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Enhanced Achilles tendon healing by fibromodulin gene transfer

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Abstract

Tendon injury is a major musculoskeletal disorder with a high public health impact. We propose a non-viral based strategy of gene therapy for the treatment of tendon injuries using histidylated vectors. Gene delivery of fibromodulin, a proteoglycan involved in collagen assembly was found to promote rat Achilles tendon repair *in vivo* and *in vitro*. *In vivo* liposome-based transfection of fibromodulin led to a better healing after surgical injury, biomechanical properties were better restored compared to untransfected control. These measures were confirmed by histological observations and scoring. To get better understandings of the mechanisms underlying fibromodulin transfection, an *in vitro* tendon healing model was developed. *In vitro*, polymer-based transfection of fibromodulin led to the best wound enclosure speed and a pronounced migration of tenocytes primary cultures was observed. These results suggest that fibromodulin non-viral gene therapy could be proposed as a new therapeutic strategy to accelerate tendon healing.

From the Clinical Editor: Tendon injury is relatively common and healing remains unsatisfactory. In this study, the effects of liposomal-based delivery of fibromodulin gene were investigated in a rat Achilles tendon injury model. The positive results observed would provide a new therapeutic strategy in clinical setting in the future.

Key words: Tendon healing; Gene therapy; Lipoplex; Polyplex

The incidence of work-related musculoskeletal disorders (WMSD) is increasing every year due to modern life. Tendon injuries represent the main pathology of WMSD and healing requires a long rest period.[1] It has been proven that growth factors like PDGF, TGF-β or VEGF can considerably accelerate this wound-healing process by regulating the inflammation phase, the production of extracellular matrix, the cell proliferation and migration.[2-5] The delivery of these growth factors has some limitations due to their short half-life time requiring repetitive injections and the extensive cost of recombinant protein production and purification.

Gene therapy is the best approach to produce growth factors *in situ* and can be exploited in tendon disorders.[6]

Transient gene expression after gene transfer by non-viral vectors would be useful for wound-healing because molecules involved in tissue repair have to be expressed only in a short-time period.[7] Synthetic vectors are chemically controlled compounds easy to handle, and show a low immunogenicity with a weak risk of transgene integration.

These last years, few reports have shown the potentiality of *in vivo* gene delivery for tendon regeneration. They mainly concern the delivery of genes encoding growth factors by using liposomes, adenoviral vectors, silica nanoparticles and electroporation.[8-11] The main effect of the expression of growth factors is the induction of tenocytes proliferation and collagen I production, but the tendon strength depends also on a good matrix assembly which relies on the collagen fibrillogenesis that depends on the activity of proteoglycans like fibromodulin (Fmod) or lumican (Lum).[12,13] Moreover, proteoglycans have been shown to enhance the repair and remodeling of injured cornea,[14] skin,[15] and ligament.[16] They have a specific expression profile in injured tendons compared to normal tissue.[17]

In this study, we investigated as nanomedicine approach whether Fmod or Lum gene transfer could improve rat Achilles

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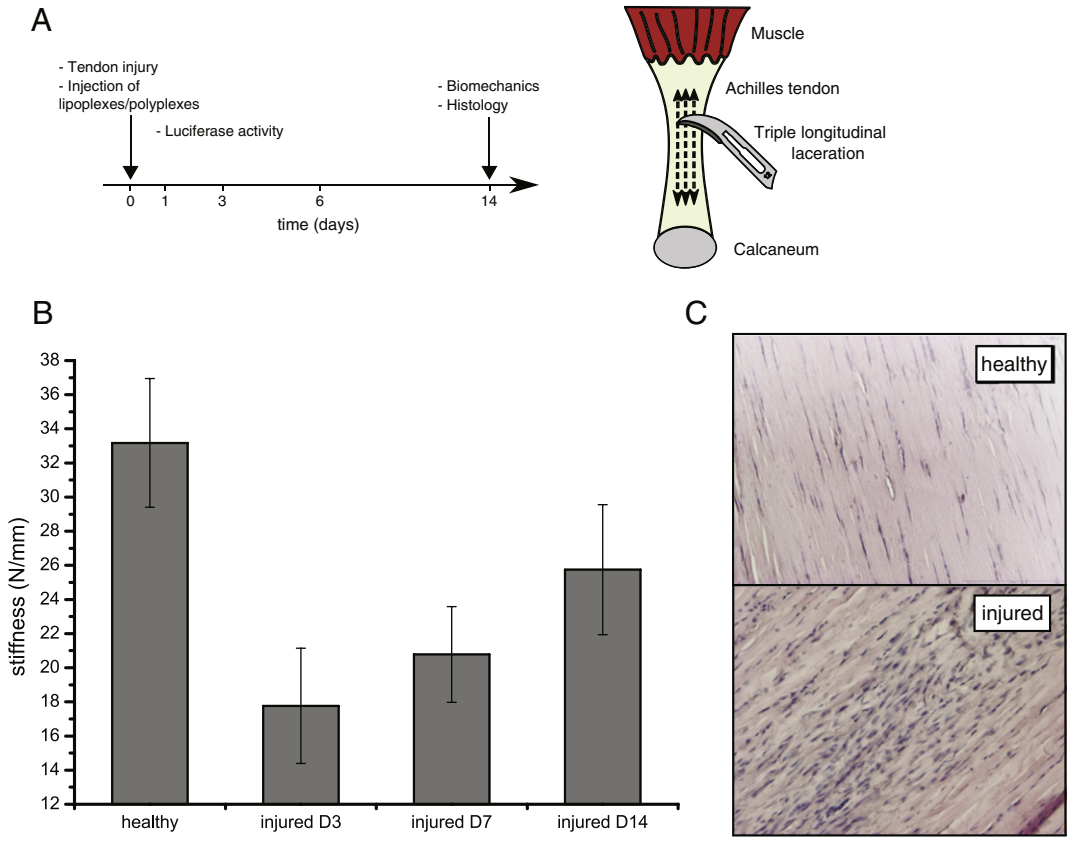

Figure 1. *In vivo* Achilles tendon healing model. **(A)** The lesion was induced at day 0 by a triple longitudinal laceration of the Achilles tendon. Then, lipoplexes or polyplexes were injected before suturing the tendon. When luciferase reporter gene was used, luciferase activity assay was performed at days 1, 3 and 6 to evaluate the gene transfer efficiency. **(B)** Stiffness of healthy and injured tendons was measured to assess the stiffness recovery kinetics after injury. At day 14 tendon stiffness was still significantly lower than healthy tendon. Data represent means ± SEM; $*P < 0.05$. **(C)** Histological analyses of Achilles tendons performed 14 days after injection showed hypercellularity in the healing region and disorganized collagen fibers induced by the lesion.

tendon healing. Gene transfer was performed either with imidazole cationic lipids (Lip100 liposomes) or histidylated linear polyethyleneimine (PTG1) in Achilles tendons *in vivo* and in primary cultures of tenocytes. Wound-healing effect was assessed by histological analyses and stiffness measurements after transfection of fibromodulin. Finally, we performed *in vitro* experiments including wound-healing, cell proliferation and cell migration assays to get a better understanding of the benefic effect brought by fibromodulin gene transfer.

Methods

Plasmids

pNFCMV-Luc (pLuc), a 7.5 kb homemade plasmid DNA (pDNA) encoding the firefly luciferase under control of the strong cytomegalovirus promoter was used as a reporter gene. This plasmid has five consecutive NFκB motifs (termed NF that recognize the NFκB transcription factor) inserted upstream of the promoter. The second reporter pDNA was pMaxGFP™, a 3.5 kb plasmid encoding the eGFP gene (Lonza, Basel, Switzerland). pPDGF plasmid (Invitrogen, Cergy-Pontoise, France) was encoding the human *PDGFB* gene. Plasmids $pCEP_4$-FBM (pFmod) and $pCEP_4$-LUM (pLum) plasmids (generous gift from Dr. Peter Roughley) were encoding human fibromodulin and lumican genes, respectively.[18] pQE30, a mock plasmid (pMock) that did not encode any gene was used as control in wound-healing experiments.

Liposomes and polymers

Histidylated liposomes (Lip100) were prepared by mixing *O*,*O*-dioleyl-*N*-[3*N*-(*N*-methylimidazolium iodide)propylene] phosphoramidate and *O*,*O*-dioleyl-*N*-histamine phosphoramidate. These lipids were synthesized as previously described.[19,20] Histidylated linear polyethylenimine (PTG1) was produced by Polytheragene (Evry, France).[21] Lipoplexes and polyplexes were formed at vector/DNA weight ratio of 3:1 and 6:1, respectively. The detailed procedure for DNA complexes preparation is presented in supplementary methods.

Animal studies

In vivo transfection

Adult Wistar rats were bred in CBM animal facility at 22 °C for at least one week before experiments and groups were randomly made. Experiences were conducted according to the guidelines of the French Ministry of Agriculture for experiments