Course-based Undergraduate Research Experience in a Senior Cell & Molecular Biology Laboratory Course

Laura L. Atkinson
latkinson@mtroyal.ca

MOUNT ROYAL UNIVERSITY 1910

Why a Course-based Undergraduate Research Experience (CURE)?

- Experiential learning increases knowledge retention and enhances learning experience
- Increased opportunity for independent research
- Performing real science teaches resiliency
- Students gain troubleshooting and critical thinking skills
- Unexpected data provides a greater opportunity to teach

Teaching Experimental Design

- Validation of experimental system is critical to verify that you are actually measuring a real effect
- To obtain results from which conclusions can be drawn
- To use resources effectively

Critical Components

- Suitable comparisons, positive & negative controls
- Replication (biological vs.technical replicates)
- Randomization and avoidance of bias

Data Analysis - Considerations

- Understanding how a design can be analyzed
- Signal/noise ratio
- Sources of variability and reducing variability
- Risk of false positives and false negatives
- Determining the numbers needed
- T-tests and analysis of variance
- Types of experimental design and choice of design
- Assumptions behind parametric statistical tests

Assessments

- Journal club presentation of scientific article with focus on critique of experimental design
- Online lab notebook detailing purpose of each reagent and step in the experiment
- Written manuscript in the form of a publishable scientific article

References

A practical guide to course-based undergraduate research experiences. Promoting concept-driven teaching strategies in biochemistry and molecular biology. National Science Foundation (Grant number 0957205)

Costa, M. (2014) Cytoskeleton and adhesion in myogenesis. *ISRN Developmental Biology*. http://dx.doi.org/10.1155/2014/713631

Maiese, K. (2016) *Molecules to medicine with mTOR: Translating critical pathways into novel therapeutic strategies.* Retrieved from https://doi.org/10.1016/C2014-0-03321-7

Waterman, R., Heemstra, J. (2018) Expanding the CURE model: Course-based undergraduate research experience. Research Corporation for Science Advancement.

Research Question: What morphological changes do c2c12 cells undergo during the process of myogenesis?

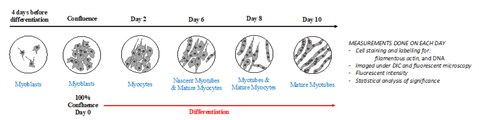

Figure 2: Experimental design of morphologic, and filamentous actin analysis done, as well as measurements taken at each day.

Key morphological events

- Change in cell shape
- Multinucleation
- Increase in mitochondrial number
- Arrangement of actin into sarcomeres

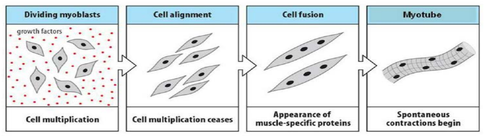

Figure 1. Morphological changes that occur in the transition of myoblasts to myotubes.

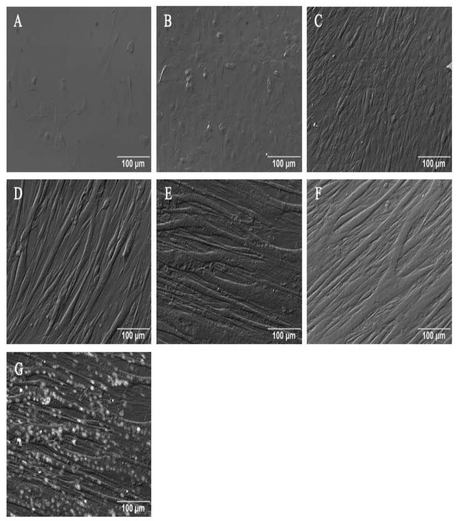

Figure 3: Morphological changes of c2c12 cells, in DIC at 20x magnification, over the course of differentiation. A Myoblasts on day -4. **B** Myoblasts at confluency. **C** Myocytes on day 2. **C** Nascent myotubes and mature myocytes on day 6. **D** Myotubes on day 8. **E** Mature myotubes on day 10. **G** Image of day 8 cells with DAPI overlay to show nuclei.

Research Question: Does filamentous actin localization and/or expression change during the process of myogenesis?

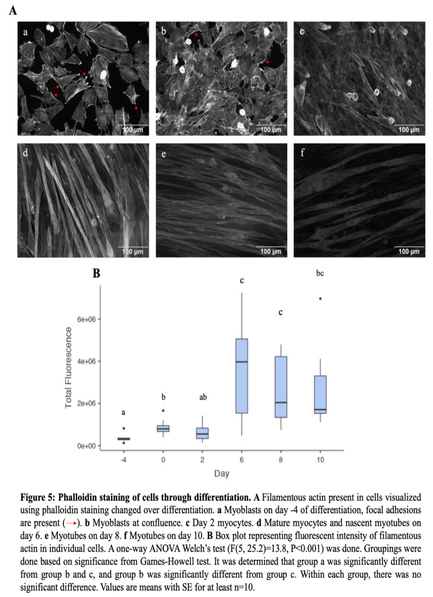

Figure 5: Phalloidin staining of cells through differentiation. A Filamentous actin present in cells visualized using phalloidin staining changed over differentiation. **a** Myoblasts on day -4 of differentiation, focal adhesions are present (→). **b** Myoblasts at confluence. **c** Day 2 myocytes. **d** Mature myocytes and nascent myotubes on day 6. **e** Myotubes on day 8. **f** Myotubes on day 10. **B** Box plot representing fluorescent intensity of filamentous actin in individual cells. A one-way ANOVA Welch's test (F(5, 25.2)=13.8, P<0.001) was done. Groupings were done based on significance from Games-Howell test. It was determined that group a was significantly different from group b and c, and group b was significantly different from group c. Within each group, there was no significant difference. Values are means with SE for at least n=10.

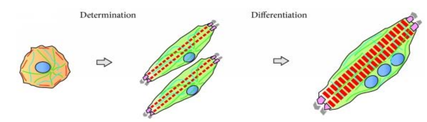

Figure 1. Proposed changes in actin localization during differentiation (Costa, 2014)

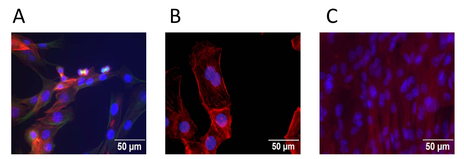

Figure 2. Filamentous actin (red) stained by phalloidin and nuclei (blue) stained with DAPI show c2c12 myoblasts undergoing mitosis (A). Actin localization around the cell periphery in myoblasts (B) and actin localization in myotubes ©.

Research Question: How does the fusion index (or multinucleation) of c2c12 cells change as they undergo myogenesis?

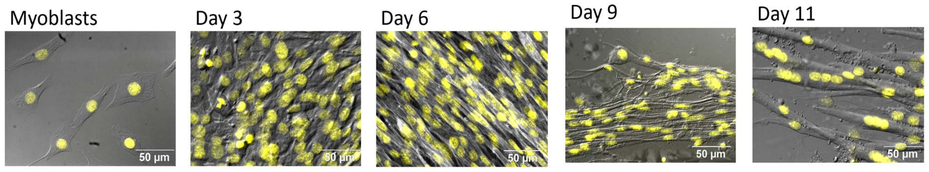

Figure 1. Sample DIC images overlaid with DAPI stained nuclei at different stages of differentiation. The slides show the progression from myoblasts to myotubes over time (in days) using the 40X objective lens. Automatic focus and exposure settings were set in cellSens software.

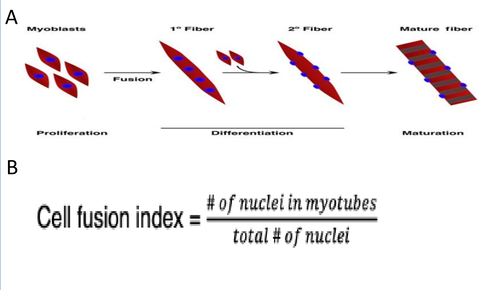

Figure 2. Process of multinucleation in myogenesis. A) Proposed model of multinucleation through fusion of myoblasts (Maiese, 2016). B) Calculation used to determine cell fusion index. Cell nuclei were counted using ImageJ software.

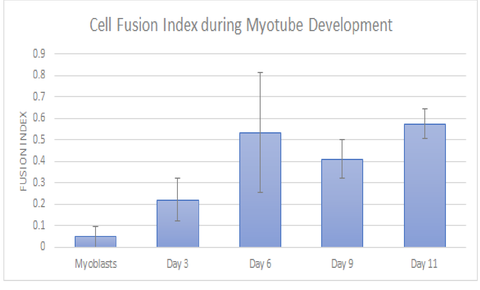

Figure 3. Fusion index is quantified at different stages of myotube development (n=2). The error bars show the standard error of the mean.

Research Question: What happens to cell area during the process of myogenesis?

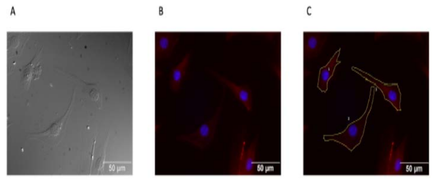

Fig 4. Detection of myoblast cell area determined using fluorescence and DIC microscopy at 20x magnification.

Phalloidin (red) detects actin, and DAPI (blue) detects cell nuclei. A) Differential Interference microscopy used to obtain a micrograph of myoblasts. B) Myoblasts viewed under fluorescence microscopy, detected using phalloidin and DAPI. C) Outline (yellow) of panel B in ImageJ to obtain the cell area used to calculate the difference in cell size.

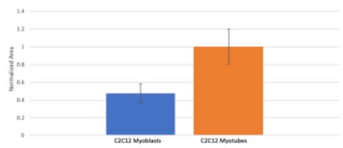

Fig 3. **Difference between Myotube and myoblast cell size.**

Myoblast cells were imaged at a 40x magnification and myotube cells were viewed at a 20x magnification. Cells were analyzed using the free hand selections tool in ImageJ and the area was obtained. Myoblasts were normalized to the myotube average size, and myotubes were normalized to a 40x magnification. Data are represented as mean +/- SEM, n=6.

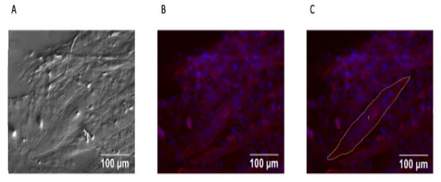

Fig 5. Detection of myotube cell area determined using fluorescence and DIC microscopy at 20x magnification.

Phalloidin (red) detects actin, and DAPI (blue) detects cell nuclei. A) Differential Interference microscopy used to obtain a micrograph of myotubes. B) Myotubes viewed under fluorescence microscopy, detected using phalloidin and DAPI. C) Outline (yellow) of panel B in ImageJ to obtain the cell area used to calculate the difference in cell size.

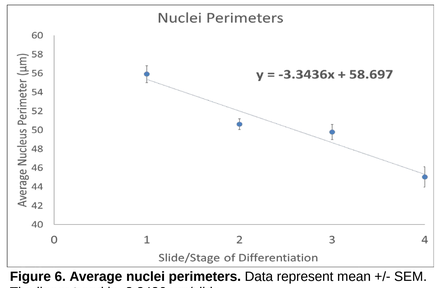

Figure 6. Average nuclei perimeters. Data represent mean +/- SEM. The linear trend is -3.3436 um/slide.

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