X-ray observation on how axial compression stimulates tibial fracture healing

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【Abstract】Objective: To validate the hypothesis that there exists an optimal axial compression stress range to enhance tibial fracture healing.

Methods: Rabbits with a surgically induced V-shaped tibial fracture were separated into 2 main groups: the control group (C Group, \( n = 6 \)) without application of any axial compression stress stimulation postoperatively and the stimulation group (S Group, \( n = 90 \)). The S Group was further divided into 20 subgroups (S11 to S54) in terms of 5 axial compression stress stimulation levels (112.8 kPa, 289.8 kPa, 396.5 kPa, 472.7 kPa, and 602.3 kPa) and 4 experimental endpoints (1, 3, 5 and 8 weeks after operation). A custom made circular external fixator was used to provide the axial compression stress of the fracture sites. Based on X-ray observation, a fracture healing scoring system was created to evaluate the fracture healing process.

Results: At 8 weeks after operation, there existed a “⌒-shape” relationship between healing score and axial compression stress stimulation level of fracture site. The optimal axial compression stress stimulation ranged from 289.8 kPa to 472.7 kPa, accompanying the best fracture healing, i.e. the fracture line became indistinct or almost disappeared, and a lot of callus jointed the two fracture ends. Meanwhile, at 5 weeks after operation, corresponding to the relatively low healing scores, there was a fracture healing performance similar to that at 8 weeks. Besides, at 1 or 3 weeks after operation, for all the axial compression stress levels (0-602.3 kPa), no obvious healing effect was found.

Conclusions: It is implied from the stated X-ray observation results in this study that the potential optimal axial compression stress stimulation and optimal fracture healing time are available. The axial compression stress level of 289.8-472.7 kPa and fracture healing time of more than 8 weeks jointly comprise the optimal axial compression stress stimulation conditions to enhance tibial fracture healing.

Key words: Tibia; Fracture healing; Stress, mechanical; Tomography, X-ray; External fixators

The effects of mechanical stress stimulation on bone fracture healing have been documented clinically for 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. For this reason, it is hypothesized in our study that there exists an optimal axial compression stress range to enhance tibial fracture healing. This research aimed to find out the optimal axial compression stress stimulation.
METHODS

Animals

This research was officially approved by the local institutional animal care and use committee. The study was conducted in 96 normal rabbits weighing (2.73±0.26) kg. The animals were numbered from 1 to 96 and housed individually in isolated cages. Food and water were provided ad libitum. All rabbits were on a 12 hours light-dark cycle.

V-shaped tibial fracture modeling

All rabbits were anesthetized with 30 g/L pentobarbital sodium delivered via marginal ear vein. During surgery, each rabbit was placed in dorsal recumbency with the right hindlimb prepared for aseptic surgery. The surgical area was clipped, prepared and draped under sterile technique. The surgical steps were as follows. (1) Apply a specially designed half circular external fixator (Figure 1) to fix the tibia to be broken. (2) Make a longitudinal incision about 15 mm outside of the lower middle right hindlimb under sterile conditions and expose the animal tibia through this longitudinal incision. (3) Cut circumferentially the periosteum and extend this incision to be about 1 mm wide. And (4) approximately transversely divide the tibia along a V-shaped fracture line using a dental drill of 0.5 mm in diameter and finally achieve the V-shaped tibia fracture model (Figure 1).

![Figure 1](image1.png)

**Figure 1.** The custom made circular external fixator of rabbit fracture tibia.

As shown in Figure 1, the custom made circular external fixator was assembled with three 5/8-circle rings (50 mm in diameter) in the proximal/middle/distal segment of the tibia, connected with 3 threaded rods. All the rings were perpendicular to the tibia axis. Two 1-mm Kirshner-wires were placed in every ring, with the intersection angle of 15°-30°. The wires were tensioned using a specially made wire tensioner. All the fixator components were made of aluminum alloy or stainless steel.

Initial axial compression stress

During the first phase of compression stimulation, 6 initial graded axial compression forces were applied to respectively stimulate the fracture ends of the broken tibia using the custom-made loading devices. The 6 initial graded axial compression forces were designed to be $0, \frac{1}{5} W_0, \frac{2}{5} W_0, \frac{3}{5} W_0, \frac{4}{5} W_0, 1 W_0$ ($W_0=22.5$ N). Also, the initial axial compression force ($F_c^i$) could be expressed by:

$$F_c^i = \frac{1}{5} W_0$$

For example, Table 1 and Figure 2 demonstrate the calibration data of the initial axial compression forces subject to the No. 62 test animal.

<table>
<thead>
<tr>
<th>Pre-defined values (N)</th>
<th>Measurement values (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0±0</td>
</tr>
<tr>
<td>4.5</td>
<td>4.96±0.64</td>
</tr>
<tr>
<td>9.0</td>
<td>8.97±0.63</td>
</tr>
<tr>
<td>13.5</td>
<td>14.61±1.85</td>
</tr>
<tr>
<td>18.0</td>
<td>15.88±1.59</td>
</tr>
<tr>
<td>22.5</td>
<td>21.96±1.97</td>
</tr>
</tbody>
</table>

![Figure 2](image2.png)

**Figure 2.** Calibration of the initial axial compression forces

Prior to the fracture simulation, CT scan was conducted to obtain the average across-section area at the potential fracture site. The range of CT scan along the tibia axis was 5 mm, covering the entire potential V-shaped fracture line. The scan interval was 2.5 mm. That is, for each tested animal tibia, 3 adjacent CT scan sections were selected and averaged to be the across-section area at the potential fracture site. Based on the graded initial axial compression force and average across-
section area at the fracture site, the following 6 initial axial compression stress levels were estimated to be 0 kPa, (112.8±17.1) kPa, (289.8±39.1) kPa, (396.5±25.7) kPa, (472.7±20.9) kPa and (602.3±28.3) kPa.

**Experimental groups**

A total of 96 rabbits were divided into 2 main groups: the control group (C Group, n=6) without application of any mechanical stimulation postoperatively and the stimulation group (S Group, n=90). The detailed experimental groups are shown in Table 2. The C Group was subdivided into 4 subgroups (C01 to C04) according to 4 experimental endpoints. There was a continuous process from C01 to C04, and only in Subgroup C04, those 6 test animals were euthanased. The S Group was further divided into 20 subgroups (S11 to S54) according to 5 axial compression stress stimulation levels and 4 experimental endpoints. Corresponding to the first experimental endpoint (1 week after operation), subgroups S11-S51 included 18 animals each and the animals were designed not to be euthanased. Corresponding to the other 3 experimental endpoints (3, 5 and 8 weeks after operation), each subgroup of S12-S54 included 6 animals, which were designed to be euthanased using carbon dioxide. The related specimens were collected for mechanical testing and histologic analysis, whose experimental data will be reported in other papers.

<table>
<thead>
<tr>
<th>Axial compression stress (kPa)</th>
<th>1 week</th>
<th>3 weeks</th>
<th>5 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>C01*</td>
<td>C02*</td>
<td>C03*</td>
<td>C04</td>
</tr>
<tr>
<td>112.8±17.1</td>
<td>S11*</td>
<td>S12</td>
<td>S13</td>
<td>S14</td>
</tr>
<tr>
<td>289.8±39.1</td>
<td>S21*</td>
<td>S22</td>
<td>S23</td>
<td>S24</td>
</tr>
<tr>
<td>396.5±25.7</td>
<td>S31*</td>
<td>S32</td>
<td>S33</td>
<td>S34</td>
</tr>
<tr>
<td>472.7±20.9</td>
<td>S41*</td>
<td>S42</td>
<td>S43</td>
<td>S44</td>
</tr>
<tr>
<td>602.3±28.3</td>
<td>S51*</td>
<td>S52</td>
<td>S53</td>
<td>S54</td>
</tr>
</tbody>
</table>

*Animals were not euthanased.

The wound was closed using nylon sutures. Surgical procedures were performed in a sterile fashion by an experienced orthopedic surgeon. Three days after operation, all animals were injected with penicillin via muscles at the rate of 2×10^5 U each time and twice a day.

**X-ray observation**

Limb X-ray observations were made at the experimental time points of 1, 3, 5, and 8 weeks after operation. When the external fixation failed due to pin slippage, bending or breakage, the affected rabbit was culled and excluded from subsequent analysis.

X-ray observation was also used to evaluate conditions of new bone formation, such as bone fracture line changes and callus growth, within the gap caused by axial compression stimulation. The fracture healing effect was calculated by means of a bone fracture healing scale standard.

**Radiographic scoring system for fracture healing**

Based on the previous literature 5-7, a radiographic scoring system for fracture healing (scales 0-6) was established to quantify the tibial fracture healing, in which approximately a score of 4 or more than 4 represented a good or excellent fracture healing. At the fracture sites, for scale 0, the fracture line is clearly visible and no callus growth is found. For scale 1, the fracture line is clearly visible, a little callus growth is found and the callus is not able to joint the two fracture ends. For Scale 2, the case is in accordance with one of the following situation. (1) The fracture line is clearly visible, a little callus growth is found and the callus has jointed the two fracture ends. (2) Lots of callus growth is found and a little internal callus has jointed the two fracture ends. And (3) the fracture line is clearly visible, lots of callus growth is found and although the callus is not able to joint the two fracture ends, the width of fracture line has decreased by more than 50%. As for scale 3, the fracture line is clearly or partially visible, lots of callus growth is found, besides lots of internal callus has jointed the two fracture ends. For scale 4, the fracture line is indistinct, lots of callus growth is found and the callus has jointed the two fracture ends. For scale 5, the fracture line has basically disappeared, lots of callus growth is found and the callus has jointed or enclosed the two fracture ends. For scale 6, the fracture line has disappeared and rechannellization of the marrow cavity is found at the original fracture site.

**Statistical analysis**

Data were presented as mean±standard deviation (SD) and statistical significance was determined by Student’s t test and coherent analysis with one-way and stepwise regression. P values less than 0.05 were regarded as statistical significance.
RESULTS

X-ray findings

X-ray findings demonstrated 2 important phenomena. (1) At 8 weeks after operation, only when the average axial compression stress=289.8 kPa, 396.5 kPa and 472.7 kPa, the best fracture healing occurred. In this research, the best fracture healing was defined as that the fracture line became indistinct or almost disappeared, and a lot of callus had jointed the two fracture ends (Figure 3). However, when the average axial compression stress=602.3 kPa, 0 kPa or 112.8 kPa, the fracture healing was poor, i.e. bone fracture line was still clearly or partly visible (Figure 4). And (2) at 1 or 3 weeks after operation, for all the axial compression stress levels (0-602.3 kPa), no obvious healing effects were found.

Figure 3. X-ray findings of No. 62 rabbit during 8-week tibial fracture healing (average axial compression stress=472.7 kPa; healing score=5)

Figure 4. X-ray findings of No. 59 rabbit during 8-week tibial fracture healing (average axial compression stress=0 kPa; healing score=2)

Tibial fracture healing scores

Figure 5 indicates the tibial fracture healing scores of all subgroups. Scores of 4 or more were acceptable. From Table 3, we find the detailed experimental results as follows. (1) At 8 weeks after operation, there existed a “⌒-shape” relationship between healing score and axial compression stress of bone fracture. The optimal stress stimulation levels ranged from 289.8 kPa to 472.7 kPa, accompanying the best fracture healing. (2) At 5 weeks after operation, there also existed a “⌒-shape” relationship between healing score and axial compression stress, similar to the healing effects at 8 weeks.
after operation. But the corresponding healing scores were lower ($P=0.838$). And (3) at 1 or 3 weeks after operation, however, healing scores showed significant difference between the 1-week or 3-week subgroups and the 8-week subgroups (1-week vs 8-week, $P=0.001$; 3-week vs 8-week, $P=0.0468$). At the two time points, for all axial compression stress levels (0-602.3 kPa), no obvious healing effects were found.

Table 3. Rabbit tibial fracture healing scores ($n=96$)

<table>
<thead>
<tr>
<th>Axial compression stress (kPa)</th>
<th>1 week</th>
<th>3 weeks</th>
<th>5 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0±0**</td>
<td>1.33±0.816* ^</td>
<td>1.83±0.410*</td>
<td>2.00±0.632</td>
</tr>
<tr>
<td>112.8±17.1</td>
<td>0±0**</td>
<td>1.50±0.577 ^</td>
<td>2.00±0.000</td>
<td>2.50±0.577</td>
</tr>
<tr>
<td>289.8±39.1</td>
<td>0±0**</td>
<td>2.25±0.500 ^</td>
<td>4.00±1.220</td>
<td>4.40±0.894</td>
</tr>
<tr>
<td>396.5±25.7</td>
<td>0±0**</td>
<td>2.00±0.000 ^</td>
<td>3.22±1.090</td>
<td>4.00±1.225</td>
</tr>
<tr>
<td>472.7±20.9</td>
<td>0±0**</td>
<td>2.00±0.000 ^</td>
<td>3.67±1.580</td>
<td>4.00±0.000</td>
</tr>
<tr>
<td>602.3±28.3</td>
<td>0±0**</td>
<td>2.00±0.000 ^</td>
<td>2.50±0.550</td>
<td>3.00±0.545</td>
</tr>
</tbody>
</table>

*Animals were not euthanased; $^*P=0.001$ and $^\#P=0.0468$, compared with corresponding scores at the 8 weeks.

Figure 5. The relationship between healing scores and axial compression stress during 8-week tibial fracture healing.

**DISCUSSION**

**Experimental fracture models**

Fracture healing has been studied with several kinds of animals and different types of fractures in different bones, depending on the study aims. The marked variation of methods used in fracture healing studies shows that it is rather difficult to standardize the experimental fractures. The major diaphyseal fracture models are on mice, rats, rabbits, dogs, sheep, goats, cats and calves, but the most commonly used are rats, rabbits, dogs and sheep. Although the bones of some animals, such as rats and sheep, differ physiologically from human bones, for example, they do not undergo normal Haversian remodellation, they have been widely used in orthopaedic researches. Results obtained from animal experiments cannot be extrapolated directly to humans, and studies on higher animals like dogs and monkeys are still needed. The challenges in experimental fracture models pertain to the stability of fixation and reproducibility. There have been 4 major fracture-producing methods for diaphyseal fracture models, including manual fracture, three-point bending method, a guillotine-like fracture apparatus and osteotomy. Immobilization or fixation of the fracture site determines the amount of cartilaginous callus formation and the mode of healing.

**Experimental fracture healing**

Fracture healing is a complex biological process, which is substantially influenced by the mechanical properties of the osteosynthesis. A rigid system can induce nonunion, delayed healing, or disuse osteoporosis through stress shielding, whereas a system too flexible can produce malunion, nonunion, and pin-bone interface problems. In the latest 40 years, Ilizarov developed a new technique to treat those difficult fracture problems. This method is based on the original biologic principles and the use of a circular external fixator that transfixes the bone with thin, pretensioned Kirschner wires. Even now this external fixation is an accepted method for treating open fractures of the lower limb.

This study was developed to further investigate those difficult fracture problems. We hypothesized that there existed an optimal axial compression stress range to enhance tibial fracture healing. In order to validate this hypothesis, an open fracture model of the rabbit tibia was established to examine the distinctive patterns of bone fracture healing. It is implied from the stated X-ray observation results that the potential optimal mechanical stress stimulation and optimal fracture healing time are available. In detail, the axial compression stress level of 289.8-472.7 kPa and fracture healing time of more than 8 weeks jointly comprised the optimal axial compression stress stimulation conditions to enhance tibial fracture healing.

The major limitation of this study is that it only addresses the initial axial compression stimulations. Although the spring properties of Kirschner-wires is helpful to provide a small dynamic axial stress stimulations, we can not measure this dynamic stimulations, and hence we can not adjust quantitatively the axial compression stress at the fracture site during the entire...
tibial fracture healing.

REFERENCES


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