VITROCELLOMICS

FP6 EU STREP Project

Reducing Animal Experimentation in Preclinical Predictive Drug Testing by Human Hepatic *In Vitro* Models Derived from Embryonic Stem Cells

Start: 1 January 2006
Duration: 3 years
EC contribution: 2.942.000 Euro
Contractors: 9
Coordinator: Carl-Fredrik Mandenius,
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Karolinska Institute, Sweden
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Charité Berlin, Germany
Cellartis, Sweden
Pharmacelsus, Germany
AstraZeneca, Sweden
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Overall objectives

- **Replacing animals in preclinical pharmaceutical development by human cell culture systems**

- **Supporting the predictability of the drug discovery and development process by allowing more reliable and relevant testing in the preclinical phase and hinder weak lead candidates to enter clinical phases with innovative human hepatic cell systems**

- **Delivering an *in vitro* testing system with adjacent methodology pertinent for validation in GLP/SOPs environment for absorption, metabolism, and toxicity**

- **The ultimate aim is to reduce or even totally abolish the use of animals in drug testing, refine the model system under consideration and to replace the animals currently used**

Specific technology-related objectives

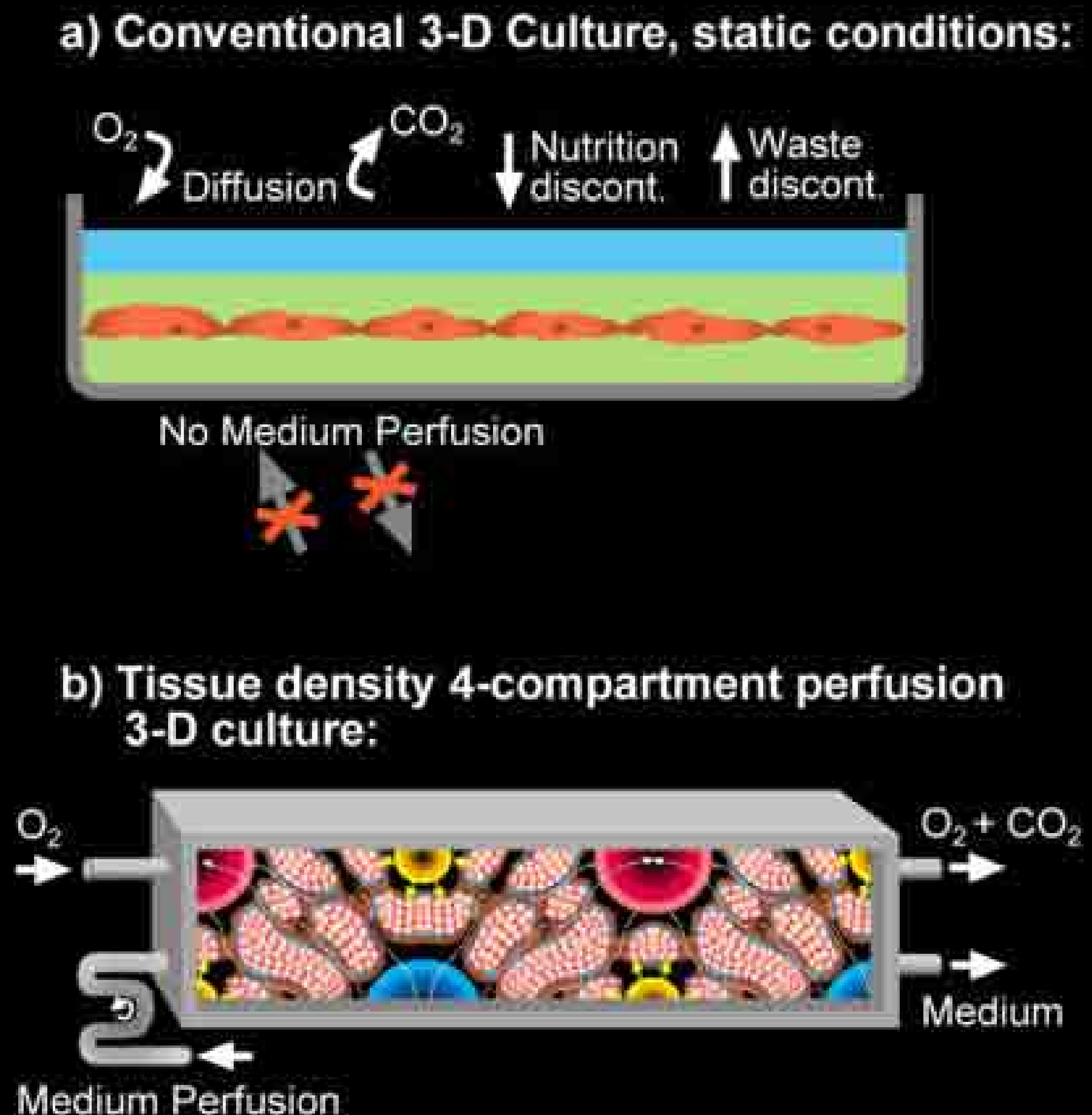
- **Establishing relevant hES cell derived hepatocyte cultures that allow a more predictable preclinical lead testing program to be carried out**

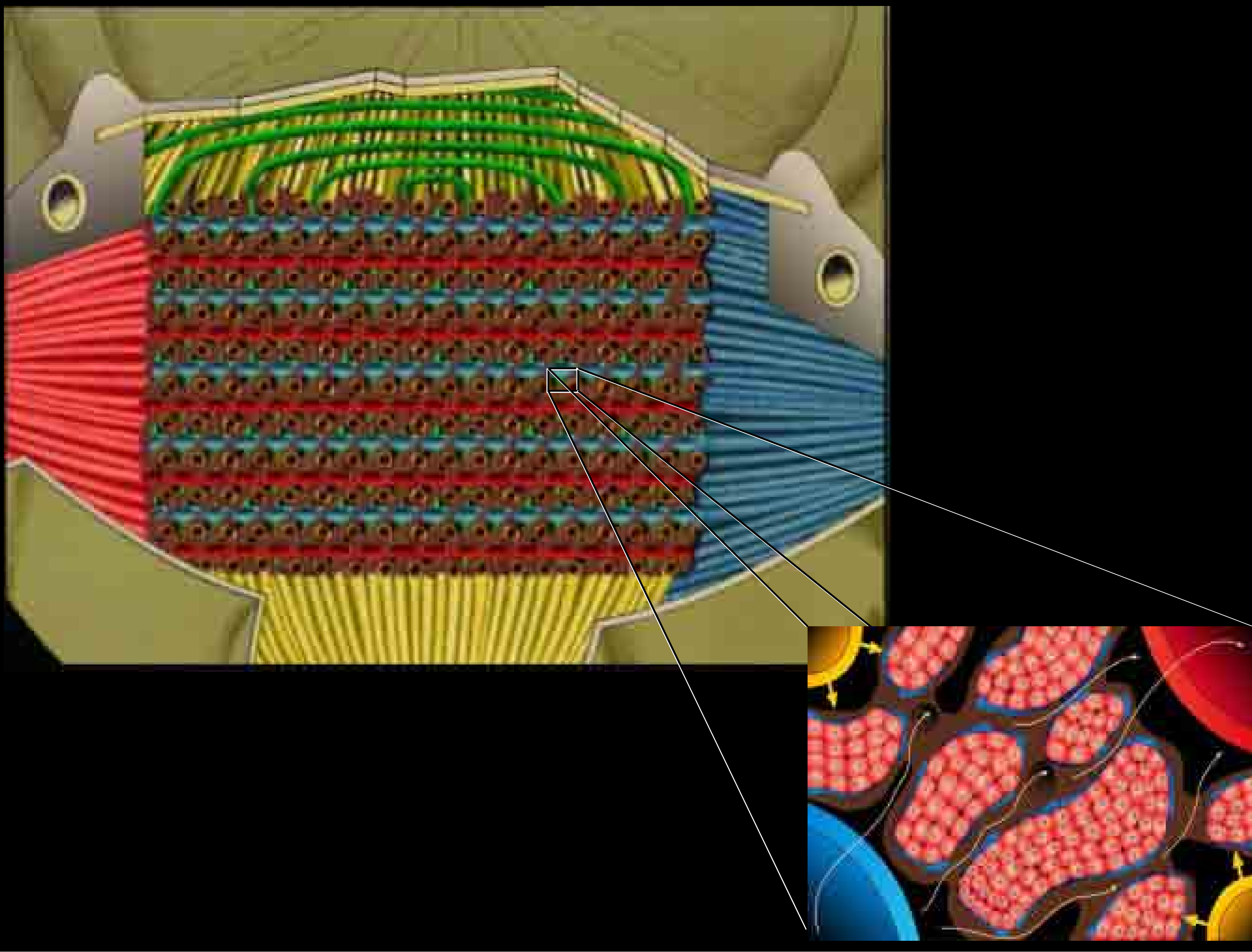
- **Development of a 3-dimensional *in vitro* model for long-term studies of drug metabolism and toxicology that mimics the *in vivo* tissue or organ cyto-architecture and function**

- **Establishing a versatile cell lab platform based on the developed cell lines and cultivated in advanced miniaturised bioreactor systems with non-invasive measurement techniques for *in vitro* testing of metabolism, toxicology and absorption**

- **To quantify metabolism by *in vitro* assay methods**

Bio-Engineering *in vitro* with fidelity to *in vivo* - *Stem cell tissue models for therapeutics*

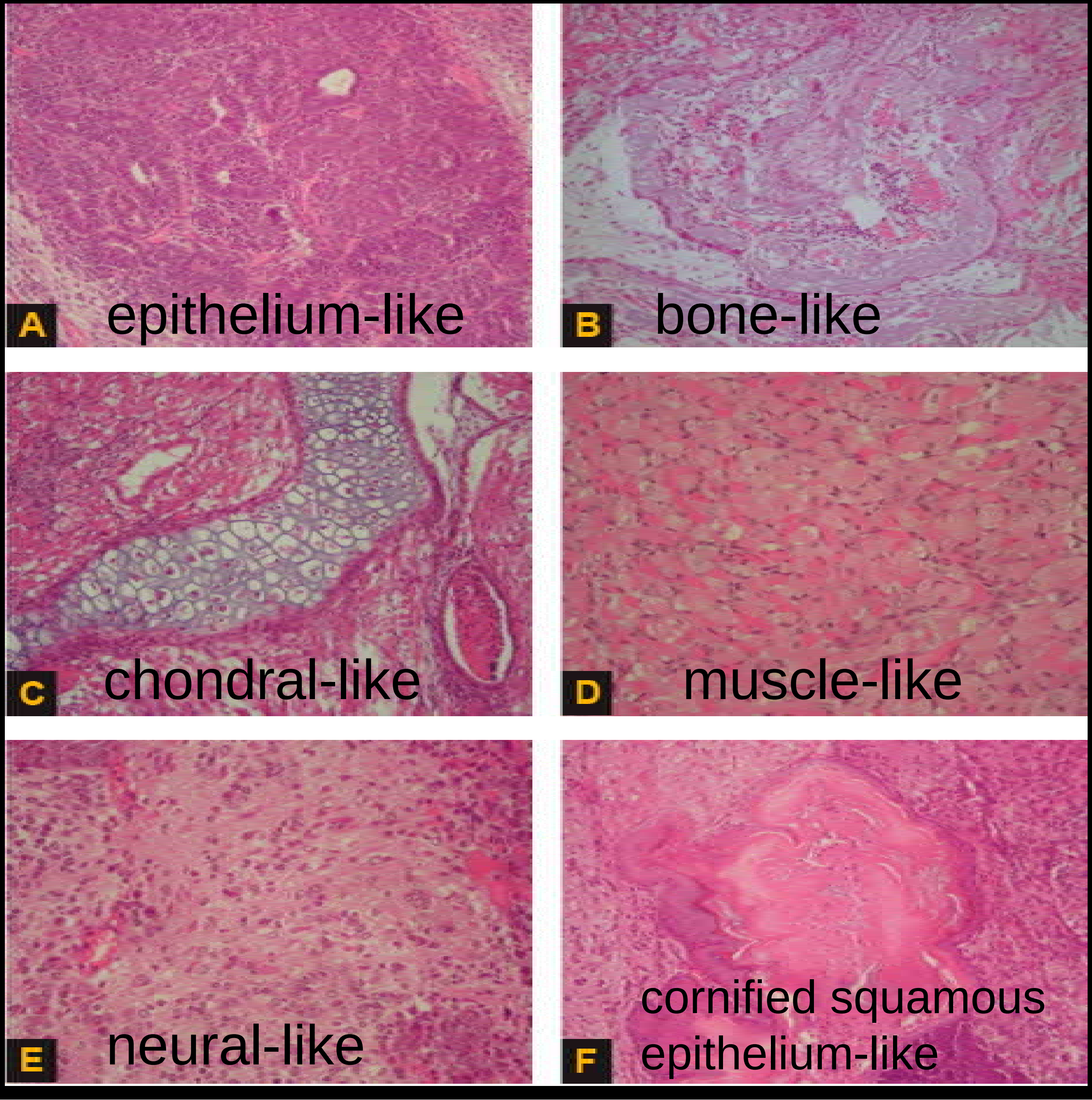




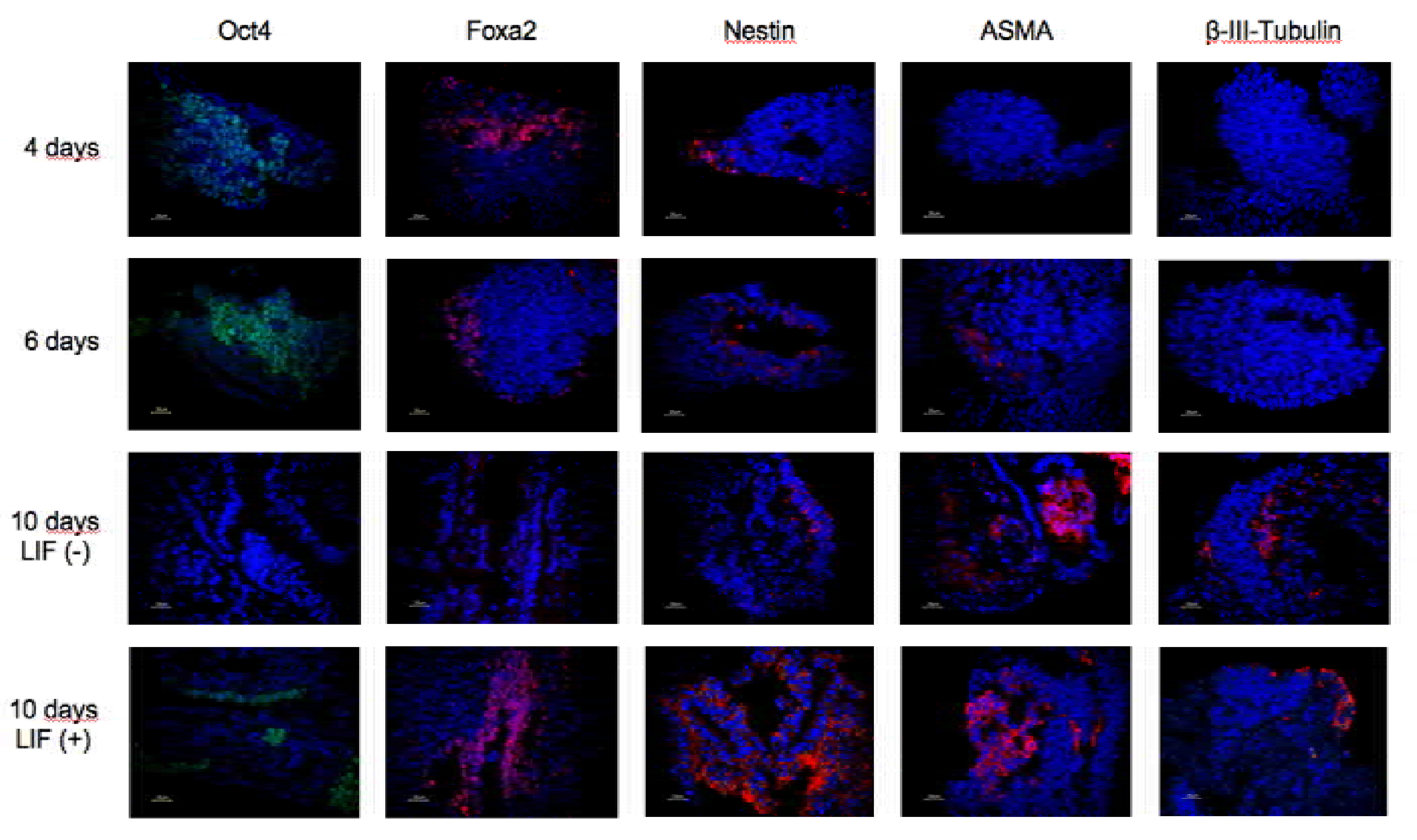
Mice embryonic stem cell expansion prior to differentiation - scale up technologies

10^7 inoculated mice ESC cells (129/SVEC) were expanded to 10^9 mESC within 10 days (+ feeder cells and LIF): hollow fibers of the bioreactor before (upper part) and after 10 days of mESC culture (lower). The embryonic stem cells completely fill the cell compartment of the bioreactor.

Expanded mESC can form teratomas



mESC spontaneous differentiation in vitro



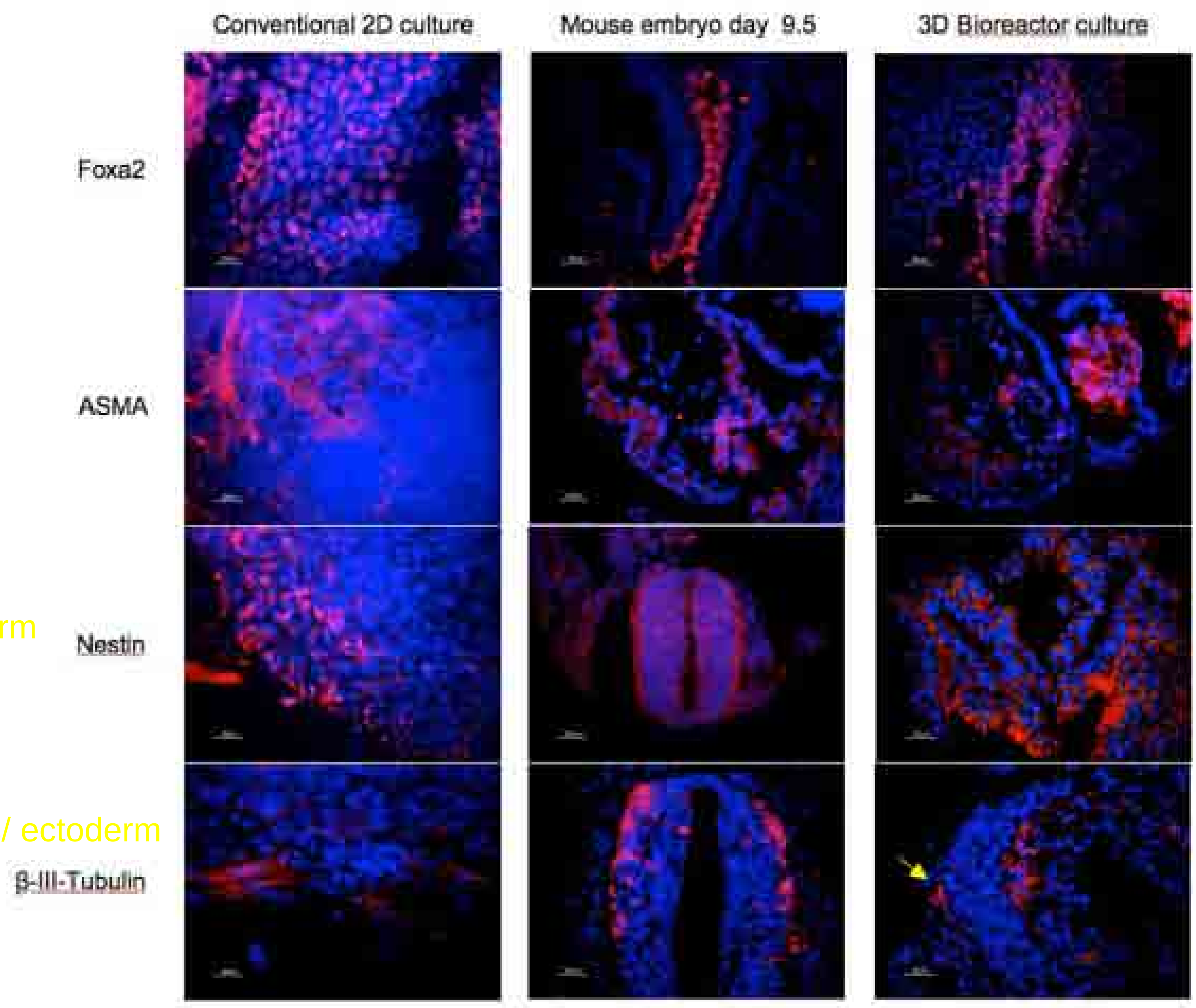
mESC differentiation in vivo / in vitro

gut tube/ endoderm

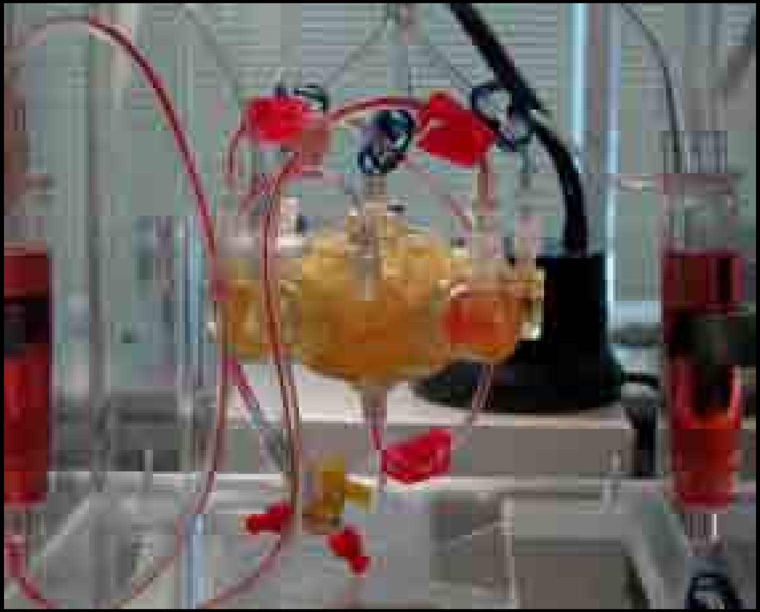
cardiac/ mesoderm

neural tube/ ectoderm

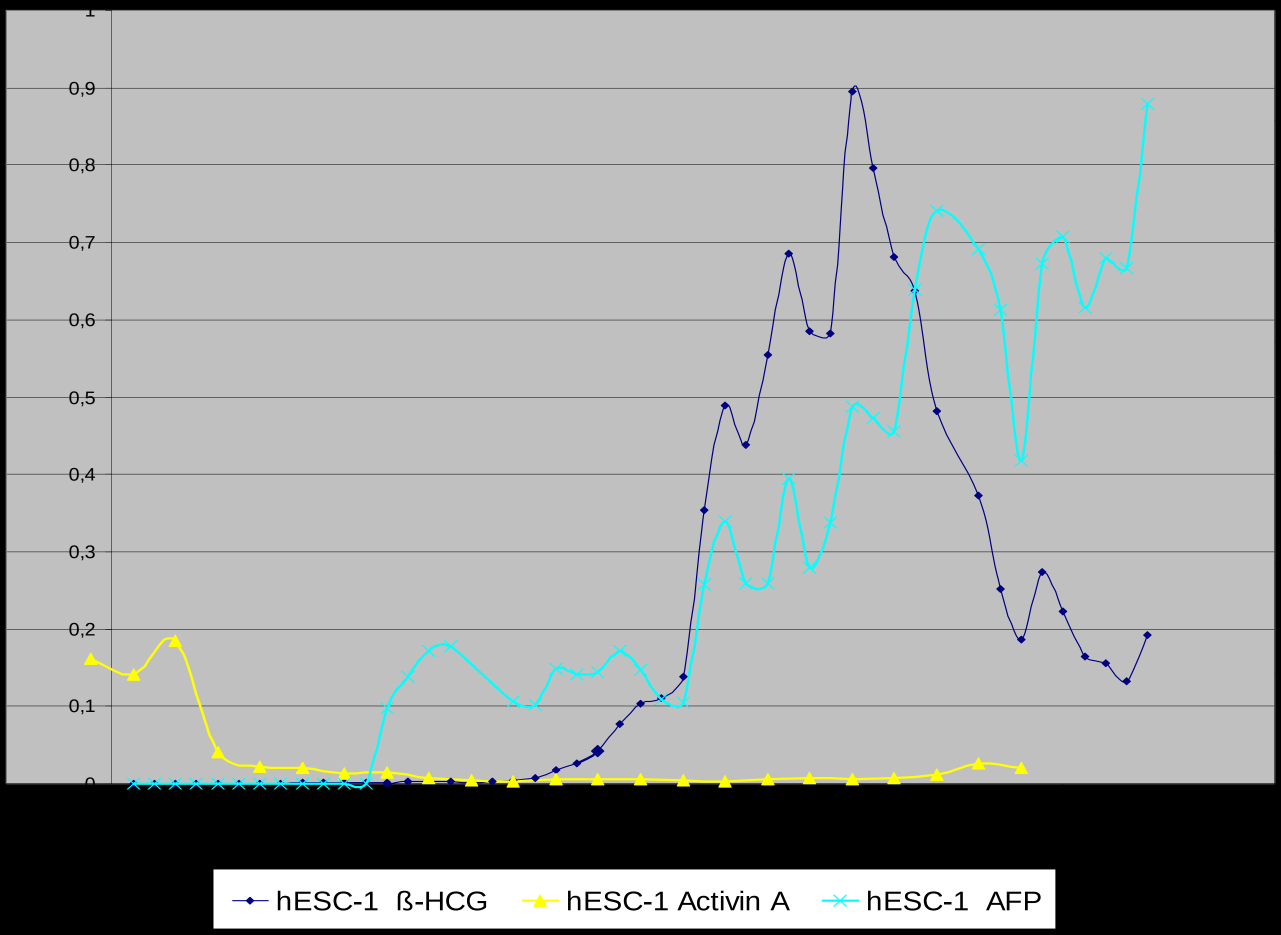
postmitotic neurons/ ectoderm



Spontaneous Differenitation of hESC in vitro

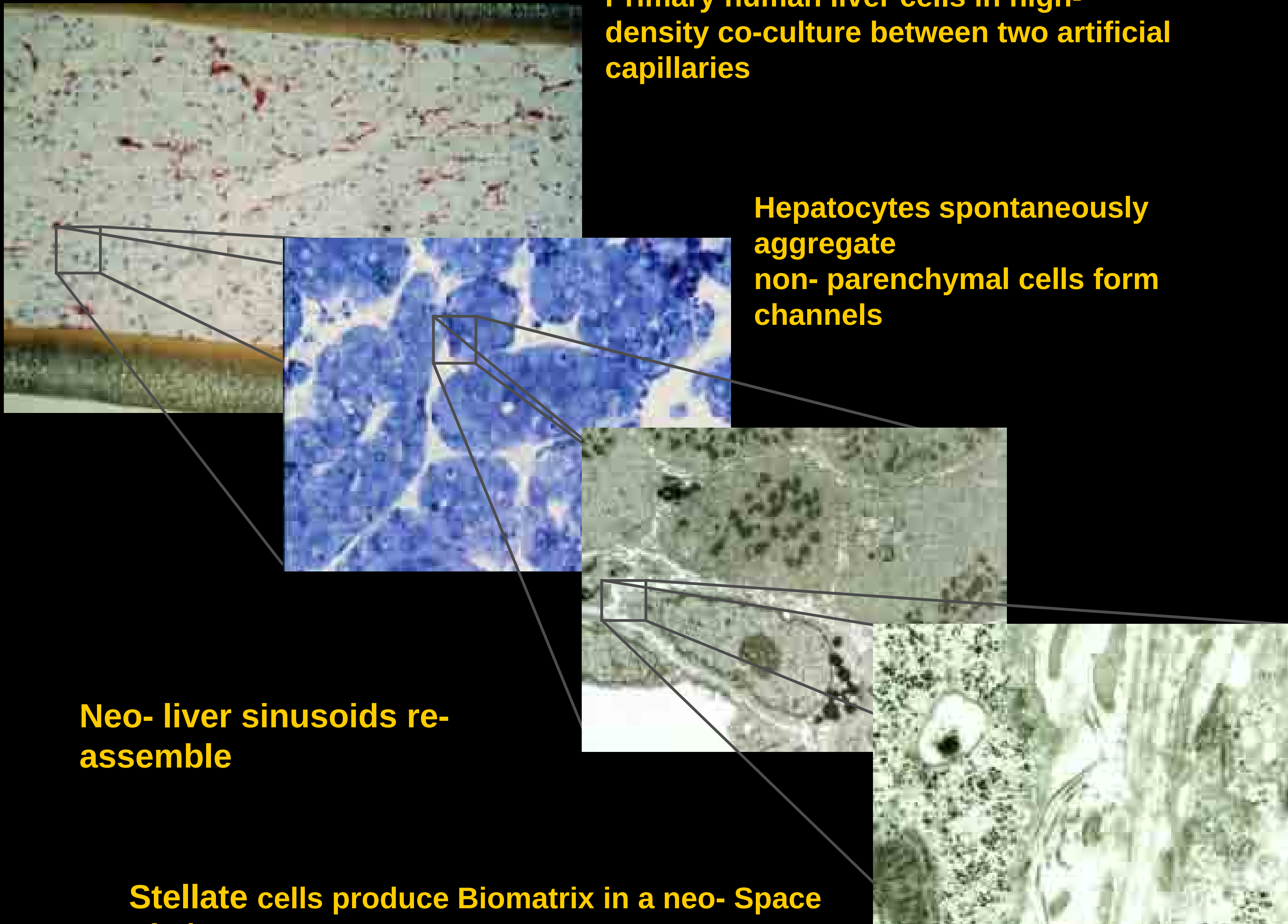


AFP, ß-HCG, Activin A release - SA 002



Tecnology developent hepatic cell applications

Primary human liver cells in high-density co-culture between two artificial capillaries



Hepatocytes spontaneously aggregate
non- parenchymal cells form channels

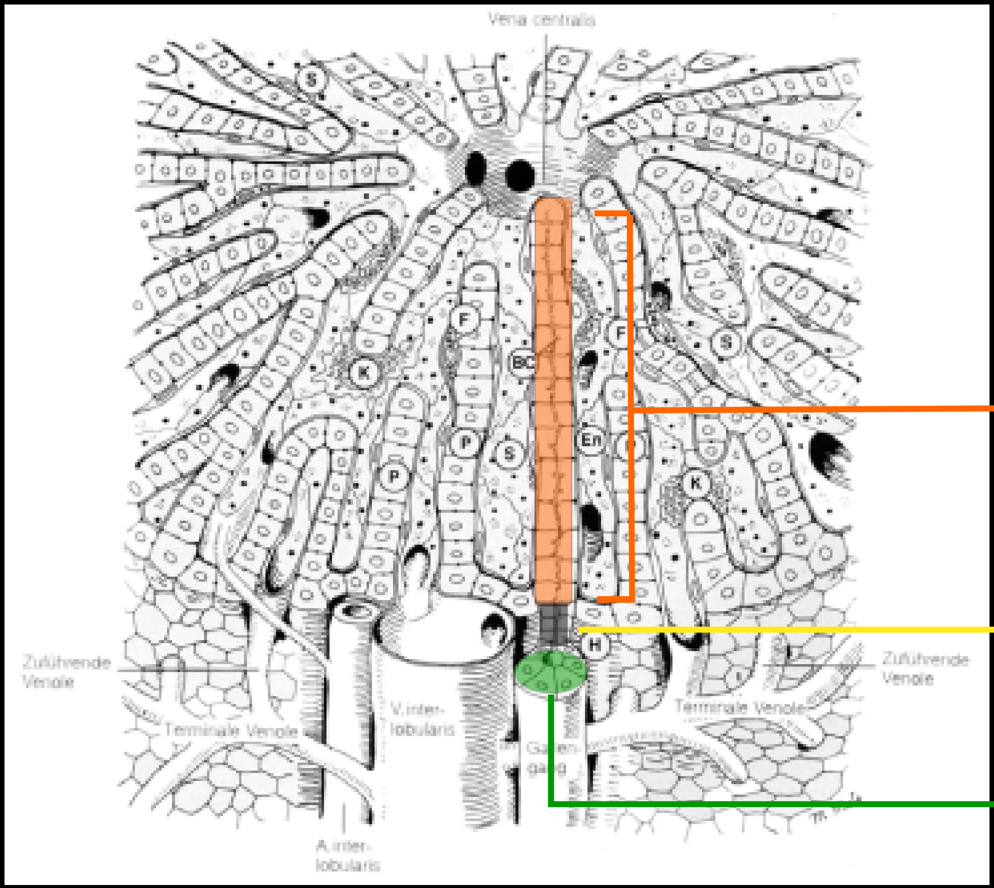
Neo- liver sinusoids re-assemble

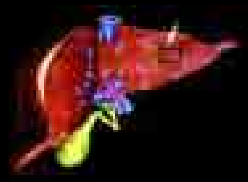
Stellate cells produce Biomatrix in a neo- Space of Disse

Gerlach et al.; J Invest Surg 2003, 16: 83-92

The stem cell niche and the importance of 3-D environment for *in vitro* progenitor cell research

Canals of Hering, bipotent hepatic progenitors





Liver lobe

Liver lobuli

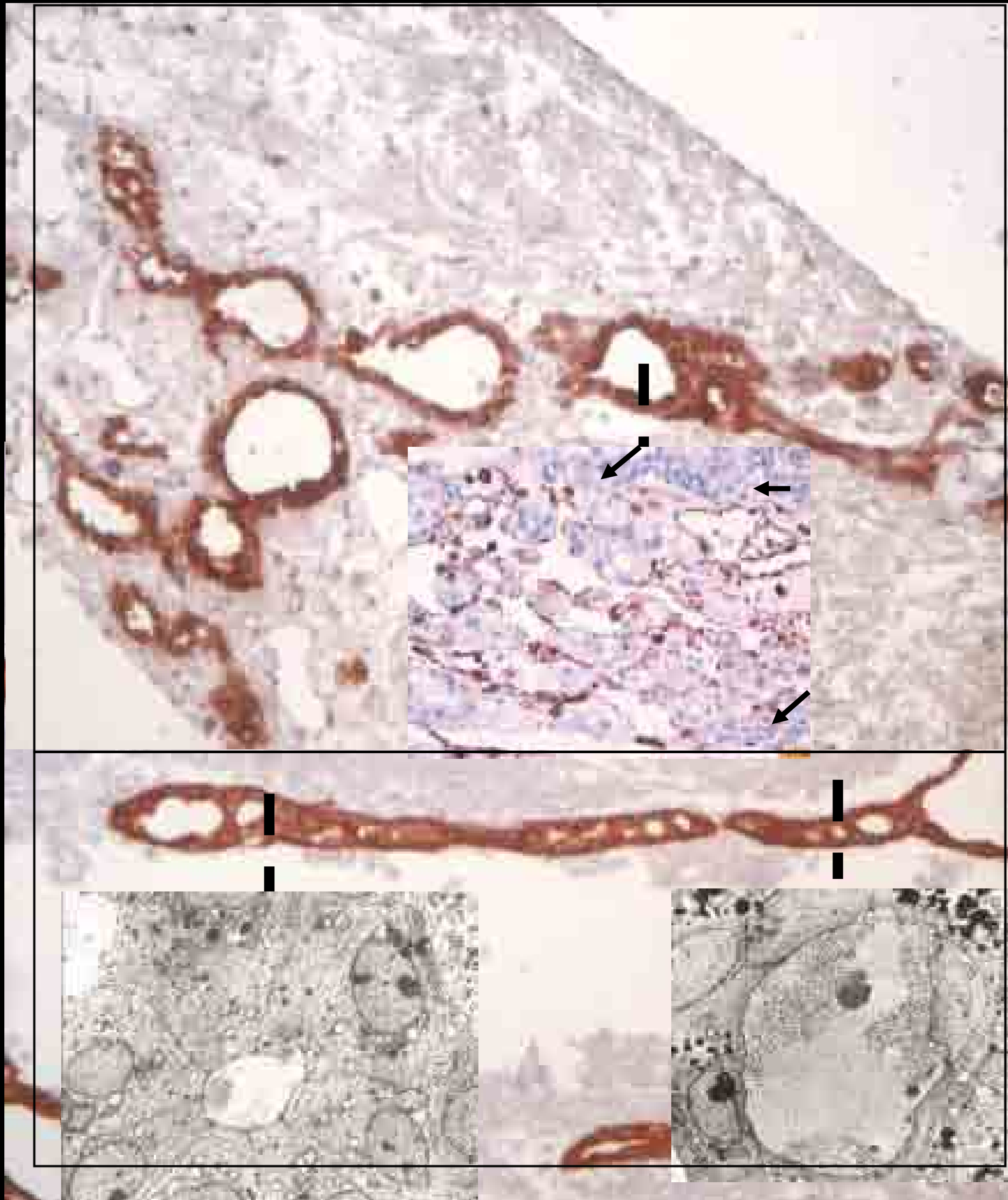
Liver sinusoid

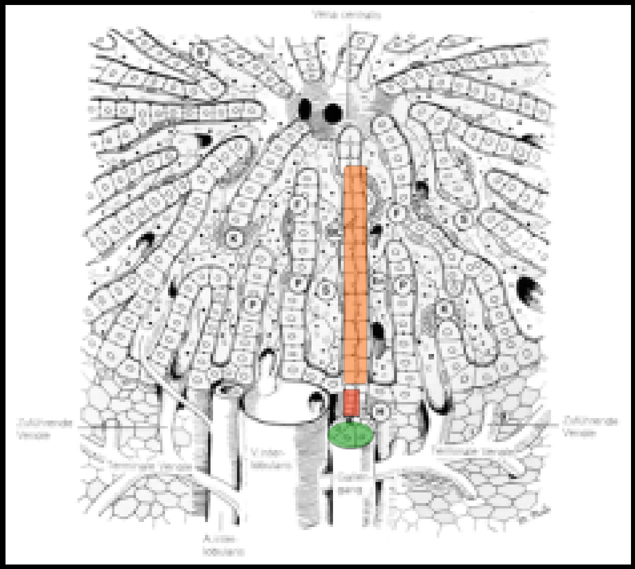
Parenchymal epithelium: hepatocytes

Canals of Hering: bipotent progenitor cells

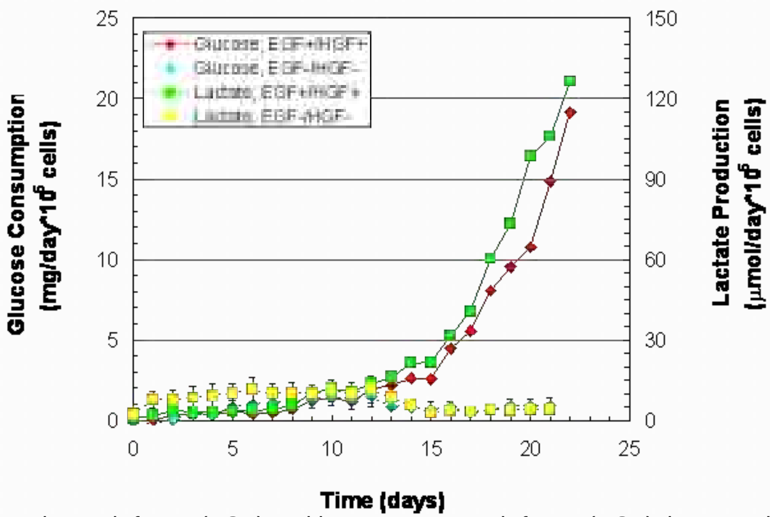
Bile ducts: biliary cells

Adult liver progenitors
re-assemble to
Canal of Hering-like
structures in the bioreactor



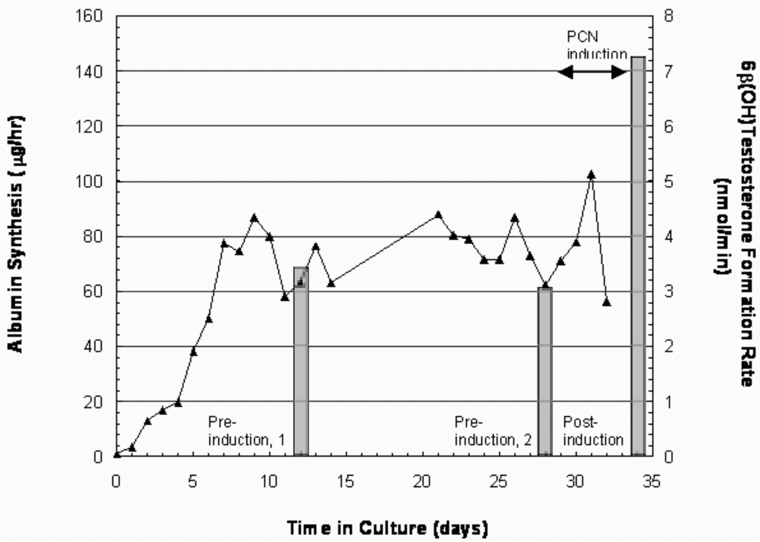


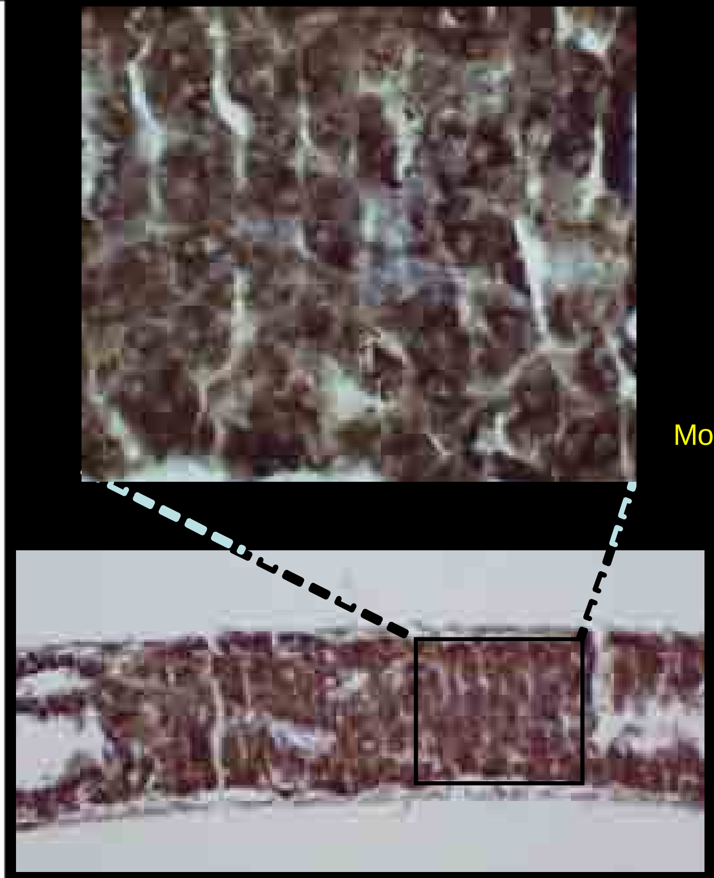
Metabolism of Human Fetal Liver Cells during Bioreactor Expansion



Epidermal growth factor (EGF) and hepatocyte growth factor (HGF), increased glucose consumption and lactate production, suggesting cell proliferation.

P-450 Enzyme induction of Fetal Liver Cells after Bioreactor Expansion





Mouse fetal liver cells

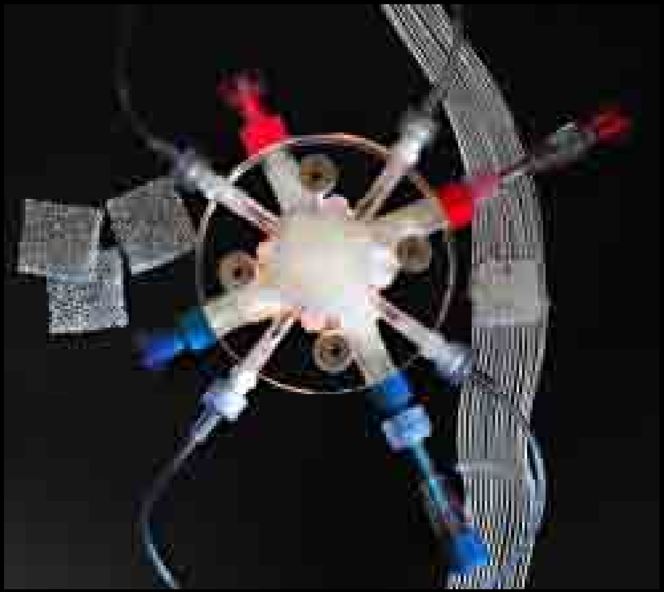
Albumin hepatocytes

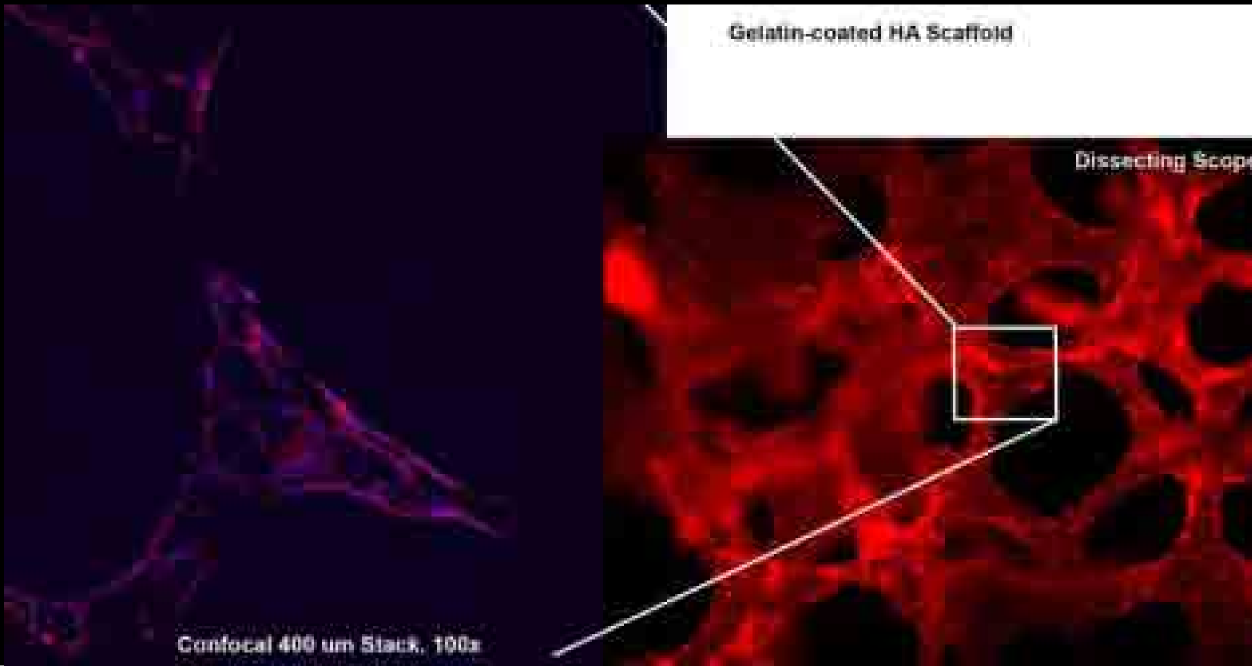
on Culture dish

Bio-artificial liver - clinical translation ?

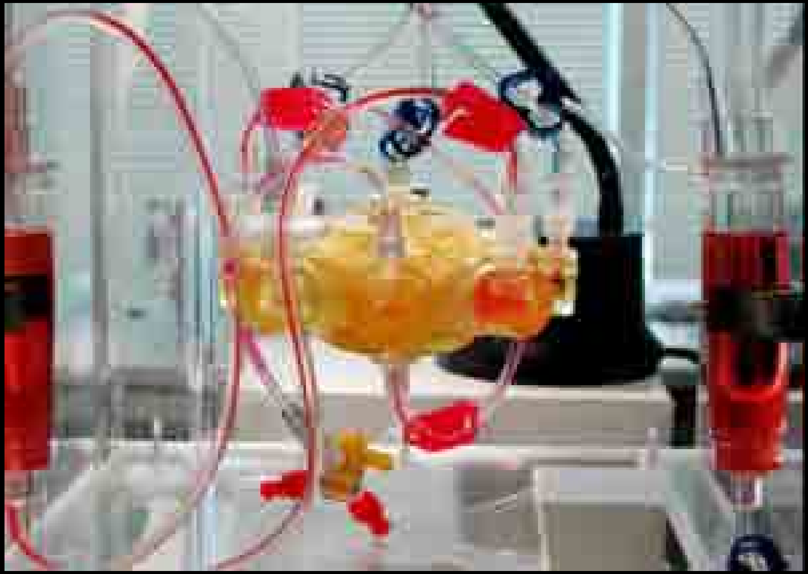
Further Examples translation projects

Bone marrow stem cell bioreactor

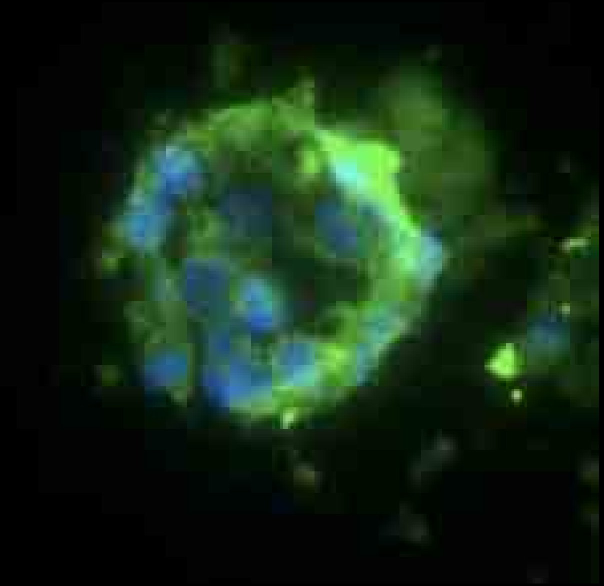




3D in vitro culture model for pancreatic islets beta-cell precursors

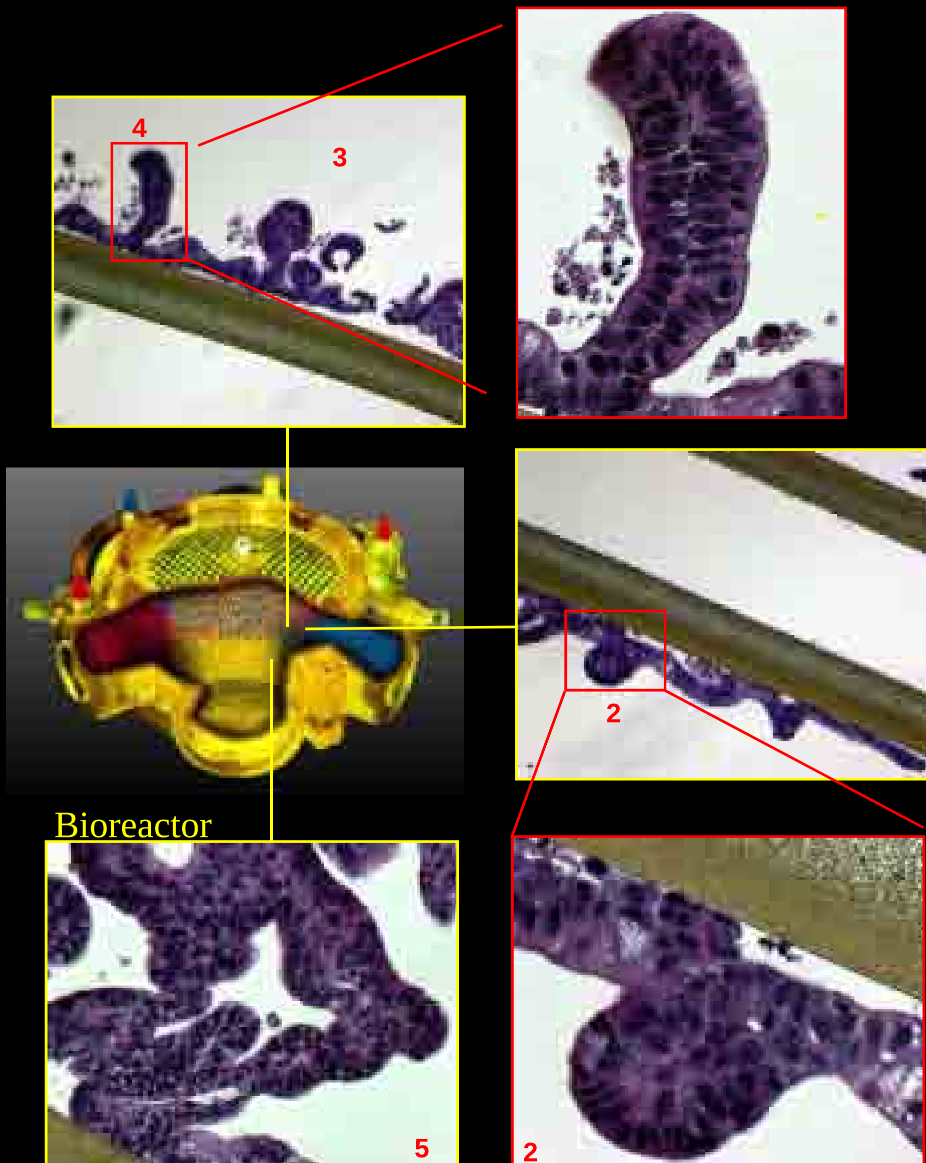


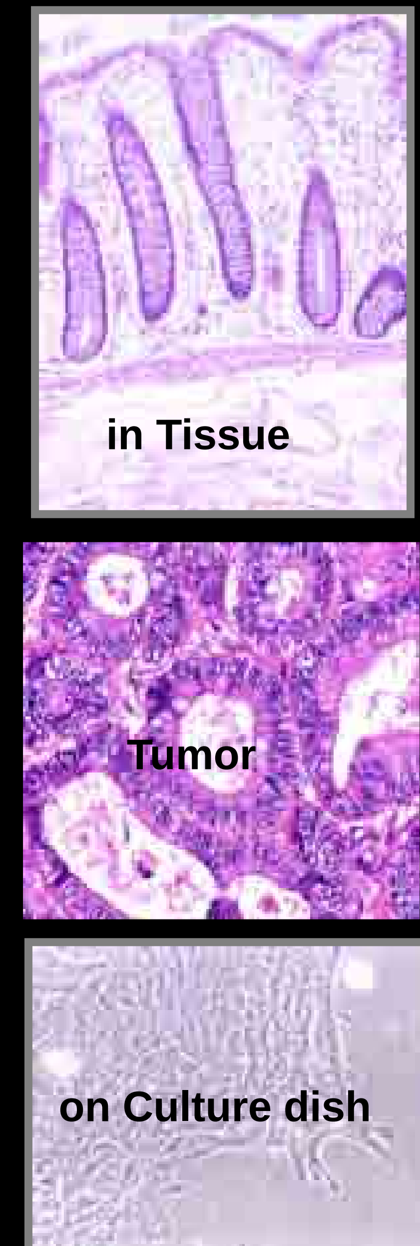
in Bioreactor



Insulin (green) Nuclei (blue)

Human colon cancer stem cells in vitro model





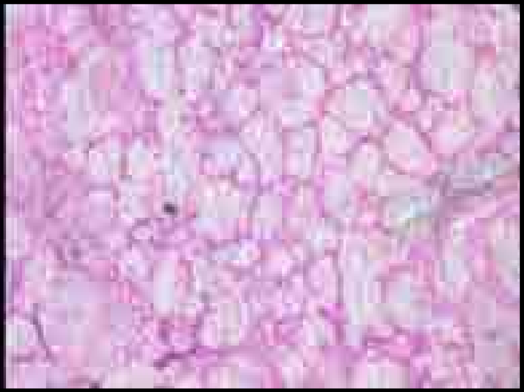
Crypt Dev. from Metastatic Colon Cancer Tu-12 (E. Lagasse)

Fat cell *in vitro* differentiation of human adipose precursors

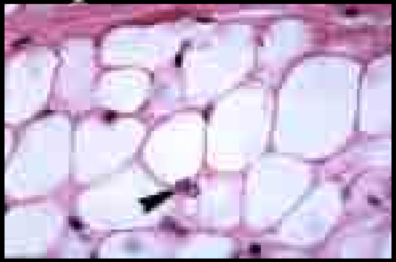
2D Culture

3D Bioreactor

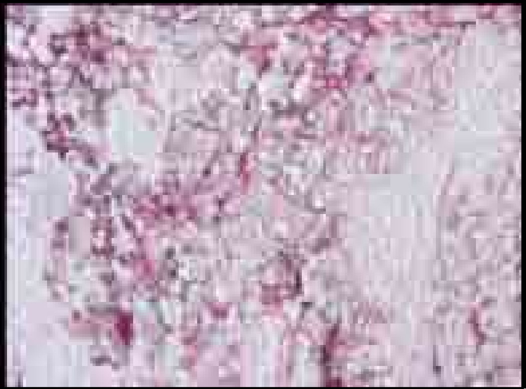
Normal Mammalian Adipose Tissue



H&E



Oil Red



Masson’s Trichrome