Modelling of Animal Cells for Research

-Hepatocytes-

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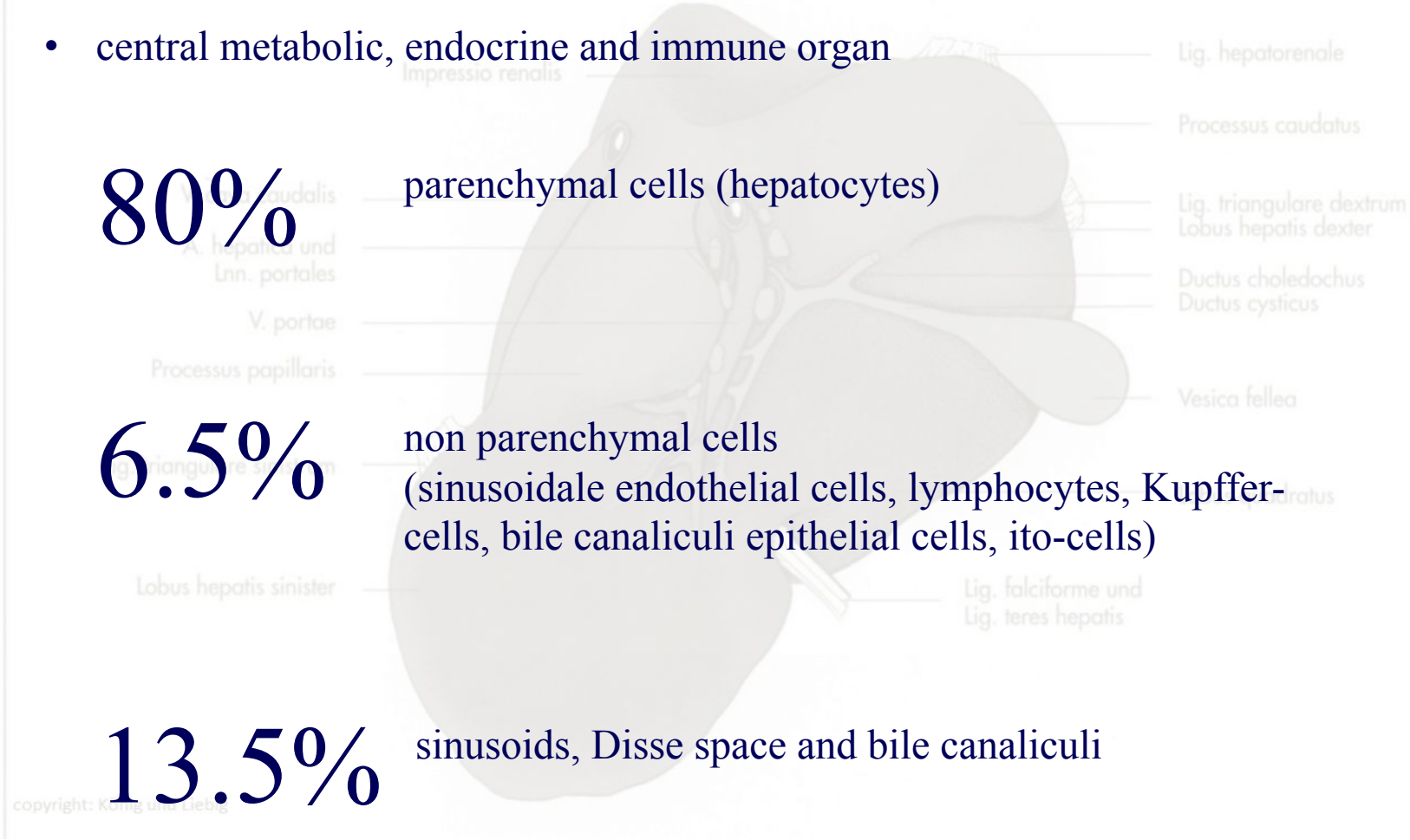
Liver

- central metabolic, endocrine and immune organ

80% parenchymal cells (hepatocytes)

6.5% non parenchymal cells
(sinusoidale endothelial cells, lymphocytes, Kupffer-cells, bile canaliculi epithelial cells, ito-cells)

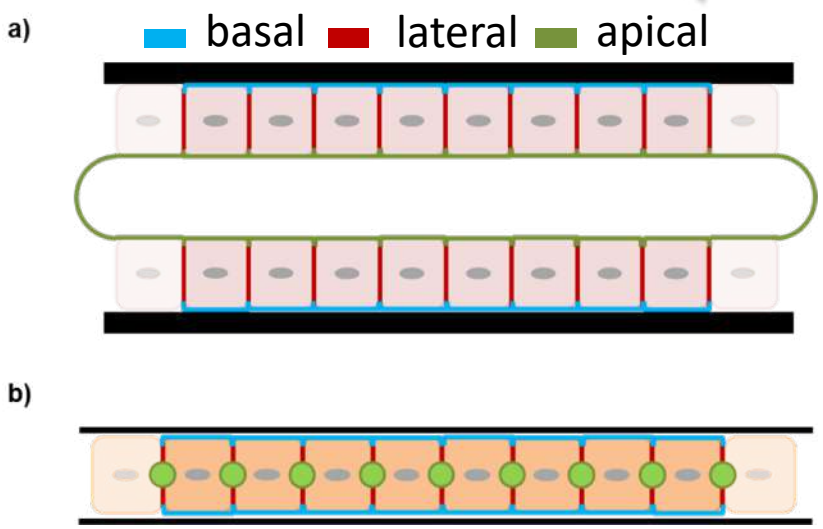
13.5% sinusoids, Disse space and bile canaliculi

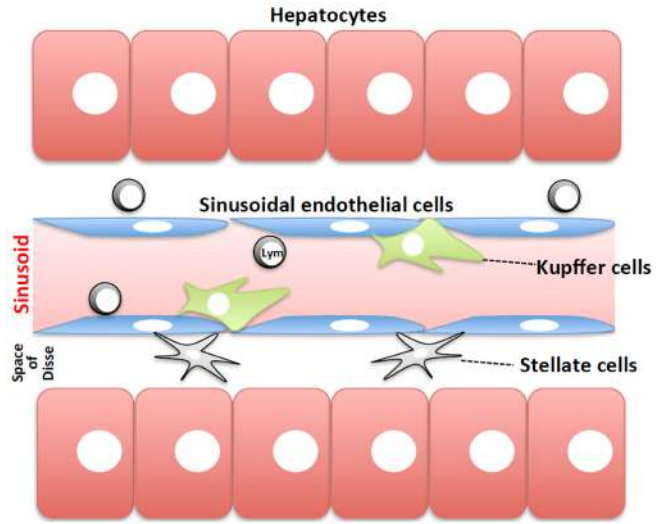


Modelling of hepatocytes

Parenchymal cells

Non parenchymal cells





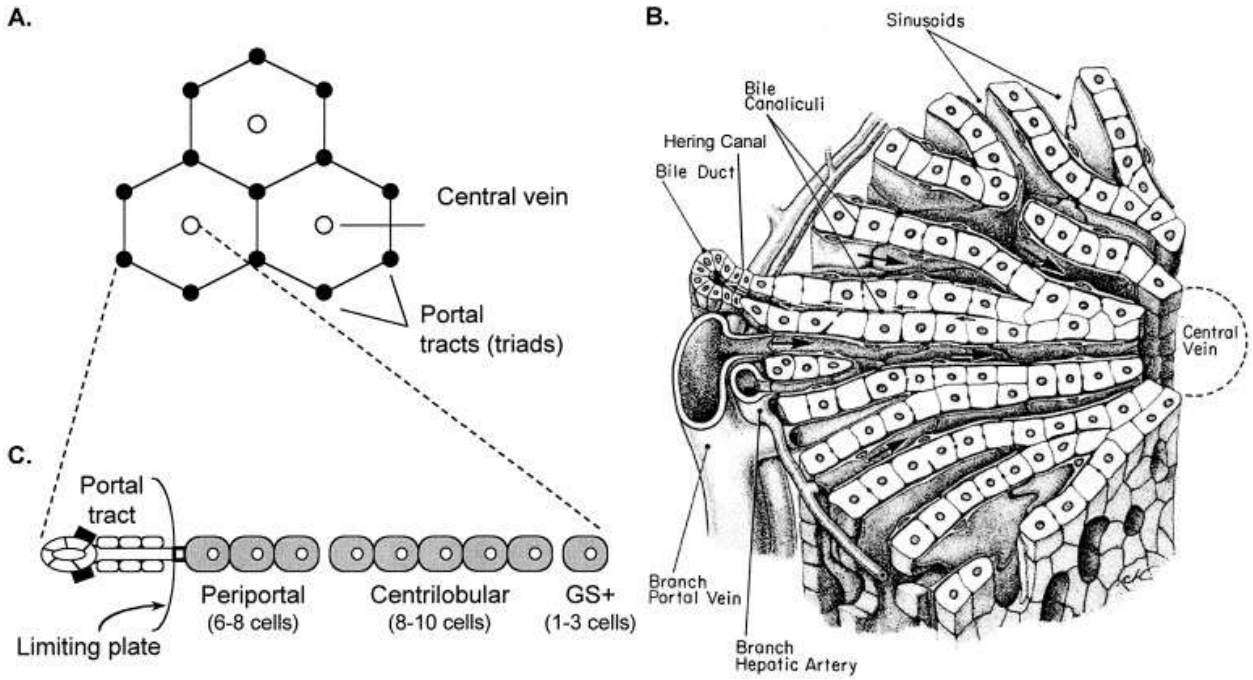
a) epithelial cells
b) hepatocytes (single layer, 2 basal sides with contact to the Disse space; apical sides → bile canaliculi)

Treyer und Müsch 2013, Barnes et al. 2014, Tsutzi and Nishiguchi 2014

Modelling of hepatocytes

periportal vs. periportal

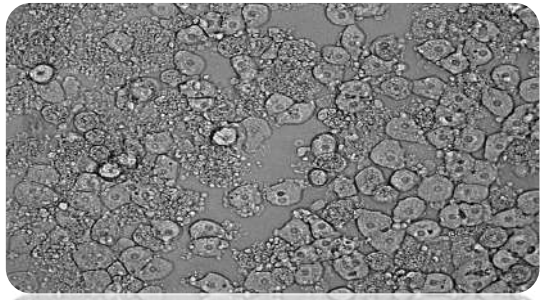
- periportal hepatocytes → ↑enzymes involved in **glycogen degradation** and **gluconeogenesis**
- perivenular hepatocytes → ↑ content of enzymes involved in **glycolysis** and **glycogen synthesis**



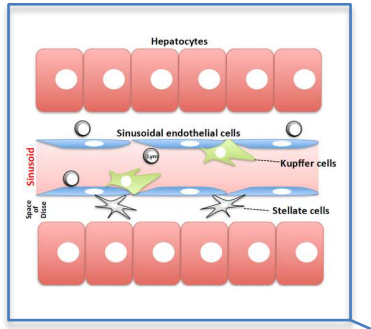
Hepatocyte ≠ Hepatocyte

Options of Modelling Hepatocytes for Research

Monoculture

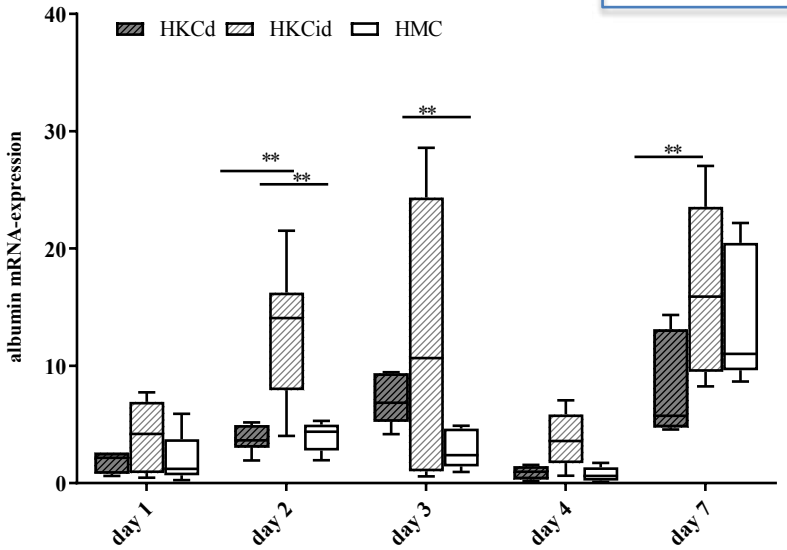


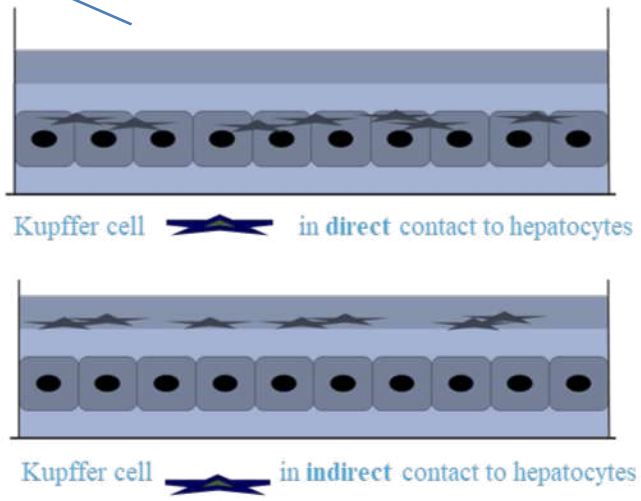
primary bovine hepatocytes in monoculture



Co-Culture

primary bovine hepatocytes in co-culture with Kupffer cells (fluorescence labeled phagocyted latex beads)

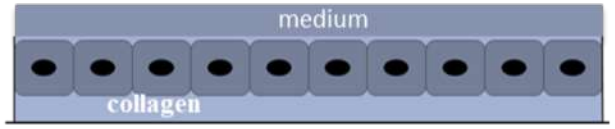




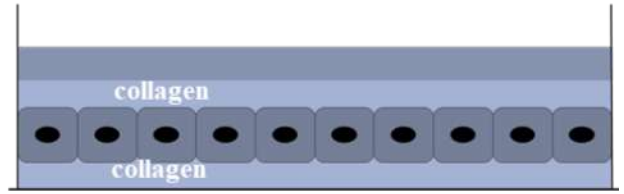
Witte et al. 2017, Fischbach et al. 2019

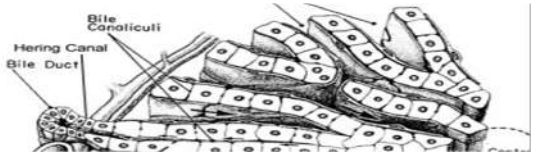
Options of Modelling Hepatocytes for Research

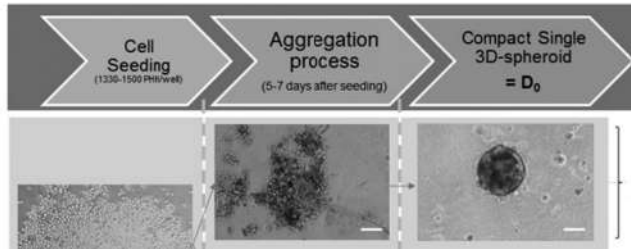
- Monolayer
- Sandwich
- 3DCulture Organoids
- 3D hepatic models Organ-on-a-chip

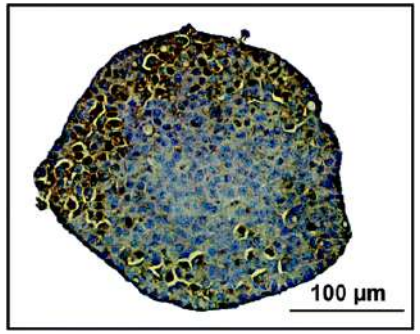


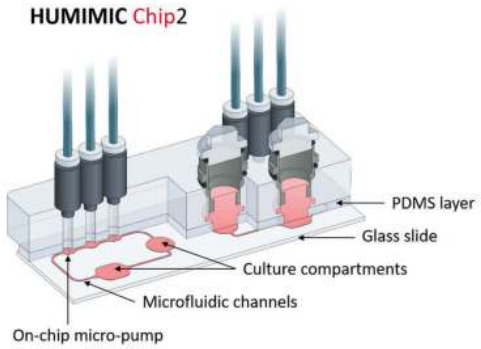
collagen = main protein of extracellular matrix











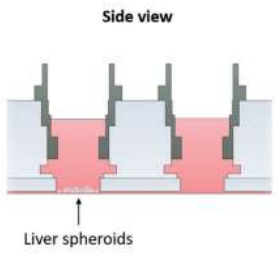
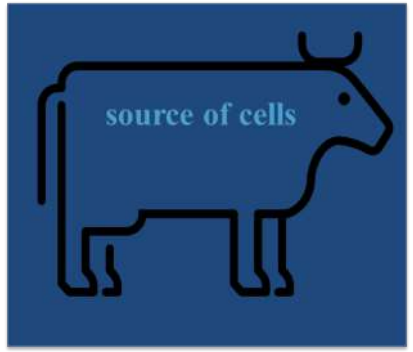


FIGURE 1 Scheme of the experimental setup of the HUMIMIC Chip2® (copyright by TissUse GmbH). The actual chip consists of three layers: a standard microscopic glass slide, a polydimethylsiloxane (PDMS) layer with channels and culture compartments and an adapter plate made of polycarbonate [Colour figure can be viewed at wileyonlinelibrary.com]

Gaskell et al. 2016, Baze et al. 2018, Görgens et al. 2021

Options of Modelling Hepatocytes for Research



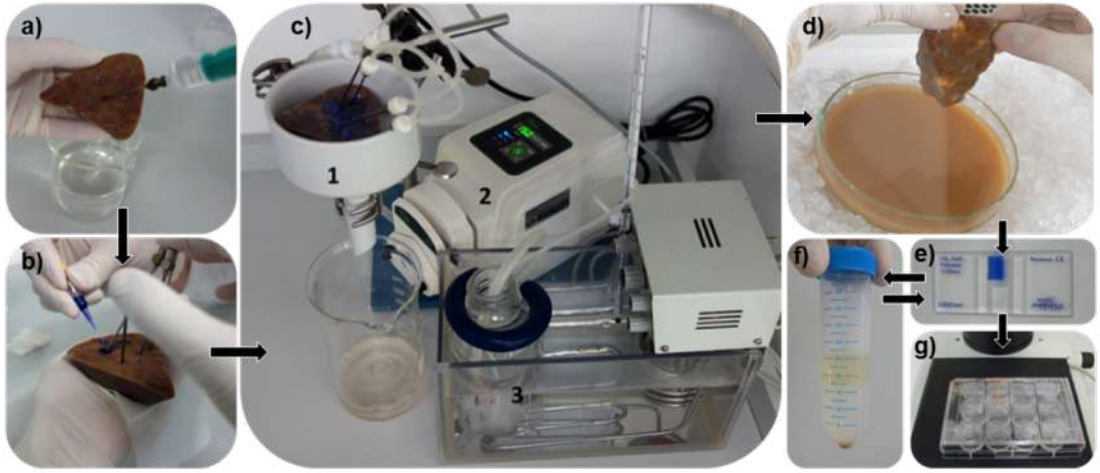
primary cells

-ex vivo culture of cells freshly obtained

- proliferation limited (growth arrest in a replicative senescence)

- 100 Mio cells →no protocol for cryopreservation of bovine hepatocytes

Two-step-collagenase digestions and subsequent percoll density gradient

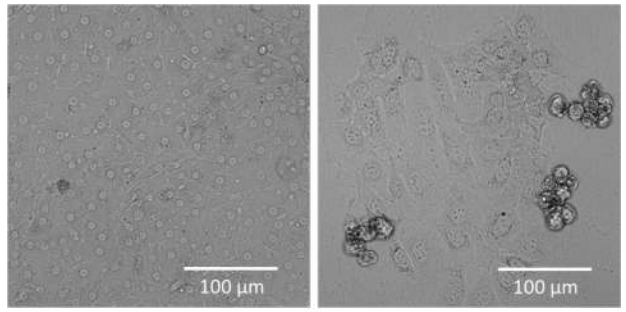


Ehrhardt and Schmicke 2012, Witte et al. 2019, Chong et al. 2021, Fischbach et al. 2018

Options of Modelling Hepatocytes for Research

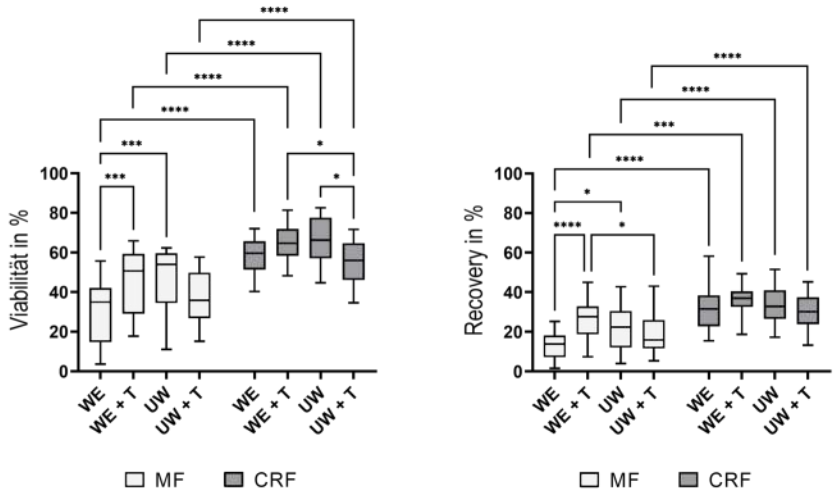
Cryopreservation of hepatocytes

- CRF is necessary prevent the formation of intracellular ice crystals and osmotic stress
- addition of trehalose (non-penetrating cryoprotective agent) increased viability of bovin hepatocytes compared to DMSO
- recovery rate of hepatocytes is low → downregulation of β1-integrin → less cell-matrix interactions on collagen



freshly isolated primary bovine hepatocytes

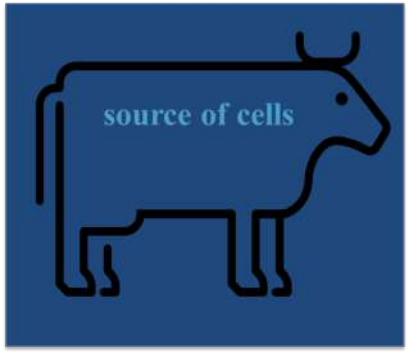
William's E Medium + Trehalose after CRF



MF= manual freezing, CRF= controlled rate freezing

Andres et al. 2023, BovReg WP1, Task 1.1

Options of Modelling Hepatocytes for Research

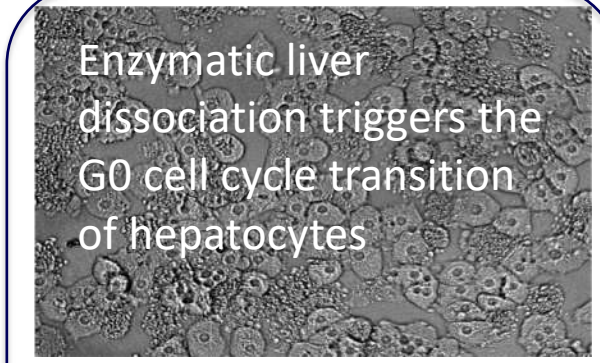


primary cells

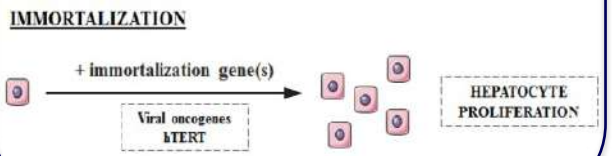
Primary cell culture is the ex vivo culture of cells freshly obtained from a multicellular organism.

cell line immortalized

Immortalized cell lines → cells that have been manipulated to proliferate indefinitely → cultured for long periods of time



Immortalization genes (SV40 or hTERT) can be artificially overexpressed in hepatocytes → leading to controlled cell proliferation

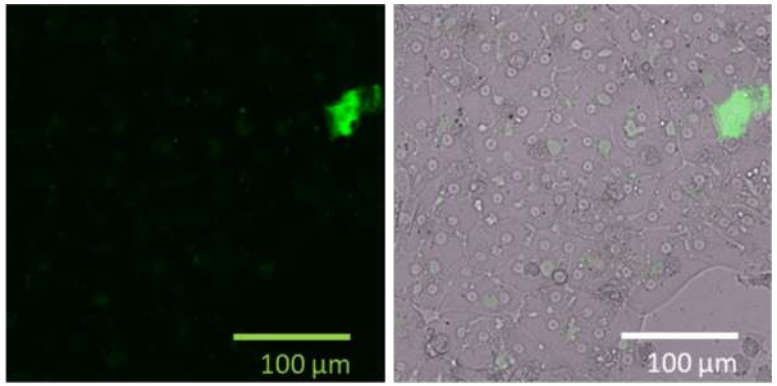


Ramboer et al. 2014

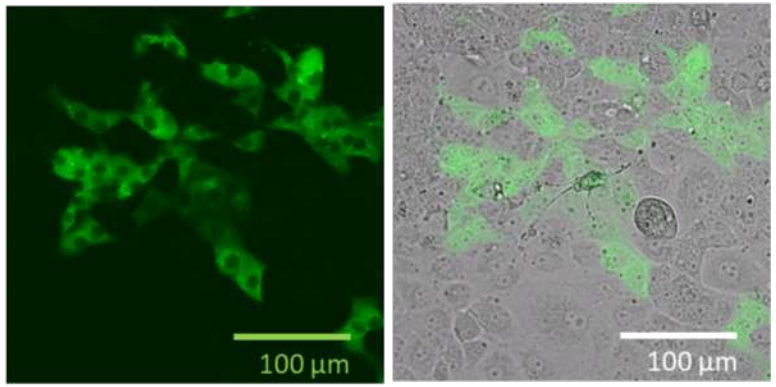
Options of Modelling Hepatocytes for Research

Lipo-transfection of hTERT

- primary bovine hepatocytes were successfully transfected with the **Lipofectamine® 2000 reagent** (protocols established)
- the single transfection of **hTERT** did not lead to unlimited cell division in bovine hepatocytes
- additional growth factors and/or transfection of oncogenes are necessary



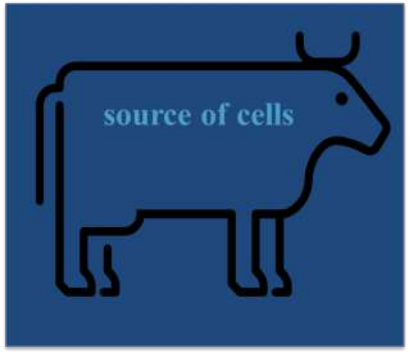
Bovine hepatocytes after transfection hTERT



Madin-Darby bovine kidney-cells, transfection control

Andres 2021, BovReg WP1, Task 1.1

Options of Modelling Hepatocytes for Research



primary cells

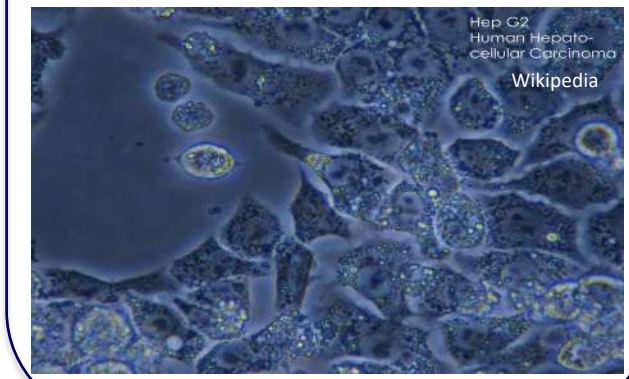
Primary cell culture is the ex vivo culture of cells freshly obtained from a multicellular organism.

cell line immortalized

Immortalized cell lines are cells that have been manipulated to proliferate indefinitely and can thus be cultured for long periods of time.

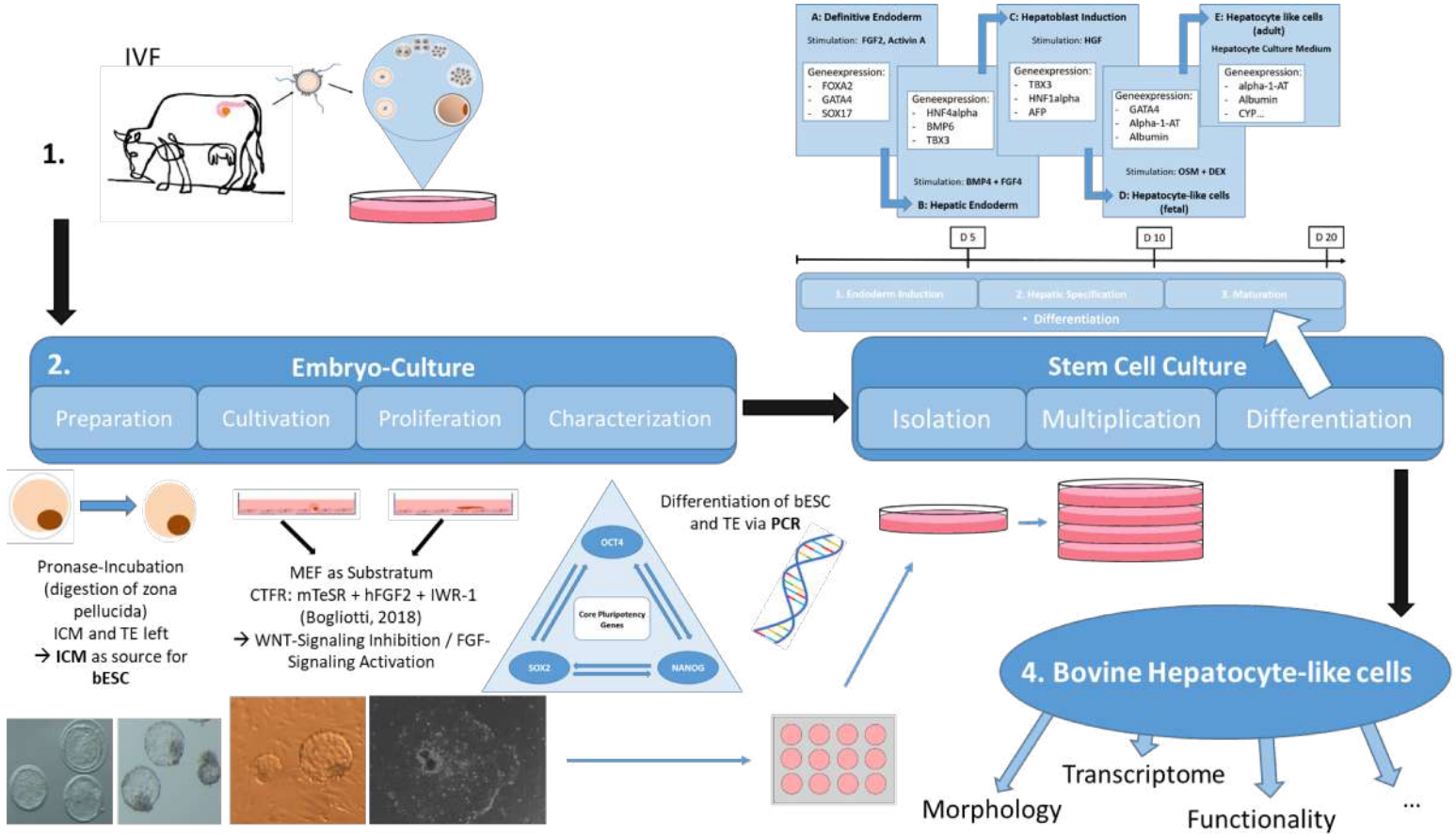
tumor originated cell line

e.g. HepG2 cells isolated from a hepatocellular carcinoma of a 15-year-old male with liver cancer in 1975; applicable in 3D cell culture



Chong et al. 2021, Fischbach et al. 2018

Options of Modelling Hepatocytes for Research



BovReg *PARTNERS*

Thank you for your attention

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