P 1-94

Slow releasing platelet derived growth factor could improve tendinitis; in vitro study.

Seok Kang[1*†], Joon Shik Yoon[1], Sung Eun KiM[2], Hyuk Sung Choi[1]

Korea University Guro Hospital, Department of Rehabilitation Medicine[1], Korea University Guro Hospital, Department of Orthopedic Surgery and Rare Diseases Institute[2]

Objective
It has been reported that the platelet derived growth factor (PDGF) could have treatment effect in tendon or ligament injury. However, the PDGF has limitations of short half-life and low affinity to tissue. The poly(lactic-co-glycolic acid) (PLGA) polymer is a FAD-approved material for the drug delivery and the tissue engraft scaffold. The PLGA microsphere with surface modification using heparin-dopamin (hep-PMS) has an ability of slow releasing. We fabricated the slow releasing PLGA microsphere impregnated with PDGF (PDGF/Hep-PMS). We aimed to investigate whether the PDGF/Hep-PMSs could suppress the inflammatory response in lipopolysaccharide (LPS)-stimulated tenocytes in vitro.

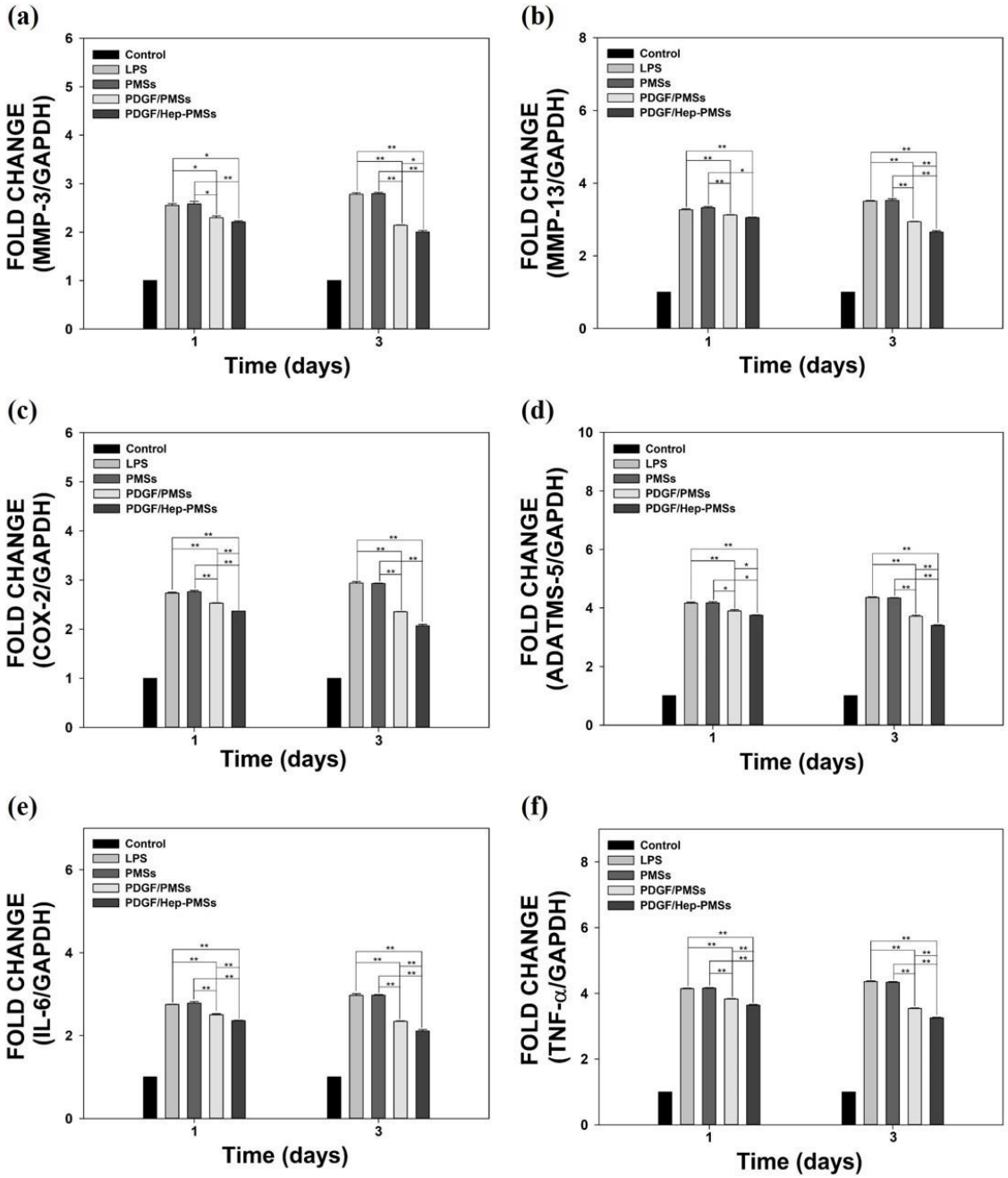
Method
To impregnate the PDGF on Hep-PMS, the Hep-PMSs were immersed into 0.1 M MES buffer (pH 5.6) and gently shaken 30 min to active highly negative-charged heparin on Hep-PMSs. Highly positive-charged PDGF at concentration of 500 ng/mL was added to 0.1 M MES buffer (pH 5.6) containing Hep-PMSs. Tenocytes were isolated from the rotator-cuff tendon of New Zealand white rabbit. The tenocytes (1 x 10^5 cells/well) were seeded on 10 mg of PMSs, PDGF/PMSs, and PDGF/Hep-PMSs in 24-well tissue-culture plates and maintained in Dulbecco's modified Eagle's medium. At 1, 3, and 7 days, each sample was rinsed with PBS and CCK-8 proliferation kit reagents were added and incubated for 1 h. Reagents were transferred to 96-well plates and optical density was measured with a Flash Multimode Reader at 450 nm. To demonstrate the anti-inflammatory effects of PMSs, PDGF/PMSs, and PDGF/Hep-PMSs, pro-inflammatory cytokines on cells grown on each sample after LPS activation were measured by real-time polymerase chain reaction (PCR).

Results
There were no significant differences in cell proliferation between any groups on day 1. At 3 days, the differences in tenocyte proliferation were significant between tenocyte grown on PDGF/PMSs and PMSs and between tenocytes on PDGF/Hep-PMSs and PMSs. At 7 days, there were significant differences in tenocyte proliferation cultured on PDGF/PMSs or PDGF/Hep-PMSs compared to PMSs. No mRNA expression of pro-inflammatory cytokines was detected in LPS-stimulated cells grown on PMSs compared to in the positive control group on days 1 and 3. However, PDGF/PMSs or PDGF/Hep-PMSs showed significantly decreased MMP-3, MMP-13, COX-2, ADAMTS-5, IL-6, and TNF-α expression levels relative to in LPS-activated tenocytes on days 1 and 3. Moreover, the

mRNA levels of MMP-3, MMP-13, COX-2, ADAMTS-5, IL-6, and TNF-α in LPS-stimulated tenocytes on PDGF/Hep-PMSs were significantly decreased compared to those of all other groups.

Conclusion

The PDGF/Hep-PMSs could suppress the mRNA levels of pro-inflammatory cytokines.ocal delivery of PDGF. Localized and slow delivery of PDGF using heparinized-porous microspheres is a promising therapeutic injectable material for controlling tendinitis.



Relative mRNA levels of pro-inflammatory cytokines, including (a) MMP-3, (b) MMP-13, (c) COX-2, (d) ADAMTS-5, (e) IL-6, and (f) TNF-α in LPS-stimulated tenocytes grown on PMSs, PDGF/PMSs, and PDGF/Hep-PMSs on days 1 and 3. Data are presented as the mean ± SD (n = 5). *P < 0.05 and **P < 0.01.