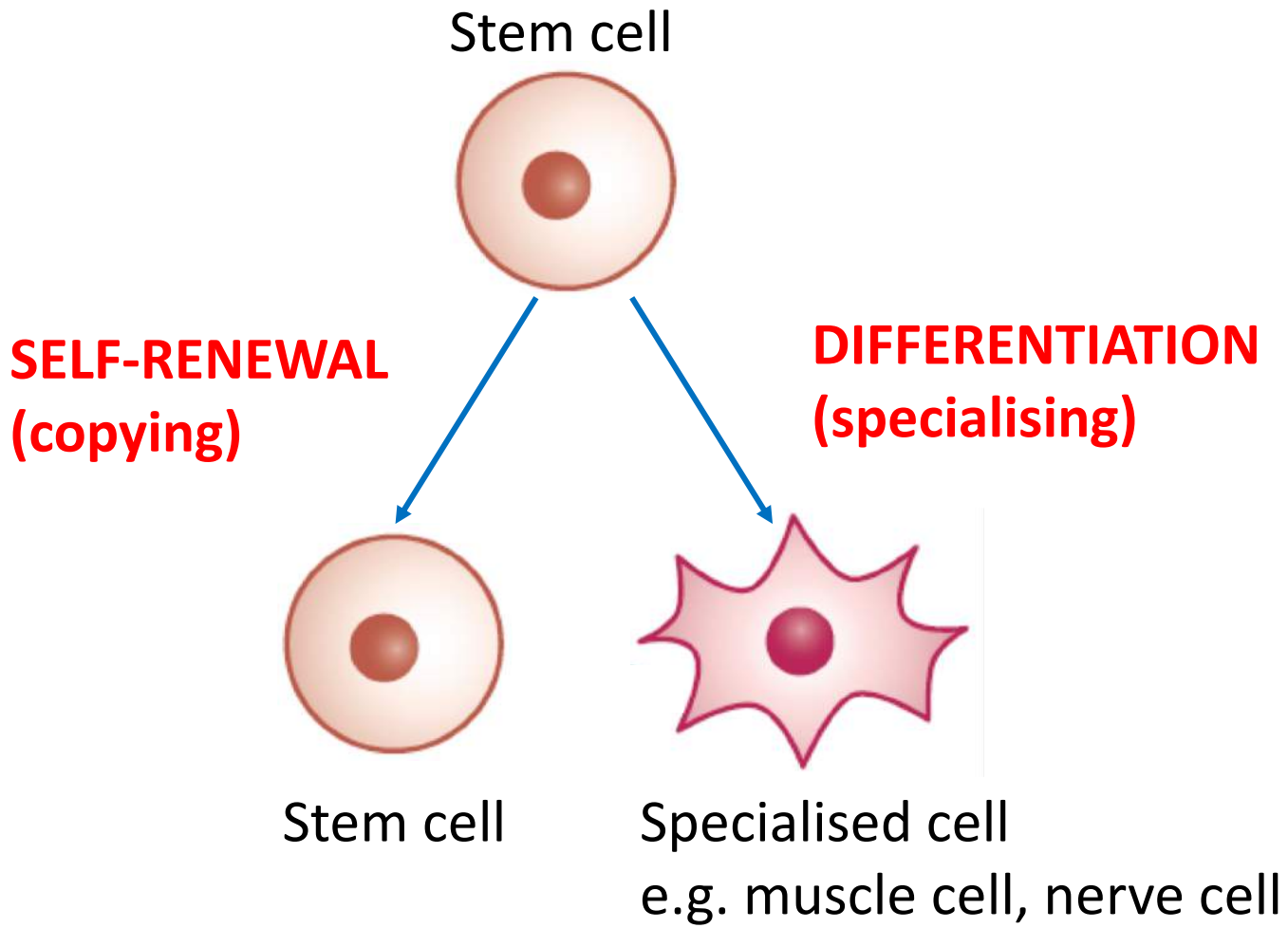
Leids Universitair Medisch Centrum

Chemically defined culture and differentiation of human pluripotent stem cells: where are we at?

3R Symposium on In Vitro Practices
1st June 2018

Richard Davis
Eindhoven

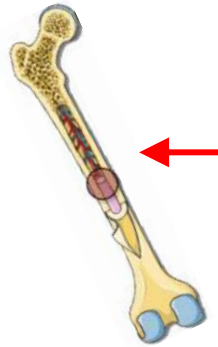
The Two Paths of a Stem Cell



Types of Stem Cells

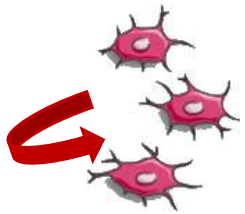
Tissue Stem Cells

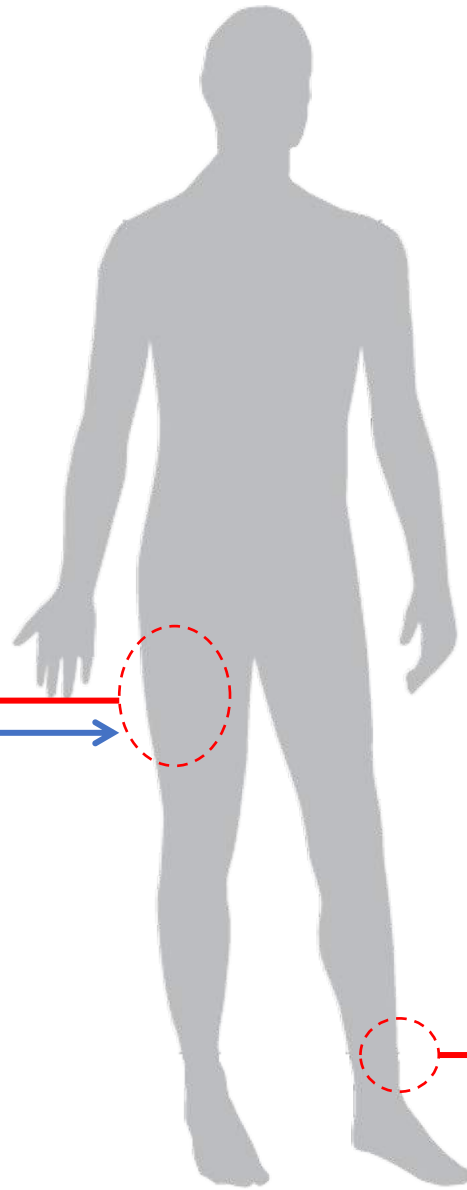
throughout body (e.g. bone marrow, lung, liver, intestine)



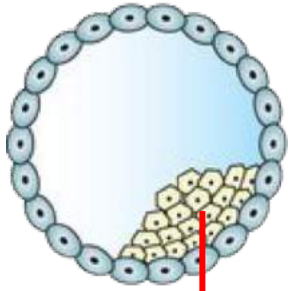
Adult stem cells

Form cell types of the tissues from which they are derived





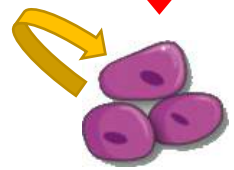
Pluripotent Stem Cells



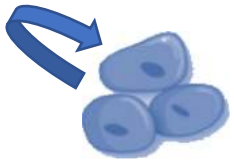
from blastocyst (human embryo)

cells inside = 'inner cell mass'

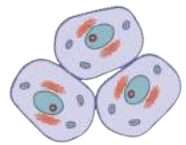
Can form all cell types in the body



Embryonic stem cells (ESCs)



Induced pluripotent stem cells (iPSCs)

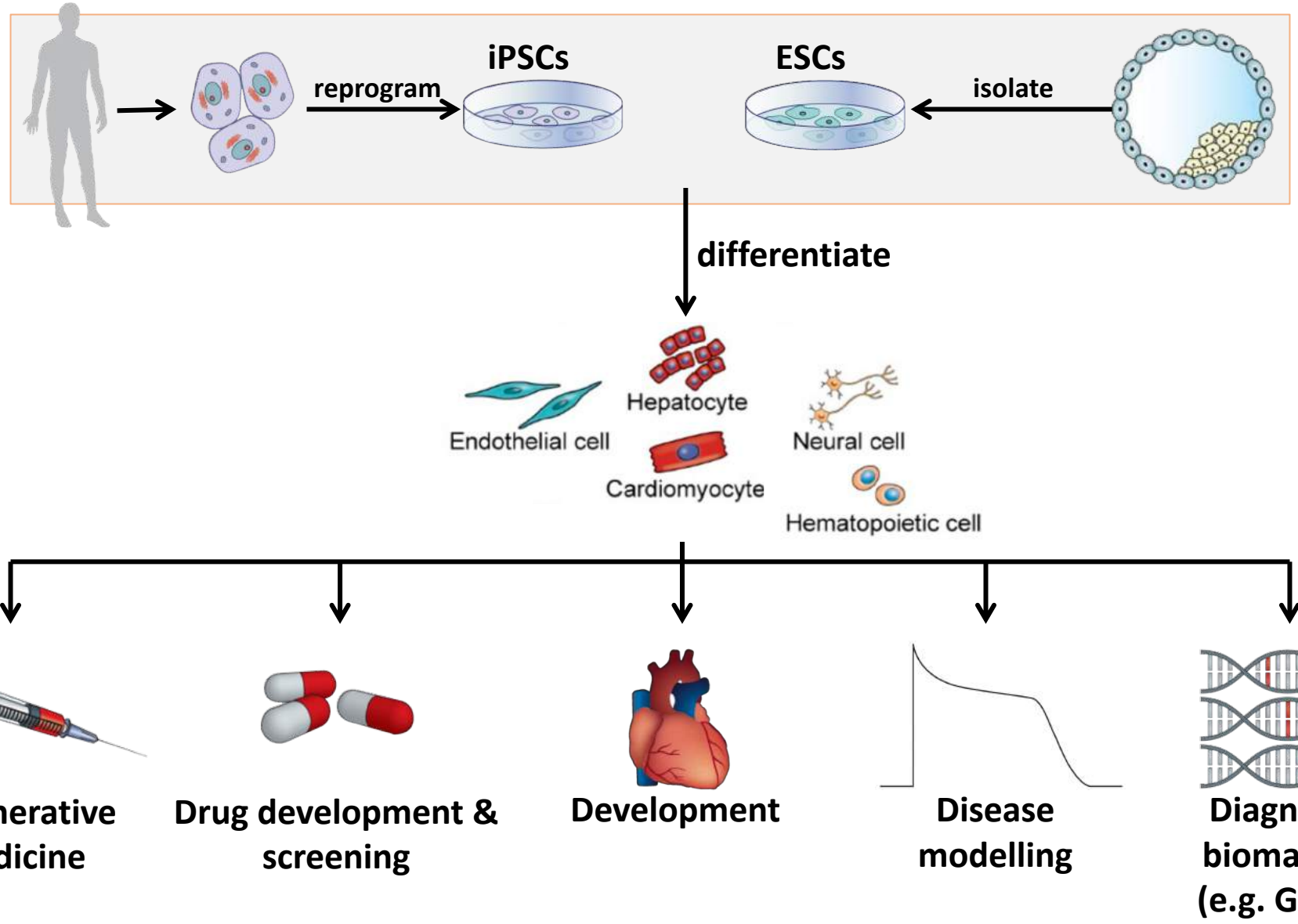


From skin, blood, urine etc.

'Reprogramming'

(Adapted from C. Mummery)

Pluripotent Stem Cell Applications



Why the push for "serum-free" culturing of hPSCs?

Not just "serum-free" but "chemically-defined"

Reproducibility of results

- Animal-derived products – ill-defined
- Batch-to-batch variation affects on both cell growth pattern and differentiation
- Already line-to-line variability in terms of differentiation efficiency

For use of hPSCs to understand development

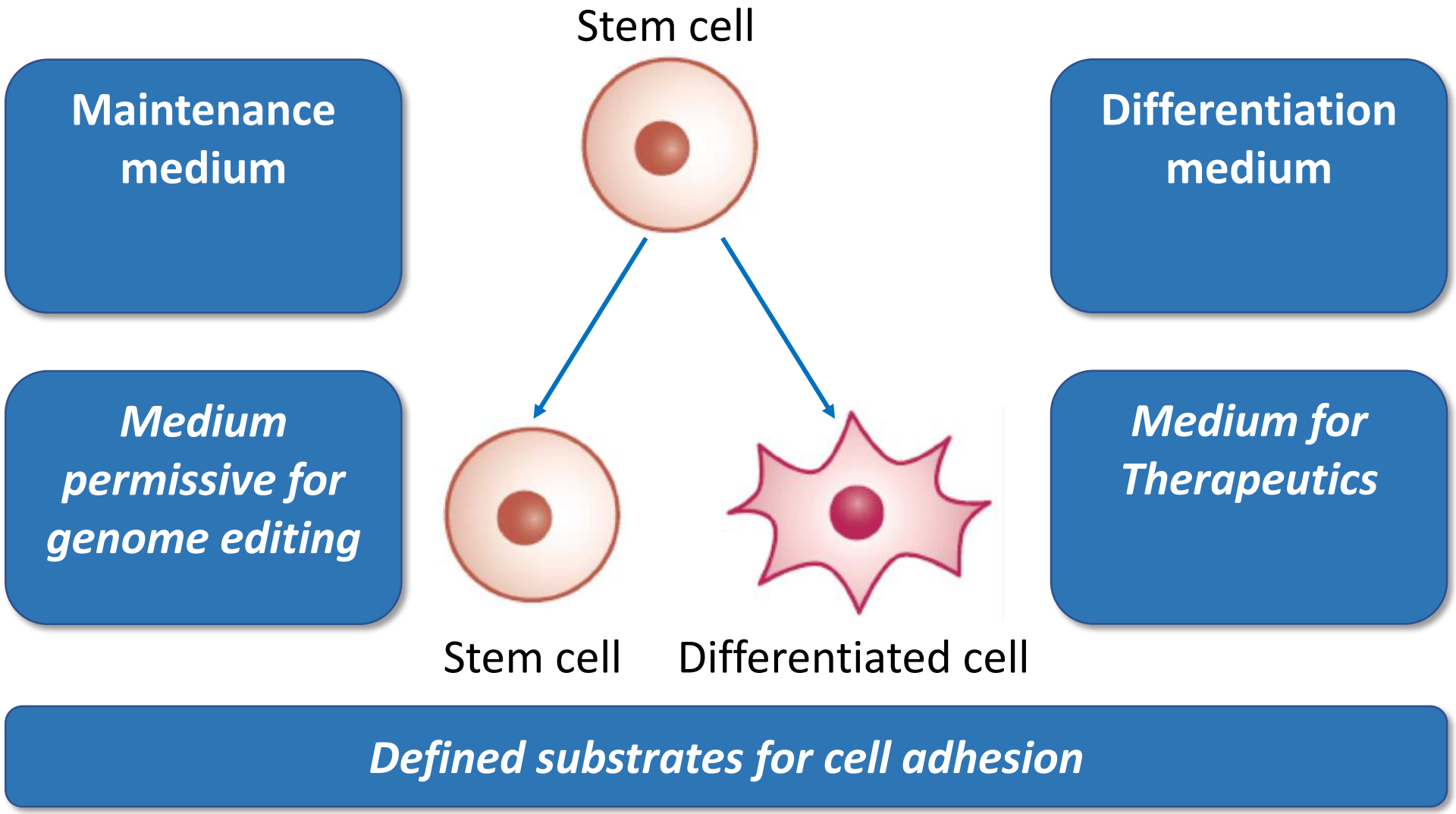
- Unknown factors can interfere with hormone and/or growth factor effects

Clinical applications

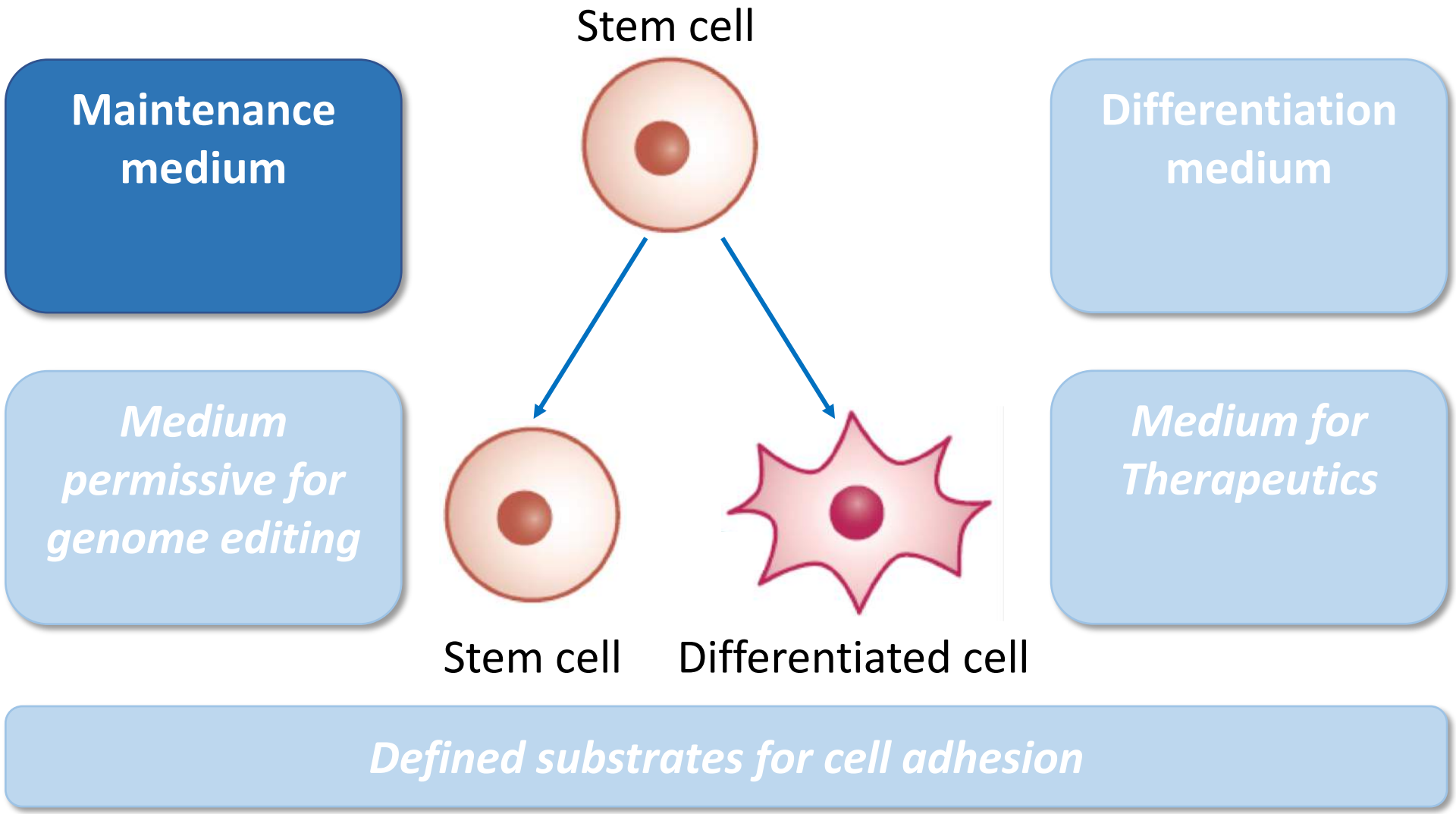
- Not GMP-compliant – risk of viral, mycoplasma / prion contamination
- Incorporation of animal proteins that can provoke immune responses (*non-human sialic acid (Neu5Gc)*)

Ethical & moral considerations

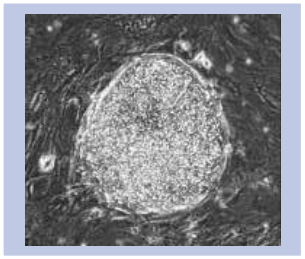
Different culture requirements for the maintenance and differentiation of hPSCs



Different culture requirements for the maintenance and differentiation of hPSCs



Human PSC Culture Medium Milestones



Primed State

Naïve State

Thomson *et al.*
DMEM
FBS
MEF

Amit *et al.*
KO-DMEM
KSR
FGF2
MEF

Xu *et al.*
Conditioned medium
Matrigel

Amit *et al.*
KO-DMEM
KSR
FGF2
TGFB1
Matrigel

Xu *et al.*
XVIVO-10
(contains BSA)
High FGF2
Matrigel

Xu *et al.*
DMEM/F12
KSR
High FGF2
BMP4 inhibitor
Matrigel

Vallier *et al.*
IMDM/F12
BSA
FGF2
Activin A
Matrigel

Li *et al.*
XVIVO-10
(contains BSA)
High FGF2
Matrigel

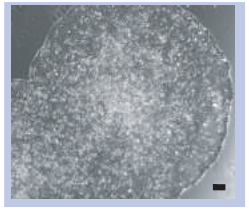
2007 – Generation of human iPSCs

StemPro hESC

Wang *et al.*
DMEM/F12
BSA
FGF2
Activin A
Heregulin
Matrigel

Ludwig *et al.*
DMEM/F12
HSA
High FGF2
TGFB1
Laminin

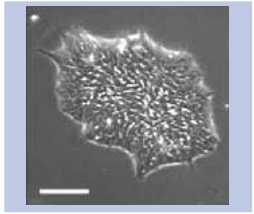
TeSR1



Hanna *et al.*
Oct4^{+}, *Klf4*$^{+}$, *Klf2*$^{+}$
DMEM/F12/Neurobasal
BSA
N2
B27
LIF
GSK3Bi
ERK1/2i
Forskolin
MEF

Chen *et al.*
DMEM/F12
AA 2-P
FGF2
TGFB1
Vitronectin

E8



Ware *et al.*
DMEM/F12
KSR
FGF2
GSK3Bi
ERK1/2i
MEF

Gafni *et al.*
KO DMEM
BSA
N2
B27
FGF2
TGFB1
LIF
GSK3Bi
ERK1/2i
JNKi
p38i
MEF

Carter *et al.*
DMEM/F12
KSR
NME7p
MN-C3

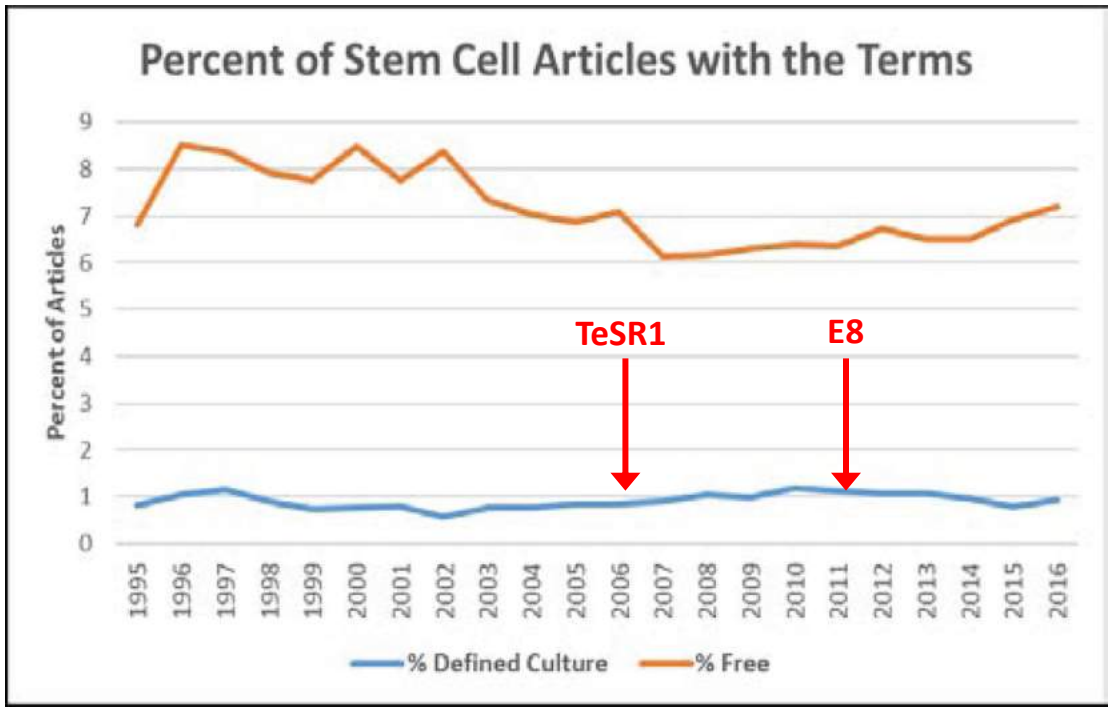
Theunissen *et al.*
DMEM/F12/Neurobasal
BSA
N2
B27
FGF2
Activin A
LIF
GSK3Bi
ERK1/2i
ROCKi
BRAFi
SRCi
MEF

1998 2000 2002 2004 2006 2008 2010 2012 2014 2016

FBS elimination	FGF2 addition	MEF elimination	Conditioned medium elimination	High FGF2	TGFB1/ Activin A addition	KSR elimination	HSA/BSA elimination	Small molecule inhibitors	FGF2 elimination

(Schuldt et al, Cardiac Regeneration, 2017)

Uptake of these media by the research community



(Vecchi & Wakatsuki, *Arch Stem Cell Res*, 2016)

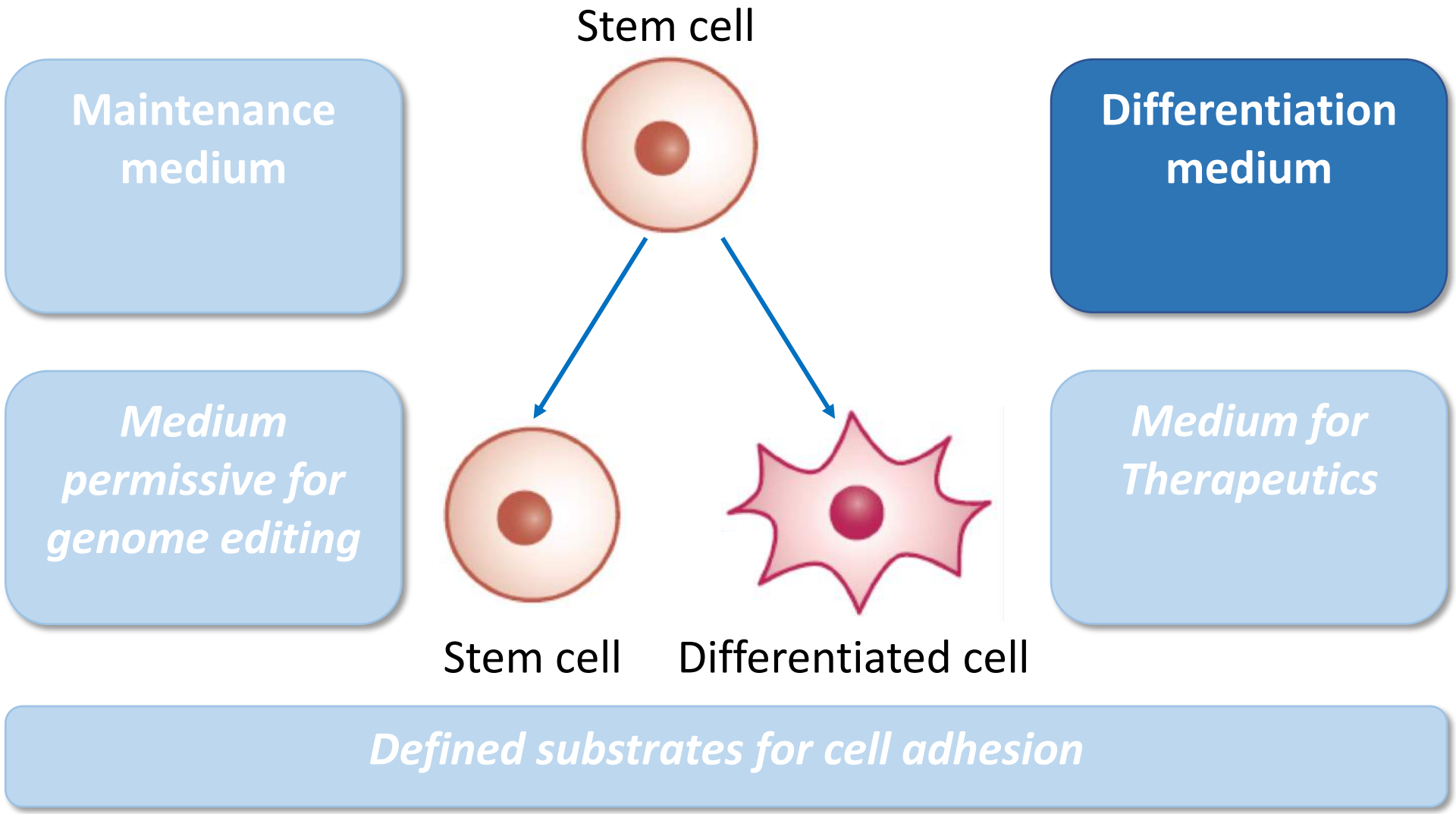
- Difficult to get a clear picture, but possibly uptake has been slow.

Possible reasons:

- Difficult to adapt downstream processes (e.g. genetic modification) to the new maintenance media
- **COST**

  - mTeSR1 – €300 per 500 ml
  - TeSR1 (homemade) – complex formulation
  - E8 – ~€225 per 500 ml
  - E8 (homemade) - ~€100 per 500 ml; however QC tradeoff

Different culture requirements for the maintenance and differentiation of hPSCs

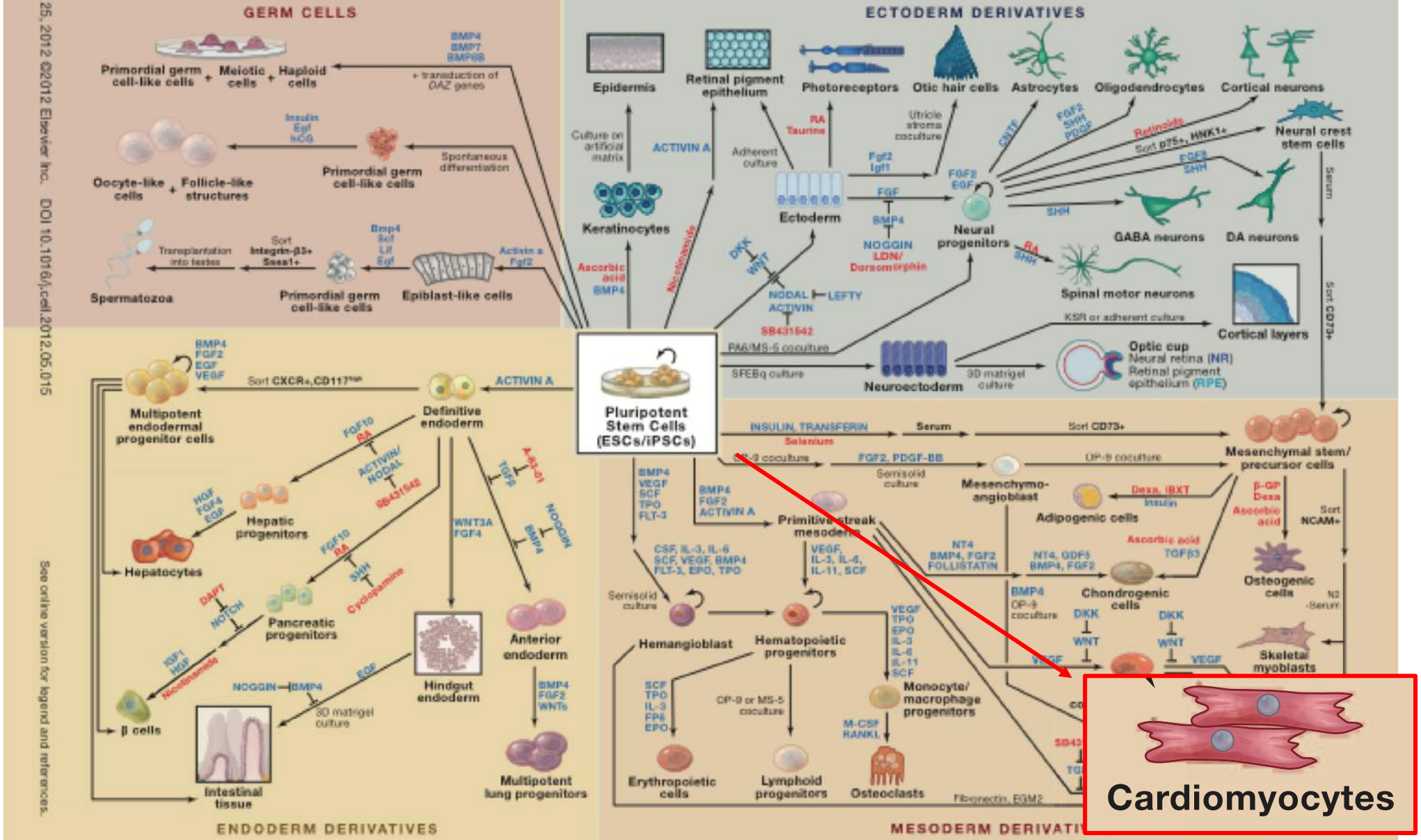


1174 Cell 149, May 25, 2012 ©2012 Elsevier Inc. DOI 10.1016/j.cell.2012.05.015

SnapShot: Directed Differentiation of Pluripotent Stem Cells

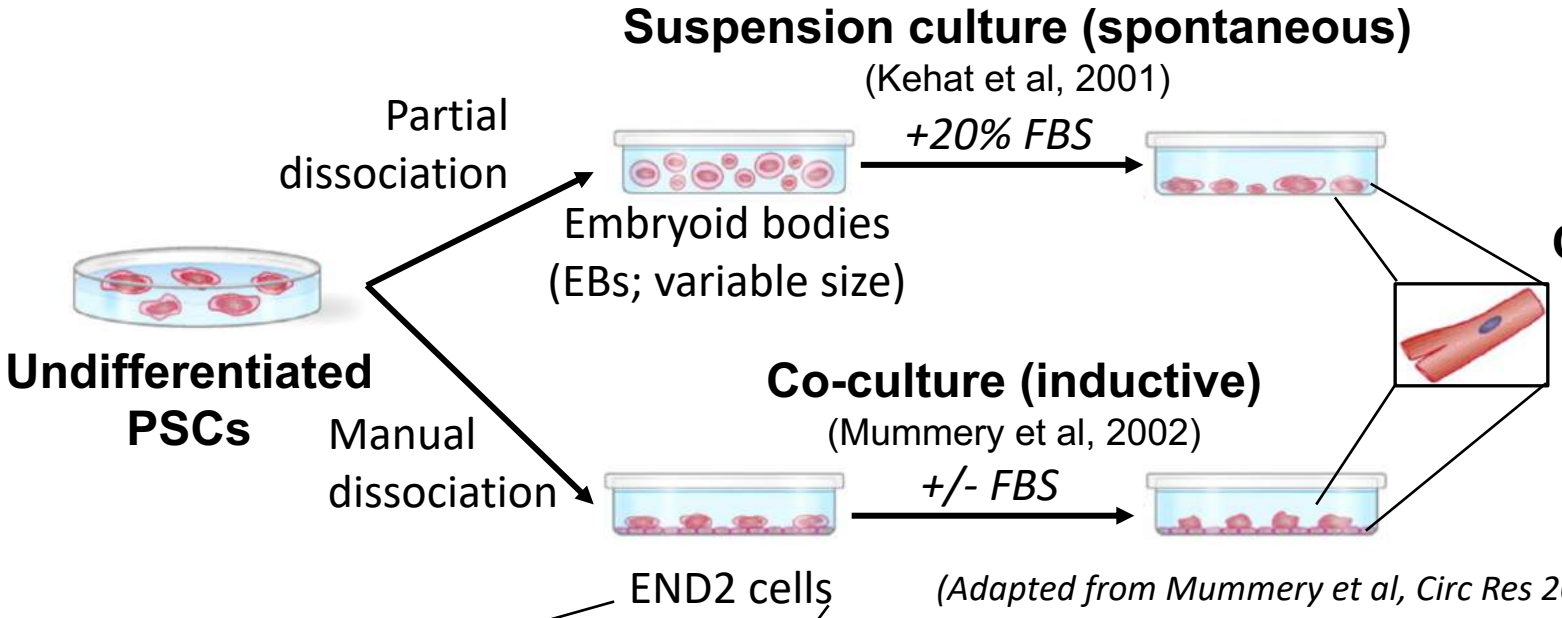
Cell

Luis A. Williams, Brandi N. Davis-Dusenbery, and Kevin C. Eggan
HHMI, Harvard University, Cambridge, MA 02138, USA

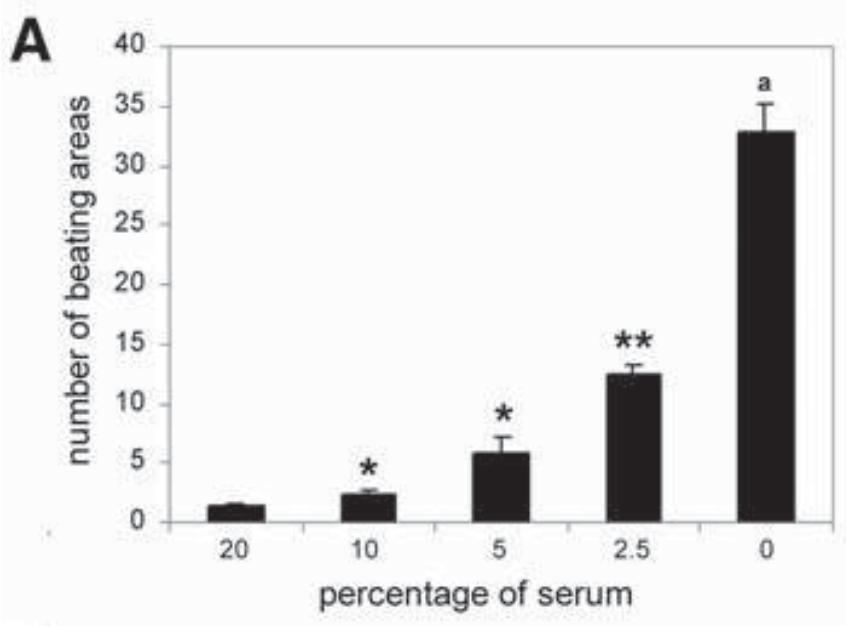


See online version for legend and references.

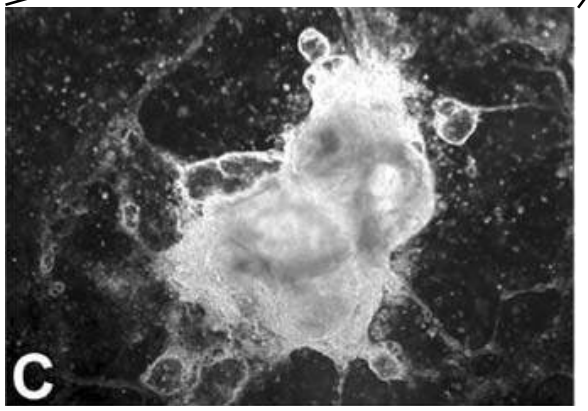
Differentiation of hPSCs to Cardiomyocytes version 1 – spontaneously or via END2 co-culture



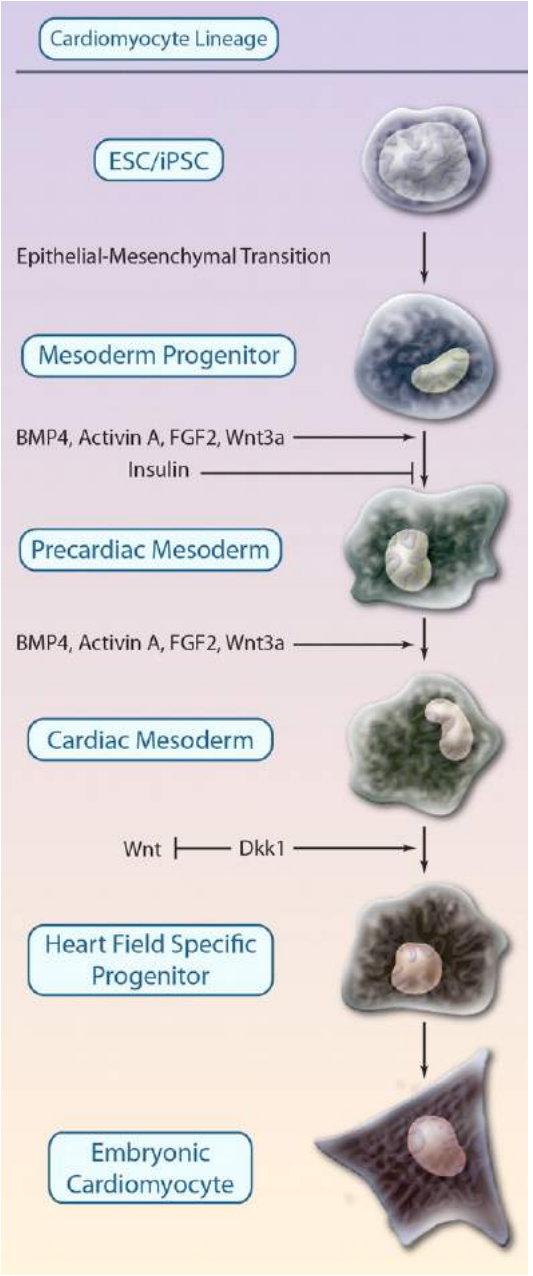
- Efficiency quite variable
- Low % of CMs obtained



(Passier et al, 2005)



hPSC differentiation mirrors embryonic development



(Mummery et al, Circ Res 2012)

- Most efficient in vitro differentiation procedures of human PSCs mimics the sequential stages of embryonic cardiac development.
- Signalling pathways with key roles in embryonic cardiac development also responsible in differentiating PSCs.

A "neutral" differentiation medium is required to assess the role of various agonist and antagonist factors in directing differentiation

Development of APEL – a serum-free culture medium for differentiating hPSCs (Ng, Davis et al. Nat Prot, 2008)

- Based on a serum-free medium developed for mESC differentiation (Johansson & Wiles, 1995)

BPEL: Serum-free medium containing BSA

Deionised **B**SA
Inclusion of **P**VA (BSA: PVA 1:1)
Inclusion of **E**ssential **L**ipids & synthetic cholesterol

→ Neural cell types

HPEL: Human serum albumin substituted for BSA

HSA; PVA; lipids & cholesterol

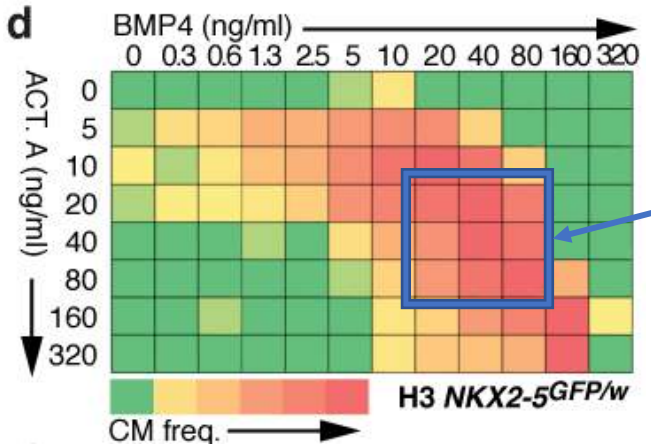
APEL: Serum-free medium containing recombinant human proteins

Recombinant human albumin (**A**lbucult), insulin, transferrin, essential lipids, cholesterol +/- polyvinylalcohol (PVA)

- Blood cell types
- Pancreatic cells
- Kidney cell types
- Cardiac cells
- Thymic cells
- Lung cell types

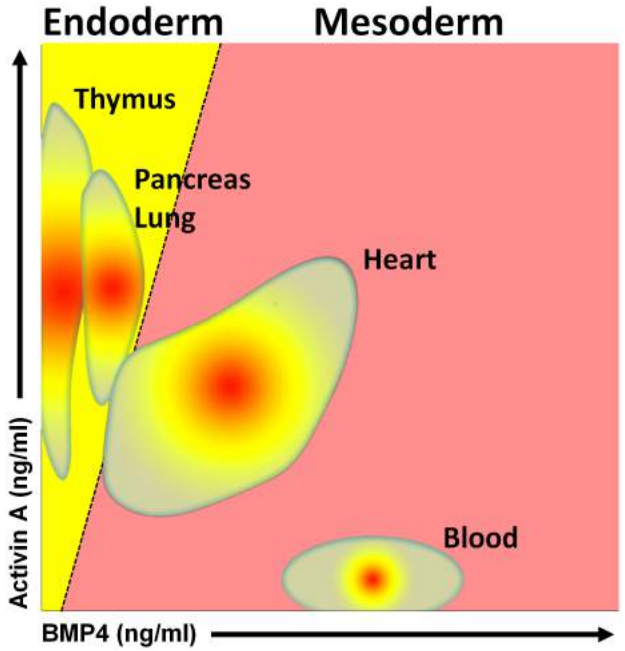
APEL allows the identification of optimal cytokine concentrations and ratios for differentiating hPSCs

Cardiomyocyte differentiation

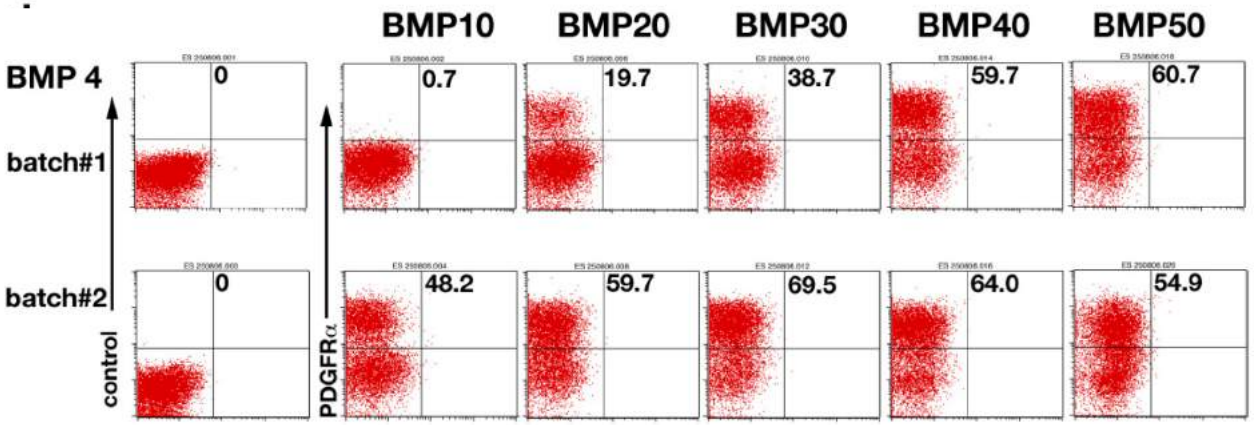


Optimal BMP4:Activin A concentration between 20:20 – 40:40 ng/ml

(Elliott et al, Nat Meth 2011)



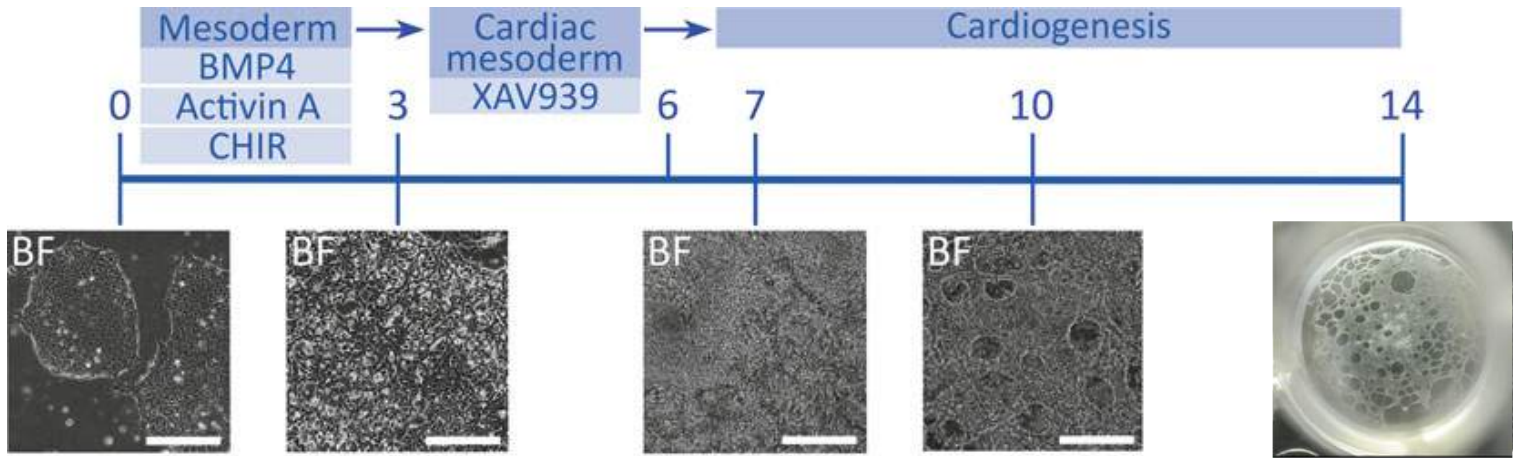
(R. Jenny, *The generation of human lung progenitors from human embryonic stem cells*)



(Ng, Davis et al, Nat Protoc 2008)

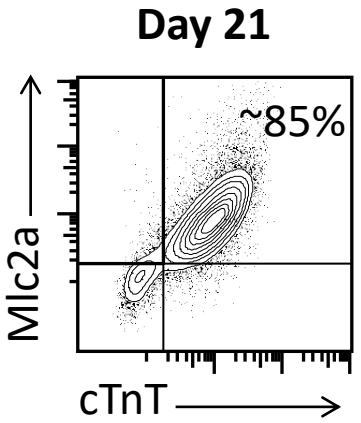
- Differentiations in APEL can reveal variability in growth factor activity between different batches

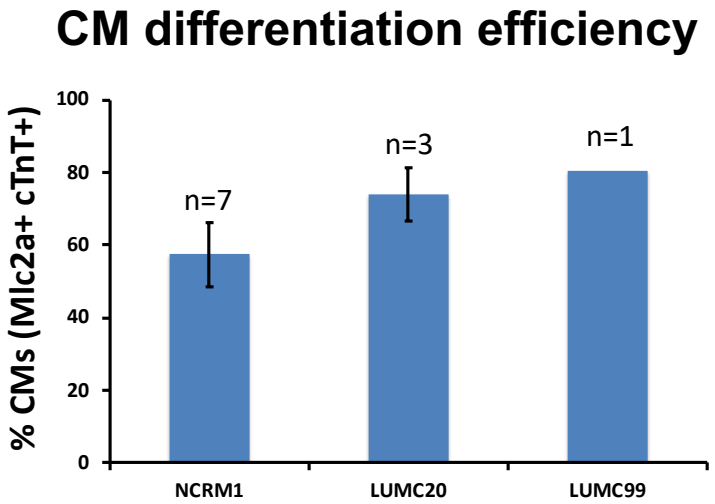
BPEL enables efficient generation of hPSC-derived CMs

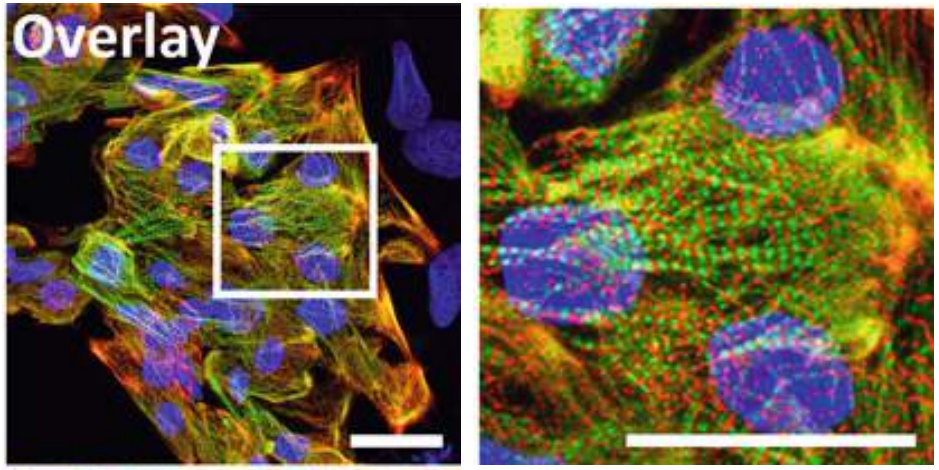


(van den Berg et al. Meth Mol Biol, 2015)

- Reduced insulin for CM differentiation
- More economic version of APEL
- Batch-to-batch variability minimized if premium-grade BSA used



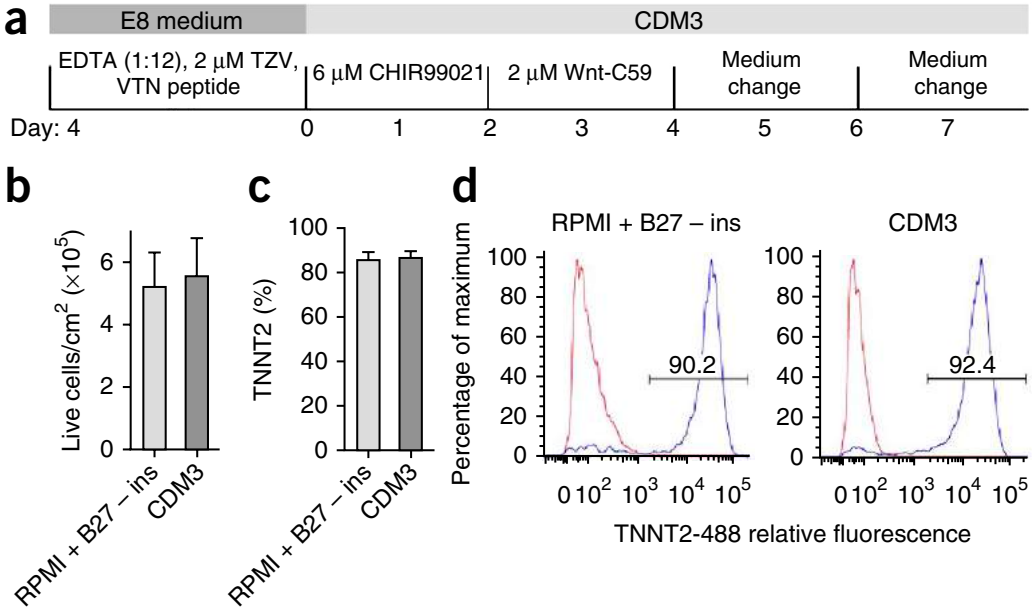




TNNI3; ACTN2; DAPI

A minimal cardiac differentiation media consisting of just 3 components – Burridge et al. Nat Meth, 2014

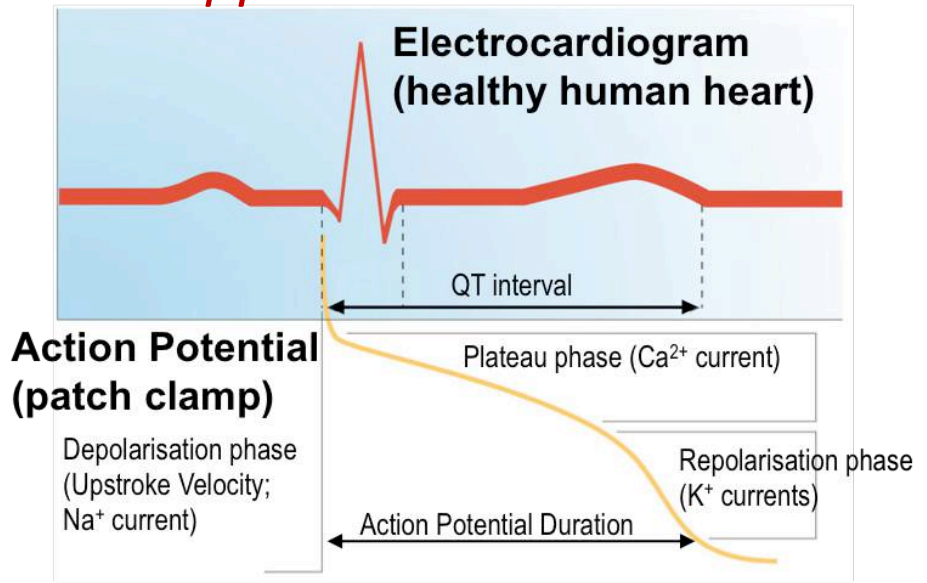
- Sequentially removed 21 components based on published differentiation media
- Identified 3 essential components: RPMI 1640 medium, ascorbic acid and BSA
- Can replace BSA with recombinant human albumin



Burridge et al. Nat Meth, 2014

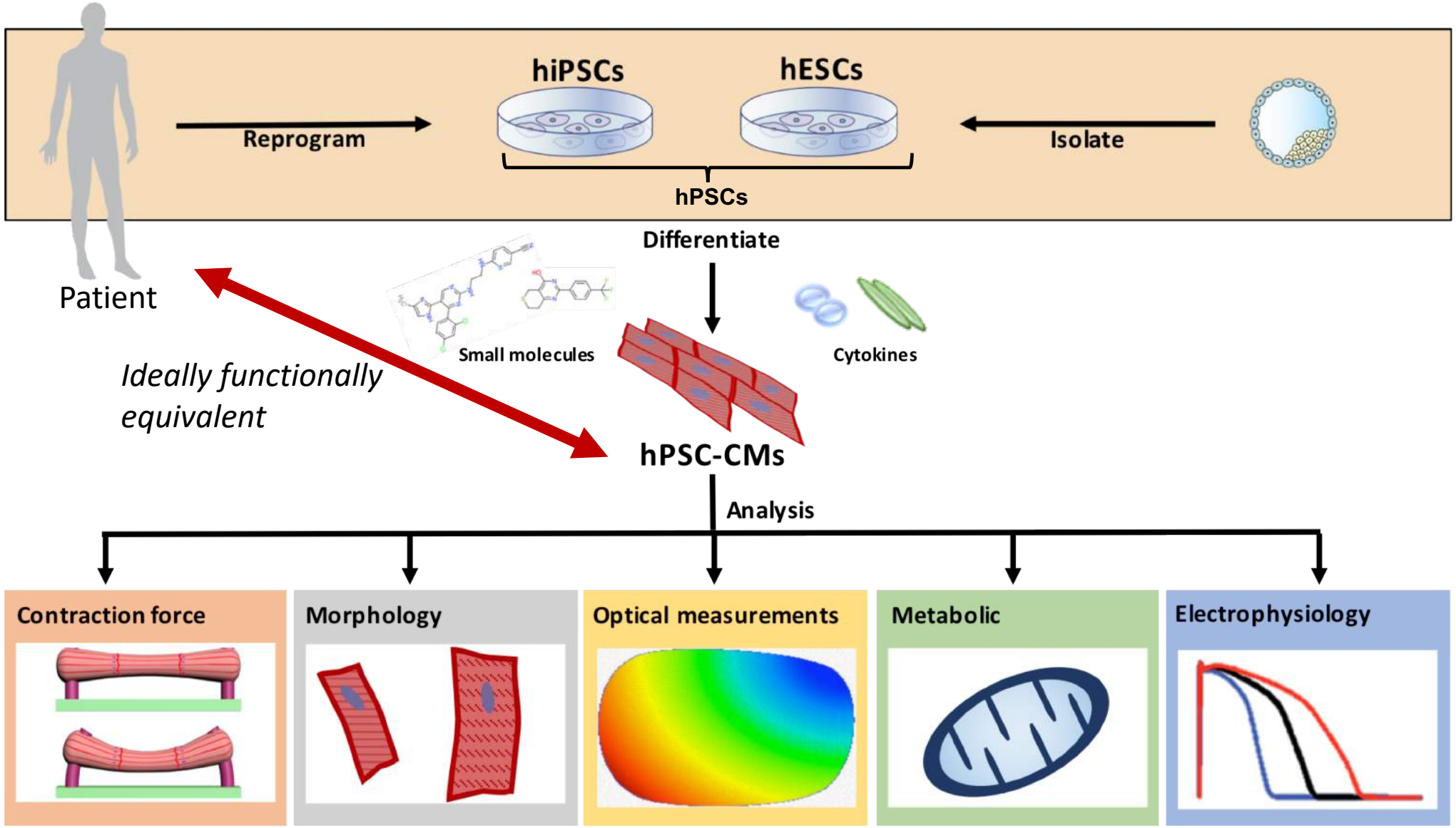
- Differentiation just with small molecules

- *Potential issue is the maturity of the CMs for downstream applications*



- **hPSC-CMs in all differentiation media are still less mature than adult CMs**

Functional assays using hPSC-derived cardiomyocytes



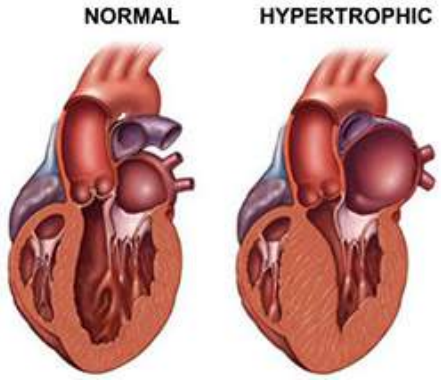
Caveats:

- Maturity of cells
- Lack in vivo context

(Brandão et al, *DMM*, 2017)

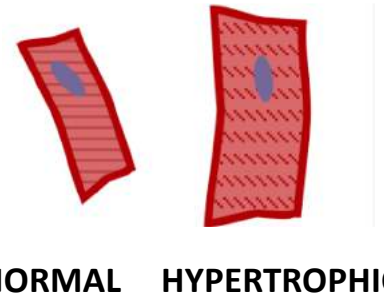
Maturation of hPSC-CMs can be required to reveal disease phenotypes

Hypertrophic cardiomyopathy



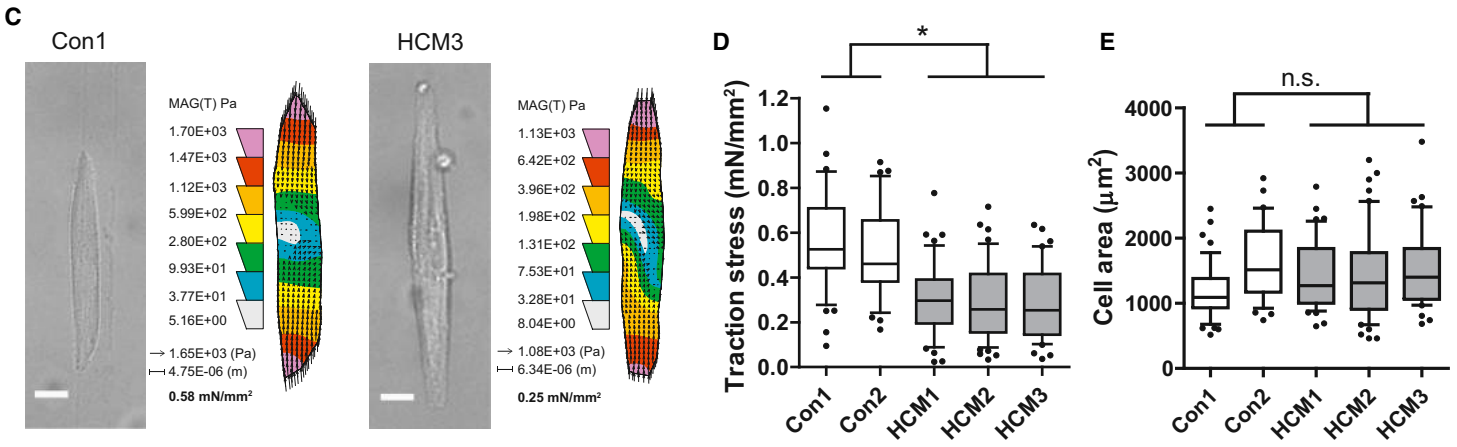
- Genetic-based disease
- Common cause of sudden cardiac death (SCD) in young people
- Thickening of the ventricle wall
- Reduced blood flow
- Lead to arrhythmias & SCD

CARDIOMYOCYTES



- Enlarged
- Structurally disorganised
- Impaired contractility
- Impaired Ca^{2+} handling

Modified BPEL media improves the maturity of PSC-CMs revealing a contractility defect in the HCM patient lines



(Birket et al, Cell Rep 2015)

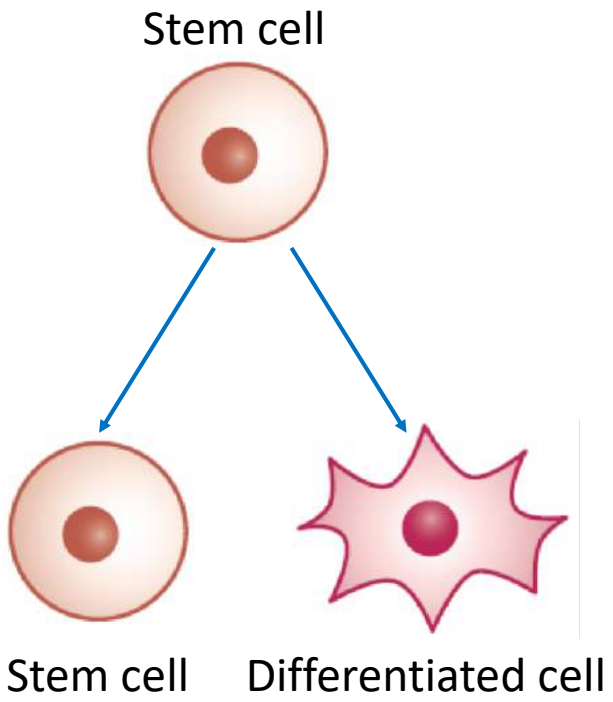
Summary – where are we at?

Maintenance medium

- Completely-defined, serum-free media available
- Suitable for clinical use
- Understanding pluripotency has been a key driver in media development
- Ongoing development: media to improve efficiency of certain techniques (e.g. gene targeting)

However:

- Adoption rate of these media within stem cell community ???
- Cost – commercial



Differentiation medium

- Serum-free (completely-defined) media available
- Enables differentiation methods to be more precisely tuned for specific cell types
- Still requires development

Challenges:

- Maturity of differentiated cells
- Reproducibility (intra- & interline variability)
- Costs (media; cytokines)
- Compatibility with completely-defined maintenance media

Acknowledgements

Cardiac Differentiation

Mummery Group (*LUMC*)

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- Marcelo Ribeiro (*U. Twente*)
- Christine Mummery

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- Karina Brandão
- Lettine van den Brink
- Mervyn Mol
- Duncan Miller

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- David Elliott
- Andrew Elefanty
- Ed Stanley

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