The regulation of tendon stem cell differentiation by the alignment of nanofibers

茵梓，陈晓，陈佳林，沈炜亮，欧阳宏伟

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Introduction

Tendon injuries are common, especially during sports and other rigorous activities. Recently, identification of tendon stem/progenitor cells (TSPCs) which were demonstrated to be self-renewal and possess regenerative capabilities opens new possibilities for treating damaged tendon tissue [Nat Med.2007]. Tendon is a unique connective tissue composed of parallel nano-grade collagen fibers. The effect of this tissue-specific matrix orientation on stem cell differentiation has not been investigated. This study aimed to determine the effects of nano-topography on the differentiation of human TSPCs and develop a biomimetic scaffold for tendon regeneration.

Materials and methods

Fabrication of nanofibrous scaffold by electrospinning.

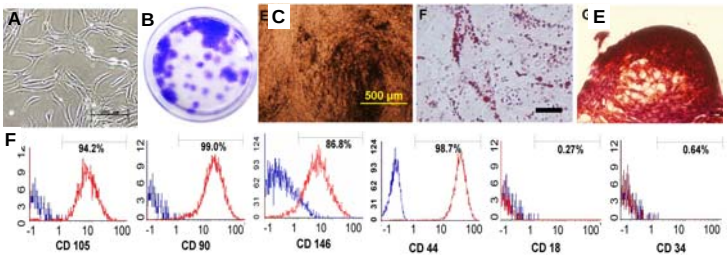
Cell viability by SEM and confocal microscopy.

Cell differentiation:

- ALP and alizarin red S staining
- Real time PCR
- Histology

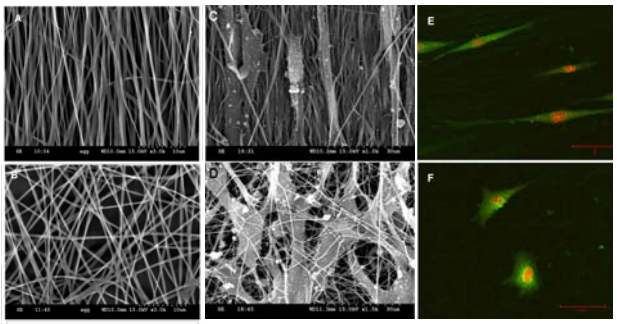
Results

Stage I. Isolation and characterization of hTSPCs



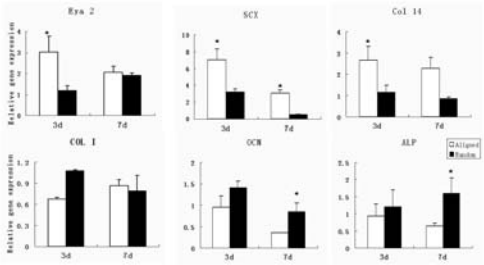
(A) HTSPC morphology. (B) Colonies fomation shows self-renewal capacity of hTSPCs. (C) osteogenesis, (D) adipogenesis and (E) Chondrogenesis of hTSPCs (F) Flow cytometry analysis of the expression of indicated cell surface markers related to mesenchymal stem cells in hTSPCs.

Stage II. Fabricate nanofibers and morphology of hTSPCs on nanofibrous scaffold.

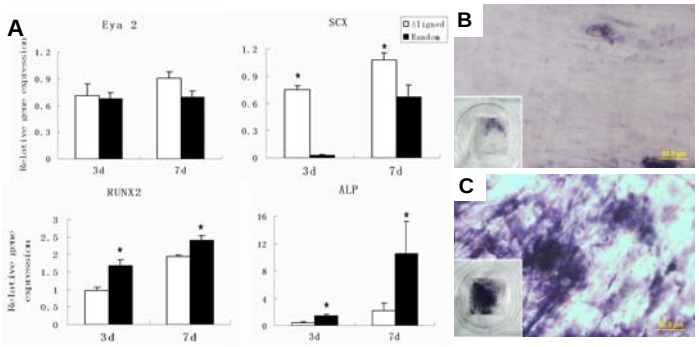


SEM results of aligned (A) and randomly-oriented (B) nanofibrous scaffold. hTSPCs were spindle-shaped and well orientated on the aligned nanofibers (C) and (E).The cells on the randomly-oriented nanofiber scaffolds exhibited a stellate-patterned phenotype (D) and (F).

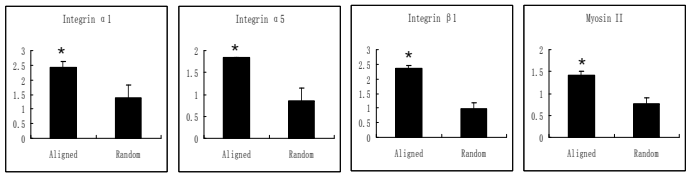
Stage III. Cell differentiation induced by PLLA nanofibrous scaffold.



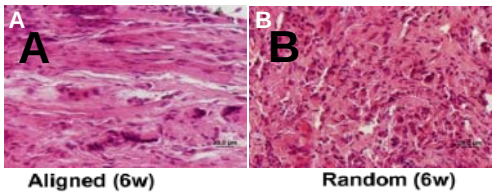
The expression of tendon-specific genes was significantly higher in hTSPCs growing on aligned nanofibers than those on randomly oriented nanofibers



(A) The expression of tendon-specific genes was still significantly higher in hTSPCs growing on aligned nanofibers than those on randomly oriented nanofibers under osteogenic media. The randomly oriented cell express more osteogenic genes, such as runx2, ALP. ALP staining show the randomly oriented nano-scaffold induced osteogenesis (B), while the aligned scaffold hindered the process (C).



That aligned cells expressed higher levels of integrin α 1, α 5 and β 1 subunits and myosin II B ($p < 0.05$) than random ones indicates the nanotopography induced differentiation may be through mechanotransduction pathway.



Typical HE staining shows aligned scaffold induced tendon-like tissue *in vivo* (A).

Conclusion

The present study is the first demonstration that an aligned nanofibrous scaffold induces teno-lineage differentiation of human tendon stem cells and could serve as an optimal scaffold for tendon regeneration. More importantly, this matrix-specified lineage resisted the power of osteogenic induction medium. Furthermore, the aligned nanofibrous scaffold influenced the differentiation of hTSPCs through an integrin-mediated mechanotransduction pathway.