Tissue cultures, organ culture, whole embryo culture and stem cell culture

Types Of Tissue Culture :

There are three main methods of initiating a culture.,

(1) **Organ culture** implies that the architecture characteristic of the tissue *in vivo* is retained, at least in part, in the culture. Toward this end, the tissue is cultured at the liquid–gas interface (on a raft, grid, or gel), which favors the retention of a spherical or three-dimensional shape.

(2) In **primary explant culture**, a fragment of tissue is placed at a glass (or plastic)–liquid interface, where, after attachment, migration is promoted in the plane of the solid substrate.

(3) ***Cell culture*** implies that the tissue, or outgrowth from the primary explant, is dispersed (mechanically or enzymatically) into a cell suspension, which may then be cultured as an adherent monolayer on a solid substrate or as a suspension in the culture medium.

Because of the retention of cell interactions found in the tissue from which the culture was derived, organ cultures tend to retain the differentiated properties of that tissue. They do not grow rapidly (cell proliferation is limited to the periphery of the explant and is restricted mainly to embryonic tissue).

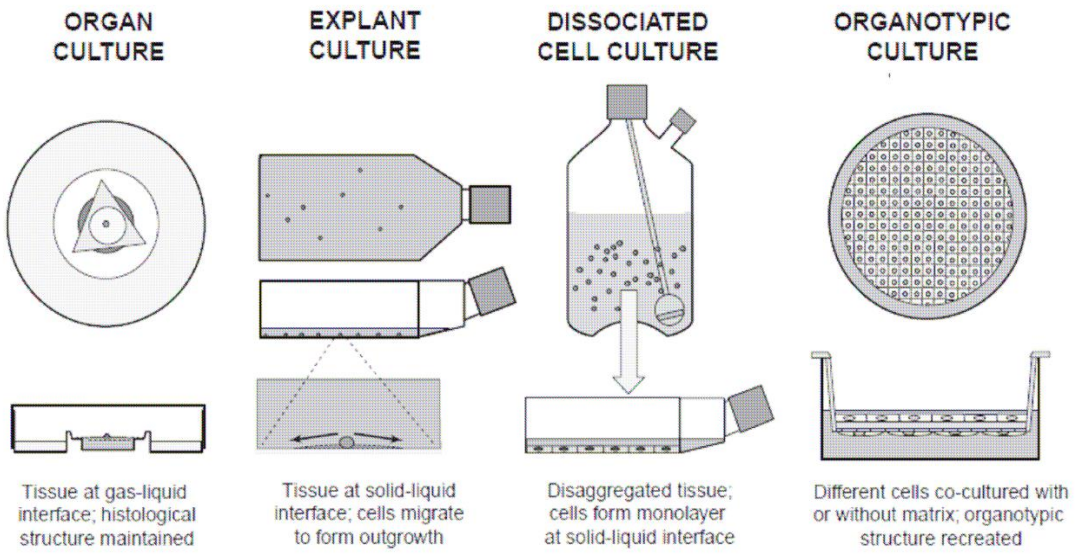


Fig. 1.3. Types of Tissue Culture.

TABLE 1.4. Properties of Different Types of Culture

Category	Organ culture	Explant	Cell culture
Source	Embryonic organs, adult tissue fragments	Tissue fragments	Disaggregated tissue, primary culture, propagated cell line
Effort	High	Moderate	Low
Characterization	Easy, histology	Cytology and markers	Biochemical, molecular, immunological, and cytological assays
Histology	Informative	Difficult	Not applicable
Biochemical differentiation	Possible	Heterogeneous	Lost, but may be reinduced
Propagation	Not possible	Possible from outgrowth	Standard procedure
Replicate sampling, reproducibility, homogeneity	High intersample variation	High intersample variation	Low intersample variation
Quantitation	Difficult	Difficult	Easy; many techniques available

TABLE 1.5. Subculture

Advantages	Disadvantages
Propagation	Trauma of enzymatic or mechanical disaggregation
More cells	Selection of cells adapted to culture
Possibility of cloning	Overgrowth of unspecialized or stromal cells
Increased homogeneity	Genetic instability
Characterization of replicate samples	Loss of differentiated properties (may be inducible)
Frozen storage	Increased risk of misidentification or cross-contamination

TABLE 5.1. Tissue Culture Equipment

Basic requirements
Laminar-flow hood (biohazard if for human cells)
Incubator (humid CO_2 incubator if using open plates or dishes)
5% CO_2 cylinder (for gassing cultures)
Liquid CO_2 cylinders, without siphon (for CO_2 incubator)
Balance
Sterilizer (autoclave, pressure cooker)
Refrigerator
Freezer (for −20°C storage)
Inverted microscope
Soaking bath or sink
Deep washing sink
Pipette cylinder(s)
Pipette washer
Still or water purifier
Bench centrifuge
Liquid N_2 freezer (~35 L, 1,500–3,000 ampoules)
Liquid N_2 storage Dewar (~25 L)
Slow-cooling device for cell freezing (*see* Section 20.3.4)
Magnetic stirrer racks for suspension cultures
Hemocytometer

The Substrate -Attachment And Growth :

The majority of vertebrate cells cultured *in vitro* grow as monolayers on an artificial substrate. Hence the substrate must be correctly charged to allow cell adhesion, or at least to allow the adhesion of cell-derived attachment factors, which will, in turn, allow cell adhesion and spreading.Cells shown to require attachment for growth are said to be *anchorage dependent*; cells that have undergone transformation frequently become *anchorage independent* and can grow in suspension when stirred or held in suspension with semisolid media such as agar.

Substrate Materials:

***Glass*.** This was the original substrate because of its optical properties and surface charge, but it has been replaced in most laboratories by synthetic plastic (polystyrene), which has greater consistency and superior optical properties.

***Disposable plastic*.** Single-use sterile polystyrene flasks provide a simple, reproducible substrate for culture. They are usually of good optical quality, and the growth surface is flat, providing uniformly distributed and reproducible monolayer cultures. As manufactured, polystyrene is hydrophobic and does not provide a suitable surface for cell attachment, so tissue culture plastics are treated by corona discharge, gas plasma, or γ -irradiation, or chemically, to produce a charged, wettable surface.

Although polystyrene is by far the most common and cheapest plastic substrate, cells may also be grown on polyvinylchloride (PVC), polycarbonate, polytetrafluorethy-lene (PTFE; Teflon), Melinex, Thermanox (TPX), and a number of other plastics.

Culture vessel characteristics:

Culture vessel			
Multiwell plates	**Petri dishes**	**Flasks**	
Microtitration	3.5-cm diameter	10 cm^2 (T10)	
Microtitration	5-cm diameter	25 cm^2 (T25)	
4-well plate	6-cm diameter	75 cm^2 (T75)	
6-well plate	9-cm diameter	175 cm^2 (T175)	**Stirrer bottles**
12-well plate		225 cm^2 (T225)	500 mL (unsparged)
24-well plate		Roller bottle	5000 mL (sparged)

Media:

Eagle's Minimal Essential Medium (MEM) [Eagle, 1959] is the commonly used one supplemented with calf, human, or horse serum, protein hydrolysates, and embryo extract. Other cell lines includes L929 cells, HeLa, etc.

Isolation and propagation of cells of a specific lineage may require a selective serum-free medium, whereas cells grown for the formation of products, as hosts for viral propagation, or for non-cell-specific molecular studies rely mainly on Eagle's MEM [Eagle, 1959], Dulbecco's modification of Eagle's medium, DMEM [Dulbecco & Freeman, 1959], or, increasingly, RPMI 1640 [Moore et al., 1967], supplemented with serum. Industrial-scale production techniques now use serum-free media, to facilitate downstream processing and reduce the risk of adventitious infectious agents.

PHYSICOCHEMICAL PROPERTIES :

pH :

Most cell lines grow well at pH 7.4. The optimum pH for cell growth varies relatively little among different cell strains, some normal fibroblast lines perform best at pH 7.4–7.7, and transformed cells may do better at pH 7.0–7.4

Phenol red is commonly used as an indicator. It is red at pH 7.4 and becomes orange at pH 7.0, yellow at pH 6.5, lemon yellow below pH 6.5, more pink at pH 7.6, and purple at pH 7.8.

CO_2 and Bicarbonate :

Carbon dioxide in the gas phase dissolves in the medium, establishes equilibrium with HCO_3^- ions, and lowers the pH.

Buffering :

Culture media must be buffered under two sets of conditions:

(1) open dishes, wherein the evolution of CO_2 causes the pH to rise and

(2) overproduction of CO_2 and lactic acid in transformed cell lines at high cell concentrations, when the pH will fall. A buffer may be incorporated into the medium to stabilize the pH.

Oxygen :

The other major significant constituent of the gas phase is oxygen. Whereas most cells require oxygen for respiration *in vivo*, cultured cells often rely mainly on glycolysis, a high proportion of which, as in transformed cells, may be anaerobic.

Cultures vary in their oxygen requirement, the major distinction lying between organ and cell cultures. Although atmospheric or lower oxygen tensions are preferable for most cell cultures, some organ cultures, particularly from late-stage embryos, new-borns, or adults, require up to 95% O_2 in the gas phase .

Osmolality :

Most cultured cells have a fairly wide tolerance for osmotic pressure .As the osmolality of human plasma is about 290 mosmol/kg, it is reasonable to assume that this level is the optimum for human cells *in vitro*, although it may be different for other species.

Temperature :

The optimal temperature for cell culture is dependent on

(1) the body temperature of the animal from which the cells were obtained,

(2) any anatomic variation in temperature

(3) the incorporation of a safety factor to allow for minor errors in regulating the incubator. Thus the temperature recommended for most human and warm-blooded animal cell lines is 37° C.

BALANCED SALT SOLUTIONS

A balanced salt solution (BSS) is composed of inorganic salts and may include sodium bicarbonate and, in some cases, glucose.

COMPLETE MEDIA :

The term *complete medium* implies a medium that has had all its constituents and supplements added and is sufficient for the use specified. It is usually made up of a defined medium component, some of the constituents of which, such as glutamine, may be added just before use, and various supplements, such as serum, growth factors, or hormones. Defined media range in complexity from the relatively simple Eagle's MEM [Eagle, 1959], which contains

essential amino acids, vitamins, and salts, to complex media such as medium 199 (M199), CMRL 1066, MB 752/1 ,RPMI 1640 ,and F12 and a wide range of serum-free formulations . The complex media contain a larger number of different amino acids, including nonessential amino acids and additional vitamins, and are often supplemented with extra metabolites (e.g., nucleosides, tricarboxylic acid cycle intermediates, and lipids) and minerals.

ORGAN CULTURE:

Organ culture is a development from tissue culture methods of research, the organ culture is able to accurately model functions of an organ in various states and conditions by the use of the actual *in vitro* organ itself.

Parts of an organ or a whole organ can be cultured in vitro. The main objective is to maintain the architecture of the tissue and direct it towards normal development. In this technique, it is essential that the tissue is never disrupted or damaged. It thus requires careful handling. The media used for a growing organ culture are generally the same as those used for tissue culture. The techniques for organ culture can be classified into (i) those employing a solid medium and (ii) those employing liquid medium.

Methodology

Embryonic organ culture is an easier alternative to normal organ culture derived from adult animals. The following are four techniques employed for embryonic organ culture.

Plasma clot method

The following are general steps in organ culture on plasma clots.

1. Prepare a plasma clot by mixing 15 drops of plasma with five drops of embryo extract in a watch glass.
2. Place a watch glass on a pad of cotton wool in a petri dish; cotton wool is kept moist to prevent excessive evaporation from the dish.
3. Place a small, carefully dissected piece of tissue on top of the plasma clots in watch glass.

The technique has now been modified, and a raft of lens paper or rayon net is used on which the tissue is placed. Transfer of the tissue can then be achieved by raft easily. Excessive fluid is removed and the net with the tissue placed again on the fresh pool of medium.

Agar gel method

Media solidified with agar are also used for organ culture and these media consist of 7 parts 1% agar in BSS, 3 parts chick embryo extract and 3 parts of horse serum. Defined media

with or without serum are also used with agar. The medium with agar provides the mechanical support for organ culture. It does not liquefy. Embryonic organs generally grow well on agar, but adult organ culture will not survive on this medium.

The culture of adult organs or parts from adult animals is more difficult due to their greater requirement of oxygen. A variety of adult organs (e.g. the liver) have been cultured using special media with special apparatus (Towell's II culture chamber). Since serum was found to be toxic, serum-free media were used, and the special apparatus permitted the use of 95% oxygen.

Raft Methods

In this approach the explant is placed onto a raft of lens paper or rayon acetate, which is floated on serum in a watch glass. Rayon acetate rafts are made to float on the serum by treating their 4 corners with silicone.

Similarly, floatability of lens paper is enhanced by treating it with silicone. On each raft, 4 or more explants are usually placed.

In a combination of raft and clot techniques, the explants are first placed on a suitable raft, which is then kept on a plasma clot. This modification makes media changes easy, and prevents the sinking of explants into liquefied plasma.

Grid Method

Initially devised by Trowell in 1954, the grid method utilizes 25 mm x 25 mm pieces of a suitable wire mesh or perforated stainless steel sheet whose edges are bent to form 4 legs of about 4 mm height.

Skeletal tissues are generally placed directly on the grid but softer tissues like glands or skin are first placed on rafts, which are then kept on the grids.

The grids themselves are placed in a culture chamber filled with fluid medium up to the grid; the chamber is supplied with a mixture of O_2 and CO_2 to meet the high O_2 requirements of adult mammalian organs. A modification of the original grid method is widely used to study the growth and differentiation of adult and embryonic tissues.

Limitations

- Results from organ cultures are often not comparable to those from whole animals studies, e.g. in studies on drug action since the drugs are metabolized in vivo but not in vitro.

Applications Of Cell Culture:

1. The mitotic process and its modification by stimulants or suppressors have been studied in many cell types.
2. Visible light has some inhibitory effects upon living cells. The lethal effects of X-

irradiation can be quantified on mouse cells ,and the effects of radiation upon cell constituents and upon DNA and RNA synthesis can be studied.

3. Differentiation at the cellular level has mostly been studied in organ, rather than cell, cultures.
4. The uses of tissue culture in the study of cancer can be studied.
5. Comparison of enzyme activities in cells in culture with those from the mouse have been made.

STEM CELLS CULTURES:

Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide (through mitosis) to produce more stem cells. They are found in multicellular organisms. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells—ectoderm, endoderm and mesoderm (see induced pluripotent stem cells)—but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

There are three known accessible sources of autologous adult stem cells in humans:

1. Bone marrow, which requires extraction by *harvesting*, that is, drilling into bone (typically the femur or iliac crest).
2. Adipose tissue (lipid cells), which requires extraction by liposuction.
3. Blood, which requires extraction through apheresis, wherein blood is drawn from the donor (similar to a blood donation), and passed through a machine that extracts the stem cells and returns other portions of the blood to the donor.

Stem cells can also be taken from umbilical cord blood just after birth. Of all stem cell types, autologous harvesting involves the least risk. By definition, autologous cells are obtained from one's own body, just as one may bank his or her own blood for elective surgical procedures.

Adult stem cells are frequently used in medical therapies, for example in bone marrow transplantation. Stem cells can now be artificially grown and transformed (differentiated) into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves. Embryonic cell lines and autologous embryonic stem cells generated through Somatic-cell nuclear transfer or dedifferentiation have also been proposed as promising candidates for future therapies.

Stem Cells are different from other cells because:

1. They can continue to divide for long periods of time: Most cells such as skin cells cannot replicate themselves after a certain period of time. Stem cells are self-sustaining by replicating themselves for a much longer period of time.

2. **They are unspecialized:** Specialized cells have specific capabilities that allow them to perform certain tasks. For example a red blood cell contains hemoglobin that allows it to carry oxygen. Stem cells have unspecialized capability and do not have tissue- specific structures to perform specialized functions.

3. They can give rise to specialized cells: Stem cells go through a process called **differentiation** and create special types of cells (muscle, nerve, skin, etc.).

Embryonic stem cells

Embryonic stem cells are the cells within the protective layer of the blastocyst. They are pluripotent, which means they can develop into any of the cells of the adult body. Researchers believe that, because they are pluripotent, and easy to grow, they have the best potential for replacing damaged or lost tissue or body parts.

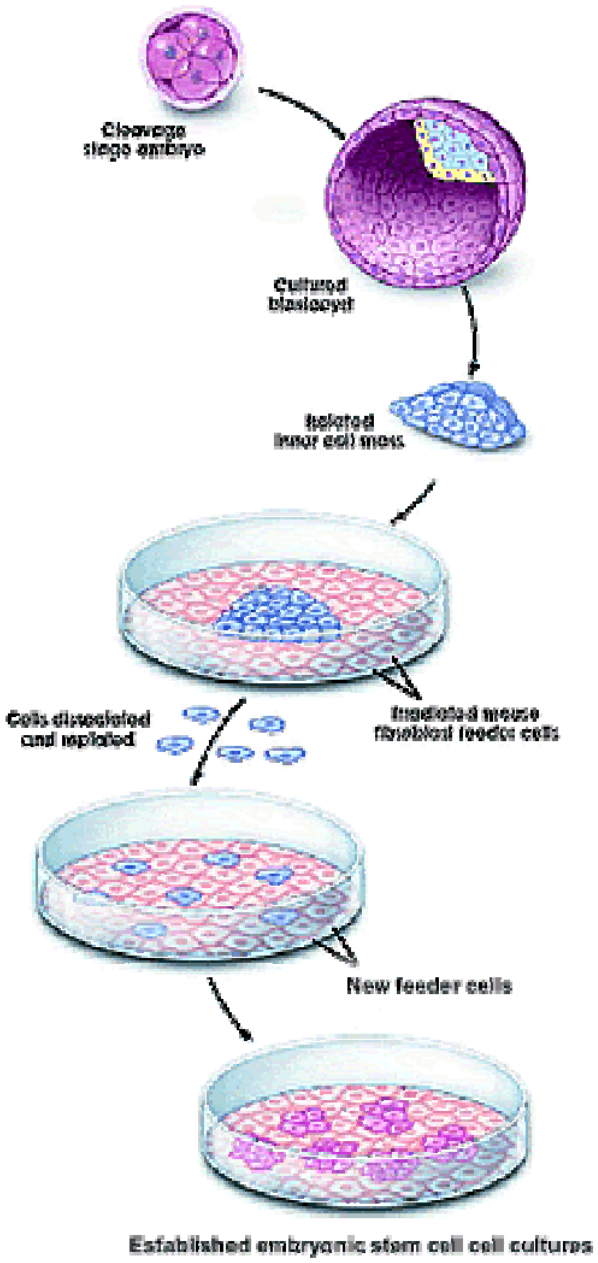
Adult stem cells

Also known as **progenitor cells** or **somatic stem cells**, adult stem cells are located, in small quantities, throughout the body and generate specialized cells for the area they are located. These cells do not renew themselves as well as embryonic stem cells. Still, if these cells are put in a different environment, they may produce a different type of cells from the originating cell.

Stem cell research is an active area of inquiry and scientists are discovering new characteristics of stem cells every day. For example, recent research indicated that multipotent stem cells from one type of tissue (blood) might actually have the ability to generate cells for a different type of tissue (nerve).

Scientists are continuing to search for new sources of adult stem cells. Some of the locations where stem cells have been located include: bone marrow, skin, liver, blood, and the brain. Some adult stem cells, which have already been used to treat illnesses, include **hematopoietic** stem cells and umbilical cord blood stem cells.

Hematopoietic stem cells are located in the bone marrow and form blood cells. They have been successfully used to treat blood disorders for younger patients. **Umbilical cord blood** stem cells are located in the blood of the umbilical cord after birth. Umbilical cord stem cells are similar to hematopeitetic stem cells in adults, but they are less mature and have much more potential to differentiate into various types of cells.



WHOLE EMBRYO CULTURE

Whole embryo culture appears to be an excellent method to screen chemicals for teratogenic hazard. Compared to *in* vivotesting it is cheap and rapid and does not involve experimentation on live adult animals. Also in the important area of risk estimation whole embryo culture offers distinct advantages over *in vivo* teratogenicity testing. Adverse embryonic outcomes (malformations or embryotoxicity) are directly related to the serum concentration of the compound being tested and can be compared to the serum concentration in the human. A similar comparison is not possible after *in vivo* testing because for most compounds there are major pharmacokinetic differences between humans and experimental animals. *In vivo* testing is also limited by the possibility that metabolites that occur in the human do not occur in the test

animal. This problem can be overcome in the *in vitro* system by adding the metabolite directly atthe desired concentration either with or without the parent compound. There is only one major disadvantage to *in vitro* testing and that is the limited period of embryogenesis that is undertaken in the commonly used culture system. This restricts the range of malformations that can be induced and may render the testing system unsuitable for compounds that are likely to exert their major toxicological effect late in gestation. Any evaluation of whole embryo culture for hazard and risk assessment in teratology must take into account the limited value of currently used *in vivo* methods. Over 2000 chemicals have been reported to be teratogenic in experimental animals exposed *in vivo*. In comparison only about 20 chemicals are known to cause birth defects in the human. This large number of *in vivo* false positive cannot easily be distinguished from true-positives. In this respect *in vivo* testing is severely deficient. The embryo culture testing system would also be expected to produce many falsepositives; but by comparing effective drug concentrations with human therapeutic concentrations they can be differentiated from true-positives. The most serious deficiency for an *in vivo* or *in vitro* teratogenicity testing system would be false-negatives. This has not been a problem in the validation of *in vitro* testing so far (except perhaps procarbazine), but difficult drugs such as thalidomide were not included. Thalidomide remains an important index chemical because it is not teratogenic in rats or mice but is teratogenic in the rabbit and human. It is likely that these species differences are due to metabolic differences between species and it is possible that if the proximate *teratogen/s* of thalidomide were identified they would be teratogenic in rat embryo culture. Whole embryo culture remains a very powerful technique that should continue to contribute to the determination of the safety of drugs and other chemicals during pregnancy.

REFERENCES

1. Carrel, Alexis and Montrose T. Burrows (1911). "Cultivation of Tissues in Vitro and its Technique". Journal of Experimental Medicine. 13 (3): 387–396. doi:10.1084/jem.13.3.387. PMC 2125263. PMID 19867420.

2. Steinhardt, E; Israeli, C; and Lambert, R.A. (1913) "Studies on the cultivation of the virus of vaccinia" J. Inf Dis. 13, 294–300

3. "Lab-grown bladder shows big promise", New Scientist, 2006, issue 2546, https://www.newscientist.com/channel/health/mg19025464.200-labgrown-bladder-shows-big-promise.html

4. Atala, Anthony; Bauer, Stuart B; Soker, Shay; Yoo, James J; Retik, Alan B (April 2006). "Tissue-engineered autologous bladders for patients needing cystoplasty". The Lancet. 367 (9518): 1241–1246

5. Max-Planck-Gesellschaft (2012, January 27). Heart of silk: Scientists use silk from the tasar silkworm as a scaffold for heart tissue. ScienceDaily. Retrieved January 29, 2012, from https://www.sciencedaily.com/releases/2012/01/120127135943.htm

6. Plant Tissue Culture. 100 years since Gottlieb Haberlandt. Laimer, Margit; Rücker, Waltraud (Eds.) 2003. Springer ISBN 978-3-211-83839-6

7. Martin, Bernice M. "Routine Cell Culture." Tissue Culture Techniques: An Introduction. Boston: Birkhäuser, 1994. 29-30. Print.

8. Simon, Eric M. (1988). "NIH PHASE I FINAL REPORT: FIBROUS SUBSTRATES FOR CELL CULTURE (R3RR03544A) (PDF Download Available)". ResearchGate. Retrieved 2017-05-22.

9. Reece, Jane B., Lisa A. Urry, Michael L. Cain, Steven A. Wasserman, Peter V. Minorsky, and Robert B. Jackson. "Angiosperm Reproduction and Biotechnology." Campbell Biology. 9th ed. San Francisco, CA: Benjamin Cummings, 2011. 860. Print.

10. Irfan Magsood, M; M. M.; Bahrami, A. R.; Ghasroldasht, M. M. (2013). "Immortality of cell lines: Challenges and advantages of establishment". Cell Biology International. 37 (10): 1038–1045.

11. Thomson, JA; Itskovitz-Eldor J; Shapiro SS; Waknitz MA; Swiergiel JJ; Marshall VS; Jones JM (November 6, 1998). "Embryonic stem cell lines derived from human blastocysts". Science. 282 (5391): 1145–1147. Bibcode:1998Sci...282.1145T.

12. Walasek, MA; van Os R; de Haan G (August 2012). "Hematopoietic stem cell expansion: challenges and opportunities". Ann N Y Acad Sci. 1266 (1): 138–150. Bibcode:2012NYASA1266..138W.

13. Takahashi, Katzutoshi; Shinya Yamanaka (August 25, 2006). "Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors". Cell. 126 (4): 663–676.

14. Park, IH; Arora, N; Huo, H; Maherali, N; Ahfeldt, T; Shimamura, A; Lensch, MW; Cowan, C; Hochedlinger, K; Daley, GQ (September 5, 2008). "Disease-specific induced pluripotent stem cells". Cell. 134 (5): 877–886

15. George, Robert P; Alfonso Gomez-Lobo (2005). "The Moral Status of the Human Embryo". Perspectives in Biology and Medicine. 48 (2): 201–210.

16. Cohen, Cynthia B (June 25, 2007). Renewing the Stuff of Life: Stem Cells, Ethics, and Public Policy. Oxford University Press. ISBN 9780195305241.