SCIENTIFIC ARTICLE

The Effects of Autologous Platelet-Rich Fibrin on Flexor Tendon Healing in a Rabbit Model

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Purpose Platelet-rich plasma containing large amounts of growth factors is purported to increase repaired flexor tendon strength. However, the use of bovine thrombin has the risk of antibody formation. We evaluated the effects of the newer generation autologous platelet-rich fibrin (PRF) on flexor tendon healing.

Methods We performed surgical repair of 32 flexor tendons from the index and ring digits of the hind paws of 8 New Zealand white rabbits. In the PRF group, the PRF membrane was either wrapped around or interposed between the repair sites. At 3 weeks after surgery, the tested tendons were subjected to range of motion analysis, cross-sectional area measurement, biomechanics testing, and histological analysis.

Results The results showed no significant increase in range of motion in the PRF group compared with the control group, but there was a significant increase in cross-sectional area of the tendons in the PRF group. The biomechanical testing suggested that the control had a higher load to failure and stress to failure but similar stiffness and modulus to the PRF group.

Conclusions The PRF did not have a major influence on cellular organization. It also had an undesirable effect on the biomechanical properties of repaired flexor tendons.

Clinical relevance The findings of this study suggest PRF may, in certain situations, hinder rather than enhance, the healing for repaired flexor tendons. *(J Hand Surg Am. 2017;■(■):1.e1-e7.*

Key words Flexor tendon healing, platelet-rich fibrin, platelet concentrates, tendon repair.

Online Exclusive

Acute flexor tendon injuries are common.[1] Early mobilization is limited by the strength of repair and the rate of healing. Delayed mobilization increases the risk of progressive adhesion in healing tendons. Currently, the strength of tendon repair is limited by the suture material used and the number of strands that can be safely placed in the tendons. Emerging studies on biological treatment has shown promise in tendon healing. Growth factors can be delivered through bone marrow–derived mesenchymal stem cells or platelet concentrates to modulate angiogenesis and cell proliferation.[2,3]

Since the introduction of platelet-rich plasma (PRP), it has been shown to affect bone and tendon regeneration.[3,4] However, the use of bovine thrombin has the risk of antibody formation and its restriction in certain countries has led to the development of platelet-rich fibrin (RPF) by Dohan et al.[5] The newer-generation

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PRF is completely autologous as well as easier and less costly to obtain.[5] In addition to the simplified preparation, PRF has superior healing properties compared with PRP. The high-density fibrin network provides an optimal matrix for cell migration and allows prolonged release of growth factors.[5] The application of PRF membrane in maxillofacial and periodontal surgery has been studied with promising results.[6] However, the effects of PRF on tendon healing are not known.

This study investigated the effects of autologous PRF on flexor tendon healing in a rabbit model. The PRF membrane was wrapped around or interposed between the primary tendon repair sites. We hypothesized that the application of PRF would expedite tendon healing with increased range of motion (ROM) and improve both strength of repair and cellular organization.

MATERIALS AND METHODS

Animal model

Our Institutional Animal Care and Use Ethics Committee reviewed and approved all experimental procedures and protocols used in this study. We used the flexor digitorum profundus of the index and ring finger hind paws of 8 female New Zealand white rabbits weighing 2.5 to 3 kg. A randomization table assigned the digits to the control or the PRF groups. We assessed tendon healing in 2 parts, both at 3 weeks after surgery. In part I, 16 flexor tendons in total, with 8 in the control and 8 in the PRF groups, were tested for ROM and cross-sectional area before subjecting them to biomechanical testing. In part II, the histological properties were studied in 16 tendons in total with 4 in the control, 8 in which the PRF was wrapped around the repair site, and 4 in which the PRF was interposed into the repair site.

Sample size calculation

Before the study, a sample size estimate was performed. We applied an allocation ratio of 1 and assumed an alpha error of 0.05. A sample size of 8 was calculated to provide 80% power to detect a difference of 8 N in mean load to failure and an SD of 5 N.

Preparation of PRF

We prepared PRF based on the protocol developed by Dohan et al.[5] We collected 10 mL of venous blood from the rabbit's ear without anticoagulant and immediately centrifuged the tubes using a bench-top centrifuge at 2,700 revolutions per minute for 12 minutes at room temperature (Fig. 1). The activation of the platelets triggers a coagulation cascade forming a platelet-containing fibrin clot in between the top layer of acellular plasma and the bottom layer of red blood cells.[5] The red blood cell base was discarded and the remaining PRF clot was placed on the grid of the PRF box (Process Ltd., Nice, France) and covered with the compressor and lid (Fig. 2) for 1 minute to produce a 1-mm-thick membrane. Platelet counts in whole blood and in the blood collection tubes after PRF extractions were done using an automated counter (Hemavet950; Drew Scientific, Miami Lakes, FL). In an *in vitro* study of the mechanical properties of PRF membrane, Young's modulus of elasticity was found to be 0.35 GPa and the hardness was 10.67 MPa.[7] In our study, the PRF membrane held sutures well and tolerated gentle manipulation and stretching. Round-bodied needles were used to minimize trauma to the membrane.

Animal surgery

We anesthetized the rabbits with an intramuscular injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) and supplemental local anesthesia with 2.5 mL 1% lidocaine (Xylocaine). The flexor digitorum profundus in the middle of zone II was sharply divided using a surgical blade. The tendon ends were repaired with a 2-strand modified Kessler technique using Prolene 5-0 sutures (Ethicon, Somerville, NJ). No epitendinous sutures were placed owing to the small size of the tendons. For the experimental digits, we wrapped the PRF membrane around the repair site and tagged with 2 Prolene 6-0 interrupted sutures (Fig. 3). For part II of the study, we interposed PRF membrane between the tendon repair ends in 4 tendons. Similarly, tendons were repaired with a 2-strand repair with no epitendinous sutures (Fig. 4). To unload the repair, we divided the tendons proximally at the common tendon origin. After surgery, we provided *ad lib* feeding and allowed the rabbits to move freely within the cage.

Range of motion analysis

Range of motion testing was done.[8] After the rabbits were sacrificed, the digits were immediately isolated and mounted onto a custom-designed apparatus at the proximal phalanx to allow flexion of the proximal and distal interphalangeal joints (Fig. 5). We attached a 0.98-N tensile load to the proximal end of the repaired tendon and measured the combined angular rotation of the proximal and distal interphalangeal joints in degrees.

Cross-sectional area

A laser micrometer (Keyence Corporation, Osaka, Japan) measured the cross-sectional area of the tendon repair site.[9] Two aligned laser beams scan the edges around the repair site and the cross-sectional area is reconstructed and calculated (Fig. 6).