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Introduction

External stimuli are required to induce determination and differentiation of muscle progenitor cells into skeletal muscle cells *in vitro*. Possible stimuli are:

- Biochemical → Differentiation medium
- Biophysical → Electrical stimulation [1]
  → Substrate stiffness [2]

The effects of electrical stimulation (ES) were systematically investigated on 2D cultures of C2C12 murine skeletal myoblasts. The influence of ES on cells growing on flexible substrates (Flexcell Int.) was compared to standard tissue culture substrates. Furthermore, a 3D scaffold system was investigated for alignment of myotubes, since this is a prerequisite for muscle maturation. The main questions within this research were:

- *Does ES of muscle cells growing on a flexible substrate influence maturation, compared to standard tissue culture substrates?*
- *Do muscle cells align to polymer fibers in a scaffold?*

Material and methods

(2D) C2C12 myoblasts were cultured in standard growth medium and biochemically differentiated using 2% HS containing medium. Bipolar electrical field stimulation was realized by the C-Pace set-up (figure 1).

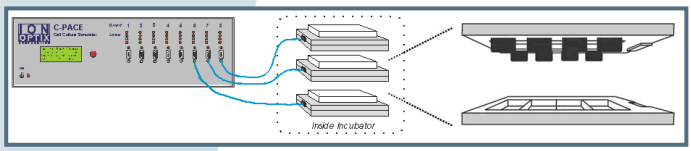

Figure 1 *Multi-channel fieldstimulation set-up (C-Pace, IonOptix Corporation)*

Cells were cultured in collagen type I coated standard 6-well and Flexwell dishes. The ES protocol (figure 2) was introduced after 48h of culturing in differentiation medium. (3D) PGA scaffolds were seeded with C2C12 myoblast within a fibrin gel, and grown in differentiation medium for 7 days.

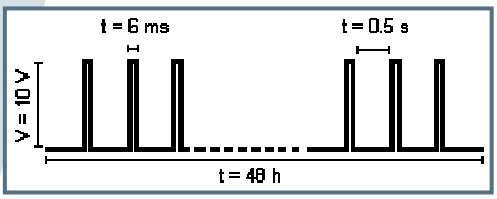

Figure 2 *Electrical stimulation protocol [1]*

Results

After ES, myotubes were contracting and showed different morphologies on standard 6-well dishes compared to Flexwell dishes. Myotubes on Flexwell dishes developed premature cross-striations (arrow figure 3).

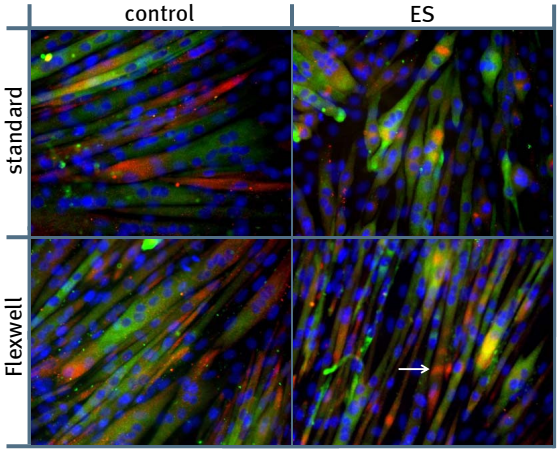

Figure 3 *C2C12 myotubes after 48h of ES on standard 6-well and Flexwell dishes. Cells were stained for α-sarcomeric actin, MHC and nuclei.*

Future analyses for quantification of myotube maturation include qPCR; expression of myogenic regulatory factors, myosin isoforms and α–sarcomeric actin will be evaluated.

Cytoskeletal staining (phalloidin) of tissue engineered skeletal muscle constructs with PGA, revealed that myotubes aligned and attached parallel to the polymer fibers within 7 days of culturing.
The developed 3D model system can be combined with the ES set-up for future experiments.

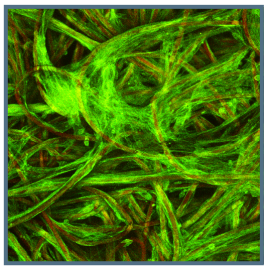

Figure 4 *C2C12 myotubes in PGA scaffold. Staining: PGA autofluorescence, and phalloidin-FITC.*

Conclusion

We have strong indications that myotubes are more mature after 48h of ES on Flexwell substrates, compared to standard tissue culture substrates. Since myotubes aligned parallel to PGA fibers in a scaffold, possibilities for aligned electrospun scaffolds are generated to stimulate skeletal muscle maturation.

References

[1] Thelen, M. H., et al. (1997). *Biochem.J. 321 (Pt 3): 845-848.*
[2] Engler, A. J., et al. (2004). *J Cell Biol 166(6): 877-87.*