Evaluation of the Effect of Platelet-Rich Fibrin on Long Bone Healing: An Experimental Rat Model

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abstract

Pseudoarthrosis, or nonunion, of the long bones is a challenging medical condition for orthopedic surgeons to treat. Therefore, healing enhancer materials are commonly used. The authors investigated whether platelet-rich fibrin accelerates long bone healing by comparing radiological and histological findings in a rat model of open femoral fracture. Platelet-rich fibrin is a current biomaterial that contains many growth factors and platelets. There are no studies in the literature investigating the effects of platelet-rich fibrin on fracture healing. Sixteen mature male rats were divided into 2 groups. In both groups, an open femoral fracture was created. The platelet-rich fibrin was obtained by centrifuging blood collected from the rats. Rats in the study group were treated with sterile platelet-rich fibrin, and those in the control group were administered saline. The rats were killed at the end of 4 weeks and examined histologically and radiologically. The radiographic and histological scores of the 2 groups differed significantly ($P<.05$). These results indicate that platelet-rich fibrin is an efficient biomaterial in fracture healing and that it increases the amount of osseous tissue formation. Platelet-rich fibrin does not cause an allergic reaction, is cost-effective, and is easy to obtain. Additional studies are necessary to determine whether platelet-rich fibrin accelerates the fracture healing process or induces a better quality of fracture healing. [Orthopedics. 2017; 40(3):e479-e484.]

The rebuilding of lost or damaged tissues remains an important medical issue. The limitations of current strategies for the treatment of fracture have caused difficulties in the bone healing process. Bone is a metabolically dynamic biological tissue that comprises active cells integrated into a rigid framework. Bone healing is a unique regenerative process that includes mesenchymal cell condensation, chondrogenesis, angiogenesis, and bone formation. It begins with an inflammatory reaction followed by formation of a soft and then a hard callus. Interactions among the extracellular matrix, growth factors, and osteoprogenitor cells contribute to bone remodeling. The 3 distinct but overlapping phases of fracture healing are the early inflammatory phase, the repair phase, and the late remodeling phase. During this last phase, the hard callus is transformed to yield cortical and trabecular bone. Fracture healing is also affected by different systemic and local variables that influence restoration of the original physical and mechanical properties of the injured tissue. It requires the interaction of several physiological and biomechanical steps that reflect a wide range of activities at the molecular and cellular levels. Understanding the multiple complex processes underlying fracture healing will ensure an effective therapeutic approach. Bone healing, and specifically the biochemical, biomechanical, cellular, hormonal, and

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The authors have no relevant financial relationships to disclose.

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Received: November 1, 2016; Accepted: January 30, 2017.

doi: 10.3928/01477447-20170308-02
pathological components, can be studied in a fracture healing model.

Several alternative healing methods have been developed during the past few decades. Of these, platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) have been used to accelerate the healing process. Platelet concentrates, prepared from peripheral blood, were introduced in the 1990s; since then, their use in clinical practice has steadily increased. The positive results obtained with PRP and PRF correlate with the fact that platelets are active growth factor–secreting cells that initiate the wound-healing process.10,11 Specifically, connective tissue healing, bone healing, fibroblast mitogenesis, angiogenesis, macrophage activation, and cell proliferation.12 Platelet-rich fibrin is a second-generation platelet-rich biomaterial13,14 that was developed in France by Choukroun et al15,16 in 2001 and introduced by Dohan et al17,18 in 2006. It is rich in leukocytes; cytokines; fibrin; circulating stem cells; the proinflammatory cytokines interleukin-1β, interleukin-6, and tumor necrosis factor-α; the anti-inflammatory cytokines interleukin-4 and interleukin-10; vascular endothelial growth factor; insulin-like growth factor-1 and insulin-like growth factor-II; and platelet-derived growth factor.18 The successful preparation of PRF depends on the speed of blood collection and centrifugation. Slower processes will result in the polymerization of fibrin, leading to a smaller fibrin clot. Unlike PRP, PRF contains the fibrin clot matrix (PRF-M) and does not immediately dissolve but rather takes shape slowly, similar to the process of blood clot formation. The potential of PRF-M in bone and soft tissue regeneration, without induction of an inflammatory reaction, has been demonstrated in several studies.19-22 However, few studies have investigated the effects of PRF on long bone healing. Thus, in this study, the authors analyzed the histopathological and radiological results of PRF application in a rat model of femoral fracture.

**MATERIALS AND METHODS**

**Surgical Procedure**

The experimental protocols were approved by the Dumlupınar University Animal Care Committee and the study was approved by the Ethics Committee of Dumlupınar University. The study was conducted in accordance with National Institutes of Health guidance for the care and use of laboratory animals. The study included 16 mature male Wistar albino rats weighing 325±25 g. The rats were housed in individual cages in a controlled environment at a room temperature of 23°C±2°C and a humidity of 40% to 70% in a 12-hour light–dark cycle. The rats had access to food and water ad libitum. Antibiotic prophylaxis consisting of 40 mg/kg of cefazolin was administered before anesthesia.

The rats were randomly divided into experimental and control groups. Animals in both groups were taken to the operating room following the necessary monitoring and preparation processes. The dose of anesthesia was adjusted for each rat according to body weight, as determined by measurements on an electronic scale. Intraperitoneal anesthesia consisted of 50 mg/kg of ketamine and 10 mg/kg of xylazine. The surgeries were performed by one surgeon (T.C.D.). The left thigh of each rat was prepared with povidone-iodine and the surgical area was covered with a sterile cover. A 2-cm incision was made on the lateral aspect of the femur and an open mid-shaft femoral fracture was created with an oscillatory saw. The femoral canal was cavitated.
with an 18-gauge needle, after which a motor-driven drill was used to place an 0.8-mm K-wire into the medullary canal, close to the upper end of the femur. Then the K-wire was cut and advanced such that it was close to the articular surface. Previously obtained sterile PRF was applied to the bone, the muscle structures were closed with absorbable suture, and the skin incision was closed with a 4-0 nonabsorbable suture. The same procedure was used in the control rats, but saline was administered instead of PRF.

Postoperative pain was managed by the subcutaneous application of buprenorphine hydrochloride. The rats were given water and standard food ad libitum. The study was completed at the end of 28 days, at which time all of the rats were killed. The left femurs were prepared for radiological and histological evaluations. The left limb was removed entirely, without damaging the callus tissue, to avoid altering the results of the experiment.

**PRF-M Preparation**

Blood taken from 4 rats was centrifuged at 3000 rpm for 10 minutes without added anticoagulant. The PRF matrix was removed with a thin, flat-ended tool and separated from the lower layer. The obtained PRF consisted of a yellow fibrin section (Figure 1) mainly constituting the shaft, and a red section located under the yellow fibrin and the buffy coat section, with a large number of platelets trapped between these 2 structures.17

**Radiological Evaluation**

Anteroposterior radiographs were obtained and evaluated by 2 independent orthopedic surgeons. The findings were classified according to the method of Goldberg et al23: 1, no fracture healing; 2, moderate healing; and 3, complete healing (Figure 2).

**Histological Evaluation**

Histological evaluations were performed in the clinical pathology unit of the authors’ hospital. Following the radiological analysis, the entire femur was fixed in 10% formalin, decalcified, and then fixed in 10% acetic acid, 0.85% sodium chloride, and 10% formalin for 72 hours. After extraction of the K-wire, samples from the distal and proximal sections of the fracture line were embedded in paraffin blocks and cut to yield 4- to 5-mm longitudinal sections, which were stained with hematoxylin-eosin, Masson’s trichrome, and CD34 for immunohistochemical examination (Figures 3-4). The slides were examined at different magni-

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**Figure 3**: Histological and immunohistological assessments of the platelet-rich fibrin group: hematoxylin-eosin (a, original magnification ×40; b, original magnification ×100), Masson’s trichrome (c, original magnification ×100; d, original magnification ×40), and CD34 (e, original magnification ×400; f, original magnification ×400) staining.

**Figure 4**: Histological and immunohistological assessments of the control group: hematoxylin-eosin (a, original magnification ×100; b, original magnification ×400), Masson’s trichrome (c, original magnification ×100; d, original magnification ×100), and CD34 (e, original magnification ×40; f, original magnification ×100) staining.
fications by 2 pathologists using a light microscope (Olympus BX51; Olympus, Tokyo, Japan). Both pathologists were blind to the therapy and control groups. The findings were scored using the scale described by Huo et al.24

Statistical Analysis
SPSS version 22.0 software (IBM Corporation, Armonk, New York) was used to evaluate the data. Conformity of the data to a normal distribution was tested with the Shapiro-Wilk test. Data from 2 independent groups were compared using the Mann-Whitney U test and Monte Carlo simulation. The Wilcoxon signed rank test was used with the Monte Carlo simulation for 2 repeated measurements of dependent variables. The Bland and Altman and concordance coefficient methods were used to evaluate the conformity and concordance of and the differences between the evaluations of the orthopedic surgeons. Quantitative data were expressed as the median (maximum–minimum range) and categorical data were expressed as number and percentage. The data were analyzed at a 95% confidence level. P<.05 was considered statistically significant.

RESULTS
Radiological Data
The median Goldberg classification scores determined by the 2 orthopedists were 1.29 and 1.43 in the control group and 2.75 and 2.75 in the PRF group (Table). The difference in radiographic scores between the groups was statistically significant (P<.05) (Figure 5).

Histological Data
The median histological score was 3.50 in the control group and 7.29 in the PRF group (Table). The difference was statistically significant (P<.05) (Figure 5).

DISCUSSION
The authors used an animal model of femoral fracture to demonstrate that PRF promotes long bone healing. Methods to improve bone healing, including following fracture, that accelerate healing as well as fusion have been examined in several studies.25,26 The complex, immune-mediated process of fracture healing involves many local, systemic, and environmental factors, such as the type and size of the wound caused by the trauma, the involvement of vascular tissue, and the amount of bleeding. Angiogenesis is also an important process in fracture healing, as it affects endochondral and intramembranous repair pathways. Athanasopoulos et al27 showed that anti-angiogenesis agents impaired bone healing by causing defective granulation tissue formation, cartilage tissue differentiation, and endochondral ossification. Vascular endothelial growth factor plays a role in bone trauma by stimulating bone mineralization and callus formation. Both PRP and PRF promote bone healing. Gassling et al28 showed that PRF supports the immune system, consistent with the current finding of a lack of infection in the experimental

<table>
<thead>
<tr>
<th>Score</th>
<th>Control (n=7)</th>
<th>Platelet-Rich Fibrin (n=8)</th>
<th>Total (N=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goldberg classification</td>
<td>3.50 (4 to 2)</td>
<td>7.29 (8 to 6)</td>
<td>6 (8 to 2)</td>
<td>&lt;.001</td>
</tr>
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<td>Orthopedic surgeon 1</td>
<td>1.29 (2 to 1)</td>
<td>2.75 (3 to 2)</td>
<td>2.10 (3 to 1)</td>
<td>.002</td>
</tr>
<tr>
<td>Orthopedic surgeon 2</td>
<td>1.43 (2 to 1)</td>
<td>2.75 (3 to 2)</td>
<td>2.18 (3 to 1)</td>
<td>.003</td>
</tr>
<tr>
<td>Diff. (1–2)</td>
<td>-0.14 (0 to -1)</td>
<td>0 (1 to -1)</td>
<td>-0.08 (1 to -1)</td>
<td>.944</td>
</tr>
</tbody>
</table>

*aMann Whitney U test and Monte Carlo simulation.
*bWilcoxon signed rank test and Monte Carlo simulation.

Figure 5: Descriptive statistical analysis of the histological (a) and radiological (b, c) scores of rats in the control and platelet-rich fibrin (PRF) groups.


27. Athanasopoulos AN, Schneider D, Keiper T, et al. Vascular endothelial growth factor

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**References**


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