# Long tendon constructs can be fabricated from rabbit adipose derived stem cells (rADSCs) and a collagen type I gel by cyclic stretching

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**Background and Introduction:**
Severely destroyed tendon tissue is often difficult to reconstruct. If the defect is too large in size direct suture of the tendon is not possible and it needs to be repaired by transplantation or transposition of other tendons. Since functional motor units have to be sacrificed to repair the defect there is the need for alternative sources of appropriate tissue.

**Rational:**
The field of Tissue engineering offers new methods for the reconstruction of damaged tissue. Progenitor cells of the adipose have proven to be an efficient cell source to create tissue-like complexes that regenerate tissue defects in vitro and in vivo. [1,2,3] These cells are multipotent, easy to obtain and to cultivate, and can be differentiated into the desired cell type. The authors investigated if useful tissue for tendon reconstruction could be fabricated from rabbit adipose derived stem cells (rASCs) in a collagen type I gel by cyclic stretching.

**Methods:**
Adipose was obtained from the inguinal fat pad of male New Zealand White Rabbits. To isolate rASCs the adipose tissue was minced, digested with collagenase, and cells were propagated in culture. Passages 3 or 4 were used for the experiments. Tendon constructs were fabricated by dispersing rASCs in a collagen type I gel (ArsArthro AG, Esslingen). Polymerization of the cell containing gel ($1x10^6$ cells/ml) occurred in special designed glass-cylinders with defined measurements. The constructs were transferred to a bioreactor and exposed to cyclic stretching for 5 weeks (Fig. 1). The stretching protocol included a stretching time of 8 hours followed by a 16 hours resting period. Stretching distance was 1 cm and stretching frequency 0.5 Hz. Non-stretched constructs served as control.

**Results:**
Tendon-like constructs with a length of 10 cm could be fabricated. They showed an increased opacity and a decreased diameter compared with the control. Histological analyses displayed longitudinal oriented, spindle-shaped cells, an organized dense matrix, and parallel collagen fibers. PCR analysis showed increased mRNA syntheses (collagen type I, III, fibronectin) compared with the control. Structural analysis showed a paratenon-like smooth surface and a parallel orientation of the collagen fibers of the stretched constructs (Fig.2 and 3).

**Discussion and Conclusion:**
Long tendon-like structures that display features comparable to regular tendon tissue could be fabricated using rASCs, a collagen type I gel, and a cyclic stretching bioreactor. The use of adipose derived progenitor cells seems to have a high potential for tendon tissue engineering. Further biomechanical studies and animal studies will investigate the potential clinical application of these long tendon constructs for tendon regeneration.

**References:**
[1] P.A. Zuk et al. (2001) *Tissue Eng* **7**:211-28
[2] J.L. Dragoo et al. (2003) *J Bone Joint Surg Br* **85**:740-7
[3] S. Nathan et al. (2003) Tissue Eng. **9**:733-44.

**ACKNOWLEDGEMENTS:**
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**Figures**

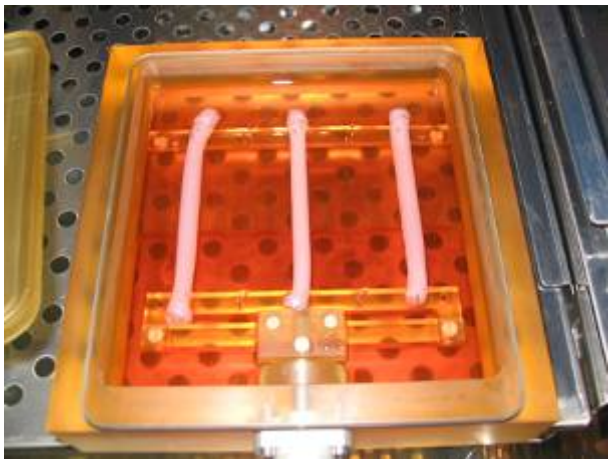

Fig 1: Three tendon constructs after transfer into the bioreactor for cyclic stretching.

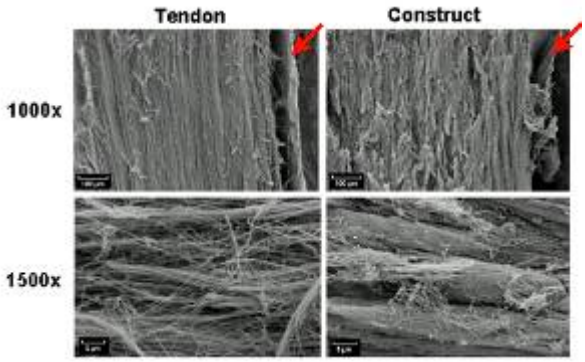


Fig. 3: Scanning Electron Microscopy of a natural tendon and the stretched construct. Note parallel alignment of collagen fibres and paratenon like structure marked with red arrows (first column: natural tendon, second column: stretched constructs, upper row: magnification 1000x, lower row: magnification 1500x).

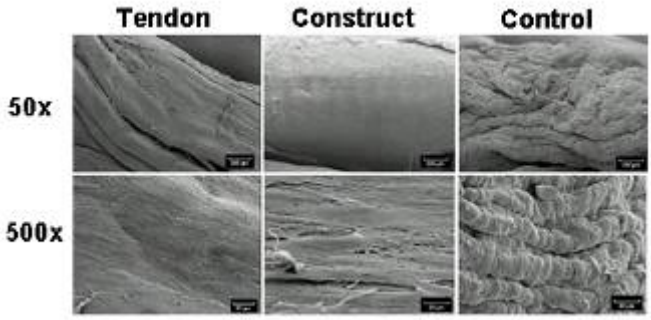


Fig 2: Scanning Electron Microscopy of the surface from a natural tendon, the stretched construct, and the control (first column: natural tendon, second column: stretched construct, third column: control, upper row: magnification 50x, lower row: magnification 500x)