CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

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Project Number

31746

Project Title

The Effects of hESC Mother Colony Partitioning on the Pluripotency of Daughter hESC Colonies

Abstract

Objectives/Goals

Propagation of stem cell lines plays a significant role in stem cell research. The use of mechanical means in passaging hESC colonies is preferred, as the probability of aneuploidy in daughter colony cells is minimized. Highly pluripotent daughter colonies are produced when the differentiated material transferred to them from the mother colony is reduced. A proposed methodology accomplishes this reduction by excluding from the propagation to the daughter colonies a larger area around the button of the mother colony. Although the number of transferred cells is smaller, the potential exclusion of differentiated cells leads to daughter hESC colonies with higher pluripotency.

Methods/Materials

A twenty four well plate to receive (HES3) colony fragments was prepared with the appropriate density MEF feeder layer. Each mother colony was divided into two parts. One part was partitioned by radially sectioning the mother hESC colony excluding a tightly defined area surrounding the button. The other part was partitioned by radially sectioning the mother hESC colony excluding a larger area surrounding the button. The resulting fragments were placed in the well plate and were incubated (36.6Cand 5% CO(2)).for seven days, following a daily medium change procedure. At the end of the seventh day, the daughter colonies growing on the plate were fixed and imaged. Pictures of the mother and daughter hESC colonies were taken under a compound stereo microscope and a number of qualitative and quantitative criteria were employed to assess the level of pluripotency maintained by the daughter hESC colonies.

Results

Three mother hESC colonies were partitioned using the traditional and the proposed partitioning scheme. Visual inspection of the daughter colonies and statistical analysis of the colony scores revealed that the proposed partitioning method of the mother hESC colony provided better daughter colonies than the traditional partitioning method.

Conclusions/Discussion

The results demonstrated that the less potentially differentiated material that is passaged from the parent to daughter colonies, the higher the colony grade of the daughter colony. Therefore, when passaging cells, it is necessary to avoid potentially differentiated material by cutting, not just around the button of the mother colony, but at a radius excluding the neighborhood of the button as well.

Summary Statement

The goal of the project is the production of high quality stem cell colonies for clinical research and application in regenerative medicine.

Help Received

The experimental work was performed during my summer internship at the Stem Cell Core facility of the Keck School of Medicine at the University of Southern California under the mentorship of Dr. Victoria Fox.

Preliminary