Journal of Orthopaedic Translation 24 (2020) 183–189

Contents lists available at ScienceDirect

Journal of Orthopaedic Translation

journal homepage: www.journals.elsevier.com/journal-of-orthopaedic-translation

Original Article

The effect of thermosensitive hydrogel platelet–rich–plasma complex in the treatment of partial tear of anterior cruciate ligament in rat model

Yue Li [a], Sai C. Fu [b,c], Yau C. Cheuk [b,c], Tim-Yun Ong [b,c], Hua Feng [a], Shu-Hang Yung [b,c,*]

[a] Sports Medicine Service, Beijing Jishuitan Hospital, Beijing, China
[b] Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, China
[c] Lui Che Woo Institute of Innovative Medicine, The Chinese University of Hong Kong, Hong Kong, China

ARTICLE INFO

Keywords:
Anterior cruciate ligament
Hydrogel
Partial tear
Platelet-rich plasma

ABSTRACT

Background/Objective: The treatment of anterior cruciate ligament (ACL) partial tear is controversial. The reconstructive surgery is invasive while the prevalence of subsequent insufficiency after conservative treatment has been reported to range from 11% to 62%. Therefore, a new method that promotes tissue regeneration is needed. The aim of this study was to observe the healing of ACL partial tear biomechanically and histologically after the administration of a thermosensitive hydrogel platelet-rich-plasma (PRP) complex.

Methods: The complex was prepared according to a previously published protocol. One hundred and fifty 12-week-old male Sprague-Dawley rats were included and they were allocated into 4 groups. Lesion control group (Group 1), treatment group (Group 2), gel-only group (Group 3) and intact group (Group 4). Biomechanical testing, histological analysis (H&E and immunohistochemical staining) and scoring was performed.

Results: On gross observation, the treatment group showed a continuous ACL with slightly thickened synovium or a partially healed ACL at 6-week follow up. In the biomechanical testing at 6 weeks after surgery, the failure load of the treatment group was significantly superior when compared with the lesion control group (52.7±10.8N vs. 41.6±7.8N, $p<0.01$), but the failure load was not restored to level of the intact group (52.7±10.8N vs. 61.5±9.1N, $p=0.037$). The maturity index of wound sites showed no significant inter-group differences at any timepoints. However, an increased expression of vascular endothelial growth factor (VEGF) and pro-collagen I was detected.

Conclusion: The thermosensitive hydrogel-PRP was shown to be effective in enhancing the healing of ACL partial tear in the rat model, and potentially this complex can be used as a treatment for patients with ACL partial tear.

The translational potential of this article: The thermosensitive hydrogel-PRP is potentially translated to clinical use to treat patients with ACL partial tear by injection under arthroscopy or ultrasound guiding.

Introduction

Among anterior cruciate ligament (ACL) tears, 5–15% are partial tears [1]. The prevalence of subsequent insufficiency of ACL has been reported to range from 11% to 62% in cases of nonoperatively treated partial tears in adults [2]. In a study by Bak et al. [3], only 30% of patients with partial ACL tears were able to return to the preinjury level of sporting activity at 5 years follow-up.

For the management of ACL partial tear, different nonoperative and operative treatment methods, such as immobilisation or bracing, single-bundle repair [4], and reconstruction of the ACL have been proposed [5]. However, nonoperative treatment resulted in a high risk of deterioration while operative treatment came with extra comorbidity. Therefore, until now, there was no consensus on the management of ACL partial tear.

This leads to a search for a method that balances both the recovery of ACL function and also prevents comorbidities. It has been discovered that platelet-rich plasma (PRP) is effective in the treatment of soft tissue injuries, such as hamstring strain, meniscal lesion, and rotator cuff tears [6]. Therefore, it is reasonable to assume that PRP can also be used as a bioenhancer for ACL healing. However, on the contrary, it has been reported that the presence of urokinase plasminogen activator found in the synovial fluid after injury played a key role in the failure of the fibrin–-platelet provisional scaffold formation hence compromising the healing of ACL [7–9]. With this dilemma, a material that protects the PRP from

* Corresponding author. Room 74029, 5/F, Lui Che Woo Clinical Science Building, Prince of Wales Hospital, Shatin, NT, Hong Kong, China.
E-mail address: patrickyung@cuhk.edu.hk (S.-H. Yung).

https://doi.org/10.1016/j.jot.2019.12.009
Received 1 September 2019; Received in revised form 19 December 2019; Accepted 22 December 2019
Available online 22 January 2020

being compromised by synovial fluid while sustainably releases it to the injury site would be required.

To protect the healing process from the interference from the synovial fluid, some authors proposed composite hydrogel, collagen sponge [9, 10], or implanted scaffold [11] to provide the ACL graft a stable chemical and physical environment. However, the placement of these materials must be meticulous, or the material may be displaced, leading to a failure of treatment. A thermosensitive hydrogel, monomethoxypoly (ethylene glycol)-co-poly (lactic-co-glycolic acid), known as mPEG-PLGA, was proposed to protect the lesion site from the "hostile" synovial fluid [12]. The mPEG-PLGA gelates around body temperature, so the hydrogel can easily be injected as a solution onto the injured ACL. The instant gelation of the hydrogel on the injured ACL at body temperature can eliminate the risk of displacement. In terms of drug-release, the mPEG-PLGA was shown to steadily release teicoplanin with a diffusion-controlled mechanism, which lasted up to 4 weeks in the treatment of osteomyelitis [12].

The purpose of this study was to observe the effect of the mPEG-PLGA-PRP complex on the healing of ACL partial tear in a rat model. We hypothesised that the mPEG-PLGA-PRP complex could restore the ACL partial tear to normal state both histologically and mechanically.

Methods

The animal experiments in this study were approved by the Animal Experimentation Ethics Committee at The Chinese University of Hong Kong (Ref No. 16-194-MIS).

Study design

One hundred and fifty 12-week-old male Sprague–Dawley rats were included. Their mean body weight was 439.5 g (range, 390–550 g), and they were allocated into 4 groups. The lesion control group (Group 1) was evaluated at time 0, 2 weeks, and 6 weeks postoperatively. The treatment group (Group 2) and the gel-only group (Group 3) were evaluated at 2 weeks and 6 weeks postoperatively. The sample size in each of the 7 groups stated was 20, and they were equally divided for histological and mechanical evaluations. The sample size of the intact group (Group 4) was 10, and only mechanical testing was performed. Group 1 was treated with an injection of 1 ml 0.9% saline into the knee joint; Group 2 was treated with the injection of 1 ml mPEG-PLGA-PRP complex, as described below. Group 3 was treated with an injection of 1 ml mPEG-PLGA hydrogel on the natural ACL. Group 4 received no treatment at all.

Preparation of mPEG-PLGA-PRP complex

The mPEG-PLGA diblock copolymer was used to fabricate the temperature-sensitive hydrogel. The molecular weight ratio of mPEG to PLGA that we used was 550:1105. The sol-to-gel-to-sol behavior curves at different concentrations were described well by Peng et al. [12]. The mPEG-PLGA diblock copolymer was dissolved and blended in 1 × PBS buffer to a weight percentage of 25%. The buffer was stored in a 4 °C room for 14 days.

After anesthesia, whole blood was drawn from the femoral vein of the index rat into a tube containing 300 μL sodium citrate immediately prior to surgery. Three cubic centimeters of blood was drawn from each animal. The blood was centrifuged to isolate the PRP fraction at 2100G, 22 °C for 3 min. The supernatant was then transferred to another tube and centrifuged at 4150G, 22 °C for 6 min. After all the supernatant was removed, 100 μL was added back to the tube and mixed with the precipitate. This resulted in an approximately 10X enrichment of the platelet concentration of the blood.

The PRP was then added to the 100 μL hydrogel, making the PRP: hydrogel ratio at 1:1 by volume for the complex. The overall concentration of the hydrogel was approximately 12.5%. The gelation temperature was around 36 °C, and the complex was kept on ice until use.

Surgical technique

Male Sprague–Dawley rats (12 weeks old) were used in this experiment. After general anesthesia with an intraperitoneal injection of a mixture of xylazine, ketamine, and 0.9% saline (ratio 2:3:3, at a dose of 0.2 ml/100 g body weight), the lower limb was shaved. A 1.5 cm incision was made to expose the patellar tendon. The patellar tendon was displaced, and fat pad excised to expose the ACL. The ACL lesion was created by penetrating through the ACL tissue with a 30 G syringe needle (φ = 0.3 mm), at the femoral insertion site of ACL vertically to the orientation of ACL (Fig. 1).

The mPEG-PLGA-PRP complex (treatment group), mPEG-PLGA hydrogel (gel-only group), or saline (lesion control group) was then injected into the lesion (Fig. 2). The complex gelated within 30 s. The patella was reduced, and the tissue was sutured in layers. The rats were allowed free cage movement and Temgesic was administered subcutaneously as an analgesic every 24 h for 3 consecutive days postoperatively. At the designed endpoints, the animals were sacrificed with an intraperitoneal injection of overdose pentobarbital. The index knees were harvested for further evaluation.

Biomechanical testing

All specimens were dissected free of patella, skin, muscle, tendons, meniscus, and ligaments except ACL. The femur and the tibia were cut 20 mm from the joint line for fixation in an adhesive polymer (1:1:2 of UREOL 5202-1A, UREOL 5202-1B, Filler DT-082. Ciba Specialty Chemicals, Cambridge, UK). The samples were then mounted to a mechanical testing machine (H25KM, Tinus Olsen, PA, USA) via custom-made jigs with a 50 N load cell (H25KM, Tinus Olsen) (load measurement accuracy: +0.5% of max. load). The jigs were positioned to keep the knee in 60° knee flexion with natural varus of rat knee at approximately 10°. The tensile test for failure load was carried out at a crosshead speed of 60 mm/min with a 250 N load cell until an abrupt drop in loading was detected. Failure load was measured as the maximum force until graft failure, and the mode of failure was recorded. The stiffness of the sample was measured as the slope at linear region of the force–displacement curve.

Histological analysis and scoring system

All specimens were fixed, decalcified, and embedded at 0° of knee flexion. Paraffin sections, 5 μm thick, along the sagittal plane of the knee, were collected in groups of 20 consecutive sections. Sections with the most ACL tissue from each specimen were chosen for hematoxylin and eosin (H and E) staining and another two sections for immunohistochemical staining for the detection of vascular endothelial growth factor (VEGF) and pro-collagen I.

Proteins of interest were detected by immunohistochemistry using a monoclonal antibody (VEGF and pro-collagen I: Abcam, Cambridge, MA). Antigen retrieval was performed by digesting the sections with 0.1% trypsin solution at 37 °C for 20 min. Subsequently, endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide at room temperature for 30 min. The nonspecific sites were blocked with 20% goat serum at room temperature for 30 min. The samples were incubated with the mouse monoclonal antibodies to VEGF and pro-collagen I overnight at 4° [13]. All sections were examined under bright field and polarised illumination (Leica Microsystems, Germany). The wound sites of all chosen sections were scored by two independent examiners according to a scoring system developed by Murray et al. [9], the maturity index for the ligament. The index used three criteria to describe changes during the process of ligament wound healing: cellularity (number, type, nuclear aspect ratio, orientation, and organisation of cells), vascularity (number, type, and organisation of vessels), and collagen (bundle width, orientation, and presence of crimp).

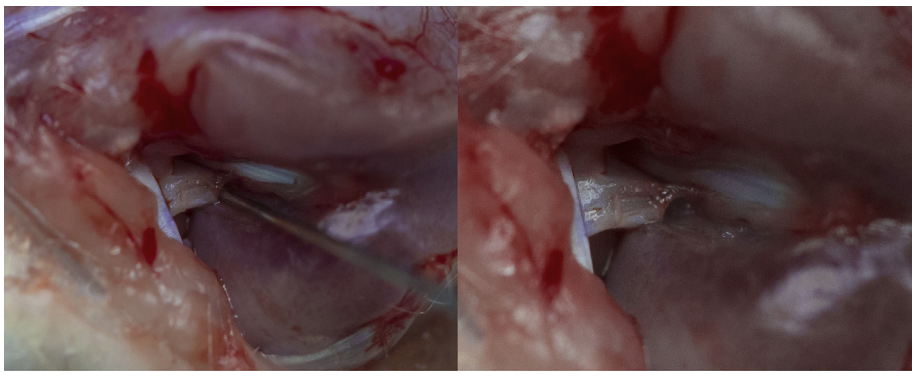

Figure 1. The lesion was created by a 30 G syringe needle (φ = 0.3 mm) that penetrated through the ACL tissue at the femoral insertion site of ACL vertically to the orientation of ACL.

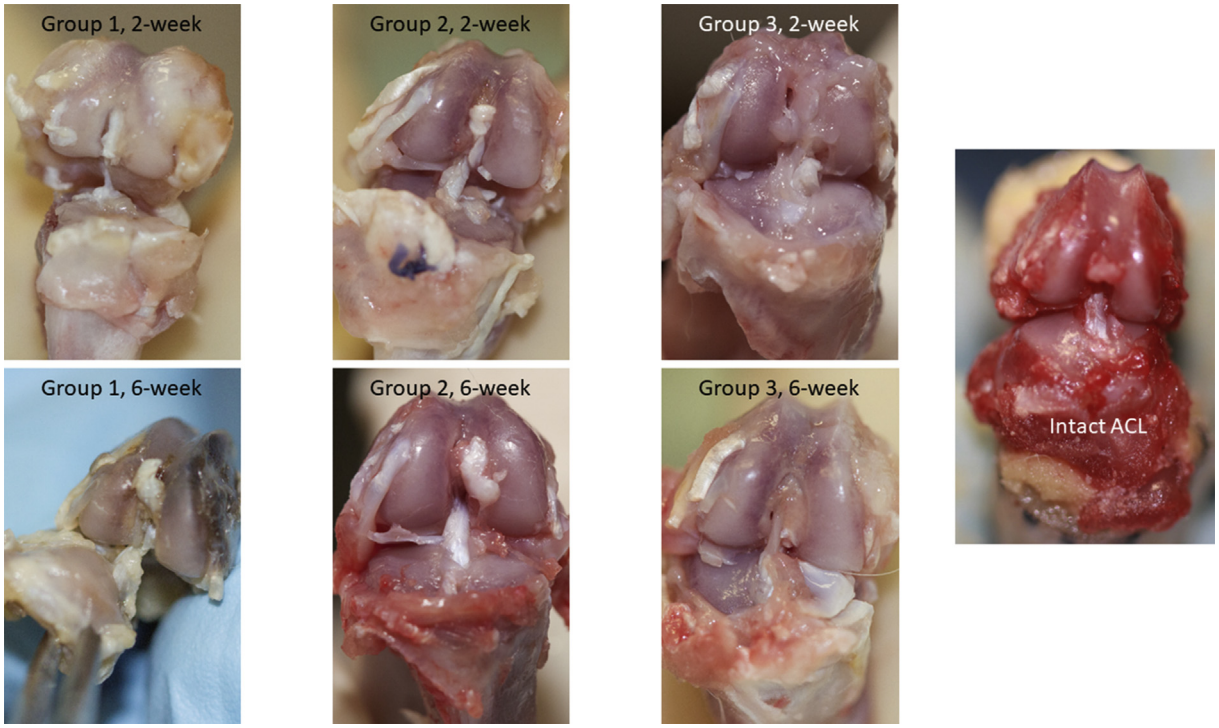

Figure 2. The gross observation of the samples. Group 1, ACLs seemed to be torn and covered with synovium with an anteriorly dislocated knee and severe thickening of synovium at both 2nd and 6th week. In addition, either a meniscus tear, chondral lesion, or a tibiofemoral osteophyte formation was also found; In Group 2, although slightly thickened synovium was observed, a continuous ACL or partially healed ACL can also be observed; In Group 3, similar to Group 1, ACLs seemed to be partially absorbed with few remnant tissues that were enveloped by synovium; In Group 4, all cases were intact ACLs.

Statistical analysis

Statistical analysis was done with Statistical Package for Social Science (SPSS) 16.0 (SPSS Inc, Chicago, USA). For histological scoring, nonparametric Mann–Whitney U tests were used for comparisons between time points and between different groups. After checking of normal distribution by Kolmogorov–Smirnov test, data of failure load and stiffness were analysed by ANOVA and posthoc Turkey's HSD. A significant difference was determined at $p < 0.05$.

Results

Gross observation inside the knee joint

At a 6-week follow up, ACLs showed different presentations on gross observation. In Group 1, the ACLs were mostly torn and covered with synovium in the majority of cases. In one case, an anteriorly dislocated knee and severe thickening of synovium were observed. Meniscus tear, chondral lesion, or a tibiofemoral osteophyte formation were also found in subjects. In Group 2, although slightly thickened synovium was observed in some subjects, a continuous ACL or partially healed ACL was observed. In Group 3, similar to Group 1, ACLs were partially absorbed with few remnant tissues enveloped by synovium; In Group 4, all cases were intact ACLs (Fig. 2).

Biomechanical properties of ACL

At time zero, the failure load of the lesion control was significantly lower than the intact group (41.6 ± 7.8 N vs. 61.5 ± 9.1N, $p < 0.01$), although there was no significant difference in stiffness (36.3 N/m vs. 30.6 N/m, $p = 0.197$). At 6 weeks, the failure load of the treatment group was significantly superior when compared with the lesion control group

Table 1
Summary of biomechanical properties of femur-ACL-tibia complex[a].

Group	Stiffness (N/m)	Tensile Strength (N)
Group 1–0wk	36.3 ± 11.6	41.3 ± 7.5[b]
Group 1–2wks	47.9 ± 10.4[c]	46.9 ± 7.2
Group 1–6wks	30.1 ± 7.7	40.3 ± 12.5[b]
Group 2–2wks	32.1 ± 13.8	41.6 ± 8.1[b]
Group 2–6wks	35.9 ± 10.8	50.1 ± 11.7
Group 3–2wks	36.2 ± 13.8	45.9 ± 13.5
Group 3–6wks	33.6 ± 11.5	35.0 ± 12.9[b]
Group 4	35.2 ± 12.1	61.9 ± 8.5[c]

wks, weeks; ACL, anterior cruciate ligament.
[a] All data were expressed as mean ± standard deviation.
[b] Data were significantly lower than the data of Group 2–6wks.
[c] Data were significantly higher than the data of Group 2–6wks.

(52.7 ± 10.8 N vs. 41.6 ± 7.8 N, p < 0.01), but the failure load was not restored to the level of the intact group (52.7 ± 10.8 N vs. 61.5 ± 9.1 N, p = 0.037). The biomechanical data and column figures are shown in Table 1 and Fig. 3.

Histologic scoring of ligamentous healing

Out of the 32 samples, 6 were excluded from the analysis due to surgical complications and malalignment during embedding. The sample size for the histological evaluation was illustrated in Table 2. At 2 weeks postoperation, there was moderate to high cellularity and vascularity in the PRP-hydrogel group, when compared with the no-treatment control and gel-only groups. At 6 weeks postoperation, a similar trend was observed, and the cellularity, collagen alignment, and vascularity had not returned to the normal level. Results from the histological scoring were summarised in Table 2 and Fig. 4. Kruskal–Wallis test showed no significant difference between groups in both the total score and subscores ($p > 0.05$).

Immunohistochemical findings

In Group 1 and 3, procollagen I and VEGF were not identified in the wound site at 2- and 6-week time points. In Group 2, at 2-week time point, there was no VEGF present at the wound site. However, at 6-week time point, increased expression of VEGF was observed when compared with Group 1 and 3 (Fig. 5). In Group 2, although the procollagen I was only present after 2 weeks, more oriented and mature procollagen I was detected at 6-week follow-up (Fig. 6).

Discussion

It has been demonstrated by several preclinical and clinical studies [14–22] that the natural regeneration of a torn ACL is difficult. A randomised controlled trial reported no difference in outcomes after primary repair versus conservative treatment of ACL tears [19]. The main factors responsible for ligament healing can be divided into intrinsic and

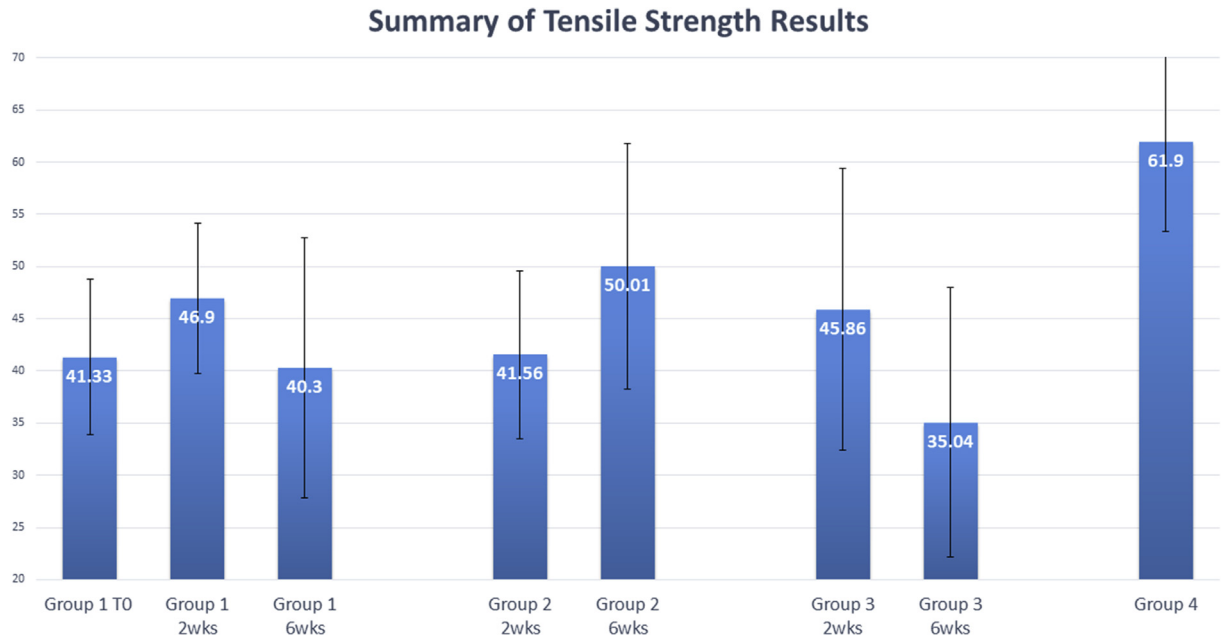

Figure 3. At time zero, the failure load of lesion control was significantly lower than intact group (41.6 ± 7.8 N vs. 61.5 ± 9.1 N, $p < 0.01$), although no significant difference in stiffness (36.3 N/m vs 30.6 N/m, $p = 0.197$). At 6 weeks, the failure load of the treatment group was significantly improved compared to the lesion control group (52.7 ± 10.8 N vs. 41.6 ± 7.8 N, $p < 0.01$), but still not restored to the intact state (52.7 ± 10.8 N vs. 61.5 ± 9.1 N, $p = 0.037$).

Table 2
Histological scoring of wound sites for effects of PRP hydrogel on ACL partial tear[a].

Group	Time postoperation	Cellularity subscore (max. 10)	Collagen subscore (max. 12)	Vascularity subscore (max. 6)	Total score (max. 28)
No treatment	2 weeks (n = 3)	5.0 (5.0–7.0)	6.0 (0–10.0)	3.0 (3.0–4.0)	14.0 (9.0–17.0)
	6 weeks (n = 4)	5.5 (5.0–7.0)	7.0 (6.0–8.0)	1.5 (0–4.0)	14.5 (14.0–15.0)
PRP-hydrogel	2 weeks (n = 4)	5.0 (5.0–5.0)	6.0 (2.0–6.0)	3.5 (3.0–4.0)	14.5 (10.0–15.0)
	6 weeks (n = 6)	5.0 (4.0–7.0)	6.0 (6.0–10.0)	3.5 (0.0–4.0)	14.5 (11.0–18.0)
Hydrogel only	2 weeks (n = 4)	6.0 (6.0–6.0)	6.0 (6.0–8.0)	3.5 (3.0–5.0)	16.0 (15.0–18.0)
	6 weeks (n = 4)	5.0 (5.0–6.0)	6.0 (6.0–6.0)	4.5 (3.0–5.0)	16.0 (14.0–16.0)

No significant inter-group difference in the total score and subscores was detected.
[a] The median score with a range from each experimental group was presented. A higher score indicates better healing.

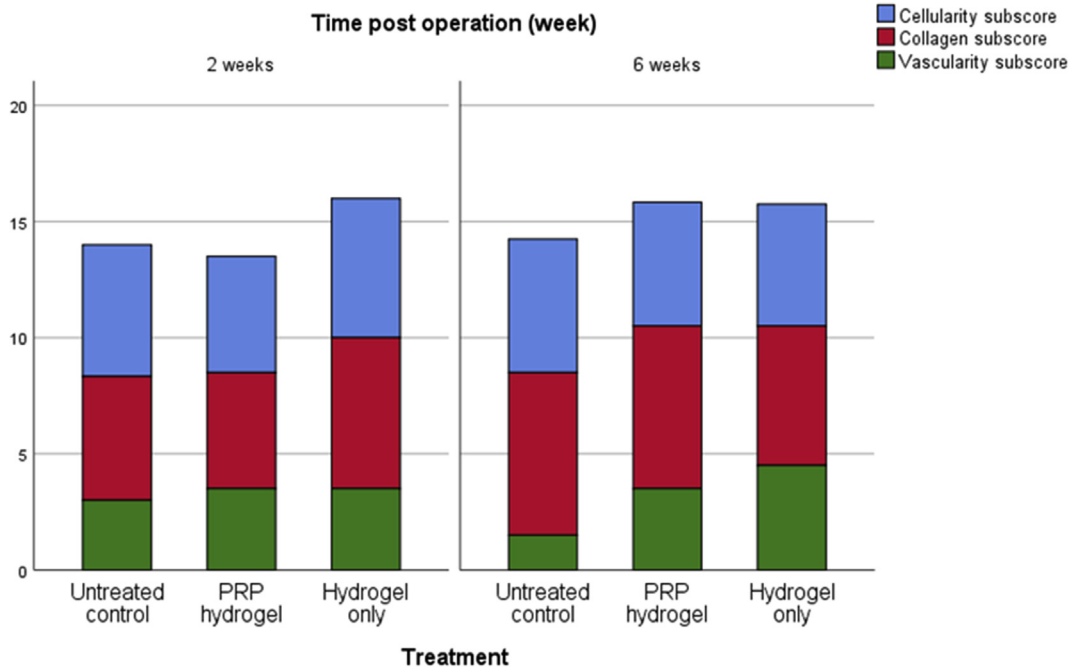

Figure 4. At 2 weeks postoperation, there was moderate to high cellularity and vascularity in the PRP-hydrogel group, as compared to no treatment control and gel only groups. At 6 weeks postoperation, a similar trend was observed.

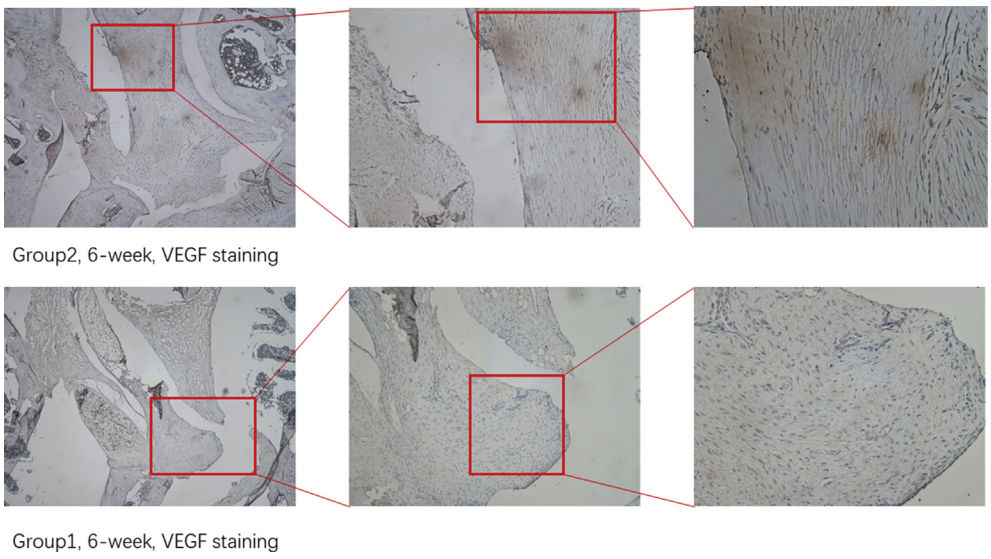

Figure 5. In Group 1 and 3, procollagen I and VEGF were not identified in the wound site at 2 and 6-week time points. In Group 2, at 2-week time point, there was no VEGF present at the wound site. However, at 6-week time point, an increased expression of VEGF was observed compared to Group 1 and 3.

extrinsic factors. The intrinsic factors referred to the healing capacity while the extrinsic factors referred to the healing environment. In an experimental study by Deie et al. [23], the intrinsic healing capacity of ACL was found to be as high as the semitendinosus tendon. In studies comparing the healing process of intra- and extra-articular ligament, they demonstrated that the response to injury is similar for both, but there is a lack of tissue bridging the rupture site of the intra-articular ligament, and the disruption of the inflammatory cascade leads to cell immigration and tissue remodelling by plasmin in the synovial fluid [9,19]. Therefore, the poor regeneration of ACL appeared mostly attributed to the extrinsic factors.

A novel biodegradable thermosensitive drug carrier (mPEG-PLGA) was first developed and optimised by Peng et al. [12,24] and Hu et al. [25]. According to their findings, the copolymer used in the present study gelates at around 36 °C, and degrades by 70% within 31 days. In Peng et al.'s research [12], the release of teicoplanin from the gel was steady and lasted up to 4 weeks. At day 31, the total amount of teicoplanin release was 70%. The near-linear release of the antibiotic from the degradable mPEG-PLGA made it a suitable drug-release system. The thermosensitive hydrogel has several potential advantages. First, with the sol-to-gel system, the hydrogel is injectable on delivery; thereafter, the gelation occurs within 30 s at around body temperature. This allows for the application of the hydrogel in a minimally invasive but efficient way. Second, the formulation and preparation of the thermos-gelling system is free of cytotoxicity. In a study by Hu et al. [25]., one month after the injection of gel into the rabbit's eyes, the HE staining showed no abnormal histology for retina tissues. In a comparison between the treated and untreated groups, no morphological or inflammatory

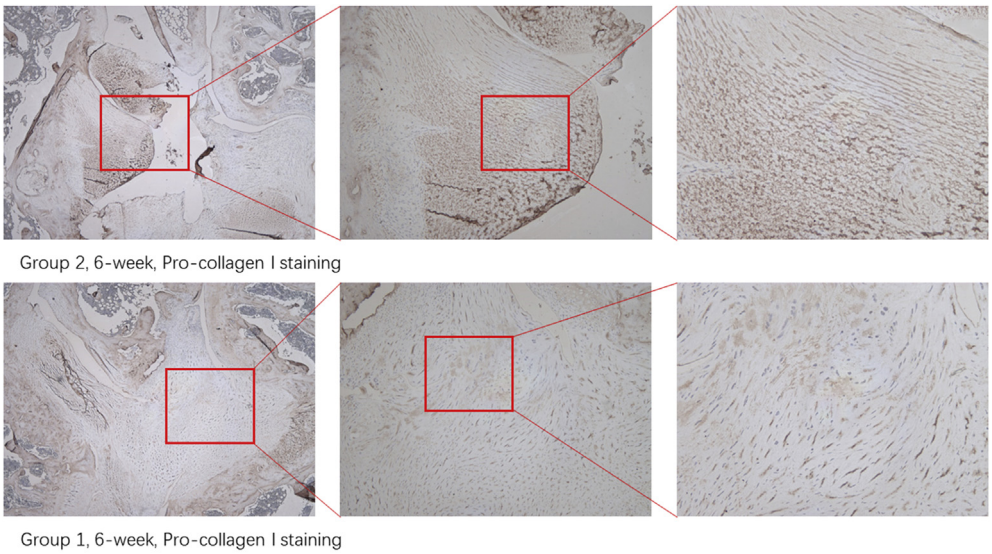

Figure 6. At 2 weeks postoperation, the procollagen I was merely present. At 6 weeks postoperation, more oriented and mature procollagen I was detected.

changes were detected. Furthermore, Peng et al. [24] injected hydrogels with 20, 25, and 30 wt% formulations intracutaneously, and demonstrated that injection of the BOX hydrogel in rabbits did not show any skin irritation over 3 days. Third, as shown by previous studies [12, 24–26], the system is of high encapsulation rate for bioactive molecules. Last, the degradation was also evaluated in vitro, and the gel permeation chromatography analysis showed up to 70% of the index copolymers were degraded within the 31-day period via hydrolysis of ester linkages [12].

A few preclinical studies focusing on the regeneration of ACL partial tear has been published [9,15,20–22,27]. Kondo et al. [22] treated midsubstance-laceration type ACL partial tear with transforming growth factor–β1 mixed with 0.2 mL fibrin sealant. The biomechanical results of the study group were significantly superior to the control group. However, the results were not restored to the normal state, which was consistent with the present study. In the study by Murray et al. [9], the H and E staining and immunohistochemical findings of the wound site at 3 and 6 weeks revealed similar distributions of protein when compared with the treated, centrally located ACL partial tear subjects. Oe et al. [20] compared ACL regeneration between rat groups subjected to intra-articular injection of fresh whole-bone marrow cells (BMCs), cultured mesenchymal stem cells (MSCs), or saline. It was shown that the level of transforming growth factor-β1 of both BMCs and MSCs group was significantly increased when compared with the saline group. In contrast to the findings of the present study, the tensile strength in the BMC group was restored to a near-normal level, whereas a recent 12-month follow-up study on the regeneration of canine partial cranial cruciate ligament tear treated with single intra-articular application of PRP-collagen demonstrated a high likelihood ratio of 19.5 ($P = 0.0015$) for progressing to a complete tear within 12-months of diagnosis [28]. Clinical studies regarding the bioenhanced regeneration of ACL partial tear was also divergent [29]. In most studies, the subjective evaluation was significantly improved at the final follow up, while the reoperation rate varied greatly from 8.9% to 36% [17,30–32]. It should be noted that either the animal researches or clinical studies mentioned above had encapsulated the bioenhancer with thermosensitive hydrogel, which solidified at body temperature, for preventing the disruption of the inflammatory cascade by the synovial fluid, but as far as we were concerned, this is the first study to adopt the thermosensitive hydrogel-PRP to enhance the regeneration of ACL partial tear. The advantage of this mPEG-PLGA-PRP complex was bifold. First, as mentioned above, this complex was capable of gelation at the body temperature of rat, which was beyond the property of saline or collagen that was utilised as a medium or capsule of a bioenhancer. Second, PRP has already been used in clinical practice for the treatment of humeral lateral epicondylitis, tendinopathy, or other sports-related injuries [26]. PRP can be easily produced from the patients' venous blood by the commercialised facility. Although MSCs, transforming growth factor-β1 or other cytokines, were as effective as PRP, the extraction of these factors was much more complicated than PRP. As PRP works by a similar mechanism, the induction of cell proliferation and angiogenic factors, it seemed that PRP is more cost-effective.

In the present study, the outcome of the subjects in the hydrogel-PRP group was biomechanically superior to the hydrogel group, and the functional healing markers procollagen I and vascular endothelial growth factor (VEGF), were detected in the study group within the wound site; it appeared that platelets and their subsequent release of growth factors is critical to a functional healing response in the ACL partial tear [33]. This finding was supported by several basic studies, which showed that PRP contained growth factors, such as VEGF, insulin-like growth factor-1 (IGF-1), transforming growth factor-1 (TGF-1), and platelet-derived growth factor type BB (PDGF-BB). These growth factors may benefit the healing response by the induction of cell proliferation and angiogenic factors [26,34]. There are three phases of tendon healing, the initial inflammatory phase, the proliferative phase, and the remodelling phase [35]. The final stage of the remodelling begins approximately 6 weeks after injury with a decrease in the cellular and vascular content of the callus tissue, and an increase in collagen type I content and density [36]. The time point of the final remodelling phase may explain the incomplete restoration of tensile strength in the study group of the present study.

The maturity index of the wound site ranged from 11 to 18 in the treatment group, which was not significantly different from the control group. This finding was consistent with Murray's results [9]. Since the index was designed to evaluate the maturity of tissue, and the maturation may proceed without the effect of the hydrogel-PRP. As reported by Murray et al. [9], other than the incorporation of cellular, vascular, and collagen property, it is the filling percentage of the wound site that determined the outcome of maturation.

The first limitation of the present study is that we did not observe the mechanism of PRP in enhancing ACL partial tear healing at the genetic level. Second, it should be acknowledged that the molecular weight of teicoplanin and BMP-2 that were previously used with mPEG-PLGA hydrogel was relatively small, so the releasing property of PRP might be different. Moreover, the follow-up time of six weeks is relatively short, as the final remodelling phase usually completes in no less than 3 months. Last, although the maturity index is a reliable system for evaluating tissue

maturation, a revised system that contains tissue maturation, degeneration, and regeneration may be required for the assessment of wound healing. Furthermore, in the current system, vascularity and cellularity score have time limits. In the early stages, increased cellularity and vascularity may have a positive effect on promoting healing, but if vascularity and cellularity remain high at the remodelling and maturation phase, it is not beneficial for the regeneration of ligament-like tissue. So, the revised scoring system should be stage-specific, and only some items apply to the early healing stages.

In conclusion, the thermosensitive hydrogel-PRP is effective in enhancing the healing of ACL partial tear in a rat model. The tensile strength of the treatment group significantly improved when compared with the control group, but not restored to the level of the normal state, and more VEGF and pro-collagen I were detected in the treatment group at 6-week follow-up. All groups shared similar levels of tissue maturation.

In terms of clinical relevance, the thermosensitive hydrogel-PRP can be a potential treatment for patients with ACL partial tear if the biosafety and ethics issue can be approved. The application of this complex will transform the treatment of ACL partial tear from a treatment dilemma to a time-saving and economic case as ultrasound or arthroscopy-guided injection.

Conflict of interest

The authors have no conflicts of interest to disclose in relation to this article.

Acknowledgements

During the experiment, Dr. Guangping Li, who is a researcher of the department of pathology of Beijing Jishuitan Hospital offered valuable help in the preparation and processing of slides.

References

[1] Colombet P, Dejour D, Panisset JC, Siebold R, French Arthroscopy S. Current concept of partial anterior cruciate ligament ruptures. Orthop Traumatol Surg Res 2010;96(8 Suppl):S109–18.
[2] DeFranco MJ, Bach Jr BR. A comprehensive review of partial anterior cruciate ligament tears. J Bone Joint Surg Am 2009;91(1):198–208.
[3] Bak K, Scavenius M, Hansen S, Norring K, Jensen KH, Jorgensen U. Isolated partial rupture of the anterior cruciate ligament. Long-term follow-up of 56 cases. Knee Surg Sport Traumatol Arthrosc 1997;5(2):66–71.
[4] Sommerlath K, Odensten M, Lysholm J. The late course of acute partial anterior cruciate ligament tears. A nine to 15-year follow-up evaluation. Clin Orthop Relat Res 1992;(281):152–8.
[5] Papalia R, Franceschi F, Zampogna B, Tecame A, Maffulli N, Denaro V. Surgical management of partial tears of the anterior cruciate ligament. Knee Surg Sport Traumatol Arthrosc 2014;22(1):154–65.
[6] Mlynarek RA, Kuhn AW, Bedi A. Platelet-rich plasma (PRP) in orthopedic sports medicine. Am J Orthoped 2016;45(5):290–326.
[7] Murray MM, Fleming BC. Biology of anterior cruciate ligament injury and repair: kappa delta ann doner vaughn award paper 2013. J Orthop Res 2013;31(10):1501–6.
[8] Murray MM, Spindler KP, Abreu E, Muller JA, Nedder A, Kelly M, et al. Collagen-platelet rich plasma hydrogel enhances primary repair of the porcine anterior cruciate ligament. J Orthop Res: Official Publication of the Orthopaedic Research Society 2007;25(1):81–91 [eng].
[9] Murray MM, Spindler KP, Ballard P, Welch TP, Zurakowski D, Nanney LB. Enhanced histologic repair in a central wound in the anterior cruciate ligament with a collagen-platelet-rich plasma scaffold. J Orthop Res 2007;25(8):1007–17.
[10] Fleming BC, Magarian EM, Harrison SL, Paller DJ, Murray MM. Collagen scaffold supplementation does not improve the functional properties of the repaired anterior cruciate ligament. J Orthop Res: Official Publication of the Orthopaedic Research Society 2010;28(6):703–9 [eng].
[11] Murray MM, Flutie BM, Kalish LA, Ecklund K, Fleming BC, Proffen BL, et al. The bridge-enhanced anterior cruciate ligament repair (BEAR) procedure: an early feasibility cohort study. Orthop J Sports Med 2016;4(11). 2325967116672176.
[12] Peng KT, Chen CF, Chu IM, Li YM, Hsu WH, Hsu RW, et al. Treatment of osteomyelitis with teicoplanin-encapsulated biodegradable thermosensitive hydrogel nanoparticles. Biomaterials 2010;31(19):5227–36.
[13] Song GY, Zhang J, Li X, Li Y, Feng H. Biomechanical and biological findings between acute anterior cruciate ligament reconstruction with and without an augmented remnant repair: a comparative in vivo animal study. Arthroscopy : The Journal of Arthroscopic & Related Surgery : Official Publication of the Arthroscopy Association of North America and the International Arthroscopy Association 2016;32(2):307–19.
[14] Gobbi A, Whyte GP. Long-term outcomes of primary repair of the anterior cruciate ligament combined with biologic healing augmentation to treat incomplete tears. Am J Sports Med 2018. 363546518805740.
[15] Dallo I, Chahla J, Mitchell JJ, Pascual-Garrido C, Feagin JA, LaPrade RF. Biologic approaches for the treatment of partial tears of the anterior cruciate ligament: a current concepts review. Orthop J Sports Med 2017;5(1). 2325967116681724.
[16] Cook JL, Smith PA, Bozynski CC, Kuroki K, Cook CR, Stoker AM, et al. Multiple injections of leukoreduced platelet rich plasma reduce pain and functional impairment in a canine model of ACL and meniscal deficiency. J Orthop Res : Official Publication of the Orthopaedic Research Society 2016;34(4):607–15 [eng].
[17] Seijas R, Ares O, Cusco X, Alvarez P, Steinbacher G, Cugat R. Partial anterior cruciate ligament tears treated with intraligamentary plasma rich in growth factors. World J Orthop 2014;5(3):373–8 [eng].
[18] Gupta A, Sharif K, Walters M, Woods MD, Potty A, Main BJ, et al. Surgical retrieval, isolation and in vitro expansion of human anterior cruciate ligament-derived cells for tissue engineering applications. J Vis Exp: J Vis Exp 2014(86).
[19] Vavken P, Murray MM. The potential for primary repair of the ACL. Sports Med Arthrosc 2011;19(1):44–9 [eng].
[20] Oe K, Kushida T, Okamoto N, Umeda M, Nakamura T, Ikehara S, et al. New strategies for anterior cruciate ligament partial rupture using bone marrow transplantation in rats. Stem Cells Dev 2011;20(4):671–9.
[21] Kanaya A, Deie M, Adachi N, Nishimori M, Yanada S, Ochi M. Intra-articular injection of mesenchymal stromal cells in partially torn anterior cruciate ligaments in a rat model. Arthroscopy : The Journal of Arthroscopic & Related Surgery: Official Publication of the Arthroscopy Association of North America and the International Arthroscopy Association 2007;23(6):610–7.
[22] Kondo E, Yasuda K, Yamanaka M, Minami A, Tohyama H. Effects of administration of exogenous growth factors on biomechanical properties of the elongation-type anterior cruciate ligament injury with partial laceration. Am J Sports Med 2005;33(2):188–96.
[23] Deie M, Ochi M, Ikuta Y. High intrinsic healing potential of human anterior cruciate ligament. Organ culture experiments. Acta Orthop Scand 1995;66(1):28–32.
[24] Peng KT, Hsieh MY, Lin CT, Chen CF, Lee MS, Huang YY, et al. Treatment of critically sized femoral defects with recombinant BMP-2 delivered by a modified mPEG-PLGA biodegradable thermosensitive hydrogel. BMC Muscoskelet Disord 2016;17:286.
[25] Hu CC, Chaw JR, Chen CF, Liu HW. Controlled release bevacizumab in thermoresponsive hydrogel found to inhibit angiogenesis. Bio Med Mater Eng 2014;24(6):1941–50.
[26] Chen X, Jones IA, Park C, Vangsness Jr CT. The efficacy of platelet-rich plasma on tendon and ligament healing: a systematic review and meta-analysis with bias assessment. Am J Sports Med 2018;46(8):2020–32.
[27] Kobayashi D, Kurosaka M, Yoshiya S, Mizuno K. Effect of basic fibroblast growth factor on the healing of defects in the canine anterior cruciate ligament. Knee Surg Sport Traumatol Arthrosc : Official Journal of the ESSKA 1997;5(3):189–94.
[28] Sample SJ, Racette MA, Hans EC, Volstad NJ, Schaefer SL, Bleedorn JA, et al. Use of a platelet-rich plasma-collagen scaffold as a bioenhanced repair treatment for management of partial cruciate ligament rupture in dogs. PLoS One 2018;13(6). e0197204.
[29] Li Y, Fu SC, Cheuk YC, Song G, Feng H, Yung SH. The non-reconstructive treatment of complete ACL tear with biological enhancement in clinical and preclinical studies: a systematic review. Asia-Pacific Journal of Sports Medicine, Arthroscopy, Rehabilitation and Technology 2018;14:10–6.
[30] Gobbi A, Whyte GP. Long-term outcomes of primary repair of the anterior cruciate ligament combined with biologic healing augmentation to treat incomplete tears. Am J Sports Med 2018;46(14):3368–77.
[31] Eggli S, Roder C, Perler G, Henle P. Five year results of the first ten ACL patients treated with dynamic intraligamentary stabilisation. BMC Muscoskelet Disord 2016;17:105 [eng].
[32] Steadman JR, Matheny LM, Briggs KK, Rodkey WG, Carreira DS. Outcomes following healing response in older, active patients: a primary anterior cruciate ligament repair technique. J Knee Surg 2012;25(3):255–60 [eng].
[33] Murray MM, Magarian E, Zurakowski D, Fleming BC. Bone-to-bone fixation enhances functional healing of the porcine anterior cruciate ligament using a collagen-platelet composite. Arthroscopy : The Journal of Arthroscopic & Related Surgery: Official Publication of the Arthroscopy Association of North America and the International Arthroscopy Association 2010;26(9 Suppl):S49–57 [eng].
[34] Spindler KP, Kuhn JE, Freedman KB, Matthews CE, Dittus RS, Harrell Jr FE. Anterior cruciate ligament reconstruction autograft choice: bone-tendon-bone versus hamstring: does it really matter? A systematic review. Am J Sports Med 2004;32(8):1986–95.
[35] Salamanna F, Veronesi F, Maglio M, Della Bella E, Sartori M, Fini M. New and emerging strategies in platelet-rich plasma application in musculoskeletal regenerative procedures: general overview on still open questions and outlook. BioMed Res Int 2015;2015. 846045.
[36] Fu SC, Cheuk YC, Yung SH, Rolf CG, Chan KM. Systematic review of biological modulation of healing in anterior cruciate ligament reconstruction. Orthop J Sports Med 2014;2(3). 2325967114526687.