Culturing Embryonic and Adult-Derived Stem Cells:
Introduction and Key Applications

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Outline

- Introduction to stem cells
- Embryonic stem cells
- Alternatives to embryonic stem cells
- Introduction to human embryonic stem cell culture
- Adult stem cells
- Introduction to adult stem cell culture and differentiation
What Does It Mean To Be a Stem Cell?

Stem cells are the foundation for every organ, tissue, and cell in the human body.

A single cell that can replicate itself, or...

differentiate into many cell types.
Different Types of Stem Cells

**Sources of cells**

**Types of stem cells**

**Embryonic**
- self-renew
- differentiate into all tissue types

**Adult**
- found in tissue
- self-renew
- differentiate into cells of the same lineage

**Progenitor**
- derived from stem cells
- can not self-renew
- only differentiate into cells of the same lineage
Embryonic and Adult Stem Cells

**Embryonic**

- **Totipotent**
  - Can form any tissue, including placenta

- **Pluripotent**
  - Can form any tissue in the embryo but not the placenta

**Adult**

- **Multipotent**
  - Can form multiple cell types within a particular tissue, organ or physiological system

Potential Uses for Stem Cells

- Identify drug targets and test potential therapeutics
- Study cell differentiation
- Understanding prevention & treatment of birth defects
- Cultured Pluripotent Stem Cells
- Tissues/Cells for Transplantation

- Bone marrow for leukemia & chemotherapy
- Nerve cells for Parkinson's & Alzheimer's disease
- Heart muscle cells for heart disease
- Pancreatic islet cells for diabetes

Schematic adapted from http://stemcells.nih.gov/index.asp
Current and Future Clinical Stem Cell Applications

• **Blood disease**
  - Hematopoietic stem cells have been used for bone marrow transplantation for over 20 years.

• **Spinal cord injury**
  - Geron is awaiting FDA approval to begin clinical trial with hESC-derived oligodendrocyte progenitor cells.

• **Type II diabetes treatment**
  - Restore glucose-responsive insulin-secreting cells either by transplantation of stem cell-derived cells or reprogramming of existing cells.
Characteristics of Embryonic Stem (ES) Cells

- Undifferentiated/non-committed
- Self renewal
- Pluripotency

How Were ES cells First Isolated?

- ES cells were first derived in 1981 from a MOUSE embryo


- Protocols for hES cell culture were optimized from mouse ES cells and other embryonic stem cells’ culturing practices.

- hES cells were isolated by transferring the inner cell mass of a 3-5 day old embryo onto a mouse fibroblast feeder layer.
Potential Alternatives to ES Cells

Somatic cell nuclear transfer (SCNT)
- The nucleus from an adult cell is transferred to an enucleated egg (the nucleus was removed)
- **Advantages** over ES cells
  - No embryo derived cells
  - Well characterized system in mice
  - No Federal funding restrictions
  - Potential for generating stem cells from any individual
- **Challenges**
  - Limited success in primates
  - Human egg donation
  - Labor intensive

Induced pluripotent stem cells (iPS)
- Human cells are infected with genes that make them behave like hES cells
- **Advantages** over ES cells
  - No embryo derived cells
  - Adult cells only
  - No Federal funding restrictions
  - Potential for generating stem cells from any individual
- **Challenges**
  - VERY new technology (2007)
  - The infection process makes the genes integrate randomly into the DNA. (Potential for cancer in clinical applications.)
iPS Cells for Studying Human Disease

- Potential for studying human disease
  - Basic biology and drug screening

  - Amyotrophic lateral sclerosis (ALS)
  - Parkinson disease (PD)
  - Huntington disease (HD)
  - Juvenile-onset, type 1 diabetes mellitus (JDM)
Key Challenges with hES Cell Culture

- Lack of defined culturing environment with a standard protocol
- Spontaneous differentiation
- Scaling up cultures
- Efficient transfection of hES cells
- Simulating physiological conditions *in vitro*
- Time required for full characterization of new culturing condition: *in vitro* and *in vivo*
- Difficulty in comparing data from different laboratories
A Decade of Developments in hES Cell Culture Environments

- Started with mES cell culture conditions
- MEF to human feeders, hES cell-derived feeders
- BD Matrigel™ Matrix/ECMs with MEF-CM
- ECMs + hES cell media (with soluble growth factors in media to control differentiation)
  - BIO (GSK3 inhibitor – wnt pathway)
  - High bFGF
  - Noggin +/- bFGF
  - Activin A
  - TGFβ
- ECMs + Defined media (with some animal components)
- ECMs + Animal-component free defined media

- Ultimate Goal = Completely Animal-Component Free Defined culture environment
hES cells are typically cultured on mouse embryonic fibroblast (MEF) feeder layers.

Limitations of Mouse Embryonic Fibroblast (MEF) Feeder Layer Systems

- **MEF Issues**
  - Labor intensive to maintain two cell types
  - Variation of different MEF lots

- **Contamination**
  - Potential contamination of animal pathogens from mouse feeders is a major concern when trying to move into therapeutic applications

- **Downstream Manipulation**
  - Colonies that form on feeder layers are compact and difficult to genetically manipulate and transfec
  - Transfection efficiency of hESCs is low. Quenching can occur from the feeder layers
  - Difficult to isolate DNA / RNA due to potential cross-contamination from feeder layers
Limitations of Mouse Embryonic Fibroblast (MEF) Feeder Layer Systems (Cont’d)

• **Standardization**
  – There is no widely accepted standard protocol which could result in major issues when moving into the therapeutic arena
  – Difficulty in comparing data from different laboratories exists due to the absence of standard hESC culture practices
  – Cells undergo spontaneous differentiation on a MEF feeder layer which makes comparisons from culture to culture difficult
Feeder-free hES Cell Culture

• First documented in 2001 (Xu, et al. Nature Biotech. 24:185)

• BD Matrigel™ Matrix-coated surface used with mouse embryonic fibroblast feeder layer conditioned media (MEF-CM)

• Multiple media conditions and defined media have been used successfully in combination with BD Matrigel Matrix-coated surface for culturing hES cells
BD Matrigel™ Matrix: a reconstituted basement membrane

basal lamina = basement membrane
BD Matrigel™ Matrix = reconstituted basement membrane

Figure: Molecular Biology of the Cell (3rd Edition).
Interaction of Cells with Basal Lamina ECM

- The ECM interacts with cells via cell surface receptors such as integrins
- Reservoir for growth factors
- Substrate for cell attachment and spreading, contact guidance for cell migration, and a scaffold for building tissues
- Influences morphology of cells
- May be associated with particular patterns of cell differentiation and proliferation

Figure: *Nature Reviews Cancer* 3:422 (2003).
BD Matrigel™ Matrix: a reconstituted basement membrane

Purified preparation from EHS mouse tumors

Composition:

- Laminin ~ 60%
- Collagen IV ~ 30%
- Entactin ~ 8%
- Heparan sulfate proteoglycan (perlecan)
- Growth factors (e.g., PDGF, EGF, TGF-β)
- Matrix metalloproteinases

Not a defined substrate
A Complete Culturing Environment for Human ESCs

Media + Surfaces = Complete Cell Environments

BD Biosciences, StemCell Technologies, and the WiCell™ Research Institute have established a strategic collaboration to develop optimized, feeder-independent cell culture environments for hES cell research.

- mTeSR™1 Maintenance Medium
  *from StemCell Technologies*

- BD Matrigel™ hESC-qualified Matrix
  *from BD Biosciences*
BD Matrigel™ hESC-qualified Matrix

- Optimized surface for hES cell culture
- Qualified as mTeSR™ 1-compatible
- 5 mL vial – can aliquot and store
- Coats 50-60 six well BD Falcon™ Multiwell Plates

Available at bdbiosciences.com/stemcellsource

Undifferentiated hES Cell Colony

- Compact and dense H9 colonies on MEF feeders
- Spread-out and monolayer-like colonies on BD Matrigel Plates
Markers of Undifferentiated hES Cells on MEF-CM & mTeSR™1

OCT4 + Hoechst33342

H9 on BD Matrigel™ hESC-qualified Matrix
Undifferentiated Marker Expression
FACS Analysis

H9 cells grown on BD Matrigel™ hESC-qualified Matrix in mTeSR™ 1 for 5 passages

98% OCT4 positive cells

94% SSEA4 positive cells
Comparison of BD Matrigel™ Matrix vs. other ECM Proteins

- BD Matrigel™ Matrix is equivalent or better than most single extracellular matrices (ECMs) tested:
  - Laminin equivalent to BD Matrigel Matrix (Xu, et al. 2001)
  - Laminin, collagen, fibronectin, and vitronectin were combined for optimal ECM complex for culturing hES cells (Ludwig et al. 2006)
  - Fibronectin and collagen combination is required to match the performance of BD Matrigel Matrix (Lu, et al. 2006)

- Pure ECM often much more expensive to use

- ECM combination requires more steps and time to coat
Alternative Surface for ES Cell Culture

BD™ Laminin/Entactin Complex High Concentration

• Major component of basement membrane in Engelbreth-Holm-Swarm (EHS) mouse tumors
Comparison of Different Surfaces and Media by Immunofluorescence

BD™ Laminin/Entactin Complex
High Concentration

mTeSR™1

MEF-CM

BD Matrigel™ hESC-qualified Matrix

OCT-4 expression in H9 cells
Embryoid Body (EB) Formation from hES Cells

Phase contrast image of Embryoid bodies formed by H9 cells grown on different surfaces.
Neurons and Cardiomyocytes from H9-derived Embryoid Bodies

EBs derived from H9 cells cultured on BD™ Laminin/Entactin Complex High Concentration for 32 passages in mTeSR™1 media
Summary of hES Cell Culture Conditions

- **Traditional method – Mouse Embryonic Fibroblast (MEF) feeder layer**
  - Use MEF feeder layer as a substrate that provides essential growth and attachment factors

- **Alternative feeder layer method – human foreskin fibroblast feeder layer**
  - Undefined media and surface components, but no animal-derived factors

- **Feeder-free hESC culture**
  - BD Matrigel™ or extracellular matrix (ECM) proteins are used as a substrate
  - May use conditioned media from MEFs or a defined media

- **Complete hESC environment**
  - **BD Matrigel hESC-qualified Matrix + mTeSR™1 Maintenance Medium for Human Embryonic Stem Cells**
    - Pre-qualified system saves significant time and resources
    - No need to test lots of BD Matrigel Matrix to determine if they will sustain undifferentiated growth of hESCs
    - The media is completely defined
Embryonic vs. Adult Stem Cells

• Embryonic Stem Cells
  – Pluripotent
  – Relatively easy to grow in culture

• Adult Stem Cells
  – Multipotent
  – Difficult to isolate, purify and maintain in the undifferentiated state
Characteristics of Adult Stem Cells

- **Adult stem cells are found in many tissues**
  - They are undifferentiated cells found among differentiated cells.

- **Their primary role in the body is to maintain and repair the tissue in which they are found.**

- **Adult stem cells are multipotent, not pluripotent**
  - **Pluripotent**: can differentiate into any cell type in the embryo
  - **Multipotent**: can differentiate into a subset of cell types, but NOT a complete organism

- **Adult stem cells may exhibit plasticity**
Plasticity is the ability of stem cells from one adult tissue to generate the differentiated types of another tissue.

http://stemcells.nih.gov/info/basics/basics4.asp
Adult stem cells

- Hematopoietic
- Mesenchymal
- Neuronal
Hematopoietic Stem Cells (HSCs)

- **Source:** bone marrow
- **Differentiation pathways**
  - Ex. T cell, B cell, Erythrocyte
- **Culture conditions**
  - Maintenance of undifferentiated HSCs
    - Ex. stem cell factor (SCF), IL-3, IL-6
  - Differentiation
    - Ex. EPO, G-CSF
Mesenchymal Stem Cells (MSCs)

• Source: umbilical vein, bone marrow, adipose tissue, human embryonic stem cells

• Differentiation Pathways
  – Ex. osteogenic, chondrogenic, adipogenic

• Culture Conditions
  – Maintenance of undifferentiated MSCs
    • Culture surface: Tissue culture (TC)-treated cellware
      • Better yield on BD Falcon™ TC Flasks (Sotiropoulou, et al. Stem Cells 24:462 [2006])
  – Differentiation
    • Ex. FGF, EGF, ITS+ Premix, TGF-β, Hydrocortisone
Neural Stem Cells (NSCs)

• Source: brain cortices, differentiated from embryonic stem cells

• Differentiation pathways
  – Ex. neuron, astroglial

• Culture conditions
  – Maintenance for undifferentiated cells
    • Neurospheres
    • Media components: ex. EGF, FGF
  – Differentiation
    • Surface: poly-L-ornithine/laminin, BD™ PuraMatrix™ Peptide Hydrogel, BD Matrigel™ Matrix
    • Media components: ex. FGF, BDNF
Analysis of hESC-derived self-renewing neural stem cells (NSC) by IF and FACS

hESC → Embryoid bodies → Neural rosettes → Neural stem cells → Neurons

7 days → 17-20 days → 23-25 days → 30-44 days

Sox2  Nestin  Ki67  Hoechst

CD

EF

Sox2: hESC, NSC
Nestin: NSC
Oct3/4: hESC
Ki67: proliferation
Hoechst: Cell nuclei

BD
Summary

- **Embryonic stem cells**
  - Pluripotent
  - Alternatives: SCNT and iPS cells
  - Multiple culture methods
    - Feeder layer
    - BD Matrigel™ Matrix
    - Defined ECM

- **Adult stem cells**
  - Multipotent
  - Culture environment (surface and media) specific to cell type and differentiation pathway
hES and iPS cells

References – Adult Stem Cells

HSCs


MSCs


NSCs

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