


Culturing and production of hES cells


Presenter: Johan Hyllner

- Introduction to Cellartis AB
- hESC culture and morphology
- Mechanical passaging
- Single cell enzymatic dissociation (SCED) passaging
- Feeder-free passaging and up-scaled manufacturing





Cellartis – The Company


- Swedish/UK based company founded in early 2001, 63 employees
- A consortium of Scandinavian venture capital investors are the major owners: InnovationsKapital, Inter Ikea and Biofund (> 75%)
- Revenue generated from product sales, R&D collaborations with multinationals and research grants
- Partner in > 50 M€ stem cell research programs funded by EU and a ~15 M€ program on hESC in the U.K.
- Strategy
 - Primary focus on the drug discovery segment of the market
 - To operate the company on a quality level and with a platform that permits Cellartis to participate in the area of regenerative medicine. NovoNordisk collaboration: to find a cure for diabetes.
 - Cellartis business is based on solid science of highest quality and extensive collaborations with Academic and Industrial Partners



Our Facilities in Sweden and Scotland

Extensive QA-validated mass production

<p>Cellartis UK (Dundee)</p> <ul style="list-style-type: none"> • Focus on up-scaled production • "Tailor-made" for hES cell work • Clean-rooms • Three laboratories that comply with GMP production (EU standard) 	<p>Cellartis Sweden (Göteborg)</p> <ul style="list-style-type: none"> • "Tailor-made" for hES cell work • Clean-rooms • Three laboratories that comply with GMP production (EU standard) • One laboratory that complies with GMP production (US standard) 
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The Cellartis hESC platform

A broad base

- >30 Cellartis cell lines (NIH and UK stem cell registry) + collaborative lines
- Subclones
- Multiple culturing techniques (including feeders, feeder free, enzymatic passaging)
- Cellartis hES cell lines have been approved for research use in all major markets such as USA, Japan, Germany, UK, France etc
- First derivation of a truly xeno-free hES cell line
- Preparation of working cell banks (>100 vials) = LOT
- Upscaling technologies

Adewumi et al., *Nature Biotechnology* 2007, 25:803-816
Eklström et al., *Stem Cells* 2007, 25:1690-1696
Synnregren et al., *Stem Cells*, 2007, 25:473-480
Bábikova et al., *Genome Research* 2006, 16:1075-1083
Eklström et al., *Stem Cells* 2006, 24:2170-2176
Calsander et al., *Chrom Res* 2006, 14:131-137
Heins et al., *J Biotechnol* 2005, 122:511-520
Maitra et al., *Nature Genetics* 2005, 37:1099-1103
Noaksson et al., *Stem Cells* 2005, 23:1460-1467
Darrifors et al., *Stem Cells* 2005, 23: 483-489
Sjögrén-Jansson et al., *Dev Dyn*, 2005, 233:1304-1314
Sjögrén et al., *Reprod Biomed Online*, 2004, 9:326-329
Heins et al., *Stem Cells* 2004, 22:367-376



Cellartis EU collaborations

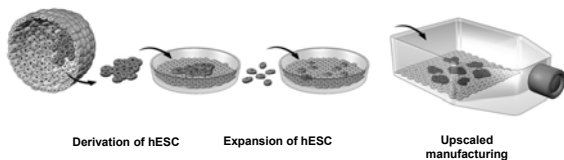
- BetaCellTherapy
23.8 M€ / 5 Yr
- Vitrocellomics finalised 2009
3.7 M€ / 3 Yr
- HeartRepair
13 M€ / 4 Yr
- △ Siems
3.5 M€ / 3 Yr
- △ InvitroHeart
4.4 M€ / 3 Yr
- △ Carcinogenomics
12 M€ / 5 Yr
- ◇ ESNATS
15.5 M€ / 5 Yr
- ◇ Hyperlab
3.9 M€ / 3 Yr



Coquimbo, Chile



Establishment, expansion and upscaling of hES cells



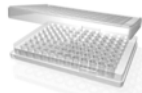
Culture of hES cells

Culture methods

- Cell clusters
- Single cells (difficult)
- Manual dissection
- Enzymatic digestion
 - Trypsin, Tryple Select
 - Collagenase
 - Dispase
 - EDTA

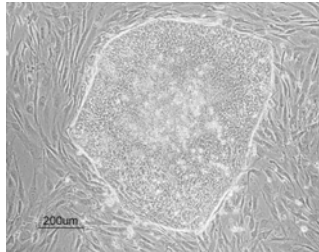


- Culture dishes (IVF, petri)
- Flasks (T25, T75, T150 etc)
- Multiwell plates (6w, 12w, 24w 48w, 96 w, 384w)

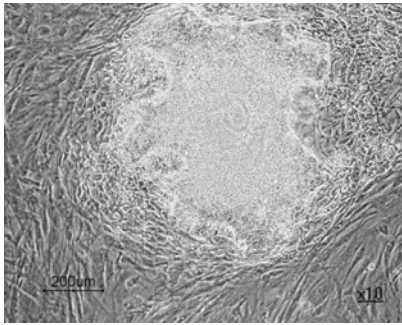


Undifferentiated hES cell colony cultured on a layer of feeder cells

- Mitotically inactivated feeder cells (mouse embryonal fibroblasts)
- Mitomycin C / irradiation
- Push away feeder cells
- Feeder density
- Feeder passage number
 - p2 or p3



Differentiated hESC colony

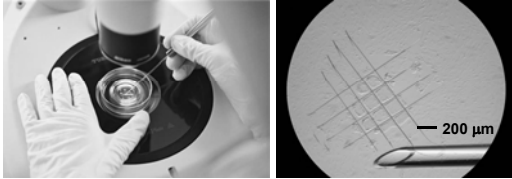


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Mechanical passaging

Manual cutting of hES colonies

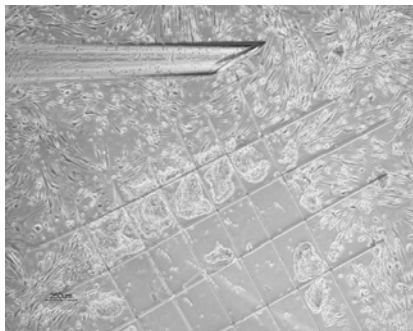
The cells are routinely grown on mitotically inactivated mouse embryonic fibroblasts (MEFs), and passed every 4-5 days by manual dissection into smaller cell clusters.



- Used for hES cell cultures (>150 passages)
- Selection of cells could be performed
- Low frequency of caryotypic abnormalities
- Laboursome, low yield, operator variability

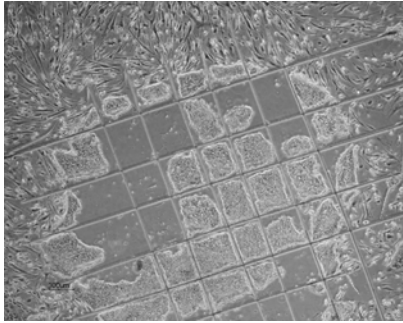
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Cutting hESC colonies



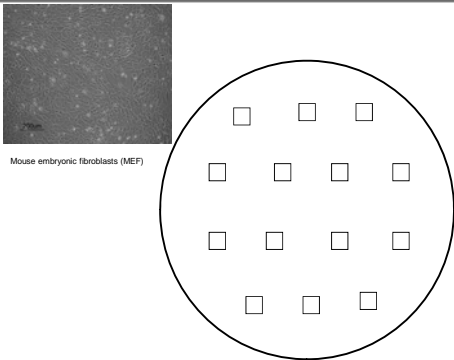
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Cutting hESC colonies



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Placement of cut cell clusters on new MEF cells

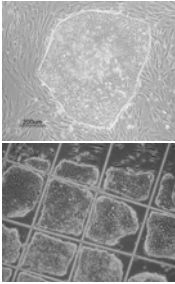


Mouse embryonic fibroblasts (MEF)

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Characterisation program at Cellartis
of working stem cell banks (LOTs)

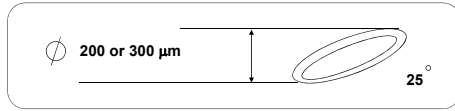
- > Morphology
- > Thawing recovery rate of frozen LOT:s
- > Stem cell markers: SSEA-3, SSEA-4, SSEA-1, TRA-1-60, TRA-1-81, Oct-4 and hES-Collect™
- > ALP activity and telomerase expression
- > Cytogenetic analysis (Karyotyping, FISH etc.)
- > *In-vitro* and *in-vivo* pluripotency
- > Mycoplasma and sterility control
- > Control for human viruses
 - > Human Immunodeficiency Virus type 1 and 2
 - > Hepatitis B and C
 - > Cytomegalovirus,
 - > Herpes Simplex Virus type 1 and 2
 - > Epstein-Barr Virus
 - > Human Papilloma Virus



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Stem Cell Cutting Tool

Advantages over drawn-out capillaries

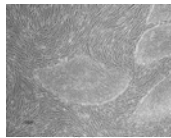


- Higher quality of glass
- Last a whole session
- Can be used for both cutting and transfer
- Same angle of edge every time
- Can be used for size approximations
- Sterile and tested for embryo toxicity

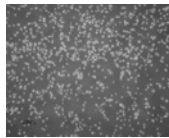
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Improved culture methods

Industry & clinical applications require large volumes



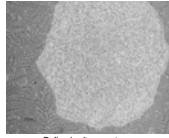
Improved feeder-systems



Enzymatic passaging



Flask / Multiwell culture



Defined culture systems

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Amount of hES cells needed?

5×10^9 undifferentiated hES cells required to produce enough cardiomyocytes to repair a heart damaged by infarction.

Mummery C.L., *Nature*, 2005

25 000 – 50 000 cm^2 = 143-286 x T175 flasks

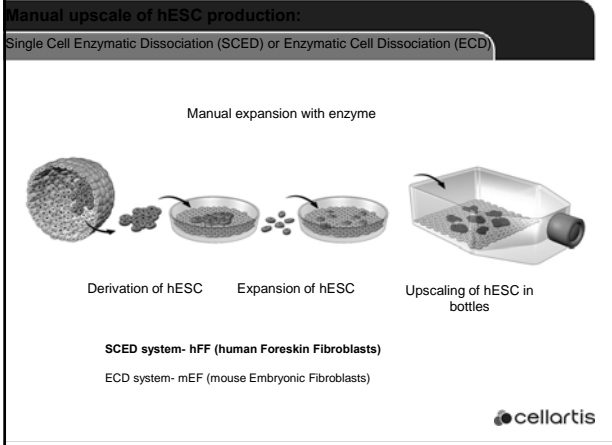
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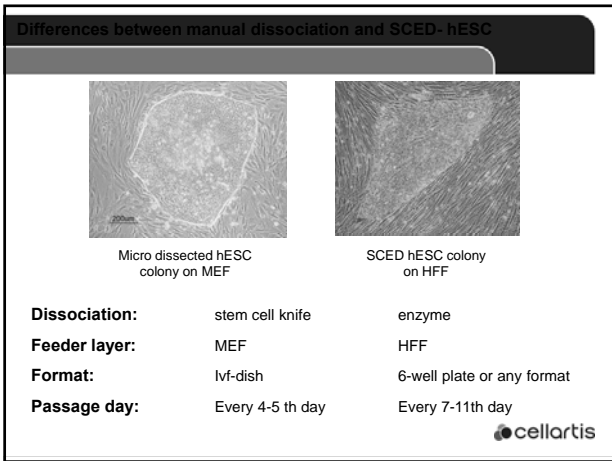
25 000 IVF dishes

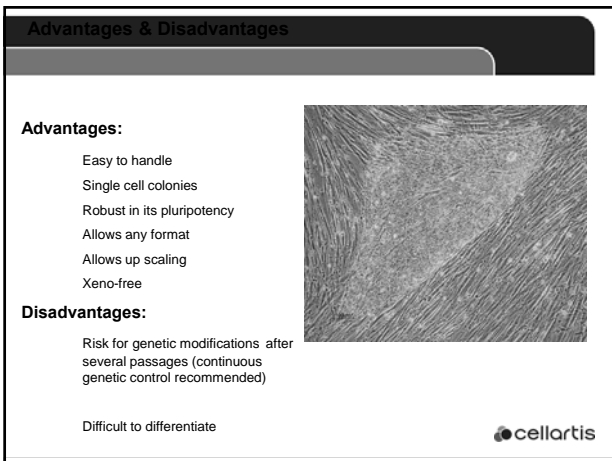


- Scale up culture
- More efficient protocols

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Up-scaling of hESC production:

Single Cell Enzymatic Dissociation (SCED)

Routine Split ratio 1:4 - 1:40 Passage interval 7-11 days

Advantages:
 Easy to handle
 Single cell colonies
 Robust
 Allows any formats
 Allows up scaling
 Xeno-free

Disadvantages:
 Risk for genomic aberrations after long term passaging

Ellerström et al, Stem Cells 2007

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SCED

Characterization of cells cultured in the SCED system

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Scaled up hES cell culture

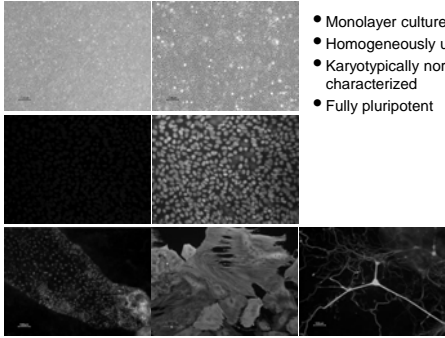
No supporting feeder layer

- Feeder free culture
 - Matrix
 - Undefined (e.g. Matrigel, gelatin)
 - Defined (e.g. collagen, fibronectin, laminin)
- Difficulties to grow on plastic only
- Conditioned medium
 - Reproducibility
 - Cell source (mEF, hFF etc)
- Undefined factors (e.g. serum)
- Defined medium
- Cluster passage vs. single cell passage
 - Single cell passage necessary for quantitative screening
- Limited long term experience
 - Epigenetic changes
 - Directed differentiation from feeder free cultures
 - Chromosomal integrity

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Upscaling of hESC: Cellartis FF (feeder-free) hES cell cultures

confluent monolayer culture of hES cells

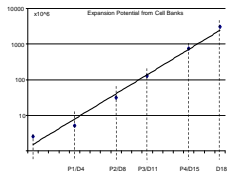


- Monolayer culture of hES cells
- Homogeneously undifferentiated
- Karyotypically normal and characterized
- Fully pluripotent

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Large scale production and distribution of hES cells

From one vial to a billion cells in 2 weeks

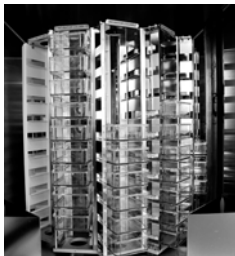


- Large scale automated production from fully characterized Cell Banks
- Shipped fresh "Ready to plate" or "Plated"



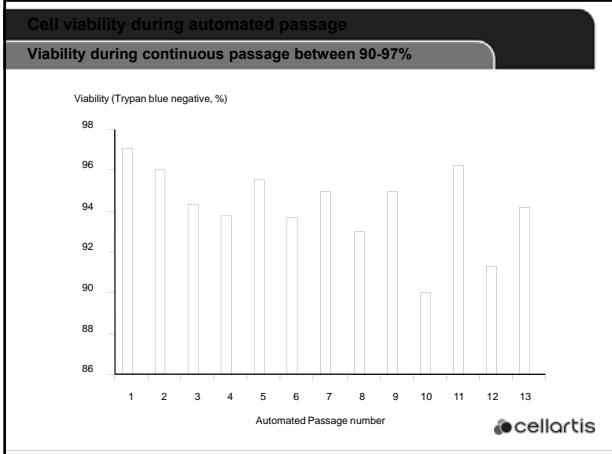
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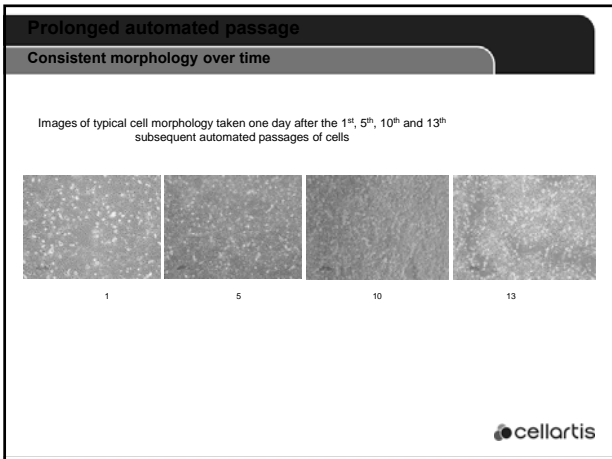
hES Cell culture automation in Dundee



- 10^9 cell capacity
- 96w, 384w plate seeding

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hESC in clean rooms ≠ GMP

End product meets preset specifications

- Donation
- Procurement
- Testing/Quality control
- Processing/Manufacturing
 - Traceability
 - Preservation
 - Storage
- Distribution
- Release criteria
 - SOPs

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GMP –hES cells

- Animal components acceptable from a regulatory perspective in some territories
- Does not necessarily ensure the highest quality or most optimal cells for specific applications
- The benefit of GMP is that cells are produced in a specified and reproducible way, ensuring safety
- GMP is a requirement for GCP
- Not necessary for general R&D, drug discovery or toxicity testing

Quality System at Cellartis AB

Quality System

