indirect measure of bone mineral content (BMD), although it involves several limitations (Markel & Chao 1993). In spite of these limitations, good precision and accuracy have been reported for calibrated radiographic absorptiometry (Yang *et al.* 1994). Dual-energy X-ray absorptiometry (DXA) is the method of choice for defining BMC more accurately. However, it is also a projectional method, which may have some of the limitations of radiographic photodensitometry. Computed tomography has been applied to the volumetric evaluation of callus size (den Boer *et al.* 1998, Nakamura *et al.* 1998) and mineral density (Markel *et al.* 1990, Aronson & Shen 1994, den Boer *et al.* 1998, Reichel *et al.* 1998, An *et al.* 2000). Peripheral quantitative computed tomography (pQCT), introduced in 1976 (Rüegsegger *et al.* 1976), is a method for the assessment of volumetric bone mineral density (BMD), bone mineral content (BMC) and bone crosssectional geometry (Louis *et al.* 1996, Takada *et al.* 1996). Even though it was developed for human studies, pQCT has proven an effective and highly precise tool for evaluating the densitometric and geometric properties of bone in experimental animal studies, too (Gasser 1995, Jämsä *et al.* 1998a, Jämsä *et al.* 1998b, Ferretti 2000). PQCT has also been used to evaluate the healing callus of sheep tibia (Augat *et al.* 1997).

Biological markers of bone healing and bone formation can be detected from serum. The indicators of bone resorption include elevated levels of tartrate-resistant acid phosphatase (two subtypes) (Halleen *et al.* 2000) and pyridinoline in plasma and elevated levels of fasting calcium and hydroxyproline, deoxypyridinium crosslinks and hydroxylysine glycosides in urine (Garnero & Delmas 1996, Kleerekoper 1996, Russell 1997, Garnero *et al.* 2000). Further markers of bone formation are increased levels of 21 osteocalcin, procollagen peptides and bone-specific alkaline phosphatase in serum (Garnero & Delmas 1996, Kleerekoper 1996, Russell 1997, Rogers *et al.* 2000). Other methods or special devises have been used for evaluating fracture healing, such as MRI and quantitative radiographs, depending on the scope of the current study.

5 Conclusions

(1) The authors had described an open tibial fracture model of the midshaft tibia that showed distinctive patterns of bone fracture

healing.

(2) It was implied from the stated x-ray observation results that the potential optimal mechanical stress stimulation and optimal fracture healing time were available. In detail, the mechanical stress level of 2.90-4.73 kgf/cm2 and fracture healing time of more than five(5) weeks comprised the optimal mechanical stress stimulation conditions to enhance tibial fracture healing.

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