Cells and Tissues Discussion Panel

Chair: Christine Mummery (LUMC)

Panel members: Paula Alves (IBET), Jochen Kuehnel (Beiesdorf), Oliver Frey (InSphero)

Topics to include but not limited to:

- cell sources
- SOPs and protocols
- performance standards
- functionality assessment
- quality management
- repeatability

Microphysiological and Organ-on-Chip systems

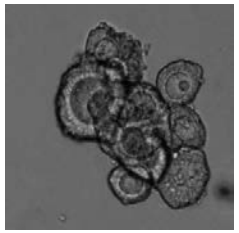
→ *Recapitulating complex human biology in vitro*

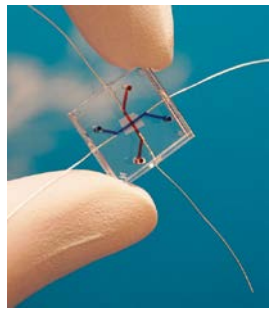
High throughput cell assays

- ✓ Human genetic background
- ✓ Standardized & automated
- ✓ Cost efficient
- ✗ No perfusion
- ✗ No mechanical forces
- ✗ Cell to media ratio too low
- ✗ 2D **(mono-)cultures** of (cancer) cells on hard plastic

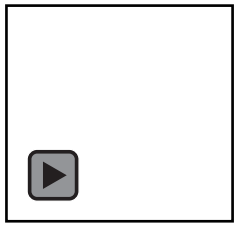
Organ on Chip

Mini-gut





Mini-heart



Microphysiological Systems

- ✓ Human engineered tissues
- ✓ *In vivo* structure & function
- ✓ Microphysiological environment
- ✓ **Human immune aspects**

Animal models

- ✓ Complex tissue architecture
- ✓ Circulation
- ✓ Systemic effects
- ✗ Different physiology
- ✗ **Different immune system**
- ✗ Specific environment & diet
- ✗ Strain-specific results due to (in)breeding

Credit Peter Loskill, Fraunhofer

Human Cell Sources

- Primary cells: from tissue biopsies, waste surgical tissue, body fluids (blood, amniotic fluid, bone marrow, cerebral spinal fluid, lung lavage etc

closely resemble tissue of origin but show batch-to-batch variability, show limited lifespan in culture, require broad informed consent documentation, rapidly lose tissue-specific phenotype in culture and may be difficult to access

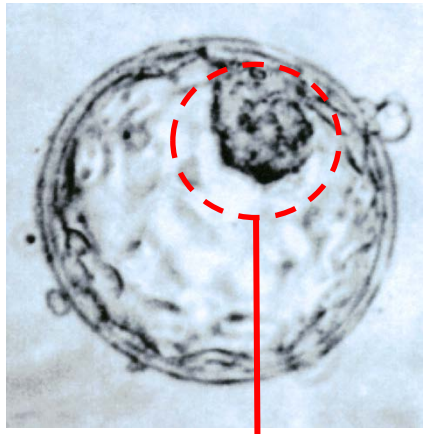
- Immortalized or cancer cell lines

expand easily in culture to extremely large batch sizes but may not reflect healthy tissue phenotype and show genetic instability

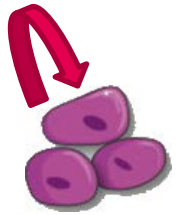
- Adult- or pluripotent stem cells

expand indefinitely in culture, broad informed consent often available, make many cell types of the body but may show line-to-line vardiability and differentiated derivatives may be immature

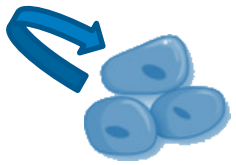
Three kinds of human stem cells



Derived from human embryos



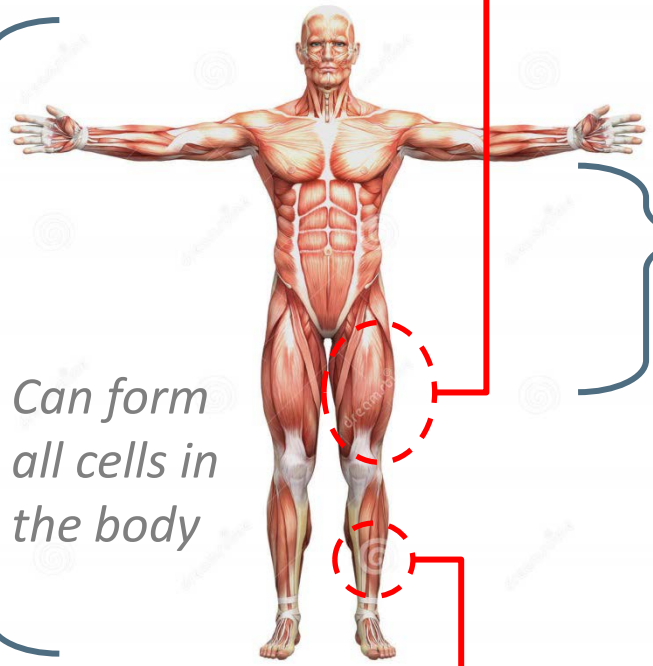
Pluripotent embryonic stem cells



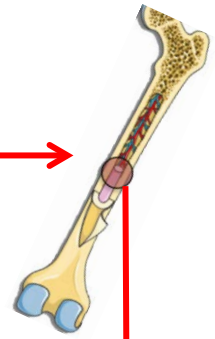
Induced pluripotent stem cells

'Reprogramming'

From skin, blood, urine etc.

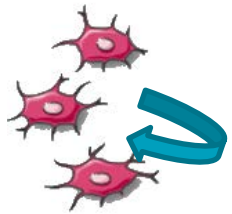


Can form all cells in the body



From bone marrow, gut, lung, liver, pancreas (as organoids) etc

Form cell types of the tissues from which they are derived



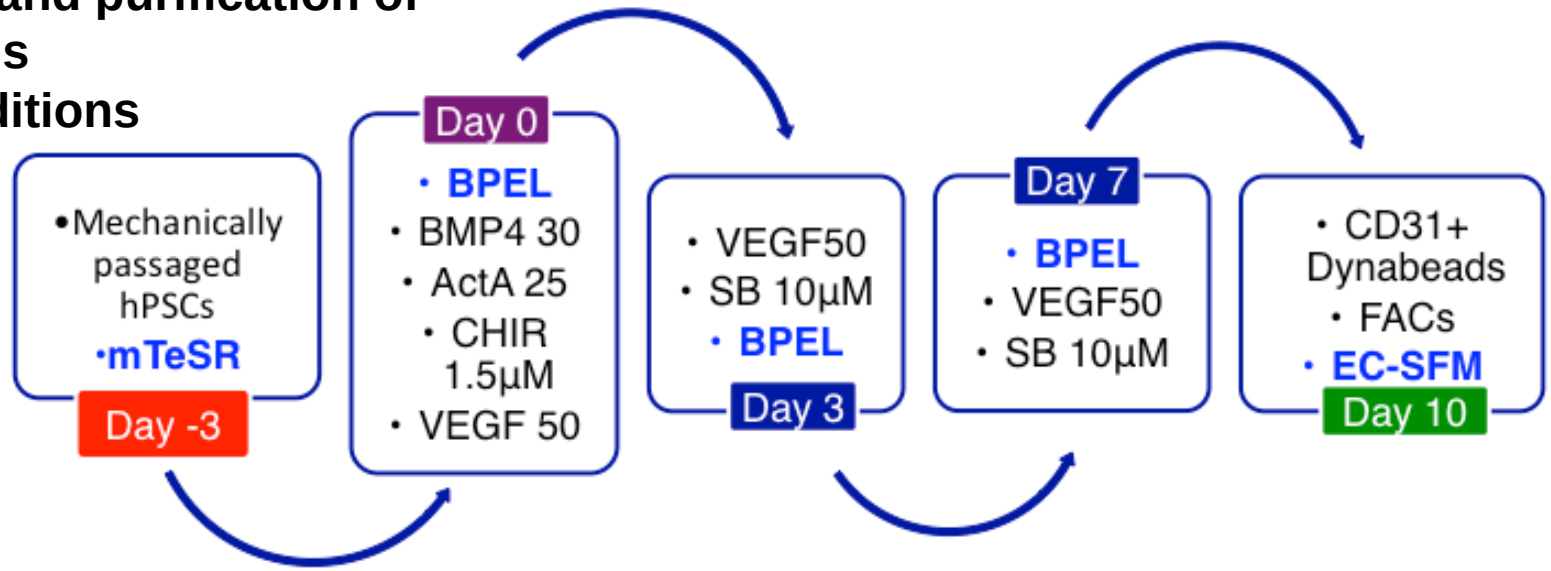
Endothelial cells (ECs) as an example

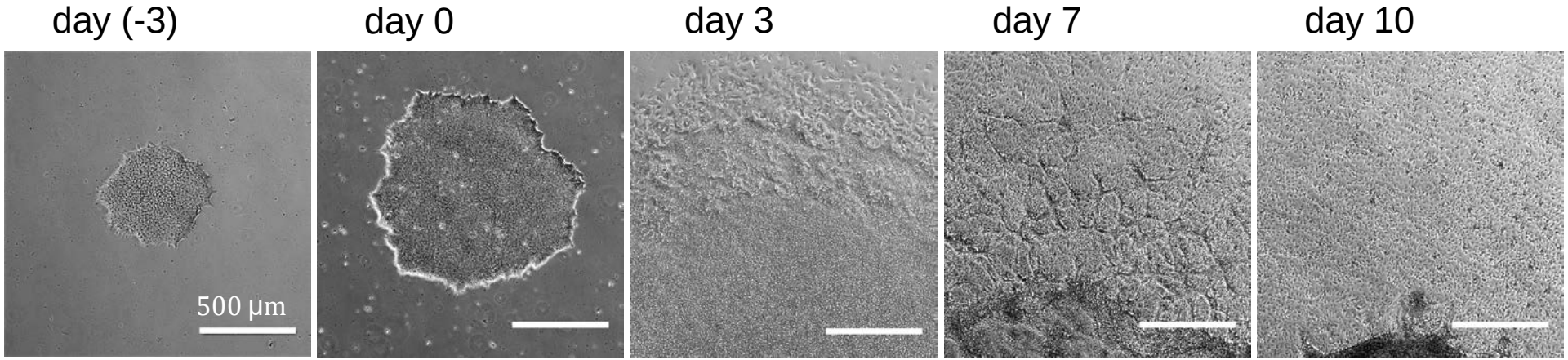
- Primary sources : human umbilical vein ECs (HUVECs), human microvascular dermal ECs (hMDECs), human blood outgrowth ECS (hBOECs)

- Stem cells : human pluripotent stem cell ECs (hPSC-ECs)

Vascular differentiation of hPSCs

Differentiation and purification of endothelial cells in defined conditions

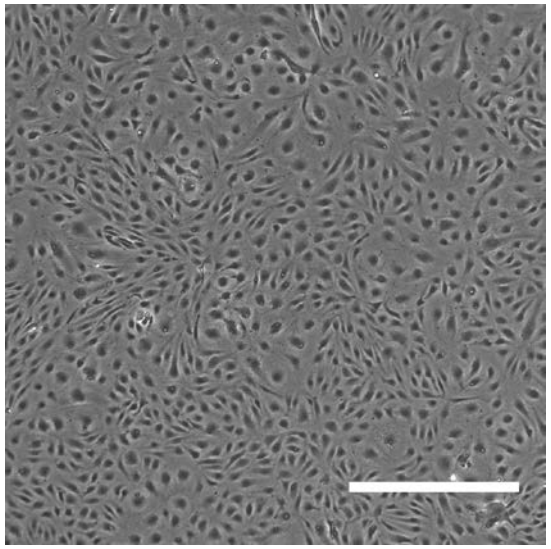




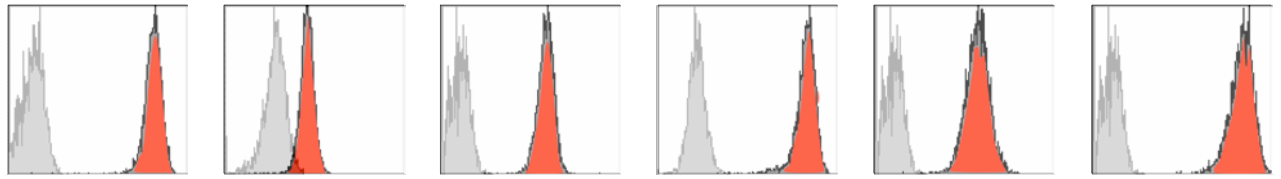
Orlova et al., Nat Prot 2014

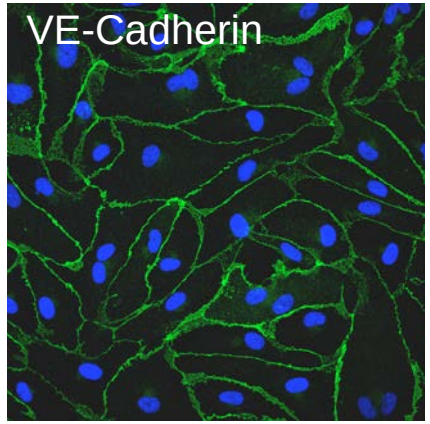
Characteristics of endothelial cells derived from hiPCSs

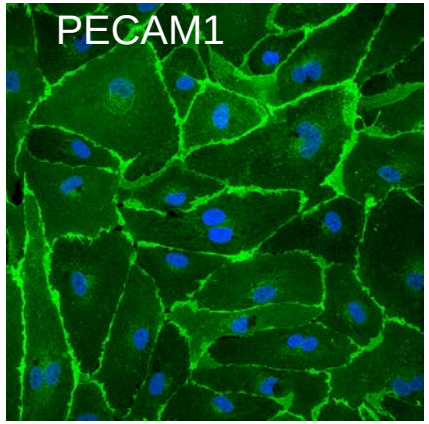
CD31+

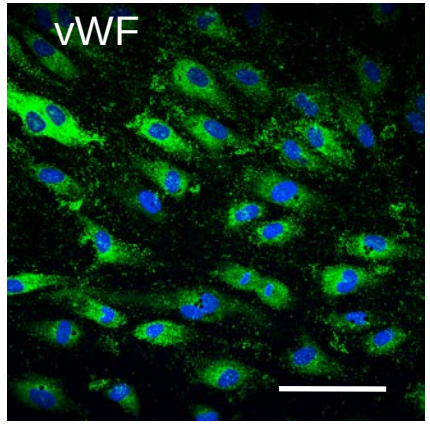


PECAM1 VE-Cadherin KDR CD34 CD73 ENG



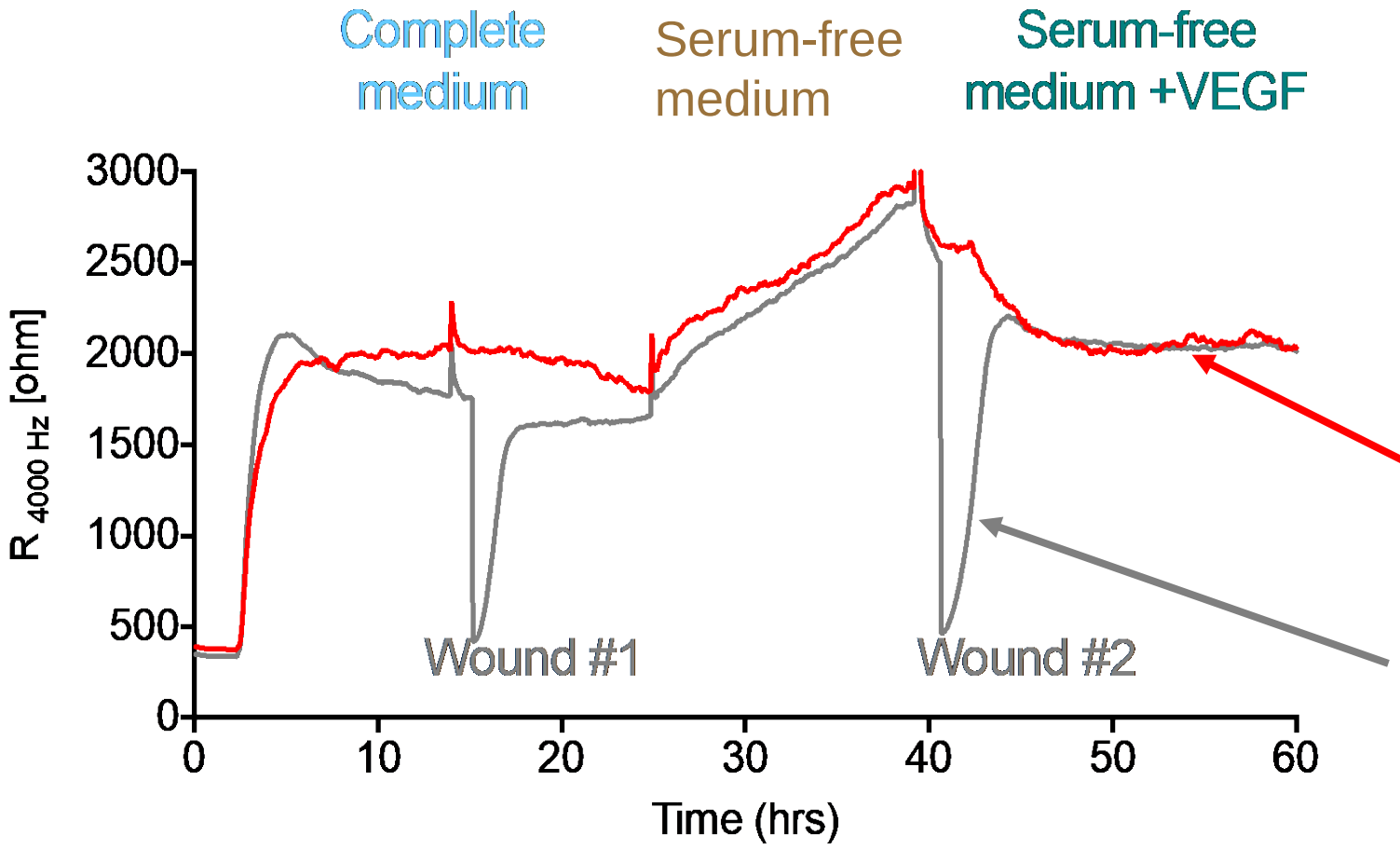


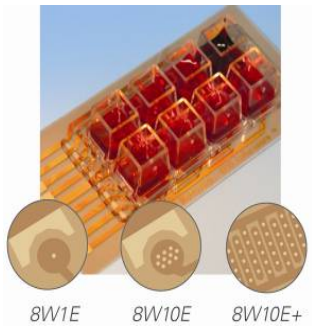


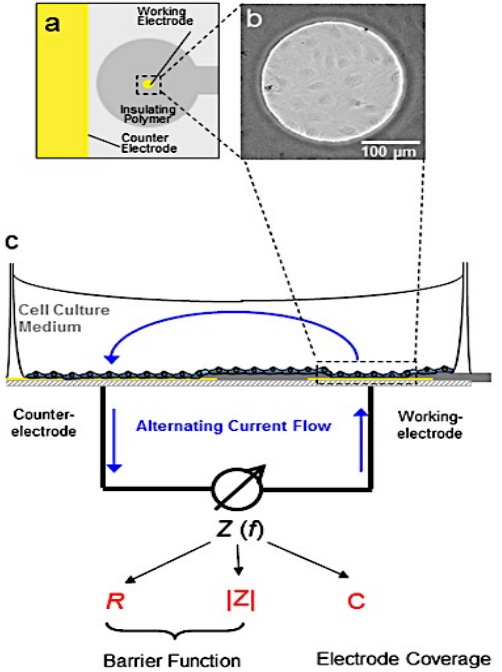


100% pure CD31+ endothelial cell populations can be cryopreserved and stored

Assessment of hPSC-ECs Barrier Function: Electrical Cell-Substrate Impedance Sensing (ECIS)







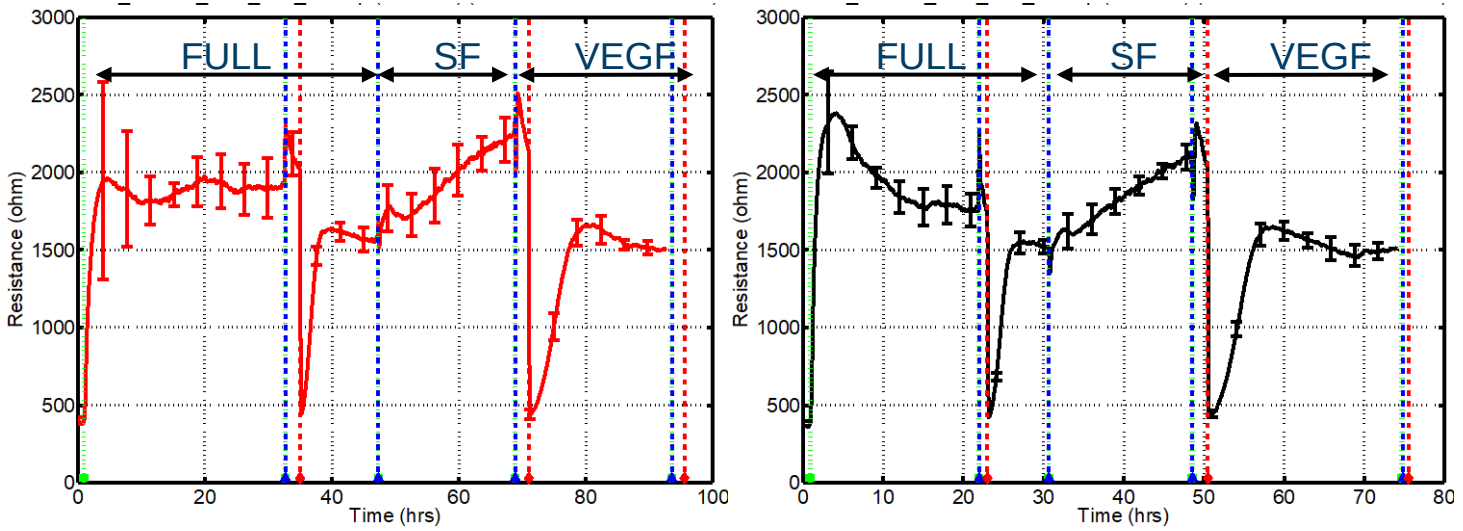
(i) Barrier (R_{4000}Hz) :
Complete medium

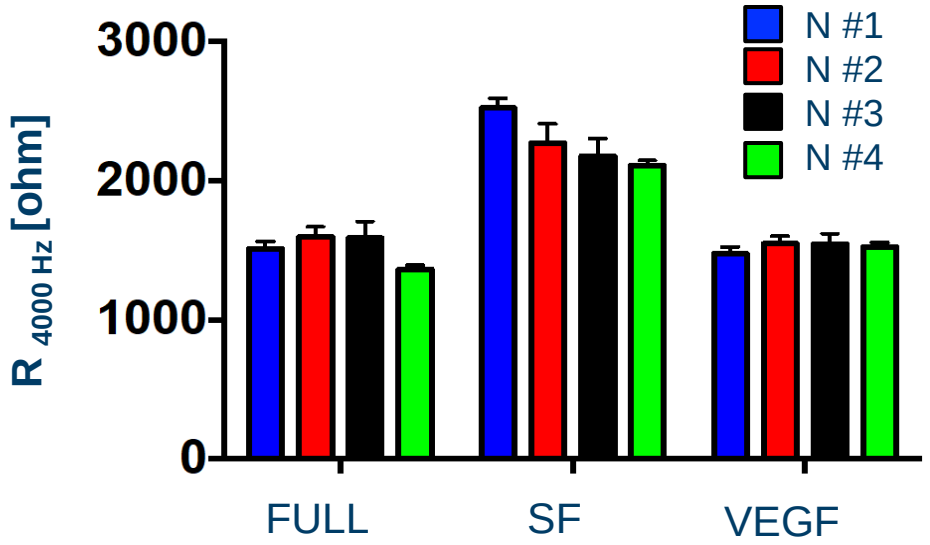
(ii) Barrier (R_{4000}Hz) :
Serum-free medium
-/+VEGF or other compounds

(iii) Real-time wound healing assay: migration time

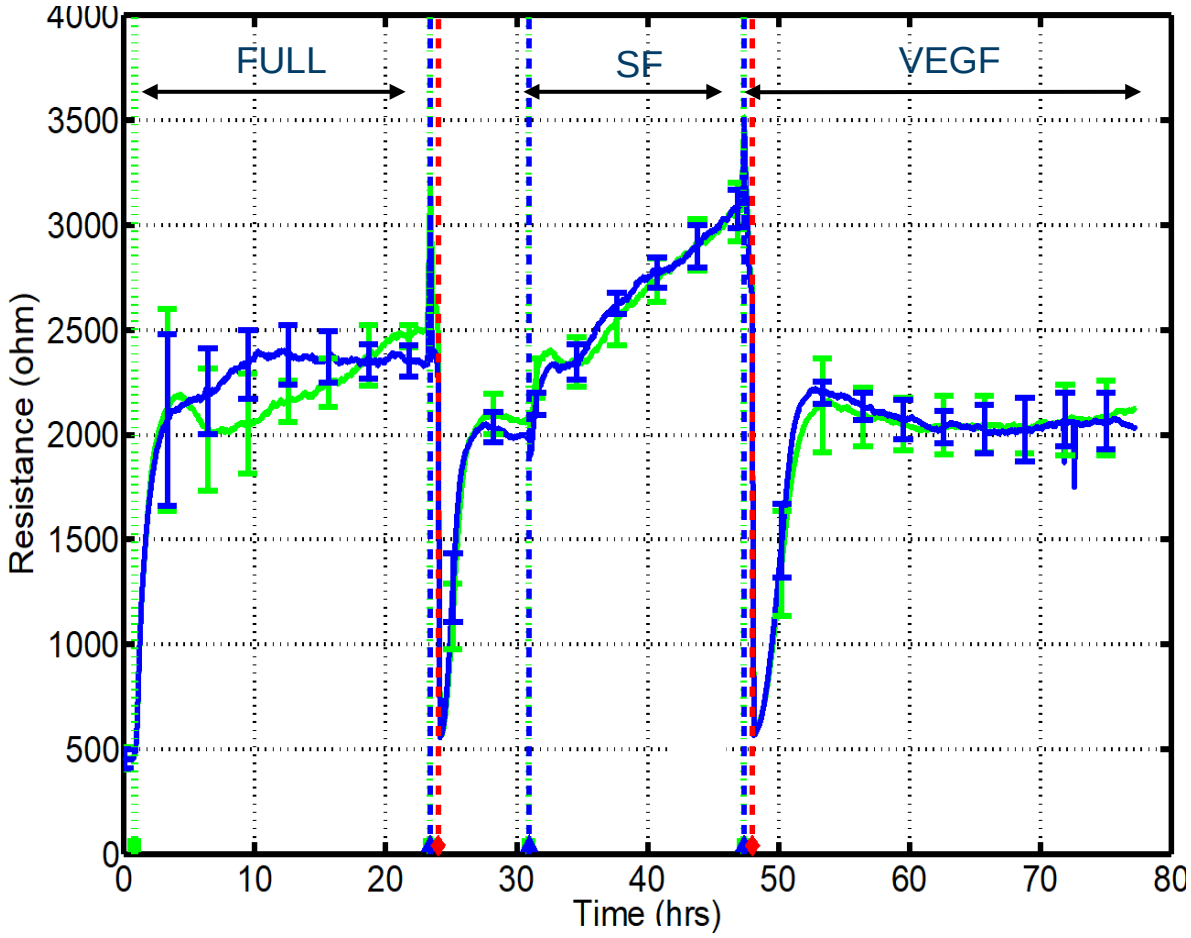
Halaidych et al., Stem Cell Reports 2018

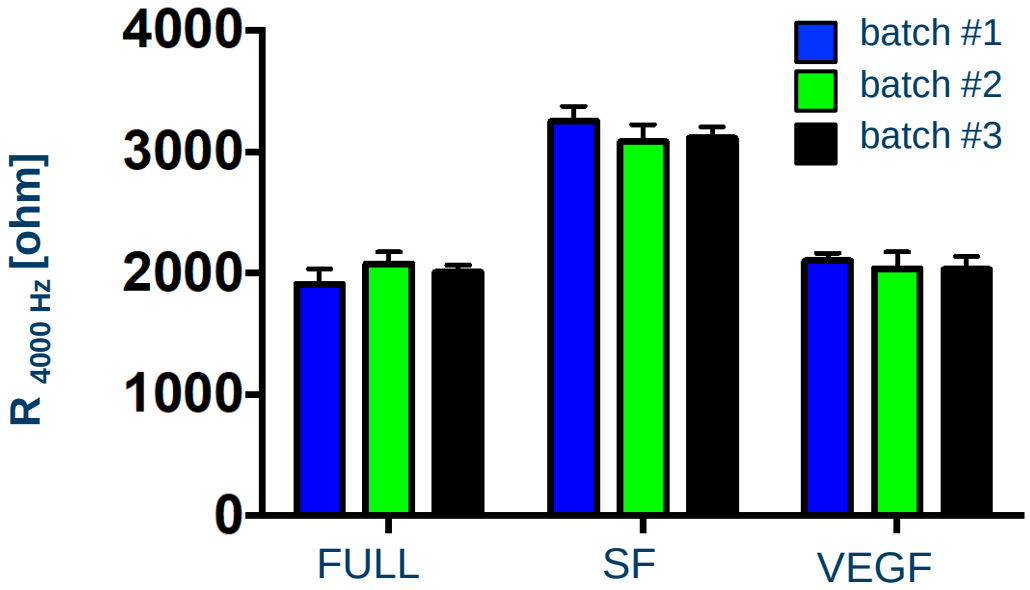
ECIS: independent biological experiments with the same batch of hiPSC-ECs



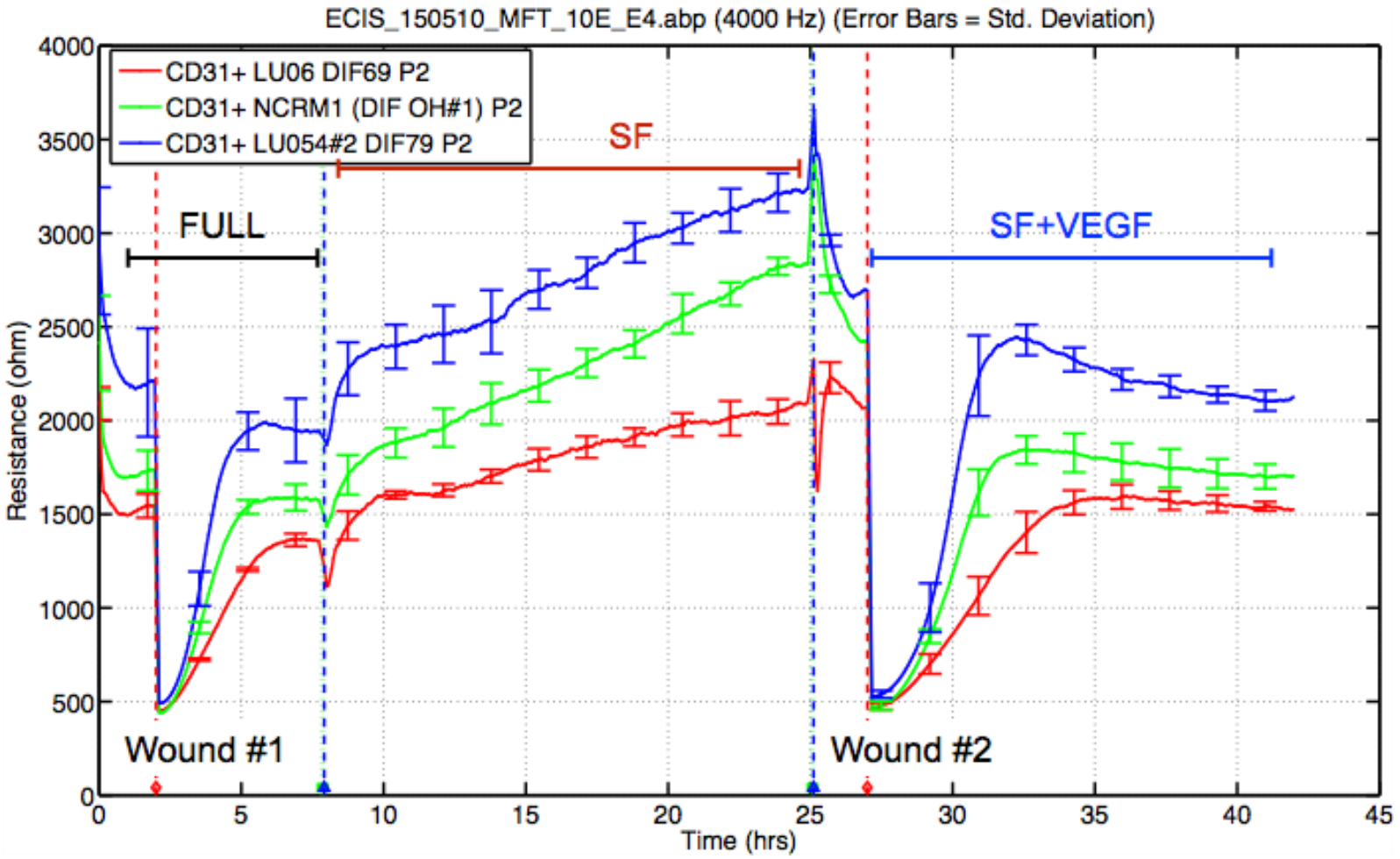


ECIS: independent batches of hiPSC-ECs isolated from the same control line





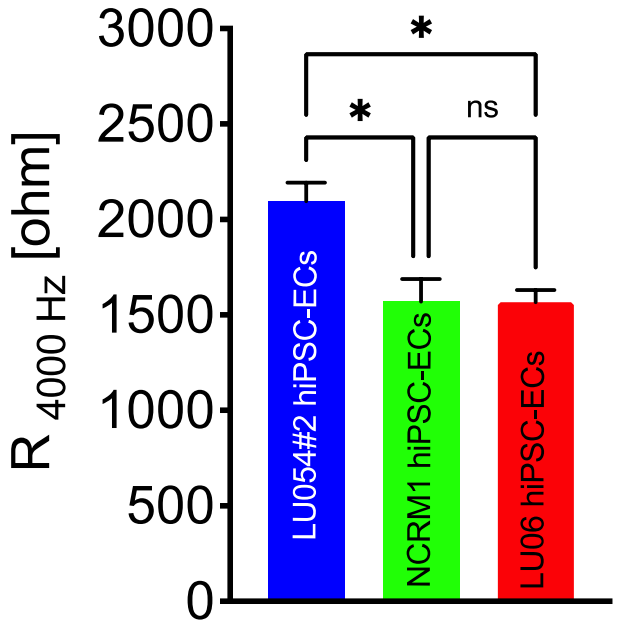
ECIS: hiPSC-ECs isolated from different control hiPSC lines



LU054#2 hiPSC-ECs (SeV)

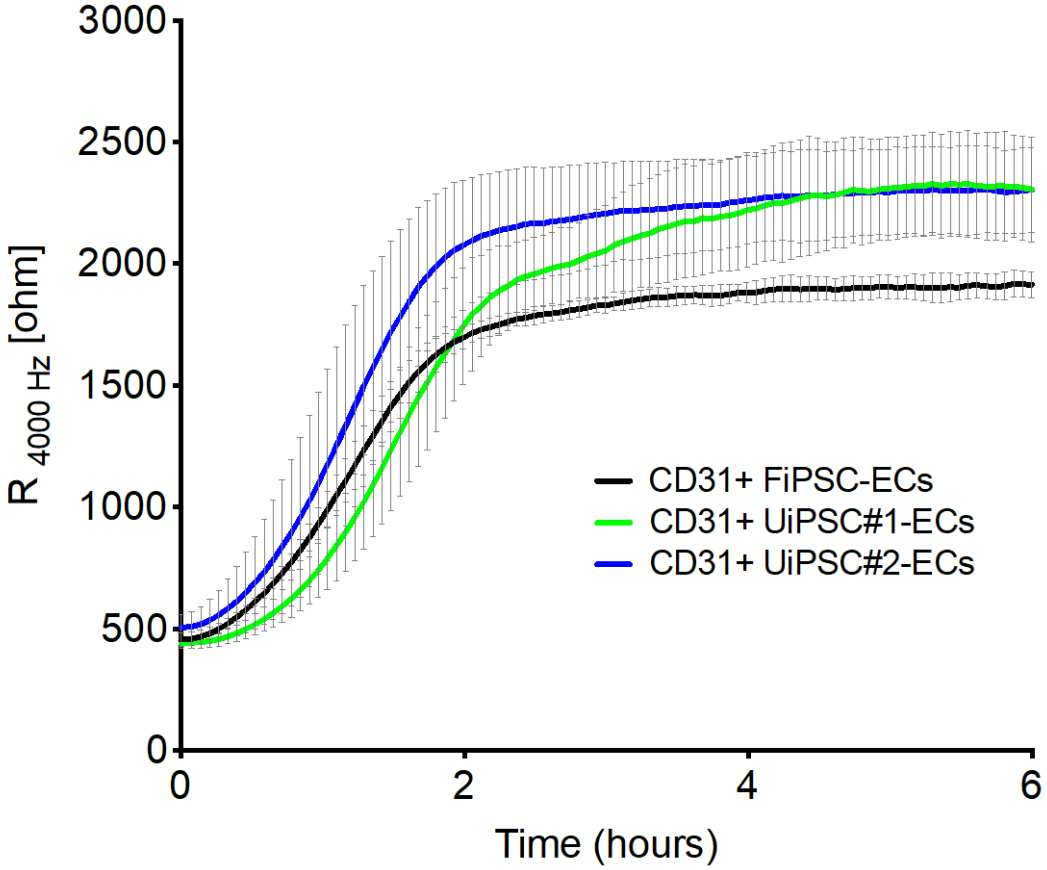
NCRM1 hiPSC-ECs (Epi)

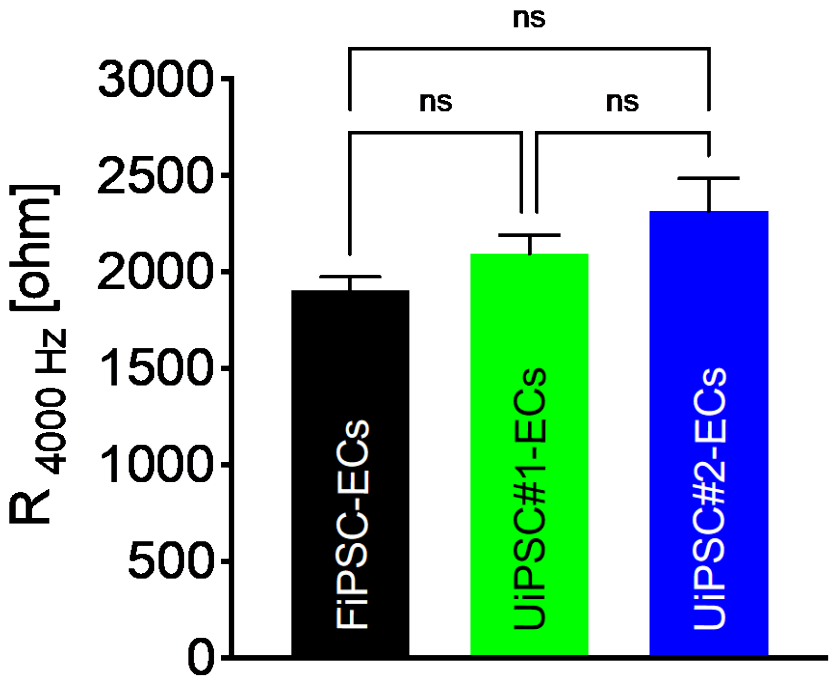
LU06 hiPSC-ECs (LV)



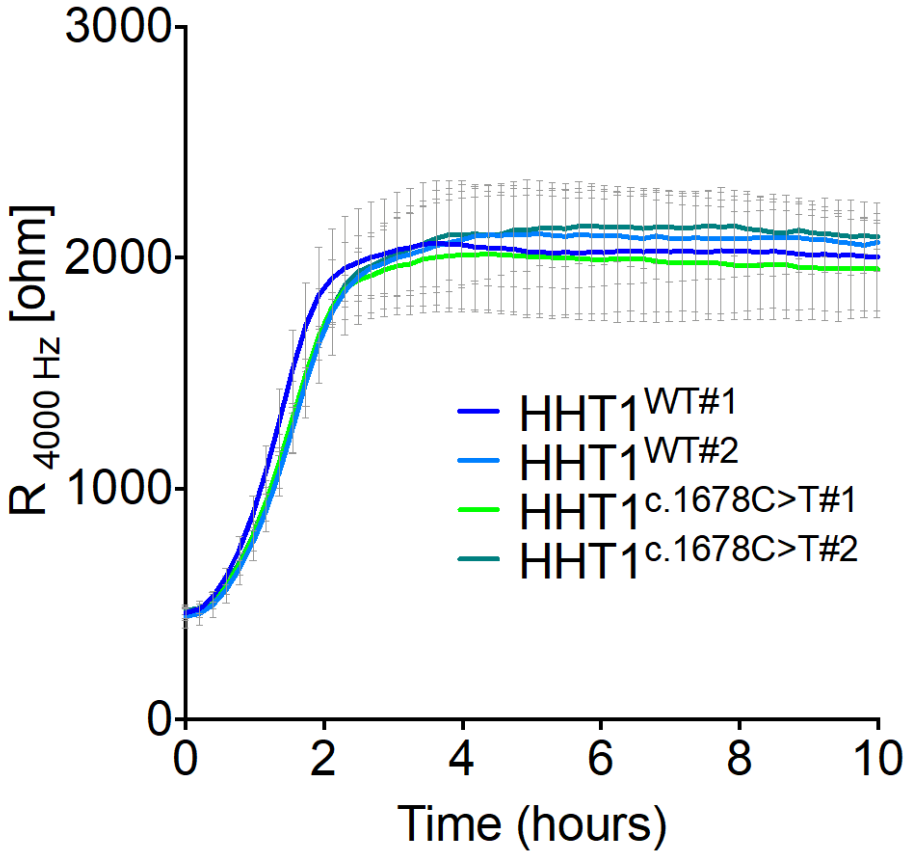
FULL

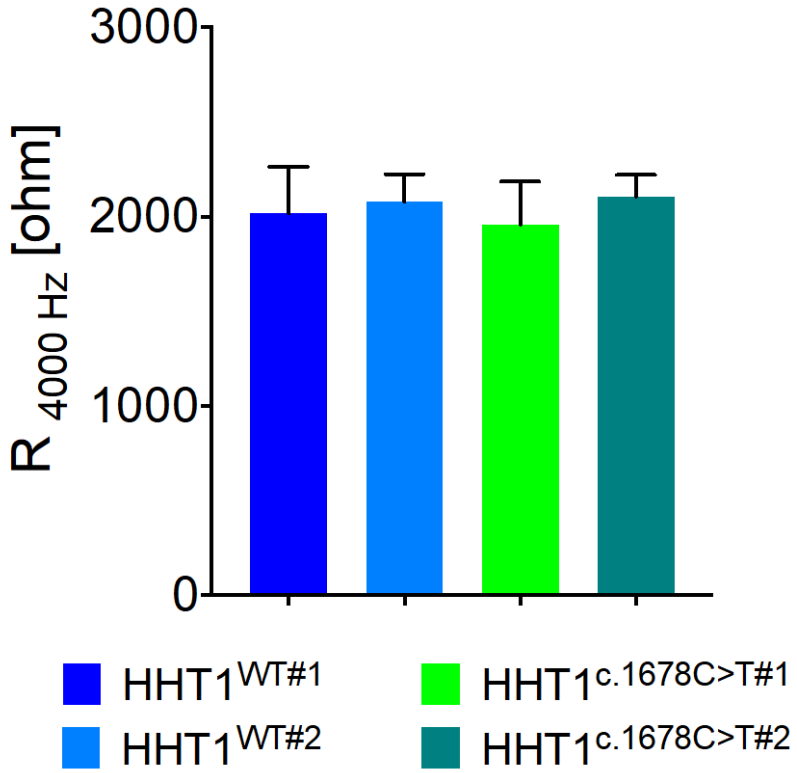
ECIS: hiPSC-ECs isolated from different control hiPSC lines (SeV reprogrammed)





ECIS: isogenic HHT1-iPSCs derived ECs





Summary

For *all* cell types

- cell sources
- SOPs and protocols
- performance standards
- functionality assessment
- quality management
- repeatability