SERUM DEPRIVATION COUNTERACTS PHENOTYPIC DRIFT OF TENDON CELLS

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Introduction

Current gaps in basic knowledge on tendon tissue healing and remodeling combined with limited availability of tendon-derived cells (TDCs) have necessitated *in vitro* TDC expansion. However, required culture conditions conflict with the physiological relevance of TDCs. High fetal bovine serum (FBS)- and glucose levels as well as random cell morphology mimic pathological tissue and cause cellular phenotypic drift [1], a phenomenon that is also induced by those medium components in *ex vivo* tendon cultures [2]. Therefore, the aim of this research was to determine whether phenotypic drift of *in vitro* expanded TDCs could be reversed by adapting cell morphology, FBS- and/or glucose levels, and whether the medium component(s) with the highest impact could prevent cellular phenotypic drift in *ex vivo* tendon fascicles.

Methods

Passage 4 mouse tail TDCs were seeded on aligned (AL) or random (RA) microcontact printed collagen I patterns (fig. 1) in standard medium, which was switched to high- (HG) or low-glucose (LG) DMEM -FBS at sub-confluency (t = 0d). TDC phenotype was monitored over 7d by measuring levels of marker genes for all mesenchymal lineages using qPCR, with TDCs analyzed directly after isolation as native control (fold change = 1).
Similarly, mouse tail tendon fascicles were cultured in HG-DMEM +/-FBS. mRNA was sequenced after 6d, comparing mRNA levels of the mesenchymal lineages to those in freshly isolated fascicles (fold change = 1).

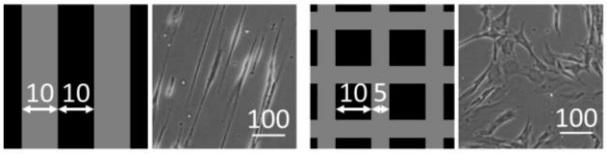

Figure 1: schematic of aligned (left) and random (right) microcontact printing patterns, grey: collagen I, black: non-printed areas; with resulting phase contrast microscopy images of seeded TDCs. All sizes are in µm.

Results

Most strikingly, TDC expansion until t = 0d resulted in a 500-fold decrease of Tenomodulin (Tnmd). Subsequent serum deprivation for 7d almost restored these levels to native. Also for the other lineages, serum deprivation had a significantly larger effect on convergence towards native gene expression levels than glucose level and morphology (fig. 2).

In *ex vivo* tendons, Tnmd dropped 15-fold in +FBS, and remained close to native in -FBS, a pattern that was observed for the majority of the lineages (fig. 3).

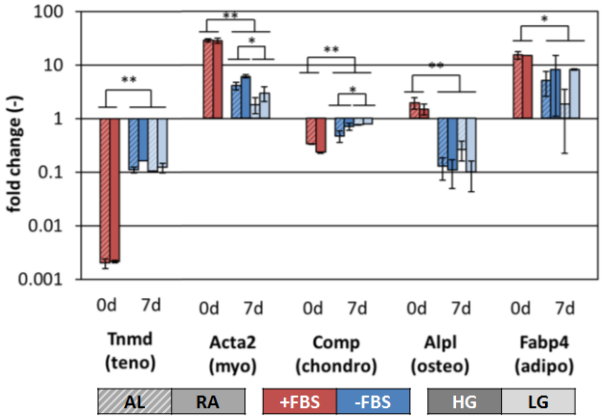

Figure 2: relative levels of phenotypic marker genes in in vitro *TDCs, before and after serum deprivation.*

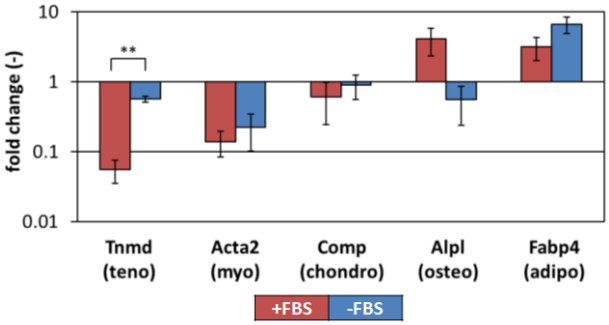

Figure 3: relative levels of phenotypic marker genes in tendon explant cultures, with and without serum deprivation.

Discussion

Previously, high FBS- and glucose levels as well as random cell morphology have been shown to induce phenotypic drift in TDCs. However, in this research, effects of these conditions were compared proportionately for the first time, while aiming at reversing rather than preventing phenotypic drift. Results indicated serum deprivation to overrule glucose level and morphology regarding convergence of phenotypic marker gene expressions towards – and potentially even completely recovering – native levels. These results could be translated to *ex vivo* tendon cultures, where most phenotypic marker gene levels were closer to native in serum-deprived medium.

References

1. Yao et al. Tissue Eng, 12:1843-1849, 2006.
2. Wunderli et al. J Orthop Res, 36:1383-1390, 2018.