

New Human Embryonic Stem-Cell Lines — More Is Better

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It is rare that a field of scientific research can simultaneously represent a domain of fundamental discovery in human biology and potentially have major effects on human health and the quality of life. Human embryonic stem cells serve not only as a resource for basic research, but also as the starting material for the development of cell-based therapies. Formidable scientific challenges face those who aim to develop cell-based therapies. It will take the efforts of many scientists and clinicians in a variety of disciplines to bring this endeavor to fruition. Of immediate importance and concern is whether or not we have sufficient starting material for the initial phases of this research.

Embryonic stem cells are derived from the inner cell mass of blastocysts, the preimplantation stage at which the developing mammalian embryo is implanted in the uterine endometrium (see Figure). The derivation of mouse embryonic stem cells was first reported in 1981^{1,2}; Thomson et al. reported the derivation of human embryonic stem cells in 1998,³ and their general procedure has been used by others. To isolate human inner cell mass, cleaving preimplantation-stage embryos are cultured to the blastocyst stage; the blastocysts are treated with a weak acid to remove the acellular zona pellucida that covers the surface of the conceptus, and the trophoblast cells are lysed (through immunosurgery) in the presence of antihuman antiserum (usually antibodies to human red cells) and guinea pig complement. The isolated inner cell masses are then cultured, in the presence of culture medium with growth factors, on a feeder layer of mouse embryonic fibroblasts (after the feeder cells have been irradiated or treated with mitomycin to stop them from dividing).

The need for feeder cells is an annoying obstacle to the convenient growth and efficient study of embryonic stem cells in the laboratory. Moreover, the use of mouse feeder cells raises the specter of the interspecies transfer of viruses. Therefore, one goal of research on embryonic stem cells is to find a way to

derive and culture cell lines without the use of feeder layers. In the meantime, the use of feeder cells of human origin addresses the concern about the interspecies transfer of viruses, although intraspecies transfer remains possible.

The slow growth rate of embryonic stem cells (most have a doubling time of 32 hours or more) is often the limiting factor in experiments using these cells. For this reason, initial passages are routinely performed by means of mechanical dissociation, which, though time-consuming, results in higher plating efficiency than dissociating the cells with enzymes. Some human embryonic stem-cell lines are maintained solely by means of mechanical dissociation, especially when large numbers of cells are required for an experiment.

The defining characteristics of embryonic stem-cell lines are in the process of being established.⁴ Key properties include continuous passage; clonability; chromosome stability; the expression of a variety of molecular, biochemical, and antigenic markers; and the ability to differentiate into various types of tissue and to form tumors (teratomas) after being grafted into immunocompromised animals.

It is not known how many human embryonic stem-cell lines exist in laboratories throughout the world, but in the United States, much attention has focused on the lines that were derived before August 9, 2001, at 9:00 p.m., since only research performed with the use of these lines is eligible for federal funding. There are 15 such lines identified on the National Institutes of Health (NIH) Registry. Most of these lines are expensive, and the Material Transfer Agreements for some of them contain stringent requirements.

The article by Cowan et al. in this issue of the *Journal*, which reports the derivation of 17 new lines of human embryonic stem cells, is therefore an important contribution. In a tour de force, Douglas Melton of this research group mustered resources from the Howard Hughes Medical Institute, the Juvenile Di-

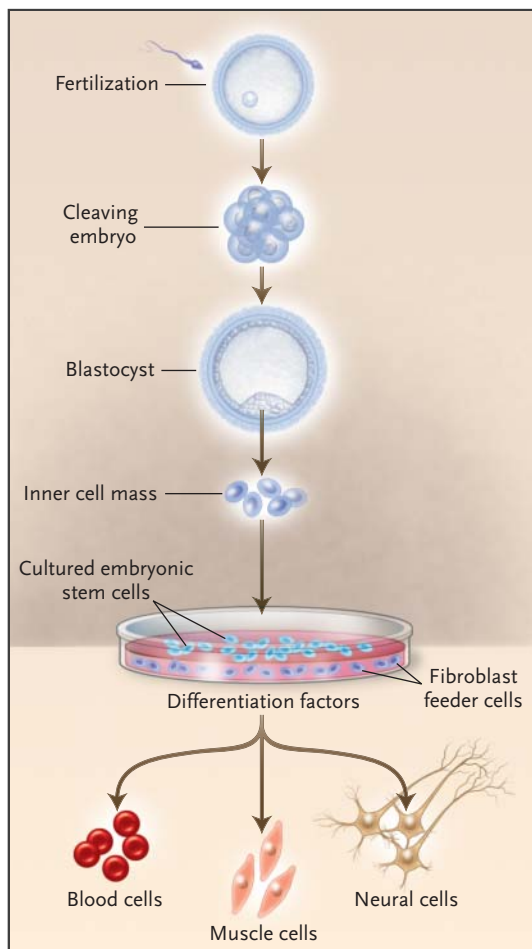


Figure. Derivation of Embryonic Stem Cells.

Embryonic stem cells are derived from the inner cell mass of the blastocyst; cells composing the inner cell mass are isolated and then plated on culture medium, below which is a layer of feeder cells.

abetes Research Foundation, and Harvard University in order to produce new human embryonic stem-cell lines that are more readily available to investigators, although research using these lines is ineligible for NIH funding at present. The lines were derived with

the use of trypsin for passaging and were grown in the presence of penicillin and streptomycin, all of which should make for easier handling in the laboratory. (Most human embryonic stem-cell lines currently available are intolerant of antibiotics.) These cell lines express the accepted embryonic stem-cell markers and demonstrate differences in doubling times — ranging from 24 to 72 hours. The shorter doubling times may well be important, given the limitations that slow growth rates place on experiments.

The lines were established and are grown on layers of mouse feeder cells, so their usefulness in humans may be limited,⁵ although the Food and Drug Administration has not ruled out the clinical use of cell-based therapies derived from lines grown on feeder layers from other species. Perhaps when the critical factor or factors produced by the feeder layers have been identified, we can count on the same team to derive new cell lines that can be grown without the need for feeder layers. Of course, it is only through research involving embryonic stem-cell lines that such factors may be discovered — once again underscoring the importance of the contribution made by Cowan et al.

Dr. Gearhart reports that Johns Hopkins University holds in escrow for him stock in the Geron Corporation. He also reports having received lecture fees from Johnson & Johnson.

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1. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981;292:154-6.
2. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci U S A* 1981;78:7634-8.
3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282:1145-7. [Erratum, *Science* 1998;282:1827.]
4. Brivanlou AH, Gage FH, Jaenisch R, Jessell T, Melton D, Rosant J. Stem cells: setting standards for human embryonic stem cells. *Science* 2003;300:913-6.
5. Dawson L, Bateman-House AS, Mueller Agnew D, et al. Safety issues in cell-based intervention trials. *Fertil Steril* 2003;80: 1077-85.