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# The gelling effect of platelet-rich fibrin matrix when exposed to human tenocytes from the rotator cuff in small-diameter culture wells and the design of a co-culture device to overcome this phenomenon

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## Objectives

**Platelet-rich fibrin matrix (PRFM) has been proved to enhance tenocyte proliferation but has mixed results when used during rotator cuff repair. The optimal PRFM preparation protocol should be determined before clinical application. To screen the best PRFM to each individual's tenocytes effectively, small-diameter culture wells should be used to increase variables. The gelling effect of PRFM will occur when small-diameter culture wells are used. A co-culture device should be designed to avoid this effect.**

## Methods

**Tenocytes harvested during rotator cuff repair and blood from a healthy volunteer were used. Tenocytes were seeded in 96-, 24-, 12-, and six-well plates and co-culture devices. Appropriate volumes of PRFM, according to the surface area of each culture well, were treated with tenocytes for seven days. The co-culture device was designed to avoid the gelling effect that occurred in the small-diameter culture well. Cell proliferation was analyzed by water soluble tetrazolium-1 (WST-1) bioassay.**

## Results

**The relative quantification (condition/control) of WST-1 assay on day seven revealed a significant decrease in tenocyte proliferation in small-diameter culture wells (96 and 24 wells) due to the gelling effect. PRFM in large-diameter culture wells (12 and six wells) and co-culture systems induced a significant increase in tenocyte proliferation compared with the control group. The gelling effect of PRFM was avoided by the co-culture device.**

## Conclusion

**When PRFM and tenocytes are cultured in small-diameter culture wells, the gelling effect will occur and make screening of personalized best-fit PRFM difficult. This effect can be avoided with the co-culture device.**

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**Keywords:** Rotator cuff tears, Tenocytes, Platelet-rich fibrin matrix, Co-culture, Gelling effect

## Article focus

- The optimal platelet-rich fibrin matrix (PRFM) preparation protocol for different stages of rotator cuff tears in different patients is unknown.
- A screening method for personalized best-fit PRFM should be developed to test as many tenocytes/PRFM interactions as possible.
- The gelling effect of PRFM occurs when small-diameter culture wells are used.

## Key messages

- The gelling effect of PRFM occurs in small-diameter culture wells, but not in large ones.
- The design of the co-culture device could avoid the gelling effect of PRFM and facilitate tenocyte/PRFM studies.

- This platform could be further used to screen personalized best-fit PRFM preparation protocols according to disease stage and severity.

## Strengths and limitations

- The *in vitro* positive effects were not confirmed in all the *in vivo* studies because of the many variables affecting the success rate in a complex scenario where both PRFM and lesion site play a crucial role.
- The co-culture design provides the possibility of different cell interactions with PRFM in the same environment since there are two 'cell chambers'. Different cells could be co-cultured together to observe their interactions, which mimic real conditions.
- Only transforming growth factor (TGF)-β and interleukin (IL)-1β were analyzed in this study. Further work should be focused on analyzing different growth factors at different timepoints.

## Introduction

Platelet-rich plasma (PRP) is an autologous blood-derived product that includes many growth factors.[1] Among them, platelet-rich fibrin matrix (PRFM) is especially appealing. This preparation creates a product with a gel-like structure that allows for gradual elution of growth factors, which could be applied between the tendon-bone interface during rotator cuff repair. PRFM can also serve as a biomimetic scaffold, acting as a reservoir of growth factors and cytokines. The molecules essential for tendon repair, such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF)-1, are functional and abundant in PRFM scaffolds.[2] With these bioactive molecules, enhanced tenocyte growth and proliferation, increased extracellular matrix production, and upregulation of tenocyte gene expression could happen in PRFM scaffolds. Since rotator cuff repairs are always limited by a paucity of vascularity, it is important to have a sufficient supply of nourishment and growth factors in developing cell-based therapeutic approaches.

Although PRFM has been proved to help tenocyte proliferation, the efficacy of its clinical use during rotator cuff repair has shown controversial results.[3-5] These inconsistent results were attributed to the fact that PRFM preparations are not consistently defined and are obtained through numerous methods. The component concentrations, activation method, application state, number, and preparation method have not been well controlled.[6]

On the other hand, the age and sex of each individual from whom tenocytes are harvested may contribute to these complicated situations.[7] Therefore, a standardization should be developed based on the preparation of PRFM according to each patient's clinical condition and when PRFM augmentation is considered.

In order to screen for the best-fit correlation between different preparations of PRFM when exposed to tenocytes from patients of a different age and sex, as many tenocytes as possible should be sub-cultured to provide an adequate amount for further cross-matching of PRFM/tenocyte interactions. However, multiple passages of tenocytes lead to phenotypic drift, and this poses a problem in tenocyte research because freshly cultured tenocytes are not readily available in sufficient quantities. Studies have shown that there is the potential for tenocyte phenotypic drift after prolonged maintenance in monolayer cell culture.[8] Therefore, only cells within the first three passages would be recommended for tenocyte studies.[9] In order to screen as many PRFM/tenocyte stimulation conditions as possible, and to avoid phenotypic drift of tenocytes, smaller-diameter culture wells should be used because the cell seeding density of each experiment should be the same.

However, bioactive factors such as adhesive proteins, clotting, and fibrinolytic factors may come with the production of PRFM and produce some adverse effect other than growth factors when co-cultured with tenocytes. Hoppe et al[10] found that the gelling effect of PRP would be a major problem for tenocyte culturing. However, most of the published studies regarding PRP and tenocytes did not disclose the gelling effect. Among them, conventional six-well plates were used for co-culture of tenocytes and PRPs.[11,12]

We hypothesized that the gelling effect occurs depending on the size of the culture wells used during tenocyte and PRFM studies. The gelling effect may occur in small-diameter culture wells rather than in large ones. A special co-culture platform should be designed to avoid the gelling effect. This platform could be used to screen the best preparation protocol of PRFM when co-cultured with tenocytes from the rotator cuff tear of individuals of a different age and sex, with different chronicity, and size of tear. The optimal PRFM formulation, platelet activation status, and dosing regimen of each individual could be determined by this platform, making personalized medicine possible.

## Materials and Methods

**Isolation of human tenocytes.** Human tenocytes were isolated from a torn edge of the supraspinatus tendon of a 73-year-old female patient, which was approved by the Institutional Review Board at the Chang Gung Memorial Hospital, Taiwan (IRB number 201601492A3). Tendon samples were treated as previously described by Chiu et al.[13]

**Seeding tenocytes into culture wells.** A total of two $10^4$ tenocytes/$cm^2$ were seeded in conventional six-, 12-, 24-, and 96-well plates (Corning Multiple Well Plates, Corning, New York) with different diameters (six-well plates, bottom diameter/growth area, 34.8 mm/9.5 $cm^2$; 12-well plates, 22.1 mm/3.8 $cm^2$; 24-well plates, 15.6 mm/1.9 $cm^2$; 96-well plates, 6.4 mm/0.32 $cm^2$) and co-culture devices (growth area, 0.67 $cm^2$).

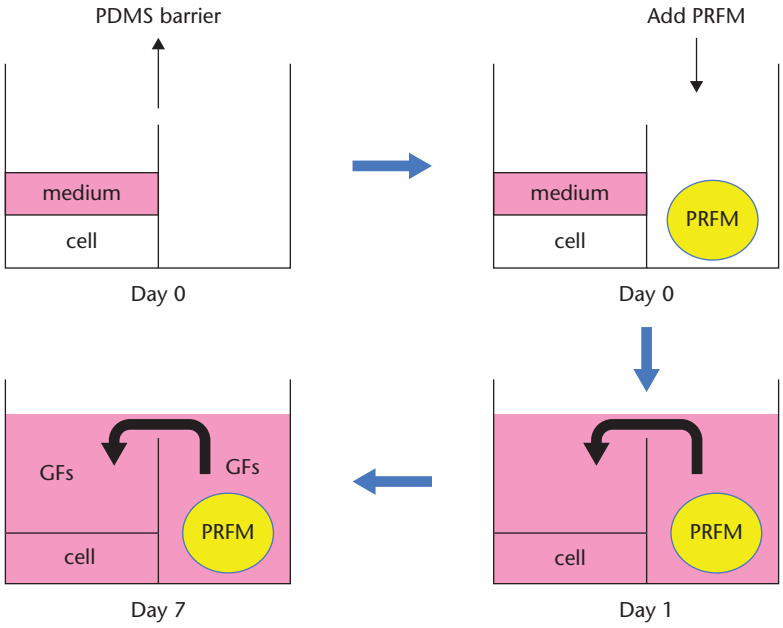


Fig. 1

The concept of a co-culture device to avoid the gelling effect. Top left) cell seeding in the cell chamber. Top right) platelet-rich fibrin matrix (PRFM) added into another chamber (PRFM chamber) without cell seeding. PRFM gelling may not affect the cell in the chamber because of the polydimethylsiloxane (PDMS) barrier. Bottom right) add culture medium into the PRFM chamber until it crosses over the PDMS barrier. Bottom left) growth factors in PRFM with lighter gravity dispersed in the culture medium are disseminated to the cell chamber to stimulate tenocyte proliferation. GF, growth factor.

**PRFM preparation.** The PRFM was prepared with the modified technique described by Choukroun et al.[14] A blood sample was collected from a healthy volunteer. Two sets of 9 ml blood and 1.25 ml citrate phosphate dextrose adenine-1 were mixed and agitated gently in a plain tube (BD Diagnostics, Franklin Lakes, New Jersey). PRFM was prepared using a two-step centrifugation with a table-top centrifuge machine, in which step one centrifugation (separating centrifugation) used 1500 rpm for five minutes and step two centrifugation (condensation centrifugation) used 6300 rpm for 15 minutes. Calcium chloride ($CaCl_2$) was added after the second centrifugation step and resulted in the formation of a dense fibrin matrix. Intact platelets were subsequently trapped in the fibrin matrix. To prepare PRFM-conditioned media, the PRFM were then soaked in 10 ml of serum-free Dulbecco's Modified Eagle's Medium (Gibco, Thermo Fisher Scientific, Waltham, Massachusetts) in a centrifuge tube. The conditioned medium was then collected for further use. A 1 ml sample of each native blood specimen and each preparation was analyzed by a complete blood counter (Sysmex XT-1800i automated hematology analyzer; Sysmex Corp., Kobe, Japan). The platelet ratio in PRFM was defined as the platelet number of the prepared PRFM divided by the platelet number of the whole blood sample.[15]

**Design and fabrication of the co-culture device.** To eliminate the gelling effect of PRFM, the idea of a special co-culture system was developed as shown in Fig. 1 The basic concept of the system is to separate the higher weight materials (e.g. adhesive proteins, clotting, and fibrinolytic factors) that cause a gelling effect from growth factors produced within PRFM, which is supposed to be distributed in the culture medium.

A culture well of the co-culture device was 15.6 mm in diameter and 7 mm in height, as shown in Fig. 2a. It was composed of three sub-chambers separated by a barrier of 3 mm in height and 1 mm in width. The co-culture device consisted of a glass substrate and polydimethylsiloxane (PDMS) layer (Sylgard 184; Dow Corning, Midland, Michigan). Two of the sub-chambers are for cell seeding (cell chamber), and the other for the PRFM. First, PRFM was added to the PRFM chamber to avoid the gelling effect (Fig. 2b). After the PRFM consolidated with a gel-like form, a culture medium (Gibco, Thermo Fisher Scientific) was added into the PRFM chamber until the fluid level crossed over the separation PDMS barrier (Fig. 2c). Using this method, growth factors dispersed in the culture medium would be disseminated to the cell seeding chamber to stimulate tenocyte proliferation.

**Treatment of tenocytes with PRFM in different size culture wells and co-culture device.** After cell attachment for 24 hours, tenocytes were treated with 10 µl of PRFM in six-, 12-, 24-, and 96- conventional culture well plates and the co-culture device. In conventional culture wells, PRFM was added directly into culture wells along with tenocytes. In the co-culture device, PRFM was added into the PRFM chamber first (Fig. 2b). After consolidation of PRFM within 24 hours, 120 µl of culture medium was applied to top up the PRFM chamber until it passed over the PDMS

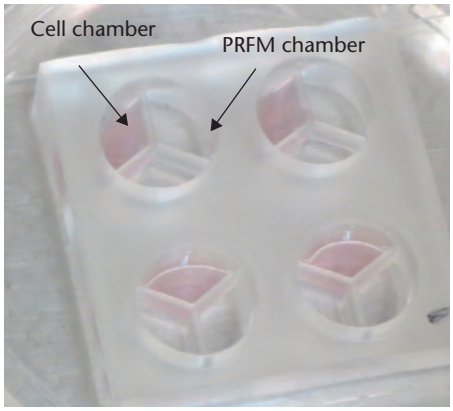


Fig. 2a

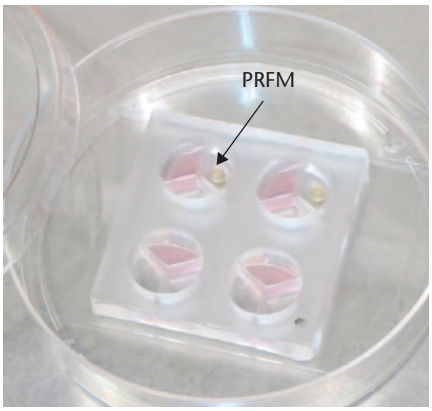


Fig. 2b

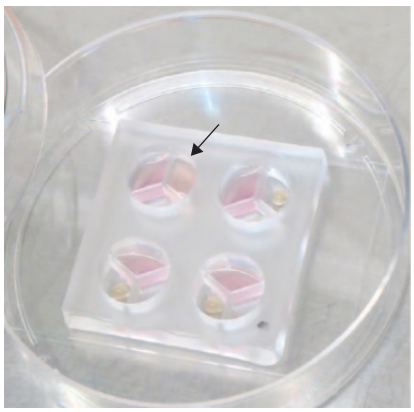

Fig. 2c

The co-culture device design. a) Tenocytes seeded in the cell chamber. b) Platelet-rich fibrin matrix (PRFM) was added into the PRFM chamber to avoid the gelling effect. c) Culture medium (arrow) was added into the PRFM chamber until the fluid level crossed over the separation polydimethylsiloxane (PDMS) barrier, causing growth factors dispersed in the culture medium to be disseminated to the cell chamber to stimulate tenocyte proliferation.

**Table I.** Primers for reverse-transcription polymerase chain reaction to determine tenocyte gene expression.

| Gene | Primer sequence | Length (bp) |
|---|---|---|
| GAPDH | Sense: GAGTCCACTGGCGTCTCCAC<br>Antisense: GGTGCTAAGCAGTTGGTGGT | 188 |
| Type I collagen | Sense: GGCCCAGAAGAACTGGTACA<br>Antisense: GGCTGTTCTTGCAGTGGTAG | 200 |
| Type III collagen | Sense: CCAGGAGCTAACGGTCTCAG<br>Antisense: CAGGGTTTCCATCTCTTCCA | 103 |
| Decorin | Sense: TGCTGTTGACAATGGCTCTC<br>Antisense: GCCTTTTTGGTGTTGTGTCC | 192 |
| Tenascin-C | Sense: TCAAGGCTGCTACGCCTTAT<br>Antisense: GTTCTGGGCTGCCTCTACTG | 230 |
| Scleraxis | Sense: CCTGAACATCTGGGAAATTTTAC<br>Antisense: CGCCAAGGCACCTCCTT | 111 |

GAPDH, glyceraldehyde 3-phosphate dehydrogenase

barrier, causing exchange of culture medium between the cell chamber and PRFM chamber (Fig. 2c). Hence, tenocytes cultured in the cell chamber could receive PDGFs by diffusion. Non-treated cells were used as a control. The treatment lasted for seven days.

Since the diameter of each culture well was different, we added different volumes of PRFM according to the growth area of each well. Approximately 300 µl of PRFM was added directly to the conventional six-well plate, 120 µl in the 12-well plate, 60 µl in the 24-well plate, 10 µl in the 96-well plate, and 20 µl in the PRFM chamber of the co-culture device. After consolidation of PRFM, culture medium was added into the PRFM chamber as previously described. All experiments were performed in triplicate.

**Cell proliferation assay: WST-1.** Cell proliferation was analyzed 24 hours after PRFM exposure with a water soluble tetrazolium-1 (WST-1) kit (Roche, Basel, Switzerland) as previously described.[13]

**Quantitative real-time polymerase chain reaction assay.** Ribonucleic acid (RNA) was isolated from cells in different culture wells and co-culture device using TRIzol Reagent (Invitrogen, Carlsbad, California) as previously described.[9] TaqMan Gene Expression Assays (Thermo Fisher Scientific) were obtained for the following genes: type I collagen, type III collagen, tenascin-C, decorin, and scleraxis, relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the endogenous control (Table I).

**Growth factor analysis.** During the cell culture period, the spent medium was collected for the analysis of growth factors; transforming growth factor (TGF)-β and interleukin (IL)-1β were chosen for the analyses. Active TGF-β and IL-1β concentrations were determined using enzyme-linked immunosorbent assay (ELISA) in duplicate aliquots of all samples with the Quantikine Human Immunoassay kits (R&D Systems, Minneapolis, Minnesota).

**Statistical analysis.** Each experiment was performed in triplicate. To compare readouts of WST-1 cell proliferation assays among different culture conditions, analysis of variance, followed by Tukey multiple comparison tests, was used. Differences were considered statistically significant when p-values were less than 0.05. All statistical analyses were performed with the SPSS program (version 21.0; IBM, Armonk, New York).

## Results

**Characteristics of PRFM.** The prepared PRFM were red blood cell (RBC)- and white blood cell (WBC)-poor PRPs (Mishra type 4A).[16] The mean platelet, RBC, WBC, and differential counts are shown in Table II. Transforming growth factor-β and IL-1β concentrations are also presented in Table II.

**Tenocytes treated with the same volume of PRFM.** After PRFM was produced by activation of $CaCl_2$, 10 µl of it was added to different sizes of culture wells and co-culture systems. The relative quantification (condition/control) of WST-1 assay on day seven revealed a significant decrease in tenocyte proliferation in small-diameter culture wells (96 and 24 wells) because of gelling material formation in these wells (Fig. 3), though not in large-diameter culture wells (12 and six wells).

**Table II.** Characteristics of platelet-rich fibrin matrix (PRFM) used.

| Characteristic | Baseline; SD | PRFM; SD | Ratio compared with baseline; SD |
|---|---|---|---|
| Platelet count × $10^3$/μl | 251.75; 31.82 | 1648; 594.21 | 6.46; 1.92 |
| RBC count × $10^6$/μl | 5.21; 0.68 | 0.17; 0.05 | 0.03; 0.01 |
| WBC count × $10^3$/μl | 7.89; 0.99 | 2.91; 0.97 | 0.37; 0.13 |
| **Growth factor concentration, ng/ml** | | | |
| TGF-β1 | 166.19; 87.90 | 230.69; 151.33 | N/A |
| IL-1β | 0.06; 0.01 | 0.07; 0.01 | N/A |

RBC, red blood cell; WBC, white blood cell; TGF, transforming growth factor; IL, interleukin; N/A, not applicable

Fig. 3

The gelling effect (arrow) of platelet-rich fibrin matrix (PRFM) occurred in the 96-well plate.

On the other hand, tenocytes in a co-culture system revealed significantly increased proliferation when compared with the control group. This condition illustrated the detrimental gelling effects of PRFM when exposed to tenocytes in smaller-diameter culture wells, though not in larger ones or co-culture devices (Fig. 4a).

**Tenocytes treated with different volumes of PRFM according to the surface area of each culture well.** Different amounts of PRFM were added to culture wells of different sizes and co-cultured according to their surface area. The relative quantification (condition/control) of WST-1 assay on day seven again revealed a significant decrease in tenocyte proliferation in small-diameter culture wells (96 and 24 wells), but not in large-diameter culture wells (12 wells). When 300 μl of PRFM was added to the six-well plate, the WST-1 assay had a significant increase compared with that of the control group, which implied that PRFM had stimulated the proliferation of tenocytes. This phenomenon of PRFM stimulating tenocyte proliferation has been highlighted in two studies (Fig. 4b).[17,18] When 20 μl of PRFM was added to the co-culture device, the same stimulating phenomenon of PRFM on the tenocytes was observed. This implied that, with the special co-culture design, the gelling effect was avoided even in small-diameter culture wells, and PRFM could work as an augmentation factor to enhance tenocyte proliferation (Fig. 4b).

**Microscopic view of tenocytes treated with PRFM in the conventional culture well and co-culture device.** The gelling effects of PRFM containing adhesive proteins, fibrinogen, and platelet aggregates were observed in conventional culture wells (96, 24, 12, and six wells) (Fig. 5a). When PRFM was added to the PRFM chamber of the co-culture device, rather than directly into the cell chamber as previously described (Fig. 2b and 2c), contents with heavier weight, such as adhesive proteins and platelet aggregates, were less present in the cell chamber. In contrast, lighter materials, such as TGF-β and IL-1β, would be dispersed in the culture medium and disseminated to the cell chamber to enhance tenocyte proliferation (Fig. 5b).

## Discussion

In this study, we confirmed that the gelling effect appears in small-diameter culture wells but not in large-diameter wells. A special co-culture platform was designed to avoid the gelling effect. This platform could be further used to screen the best preparation protocol of PRFM when co-cultured with tenocytes from different individuals.

Emerging strategies have been suggested to improve the biological environment around the repair site because of the high re-tear rate of open, mini-open, or arthroscopic rotator cuff repairs.[19-22] PRP is promoted as an ideal biological autologous blood-derived product.[23] PRFM, a subset of the PRP family, has been shown to have an advantage over PRP in that its structural integrity allows growth factors to be localized and released in a controlled way at the tendon-bone interface, and promotes collagen organization, fibre pattern alignment, tenocyte proliferation, and type I collagen synthesis.[24]

However, in contrast with the positive potential of PRFM reported in basic research literature, clinical outcomes have reported controversial results.[6] Gumina et al[4] used PRFM on the tendon repair site. They found a 14% re-tear rate in isolated posterosuperior cuff tears in the PRFM group compared with a 50% re-tear rate in the control group at 13 months. Castricini et al[3] performed a randomized double-blind trial to evaluate the effect of the same PRFM formulation used during rotator cuff repair and found no significant difference in the Constant score

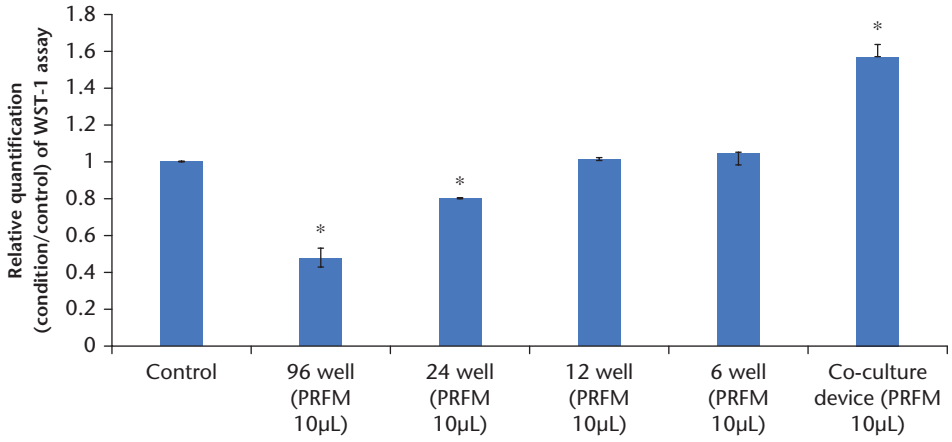


Fig. 4a

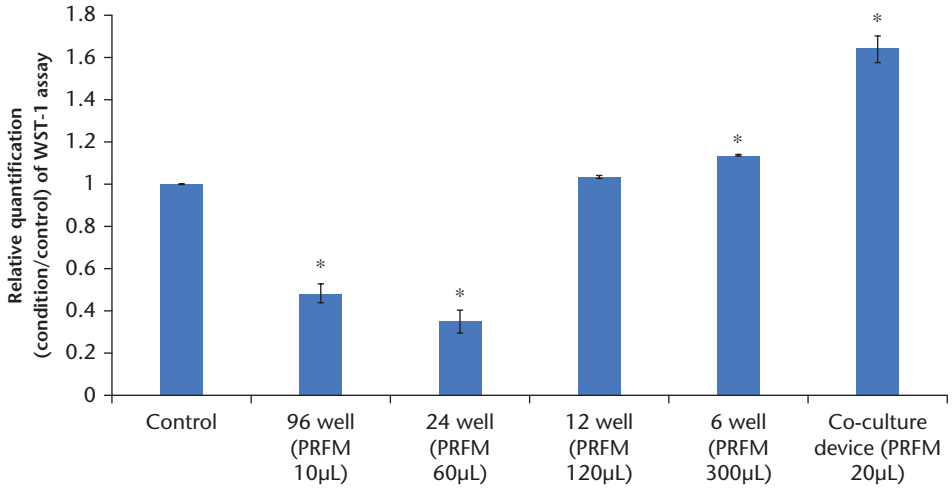


Fig. 4b

Tenocytes treated with the same and different volumes of platelet-rich fibrin matrix (PRFM). a) Tenocytes treated with 10 μl PRFM in different culture wells. The PRFM gelling effect was observed in small-diameter culture wells (96- and 24-well plates) but not in large-diameter culture wells (12- and six-well plates) or co-culture devices. There was a significant decrease in tenocyte proliferation in small-diameter culture wells. b) Tenocytes treated with different volumes of PRFM according to the surface area of each culture well. The PRFM gelling effect was observed in small-diameter culture wells (96- and 24-well plates) but not in large diameter culture wells (12- and six-well plates) and co-culture device. There was a significant decrease in tenocyte proliferation in small-diameter culture wells and a significant increase in tenocyte proliferation in the six-well plate and co-culture device. *p-value < 0.05.

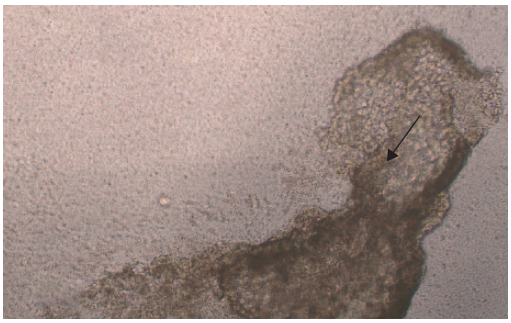

Fig. 5a

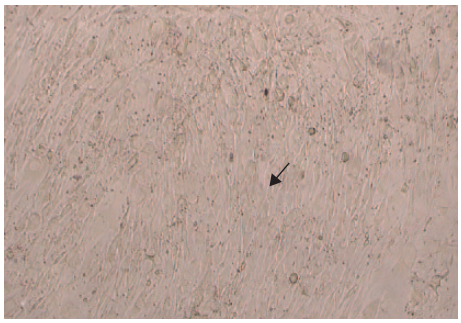

Fig. 5b

Platelet-rich fibrin matrix (PRFM) in the conventional culture well and co-culture device on day seven. a) Platelet aggregates (arrow) were found in the conventional 96-well plate. Decreased tenocyte proliferation was caused by the gelling effect of PRFM due to direct contact of tenocytes and PRFM. b) The PRFM gelling effect was avoided by the use of the co-culture device. Tenocytes (arrow) were more organized in the co-culture device than in the conventional 96-well plate.

when compared with controls. In contrast, Rodeo et al[5] published their results of applying PRFM to the tendon-bone interface at the time of rotator cuff repair and suggested that PRFM may have a negative effect on

tendon-to-bone healing. One important reason for this might be the difference in PRFM used in different studies. The other reason may be that tenocytes harvested from people of a different age and sex had different characteristics.[7,25] Klatte-Schulz et al[26] published in 2013 that tenocytes of older female donors had a weaker stimulation potential compared with those from younger female and older male donors.

It is not practical in routine practice to standardize the preparation and application of PRFM, let alone determine the best PRFM for each individual with a different age and sex. Therefore, an *in vitro* platform to screen the best PRFM stimulations to tenocytes from different individuals provides opportunities to identify a personalized best-fit treatment. To achieve this goal, as many tenocytes as necessary should be provided for a thorough screening. However, damaged tendons from the torn rotator cuff have a very limited capacity for regeneration.[27] Subculture is warranted to provide enough tenocytes to screen the best-fit PRFM preparation. It is known that multiple passages of cells lead to phenotypic drift. Gene expression of types I and III collagen for cultured tenocytes significantly decreased after two passages. As a precaution, only cells within the first three passages should be used for *in vitro* monolayer cell models.[9] This poses a problem in tenocyte research because freshly cultured tenocytes are not readily available in a sufficient amount. Hence, a smaller-scale culture system should be used in order to reduce the quantity of tenocytes needed. According to our results, the gelling effect was observed when smaller-diameter culture wells (96- and 24-well plates) were used but not in larger-diameter culture wells (12- and six-well plates) and the co-culture device. Hoppe et al[10] proposed that the gelling effect of PRP was a major problem of tenocyte culture, but this phenomenon was not well observed by most PRP studies.[10] One possible explanation is that these studies used relatively larger-diameter plates, such as a six-well plate.[11,12,28] When smaller-diameter culture wells were used, the gelling effects would create an unwanted detrimental effect and hamper the stimulating properties believed to facilitate tenocyte proliferation. With the design of the co-culture device, the unwanted part of PRFM (adhesive proteins, fibrins, fibrinogens) with greater weight would be precipitated in the PRFM chamber, and growth factors that really stimulated tenocyte proliferation would be dispersed to the cell chamber, because they were much lighter.

Our study has several limitations. First, the *in vitro* positive effects were not confirmed in all of the *in vivo* studies because of the many variables affecting the success rate in a complex scenario where both PRFM and lesion site play a crucial role. However, conventional cell culture methods could not represent 'real life' either, which is the inherited limitation of all *in vitro* studies. In contrast, the co-culture design provides the possibility of different cell interactions with PRFM in the same environment since there are two 'cell chambers'. Different cells (e.g. tenocytes and bone-marrow-derived mesenchymal stem cells harvested from the proximal humerus during cuff repair) could be co-cultured to observe their interactions, which mimic real conditions when cuff repair is carried out along with multiple channeling techniques of the greater tuberosity.[29] Second, the objective of rotator cuff repair is to re-establish the normal insertion site of the tendon-bone junction. This study only provides a platform to see how PRFM stimulates tenocyte proliferation. The use of monolayer cultures does not reproduce the physiological environment of tenocytes, but high cell concentrations are necessary for appropriate collagen synthesis to take place, especially in the tendon-bone junction of the rotator cuff. Third, we only analyzed TGF-β and IL-1β because of their anabolic and catabolic roles in tendon healing. There are many growth factors in PRFM affecting tendon healing and regeneration. Further work should be focused on analyzing different growth factors, collecting from spent medium as much as possible at different timepoints during the culture. In that way, the interaction of different cells and growth factors could be recorded for further analysis. The design of the co-culture device, with multiple cell chambers developed according to the concept of the index study, not only helps us to avoid the PRFM gelling effect in a small-scale culture device but also provides a chance to culture different cells and PRFM in the same environment, mimicking the complicated *in vivo* conditions. Fourth, the *in vitro* individual best-fit PRFM preparation may not be applied simultaneously with rotator cuff repair because it takes around three weeks for tenocyte isolation and co-culture with PRFM in the co-culture device. However, the optimal timing and dosing frequency of PRFM to enhance tendon healing are still controversial. To date, all previous PRP-augmented rotator cuff studies have used zero delivery at the time of surgical repair but with mixed results.[30] Therefore, the best time for biological augmentation in the tendon healing cascade should be further investigated.

In conclusion, when PRFM and tenocytes are cultured in a small-diameter culture well, the gelling effect will occur and make screening of personalized best-fit PRFM difficult. This effect can be avoided with the design of the co-culture device, which makes it possible to investigate the relationship between different growth factor kinetics and cell proliferation in a small-diameter culture well.

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**Author contributions**

- C-H. Chiu: Created the ideas, Formulated the research goals, Wrote the paper.
- P. Chen: Performed the statistics, Provided advice regarding further clinical application of the co-culture platform for elderly people with rotator cuff tears.
- W-L. Yeh: Provided the study material on tenocytes culture.
- A. C-Y. Chen: Provided the study material on tenocytes culture.
- Y-S. Chan: Provided the experience in platelet-rich fibrin matrix preparation.
- K-Y. Hsu: Provided the experience in platelet-rich fibrin matrix preparation.
- K-F. Lei: Provided the technical support of co-culture design.

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**Conflict of interest statement**

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- There were no competing interests among all authors.

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- The study was approved by the Institutional Review Board at the Chang Gung Memorial Hospital, Taiwan (IRB 201601492A3).