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Adipose-derived stem cells enhance primary tendon repair: Biomechanical and immunohistochemical evaluation☆

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Summary *Background:* Primary tendon repair aims at increased tensile strength at the time of mobilisation. Tendon repair and regeneration using mesenchymal stem cells have been described in different studies; however, adipose-derived stem cell (ASC) use for tendon regeneration and repair has recently been taken into consideration. In this study, we sought to determine whether ASCs would be beneficial in primary tendon healing.
Materials and methods: Both the Achilles tendons of rabbits ($n = 6$) were incised and consequently repaired. To the left side was applied platelet-rich plasma (PRP) gel and to the right side autologous ASC-mixed PRP. The tensile strength was measured on the 4th week. The samples were taken for immunohistochemical evaluation of collagen type I, transforming growth factor beta (TGF-β) 1, 2, 3, fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF).
Results: The tensile strengths in control and experimental groups were found out to be 29.46 ± 3.66 and 43.06 ± 3.80 kgf. Collagen type I, FGF and VEGF levels were statistically higher, whereas TGF-β1, 2, 3 were lower in the experimental group.
Conclusion: ASCs enhance primary tendon healing; however, the complex interaction and the cascades by which ASCs could increase collagen type I, FGF and VEGF and decrease TGF-β levels should further be investigated.

☆ Meetings at which this study was presented:
1) *6th Annual Meeting of the International Federation of Adipose Therapeutics and Science (IFATS)*, 24–26 October, 2008, Toulouse, France.
2) *54th Annual Meeting of Plastic Surgery Research Council*, 27–30 May, 2009, Pittsburg, USA.

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A damaged tendon never restores the biological and biomechanical properties of normal tendon completely. The collagen fibrils remain thinner, with a reduction in the biomechanical strength of the tendon.[1] The tendons heal by way of both extrinsic and intrinsic mechanisms. The exact nature of tendon healing remains unknown. The weakest point of tendon healing and the most likely time for rupture after repair is 5–10 days postoperatively. There is also an increased rate of rupture 6 weeks postoperatively when strengthening exercises may exceed the tensile strength of the repair. The goal of rehabilitation after tendon repair is to achieve optimal function and gliding, while preventing tendon rupture.[2] Thus, there have been different studies on how to increase the strength of the repaired tendon and the clinical and experimental research mainly focussed on the type of sutures and repair techniques.[3]

Previous studies on tendon healing have implicated basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and transforming growth factor beta subfamily (TGF-β) in the tendon-healing process.[4,5] TGF-β is a superfamily of growth factors with a wide range of functions including fibroblast and macrophage recruitment, stimulation of collagen production, down-regulation of proteinase activity and increase in metalloproteinase inhibitor activity.[6,7] Recent research has described TGF-β as a key cytokine in the pathogenesis of fibrosis and scar formation, resulting from excessive disordered collagen deposition.[8] Therefore, any treatment modality that can increase TGF-β levels in tendon healing might enhance the collagen deposition; however, the adhesions would also be increased.[9]

Regenerative medicine has established a new promising experimental and clinical epoch.[10] The differentiation of postnatal somatic stem cells or mesenchymal stem cells (MSCs) to different cell lineages and tissues including tendons were described. In addition, the MSCs, mainly bone marrow-derived stem cells (BSCs), were proven to enhance tendon healing.[11–13]

Adipose-derived stem cells (ASCs) recently presented by Zuk et al.[14] were shown to be as effective as the other MSCs by their multipotency and proliferative efficiency.[15–18] Therefore, in this study we sought to determine whether ASCs would be beneficial in primary tendon healing.

Materials and methods

Isolation and preparation of ASCs

Ten-week-old white Japanese rabbits (Saitama Experimental Animals Supply Corporation Ltd., Saitama, Japan) were anaesthetised with sodium pentobarbital (Nembutal; Dainippon Sumitomo Pharma Co. Ltd., Osaka, Japan) at 35 mg kg^{-1} and shaved. ASCs were harvested and processed according to our established protocol.[16–18] The inguinal fat pads were excised and extensively washed with phosphate-buffered saline (PBS) (Gibco-BRL, Grand Island, NY, USA). They were then finely minced, and incubated on 100-mm^2 tissue culture plates (Becton-Dickinson, Franklin Lakes, NJ, USA) with Dulbecco's Modified Eagle Medium (DMEM) (Gibco-BRL, Grand Island, NY, USA), 10% foetal bovine serum (Gibco-BRL, Grand Island, NY, USA) and 1% antibiotic–antimycotic (Gibco-BRL, Grand Island, NY, USA) for 1 h. The tissue was then rinsed three times in PBS for 5 min followed by digestion with 0.15% collagenase (Wako, Osaka, Japan) and vigorous shaking for 30 min at 37 °C in a 50-cc centrifuge tube. Next, an equal volume of control medium was added to neutralise the collagenase. Then, the cell suspension was centrifuged at 1300 rpm (260 *g*) for 5 min and the cell pellet was resuspended with a control medium. After cell counting using trypan blue, the cells were plated at 100-mm^2 tissue culture plates and maintained in the control medium at 37 °C in 5% carbon dioxide. The medium was replaced every 3 days. The cells were harvested at 80–90% confluence and the cultured cells were detached from the culture dishes with 0.25% trypsin/ethylene diamine tetraacetic acid (EDTA) (Gibco-BRL, Grand Island, NY, USA), neutralised with culture medium and then passaged into 1:3. Autologous ASCs of passage 3 isolated from each rabbit were used in the subsequent *in vivo* studies. There were 1×10^7 cells in 1 ml for in the ASC group.

1,1′-Dioctadecyl-3,3,3′,3′tetramethylindocarbocyanine labelling of ASCs

1,1′-Dioctadecyl-3,3,3′3′tetramethylindocarbocyanine (DiI) was dissolved in 99% ethanol at a concentration of 25% and stored at −20 °C for use. Cells were labelled with fluorescent DiI (Molecular Probes, Eugene, OR, USA) according to the manufacturer's recommendations; cells in suspension were incubated with DiI at a concentration of 2.5 μg ml^{-1} in PBS for 5 min at 4 °C.

Platelet-rich plasma (PRP) preparation

Platelet-rich plasma (PRP) preparation was performed, as described previously.[16,19] Thus, 10 ml of whole blood from each 10-week-old, white, male, Japanese rabbit was drawn preoperatively with a 23-gauge needle (TERUMO, Tokyo, Japan) into tubes containing 3.8% sodium citrate. The blood was first centrifuged in a standard laboratory centrifuge machine (Kubota 3740, Tokyo, Japan) for 10 min at 2400 rpm (450 *g*). The supernatant plasma was collected along with the buffy coat, which consists of platelets and leucocytes, into a neutral tube with a long cannula. A second centrifugation at 3600 rpm (850 *g*) was performed for 15 min to concentrate the platelets. The infranatant containing the buffy coat was resuspended with 1.3 ml of the residual plasma to prepare the final PRP product. PRP gelation was activated with a 10% calcium chloride solution immediately before the administration *in vivo*. Autologous PRP preparations were made from each rabbit and applied to the same rabbit.

Experimental model

All animal procedures were performed in accordance with the guidelines of the Nippon Medical School Animal Care and Use Committee (Approval Number: 20-042). Following the anaesthesia with sodium pentobarbital at 35 mg kg^{-1}, both of the Achilles tendons were exposed. The tendons were incised with a scalpel (No: 15, Feather Co. Ltd.,