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Funding Source: Life Sciences Undergraduate Research Opportunity Program

Optimization of protocols to propagate and passage undifferentiated and differentiated mouse embryonic stem cells

The transplantation of neuralized stem cells holds the promise of providing neurotrophic effects to individuals suffering from some neurodegenerative disorders. Several current neuralization protocols require the formation of embryoid bodies to obtain a population of neural stem cells. Embryoid bodies (EBs) are round, free-floating aggregates of a heterogeneous population of ES cells undergoing differentiation. The addition of neuralization factors, such as retinoic acid (RA), induces the EB cells toward a neural fate. However, the EBs are only useful if the cells they consist of can survive dissociation. One way of forming EBs is to trypsinize cultured ES cells and transfer them to an uncoated plate with Embryonic Stem cell Induction Media. Different protocols have suggested the use of various trypsin concentrations, ranging from 0.05% to 0.25%, for varying lengths of time. Our current lab protocol uses 0.25% trypsin for 5 minutes, but on occasion this leads to extensive loss of cells during EB dissociation. It was hypothesized that using the high trypsin concentration for an extended period of time was causing the eventual death of the neuralized cells. The goal of my experiments was to optimize the trypsinization protocol that precedes embryoid body formation and to obtain the highest yield of large EBs with the maximum amount of cell survivorship following EB dissociation. Trypsinization conditions that were analyzed included 0.05% trypsin for 5 minutes, 0.25% trypsin for 5 minutes, and 0.25% trypsin for 2.5 minutes. Our results suggest that both 0.25% trypsin for 2.5 minutes and 0.05% trypsin for 5 minutes result in a higher cell survivorship after EB dissociation than the current trypsinization protocol. Cell survivorship was determined by comparing cell counts prior to trypsinization to cell counts following EB dissociation. Future studies could include testing low oxygen tensions and different temperatures during trypsinization.

This project was completed to fulfill a Capstone requirement.