Expression of MyoD and m-Cadherin in C2C12 cells: Differentiation induced by cell-to-cell contact vs reduction in growth factors



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INTRODUCTION

- **C2C12 cells** derived from murine muscle tissue is commonly used to study myogenesis.
- Cell differentiation is part of myogenesis and can be induced through <u>cell-to-cell contact</u> or <u>reduction of growth factors</u> (GF)

Project Rationale: There is wide variation in cell culture protocols across the literature which differ greatly from American Type Culture Collection (ATCC) recommendations, despite the specific effects behind each culture condition not being clearly defined

Research Question: How will differentiation be induced by cell-to-cell contact vs reduction of GF effect C2C12 cells?

Biomarkers of myogenesis:

RESULTS

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- **MyoD** is translocated to the nucleus to act as a master regulator and transcription factor of muscle-specific genes
- **M-Cadherin** is localized to the plasma membrane to mediate cell fusion prior to myotube formation



Figure 1. Process of myogenesis as illustrated by Zammit et al. (2006).



Fig 1. Phase contrast images of myoblasts control and experimental day 7 myotubes. The top panel is a myoblast plate grown in 10% FBS. The 3 bottom panels show day 7 myotubes grown in 2% HS at 80% confluency (largest myotubes, contraction observed), 2% HS at 100% confluency, and 10% HS at 100% confluency. All images were obtained using a mobile device at 100x magnification. The purpose of these images was to visualize the effects of cell-to-cell contact and reduction of growth factors on myotube formation.

Day 7 control

Day 7 experimental







qPCR and Pfaffl





expression



m-Cadherin Expression in C2C12 cells throughout Myogenesis



had a n = 9, while all experimental groups had a n = 3, except for 2% HS at 80% confluency group had n=2. Mean +/- SEM: control: 0.944; 4.585 10%HS,100%con.: 0.787; 1.941 2% HS, 80% con.:0.826; 2.154 2% HS, 100% con.: 0.759;

2.040

10% HS at 100% confluency

MyoD

relative to GAPDH expression

in control and experimental

groups at myoblast, days 0, 4,

and 7. Gene expression was

Fig

2.

Fig 3. M-Cadherin expression relation GAPDH to in expression in control and



DISCUSSION & CONCLUSIONS

Morphology

- Earlier induction (i.e. 80% confluency) enhanced myotube formation
- Higher serum concentrations (i.e. 10% HS) promotes myotube formation but in less coordinated pattern

mediates cell fusion, allowing

myotubes to form.

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