Researchers Maintain Stem Cells Without Contaminated Animal Feeder Layers

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The growth and maintenance of human embryonic stem cells in the absence of contaminated animal products has been demonstrated by University of California, San Diego (UCSD) School of Medicine researchers in the Whittier Institute*, La Jolla, California.

Published in the April 2005 issue of the journal *Stem Cells*, the study shows that laboratory culture media enriched by a human protein called activin A are capable of maintaining human embryonic stem cells in a continuous undifferentiated state, ready for research. Undifferentiation means the stem cells have not begun the developmental path to become specific human tissue or organs.

"Our findings provide a new way to generate human stem cell lines without contamination by animal cells or products," said the study's senior author, Alberto Hayek, M.D., UCSD professor of pediatrics and director of the Islet Research Laboratory at the Whittier Institute.

Currently, stem cell lines derived from human embryos are grown and nourished in petri dish material called feeder layers that are made with animal connective tissue, primarily mouse and calf. A recent study in *Nature Medicine* ** by UCSD's Ajit Varki, M.D. showed that human embryonic stem cells grown in this animal-derived tissue become contaminated with a non-human molecule called Neu5Gc. If these stem cells were to be transplanted into people, they would provoke an immune system attack eliminating their therapeutic value.

While several laboratories have attempted to grow stem cells in alternative cultures, problems have remained. In some cases, human feeder layers were developed but this added another measure of complexity to the culture system, Hayek noted. In recent studies in Wisconsin and Massachusetts, new feeder layers were developed, but they did not entirely eliminate the use of animal products. In the Hayek study, the animal-derived feeder layers are completely eliminated. However, the petri dishes themselves are coated with laminin (an animal-derived product), and the UCSD team is continuing studies to determine if contamination occurs from this source.

For a proposed animal-free medium, the UCSD study takes advantage of a previously unidentified soluble factor this is secreted from mouse feeder layers to maintain stem cells' undifferentiated state and pluripotency, the ability to become all tissue types in the body. The scientists knew that a cocktail of various growth factors and chemicals had previously been shown to modulate cellular growth and differentiation in human pancreatic cells. Human embryonic stem cells cultured for several weeks under these conditions showed no change in cell form and structure. The team then eliminated each factor and pluripotency was assessed. At first, the results were narrowed to three molecules, with activin A, a protein that participates in cellular growth and differentiation, as well as hormone secretion, finally shown in additional testing to maintain the undifferentiated state.

The study's first author, Gillian M. Beattie, M.S., UCSD Department of Pediatrics and the Whittier Institute, said that "it will be rather simple now to develop a specific defined medium that allows for the maintenance of the human stem cells and enhances research without the problem of contaminating animal cells and their products."

In a summary to their paper, the researchers noted that "the identification of activin A as a key factor in mediating these cellular events will help to unravel the biochemical pathways responsible for 'stemness'. An increased efficiency in the generation and culture of human stem cells for potential clinical applications is timely, given the recent report of 17 newly derived stem cell lines available for non-federal research. The findings here may facilitate the derivation of new human embryonic stem cell lines without the use of animal or human feeder layers."

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The University of California has licensed this technology to Stem Tech, Inc., which plans to develop commercially viable culture media free of animal derived products.

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