^{1}H Single and Double Quantum MRI Evaluation of Tendon Regeneration Mediated by Engineered Stem Cells

K. Keinan-Adamsky[1], H. Shinar[1], G. Pelled[2], Y. Zilberman[2], D. Gazit[2], G. Navon[1]

[1]School of Chemistry, Tel Aviv University, Tel Aviv, Israel, [2]Skeletal Biotech Lab, Heberew University-Hadassah Medical Center, Jerusalem, Israel

Introduction

The common therapeutic options for tendon and ligament injuries, which consist of autografts, allografts or synthetic prosthesis, do not provide a long-term successful solution. It has recently been shown that adult mesenchymal stem cells (AMSCs) are able to differentiate into mesenchymal phenotypes including tendon/ligament forming cell as well as cartilage forming chondrocytes (1). However, the implantation of autologlous AMSCs did not provide improvement of tendon microstructure (2).

In the present work we report an observation by single and double quantum filtered (DQF) MRI, that implantation of AMSCs combined with a potent inducer of tenocytic differentiation factor (BMP2 and SMAD8) leads to a significant regeneration of the tissue. The advantage of the DQF MRI is that tendons and ligaments that have very low signal in standard MRI image are highlighted in DQF MRI allowing a follow-up of their regeneration (3). The intensity of the DQF NMR signal depends on the residual dipolar interaction of water molecules, originating from their interaction with collagen fibers. Thus DQF MRI of tendons reflects the density and order of the collagen fibers.

Methods

A partial defect of 3 mm, which is approximately 30% from the total length, was induced in Achilles tendons of Nude HSD Rhmu rats. A collagen sponge loaded with genetically engineered AMSCs, over expressing the BMP2 and SMAD8 genes, was implanted in the injured site. As a control, a sponge without engineered stem cells was implanted in another rat. The rats were sacrificed 5 weeks post implantation. The dissected legs were inserted in a 10-mm NMR tube and immersed in a fluorinated oil (Fluorinert, 3M). The MRI parameters used were: SE: 256x128, FOV= 1x1 cm, TR/TE = 1s/ 6.2 ms, slice thickness= 2 mm. DQF: 128x128, FOV= 1x1 cm, slice thickness= 2 mm, creation time τ=0.4 ms.

Results

Intact leg

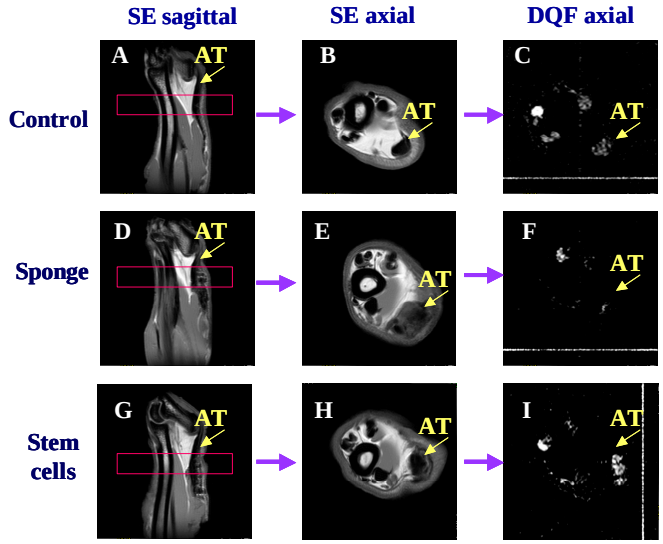
The Achilles tendon as well as the other tendons give a very low signal in both the sagittal and the axial SE images (A, B) while in the DQF image (C) they are highlighted. The bright areas in the SE images originate from other tissues such as fat and muscle that did not give rise to any DQF signal. The DQF image was obtained at a DQ creation time of 0.4 ms, which has been shown to give the maximal signal for intact tendons (3).

Injured leg with sponge implant

The Achilles tendon with the sponge, without the engineered stem cells, a month after implantation, has a non-uniform structure, as is seen in the SE images (D, E). The implantation area is observed apart from the unaffected tendon. In the DQF image (F) the Achilles tendon is not observed, indicating that either the collagen fibers are disordered or their amount is very small.

Injured leg with engineered stem cells implant

In the SE images measured 7 days after implantation (not shown), a high signal intensity, probably due to edema, is observed at the injured site. In the DQF image, the AT is not detected indicating that no collagen ordered structure are yet formed. In the SE images of the injured site, a month (G, H) after implantation, the AT is observed as a low intensity region. In the DQF image (I) the tendon is clearly observed as a bright spot with an area and intensity, which are even larger than observed for the control. The high DQF signal intensity of the Achilles tendon indicates that in the injured site there are aligned collagen fibers surpassing their amount in the healthy tissue. Histological analysis demonstrated the filling of the defect with cells closely oriented in the same axis of the rat tendon cells.



Conclusions

To the best of our knowledge, this is the first repot of healing of an AT injury by genetically engineered adult mesenchymal stem cells. DQF MRI proved the presence of collagen fibers within the injured site at a higher level than in the healthy control. These results demonstrated that the genetically modified AMSCs over expressing the active form of SMAD could serve as a regenerative platform for the repair of ligaments/tendons. ^{1}H DQF imaging is a sensitive technique for imaging ordered structures and examining structural disorders and regeneration of tendons.

References

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