Differentiation Dilemma:

How different modes of induction of differentiation affect myogenin gene expression and myotube development in C2C12 myoblasts

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Introduction	Methods
 Myogenesis involves proliferation of myoblasts, withdrawl from the cell cycle, and differentiation into multinucleated myotubes (1). Differentiation can be induced in vitro in various different ways During differentiation, various genes and myogenic regulatory factors (MRFs) are expressed, including myogenin which is often used as a 	 Murine derived C2C12 cells were induced to differentiate by either cell-to-cell contact in 10% FBS or serum deprivation by switching to 2% FBS, 10% adult horse serum (HS), or 2% HS. (see Figure 2) Myogenin gene expression was measured using quantitative PCR for the Myoblast (MB) and days 1 and 2 of differentiation.
 marker for differentiation (2). <u>Aim 1:</u> Assess the prevalence of various modes of growth/differentiation in the literature. 	 Cell fusion index (CFI), the percent of nuceli in myotubes over the total number of nuclei, was determined for 10% FBS, 10% HS, and 2% HS at day 2 Pubmed database was searched for "C2C12 differentiation" (2022-2024)
• Aim 2: Determine how the mode of induction of differentiation affects	• For each paper, the methodology for growth and differentiation of

<u>Aim 2:</u> Determine how the mode of induction of differentiation affects myogenin gene expression in C2C12 cell culture.





Figure 1. Schematic illustrating the stages of myogenesis and myogenin gene expression with differentiation induced by three primary methods. Adapted from Image courtesy of Emily Baines.



Results



Figure 3. Average cell fusion index for 10% FBS, 10% HS, and 2% HS at day 2 (n=3)

Figure 4. Confocal microscopy images of C2C12 cells on day 2 of differentiation stained for nuclei (blue) and cytoskeleton (red)

Figure 5. Myogenin gene expression for MB, days 1 and 2 of differentiation. Data represented as mean +/-SEM, n = 2-4

Mode of Differentiation	Frequency in Literature	
2% HS	336	
N/A	35	
2% FBS	18	
10% HS	6	

Discussion and Significance

- Large diversity in the modes of differentiation observed in literature
 - 2% HS is the most common mode of inducing differentiation
 - Raises concerns about **reproducibility** with differing methods.
- Modes of induction of differentiation are **not equavalent**
 - Differentiation in 2% HS accelerates myotube formation
 - Myogenin gene expression is highest on days 1 and 2 for differentiation in 10% FBS
- Myogenin gene expression does **not** track with myotube morphology at day 2 (ex. highest gene expression in 10% FBS, lowest CFI)
- Myogenin gene expression appears to be blunted in all cultures where the serum was changed to induce differentiation



Day of Differentiation



• Observed to **respond to environmental factors** and may be a **substandard** marker

for differentiation. (see right)

References and Acknowledgements

Table 1. Prevalence of

differentiation media used in literature. N/A = not specified (n = 439).

Thank you to Dr. Beth Richardson and Breanne Bali (consultation), Cara Medvedic (CFI counting), Sean Ashby (cell culture, imaging, CFI counting), and Emily Baines (infographic, consultation) (1) Burattini, S., Ferri, P., Battistelli, M., Curci, R., Luchetti, F., & Falcieri, E. (2004). C2C12 murine myoblasts as a model of skeletal muscle development: morpho-functional characterization. European journal of histochemistry : EJH, 48(3), 223-233.

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