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Minireview

# The derivation of clinical-grade human embryonic stem cell lines

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**Abstract The pluripotent nature of human embryonic stem cells (hESC) has attracted great interest in using them as a source of cells or tissue in cell therapy. However, in order to be used in regenerative medicine, the pluripotent hESC lines should be established and propagated according to good manufacturing practice quality requirements. The cultures should be animal substance free in order to exclude the risk of infections and immunogenity. They should also be genetically and epigenetically normal. The detailed molecular mechanisms of their pluripotency are still not defined. Using human feeder cells, a medium containing only human proteins, the mechanical isolation of the inner cell mass and mechanical passaging of hESC, is a safe option until a functional defined medium containing physiological concentrations of regulatory factors is available.**


*Keywords:* Human embryonic stem cells; Derivation; Culture; Good manufacturing practice

## 1. Introduction

Human embryonic stem cell lines (hESC) can be derived from the inner cell mass (ICM) of preimplantation poor quality blastocysts, obtained from in vitro fertilization (IVF) treatment, which have been donated for research and which would otherwise be discarded. Since the establishment of first hESC lines in 1998 [1], research has progressed rapidly and several studies have described the derivation and culture of new hESC lines. A high quality hESC line expresses specific cell surface markers (SSEA-4, TRA-1–60, TRA-1–81) and transcription factors (Oct-4 and Nanog), is karyotypically normal and has high telomerase and alkaline phosphatase enzyme activities [1]. Human ESCs are able to grow and self-renew unlimitedly, they can be propagated in culture for extended periods and have an ability to differentiate to multiple cell types representing all primitive embryonic germ layers; ectoderm, mesoderm and endoderm. Their differentiation potential has raised hope that these cells could be a renewable source for cell transplantation in severe degenerative diseases [2].

In order to enable the use hESC in cell transplantation in human, it is necessary to eliminate the risk of infection transmitted by retroviruses and other animal pathogens, and immunoreactions caused by animal substances in cell cultures [3]. Therefore, animal material free derivation methods, animal-free culture media and human feeder cells or animal-free substrate as coating material in the hESC culture systems should be established.

## 2. Culture of hESC on feeder cells

### 2.1. First cultures on mouse-derived feeder cells

Human ESC lines were originally derived and propagated using mouse fetal fibroblasts as feeder cells, similar to those used in derivation of mouse ESC lines [1,4]. The culture medium also contained fetal bovine serum (FBS). These animal substance containing systems bear the subsequent risk of contamination with retroviruses and other pathogens, which could be transmitted to patients. It has also been shown that the hESC cultured under such conditions contain non-human sialoproteins, which are immunogenic to humans [3].

### 2.2. Cultures on human feeder cells

Next, human fetal muscle and skin and adult fallopian tube cells were used for the culture of existing hESC lines, and human serum was successfully used in the propagation of one line [5]. We have derived new hESC lines using human foreskin fibroblast as feeder cells [6], and since then we have derived 25 hESC lines on such feeder cells. Amit et al. [7] used such cells for the maintenance of an existing hESC line. Other human feeder cells used for derivation of new hESC have been human placental fibroblasts [8,9], human endometrial cells [10], adult marrow stroma cells [11] and hESC-derived fibroblasts [12]. For the maintenance of existing lines, human feeder cells have been used by several teams (Table 1). In addition, adult skin and muscle cell could support maintenance of hESC [13]. None of these culture systems was animal substance free because almost all had used FBS in the establishment of the feeder cells and immunosurgery had been used in most isolations of the ICM (Table 1).

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*Abbreviations:* hESC, human embryonic stem cells; GMP, good manufacturing practice; ICM, inner cell mass; FBS, fetal calf serum; SR, serum replacement; ECM, extracellular matrix; IVF, in vitro fertilization

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Table 1
Recently established derivation and culture methods for human embryonic stem cells using human feeder cells or feeder-free conditions

| Substrate/feeder-cell type | Feeder-cell medium components | hESC medium components | Derivation (D)/ Maintenance (M) | Derivation method | References |
|---|---|---|---|---|---|
| Human foreskin | FBS/HS | SR, bFGF | M | – | [7] |
| | FBS | FBS, LIF | D/M | Pro/ISR | [6] |
| | FBS | SR, bFGF | D/M | Pro/ISR | [24] |
| Placenta | FBS | SR, bFGF | D/M | Tyr/Mech | [8] |
| Uterine endometrium | FBS | SR, bFGF | D/M | Pro/ISR | [10] |
| Adult marrow stroma | FBS | SR, bFGF | M | – | [11] |
| hESC derived | FBS | SR, bFGF | M | – | [25] |
| fibroblasts | FBS | SR, bFGF | M | – | [26] |
| | HS/FBS | SR, bFGF | D/M | Pro/Mech | [12] |
| Fetal muscle | HS | FBS or HS | D/M | Pro/ISR | [5] |
| Fetal skin | | | D/M | | |
| Adult fallopian tube | | | D/M | | |
| Adult skin | | | | | |
| Adult muscle | HS/FBS | FBS or | M | – | [13][a] |
| | | SR, bFGF | M | | |
| Matrigel | – | CM (MEFs), SR, bFGF | M | – | [14] |
| Matrigel | – | CM (hESC-dF), SR , bFGF | M | – | [25] |
| Matrigel | – | SR, bFGF, GSK3 inhibitor | M | – | [27] |
| Matrigel | – | SR, bFGF + noggin or high bFGF | M | | [28] |
| Matrigel | – | SR, noggin, high bFGF | M | – | [29] |
| Matrigel | – | CM(MEFs/HES)S1P, PDGF, bFGF | M | – | [30] |
| Matrigel/laminin | – | X-vivo 10, high bFGF | M | – | [23] |
| Matrigel | – | Defined, animal-free medium, bFGF, LiCl, GABA, pipecolic acid, TGFβ | D/M | Pro/ISR | [18] |
| FBS-coating | – | CDM, activin A, noudal, bFGF | M | – | [31] |
| ECM from MEFs | – | SR, Plasmanate, bFGF | D/M | Pro/ISR | [17] |
| Laminin | – | SR, activin A, KGF, NIC | M | – | [15] |
| Fibronectin | – | CM, SR, bFGF | | – | [32] |
| Fibronectin | – | SR, TGFβ, LIF, bFGF | M | – | [16] |
| HS matrix | – | CM (hESCd-F) | M | – | [33] |
| Laminin | – | X-vivo10, high bFGF | M | – | [8] |

*Abbreviations:* FBS, fetal bovine serum; HS, human serum; SR, serum replacement; bFGF, basic fibroblast growth factor; LIF, leukaemia inhibitor factors; Pro, pronase used for removal of zona; ISR, immunosurgery for removal of trophoectoderm cells; Tyr, Tyrode's acid used for removal of zona; Mech, mechanical removal of trophoectodermal cells; CM, conditioned medium; MEFs, mouse embryonic fibroblasts; hESC-dF, human embryonic stem cell derived fibroblasts; GSK3 inhibitor, glycogen synthase kinase-3 inhibitor; HES, human embryonic stem cell; S1P, sphingosine-1-phosphate; PDGF, platelet derived growth factor; LiCl, lithidium chloride; GABA, γ-aminobutyric acid; TGFβ, transforming growth factor beta; CDM, chemically defined medium; KGF, keratinocyte growth factor; NIC, nicotinamide.
[a]In this work 11 different type of human cells as feeder cells were tested.

## 3. Feeder-free culture of hESC

### 3.1. Cultures on extracellular matrix

The first feeder-free maintenance systems for existing hESC were cultures on Matrigel [14]. Matrigel is manufactured from mouse material and is composed of several extracellular matrix (ECM) components, and it contains several growth factors. A conditioned medium from fetal mouse fibroblasts allowed a non-differentiated growth of existing hESC lines on Matrigel [14] (Table 1). A non-differentiated growth up to 10 passages was reported. For possible use in human cell transplantation, such hESC are very sub-optimal. The following improvements were laminin [15] and fibronectin [16] as ECMs. Either conditioned medium from mouse fibroblasts, or combinations of growth factors (bFGF, TGFβ, activin A, Nodal, Noggin, LIF, PDGF) were used (Table 1). The differentiation on the edges of the colonies can be seen in many of these reports.

### 3.2. Feeder-free derivation

A feeder-free derivation of hESC has also been possible. ECM form mouse fetal fibroblasts was used in the first successful feeder-free derivation [17]. Such ECM is not suitable for of human cell transplantation. Matrigel was used as ECM in the first study describing chemically defined derivation of two hESC lines [18], making this system also sub-optimal regarding cell transplantation. One of these lines had karyotype 47XXY, which is common in human embryos. The second gained an extra chromosome 12 at passage level 40. Chromosomal abnormalities have been described in hESC lines earlier [19,20]. It is suspected that culturing these cells in two difficult conditions may promote chromosomal aberrations [19]. It may be that a feeder-free derivation and culture may not be optimal culture systems for transplantable cells, either, before the molecular mechanisms of self-renewal are fully known.

## 4. Culture medium for hESC, serum and serum replacement

FBS was first used in the culture medium (Table 1). It was soon replaced by serum replacement [21,22]. The widely used serum replacement (SR) (Invitrogen) promoted well the non-differentiated growth [23], but it still contained animal pro-