

Teresa Jackson

Major: Biology
University: Xavier University of Louisiana
Faculty Mentor: Dr. Mark Kirk
Mentor Department: Biological Sciences
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Development of chick embryo explant cultures as an assay system to test mammalian stem cell migration and differentiation after transplantation

Teresa Jackson, Gina Joshua, Jessica Struckhoff, Zach Whitehead, Jason Meyer, Sinead O'Connell and Mark Kirk

Stem cells are undifferentiated cells capable of achieving multiple developmental fates. The embryonic stem (ES) cell can differentiate into all cells of the body. Our stem cell research focuses on the repair and/or renewal of cells in the central nervous system with potential applications for treatment of such diseases as Parkinson's disease, ALS, Alzhiemers disease, Batten's disease, as well as other neurodegenerative disorders and injuries to the CNS. An important problem is to determine the potential of stem cells to achieve various fates after transplantation. In the present study, we developed the chick embryo explant culture as an assay system to test the ability of mouse embryonic stem cells to migrate and differentiate after transplantation. The focus of this study was to monitor the survival, migration, and incorporation of the mouse ES cells after transplantation into chick embryos and to develop an explant culture method. The explant cultures will enable repeated viewing of transplanted stem cells at various time post-transplantation. Initially to establish chick embryo explants, chicken embryos were excised from the yolk at stage 8 and transferred to agar/albumen culture dishes containing antibiotics. The chick embryos explants were incubated at 37°C in a humidified chamber to continue growth and development, and explants were viewed multiple times. To date, chick embryos survived up to 47 hours after explantation. Successful explants were obtained as determined by normal embryonic development and the presence of a normal heartbeat. For stem cell transplant experiments, green fluorescent protein (GFP)-expressing mouse ES cells were transplanted into the head region of stage 10 chick embryos while the embryos were still in the egg (i.e., in ovo). Immediately following transplantation, explant cultures were established and embryonic development was allowed to proceed for up to 28 hours (~stage 15-16). The embryos were screened using a fluorescence microscope to test for the presence and fate of transplanted mouse ES cells. Ten micrometers thick transverse sections of the embryos were examined and GFP-expressing cells were found in the lower head region around the neural tube. The transplanted ES cells appeared to contribute to embryonic mesoderm. Future experiments will test whether transplanted stem cells can incorporate into the neural tube and differentiate into neural cells.