Research Article

Effect of platelet-rich plasma and platelet-rich fibrin matrix on healing of vertical meniscal tears in a rabbit model

Recep Kurnaz¹, Orhan Balta²

¹Department of Orthopaedics and Traumatology, Eskişehir Acıbadem Hospital, Eskişehir, Turkey
²Department of Orthopaedics and Traumatology, Gaziosmanpaşa University, School of Medicine, Tokat, Turkey

ARTICLE INFO

Article history:
Submitted 23 December 2017
Received in revised form 23 May 2018
Last revision received 11 June 2019
Accepted 11 November 2019

Keywords:
Platelet rich fibrin matrix
Meniscal healing
Experimental study
Meniscal tear
Platelet rich plasma

ORCID IDs of the authors:
R.K. 0000-0002-8311-047X;
O.B. 0000-0002-4398-827X.

Abstract

Objective: This study aimed to investigate the effect of platelet-rich plasma (PRP) and platelet-rich fibrin matrix (PRFM) on the healing of vertical medial meniscal tears in a rabbit model.

Methods: The study was conducted on 72 New Zealand mature rabbits aged more than 6 months. Rabbits were randomly assigned to six groups: control (C) group, meniscal repair (MR) group, PRP group, PRFM group, MR+PRP group, and MR+PRFM group, with 12 rabbits in each group. A 5-mm full-thickness vertical tear was created in the avascular zone of the medial meniscus corpus in the right knee of all rabbits. The respective treatment for each group was given to the meniscal tear in each rabbit. Histological evaluation of healing was performed 6 and 12 weeks after surgery.

Results: Defect filling and collagen formation remained low in the C group. However, in all other groups, there was no significant difference in the 6th week. In the 12th week, similar results as those obtained in the 6th week were obtained. In the C group, there was a difference in defect filling and cell type. This difference was that the defect filling and collagen formation remained low in the 12th week. No significant difference was observed between MR, MR + PRP and MR + PRFM groups. The MR group significantly differed from the other groups in the defect-filling rate and cell type; however, the use of PRP and PRFM did not provide an obvious benefit.

Conclusion: The contradictory results obtained in previous studies emphasize the need for further research on the use of PRP in meniscal recovery and repair. We believe that if surgery is indicated, repair is absolutely necessary to improve the healing of the tissue in meniscal tears. Studies using human meniscal tissue for meniscal injury and those that evaluate clinical applications of PRP are warranted.

The most important factor affecting the success of repair is the location of the tear. The success rate of repairs in the red-red region is >95% (1). However, as the location of the tear moves away from the vascular region, the success rate reduces to 70% (2, 3). Many repair techniques and suture configurations have been developed to increase the success rate according to the shape and location of the tear (4). In the white-white region, the chance of healing is low if there are no methods to improve the healing after the repair. In such cases, improvement methods should be used. Platelet-rich plasma (PRP) is a suitable and effective alternative when growth factors need to be applied in the clinical setting. Many autologous blood products are increasingly being used to improve tissue healing in clinical practice. Nowadays, our knowledge about the growth factors that play a critical role in tissue healing is increasing. Thus, high platelet concentrate products can provide more effective benefits for tissue healing (5). PRP is a natural product that includes growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), basic fibroblast growth factor (FGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF) and endothelial cell growth factor (ECGF) (6, 7). These have demonstrated positive effects on cell proliferation, cell migration, angiogenesis, and extracellular matrix production in numerous cell types in both in vivo and in vitro models (6-10). There is no risk of blood-borne diseases because they are used as autologous products. PRP is a widely used and proven method in bone and soft tissue healing (5). For the wide clinical application of tissue repair and regeneration technologies, they should not only be scientifically effective but also be easily applicable and cost-effective. PRP has many advantages over animal-derived products and recombinant growth factors (11). There is no risk of carcinogenesis and
immunological reaction. Finally, it is much cheaper than other recombinant factors.

Few studies have investigated the efficacy of PRP on meniscal healing. Various PRP-containing growth factors may increase bone and soft tissue healing (12-14). Although there is insufficient evidence for this, it is commonly used today (15).

There are four families of PRP products based on the fibrin structure and leukocyte content: pure PRP, leukocyte and PRP (L-PRP), pure platelet-rich fibrin (PRF), and leukocyte and PRF (L-PRF) (16-18). Among these four families, L-PRF slowly releases growth factors for 7 days (17, 19). This suggests that the combination of leukocytes and fibrin matrix significantly influences the capture and release of growth factors. In addition, they help growth factors act as a combination of platelets and leukocytes in a complex fibrin matrix (20-23).

Recently, L-PRF has shown promising experimental and clinical results in trauma cases in fields such as sports medicine and orthopedics (21, 24, 25). Nevertheless, L-PRF cannot be used as an injectable product because of its viscosity. PRP has a more fluid viscosity than L-PRF; therefore, PRP can penetrate into the meniscus better. Based on the positive effects of PRP on cell proliferation, collagen synthesis, and vascularization, PRP has been used as a bioaccumulant for the regeneration of meniscal tissue (17, 26). Injection of PRP into the area with the intrasubstance tear can help the torn meniscus to heal and prevent the tear from progressing into uneven tears that require meniscus resection. However, there are limited reports on the effect of PRP on the healing of the torn meniscus.

Therefore, this study aimed to investigate the effect of PRP and platelet-rich fibrin matrix (PRFM) on the healing of vertical meniscal tears in a rabbit model. The hypothesis of this research was that PRP and PRFM would accelerate meniscal healing.

Materials and Methods

Experimental animals

This study was performed on 72 New Zealand mature rabbits aged >6 months. The rabbits were fed the same feed 1 week before the experiments and kept under the same conditions. The study was approved by the Tokat Gaziosmanpaşa University Animal Experiments Local Ethics Committee. Rabbits were randomly assigned to the following six groups: control (C) group, meniscal repair (MR) group, PRP group, PRFM group, MR+PRP group, and MR+PRFM group, with 12 rabbits in each group. The right knee joint of each rabbit was operated, and the contralateral knee was normal. The rabbit model has been used in previous studies (27, 28).

Surgical procedure

Ketamine (Alfamine, Alfasan International B.V) 10 mg/kg and xylazine (Alfazyne, 2%, Ege Vet) 8 mg/kg for anesthesia of rabbits as well as cefazolin Na (Eqizolin, İ.E. ULAGAY) 20 mg/kg for surgical antibiotic prophylaxis were administered intramuscularly.

In the control group, the right lower extremity of each rabbit was shaved following sterile staining and covering, and the skin was incised in a longitudinal anterior midline fashion to the knee joint. After medial parapatellar arthrotomy, the patella was dislocated laterally, and the knee was exposed. A 5-mm full-thickness vertical tear was created with a number 11 scalpel in the avascular zone of the medial meniscus corpus in the right knee in all rabbits. The patella was then reduced, and the incision was closed with 4/0 vicryl sutures; the skin was closed with continuous sutures using 3/0 silk. The rabbits in the control group were left without any additional intervention, and the tear was left as it was.

In the MR group, the knee joint was incised using the same surgical method. In all the rabbits, a 5-mm full-thickness vertical tear was created in the avascular zone of the medial meniscus corpus in the right knee, and the tear was repaired using vertical single suture using 4/0 PDS. The patella was then reduced, and the incision was closed with 4/0 vicryl; the skin was closed with continuous sutures using 3/0 silk (Figure 1).

In the PRP group, after the tear was formed, the patella was reduced, and the incision was closed with 4/0 Vicryl; the skin was closed with continuous sutures using 3/0 silk. Along with the PRP injection, 0.2 cc of calcium gluconate was added for platelet activation.

In the MR+PRP group, the tear was repaired with a vertical single suture using 4/0 PDS. The patella was then reduced, and the incision was closed with 4/0 vicryl; the skin was closed with continuous sutures using 3/0 silk. At the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> postoperative weeks, PRP (1 cc) obtained from each rabbit was injected into the joints. Along with the PRP injection, 0.2 cc of calcium gluconate was added for platelet activation.

In the MR+PRFM group, the defect was repaired with a vertical single suture using 4/0 PDS, and PRFM (2 cc) obtained from each rabbit was injected into their tear area in the same session.

MA I N  P O I N T S

- PRP and PRFM applications do not accelerate the healing in meniscal tears.
- It is not right to expect benefits from PRP and PRFM applications by not repairing the meniscus tear. In this study, rabbits in which meniscus tears are repaired are among the groups with the highest recovery results.
- However, no significant difference was observed in terms of healing with the application of PRP and PRFM among the meniscus repair groups.

In the PRFM group, after the tear was formed in the meniscus, PRFM (2 cc) obtained from each rabbit was in injected to their defect area in the same session.
Previous studies have reported that long-term inactivity adversely affects meniscal healing (29-31). Thus, no restriction was applied to the rabbits after surgery. For analgesia, acetaminophen was added to the drinking water of all rabbits at 1-2 mg/kg per 100 ml, and standard rabbit feed was given. In addition, cefazolin sodium 20 mg/kg was administered intramuscularly for 5 days.

**Preparation of PRP**

Blood samples were obtained from each rabbit. Using a ready-made PRP kit (Double Syringe Autologous Carrier Plasma [ACP] System, Arthrex, Inc.), 10 mL of blood was taken from the ears of rabbits under anesthesia. Each tube was centrifuged at 1500 rpm for 5 min using the single centrifuge method. After centrifugation, the remaining plasma portion was separated and a total of 4 cc of separated plasma was obtained (PRP). Platelet counting was performed in 1 cc PZP material. The remaining 3 cc of PRP was stored at −80 °C in 1-cc amounts (Figure 2).

**Preparation of PRFM**

Using a ready-made PRP kit (Double Syringe ACP System, Arthrex, Inc.), 10 mL of blood was taken from the rabbits under anesthesia. Each tube was centrifuged at 3000 rpm for 10 min with their own centrifuge instrument using the single centrifuge method. The PRFM gel was applied to the defect region using 3
mL of fibrin glue (Beriplast P® Combi-Set containing 3 mL of thrombin solution and 3 mL of fibrinogen solution) and 2 mL of calcium gluconate (Figure 3).

Sacrification of rabbits

Half of the subjects in each group were sacrificed at the 6th week after the surgery and the remaining subjects were sacrificed at the 12th week with high-dose anesthetic (pentothal sodium, Abbott). Histopathological examination was performed with disarticulation such that the tibial connections of the meniscus were not disturbed.

Histopathological examination

For histopathological examination, the right knee meniscus of each rabbit was separated and first kept in 10% neutral formalin at least for 2 days. After the tissues were washed, they were embedded in paraffin; thereafter, 4-μm sections were collected using a microtome. Finally, the sections were stained with hematoxylin and eosin. Stained sections were evaluated using a light microscope (original magnification X4, X10). In the histopathological evaluation, a semi-quantitative assessment scheme summarized in Table 1 was used by single pathologist (17, 26, 32).

Statistical analysis

Descriptive analyses were performed to obtain information about the general characteristics of the experimental groups.

Statistical analysis

Descriptive analyses were performed to obtain information about the general characteristics of the experimental groups.

When p-values were <0.05, they were considered statistically significant. One-way analysis of variance was used to compare the defect-filling rate, cell type, cellularity, collagen organization, and vascularity mean between the groups. Ready-to-use the Statistical Package for Social Sciences version 19.0 software was used in calculations (IBM Corp.; Armonk, NY, USA).

Results

In the 6th week, the defect-filling rate in the control group was <25%, no cell. There was no collagen organization, and there was fibrocartilaginous dissociation. In the MR group, the defect-filling rate was 25%–75%, and the cell type had 25%–75% chondrocyte dominance. There was hyaline or mucinous degeneration in the collagen fibers. In the PRP group, the defect-filling rate was <25%, no cell. There was no collagen organization, and there was fibrocartilaginous dissociation. In the PRFM group, the cell/extracellular matrix ratio was >0.5. The defect-filling rate was 25%–75%, and the cell type had 25%–75% chondrocyte dominance. In the MR+PRP group, the collagen fibers were organized, and hyaline or mucinous degeneration was present. The defect-filling rate was 25%–75%, with more than 75% chondrocyte dominance as
the cell type. In the MR+PRFM group, there was hyaline or mucinous degeneration with the collagen fibers organized. The defect-filling rate was 25%–75%, with >75% chondrocyte dominance as the cell type.

In the 12th week, the defect-filling rate was >75% in the C group, with 25%–75% chondrocyte dominance. There was hyaline or mucinous degeneration in the collagen fibers. In the MR group, the defect-filling rate was 75%–100%, and the

Figure 4. a-ı. (a) Group 1 (control group) example of meniscus sampled at week 6. Young connective tissue is observed in the middle and edge areas of the meniscus defect area (mag. X4), (b) Control group young connective tissue formed in the edge areas of meniscal defect and fibroblastic character of the cellular population (mag. X10), (c) Young connective tissue formed at the edge of the defect area of the control group at week 12 (mag. X10), (d) The shrinkage in the defect area and chondrocyte cellular proliferation as well as the focus of inflammation characterized by vascularization in the middle section at the 6th week in the meniscus repair only group (mag. X10), (e) Group 3 defect filling area and young connective tissue formation in the meniscus sampled at the end of week 12 (mag. X4), (f) A closer view of the area in Figure 4e. The presence of chondrocyte cells, as well as fibroblastic cells, in the young connective tissue is observed (mag. X10), (g) Group 4 appearance of the meniscus defect at the end of 12th week: defect filling rate and cellularity (mag. X4), (h) Group 5 defect area at the end of 12th week: The defect is almost completely closed and there is a collagen organization but mild inflammation and vascularization process is ongoing (mag. X10), (i) Group 6 meniscus defect area and suturation area at the end of 12th week. Defect filling is complete, cellularity is high, chondrocyte cell dominance is present but the response to sutures is evident (mag. X10)
cell type had a chondrocyte dominance of 75%–100%. The collagen fibers had an organized, homogenous eosinophilic matrix structure.

The defect-filling rate in the PRP group was 0%-75%. There was hyaline or mucinous degeneration in the collagen fibers.

The cell/extracellular matrix ratio in the PRFM group was >0.5. The defect-filling rate was 25%–100%, and the cell type had 25%–25% chondrocyte dominance. In the MR+PP group, the defect-filling rate was 75%–100%. The collagen fibers had an organized, homogenous eosinophilic matrix structure. In the MR+PRFM group, the defect-filling rate was between 75%–100%, and the cell type had a chondrocyte dominance of 75%–100%. The collagen fibers had an organized, homogenous eosinophilic matrix structure.

Histologically, mixed-type cellular proliferation was observed in most cases. Generally, in the 6th-week biopsies, besides the occasional appearance of inflammatory cells, the dominant cell type was fibroblastic, whereas in the 12th week, higher amounts of chondrocyte cells were observed. It was also observed that the process of inflammation and vascularization continued in the 12th week in the meniscal repair groups (Figure 4). This condition may be associated with increased inflammation against the suture material used.

| Table 4. Defect-filling distribution according to groups at week 12* |
|-----------------|--------------------------|-----------------|-----------------|
| (I) Group 1     | (J) Group 1              | Mean Difference | Std. Error      | p         |
| Control         | MR                       | -1.83           | .35746          | **.021    |
|                 | PRP                      | -1.00           | .57735          | .726      |
|                 | PRFM                     | -1.50           | .28868          | .082      |
|                 | MR+PRP                   | -1.25           | .38188          | .150      |
|                 | MR+PRFM                  | -1.50           | .28868          | .082      |
| MR              | Control                  | 1.83            | .35746          | .021      |
|                 | PRP                      | .83             | .54263          | .794      |
|                 | PRFM                     | .33             | .21082          | .788      |
|                 | MR+PRP                   | .58             | .32702          | .685      |
|                 | MR+PRFM                  | .33             | .21082          | .788      |
| PRP             | Control                  | 1.00            | .57735          | .726      |
|                 | MR                       | -.83            | .54263          | .794      |
|                 | PRFM                     | -.50            | .50000          | .938      |
|                 | MR+PRP                   | -.25            | .55902          | 1.000     |
|                 | MR+PRFM                  | -.50            | .50000          | .938      |
| PRFM            | Control                  | 1.50            | .28868          | .082      |
|                 | MR                       | -.33            | .21082          | .788      |
|                 | PRP                      | .50             | .50000          | .938      |
|                 | MR+PRP                   | .25             | .25000          | .969      |
|                 | MR+PRFM                  | .00             | .00000          | .        |
| MR+PRP          | Control                  | 1.25            | .38188          | .150      |
|                 | MR                       | -.58            | .32702          | .685      |
|                 | PRP                      | .25             | .55902          | 1.000     |
|                 | PRFM                     | -.25            | .25000          | .969      |
|                 | MR+PRFM                  | -.25            | .25000          | .969      |
| MR+PRFM         | Control                  | 1.50            | .28868          | .082      |
|                 | MR                       | -.33            | .21082          | .788      |
|                 | PRP                      | .50             | .50000          | .938      |
|                 | PRFM                     | .00             | .00000          | .        |
|                 | MR+PRP                   | .25             | .25000          | .969      |

*One-way analysis of variance was used for multiple comparisons
Defect filling and collagen formation remained low in the control group. However, among all other groups, there was no significant difference in the 6th week evaluations (Table 2, 3). In the 12th week, similar results as those obtained in the 6th week were observed; in the C group, there was a difference in defect filling and cell type (Table 4, 5). Defect filling and collagen formation remained low (Table 6). Meanwhile, no statistically significant difference was observed between MR, MR + PRP and MR + PRFM groups.

Microscopic analysis showed that the improvement in the C group was significantly lower than that in the other groups. Al-

### Table 5. Distribution of cell type according to groups at week 12*

| Dependent Variable | (I) Group 1 | (J) Group 1 | Mean Difference (I-J) | Std. Error | p    |
|--------------------|-------------|-------------|-----------------------|------------|------|
| Cell type          |             |             |                       |            |      |
| Control            | MR          | −1.33       | .21082                | .022       |      |
|                    | PRP         | −.50        | .50000                | 1.000      |      |
|                    | PRFM        | −1.80       | .20000                | .013       |      |
|                    | MR+PRP      | −2.25       | .25000                | .043       |      |
|                    | MR+PRFM     | −2.33       | .33333                | .259       |      |
| MR                 | Control     | 1.33        | .21082                | .022       |      |
|                    | PRP         | .83         | .54263                | .997       |      |
|                    | PRFM        | −.46        | .29059                | .901       |      |
|                    | MR+PRP      | −.91        | .32702                | .341       |      |
|                    | MR+PRFM     | −1.00       | .39441                | .661       |      |
| PRP                | Control     | .50         | .50000                | 1.000      |      |
|                    | MR          | −.83        | .54263                | .997       |      |
|                    | PRFM        | −1.30       | .53852                | .962       |      |
|                    | MR+PRP      | −1.75       | .55902                | .861       |      |
|                    | MR+PRFM     | −1.83       | .60093                | .790       |      |
| PRFM               | Control     | 1.80        | .20000                | .013       |      |
|                    | MR          | .46         | .29059                | .901       |      |
|                    | PRP         | 1.30        | .53852                | .962       |      |
|                    | PRFM        | −1.53       | .38873                | .987       |      |
|                    | MR+PRP      | 2.25        | .25000                | .043       |      |
|                    | MR          | .91         | .32702                | .341       |      |
|                    | PRP         | 1.75        | .55902                | .861       |      |
|                    | PRFM        | .45         | .32016                | .970       |      |
|                    | MR+PRFM     | −.08        | .41667                | 1.000      |      |
| MR+PRP             | Control     | 2.33        | .33333                | .259       |      |
|                    | MR          | 1.00        | .39441                | .661       |      |
|                    | PRP         | 1.83        | .60093                | .790       |      |
|                    | PRFM        | .53         | .38873                | .987       |      |
|                    | MR+PRP      | .08         | .41667                | 1.000      |      |

*One-way analysis of variance was used for multiple comparisons*

### Table 6. Distribution of the organization of collagen according to groups at week 12*

| Collagen        | Group | n  | Mean±SD | p    |
|-----------------|-------|----|---------|------|
|                 | Control | 4  | 1.75±0.5| 0.001|
|                 | MR     | 5  | 2.2±0.45|       |
|                 | PRP    | 4  | 0.5±1   |       |
|                 | PRFM   | 6  | 2.33±0.52|     |
|                 | MR+PRP | 4  | 2±0     |       |
|                 | MR+PRFM| 4  | 2±0     |       |

*One-way analysis of variance was used*
though the defect-filling rate was <25% in this group, the col-
lagen bundles were not formed in the subjects yet. In the other
groups, similar morphological features were observed in the me-
iscal repair groups in terms of defect filling, collagen organiza-
tion, and cellularity; however, they had higher scores than the non-
repaired groups. There was no significant difference among the MR, MR+PRP, and MR+PRFM groups. In this study, the MR group
was significantly different from the other groups in terms of defect-filling rate and cell type. However, the use of PRP and
PRFM did not provide an obvious benefit.

Discussion

As stated above, this study aimed to investigate the effect of PRP
and PRFM on the healing of vertical meniscal tears in a rabbit
model. The hypothesis of the research was that PRP and PRFM
would increase meniscal tear healing. However, no statistically
significant difference was observed among the groups.

Meniscal repair is considered for traumatic tears that occur close
to the peripheral and vascular area of the meniscus in young pa-
tients. The vascular supply is provided by the capillaries from
the perimeniscal capillary plexus and exudes up to 30% of the
width of the meniscus in the “red-red” region (14). Two-thirds
of the internal meniscus, “red-white,” and “white-white” regions
contain little or no nerves and blood vessels (33-35).

The meniscus has the ability to heal at the external 1/3 site be-
cause of a fibrin clot rich in inflammatory cells at the site of in-
jury. Fibrous repair tissue grows on the damaged area because of
undifferentiated mesenchymal cells (18). Owing to this mech-
anism, biological therapies aim to improve the ability of tissue
healing and repair by increasing the vascularity of the meniscus.
Various anabolic growth factors have been identified in meniscal
tissue repair and regeneration.

Interleukin-1 and epidermal growth factors increase meniscal
cell migration, and bone morphogenetic protein-2 and insul-
lin-like growth factor-I stimulate the migration of fibrochondro-
cytes from the periphery to the avascular region (10).

PRP is the promising autologous source of these and other
growth factors. However, few studies have assessed the use of
PRP in the treatment of meniscal injury or in the strengthening
of meniscal repair.

Iscida et al. investigated the use of PRP prepared using the dou-
ble-spin technique for the regeneration of meniscal tissue in
both in vitro and in vivo studies (11). In vitro cells were isolated
from rabbit tissue collected from the internal two-thirds of the
meniscus and treated with PRP or platelet-poor plasma (PPP)
at concentrations of 3%, 10%, and 30% for 48 h. Treatment with
PRP compared with that with PPP and control showed that PRP
not only increased the proliferation of the meniscal cells but also
increased in vitro glycosaminoglycan synthesis. Gene expression
analysis showed that although there was no difference in type-I
collagen, there was upregulation of biglycan and decorin with
large aggregate expression compared with that in the cultures
treated with PPP. In an in vivo study, 30 mL of PRP, PPP, or phos-
phate-buffered saline was added to freeze-dried gelatin hydrogels
and implanted in a 1.5-mm defect in the meniscus avascular re-
region in adult female rabbits.

In order to increase the effect of growth factors in meniscal heal-
ing, it is important that growth factors are exposed to the target
area for a long period (18). However, the half-life of growth fac-
tors is too short to maintain in vivo biological activity. Therefore,
it is important to deal with the short half-life of growth factors
when they are locally applied to the in vivo meniscal tear site.

Tabata et al. proposed a controlled release system, a method for
limiting the short half-life disadvantage by using biodegradable
acidic gelatin hydrogel (36). In this system, growth factors are
immobilized in gelatin hydrogels via physicochemical interac-
tion with gelatin molecules; and immobilized growth factors are
released from the hydrogel by hydrogel disintegration. Growth
factors included in the biodegradable acidic gelatin hydrogel are
slowly released and protected against desorption; therefore, the
target tissue can be exposed to growth factors continuously for
2 weeks (20, 22).

After 4 weeks of study, Ishida et al. concluded that the hydrogel
kept releasing growth factors in PRP for at least 4 weeks (11).
Animals were euthanized at the 4th, 8th, and 12th postoperative
weeks, and tissue sections were stained with hematoxylin–eosin
and safranin-O to assess tissue repair using a semi-quantitative
scoring system. At the 12th week, significantly higher scores were
observed with regard to the number of fibrochondrocytes and
extracellular matrix production in the PRP-treated tissue defects.

The sum of these in vitro and results led the authors to conclude
that PRP increases the healing properties of internal avascular
meniscus (11). In the present study, in addition to the inflam-
matory cells at the 6th week biopsies, the dominant cell type was
fibroblastic, while in the week-12, chondrocyte cells were ob-
served.

Some studies have reported that long-term inactivity adversely
affects meniscal healing. Considering these studies, no restric-
tion was applied to the rabbits after surgery (25, 26, 29).

Narita et al. also used biodegradable gelatin hydrogel for PRP
presentation on horizontal meniscal tears (26). They found that
FGF-2 with GH significantly stimulated the proliferation of me-
iscal cells and inhibited meniscal cells by up to 4 weeks before
they died, thereby increasing meniscal cell density and enhanc-
ing meniscal repair in the rabbit model. However, neither study
(11, 26) directly measured the amount of growth factors released
from platelets in vivo; therefore, it is unclear how long the bio-
logical activity of the growth factors lasted. The present results
showed no difference between the control group and the PRP group. This may be due to the premature burst release in meniscus tears and desorption of growth factors. In this study, the amount of growth factors released from the platelets was not directly measured; therefore, it is unclear whether the growth factors are appropriately released and activated. In addition, we are unaware as to how long the growth factors remain biologically active. There is limited information regarding the in vivo release properties of growth factors because previous studies also did not directly measure the amount of growth factors in the in vivo meniscal tear model (11, 26). However, recent studies have shown that the composition of L-PRP adversely affects the mechanical properties of the fibrin skeletons and that the increased cell reduces a more preventative inflammatory environment and ultimately a cell proliferation response directly related to an inflammatory condition. This is harmful to tissue regeneration (37-39).

When Arnoczky and Warren created vascular access channels connecting the peripheral vascular region to the internal avascular region, the injury site in the avascular region could be exposed to vascular origin growth factors; they stated that it could promote meniscal regeneration (40). In horizontal tear patterns in both the C and PRP groups, there is a possibility that the vascular access channels links the peripheral vascular area with the inner avascular area of the meniscus. Hence, not only the PRP group but also the C group may be exposed to a certain growth factor level. Fibrochondrocytes are considered to migrate toward chemotactic growth factors released from both peripheral blood vessels and PRP.

Another study by Zellner et al. evaluated the use of mesenchymal stem cells (MSC) for meniscal tissue engineering (41). MSCs were implanted on an L-PRC-loaded hyaluronan–collagen composite matrix and implanted in a 2-mm defect region of the meniscus avascular region in adult male rabbits. The animals were euthanized at 6 and 12 weeks; macroscopic, histological, and immunohistochemical evaluation of the tissue was conducted. MSCs and PRP-treated defects did not show any improvement at 6 or 12 weeks compared with a cell-free implant framework (41).

In the microscopic analysis in the current study, the improvement in the C group was significantly lower than that in the other groups. Although the defect-filling rate was >25% in this group, the collagen bundles were not yet formed in the subjects. In particular, in groups with meniscal repair, defect filling, collagen organization, and cellularity have similar morphological features but higher scores than non-repaired groups. The authors presumed that the difference in our findings and those reported by Ishida et al. may be related to the larger defect size.

Shin et al. evaluated the effect of PRP on horizontal meniscal tears in an experimental rabbit model and concluded that, unlike their hypotheses, PRP did not contribute positively to the healing process (42). However, they applied PRP as a single injection in their study. In the study, three PRP injections were administered at 1-week intervals.

In a study by Griffin et al., 15 of the 35 patients who underwent meniscal repair were treated with PRP, and the remaining 20 were treated with the repair without PRP. There was no significant difference in the clinical and functional scores between the two groups (13).

The contradictory results in these studies emphasize the need for further research on the use of PRP in meniscal recovery and repair. We believe that if surgery is indicated, repair is absolutely necessary to improve the healing of the tissue in meniscal tears. Studies that use human meniscal tissue for meniscal injury and those that evaluate the clinical applications of PRP are also warranted.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Local Ethics Committee of Tokat Gaziosmanpasa University Animal Experiments (2011 HAYDEK-024).

**Author Contributions:** Concept - R.K.; Design - R.K., Supervision - R.K., O.B.; Resources - R.K., O.B.; Materials - R.K., O.B.; Data Collection and/or Processing - R.K., O.B.; Analysis and/or Interpretation - R.K., O.B.; Literature Search - R.K.; Writing Manuscript - R.K.; Critical Review - R.K., O.B.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

**References**

1. Brown GC, Rosenberg TD, Deffner KT. Inside-out meniscal repair using zone-specific instruments. Am J Knee Surg 1996; 9: 144-50.
2. Rubman MH, Noyes FR, Barber-Westin SD. Arthroscopic repair of meniscal tears that extend into the avascular zone. A review of 198 single and complex tears. Am J Sports Med 1998; 26: 87-95. [CrossRef]
3. Cannon WD Jr. Arthroscopic meniscal repair. Inside-out technique and results. Am J Knee Surg 1996; 9: 137-43.
4. Ascì M, Balta O, Kurnaz R, Eren MB, Kuyucu YE, Gunes T. “Horizontal butterfly” technique in repair of radial meniscus tears: A biomechanical study. Acta Orthop Traumatol Turc 2018; 52: 392-6. [CrossRef]
5. Sampson S, Gerhardt M, Mandelbaum B. Platelet injection grafts for musculoskeletal injuries: a review. Curr Rev Musculoskelet Med 2008; 1: 165-74. [CrossRef]
6. Ionescu LC, Lee GC, Huang KL, Mauck RL. Growth factor supplementation improves native and engineered meniscus repair in vitro. Acta Biomater 2012; 8: 3687-94. [CrossRef]
7. Hutchinson ID, Moran CJ, Potter HG, Warren RF, Rodeo SA. Restoration of the meniscus: form and function. Am J Sports Med 2014; 42: 987-98. [CrossRef]
8. Makris EA, Hadidi P, Athanasiou KA. The knee meniscus: structure-function, pathophysiology, current repair techniques, and prospects for regeneration. Biomaterials 2011; 32: 7411-31. [CrossRef]
9. McNulty AL, Guilak F. Integrative repair of the meniscus: lessons from in vitro studies. Biorheology 2008; 45: 487-500. [CrossRef]
10. Bhargava MM, Attia ET, Murrell GA, Dolan MM, Warren RF, Hanafin JA. The effect of cytokines on the proliferation and migration of bovine meniscal cells. Am J Sports Med. 1999; 27: 636-43. [CrossRef]

11. Ishida K, Kuroda R, Miwa M, et al. The regenerative effects of platelet-rich plasma on meniscal cells in vitro and its in vivo application with biodegradable gelatin hydrogel. Tissue Eng 2007; 13: 1103-12. [CrossRef]

12. Sanchez M, Delgado D, Sanchez P, et al. Platelet rich plasma and knee surgery. Biomed Res Int 2014; doi: 10.1155/2014/890630. [CrossRef]

13. TMicCarrel TM, Mall NA, Lee AS, Cole BJ, Butty DC, Fortier LA. Considerations for the use of platelet-rich plasma in orthopedics. Sports Med 2014; 44: 1025-36. [CrossRef]

14. Willits K, Kaniki N, Bryant D. The use of platelet-rich plasma in orthopedic injuries. Sports Med Arthrosc Rev 2013; 21: 225-30. [CrossRef]

15. Moraes VY, Lenza M, Tamaoki MJ, Faloppa F, Bellotti JC. Platelet-rich therapies for musculoskeletal so tissue injuries. Cochrane Database Syst Rev 2013; https://doi.org/10.1002/14651858.CD001071.pub2. [CrossRef]

16. Dohan Ehrenfest DM, Andia I, Zumstein MA, Zhang CQ, Pinto NR, Bielecki T. Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. Muscles Ligaments Tendons J 2014; 4: 3-9. [CrossRef]

17. Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammarti G. Slow release of growth factors and thrombospoedin-1 in Choukroun's platelet-rich fibrin (PRF): a gold standard to achieve for all surgical platelet concentrates technologies. Growth Factors 2009; 27: 63-9. [CrossRef]

18. Dohan Ehrenfest DM1, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) for all surgical platelet concentrates technologies. Growth Factors 2009; 27: 63-9. [CrossRef]

19. Dohan Ehrenfest DM1, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009; 27: 158-67. [CrossRef]

20. Dohan Ehrenfest DM, Bielecki T, Jimbo R, et al. Do the fibrin architecture and leukocyte content influence the growth factor release of platelet concentrates? An evidence-based answer comparing a pure platelet-rich plasma (P-PRP) gel and a leukocyte- and platelet-rich fibrin (L-PRF). Curr Pharm Biotechnol 2012; 13: 1145-52. [CrossRef]

21. El-Sharkawy H, Kantarci A, Deady J, et al. Platelet-rich plasma: implications and perspectives. Muscles Ligaments Tendons J 2014; 4: 3-9. [CrossRef]

22. Arnoczky SP, Warren RF. The microvasculature of the meniscus--an organ culture model to eliminate the influence of microvasculature and the synovium. Knee 2004; 11: 271-8. [CrossRef]

23. Werther K, Christensen IJ, Nielsen HJ. Determination of vascular endothelial growth factors and pro- and anti-inflammatory properties. J Periartic Orthop Res 2003; 21: 238-44. [CrossRef]

24. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med 2006; 34: 1774-8. [CrossRef]

25. Bielecki T, Gazdzik TS, Szczepanski T. Benefit of percutaneous injection of autologous platelet-leukocyte-rich gel in patients with delayed union and nonunion. Eur Surg Res. 2008; 40: 289-96. [CrossRef]

26. Narita A, Takahara M, Sato D, et al. Biodegradable gelatin hydrogels incorporating fibroblast growth factor 2 promote healing of horizontal tears in rabbit meniscus. Arthroscopy 2012; 28: 255-63. [CrossRef]

27. Ishima M, Wada Y, Sonoda M, Harada Y, Katsumi A, Moriya H. Effects of hyaluronan on the healing of rabbit meniscus injured in the peripheral region. J Orthop Sci 2000; 5: 579-84. [CrossRef]

28. Takeuchi N, Suzuki Y, Sagehashi Y, Yamaguchi T, Itoh H, Iwata H. Histologic examination of meniscal repair in rabbits. Clin Orthop Relat Res 1997; 338: 253-61. [CrossRef]

29. Ochi M, Kanda T, Sumen Y, Ikuta Y. Changes in the permeability and histologic findings of rabbit menisci after immobilization. Clin Orthop Relat Res 1997; 305-15. [CrossRef]

30. Bonutti PM, Weiker GG, Andrich JT. Isobutyl cyanoacrylate as a soft tissue adhesive. An in vitro study in the rabbit Achilles tendon. Clin Orthop Relat Res 1988; 229: 241-8. [CrossRef]

31. Guisasola I, Vaquero J, Forriol F. Knee immobilization on meniscal healing after surgery: an experimental study in sheep. Clin Orthop Relat Res 2002; 395: 227-33. [CrossRef]

32. Kobayashi K, Fujimoto E, Deie M, Sumen Y, Ikuta Y, Ochi M. Regional differences in the healing potential of the meniscus--an organ culture model to eliminate the influence of microvasculature and the synovium. Knee 2004; 11: 271-8. [CrossRef]

33. Day B, Mackenzie WG, Shim SS, Leung G. The vascular and nerve supply of the human meniscus. Arthroscopy 1985; 1: 58-62. [CrossRef]

34. Meaney Murray M, Rice K, Wright RJ, Spector M. The effect of cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med 2011; 39: 2135-40. [CrossRef]

35. Wilson AS, Legg PG, McNeur JC. Studies on the innervation of the peripheral region. J Orthop Sci 2000; 5: 579-84. [CrossRef]

36. Tabata Y, Nagano A, Muriruzzaman M, Ikada Y. In vitro sorption and desorption of basic fibroblast growth factor from biodegradable biomaterials. Biomed Materials 1998; 19: 1781-9. [CrossRef]

37. Tanaka M, Wada Y, Sonoda M, Harada Y, Katsumi A, Moriya H. Effects of hyaluronan on the healing of rabbit meniscus injured in the peripheral region. J Orthop Sci 2000; 5: 579-84. [CrossRef]

38. Arnoczky SP, Warren RF. The microvasculature of the meniscus--an organ culture model to eliminate the influence of microvasculature and the synovium. Knee 2004; 11: 271-8. [CrossRef]

39. Zellner J, Mueller M, Berner A, et al. Role of mesenchymal stem cells in tissue engineering of meniscus. J Biomed Mater Res A 2010; 94: 1150-61. [CrossRef]

40. Shin KH, Lee H, Kang S, et al. Effect of Leukocyte-Rich and Platelet-Rich Plasma on Healing of a Horizontal Medial Meniscus Tear in a Rabbit Model. Biomed Res Int 2015; 2015: 179756. [CrossRef]