Evaluation of the use of an autologous platelet-rich fibrin membrane to enhance tendon healing in dogs

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Objective—To examine effects of an autologous platelet-rich fibrin (PRF) membrane for enhancing healing of a defect of the patellar tendon (PT) in dogs.

Animals—8 adult dogs.

Procedures—Defects were created in the central third of the PT in both hind limbs of each dog. An autologous PRF membrane was implanted in 1 defect/dog, and the contralateral defect was left empty. Dogs (n = 4/time period) were euthanized at 4 and 8 weeks after surgery, and tendon healing was assessed grossly and histologically via a semiquantitative scoring system. Cross-sectional area of the PTs was also compared.

Results—Both treated and control defects were filled with repair tissue by 4 weeks. There was no significant difference in the histologic quality of the repair tissue between control and PRF membrane–treated defects at either time point. At both time points, the cross-sectional area of PRF membrane–treated tendons was significantly greater (at least 2.5-fold as great), compared with that of sham-treated tendons. At 4 weeks, the repair tissue consisted of disorganized proliferative fibrovascular tissue originating predominantly from the fat pad. By 8 weeks, the tissue was less cellular and slightly more organized in both groups.

Conclusions and Clinical Relevance—A PRF membrane did not enhance the rate or quality of tendon healing in PT defects. However, it did increase the amount of repair tissue within and surrounding the defect. These results suggested that a PRF membrane may not be indicated for augmenting the repair of acutely injured tendons that are otherwise healthy. (Am J Vet Res 2011;72:699–705)

Tendon injuries are a common cause of morbidity in human and veterinary medicine, and management of tendon injuries poses a considerable challenge to clinicians. The injured tendon is often refractory to treatment, heals slowly, and may remain functionally inferior to an uninjured tendon after healing. Studies have revealed that numerous growth factors, such as platelet-derived growth factor, TGF-β, insulin-like growth factor, basic fibroblast growth factor, and vascular endothelial growth factor, play critical roles in tendon healing, including mitogenesis, chemotaxis, angiogenesis, and matrix synthesis. In addition, increasing growth factor concentrations above those typically found in serum can improve both the rate and quality of tendon healing. Therefore, the use of growth factors as a potential treatment option to enhance healing of connective tissue healing holds great promise. However, questions regarding the most effective dose, choice of growth factor or a combination of growth factors, and delivery method to enhance tendon repair remain unanswered.

One potential method for delivery of growth factors as well as other beneficial bioactive molecules to an injured tendon is through the use of PRP. All of the aforementioned growth factors involved in tendon healing are contained within the α-granules of platelets. Several experimental and preclinical studies have provided promising results following the use of PRP to enhance tendon healing. Platelet-rich plasma has been defined as autologous plasma that contains a platelet concentration above the baseline values found in whole...
blood. It is typically created from citrated whole blood by use of centrifugation to separate the platelets from the other blood cells in plasma and to concentrate the platelets in plasma. In addition to providing a convenient source for high concentrations of autologous growth factors, PRP also maintains the physiologic ratios of growth factors and bioactive molecules that have been found to be ideal for wound healing.

Although platelets and their associated growth factors are important for initiating the healing cascade, perhaps of equal importance is the presence of a provisional fibrin scaffold. Fibrin provides a naturally derived scaffold to which repair cells can adhere, migrate, proliferate, and deposit matrix. Together with other plasma-derived proteins such as fibronectin and vitronectin, fibrin is able to bind to many growth factors contained within platelet α-granules, thus creating a reservoir of growth factors. Therefore, the ability to combine an increased concentration of platelets and their associated growth factors in PRP within a fibrin scaffold may provide an optimal environment for tissue repair and regeneration.

The purpose of the study reported here was to examine the effect of a PRF membrane for use in enhancing and accelerating healing of a defect in the PT of dogs. Our hypotheses were that the PRF membrane would enhance the rate (determined on the basis of the percentage of the defect filled) and histologic quality of the RT (determined on the basis of a semiquantitative evaluation of cellularity, vascularity, collagen organization, and GAG content) in the PT defect, compared with results for sham-treated PT defects.

Materials and Methods

Animals—Eight adult male Beagles were used in the study. Mean ± SD body weight of the dogs was 14.5 ± 1.9 kg. All procedures in the study were approved by the Institutional Animal Care and Use Committee at Michigan State University.

Study design—A defect was created in the central third of the PT in both hind limbs of each of the 8 Beagles. An autologous PRF membrane was implanted into 1 defect, and the defect in the contralateral PT was left empty (sham treatment) as a surgical control PT. Dogs were euthanized via IV administration of an overdose of pentobarbital at 4 and 8 weeks after surgery (4 dogs were euthanized at each time point).

PRF membrane preparation—An autologous PRF membrane was created for each dog, as indicated in the manufacturer’s instructions. Before anesthesia was induced, 18 mL of whole blood was collected from each dog via jugular venipuncture with a 20-gauge butterfly catheter and placed into two 9-mL tubes, each of which contained trisodium citrate and a separator gel. The tubes were centrifuged at 1,100 x g for 6 minutes to create a PRP supernatant. The PRF supernatant from both 9-mL...
tubes was carefully transferred by use of an 18-gauge needle and 20-mL syringe to a 35-mm-diameter flat-bottom glass vial that contained 1.0M calcium chloride. The vial was immediately centrifuged at 4,500 × g for 25 minutes while fibrin polymerization ensued. The result (as a consequence of radial centrifugation) was a dense, flat, circular, fibrin membrane suspended in a liquid serum component. Although it was not possible to measure the actual platelet concentrations in the dense fibrin constructs used in the study, it was reported in another study conducted by our laboratory group that a canine-derived PRF membrane contains significantly more growth factors than does a naturally formed blood clot of similar volume.

Surgical procedures—After premedication with morphine (0.6 mg/kg, IM) and acepromazine maleate (0.02 mg/kg, IM), the dogs were anesthetized by IV administration of thiopental. Anesthesia was maintained with isoflurane (0.3% to 3%) in oxygen. After induction and intratracheal intubation, preservative-free morphine (0.1 mg/kg) was injected into the epidural space to provide postoperative analgesia. Dogs were positioned in dorsal recumbency, and the hind limbs were prepared for aseptic surgery. The PT in each of the hind limbs was isolated and exposed via a medial parapatellar approach (Figure 1). The width of each PT was measured, and the central third of the PT was sharply incised from the distal aspect of the patella to the tibial tuberosity and resected. Care was taken to separate the retropatellar fat pad from the resected portion of the PT.

In 1 PT of each dog, a bed of 4-0 polydioxanone in a horizontal mattress pattern was placed through the remaining PT and under the defect to act as a support for the PRF membrane. The PRF membrane was rolled to form a tube, placed into the defect, and sutured to the remaining PT with nonabsorbable 5-0 nylon in a simple interrupted pattern. The layers of the surgical site were closed in a routine manner. In the contralateral sham-treated PT, the surgical site was closed following resection of the central third of the PT. The limb that was assigned to each treatment group was determined by simple randomization (coin flip). Carprofen (4 mg/kg, SC) was administered once during surgery and every 24 hours thereafter as needed for postoperative analgesia.

Postoperative regimen—After recovery from anesthesia, each dog was housed separately in a 4 × 1.5-m run and allowed unrestricted activity. Tramadol (2 to 4 mg/kg, PO, q 8 to 12 h) was administered for a minimum of 3 days after surgery. No postoperative bandage or method of immobilization was applied. The dogs were observed at least twice daily, and general condition, rectal temperature, pulse rate, respiratory rate, attitude, appetite, amount of activity, and degree of lameness were recorded. The incision sites were checked daily for signs of swelling, erythema, discharge, and dehiscence until they were completely healed.

Gross evaluation—After the dogs were euthanized, both stifle joints and PTs were exposed and grossly evaluated. The patella-PT-proximal portion of the tibia complex was harvested en bloc and placed in neutral-buffered 10% formalin. Following formalin fixation, the tendons were bisected at their midpoint in the transverse plane. Side-by-side digital photographs of the sham- and PRF membrane–treated tendons of each dog were obtained.

Histologic evaluation—After routine histologic processing, the proximal segments of the PTs from 2 dogs from each time period were sectioned in the coronal plane and the distal segments were sectioned in the transverse plane to yield 5-µm-thick slices. For the other 2 dogs from each time period, the proximal segments of the PTs were sectioned in the transverse plane and the distal segments were sectioned in the coronal plane to yield 5-µm-thick slices. Digital photographs of the transverse H&E-stained histologic sections at the midpoint of each PT were obtained, and the cross-sectional area of each PT (excluding the fat pad) was measured by converting the pixels in the digital photographs to area measurements by use of imaging software (Figure 2).

A modified version of a semiquantitative histologic scoring system was used to compare RT (the regenerated healing tissue within the defect in the central third of the PT) and TT (the native PT tissue) in the sham- and PRF membrane–treated PTs. Samples were obtained from 4 sections of each tendon, and both the RT and TT were assigned a score of 0 (normal or most typical) to 3 (most abnormal or atypical) for each of 4 variables (cellularity, vascularity, collagen organization, and GAG content). All sections were stained with H&E, except those evaluated for GAG content, which were stained with alcian blue (pH 2.5) and periodic acid-Schiff. Collagen orga-
nization was evaluated in coronal sections by use of polarized light. Histologic sections were evaluated by one of the authors (LCV), who was not aware of the source of each sample or its treatment group. The sum of the mean histologic scores for each variable was used to obtain a total histologic score for both the RT and TT for each PT.

Statistical analysis—All data were reported as mean ± SD. Mean cross-sectional areas of sham- and PRF membrane–treated PTs within each time point were compared by use of a paired Student t test. Mean cross-sectional areas of sham- and PRF membrane–treated PTs were compared over time by use of an unpaired Student t test. Means from the histologic scoring system were compared with a Wilcoxon signed rank test by use of a statistical software program. Results of a power analysis revealed that 4 dogs/time period would be sufficient to enable us to detect a difference of 33% in the quality of tissue repair at a confidence level of 95% and a statistical power of 0.8. Values of P ≤ 0.05 were considered significant for all comparisons.

Results

Animals—The dogs recovered from anesthesia without complications, and all of the dogs were ambulatory and able to bear weight following recovery from anesthesia. There were no major clinical complications related to the surgical procedures, and lameness was observed only during the first week after surgery.

Gross healing assessment and cross-sectional area—Gross examination of the PTs was performed immediately after the dogs were euthanized. At 4 weeks after surgery, the PT defect in both groups was filled in with RT, which appeared contiguous with the retropatellar fat pad. Hyperemia was evident within the surrounding PT tissue by 4 weeks after surgery but was less obvious by 8 weeks after surgery. At both time points, the PRF membrane–treated tendons had a more abundant healing response than did the sham-treated tendons (Figure 3). The cross-sectional area of the PRF membrane–treated tendons was at least 2.5-fold as great as that of the sham-treated tendons at both time points (Figure 4). At 4 weeks after surgery, the mean ± SD cross-sectional area of the PRF membrane–treated tendons (182 ± 48 mm²) was significantly (P = 0.001) greater than that of the sham-treated tendons (73 ± 47 mm²). Similarly, at 8 weeks after surgery, the mean ± SD cross-sectional area of the PRF membrane–treated tendons (216 ± 51 mm²) was significantly (P = 0.012) greater than that of the sham-treated tendons (62 ± 28 mm²). Within a treatment group, the cross-sectional area of the PRF membrane–treated and sham-treated tendons did not differ significantly (P = 0.383 and P = 0.711, respectively) over time.

Histologic evaluation of healing—The sham- and PRF membrane–treated tendons appeared histologically similar at each time point (Figure 5). After comparing the sham- and PRF membrane–treated tendons by use of the semiquantitative histologic scoring system, there was no significant difference for any of the scoring variables (cellularity, vascularity, collagen organization, or GAG content) or for the total histologic score (sum of the scores for the 4 variables) in the RT or TT at either time point (Table 1). Histologic analysis confirmed the gross observation that by 4 weeks after surgery, the defects in both groups had filled in with a hypercellular fibrovascular RT. The origin of the RT in both groups appeared to be predominantly a proliferative response arising from the retropatellar fat pad and, to a lesser extent, the paratenon (Figure 6). In addition to repair cells, it appeared that the fat pad contributed a substantial amount of the blood supply to the RT within the PT defect of both...
Discussion

Results of the study reported here did not support the hypotheses that a PRF membrane would enhance the quality and rate of healing of a defect in the central third of the PT, compared with quality and rate of healing in sham-treated defects. Although both the sham- and PRF membrane–treated defects healed with a fibrovascular RT of similar histologic quality, the cross-sectional area of the PRF membrane–treated tendons was significantly greater (at least 2.5-fold as great), compared with that of the sham-treated tendons at 4 and 8 weeks after surgery.

The increase in cross-sectional area of the PRF membrane–treated defects may have been associated with prolonged increases in concentrations of growth factors, including TGF-β1. Transforming growth factor-β1 plays a major role in the early phases of wound healing through recruitment of inflammatory cells and fibroblasts and, at later stages, through promotion of collagen production.30 Transforming growth factor-β1 is secreted from platelets as a latent precursor, whereas continuous latent activation is thought to occur up to 14 days after injury.30 Platelets may provide a long-term source of TGF-1 activity as they are thought to activate only a small fraction of the latent TGF-β1 that is released.31 Moreover, numerous studies32-34 have revealed an association between increased fibroplasia and increased concentrations of TGF-β1. Although TGF-β1 concentrations were not quantified in the PRF membrane in the present study, another recent study35 conducted by our laboratory group revealed that a PRF membrane is able to elute significantly higher concentrations of TGF-β1 and enhance proliferation of tendon cells over time, compared with results for a blood clot of similar volume. Therefore, it is possible that the significant increase in fibroplasia (and resultant increase in cross-sectional area) associated with the application of a PRF membrane in the present study could have been the result of increased concentrations (and a prolonged duration) of TGF-β1 available to the repair cells.

In addition to the chemotactic and mitogenic stimuli provided to tendon cells by increased concentrations of various growth factors in PRP,12,14,18,33,35 it has been suggested that the addition of PRP in the initial period following acute tendon injury could actually exacerbate inflammation and pain.10 Investigators in a study37 conducted on the use of PRP to treat acute skin wounds of horses found that use of PRP led to the development of excess (granulation) tissue and actually slowed wound healing. Those authors suggested that the addition of PRP in the initial period following acute tendon injury could actually exacerbate inflammation and pain.10 Investigators in a study37 conducted on the use of PRP to treat acute skin wounds of horses found that use of PRP led to the development of excess (granulation) tissue and actually slowed wound healing. Those authors suggested that the addition of PRP in the initial period following acute tendon injury could actually exacerbate inflammation and pain.

Table 1—Mean ± SD results for a semiquantitative histologic scoring system29* used to assess tendon healing at 4 and 8 weeks after surgery (n = 4 dogs/time point) performed to create a defect in the central third of the PT and sham treatment or treatment with a PRF membrane.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham 4 weeks</th>
<th>PRF 4 weeks</th>
<th>Sham 8 weeks</th>
<th>PRF 8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>2.7 ± 0.1</td>
<td>2.8 ± 0.4</td>
<td>1.6 ± 0.9</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>Vascularity</td>
<td>2.1 ± 0.5</td>
<td>2.6 ± 0.6</td>
<td>1.9 ± 0.7</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>Collagen</td>
<td>2.8 ± 0.3</td>
<td>2.3 ± 0.4</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>GAG</td>
<td>0.8 ± 0.4</td>
<td>1.1 ± 0.5</td>
<td>0.9 ± 0.5</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>Total</td>
<td>8.3 ± 0.5</td>
<td>8.6 ± 0.8</td>
<td>5.2 ± 2.3</td>
<td>6.2 ± 1.5</td>
</tr>
</tbody>
</table>

*Each of the 4 variables was scored on a scale of 0 (normal or most typical) to 3 (most abnormal or atypical); a clinically normal unoperated tendon would have a score of 0. †The RT refers to the regenerated healing tissue within the defect in the central third of the PT. ‡The TT refers to the native PT tissue.

Within a variable, values within RT and TT did not differ significantly (P > 0.05) between the sham- and PRF membrane–treated groups at either time point.

The increase in cross-sectional area of the PRF membrane–treated defects may have been associated with prolonged increases in concentrations of growth factors, including TGF-β1. Transforming growth factor-β1 plays a major role in the early phases of wound healing through recruitment of inflammatory cells and fibroblasts and, at later stages, through promotion of collagen production.30 Transforming growth factor-β1 is secreted from platelets as a latent precursor, whereas continuous latent activation is thought to occur up to 14 days after injury.30 Platelets may provide a long-term source of TGF-1 activity as they are thought to activate only a small fraction of the latent TGF-β1 that is released.31 Moreover, numerous studies32-34 have revealed an association between increased fibroplasia and increased concentrations of TGF-β1. Although TGF-β1 concentrations were not quantified in the PRF membrane in the present study, another recent study35 conducted by our laboratory group revealed that a PRF membrane is able to elute significantly higher concentrations of TGF-β1 and enhance proliferation of tendon cells over time, compared with results for a blood clot of similar volume. Therefore, it is possible that the significant increase in fibroplasia (and resultant increase in cross-sectional area) associated with the application of a PRF membrane in the present study could have been the result of increased concentrations (and a prolonged duration) of TGF-β1 available to the repair cells.

In addition to the chemotactic and mitogenic stimuli provided to tendon cells by increased concentrations of various growth factors in PRP,12,14,18,33,35 it has been suggested that the addition of PRP in the initial period following acute tendon injury could actually exacerbate inflammation and pain.10 Investigators in a study37 conducted on the use of PRP to treat acute skin wounds of horses found that use of PRP led to the development of excess (granulation) tissue and actually slowed wound healing. Those authors suggested that the development of excess (granulation) tissue could be attributable to a prolonged expression of TGF-β1 from PRP via 2 mechanisms. First, although platelets secrete approximately

Figure 6—Photographs of cross sections of representative sham-treated (A) and PRF membrane–treated (B) PTs 4 weeks after surgery. The origin of the RT (asterisk) was primarily a proliferative response arising from the fat pad (dagger) and paratenon (double dagger) in both groups, although it was more abundant in the PRF membrane–treated PTs. The tissue within the dotted lines represents the TT (ie, native PT tissue). H&E stain; bar = 4 mm.
95% of their growth factors within 1 hour after activation, they continuously synthesize small amounts of growth factors for the remainder of their life span (5 to 10 days). Second, it was determined that in contrast to other growth factors, TGF-β1 can regulate its own production by monocytes and activated macrophages in an autocrine manner, which results in a persistent expression at the wound site following a single exogenous application. These results suggest that because of the increased concentrations of growth factors contained in PRP, such preparations may be better suited for more chronic wounds in which a fresh exogenous source of growth factors would be most beneficial.

Results of a study on the spatiotemporal expression of TGF-1 after an acute PT injury (transverse incision of the medial half of the midbody of the PT) may also help explain the abundant RT detected. In that study, investigators found that the expression of TGF-β1 propagates out from the wound site to the nearby uninjured TT with time and as healing progresses. Interestingly, those authors also found that the expression of TGF-β1 is transiently enhanced over the entire length of the PT as early as 7 days after injury. Therefore, the reported spatiotemporal mechanism of TGF-β1 expression, coupled with the prolonged elevation of increased concentrations of TGF-β1 from the PRF membrane, may help explain the abundant healing tissue detected in the present study.

In the study reported here, the healing response in both the sham- and PRF membrane–treated defects appeared to arise predominately from the retropatellar fat pad. An increase in cross-sectional area in conjunction with a substantial amount of RT originating from the fat pad has been reported by numerous investigators who used similar techniques involving defects in the central third of the PT. The retropatellar fat pad is thought to provide important contributions to PT healing because of its close proximity and shared blood supply with the PT. In addition to contributing a blood supply, it is possible that the fat pad also contributes reparative progenitor cells to the PT healing process because the fat pad has been found to be a notable source of highly proliferative adipose-derived mesenchymal stem cells that are potentially able to respond to tendon injury.

Investigators in another study used a similar technique to create PT defects; they reported healing of the defects at 3 months after surgery. However, contrary to our hypotheses, the PRF membrane did not accelerate the healing or enhance the quality of the RT in a defect in the central third of the PT in dogs at the earlier time periods examined in the present study. This may have been a result of the fact that the PT defect in the present study was not of a critical size because the sham-treated defect was filled with a similar quality RT at the earliest time point examined in this study. Analysis of the results of the present study suggested that because the retropatellar fat pad appeared to provide an adequate supply of repair cells, vasculature, and growth factor stimulus in acute PT defects, the PRF membrane was not a major adjunct to the healing process in these situations. It is possible that the PRF membrane may be of more benefit in biologically compromised or chronically injured tissues in which a fresh source of bioactive molecules may be needed for longer periods to enhance the repair process. A similar conclusion was reached in a study conducted to examine the use of PRP in the healing of acute cutaneous wounds. In that study, the histologic quality of the control and PRP-treated defects also did not differ significantly.

The outcome metrics in the present study were limited to the semiquantitative histologic assessment of the RT by use of a grading scale that has been reported elsewhere. Although only 4 dogs in each group were evaluated at each time period, an a priori power analysis revealed that this number would be sufficient to detect a 33% difference in RT quality, which was believed to be clinically relevant. The lack of any functional assessment, such as biomechanical analysis of the healed tendons, could also be considered a limitation of this study. Because the dogs did not have any obvious adverse clinical signs (ie, abnormal activity or gait) related to the surgical procedure, a clinically relevant impact on joint function was not apparent. Finally, the 4- and 8-week time points used in the present study did not permit assessment of the long-term impact of a PRF membrane on remodeling of the RT.

We concluded that a PRF membrane did not enhance the rate and quality of healing for defects in the central third of the PT of dogs at 4 or 8 weeks after surgery. Although the PRF membrane induced an increased amount of fibroblastic RT, as determined by a grading scale that has been reported elsewhere, the impact on joint function was not apparent. Finally, the 4- and 8-week time points used in the present study did not permit assessment of the long-term impact of a PRF membrane on remodeling of the RT.

References

7. Haupt JL, Donnelly BP, Nixon AJ. Effects of platelet-derived growth factor-BB on the metabolic function and morphologi-