OLS
OMNI Life Science

Pluripotent Stem Cells, Spheroids, Organoids and Tissue

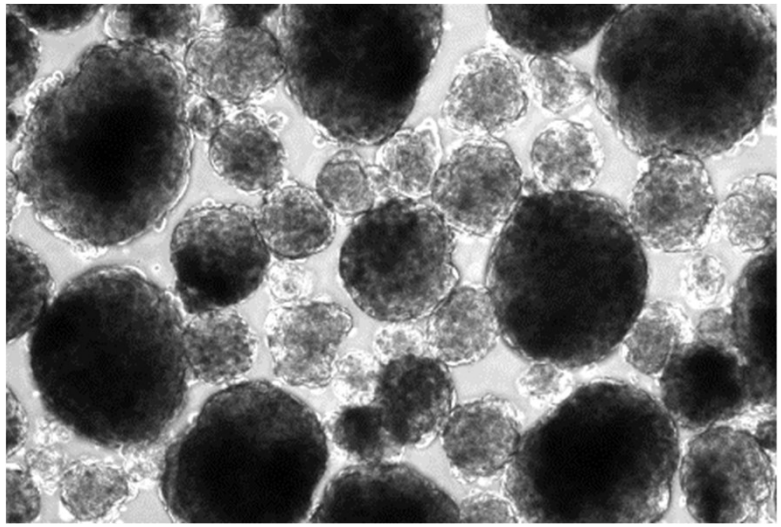
CERO – innovating 3D culture

Amir Keric & Dr. Joachim Pavel

Overview

- CERO fundamentals
  - Spheroids vs. Organoids
  - No Matrigel required

- CERO applications
  - Expansion of iPSCs
  - Mouse ESC and Cardiospheres
  - Hepatic-Spheroids
  - Organoids in CERO



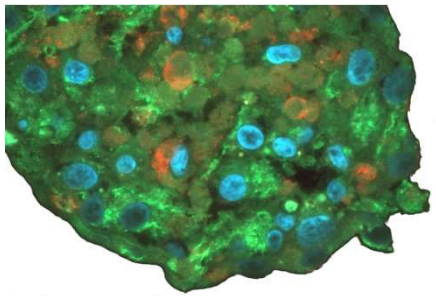
Here is CERO

- CERO - stand alone incubator
  - 4 CEROtubes
  - Individual control of bi-directional rotation
  - Controlled CO_2 & temperature
  - Online pH monitoring
  - Touch screen operation
  - Ready-to-use protocols

- CEROtubes
  - Homogenization fins
  - Vented cap
  - Flexible volume up to 50 ml
  - Disposable
  - Flat bottom

CERO - can do

- Differentiation and cultivation of Organoids & Spheroids
  - Cortical, Cardio, Hepatic, …
- Stem cell expansion and differentiation
  - iPSC & ESC, Neuro stem cells, …
- Tissue cultivation
- CERO
  - Long term cultures (up to >1year)
  - Improved viability & maturation
  - Extremely homogeneous cell aggregates
  - Improved polarity



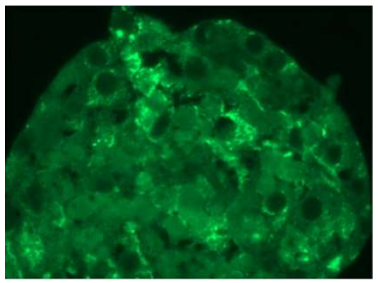
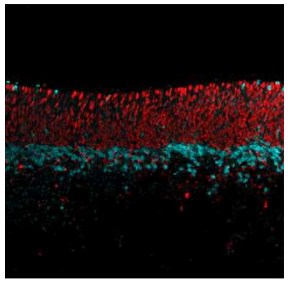
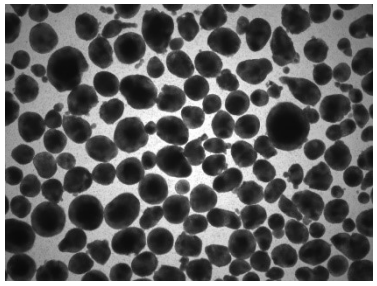
Why CERO is better!

- **No Matrigel**
- **Homogeneous conditions for:**
  - Temperature – No gradients like in normal incubators
  - CO_2 – No gradients from opening doors
- **Homogeneous distribution of oxygen and nutrition**
  - Improved viability and maturation
  - Significantly reduced apoptosis & necrosis
  - Allows experiments not possible in static cultures
- **No shear forces**
  - No impellers or “rolling” organoids in orbital shakers
- **Online Monitoring**
  - pH Monitoring - never miss the right time to change media
  - Control and track time, temperature and CO_2

CERO provides it all

Spheroids vs. Organoids in CERO

	Spheroids	Organoids
	Defined aggregates	
Structure	Unicellular	Multicellular
Cell types	Cell lines	Stem cells
Culture duration	>80 days	Several months
Complexity	Basic	High

Why Matrigel at all?

	Stem cells	Organoids
Historical use	Substrate & Nutrition	
Today use	ONLY as substrate	Embedding substrate (& differentiation factor)
Disadvantages of Matrigel	Animal origin (no clinical use)	
	Handling: Variation in aliquots, coatings & embeddings	
	Batch to Batch variation: ~2000 factors that impact cell fate	
	Tremendous cost (~400€/10ml Matrigel) & Limited shelf life	

Why add so many disadvantages, if not needed?

CERO - no Matrigel required

	3D in CERO	2D / static
Matrigel	NO	Required
Adhesion	Autoadhesion (self aggregation)	Colonies in 2D
Gravity	Minimal – dynamic suspension	Yes – physical support required

Conclusion:

Less gravity

No shear forces

No need for physical support (Matrigel)

No need for carrier (Autoadhesion)

Autoadhesion instead of Carrier or Matrigel

CERO - further advantages vs. 2D / static

	3D in CERO	2D plate / static
Attached to:	Cells only	Plastics & Substrate
Stiffness/Micro-environment	Natural Niche	Artificial (varies by brand)
Handling	Simple, reproducible, quick	High Time demand & variations in handling (day to day & operator to operator)
Media demand	Low / high yield	High / huge cost factor
Gas exchange & Nutrition supply	Dynamic, 360°	Static

CERO – reduces cost, time spend and variations

CERO – how to change media

Stop rotation &
let cells settle

Aspirate
supernatant

Add fresh media

Place in CERO &
continue rotation

4 steps / 2 minutes only / every 3-7 days

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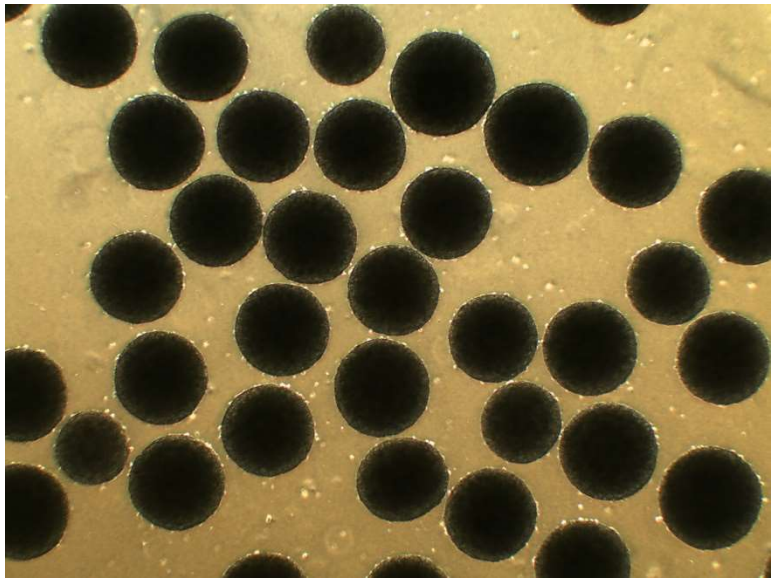
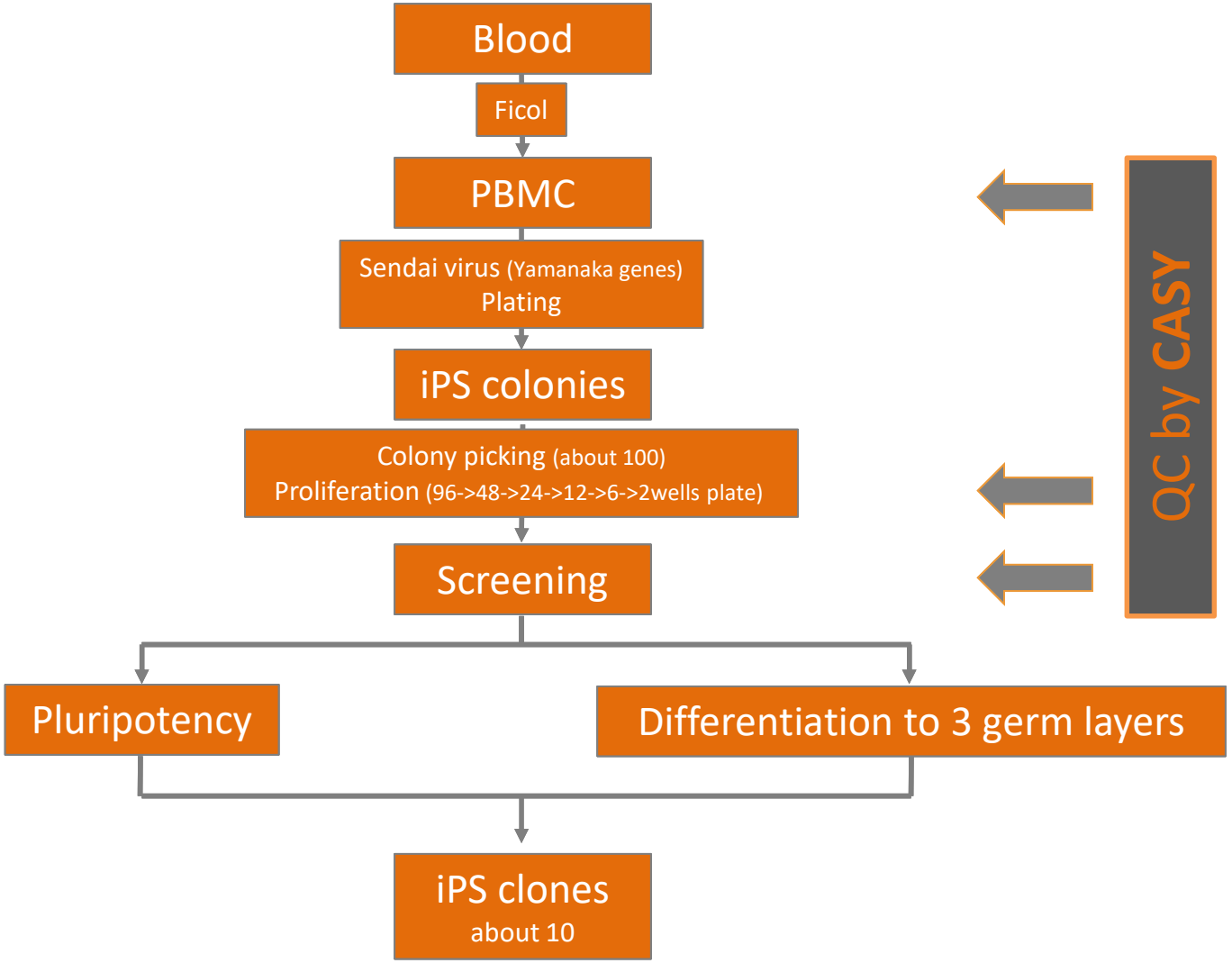
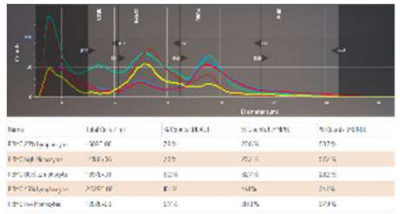
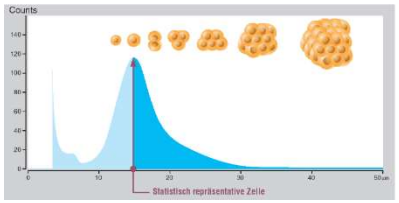


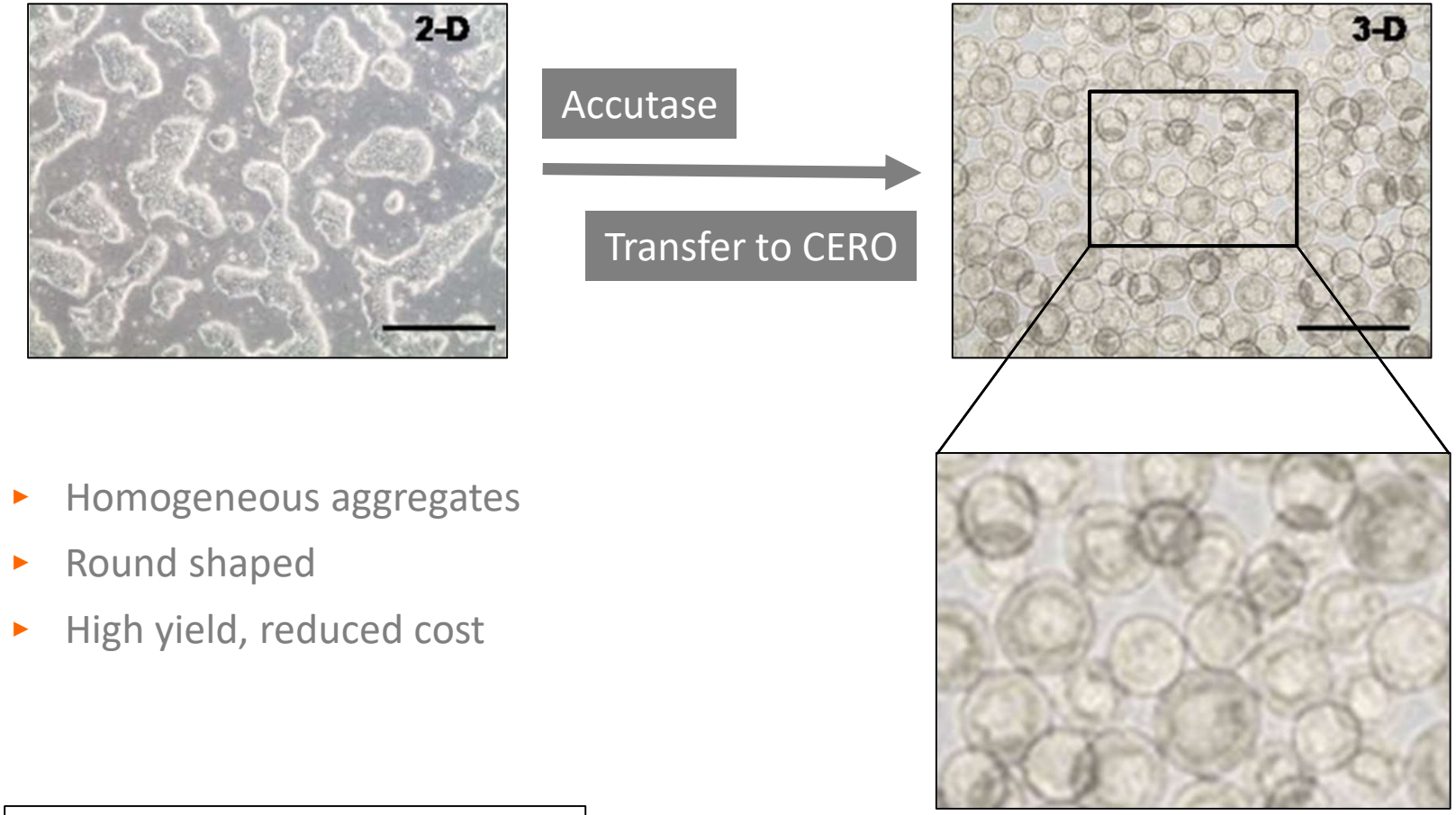
Preparation of human iPS cells







human iPS cells in CERO – Expansion



- Homogeneous aggregates
- Round shaped
- High yield, reduced cost

Elanzew, et. al.; A reproducible and versatile system for the dynamic expansion of human pluripotent stem cells in suspension; Biotechnol. J. 2015, 10, 1589–1599

hiPSCs from CERO - Cell QC

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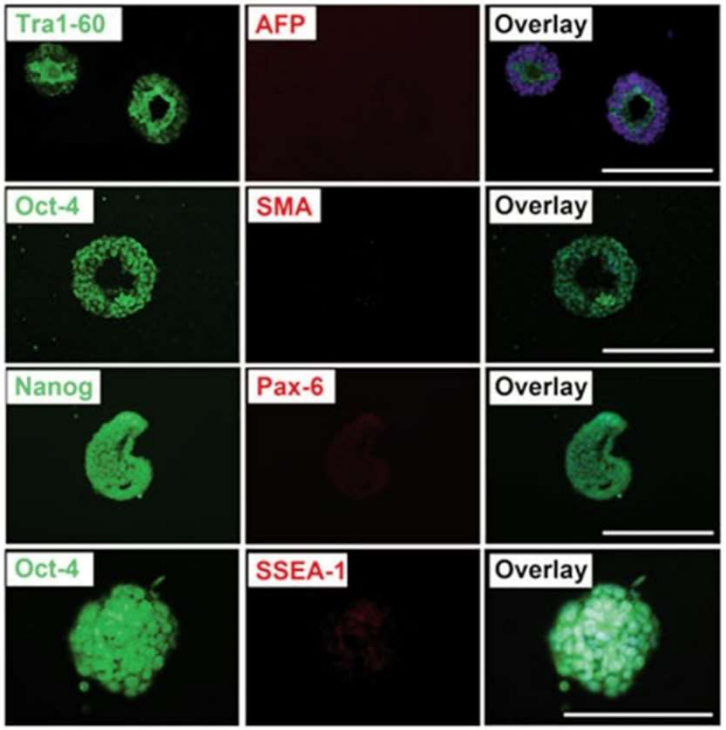
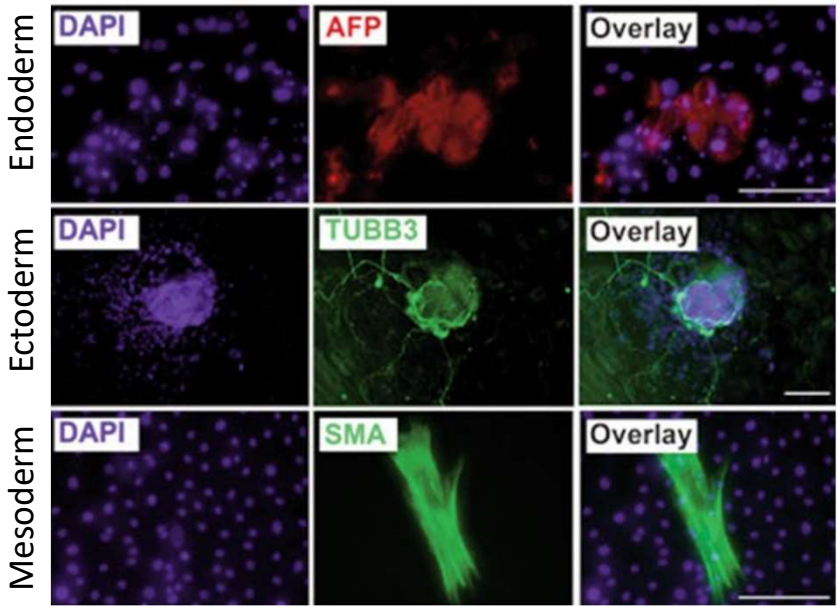
Pluripotency vs. Differentiation



- Maintain pluripotency over many passages
- No microcarriers
- Improved viability

Differentiation in germ layers



- Able to differentiate in 3 germ layers

Elanzew, et. al.; A reproducible and versatile system for the dynamic expansion of human pluripotent stem cells in suspension; Biotechnol. J. 2015, 10, 1589–1599

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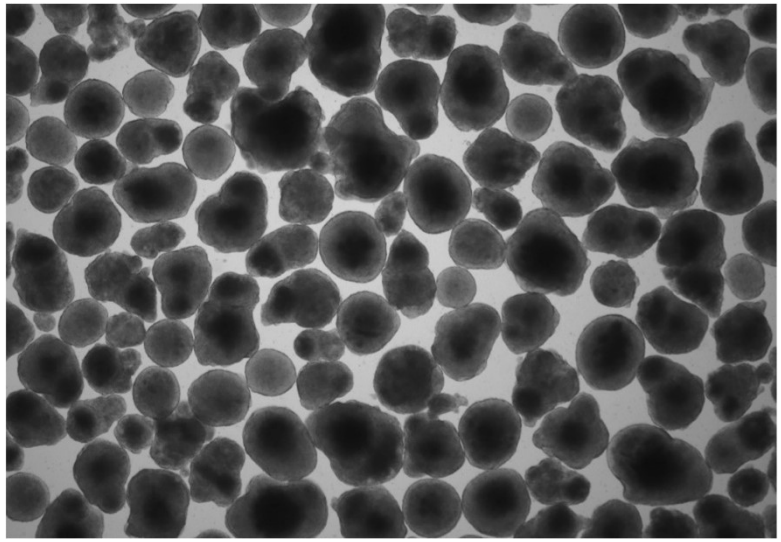
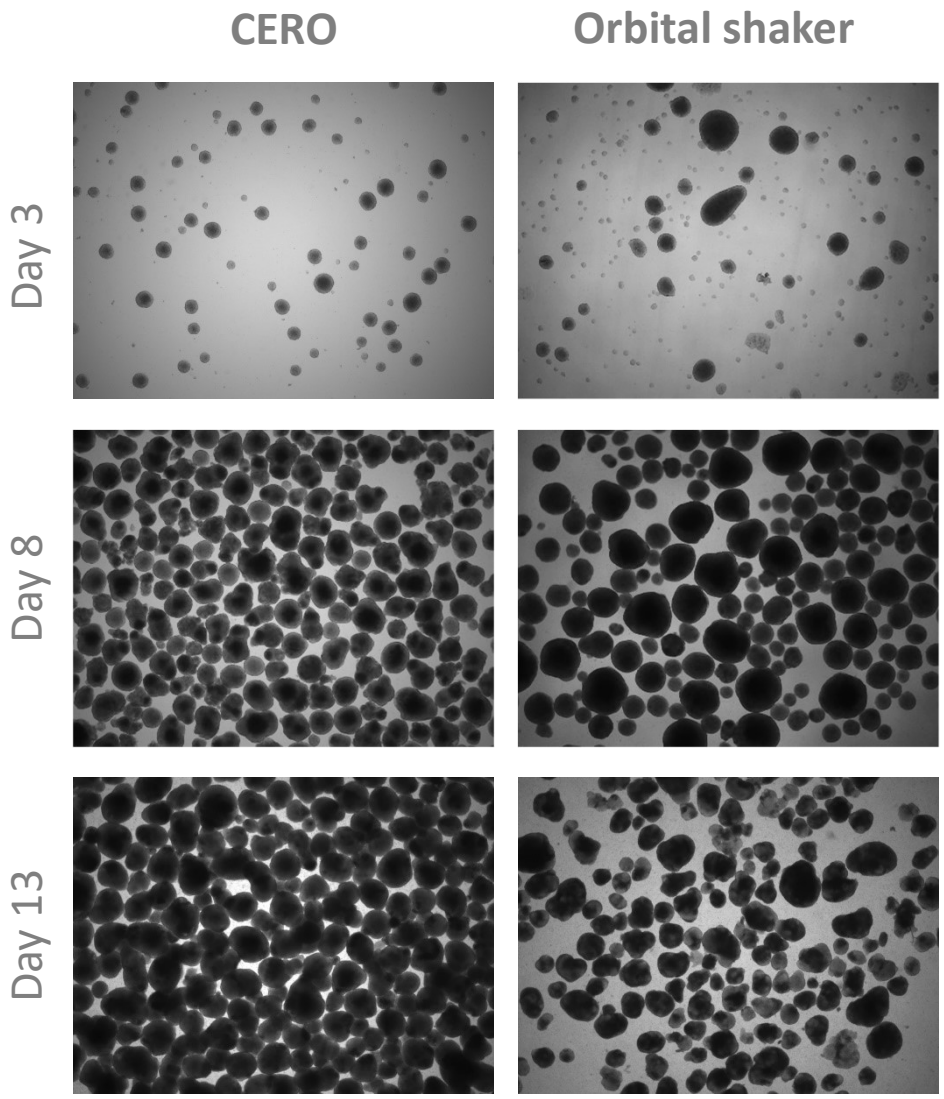


Cardiospheres - Mouse ESC expansion & differentiation



- Induction of Differentiation
- High density of homogeneous Cardiac bodies in CERO

CERO - high yield and maximum homogeneity of Cardiac bodies

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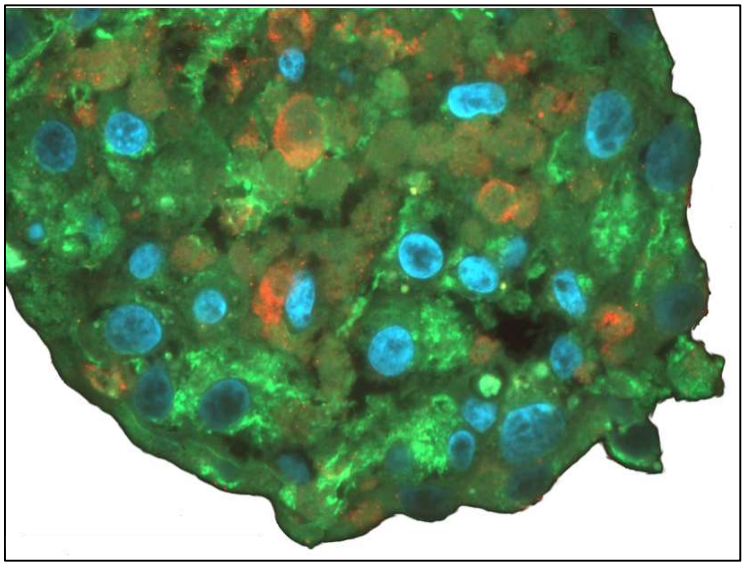
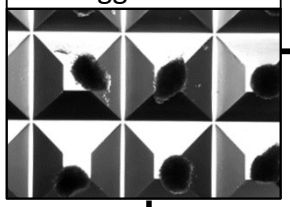
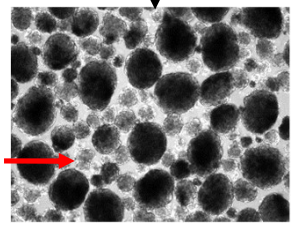


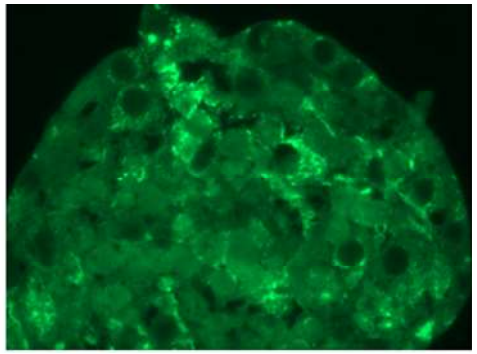
Spheroids – Example with HepG2

with CERO		without CERO
Aggrewell 	Prediff. Cells & Cell lines	Low Attachment Plate
 ECM		
Homogeneous **(~ 1000 per tube)**	Aggregates	Heterogeneous **(~96 /plate)**
minimum 80 days	Survival time	maximum 25 days

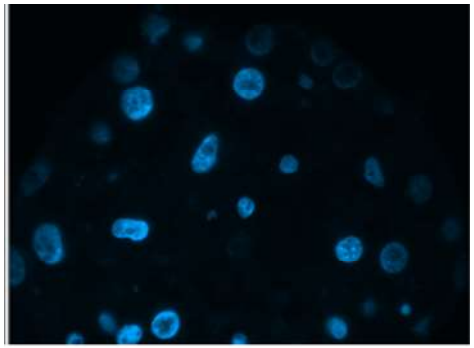
AG Heikenwälder, DKFZ Heidelberg, Germany: Establishment and validation of the 3D cultivation system “CERO” for hepatic spheroids to study viral hepatitis

Spheroids from HepaRG cells after > 80 days in CERO

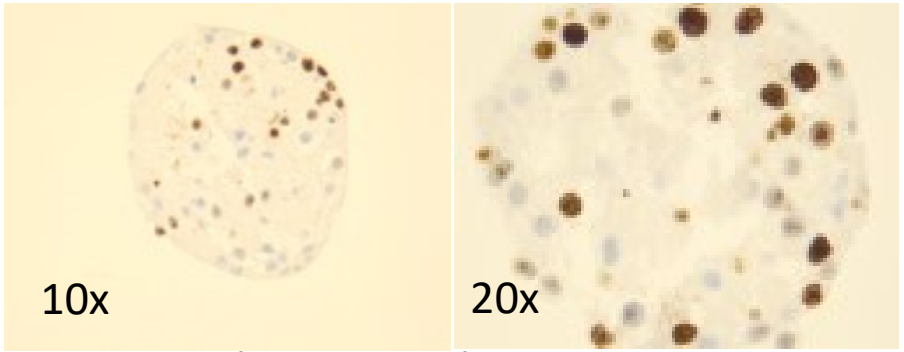
Albumin: 100% Differentiation



DAPI

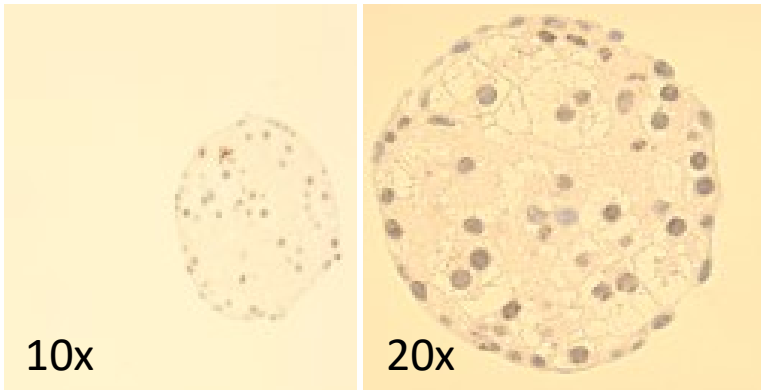


Cell proliferation marker (KI67): Proliferation ongoing



Light blue staining of DAPI plus brown of Ki67

Apoptosis marker (Casp.cl.3) : No Apoptosis

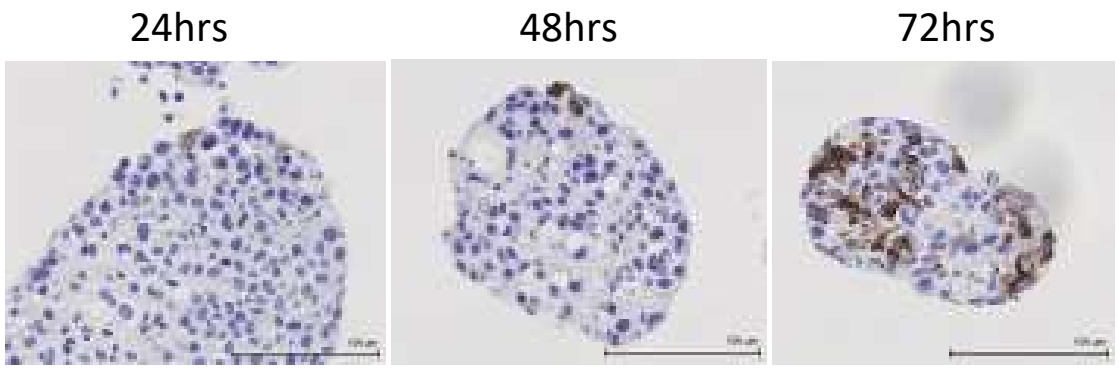


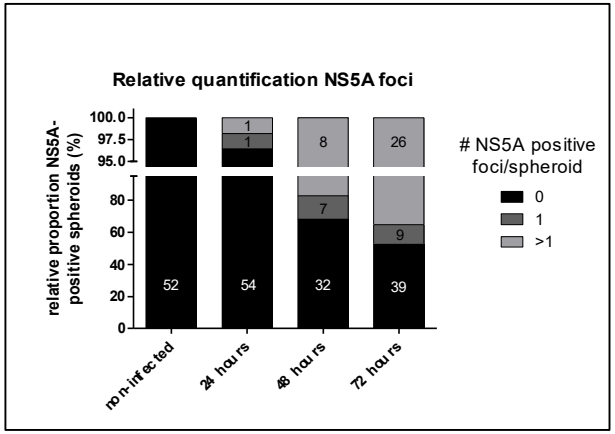
Only light blue DAPI staining

AG Heikenwälder, DKFZ Heidelberg, Germany: Establishment and validation of the 3D cultivation system "CERO" for hepatic spheroids to study viral hepatitis

CERO – enables virus infection experiments

Tracking Hepatitis C virus (HCV) infection





- Infection requires maturation of spheroids for min 20 days

  (*in 2D, spheroids start degrading after 15 to 20 days*)
- After maturation spheroids were infected with 1000 infectious particles
- Degree of infection was tracked after 24, 48 and 72 hrs. (brown areas)

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CERO – enabling human pathogenic infection studies

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Better, cheaper, faster and more homogeneous Organoids

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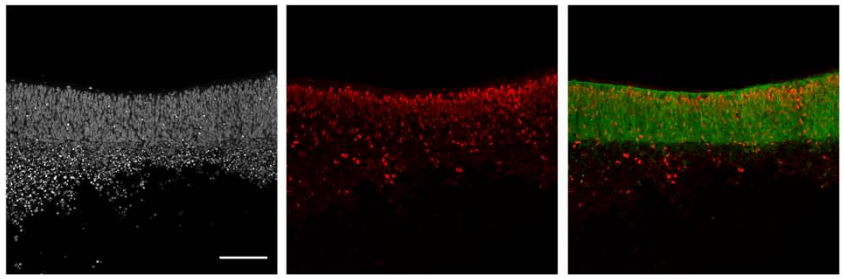
	with CERO	without CERO
Aggregates formation	**Autoadhesion**	**Low Attachement Plates**
BioMass	~ **1000 organoids** per tube	~**10-20 organoids** per well
Media consumption (per 1000 organoids)	**~30ml** every **3-7 days * 2 min**	**~300ml** every **2 days * x hours**
Conditions	**Homogeneous** - for all organoids	**Heterogeneous** - between wells & organoids
Matrigel	**NO**	**YES**
Size & Cultivation up to	**700µm and more** One year or more	**700µm max** Several months

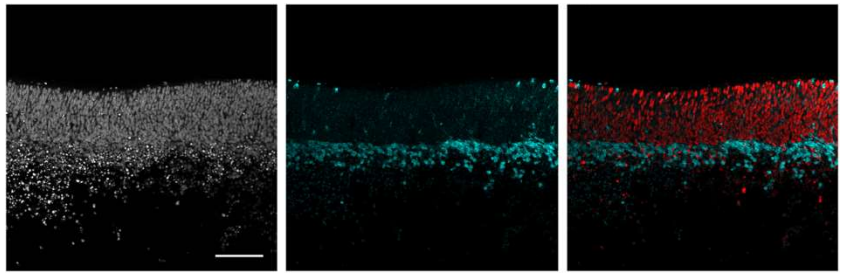
CERO – Retina Organoids

Organoids from mouse ESC (EB5 cell line)

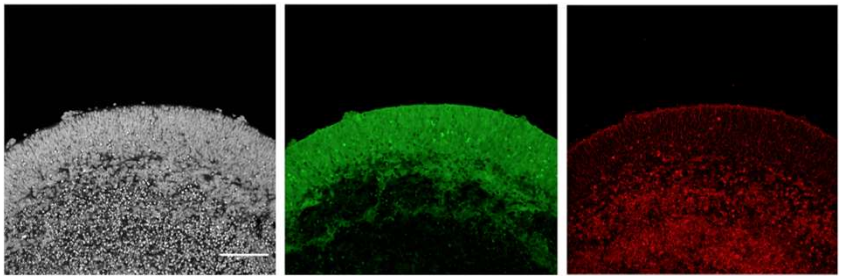
Photoreceptor and bipolar cells (day 18; **Otx2** / **GFP**)



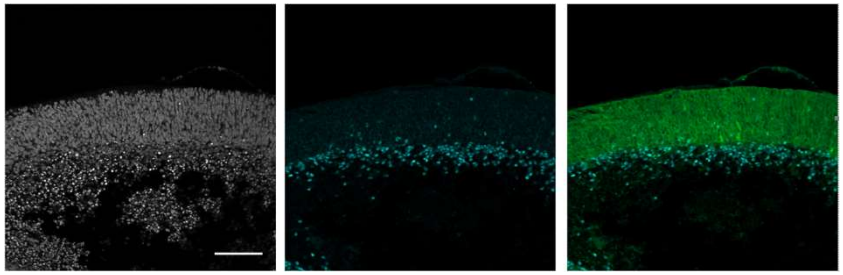
Amacrine and retinal ganglion cells (day 18; **HuC/D** / **Sox2**)



Retinal ganglion cells (day 18; **GFP** / **Brn3b**)



Amacrine and retinal ganglion cells (day 18; **Isl1/2** / **GFP**)



CERO – easy handling / fast differentiation / improved polarity

Thank you for your attention

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