

## Optimising the niche for skeletal muscle tissue engineering; role of electrostimulation in 2D and 3D cultures

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**INTRODUCTION:** Currently, skeletal muscle tissue engineering fails to result in mature, functional muscle tissue. It is hypothesized that mimicking the *in vivo* niche is needed for optimal maturation of precursor skeletal muscle cells [1]. Towards an optimal resemblance of the niche, we investigated the effect of electrical stimulation (ES) on differentiation/maturation of muscle precursor cells. Stimulation regimes were examined on C2C12 myoblasts as well as murine primary satellite cells (SCs). After careful optimization of the stimulation protocol in 2D, we translated the protocol to a 3D model system. Differentiation and maturation levels of the developed muscle constructs were investigated at functional, morphological, and transcriptional levels.

**METHODS:** C2C12 skeletal myoblasts were cultured in standard growth medium and induced to differentiate by serum deprivation. Single fibers were isolated from muscles from C57BL/6 mice, after which SCs were liberated with a 19G needle. SCs spontaneously differentiate under high serum conditions. Pulsed ES (10V, 6 ms, 2Hz) for 48h was started when myotubes had developed (respectively after 2 days differentiation in C2C12 and after 11 days in SCs). The 3D model system consisted of matrigel based bioartificial muscles (BAMs) [2] created in hydrophobic 6-well culture dishes, suitable for the C-Pace culture pacer. After ES, cultures were examined for functional parameters (contractions), morphological characteristics for muscle maturation (striations of sarcomeric  $\alpha$ -actinin or myosin) and gene expression of differentiation and maturation markers.

**RESULTS:** In monolayers of C2C12 myoblasts and SCs, results indicated that timing of ES within the differentiation process is delicate. Effects of electrical stimulation were optimal once fused myotubes had developed.

However, the effects only seemed to be temporal. The presence of cross-striations in myotubes was advanced and increased in stimulated cells versus control cells when ES was started at the right time point (Figure 1). In addition, more contractions were present in stimulated versus control cells. Quantitative PCR results also suggested that maturation was advanced in stimulated cells.

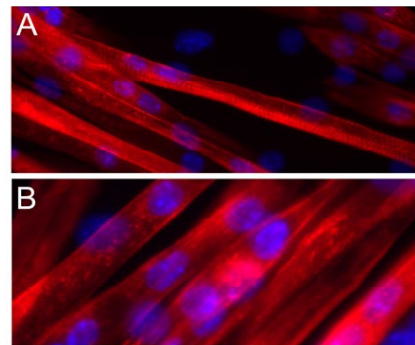


Fig 1: Cross-striations are seen in stimulated C2C12 cells (A), but not in control cells (B).

In the BAMs, the effects of ES on maturation and differentiation, as observed by development of cross-striations, were negligible compared to the effect of mere culturing in a 3D environment. Only small differences were found in the qPCR results.

**DISCUSSION & CONCLUSIONS:** For both C2C12 myoblasts as well as SCs, the effects of ES are more prominent in a 2D than in a 3D situation. We therefore propose that although ES might be useful to drive maturation of skeletal muscle precursors, confirmation in a 3D environment is essential.

**REFERENCES** <sup>1</sup>Boonen K.J.M., Post M.J. Tissue Eng.: Part B. 2008: 14: 419-431. <sup>2</sup>Gawlitta, D. et al. Ann Biomed Eng. 2007: 35: 273-284.

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