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AP Biology

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Stem Cell Lab

I. Introduction

Stem cells are the focus of tissue engineering and regenerative medicine. These stem cells have two main properties: first that they are capable of unlimited self-renewal and can produce lineages more specialized than themselves. These lineages are called lines of cells. Scientists are currently seeking ways to use stem cells in tissue therapy. The stem cell line used in this experiment is from cell cultures of a myoblastic mouse line, myoblastic meaning they can differentiate into muscle. This line is called C2C12. When C2C12 differentiates, it forms contractile myotubes and produces characteristic muscle proteins. These myotubes are long, multinuclear tubes, as they are the product of many individual cells fusing together. Certain conditions are necessary for these cell cultures to live outside the body, including: pH, temperature, Carbon dioxide and oxygen concentration, food, vitamins, minerals, surface attachments for cells, and growth factors. The proper pH, food, vitamins, and minerals are provided by the media. Oxygen and carbon dioxide is provided for as air can enter the plates, which are not air tight. The surface attachment for cells is provided in the plates and T25 flasks, and they are dislodged from their original surface from the trypsin and the slap. Growth factors are also in the media. When the media is at a low concentration, such as 1%, it prompts these C2C12 stem cells to differentiate into myotubes. Additional variables in the extra-cellular matrix can affect differentiation by affecting the growth factors, such as hormones or available nutrients.

Thus the purpose of this experiment is to determine the affect of Gatorade on C2C12 proliferation and differentiation. Gatorade is a common sports drink used for sport performance and rehydration, and it would be significant if it appeared to affect muscle differentiation, affecting the performance of athletes. Results will be measured based on the amount of proliferation of cells via confluency, or the measure of what percentage of the plate is filled with stem cells. This will be determined by observing the center of each plate (where the most cells grow) at 100x magnification. Gatorade will not significantly affect C2C12 proliferation or differentiation.

II. Materials and Methods

See lab sheet.

III. Results

Stock Solution = 0.05 mL Gatorade + 4.95 mL PBS = $\frac{0.05 \text{ mL Gatorade}}{5.0 \text{ total volume}}$ = 1% Gatorade

T25 Flask = 0.4 mL 10% media, no Gatorade

Plate 1 = 3.0 mL 1.0% media, no Gatorade

Plate 2 = 2.7 mL 1.0% media, 0.3 mL Gatorade; 0.1% Gatorade exposure

Plate 3 = 2.97 mL 1.0% media, 0.03 mL Gatorade; 0.01% Gatorade exposure

	% Confluency	% of nuclei in myotubes
Plate 1 (control)	80%	30%
Plate 2	70%	10%
Plate 3	70%	5%
T 25 Flask	80%	0%

IV. Conclusion

The Gatorade appeared to have an effect on proliferation and differentiation. The proliferation was measured by confluency percentage, or the percentage of cells filling the plate. This was done by observing the center of the plate (where the most cells grow) and estimating how much of the plate is filled with cells. (100% would mean no more cells could grow, 0% would mean there are no cells). While this is simply an estimation, it is open for error and experimental bias. As both experimenters are inexperienced, the true confluency percentage may be different than the confluency observed. However, plates two and three have a slightly lower percentage of confluency, possibly due to the presence of Gatorade. By preventing cells from growing, the Gatorade displayed toxicity. Due to the limitations of the experiment, however, there were no replicates, meaning a statistical analysis cannot be done. Also, an even confluency of cells is assumed to have been pipetted onto each plate. In reality, however, there may have been different amounts of cells originally added to each plate, thus affecting the final cell confluency.

The differentiation was quantified by looking at the nuclei. While it was possible to qualitatively observe, seeing that there was more differentiation with more tube formation and alignment in plate 1 than in plates 2 or 3, quantifying the differentiation requires a numeric measurement. When myotubes forms, the stem cells fuse, creating a multinuclear muscle cell. The differentiation was quantified by estimating the percentage of nuclei, the dark stained spheres, found in a multinuclear differentiated cell. Therefore nuclei in cells simply aligning were not counted as differentiated, while those which fused with other cells were counted as differentiated. Based on the date, Gatorade appeared to inhibit differentiation. Without replicates, however, and the biased nature of

this observation, due to the large number of nuclei, this cannot be supplemented with statistical analysis. The cells in the T25 flask did not differentiate because the media was kept at 10%, signaling the cells to remain stem cells rather than differentiate.

As with every experiment, this procedure contains much room for error, and many possible extensions. First, some possible limitations include the inexperience of the experimenters in their confluency and differentiation measures, having only one replicate, slight concentration error due to incorrect pipet usage, and insufficient/excessive trypsin time. These possible errors cause slight variations in the data caused by mistakes rather than the variable. Secondly, there are many possible extensions to this experiment. The most obvious extension is more replicates. This experiment cannot make an accurate conclusion about the data because there was only one replicate, and thus no statistical analysis can be performed. Other possible extensions include a higher concentration of Gatorade (the chosen concentrations had a seemingly insignificant effect), different flavors of Gatorade, various different stem cell lines, and performing a trypan blue exclusion assay to determine whether the cells were still alive or not.