Original Article

The use of platelet-rich fibrin with platelet-rich plasma support meniscal repair surgery[☆]Masahiko Kemmochi^{a,*}, Shigeru Sasaki^{b,c}, Masako Takahashi^d, Tomitaka Nishimura^d, Chisa Aizawa^d, Jun Kikuchi^e^a Kemmochi Orthopedic Surgery Sports Clinic, KOSSMOS Medical Corporation, 42-1 Higashi honcho, Ota, Gunma, 373-0026, Japan^b Department of Orthopaedic Surgery, Kyorin University, Tokyo, Japan^c Japan Community Health Care Organization, Yamanashi Hospital, Kofu, Yamanashi, Japan^d Kemmochi Orthopedic Surgery Sports Clinic Nurse Part, KOSSMOS Medical Corporation, Ota, Gunma, Japan^e Kemmochi Orthopedic Surgery Sports Clinic Rehabilitation Part, KOSSMOS Medical Corporation, Ota, Gunma, Japan

ARTICLE INFO

Keywords:

Platelet-rich fibrin
 Platelet-rich plasma
 Meniscal repair
 Arthroscopy
 Regenerative medicine

ABSTRACT

Introduction: Platelet-rich fibrin (PRF) is the only autologous blood product that releases growth factors and has scaffolding properties. We hypothesized that the use of PRF and Platelet-rich plasma (PRP) would improve operative results, including the recovery of function and repaired meniscus.

Materials and Methods: Seventeen patients underwent arthroscopic meniscus repair with PRF and PRP (PRF group) using our novel device for the injection of the PRF into the joint. Another five patients as a control group underwent meniscal repair without PRF and PRP (non-PRF group). The groups were compared in terms of clinical results (Tegner Activity Level Scale, Lysholm Knee Scoring Scale, and International Knee Documentation Committee [IKDC] scores) and changes in magnetic resonance imaging (MRI) findings before surgery and 6 months after surgery.

Results: The Lysholm and IKDC scores improved in all patients postoperatively. However, there was no significant differences in the postoperative score between the PRF group and the non-PRF group. Follow-up MRI findings did not clearly show improvements.

Conclusions: PRF and PRP are autologous, safe, and cost-effective sources of growth factors. Therefore, we propose a new application of PRF and PRP for autologous transplantation in meniscus repair surgery.

1. Introduction

Platelet-rich plasma (PRP) is a liquid that includes an anticoagulant, which forms a PRP clot by activation. However, PRP clots are fragile and unstable. By contrast, original platelet-rich fibrin (PRF) is a gelatinous solid body. This PRF matrix is turned into strong fibrin architecture as a PRF membrane by compression.

PRF appears to be the only material that can rigidly seal a meniscus defect while supplying growth factors. In angiogenesis, PRF acts in the stimulation phase, promoting vascular growth and increasing collagen synthesis through fibroblast proliferation.¹ PRP and PRF have different characteristics, including a difference in the release peak of the growth factors that they contain.² PRF is the only autologous fibrin matrix that releases growth factors slowly and continuously and also has scaffolding properties.^{3–5} Therefore, we aimed to assess the benefits of PRF with PRP in arthroscopic meniscal repair. We hypothesized that the use

of PRF with PRP would improve operative clinical results, including the recovery of function and repaired meniscus.

The report Platelet-Rich Fibrin Facilitates Rabbit Meniscal Repair by Promoting Meniscocytes Proliferation, Migration, and Extracellular Matrix Synthesis⁶ supports our hypothesis that PRF and PRP have great potential for the treatment of injured meniscus.

The aim of this study was to assess the benefits of PRF with PRP in arthroscopic meniscal repair.

2. Materials and methods

2.1. Processing PRF and PRP

We used leukocyte- and platelet-rich fibrin (L-PRF), called Choukroun's PRF, and leukocyte- and platelet-rich plasma (LR-PRP) in four different families of platelet concentrates.⁷

[☆] A part of this study was presented at the 32th Annual Research Meeting of Japanese Orthopaedic Association as a panel discussion at Okinawa on 27 Oct 2017.

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Fig. 1. Platelet-rich fibrin (PRF).

(a) Platelet-rich fibrin is shown in a glass cylinder after centrifuging at 400 g for 13 min (white circle). (b) The PRF matrix is shown (white circle). (c) The PRF membrane after compression by the PRF box is shown.

2.1.1. Preparation of PRF (L-PRF)

Venous blood was taken from each patient using a 21-gauge needle. According to the previously published protocol,⁸ blood samples were rapidly and gently collected without an anticoagulant in sterilized 10-mL dry glass tubes and immediately centrifuged at 400g for 13 min at room temperature (KUBOTA CORPORATION tabletop centrifuge 2420, Tokyo, Japan) (Fig. 1a). Two samples were prepared in case of production failure. After centrifuging, all procedures were performed on a clean bench. The PRF clot was removed from the tube using sterile tweezers, separated from the red blood cell base using scissors (discarding the red blood cell part), and placed in a PRF box (BS Medical, Tokyo, Japan) (Fig. 1b). The PRF clot was compressed in the PRF box to

produce a PRF membrane. Thick tube-like PRF clots were shaped or compressed to fit the size of the implantation site. Thereafter, the PRF membrane was cut vertically to form a red corpuscle residue-buffy coat-fibrin matrix. A red band coated one side of the white area, which was used as a marker during the meniscal repair (Fig. 1c).

2.1.2. Preparation of PRP (LR-PRP)

Centrifugation was performed twice using a double spin method. First, two blood samples were collected with an anticoagulant (sodium citrate solution) in sterilized 10-mL glass tubes and immediately centrifuged at 1000g for 6 min at room temperature (Fig. 2a). The centrifugation allowed blood separation into 3 distinct layers. At the

Fig. 2. Platelet-rich plasma (PRP).

(a) The first centrifuged PRP sample is shown (1000g for 6 min). (b) Aspiration of the upper layer that includes platelet-poor plasma (PPP), PRP, and some red blood corpuscles is shown (black parenthesis). (c) The upper layer of the two blood samples is transferred to a dry glass tube without anticoagulant (black parenthesis). (d) The second centrifuged PRP sample is shown (800 G, 8 min). (e) PPP that were discarded leaving (black parenthesis in Fig. 2d) just enough serum to leave the buffy coat as a middle layer and thin red blood corpuscles as a bottom layer.

bottom of the glass tube, red blood corpuscles were present. At the top of the glass tube, the acellular plasma layer was present, containing circulating plasmatic molecules and a few platelets (platelet-poor plasma [PPP]). Between these two layers was an intermediate layer with an increased platelet concentration, representing a buffy coat. Using a sterile syringe with an 18-gauge long needle, the PPP, PRP, and some red blood corpuscles were aspirated (i.e., the upper and middle layers were collected for centrifuging) (Fig. 2b). The 2 samples were transferred to dry glass tubes without an anticoagulant (Fig. 2c) and centrifuged a second time at 800g for 8 min at room temperature. The second centrifugation allowed the blood to separate into 3 distinct layers as in the first centrifugation, with a concentrate of platelets at the bottom of the tube (Fig. 2d). The PPP at the top of the glass tube was aspirated and discarded, leaving just enough serum to maintain the buffy coat as a middle layer and thin red blood corpuscles as a bottom layer (Fig. 2e). The glass tubes were then gently shaken to obtain ready-to-use PRP. We obtained approximately 1.2 cm³ of PRP from 10 cc of blood, and the two prepared samples were mixed into one glass tube. Thus, we prepared approximately 2.4 cm³ of PRP.

2.2. Generation of platelet concentrates and hematology measurements

In order to verify the concentration rate of PRP used in this method, 31 other volunteer samples prepared under the same conditions were measured using a hemocytometer manufactured by Sysmex Corporation. In order to avoid unnecessary activation, measurements of the samples were made at room temperature as soon as possible after adjustment.

2.3. Study design and patients

A prospective, interventional, non-randomized trial was conducted. The study was approved by the respective Institutional Ethics Review Boards of each author. In addition, the study was conducted in accordance with the law for ensuring the safety of regenerative medicine, after examination by the specific committees authorizing the use of regenerative medicine.⁹ All patients provided written informed consent after receiving an explanation of the potential benefits of PRP and PRF, the surgical procedure, and follow-up. No patients declined to participate in this study.

All patients were diagnosed and treated in our clinic between

Fig. 3. Platelet-rich fibrin insertion into the joint.

(a) Delivery of the PRF (black arrow) into the joint using our novel device (blue arrow) is shown. (b) PRF is inserted into the meniscal tear compartment (black arrow). (c) The inserted PRF (black arrow) is tightened by the rope.

October 2013 and April 2017. The patients had difficulties in performing activities of daily living after their injury, and although they continued their rehabilitation at our clinic, their desired level of previous daily living could not be reached. Operative indication standards were defined as follows: 1) a Tegner Lysholm Knee Scoring Scale score less than 83 points (fair) and a Tegner Activity Level Scale score over 5, or 2) a Tegner Lysholm Knee Scoring Scale score less than 65 points (poor) and a Tegner Activity Level Scale less than 5. The patients underwent standard surgical meniscal repair with PRF and PRP (PRF group) or without PRF and PRP (non-PRF group). Those which were six months after surgery were subjects of this study and cases which adapt the following items were defined to take re-arthroscopy. 1, recurrence of knee pain, 2, lysholm score 90 or less, 3, suspected of re-cleavage on MRI image, 4, other, knee intra-symptomatic symptoms as osteoarthritis.

2.3.1. PRF group

From a total of 19 patients who had undergone meniscal repair with both PRF and PRP, 17 patients (9 men and 8 women) were enrolled in the present study. Included subjects had less than Grade 2 on the Kellgren and Lawrence classification system¹⁰ and were followed up for over 6 months after surgery. The location of the meniscal tear was the anterior segment in 5 patients, middle segment in 2 patients, posterior segment in 6 patients, anterior-posterior segment in 1 patient, middle-posterior segment in 3 patients, and anterior-middle-posterior segment in 2 patients. In all patients, the loaded PRF membrane into the cleft of the tear was approximately 1 mm × 10 mm × 5 mm (about 0.2–0.3 cm³), and the injected PRP in joint was approximately 2.4 cm³.

2.3.2. Non-PRF group

The data for the control group were added retrospectively. From a total of 7 patients who underwent meniscal repair in our clinic before we received authorization for new regenerative medicine, 5 patients (3 males and 2 female) without osteoarthritis (OA) changes were selected for the control group at random. These patients underwent surgical meniscal repair sutured without PRF or PRP. The location of the meniscal tear was the anterior segment in 2 patients, the posterior segment in 2 patients, and the anterior-middle-posterior segment in 1 patient.

2.4. Arthroscopic technique

All arthroscopic procedures were performed using the all-inside technique. All surgeries were performed by the same experienced surgeon, and the same surgical procedure was performed on all patients. The arthroscopic system (Smith & Nephew, Memphis, TN) included a Dyonics 25 fluid management system as the circulation system, Clear-Trac flexible 4.5-mm diameter cannula for the output of the circulation fluid, Fast-Fix 360 for all-inside sutures, Meniscus Mender II for outside-in sutures, and our novel device for the injection of the PRF into the joint.

Anterolateral and far-anteromedial portals were created, and other anteromedial portals were added as needed. After evaluating any concomitant ligament injury and cartilage damage, the meniscus tear was identified. Synovectomy was performed to secure the field of vision. Thereafter, the meniscus was abraded using a Mitek VAPR 3 system (Johnson & Johnson, Tokyo, Japan) and unstable fragments of the meniscus were debrided using an ACUFEX hand-held instrument (Smith & Nephew, Memphis, TN), exposing the margins of the tear.

Fig. 4. The Fateful Rod System.

The novel device used to deliver platelet-rich fibrin (PRF) into the joint is shown. (a) The novel device is composed of 1 rod and 2 tubes made of a stainless steel-like metal. (b) The inside rod is the longest component, the middle tube is the second longest, and the outside tube is the shortest with gaps in-between. (c) The inner rod is inserted into the middle tube up to the part of the silicon ring stopper (blue arrow). (d) The cylindrically-shaped tip with a capacity of about 0.3 cm³. The formed PRF membrane is inserted.

Preparation of PRF and PRP was performed prior to meniscal tear suturing. Typically, the Fast-Fix 360 was used to place vertical sutures closing the cleft of the tear from the middle to the posterior horn. Tears extending from the middle to the anterior horn were repaired using the Fast-Fix 360 in combination with an outside-in technique using the Meniscal Mender II with 2-0 polydioxanone (PDS® II Suture | Ethicon).

The PRF was inserted into the cleft of the injured meniscus, using our novel device, with the PRF placed between the suture strands to secure it within the cleft. The PRF membrane was deliberately inserted into the meniscus defect so that the white band of the fibrin matrix was inserted into the bottom, and the residue of the red corpuscle was sealed to appear on the upper surface. Thus, the inserted PRF membrane could be recognized as a red spot in the sutured meniscus surface after tightening of the suture (Fig. 3a and b). Using our novel device, the PRF was gently and quickly placed into the meniscus defect, pinched between the meniscus grooves, and tightened by the Fast-Fix 360 sutures (Fig. 3c). Subsequently, the sutures were tightened, and the repair was completed in a sandwich fashion as described in a study by Kamimura and Kimura.¹¹ To fix the inserted PRF, suturing of 3–4

needles was necessary. In this case, it was enough to use 3 Fast-Fix sutures, at most, to ensure that the fistula of the ruptured area did not separate. In the case of a small fistula, it was enough to use 1 Fast-Fix suture. Even in the case of a large fistula, 1 Fast-Fix suture each was added to the fistula stump; 1 suture was added to the center if necessary. At the end of the surgery, PRP was injected into the joint while the arthroscopy was pulled out.

2.5. PRF membrane delivery system (Fateful-Rod system)

Our original device was more beneficial for performing arthroscopic meniscal repair using the all-inside technique. The novel device used to introduce the PRF into the joint target and secure the PRF firmly was composed of 1 rod and 2 tubes made of a stainless steel-like metal (Fig. 4a). The device was comprised of an inner rod, an intermediate tube, and an outer tube, with gaps in between. The inside rod was the longest component, the middle tube was the second longest, and the outside tube was the shortest (Fig. 4b). The intermediate tube with the inserted inner rod was loaded with the PRF, like a muzzle-loading gun.

	Phase I Functional recovery period								Phase II Sports preparation period								Phase III Sports return period							
Plan/weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Range of motion (Donjoy)	0°		0-30°		0-60°		0-90°		Free															
Weight bearing (Adult)	Full (Double crutch)																							
Weight bearing (Student)	Full (Double crutch)																							
Muscle training	OKC (SLR/Setting)				CKC (Heel raise)				CKC (Single heel raise)				CKC (Front/Back lunge)				CKC (Side lunge/KBW)				CKC (Single squatting)			
Balance training									Standing on one leg (Static-Dynamic)								Unstable surface (Double-Single/Static-Dynamic)							
Heart rate training									Bike (Free)				Bike (60-70% HR max)				Bike (70-80% HR max)				Bike (80-90% HR max)			
Functional training									Walking				Jogging				Running				Dash			
Plyometric training																	Jump (Double)				Jump (Single)			
Agility training																	Stepping/Twisting/Cutting							

Fig. 5. Rehabilitation Protocol.

Table 1
Demographic data.

Variable	PRF group	non-PRF group	P value
Cases (n)	17	5	–
Male/Female	9/8	3/2	n.s.
Mean age (years)	32.4 ± 16.3	20.8 ± 8.8	n.s.
Lateral/Medial meniscus	13/6	4/1	
The Kellgren and Lawrence system (Grade 0/1)	11/6	3/2	
Presence of a meniscal cyst (n)	1	0	
Mean time between beginning of symptoms and surgery (days)	60.9 ± 38.8	46 ± 30.5	
Left/Right knee	10/7	2/3	
Location of the tear	5A 2M 6P 1AP 3MP 2AMP	2A 0M 2P 0AP 0MP 1AMP	

PRF, platelet-rich fibrin; A, anterior; M, middle; P, posterior; AP, anterior-posterior; MP, middle-posterior; AMP, anterior-middle posterior.

These components (1 rod and 1 tube) were gently inserted into the outer tube. The entire device (1 rod and 2 tubes) was then inserted into the target meniscus through the contralateral portal site.

The inner rod was inserted and attached to the silicon ring stopper from the rear of the rod into the middle tube up to the silicon ring stopper (Fig. 4c). The silicon ring stopper was designed to be the tip portion of the intermediate tube so that when the inner rod was inserted, a constant volume was maintained. Therefore, the tip had a cylindrical shape with a capacity of approximately 0.3 cm³. The formed PRF membrane from the tip was inserted gently like a muzzle-loading gun so that the red band was proximal (Fig. 4d). The intermediate tube with the inner rod, which was loaded with the PRF, was gently inserted into the outer tube, and the tip was aligned and inserted into the joint from the opposite arthroscope portal to the suture meniscus. The inner rod, intermediate rod, and outer tube were inserted close to the

targeted meniscus, leaving the outer tube stationary, and allowing the intermediate tube to be inserted into the tear margin of the target meniscus (Fig. 3a). After inserting the intermediate tube into the tear section, the inner rod was pushed in and the filled PRF was pushed into the tear section (Fig. 3b). While holding the PRF with the inner rod, the outer and intermediate tubes were rotated and pulled out, and finally, the Fast-Fix 360 was fastened as the inner rod was extracted (Fig. 3c). If the filled PRF escaped from the cleft when the loop was tightened, the PRF insertion volume was reduced.

2.6. Treatment and rehabilitation protocol

All patients followed the same rehabilitation protocol. After arthroscopic meniscal repair, an extension knee brace (DJO, Ontario, Canada) was applied and patients were restricted to limited flexion for 10 weeks. Full weight-bearing was immediately permitted after the surgery for full knee extension, and patients underwent physical therapy. A knee brace with a locking mechanism every 30 degrees was used for 2 weeks. Light running was allowed after 3 months (Fig. 5).

2.7. Clinical assessment

Clinical assessments included the Lysholm Score, Tegner Activity Level Scale,¹² International Knee Documentation Committee (IKDC) Subjective Score,¹³ and magnetic resonance imaging (MRI; 0.3 T open-type instrument; HITACHI Medico Airis Bento, Tokyo, Japan) findings before surgery and 6 months after surgery. Changes in MRI findings were evaluated using the Nguyen classification system.¹⁴ We performed 3D MRI and compared preoperative and postoperative findings. In addition, the Kellgren and Lawrence system was used to classify the severity of knee osteoarthritis (OA).

3. Statistical analyses

Continuous data are reported as the mean ± standard deviation (SD). Welch's t-tests were performed for group comparisons, and paired

Table 2

The platelet concentration rate was measured by 31 samples and the leukocyte concentration rate was measured by 18 samples from No. 14 to No.31. The same number is the same specimen.

(No.)	platelet origin	PRP	ratio
1	17.1	85.1	4.98
2	18.9	96.8	5.12
3	25.7	147.5	5.74
4	23	131	5.7
5	22.6	130.6	5.78
6	14.9	71.1	4.77
7	17	92.5	5.44
8	17.2	87.2	5.07
9	22.7	98.1	4.32
10	21.3	92.9	4.36
11	12	73	6.08
12	11	90.9	8.26
13	20.3	183.8	9.05
14	16.8	93.7	5.58
15	29.4	160	5.44
16	19.7	122.3	6.21
17	16.1	109.9	6.83
18	12.2	88.7	7.27
19	19.6	70.5	3.6
20	22.5	90.4	4.02
21	21	94.8	4.51
22	33.5	175.4	5.24
23	18.7	107.4	5.74
24	16.6	99.8	6.01
25	20.5	75.7	3.69
26	15.3	96.8	6.33
27	28.4	132.3	4.66
28	18.2	106.5	5.85
29	19.4	91.2	4.7
30	22.8	118.2	5.18
31	18.3	68.5	3.74
mean $\pm$ SD	19.8 $\pm$ 4.96	105.9 $\pm$ 29.7	

(No.)	leukocyte origin	PRP	ratio
14	5200	21200	4.08
15	6300	27500	4.37
16	5300	21300	4.02
17	5200	19400	3.73
18	4500	33000	7.33
19	5600	16200	2.89
20	6300	21000	3.33
21	6300	27400	4.35
22	8600	24600	2.86
23	5000	16600	3.32
24	4600	17200	3.74
25	4800	9600	2
26	4400	15000	3.41
27	7500	22200	2.96
28	5100	18900	3.71
29	4900	14400	2.94
30	5100	15300	3
31	4800	9600	2
mean $\pm$ SD	5527.8 $\pm$ 1102.8	19466.7 $\pm$ 6112.7	

t-tests were performed to evaluate within-group changes. All statistical analyses were performed using SPSS version 22.0 for Windows (IBM Corp., Armonk, NY). A P-value of ≤ 0.05 was considered statistically significant.

4. Results

Patient characteristics are shown in Table 1. The average age of the platelet-rich fibrin (PRF) group of 17 patients (9 men and 8 women) was 32.4 ± 16.3 years (range, 15–69 years) and 20.8 ± 8.8 years (range, 13–38 years) for the non-PRF group (3 men, 2 women). The average duration of rehabilitation before the surgery was 60.9 ± 38.8

days for the PRF group and 46 ± 30.5 days for the non-PRF group (Table 1).

For the platelet-rich plasma (PRP) used in this method, mean platelet concentration was about 5.5-fold (3.4–9.1), and mean white blood cell concentration was about 3.6-fold (2.0–7.3) (Table 2).

Both groups showed significant changes (preoperative versus postoperative) in the Tegner Lysholm Knee Scoring Scale and International Knee Documentation Committee (IKDC) score ($P < 0.01$). In the non-PRF group, the Tegner Lysholm Knee Scoring Scale and IKDC score increased from 62.6 ± 6.7 and 28.5 ± 3.8 to 97.2 ± 1.8 and 91.5 ± 1.2 , respectively (paired t-tests, $P < 0.0003$, 0.000003 , respectively). In the PRF group, the Tegner Lysholm Knee Scoring Scale and IKDC score increased from 61.3 ± 11.1 and 28.9 ± 11.6 to 95.8 ± 7.1 and 87.4 ± 10.4 , respectively (paired t-tests, $P < 0.00000002$, $P < 0.0000000001$, respectively) (Table 3).

There was no significant difference between both groups in the postoperative Tegner Lysholm Knee Scoring Scale (Welch's t-tests; preoperative, $P = 0.82$; postoperative, $P = 0.69$), in the postoperative IKDC score (Welch's t-tests; preoperative, $P = 0.94$; postoperative, $P = 0.13$). No statistically significant group differences were found in the Tegner Activity Level Scale scores (Welch's t-tests; preoperative, $P = 0.21$; postoperative, $P = 0.11$) (Table 3).

Follow-up magnetic resonance imaging (MRI) performed 6-months postoperatively revealed a tendency toward healing; some did not. In addition, in the case of 3D MRI scan, there were cases in which the meniscal defect appeared to be sealed, but some cases were depicted as defects, which were not consistent. (Fig. 6). There were no case adopted as second-look arthroscopy.

5. Discussion

In the joint, normal wound healing in an avascular area is not expected due to the existence of joint fluid and a lack of blood supply. Thus, material that accelerates vascularity and the sealing of the defect in an injured meniscus would be ideal as the formation of a fibrin bridge, and vascularity in the avascular area may be expected. Importantly, vascularity in the meniscus originates from the perimeniscal capillary plexus (PCP). When a tear occurs in the peripheral third of the meniscus, a fibrin clot forms, which is rich in inflammatory cells. The PCP grows rapidly over the fibrin conduit, as well as into the fibrous scar, and contributes undifferentiated mesenchymal cells. Meniscal repair depends on the available vascular supply to provide healing factors. This is the basis for the biological enhancement of meniscal repair in avascular areas of the meniscus.¹⁵

PRP promotes additional platelet aggregation, thrombin generation, fibrin formation, and the release of several local growth factors, including PDGF, TGF- β 1, FGF, and vascular endothelial growth factor (VEGF). The release of these growth factors can promote angiogenesis and stimulate the aggregation of reparative cells. PRP has also shown remarkable healing properties for the repair of the inner avascular part of a meniscal injury in vitro and in vivo.¹ PRP treatment of an in vivo meniscus injury has been reported to accelerate fibrosis, but not the healing of meniscal cartilage.¹⁶

A previous report has suggested that PRF is superior to PRP.^{17–20} PRF can be used to promote wound healing, bone regeneration, graft stabilization, wound sealing, and hemostasis. The simplified processing technique for PRF, which does not involve complex handling, also makes PRF superior to PRP. Moreover, because the fibrin matrix is better organized, it more efficiently directs stem cell migration and the healing process.²¹

As original PRF is a gelatinous solid body, PRF can be used directly as a bell-shaped clot (PRF matrix) or as a strong membrane after compression (PRF membrane). PRF is firm enough to keep its shape during an arthroscopic operation. However, fibrin clot or activated PRP clot will not be able to keep their shape during operation. In addition, PRF is useful for tissue regeneration; it is rich in growth factors and has

Table 3
Clinical Results.

	Patients	Tegner Activity Level Scale		Lysholm Knee Scoring Scale		IKDC Score	
		Preoperative	Postoperative 6M	Preoperative	Postoperative 6M	Preoperative	Postoperative 6M
non-PRF group	A	9	9	62	97	24.1	89.7
	B	7	7	53	94	25.3	90.8
	C	5	5	70	97	27.6	93.1
	D	9	9	58	99	34.5	92
	E	9	9	70	99	31	92
	mean±SD	7.8±1.6	7.8±1.6	62.6±6.7	97.2±1.8	28.5±3.8	91.5±1.2
PRF group	F	9	9	77	100	34.5	97.7
	G	5	5	66	95	14.9	81.6
	H	9	9	62	100	17.2	98.9
	I	7	7	52	95	13.8	83.9
	J	7	7	77	100	24.1	100
	K	7	5	72	71	47.1	64.4
	L	8	8	77	100	42.5	100
	M	8	8	48	100	14.9	98.9
	N	4	4	59	93	28.7	75.9
	O	9	5	65	94	21.8	83.9
	P	5	5	48	88	19.5	72.4
	Q	7	7	48	99	28.7	93.1
	R	2	2	60	94	40.2	82.8
	S	9	9	78	100	50.6	97.7
	T	3	3	49	100	34.5	86.2
	U	2	2	52	100	19.5	87.4
	V	6	5	53	100	39.1	80.5
	mean±SD	6.3±2.3	5.9±2.3	61.3±11.1	95.8±7.1	28.9±11.6	87.4±10.4

PRF, platelet-rich fibrin; IKDC, International Knee Documentation Committee.

*P < 0.0003 ** P < 0.000003 *** P < 0.00000002 **** P < 0.0000000001.

release functions slowly of growth factors. PRF, like PRP, includes many platelet growth factors, which play an important role in wound healing. Previous in vitro studies have shown a slow release of growth factors, including TGF-β1, PDGF, and VEGF, from PRF during the first few days in a sterile medium.²²

As with most PRP techniques, the slow release of PRF is difficult to determine because of massive platelet activation, the immediate release of growth factors, and the very light fibrin network produced to sustain the concentrated injection. Indeed, the effect of the platelet cytokines, abundantly released during platelet activation and fibrin gelling, appears to be short-lived. Interestingly, PRP can release significantly higher levels of growth factors at very early time points, whereas PRF has a more gradual release of growth factors for up to 10 days.² In addition, other methods have been developed for the long-term release of growth factors.²³ The quantity of leukocytes implanted within the PRF membrane is considerable, and small lymphocytes are particularly efficient in the regulation of inflammatory reactions.²⁴

Tissue regeneration and repair processes require a harmonious reaction from various types of cells, including immune response cells, epithelial cells, fibroblasts, and stem cells, as well as other cells relevant to the tissue in question. The PRF scaffold concept appears to be an ideal source of components for the healing process.²⁵ The histologic evaluation of PRF demonstrates earlier vessel formation and tissue maturation than those for connective tissue grafts.²⁶ The three main platelet cytokines (PDGF-BB, TGFβ-1, and insulin-like growth factor 1 [IGF-1]) are abundant in PRF. These cytokines play a fundamental role in the initial healing mechanisms because of their capacity to stimulate cell migration and proliferation (particularly via PDGFs), induce fibrin matrix remodeling, and promote the secretion of a cicatricial collagen matrix (particularly via TGFβ).^{2, 23, 27}

On the basis of the above theory, we used both PRF and PRP in the meniscal repair procedure. In addition, as biological common sense, wound healing is not promoted to the extent that the growth factor level is raised. It is understood that platelet concentrate material is caused by recruitment of circulating stem cells and peripheral stem cells

via newly formed blood vessels rather than directly acting on the targeted cells and its precursor cells to regenerate. In other words, is it not more appropriate to consider para Klein theory based on harmony theory?

It is reasonable to assume that the attractiveness of PRP is its cost-effectiveness; however, the standardization of adjustment protocol and administration method and the active potential of angiogenesis greatly influence the therapeutic effect. Expensive equipment and advanced skills are unnecessary for PRF, and it is easy to adjust to it with high reproducibility. We only have to consider that standardization for the administration method.² Therefore, we are considering performing meniscal repair with only PRF in the future, too. We guess PRP and PRF are not making regeneration, but only promoting healing.

The novel PRF delivery system used in the present study was finally produced after testing many arthroscopic devices for the purpose of performing all-inside technique. Our novel device could deliver the PRF membrane to the target while protecting the PRF, with a quick and sure inter-tunnel transfer technique. Thanks to our novel device, intraoperative stress was largely reduced and the operative time was markedly shortened.

The results of the repair procedure were favorable, with no patients experiencing any problems. In addition, the Lysholm Score, Tegner Activity Level, and IKDC Subjective Scores had significantly improved 6 months after surgery compared to the preoperative scores. Although good results occurred in both groups, there was no significant difference between both groups. Moreover, the patients were able to return to former sporting activities after the surgery. However, longer follow-up periods are need in future studies.

3D MRI in some patients showed the recognized defect part of the meniscus sealed (Fig. 6b). Hence, we could not unequivocally declare complete healing with maturation. Although the clinical results after the surgery were satisfactory and good results were obtained, we plan to verify whether complete repair can occur 1 or 2 years after surgery using MRI.

The key method in the fibrin clot technique is not to bring in a direct

Fig. 6. 3D magnetic resonance imaging (MRI) findings (one platelet-rich fibrin [PRF] case).

(a) (b) Before surgery, the posterior segment appears as a defect on 3D MRI. After surgery, the defect appears to be sealed on 3D MRI. (c) (d) Before surgery, the posterior segment appears as a defect on 3D MRI. After surgery, the defect appears to be unsealed on 3D MRI.

vascular supply to a previously avascular area, but to bring the factors found within a hematoma, which normally forms at the injury site but cannot form in an avascular meniscal tear. PRF has a stronger composition and longer duration of growth-factor release than fibrin clots, which have been frequently used in meniscus repair.^{11,28} In addition to its capacity to accelerate healing, sealing with fibrin adhesive could be used for filling the defects of an injured meniscus. We think that the ideal tissue repair scaffold should be placed in contact with the meniscus defect. Moreover, scaffolds that can support the meniscus tissue, including growth factors, are preferable. Hence, given that PRF has a longer duration of growth-factor release than PRP and fibrin coagulum, PRF is considered a better material. PRF appears to be better as a filling material for meniscal restoration as well.^{23,28,29} Moreover, the use of PRF is attractive from a histologic point of view. Therefore, we believe that PRF is an ideal material for meniscal repair. According to our clinical result, even if regeneration does not occur in the meniscal tissue, the meniscus defect may be filled with fibrous tissue, and the

purpose may still be achieved.

6. Limitation

This study had several limitations.

First, the MRI resolution was limited, with a slice thickness of 2.5 mm. Although postoperative MRI evaluation has been used to evaluate surgical success,^{14,30} it does not quantitatively evaluate meniscal restoration. The MRI of a postoperatively repaired meniscus is difficult to interpret. Indeed, there is no method that quantitatively analyzes the degree of meniscus restoration via MRI. As mentioned above, MRI can provide only an evaluation of whether a restoration compartment is buried, and the 3D-MRI image is the only image expressing the continuation of the sealing effects of PRF. However, the 3D MRI image did not clearly depict the sealing of the meniscus defect with PRF and did not provide proof of PRF membrane conglutination around the meniscus (Fig. 6a–c). At the very least, however, no concerning

aggravated MRI findings were observed.

Second, the PRF had a filmy or gelatinous structure, and we could not measure the components and release duration of the growth factor included in the PRF because this is difficult and would have been expensive to ascertain. So, we had to depend on reports from research organizations, such as university hospitals, regarding the growth factors included in PRP and PRF. Fortunately, pertinent articles exist.³¹

Third, since the number of cases in the control group was small and the postoperative results of the control group were also good, it was not possible to show a significant difference between the non-PRF group and the PRF group. Finally, in our clinic, it was ethically difficult to enforce the second appearance arthroscopy. Fortunately there were no case adopted as second-look arthroscopy. Hence, we could not obtain consent for the second-look arthroscopy from patients. Thus, these limitations should be examined in the future.

7. Conclusions

The results of this repair procedure were favorable, and good results occurred in both surgical groups. However, there were no significant differences between the PRF group and the non-PRF group. Platelet concentrates, such as PRF, should be used in meniscal repair to enhance the healing process in the avascular portion of the meniscus. We believe that the use of preparations like PRP or PRF will become widespread because they are relatively easy to produce and safe to use. PRF and PRP are autologous, safe, and cost-effective sources of growth factors, and PRF can freely adapt its shape and act as a scaffold for the meniscal repair. Hence, PRF is effective as a new autologous transplantation technique for meniscus repair. Moreover, the use of PRF and PRP may be a promising alternative in meniscal surgery; however, further studies are required to support the present results.

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