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Characterisation of Differentiation in a Tissue Engineered Skeletal Muscle Model

K.J.M. Boonen, D. Gawlitta, C.V.C. Bouten
Eindhoven University of Technology, Department of Biomedical Engineering

Introduction

A 3D skeletal muscle model is relevant for research on muscular damage and disease and can also be used for optimisation of culture and stimulation conditions.
In search of an optimal in vitro muscle model, a protocol has been developed to create tissue engineered (TE) muscle. The present study was undertaken to characterise the differentiation process and maturation of these TE muscles stimulated by three differentiation media in order to optimise culture conditions.

Material and methods

C2C12 cells were suspended in a collagen/matrigel mixture and poured into coated culture dishes containing Velcro attachment points (figure 1). Each TE muscle contains 2 million cells that are stimulated to differentiate after 24 hours (s0) by switching from growth medium to fusion medium. Growth medium contains 12% foetal bovine serum, whereas fusion medium contains 2% horse serum (DM), 32ng/ml IGF-1 (DMI) or 0.4% Ultroser G (DMU).

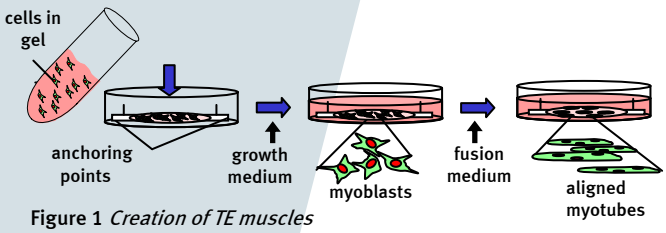

Figure 1 *Creation of TE muscles*

Muscles were sacrificed at 0, 3, 6, and 10 days after switching to fusion medium. Differentiation was assessed by measuring total protein content, creatine kinase activity and expression and localisation of actin and myosin.

Results

Morphologically, TE muscles differentiated in DM and DMU look similar, although the myotubes of TE muscles differentiated in DMU seem more regular and form faster. However, in TE muscles differentiated in DMI, no tube formation could be seen as myocytes did not fuse.

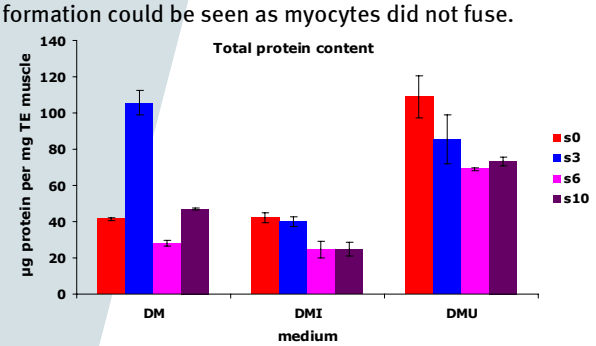

Figure 2 *Total protein content*

Total protein content decreased in all three cases after the medium shift. In DM, however, a large increase is seen prior to the decrease, which cannot be found in the TE muscles differentiated in other media (figure 2).

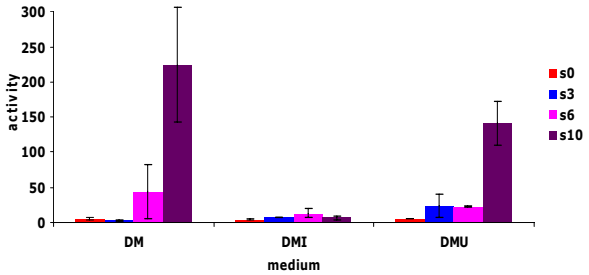

Figure 3 *Creatine Kinase activity*

Creatine kinase (CK) activity was shown to increase significantly during the culture period of the TE muscles cultured in DM and DMU. In fact, maximum levels do not even seem to be reached within 10 days, seen from the steep increase in activity from s6 to day 10. The TE muscles cultured in DMI however, show no increase at all in CK activity (figure 3).

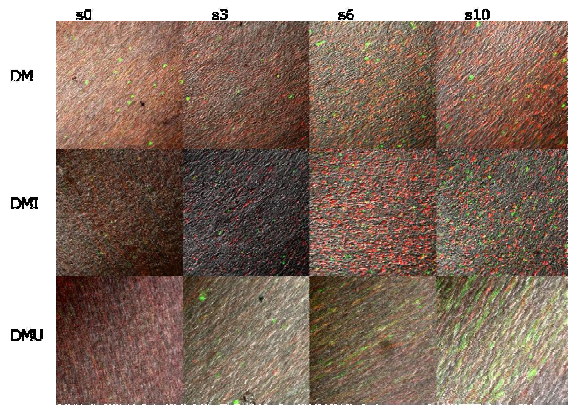

Figure 4 *Expression and localisation of myosin and actin*

Myosin heavy chain was found to increase during differentiation under influence of all three different media. Expression was seen in a punctuate pattern and no cross-striations could be found (figure 4).

Conclusions and Future studies

- This TE skeletal muscle model has been fully characterised: TE muscles differentiate best in DMU and are most mature at s10.
- Optimisation of culture and stimulation conditions will be initiated: Culture conditions without dependence on animal products will be developed for human use.