Mechanical and electrical stimulation of muscle progenitor cells

M.L.P. Langelaan, R.P. Hesselink, C.V.C. Bouten, M.J. Post, F.P.T. Baaijens
Eindhoven University of Technology, Department of Biomedical Engineering

Introduction

The Vitromeat research initiative aims to produce high quality skeletal muscle tissue for consumption in an ethical and cost efficient way. Within this project we use tissue engineering techniques in which muscle progenitor cells are differentiated towards skeletal muscle. *In vivo* these cells are subjected to mechanical as well as electrical stimulation for complete maturation. Therefore, these triggers are used in the *in vitro* cultures to stimulate the differentiation process. Mechanical stimulation induces myotube orientation and longitudinal growth by mimicking skeletal growth and active movements of an embryo *in vivo* [1]. Electrical stimulation mimics nerve stimulation during myogenesis and promotes myotube maturation [2,3]. These stimuli have been proved to be effective in C2C12 myoblasts and are under investigation in P19 embryonal carcinoma cells.

Material and methods

C2C12 myoblast were seeded in wells plates, suitable for the C-Pace electrostimulation set-up (Figure 1). Myoblasts differentiation was stimulated both by serum deprivation (2% HS) and electrostimulation for 2 days using 10 V, 6 ms pulses at 2 Hz. Thereafter, cells were analyzed by immunohistochemistry for α-sarcomeric actin, myosin heavy chain and counterstained to visualize nuclei.

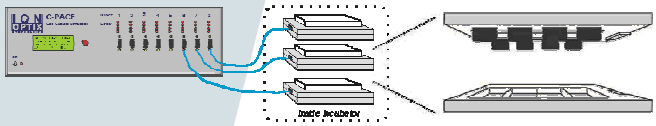

Figure 1 *C-Pace (IonOptix Co., USA) electrostimulation set-up. Bipolar pulses are transmitted to the cells in culture dishes through carbon electrodes.*

P19 cells were cultured in hanging drops with and without 1% DMSO according to the protocol shown in figure 2. Formation of skeletal muscle was analyzed morphologically.

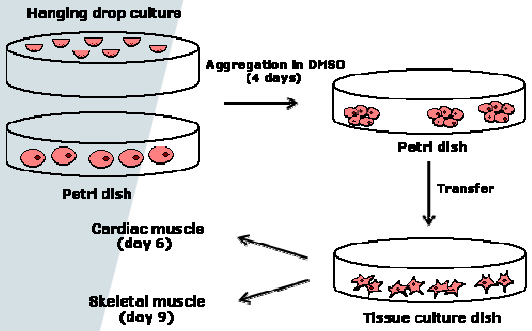

Figure 2 *Culturing P19 embryonal carcinoma cells.*

Results

After electrostimulation, C2C12 myotubes were contracting and showed clear signs of hypertrophy (Figure 3).

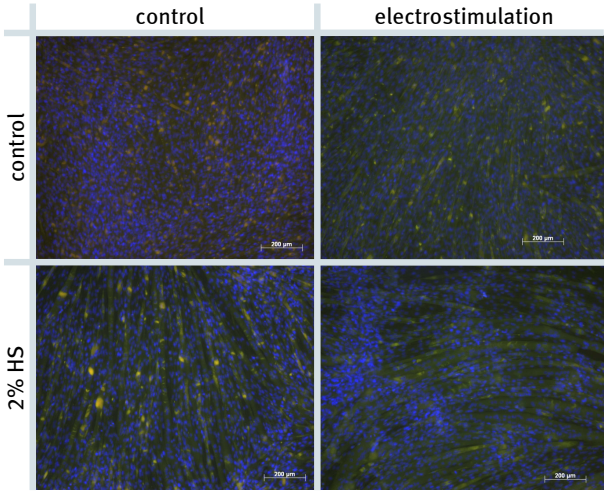

Figure 3 *C2C12 myotubes after 2 days of electrostimulation and/or serum deprivation. Cells were stained for α-sarcomeric actin (red), MHC (green) and nuclei (blue).*

Aggregating P19 cells in the presence of 1% DMSO resulted in myotube structures (Figure 4).

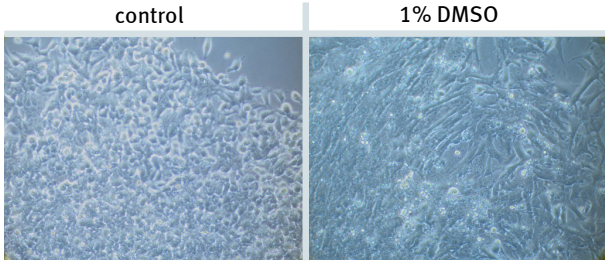

Figure 4 *Plated P19 cells. Aggregation occurred in the absence (left) and presence (right) of 1% DMSO.*

Discussion

Preliminary results indicate that the techniques used in this study are capable of directing progenitor cell differentiation towards mature skeletal muscle. Optimization of the protocols for both stimulation and culturing is required.

Future work

Experiments concerning mechanical stimulation using the Flexercell® system and electrostimulation of P19 cells will be performed in the near future.

References:

[1] Vandenburgh, H.H. et al. *Faseb J 1991,* 5(13):2860-2867
[2] Düsterhöft, S. et al. *Differentiation 1990,* 44(3):178-184
[3] Wehrle, U. et al. *Differentiation 1994,* 58(1):37-46