

The mechanism of Platelet-Rich Fibrin induced accelerated healing of meniscus injury

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INTRODUCTION: Meniscus injuries are refractory, and there are cases in which good treatment results cannot be obtained, even with sutures. Therefore, the development of new treatment methods is being sought. Currently, an autologous blood product, fibrin clot (FC), is used in clinical practice to increase the healing rate of meniscus sutures, but the results have not been satisfactory. Platelet-rich fibrin (PRF) is an easily prepared gelatinous autologous blood product with higher concentrations of growth factors than FC. In addition, it has superior sustained release properties, making it a potential new treatment for meniscus injuries (Fig 1). This study aims to evaluate the effect of PRF on meniscus injury and determine the effect of PRF on meniscus cells and synovial stem cells and their molecular mechanisms.

METHODS: In vivo, a control, FC, and PRF group were created using a rat model of meniscus defect to evaluate the effect of PRF on the meniscus injury. We created a rat model of meniscus deficiency by completely resecting the anterior half of the medial meniscus. FC/PRF was placed into the defect site, sewing with nylon sutures (Fig 1B). PRF was prepared from rat blood according to international guidelines in the literature [1] (Fig 1C). Menisci were harvested at 8 and 12 weeks postoperatively for macroscopic and microscopic evaluation to assess histologic healing of the meniscus injury. Macroscopically, the resected meniscus was set in terms of mass, area, and meniscus regeneration ratio. Microscopically, pathology was evaluated using the Modified Pauli score and the number of cells and blood vessels. Basso, Beattie, and Bresnahan's (BBB) score was used to evaluate functional motor recovery [2]. We examined the effects of PRF in vitro using meniscus cells isolated from rat meniscus and synovial stem cells isolated from rat synovium. The number of viable cells was measured by MTS assay to assess the proliferation of cells. The migration of meniscus cells was evaluated by wound healing assay. Immunoblotting and immunofluorescence were performed to evaluate the protein expression levels of type 2 collagen. The phosphorylation level of AKT was determined by immunoblotting. Inhibitory experiments were performed using MK-2206 (AKT inhibitor) and FIIN-2 (FGF receptor inhibitor). All animal care and experiments were conducted following the institutional guidelines of the Animal Committee of Mie University. Continuous and categorical variables were compared using the Student t-test and one-way analysis of variance (one-way ANOVA). All p values presented are two-sided; p values < 0.05 were considered statistically significant.

RESULTS: Initially, we evaluated the effects of PRF on meniscus healing in vivo using the rat meniscal defect model. The macroscopic evaluation at 8 and 12 weeks postoperatively showed that meniscus weight, meniscus area, and meniscus regeneration ratio were significantly increased in the PRF group compared to the control and FC groups (Fig 2A). In addition, histological examination revealed that the degree of meniscus repair (Modified Pauli score) was significantly enhanced in the PRF group compared to the control and FC groups, and cell and vessel counts were also significantly increased (Fig 2B). These data suggested PRF accelerated the healing of meniscus injury compared to the control and FC groups. We also found that PRF promoted the recovery of motor function compared to the control and FC groups (Fig 2C). Next, we examined the effects of PRF on the meniscus cell and synovial stem cell in vitro. We found that the PRF group significantly promoted the proliferation and migration of meniscus and synovial stem cells compared to the control and FC groups (Fig 3A and 3B). In addition, the PRF significantly increased the protein expression of type 1 and 2 collagen in meniscus cells compared to the Control and FC groups by fluorescence staining and immunoblotting (Fig 3C). Finally, we examined the signaling pathway associated with PRF-induced effects on meniscus cells. PRF treatment increased the phosphorylation level and induced nuclear translocation of AKT in meniscus cells (Fig 3D upper), and the inhibition of AKT or FGF receptor (FGFR) significantly suppressed the proliferative effect of PRF (Fig 3D lower), indicating the involvement of FGFR/AKT signaling.

DISCUSSION: It has been reported that during the repair process after common meniscal injuries, synovial stem cells proliferate and migrate to the site of injury to differentiate into meniscal cells. In addition, multiple growth factors may promote meniscal repair. This study shows that PRF containing various high concentrations of growth factors stimulates meniscal cells and synovial stem cells to promote the healing of meniscal injury.

SIGNIFICANCE: PRF stimulates meniscal cells and synovial stem cells to promote the healing of meniscal injury.

REFERENCES: [1] Preparing Platelet Rich Fibrin: Techniques. www.a-prf.com/en/i-prf.html/ preparing_platelet_rich_fibrin. [2] Basso DM+, J Neurotrauma. 1995.

Figure 1: Hypothesis and Method (in vivo)

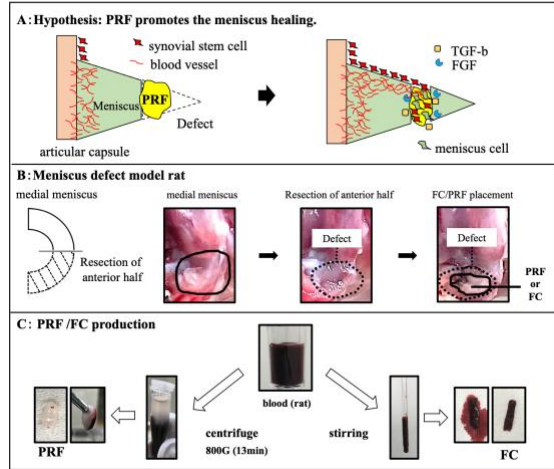


Figure 2: Effects of PRF in a rat model of meniscus injury (in vivo)

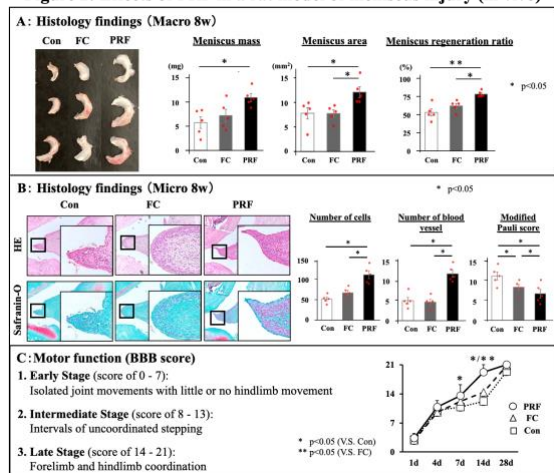


Figure 3: Effects of PRF on cells (in vitro)

