

# **Platelet-rich fibrin (PRF) accelerates the healing of Achilles tendon defect by promoting the proliferation and activation of tenocytes via FGFR/AKT and TGF- $\beta$ /SMAD3 signaling**

## **INTRODUCTION:**

For orthopedic surgeons, an Achilles tendon defect is challenging to treat, so developing new treatments is desirable. Platelet-rich fibrin (PRF), dense fibrin scaffold composed of a fibrin matrix containing many growth factors. This study aims to investigate whether PRF accelerates the healing of Achilles tendon injuries and to elucidate further the mechanisms involved.

## **METHODS:**

To create a rat model for Achilles tendon defect, a 4-mm portion of the right Achilles tendon was wholly resected and filled in the gap with PRF collected from rat blood. We assessed the healing of injured tendons through histology, motor functional recovery, and biomechanical properties. In vitro, we assessed the number of viable or proliferative cells and the migration capacity after treatment of PRF using tenocytes isolated from rat Achilles tendon. The extracellular matrix's protein or gene expression level was evaluated by immunofluorescence staining or PCR. In addition, the phosphorylated protein was evaluated by western blotting. Finally, to examine how PRFs signal to tenocytes, we performed inhibition experiments using AKT, FGFR, TGF- $\beta$ R, and SMAD3 inhibitors.

## **RESULTS:**

In the rat model for Achilles tendon defects, the number of tenocyte-like cells at the injury sites increased in the PRF group compared to the control group. Furthermore, the PRF group also increased proliferating tenocytes (SCXA+Ki-67+) and activating tenocytes (SCXA+ $\alpha$ -SMA+). Rat in the PRF group had more mature and better-aligned collagen deposits and showed an early increase in blood vessels and lymphatic vessels at the injury sites. Consistent with the histological findings, PRF improved motor function (BBB score) and the biomechanical properties of the injured Achilles tendon.

In vitro, PRF increased the number of viable and proliferative cells and promoted migratory ability in tenocytes. Also, PRF promoted the maturation of tenocytes and increased the protein and gene expression levels of collagen-I, collagen-III,  $\alpha$ -SMA, and tenascin-C. Finally, we examined how PRF transduces the signal to tenocytes. PRF induced the phosphorylation of AKT/FGFR and the nuclear translocation of AKT. Inhibition of AKT or FGF-receptor suppressed the positive effects of PRF on tenocytes. We further found that PRF promoted the phosphorylation of and the nuclear translocation of SMAD3. Furthermore, inhibition of SMAD3 or TGF- $\beta$ R suppressed the PRF-induced expression levels of the extracellular matrix, whereas the inhibition of the TGF- $\beta$ R/SMAD did not affect the proliferative effects. Consistent with in vitro data, AKT and SMAD3-positive tenocytes were increased in the PRF group in vivo.

## **CONCLUSION:**

We clarified that PRF accelerated the healing of Achilles tendon defect by promoting the growth and activation of tenocytes. Furthermore, PRF increases the proliferation ability and extracellular matrix protein expression level in tenocytes via the FGFR/AKT and TGF- $\beta$ R/SMAD3 axis, respectively.