Effectiveness of Mechanical Treatments (Periosteal Stripping VS Non-Periosteal Stripping) on Fracture Healing: In Vivo Study

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A B S T R A C T

Background: Periosteal stripping (PS) is a controversial technique in fracture management, with studies reporting both positive and negative effects on bone healing. This study aims to evaluate the effectiveness of PS and non-periosteal stripping (NPS) on fracture healing in the Sprague Dawley rat model. Methods: This study is an in vivo experimental research. Male Sprague Dawley rats (8-10 weeks old) were divided into four groups (n=10 per group): Group I: NPS, evaluation day 14; Group II: PS, evaluation day 14; Group III: NPS, evaluation day 28 and Group IV: PS, evaluation day 28. A standard fracture was created in the tibia, and PS or NPS was performed. Radiographic evaluation was performed on days 14 and 28, with the RUST score (Radiographic Union Score for Tibia) used to assess fracture healing. Statistical analysis was performed using the Kruskal-Wallis test. Results: The RUST score showed better fracture healing in the NPS group compared to PS on day 28 (p<0.05). There was no significant difference on day 14 (p>0.05). Conclusion: NPS was more effective in accelerating fracture healing in Sprague Dawley rats than PS, especially in the later stages of healing. These findings provide further evidence of the potential adverse effects of PS and highlight the importance of considering time in assessing its efficacy.

1. Introduction

Fracture healing is a complex and dynamic biological process, involving a tightly coordinated series of events to restore the structural and functional integrity of the damaged bone. This process involves complex interactions between various cell types, growth factors, cytokines, and the extracellular matrix. A deep understanding of fracture healing mechanisms is essential to develop effective therapeutic strategies to improve clinical outcomes and reduce post-fracture complications. The periosteum, the dense connective tissue membrane that surrounds the outer surface of the bone, plays a central role in the fracture healing process. The periosteum contains a heterogeneous population of osteogenic progenitor cells, including mesenchymal stem cells (MSCs), which can differentiate into osteoblasts, chondrocytes, and fibroblasts. In addition, the periosteum is also a rich source of growth factors and cytokines, such as bone morphogenetic proteins (BMPs), transforming growth factor-beta (TGF-β), fibroblast growth factors (FGFs), and vascular...
endothelial growth factors (VEGFs), which regulate various aspects of fracture healing, including angiogenesis, callus formation, and bone remodeling.1,2

Periosteal stripping (PS), a surgical technique that involves the removal of part or all of the periosteum from the bone surface, has been used in a variety of orthopedic procedures, including open fracture fixation, deformity correction, and bone lengthening. PS is believed to increase callus formation and vascularization, potentially accelerating fracture healing. However, the effect of PS on fracture healing remains a matter of debate, with some studies reporting conflicting results. Several experimental studies in animals have shown that PS can increase callus formation and vascularization in the early stages of fracture healing. This may be due to the increased release of growth factors and cytokines from the abraded periosteum, as well as increased recruitment of osteogenic progenitor cells to the fracture site. However, other studies reported that PS may hinder fracture healing at a later stage, causing delayed bone union or even nonunion. These negative effects may be due to the loss of mechanical and biological support provided by the periosteum, as well as disruption of the periosteal blood supply.3-5

The controversy surrounding the effect of PS on fracture healing has prompted further research to evaluate the effectiveness and safety of this technique. In vivo studies in animal models, such as Sprague Dawley rats, provide a valuable platform to investigate the molecular and cellular mechanisms underlying the effects of PS on fracture healing. The Sprague Dawley rat model has been widely used in orthopedic research due to its ease of handling, relatively low cost, and physiological similarity to humans.6,7 This study aims to evaluate the effectiveness of PS and non-periosteal stripping (NPS) on fracture healing in the Sprague Dawley rat model. NPS is an alternative technique that involves minimal manipulation of the periosteum, with the aim of minimizing damage to the periosteal tissue and preserving its biological integrity.

2. Methods

This study used an in vivo experimental design with a comparative approach to evaluate the effectiveness of the two techniques mechanical treatment, namely periosteal stripping (PS) and non-periosteal stripping (NPS), on fracture healing in animal models. The animal model chosen was the male Sprague Dawley rat, which is a commonly used model in orthopedic research due to its ease of handling, relatively affordable cost, and physiological similarity to humans in terms of bone healing. The research subjects were healthy male Sprague Dawley rats, 8-10 weeks old, with a body weight of between 250-300 grams. This age range was chosen because rats at this age are still in the active growth phase, so they are expected to have optimal bone healing potential. These rats were obtained from a trusted source and acclimatized in a controlled laboratory environment for at least one week before the experiment began.

The rats were randomly divided into four treatment groups, each consisting of 10 rats (n=10 per group): Group I (NPS-14): Non-periosteal stripping, evaluation on the 14th post-operative day; Group II (PS-14): Periosteal stripping, evaluation on the 14th post-operative day; Group III (NPS-28): Non-periosteal stripping, evaluation on postoperative day 28; Group IV (PS-28): Periosteal stripping, evaluation on the 28th postoperative day.

A sample size of 10 rats per group was determined based on power analysis calculations by considering the level of significance (α = 0.05), desired power (1-β = 0.80), and estimates of clinically relevant effect differences based on previous research. All surgical procedures were performed under strict aseptic conditions in a dedicated experimental animal operating room. The rats were anesthetized using a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally. After achieving an adequate level of anesthesia, the surgical area around the right tibia was cleaned and disinfected with povidone-iodine. A 1 cm long longitudinal incision was made in the skin over the right tibia. The muscles around the
tibia are carefully separated to expose the bone. A standard transverse fracture was created in the middle third of the tibial diaphysis using a 1 mm wide osteotome. Fractures were created with a single controlled blow to ensure consistency of fracture severity between subjects.

In the PS group, the periosteum was released from the bone at the fracture site for 5 mm using a periosteal elevator. Peeling is done carefully to avoid damage to the underlying bone cortex. In the NPS group, the periosteum was left intact without manipulation. After PS or NPS treatment is completed, the fracture is stabilized with external fixation using a 0.8 mm Kirschner pin. Two pins were inserted percutaneously, one each proximal and distal to the fracture site, and fixed with acrylic resin to form a stable external frame. After surgery, the rats were recovered in individual cages with soft, clean bedding. The rats were given analgesics (buprenorphine 0.05 mg/kg) subcutaneously every 12 hours for the first 3 days post-surgery to reduce pain. The rats were also given antibiotics (enrofloxacin 5 mg/kg) subcutaneously once a day for 5 days post-surgery to prevent infection. Rat cages were cleaned daily, and rats were given food and water ad libitum. The rats were closely monitored for signs of post-operative complications, such as infection, swelling, or fracture malunion.

Radiographic evaluation was performed on days 14 and 28 post-operatively using a digital radiography system. Radiographic images are taken in anteroposterior and lateral projections. The RUST score (Radiographic Union Score for Tibia) is used to quantitatively assess the rate of fracture healing. The RUST score ranges from 0 (no signs of healing) to 12 (complete bone fusion). At the end of the experimental period, namely on days 14 and 28, the rats were sacrificed with an anesthetic overdose. Fractured tibias were isolated and fixed in a 10% buffered formalin solution for 24 hours. After fixation, bone tissue samples were decalcified in 10% EDTA solution for 2 weeks. The tissue samples are then processed to make histology preparations using standard paraffin embedding techniques. Tissue sections 5 μm thick were made using a microtome and stained with hematoxylin and eosin (H&E) for general histological evaluation. Additionally, special stains such as Masson’s trichrome are used to assess new bone and collagen formation. Histological preparations were observed under a light microscope. Histomorphometric analysis was performed to measure parameters such as callus area, percentage of new bone, and trabecular thickness. Measurements were performed using image analysis software (ImageJ).

RUST score and histomorphometric data were analyzed using the Kruskal-Wallis non-parametric statistical test to compare differences between treatment groups. The Mann-Whitney U test was used to perform post-hoc comparisons if statistically significant differences were found. The significance level was set at α = 0.05. All research procedures were carried out in accordance with applicable ethical principles of animal research. The study protocol was approved by the local animal ethics committee. Maximum efforts were made to minimize the number of animals used and to reduce the pain and discomfort experienced by the animals during the experiments.

3. Results

Table 1 shows the mean RUST scores and standard deviations (presented in parentheses) for the NPS and PS groups at days 14 and 28 post-fracture. On day 14, in the NPS group, the mean RUST score was 7.2 (SD 1.3) indicating early signs of fracture healing, such as callus formation visible on radiographs. A standard deviation of 1.3 indicates individual variation in healing rates in this group. Meanwhile, in the PS group, the mean RUST score of 6.8 (SD 1.5) also indicated early signs of fracture healing but was slightly lower than in the NPS group. A standard deviation of 1.5 indicates greater individual variation in healing rates in this group compared with the NPS group. On day 28, in the NPS group, the mean RUST Score was 10.8 (SD 0.9) indicating significant progress in fracture healing. Higher scores and smaller
standard deviations indicate that the majority of individuals in this group experienced good and consistent fracture healing. Meanwhile, in the PS group: The average RUST score was 9.2 (SD 1.1) also indicating healing progress, but not as good as the NPS group. A standard deviation of 1.1 indicates smaller individual variation in healing rates in this group compared to day 14. Day 14: The difference in average RUST scores between NPS and PS was not very large (0.4), indicating that in the early stages of healing, both groups showed relatively similar healing rates. Meanwhile, on day 28: The difference in average RUST scores between NPS and PS was greater (1.6), indicating that NPS was more effective in improving fracture healing at a later stage. Overall, this table shows that NPS tends to produce better fracture healing than PS in Sprague Dawley rats, especially in the later stages of healing. However, this difference was not statistically significant on day 14 (p=0.56), but became significant on day 28 (p=0.002). This suggests that the effectiveness of NPS in improving fracture healing may be time-dependent.

Table 1. Comparison of RUST scores between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 14</th>
<th>Day 28</th>
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<tbody>
<tr>
<td>NPS</td>
<td>7.2 (1.3)</td>
<td>10.8 (0.9)</td>
</tr>
<tr>
<td>PS</td>
<td>6.8 (1.5)</td>
<td>9.2 (1.1)</td>
</tr>
</tbody>
</table>

Figure 1 shows the comparison of PS and NPS in rat fracture healing. On the 14th post-operative day, both the non-periosteal stripping (NPS) and periosteal stripping (PS) groups showed similar radiographic features. The fracture lines were still clearly visible in both groups, indicating that the bone healing process had not begun significantly. There was a little callus formation, namely new bone tissue that forms around the fracture site, but the amount was still minimal in both groups. A significant difference was seen on the 28th postoperative day. In the NPS group, callus formation was much more numerous and well organized. A clear bone bridging can be seen at the fracture site, indicating that the bone healing process has progressed further. The callus in the NPS group also appeared denser and fused with the original bone. In contrast, in the PS group, callus formation was less and less organized. The bone bridge is still minimal, and the fracture line is still clearly visible. This shows that the bone healing process in the PS group was slower than in the NPS group. A comparison of radiographic images on days 14 and 28 showed that non-periosteal stripping (NPS) was more effective in accelerating fracture healing compared with periosteal stripping (PS) in Sprague Dawley rats. On day 28, the NPS group showed more callus formation, better bone union, and more advanced fracture healing compared with the PS group.

Figure 1. Visualization of radiographic evaluation.
Table 2 shows on day 14, the NPS group showed a higher average callus area (around 5.1 units) compared to the PS group (around 4.1 units). This difference became clearer on day 28, with the NPS group achieving an average callus area of around 12.1 units, while the PS group only achieved around 10.2 units. This indicates that callus formation, which is an important early stage in fracture healing, was faster and more extensive in the NPS group compared with PS. The same pattern was seen in the percentage of new bone. On day 14, the NPS group had a slightly higher percentage of new bone (approximately 30%) compared with the PS group (approximately 25%). This difference became more striking on day 28, with the NPS group achieving a mean new bone percentage of around 55%, while the PS group only achieved around 45%. This shows that the process of mineralization and new bone formation is more active and efficient in the NPS group. Trabecular thickness, which is an important indicator of the strength and density of newly formed bone, also showed significant differences between the two groups. On day 14, the NPS group had an average trabecular thickness of approximately 0.5 units, slightly higher than the PS group (approximately 0.4 units). By day 28, this difference was even more pronounced, with the NPS group achieving a mean trabecular thickness of approximately 0.8 units, while the PS group only achieved approximately 0.6 units. This shows that the bones formed in the NPS group were not only more abundant but also denser and stronger.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callus area</td>
<td>NPS</td>
<td>5.1</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>PS</td>
<td>4.1</td>
<td>10.2</td>
</tr>
<tr>
<td>Percentage of new bone</td>
<td>NPS</td>
<td>30.0</td>
<td>55.0</td>
</tr>
<tr>
<td></td>
<td>PS</td>
<td>25.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>NPS</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>PS</td>
<td>0.4</td>
<td>0.6</td>
</tr>
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4. Discussion

The results of this study indicate that non-periosteal stripping (NPS) is more effective in accelerating fracture healing in Sprague Dawley rats compared to periosteal stripping (PS). These findings are supported by strong evidence from radiographic analysis, histomorphometry, and histological observations. The RUST score, used to assess the rate of fracture healing radiographically, showed significant differences between the NPS and PS groups at day 28 post-fracture. The NPS group had a higher mean RUST score (10.8) compared with the PS group (9.2). These differences suggest that at later stages of healing, NPS results in better bone union and more mature callus formation. Radiographic observations also support these findings. On day 28, the NPS group showed clearer bone bridges and denser calluses compared with the PS group. This shows that the process of osteogenesis, namely the formation of new bone, takes place more quickly and efficiently in the NPS group. Histomorphometric analysis provides further quantitative evidence of the effectiveness of NPS. On day 28, the NPS group showed significant increases in callus area, percentage of new bone, and trabecular thickness compared with the PS group. The greater callus area in the NPS group indicates that the callus formation process is faster and more extensive. Callus is cartilage tissue that forms around the fracture site and serves as a framework for new bone formation. The increase in callus area in the NPS group indicates a more supportive environment for the bone healing process. The higher percentage of new bone in the NPS group indicates that the process of mineralization and new bone formation is more active and efficient. The new bone formed in the NPS group also had greater
trabecular thickness, indicating that the bone formed was denser and stronger.\textsuperscript{10,11}

Histological observations on bone tissue preparations provided further insight into differences in the quality of new bone tissue between the NPS and PS groups. In the NPS group, new bone tissue was seen that was more organized and contained more osteoblasts, namely bone-forming cells. In addition, there were also more blood vessels visible, indicating a better blood supply to the fracture site. In contrast, in the PS group, the new bone tissue appeared less organized and contained more fibrous connective tissue. The number of osteoblasts and blood vessels was also less compared to the NPS group. This shows that the bone healing process in the PS group was slower and produced new bone tissue of lower quality.\textsuperscript{12,13}

The periosteum is a layer of dense connective tissue that covers the outer surface of bones, except at the joints. The inner layer of the periosteum called the cambium layer, contains osteogenic progenitor cells. These cells have the unique ability to differentiate into various types of bone cells, including osteoblasts. Osteoblasts are bone-forming cells that are responsible for synthesizing and depositing organic bone matrix, which then undergo mineralization to form new, hard bone tissue. In the fracture healing process, osteoblasts play a crucial role in the formation of callus, namely cartilage tissue which is the embryo of new bone that will connect the ends of the broken bone. When a fracture occurs, the periosteum is damaged and the osteogenic progenitor cells within it are activated. These cells then proliferate and differentiate into osteoblasts, which will begin the process of forming new bone to repair the fracture. Preservation of the periosteum during surgical procedures, such as non-periosteal stripping (NPS), allows the population of osteogenic progenitor cells to remain intact and function optimally. This can accelerate and improve the quality of fracture healing, as the availability of sufficient osteoblasts will ensure rapid callus formation and efficient new bone formation. The molecular mechanisms underlying the beneficial effects of periosteum preservation on fracture healing involve various growth factors and cytokines released by periosteal cells. These factors, such as bone morphogenetic proteins (BMPs), transforming growth factor beta (TGF-\(\beta\)), and insulin-like growth factor (IGF), play important roles in regulating osteoblast proliferation, differentiation, and activity.

In addition, the periosteum also plays a role in providing adequate blood supply to the fracture site, which is important for the survival and function of osteoblast cells. Preservation of the periosteum during fracture surgical procedures is an important strategy to optimize healing outcomes. By maintaining the population of osteogenic progenitor cells and the growth factors present therein, preservation of the periosteum can accelerate callus formation, increase new bone formation, and result in faster and better-quality fracture healing.\textsuperscript{14,15}

The periosteum has a crucial role in fracture healing not only as a source of osteogenic progenitor cells but also as a reservoir of growth factors and cytokines that regulate various biological processes important in bone healing. The periosteum contains a variety of growth factors and cytokines. Bone morphogenetic proteins (BMPs) induce the differentiation of mesenchymal cells into osteoblasts, which are bone-forming cells. BMPs also play a role in angiogenesis, namely the formation of new blood vessels that are important for the supply of nutrients and oxygen to the fracture site. Transforming growth factor-beta (TGF-\(\beta\)) has a dual role in fracture healing. In the early stages, TGF-\(\beta\) stimulates cell proliferation and callus formation. In later stages, TGF-\(\beta\) induces osteoblast differentiation and new bone formation. Insulin-like growth factors (IGFs) stimulate the proliferation and differentiation of osteoblast cells, as well as increase bone matrix synthesis. IGFs also play a role in angiogenesis and recruitment of osteogenic progenitor cells to the fracture site. Vascular endothelial growth factor (VEGF) plays an important role in angiogenesis, namely the formation of new blood vessels needed to supply nutrients and oxygen.
to the fracture site. Adequate angiogenesis is essential for optimal fracture healing. Platelet-derived growth factor (PDGF) stimulates the proliferation and migration of mesenchymal cells and plays a role in angiogenesis and bone matrix formation. Periosteal stripping (PS) involves removing part or all of the periosteum from the bone surface. This process can cause damage to the periosteum tissue and interfere with the release of growth factors and cytokines stored in it. As a result, the processes of angiogenesis, cell proliferation, and cell differentiation required for fracture healing can be hampered. Several studies have shown that PS can reduce the levels of BMPs, TGF-β, IGFs, VEGF, and PDGF at the fracture site. These decreased levels of growth factors and cytokines may explain why PS can slow fracture healing and increase the risk of complications such as nonunion. The periosteum is not only a protective layer of bone but is also an important source of growth factors and cytokines that regulate various aspects of fracture healing. Periosteal stripping (PS) can interfere with the release of these factors and hinder the healing process. Therefore, periosteum preservation should be considered in orthopedic surgical procedures to optimize fracture healing outcomes.  

The findings of this study have important implications for clinical practice. Periosteal stripping (PS) is a commonly used technique in a variety of orthopedic procedures, but the results of this study suggest that preservation of the periosteum should be considered whenever possible, especially in cases where rapid and optimal fracture healing is desired. Further studies are needed to confirm these findings in humans and to explore the molecular mechanisms underlying the beneficial effects of NPS on fracture healing. Future research could also evaluate the effectiveness of NPS in different types of fractures and in patients with certain medical conditions, such as diabetes or osteoporosis, that may affect bone healing. This study has several limitations that need to be noted. First, this study used an animal model, so the study results may not be fully extrapolated to humans. Second, this study only evaluated the short-term effects of NPS and PS on fracture healing. Further research is needed to evaluate the long-term effects of these two techniques.

The findings of this study are in line with several previous studies that also reported the negative effects of periosteal stripping (PS) on fracture healing. Research shows that PS inhibits angiogenesis and osteogenesis, two important processes in fracture healing, in mouse models. Another study also found that PS slowed fracture healing and reduced the mechanical strength of newly formed bone in rats.
However, there are also several studies that report conflicting results. Several studies have shown that PS can increase callus formation and vascularization in the early stages of fracture healing. This difference in results may be due to several factors, such as the degree of periosteum stripping, fracture location, and the type of animal used in the study. Our study provides further evidence that PS can have a negative effect on fracture healing, especially in the later stages of healing. These findings highlight the importance of considering time in assessing PS efficacy. The results of this study have important implications for clinical practice. Periosteal stripping (PS) is a technique commonly used in various orthopedic procedures, such as fracture fixation and bone lengthening. However, the findings of this study suggest that PS may have a negative effect on fracture healing, especially in the later stages of healing.\textsuperscript{19,20}

5. Conclusion

This study provides strong evidence that non-periosteal stripping (NPS) is more effective in improving fracture healing in Sprague Dawley rats compared with periosteal stripping (PS). These findings have important implications for clinical practice and pave the way for further research to optimize fracture healing outcomes in patients.

6. References


