In Vitro Characterization of an *Ex Vivo* Liver Construct

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** No financial disclosures

December 6, 2007

Overview - *ex vivo* liver constructs

- Liver Failure – Impact of the Problem
- Brief History & Rationale - Ex Vivo liver therapy
- Standards: Safety, Purity, Potency, Consistency
- Assessment techniques – past, new, & needed

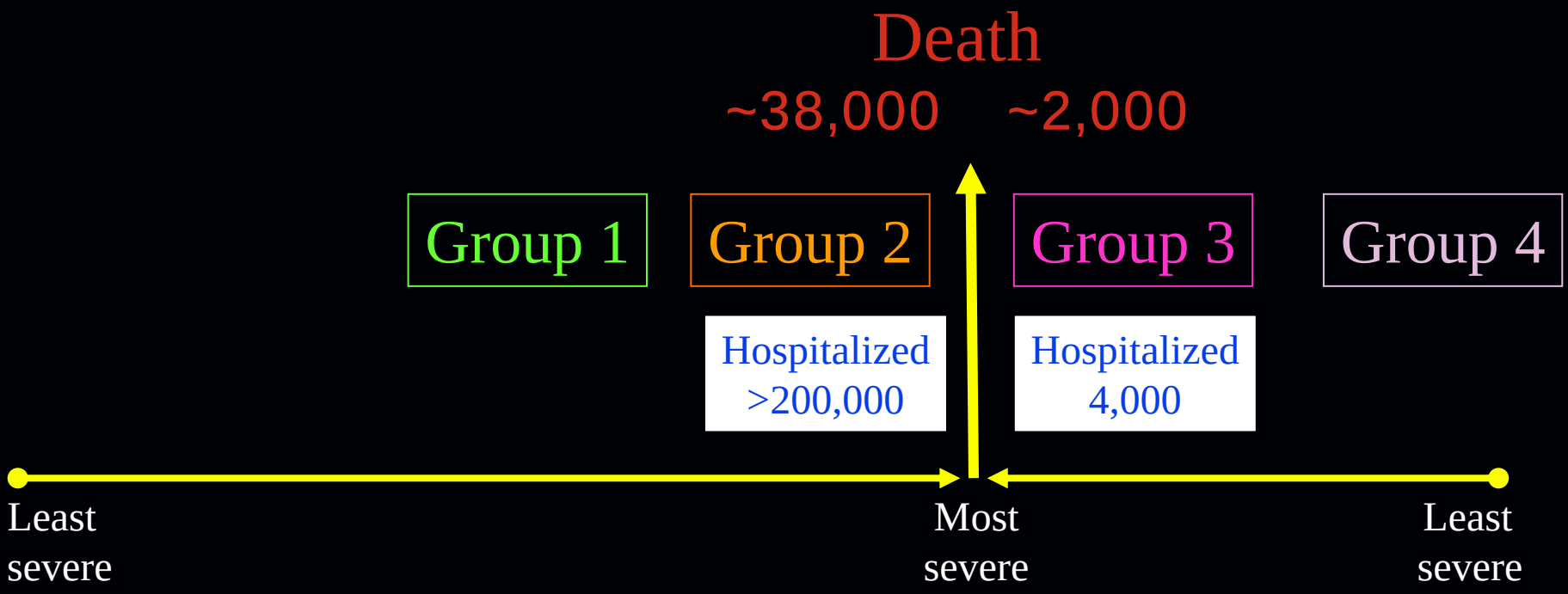
[1] 2000 Annual Report of the American Liver Foundation

Facts of Liver Disease [1]

- 8th most frequent cause of death in U.S.
  ~40,000 deaths/yr.

- Aside from Liver Transplantation, no other therapy is effective to treat end-stage liver disease
  ~6,000 transplants annually

Continuum of Severity of Liver Disease



Chronic Liver Disease
(>5 million cases)

Acute Liver Disease
(~20,000 cases/yr)

Goals of Ex Vivo Liver Therapy

- Prevent manifestations of liver failure (brain swelling, lung, kidney, SIRS)
- Bridge to Liver Transplantation
- Buy time for Spontaneous Recovery
- Improve Survival – 30 days, 1 yr

Why use Ex Vivo Liver Cell Constructs to Treat Liver Failure

- Liver Failure results from loss of liver function
- Synthetic functions (Albumin, Growth Factors, . . .)
- Regulation (amino acids, fatty acids, cytokine levels . . .)
- Selective Detoxification of protein-bound and water-soluble waste substances
- “Closed ” hepatocyte system avoids non-selective losses

Transformed vs. Primary Hepatocytes

Activity	Transformed	Primary	Comment
Protein Synthesis	√	√	Similar rates
Ureagenesis		√	Encephalopathy
P450 Enzymes		√	Drug clearance
Growth	√		
Tumor Risk	√		
Infectious Risk		√	

Closed System

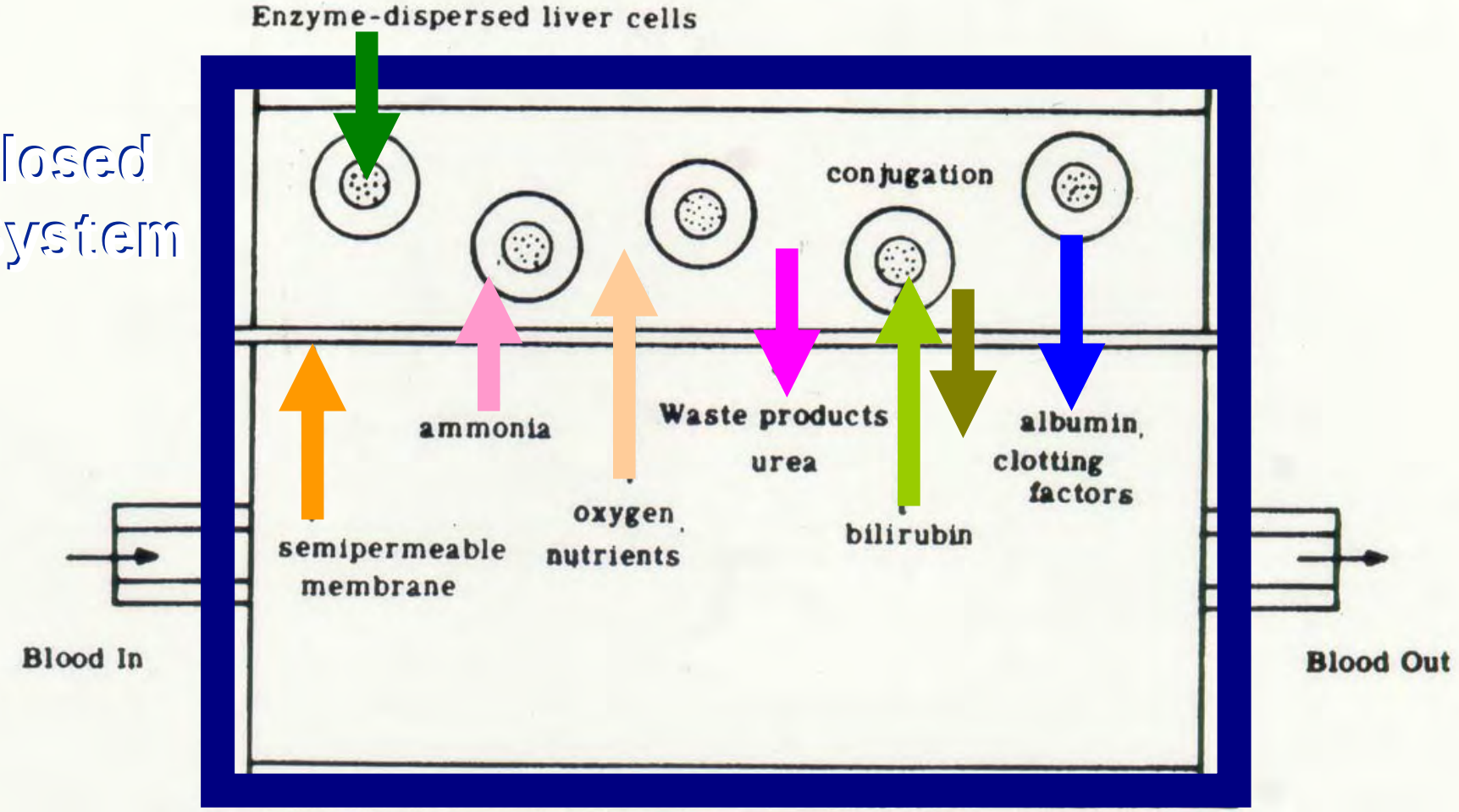


Fig. 1. Principle of bioartificial liver (BAL)

Diced Liver as Ex Vivo Liver Construct

T.S. Lie, V. Jung, F. Kachel, Ch. Höhnke, and K.S. Lee

Section of Transplantation, Dept. of Surgery, University of Bonn, Sigmund Freud Str. 25, D-5300 Bonn 1, Federal Republic of Germany

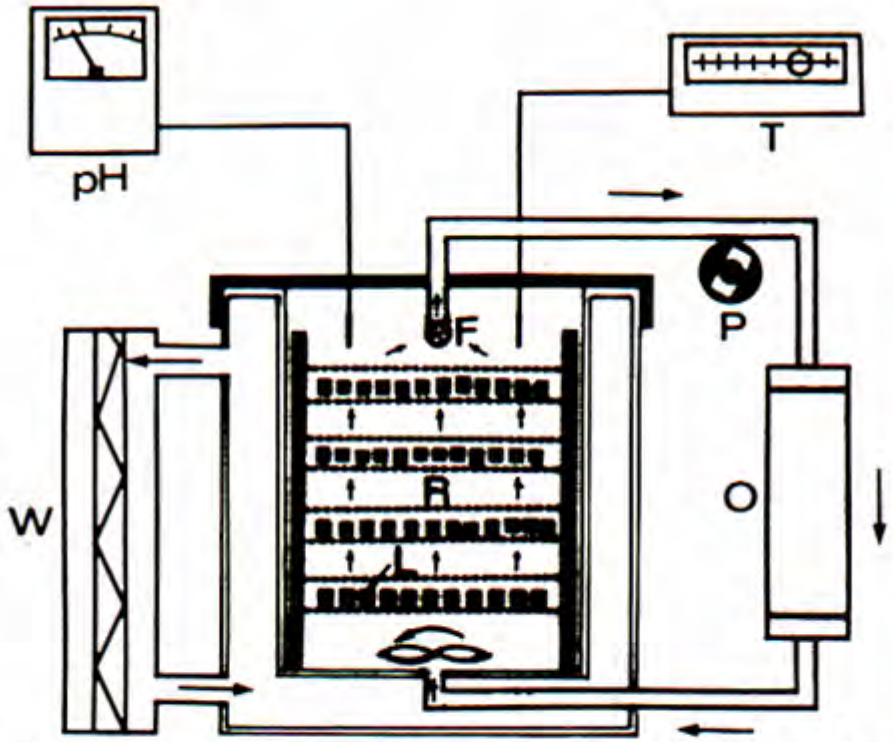
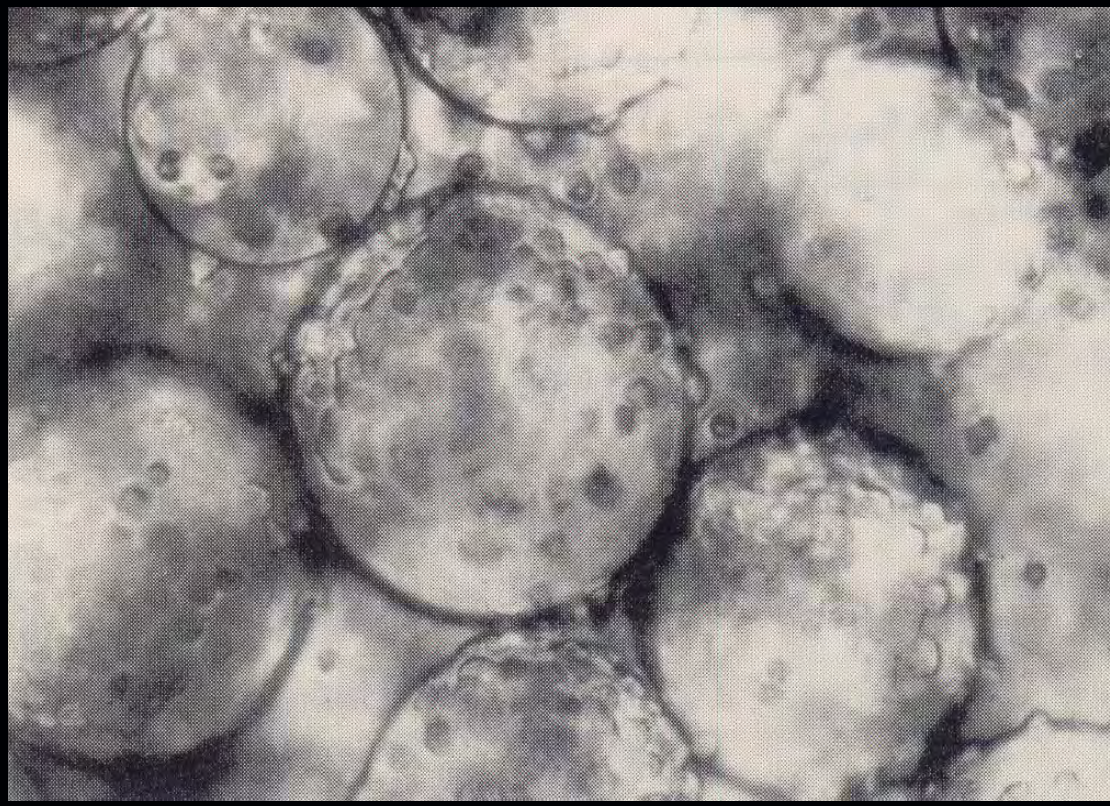


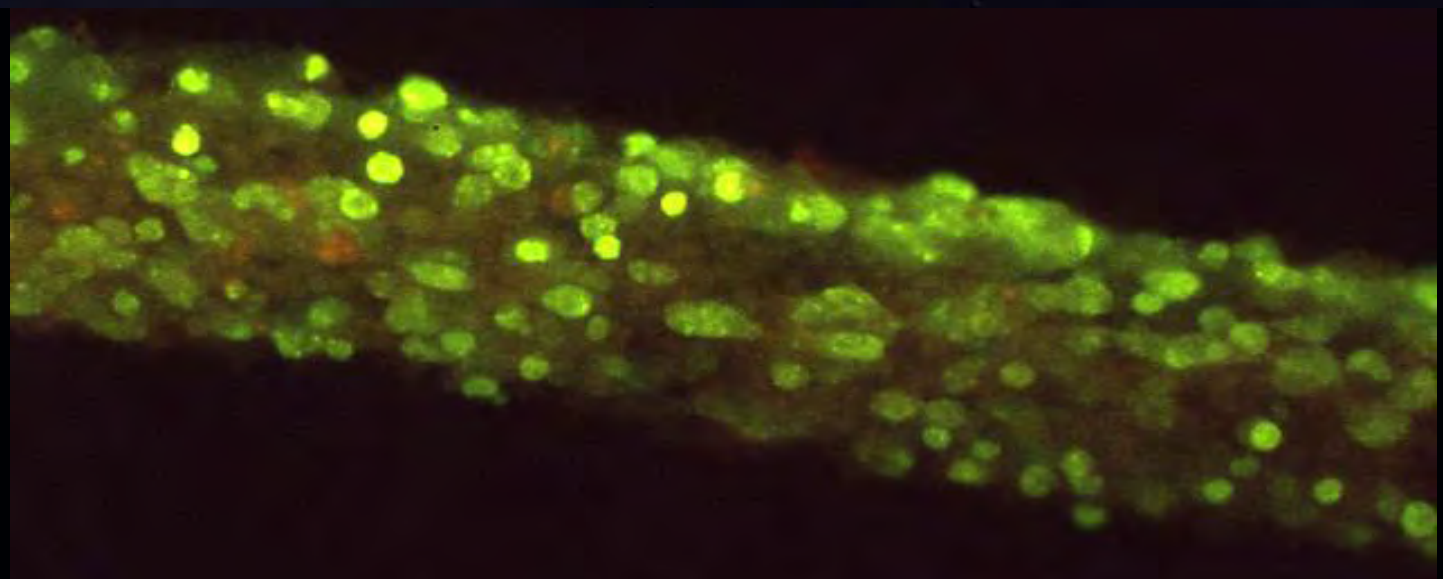
Fig. 1. Hemoperfusion circuit. *T* temperature; *W* heat exchanger; *F* filter; *P* pump; *O* oxygenator; *R* reservoir; *L* liver cubes

Liver Cells Attached to Hollow Fibers

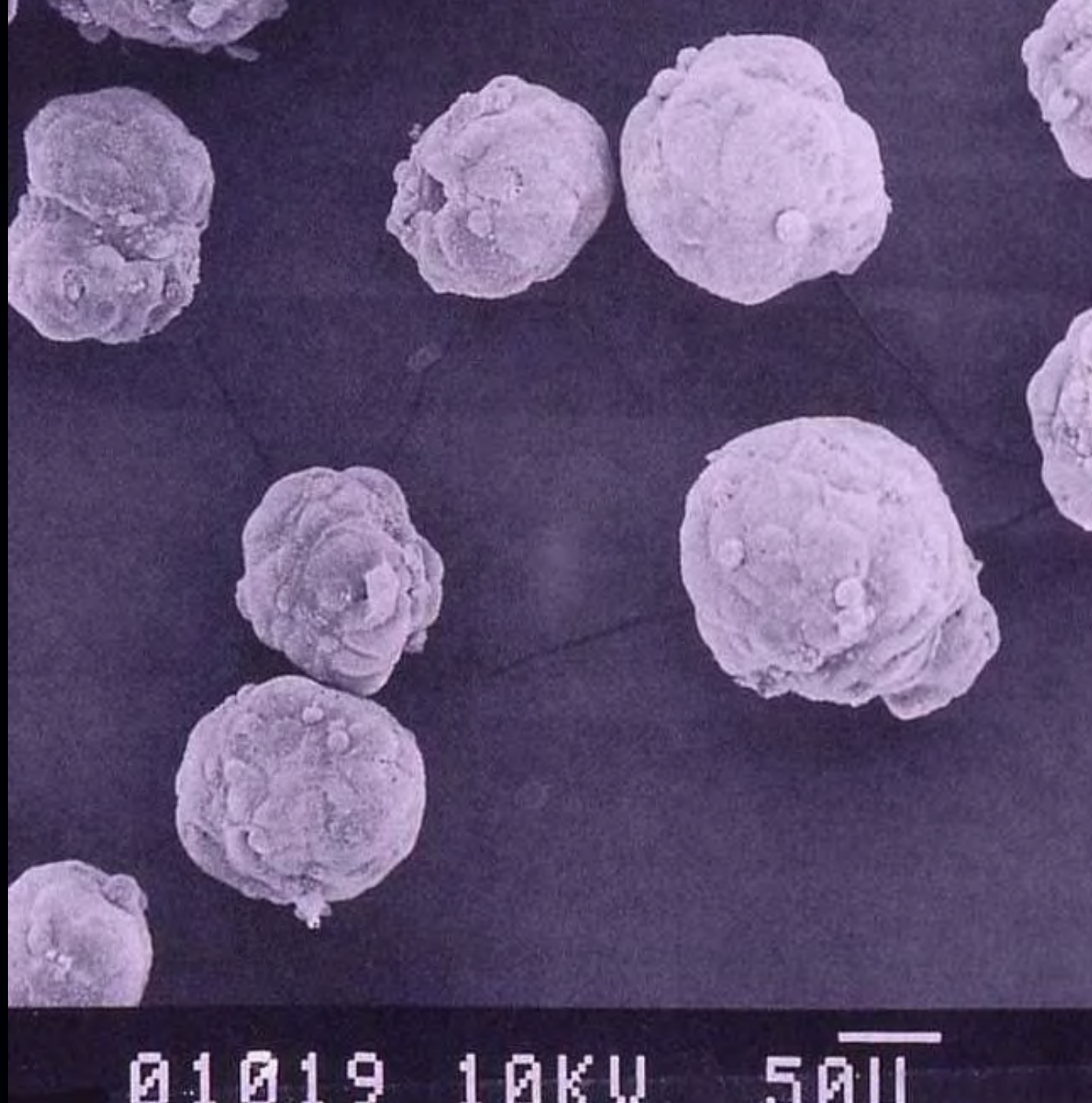
Liver Cells Attached to Microcarrier beads



Liver Cells
Entrapped
in
Collagen Gels

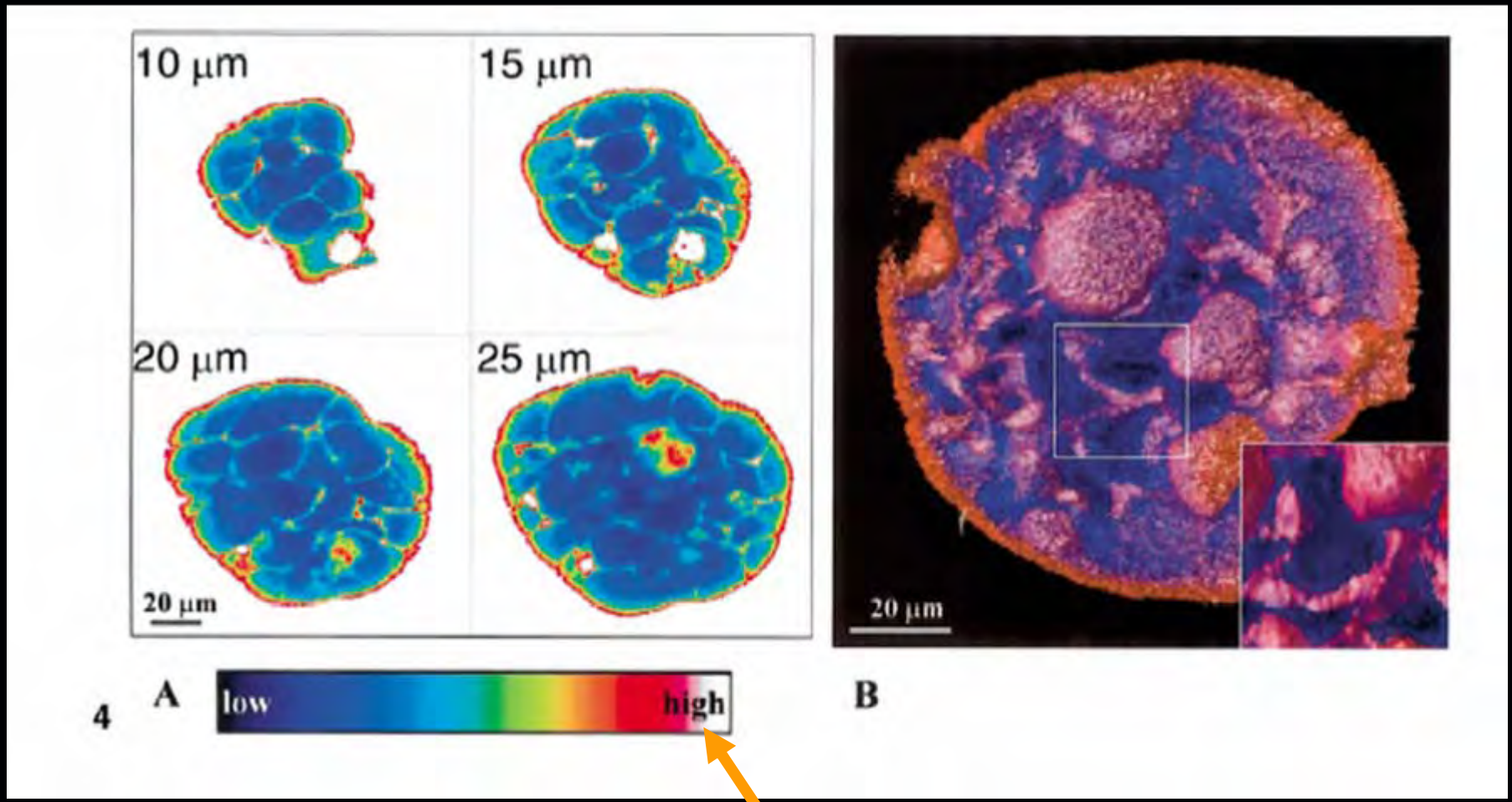


Liver Cells formed as Spheroids (aggregates)



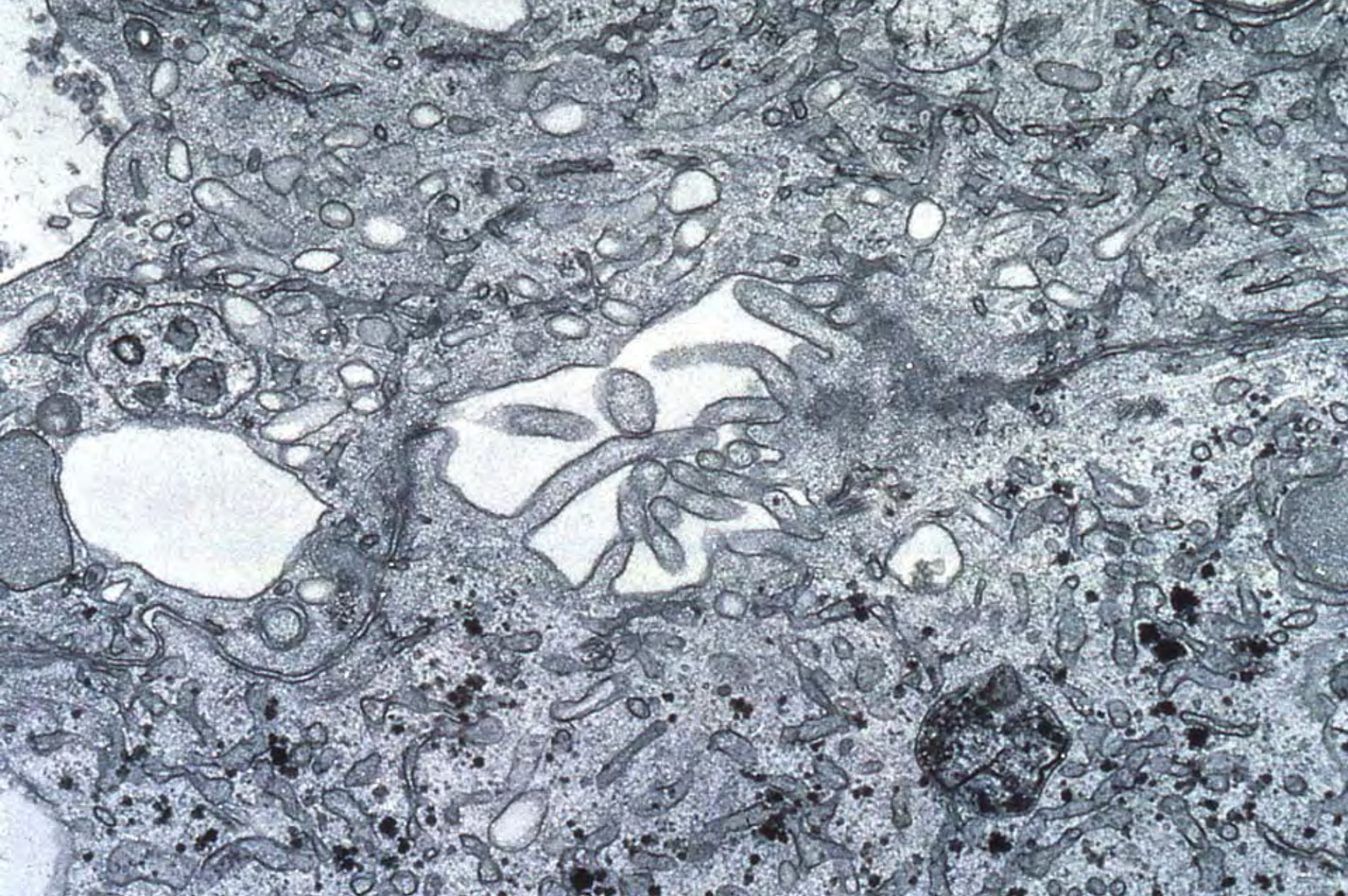
Hepatocyte Spheroids – 7 days in culture

Canaliculi Formation in Rat Hepatocyte Spheroids – 4 days **



** Experimental Cell Research **274**, 56–67 (2002)

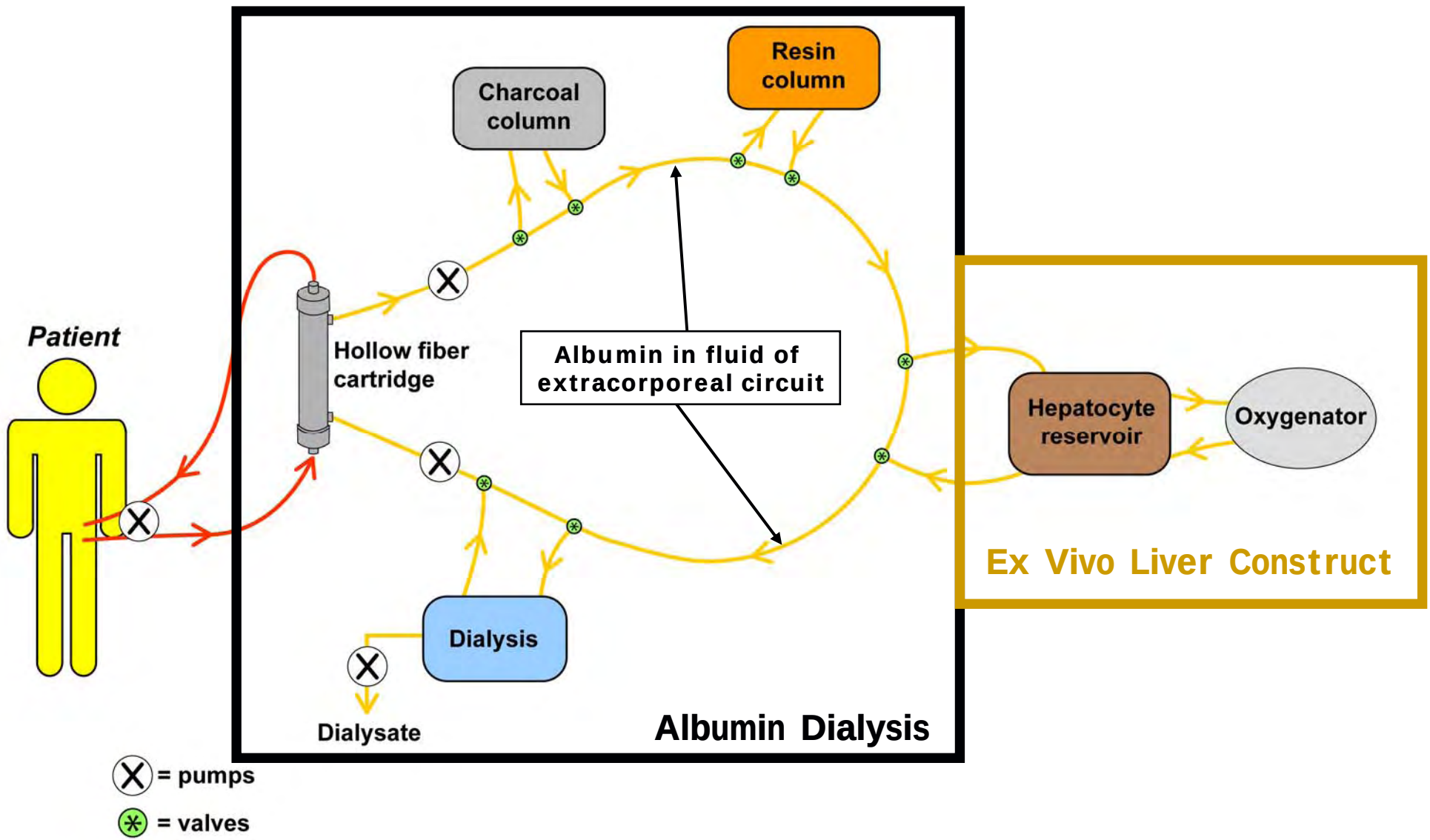
Dextran Beads – 4400kD



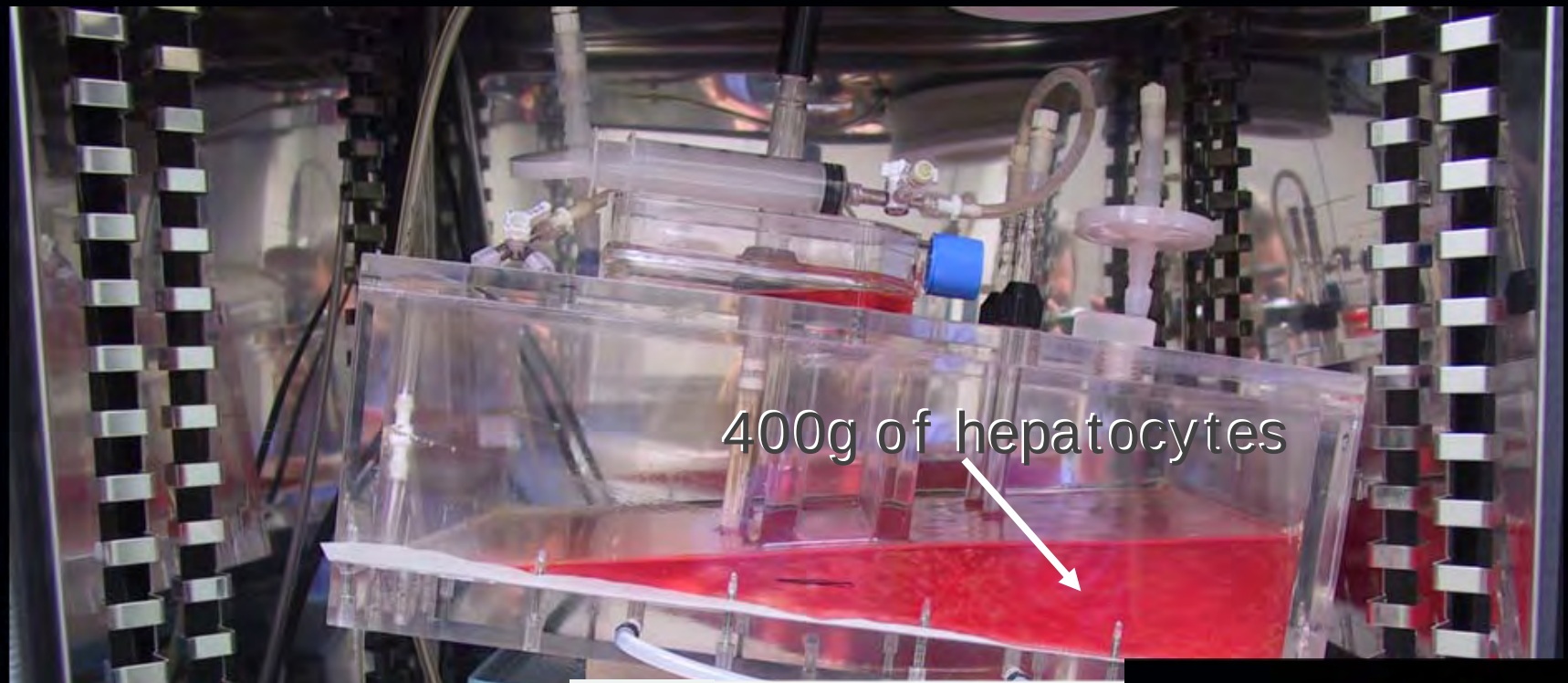
Ex Vivo Liver Support Systems

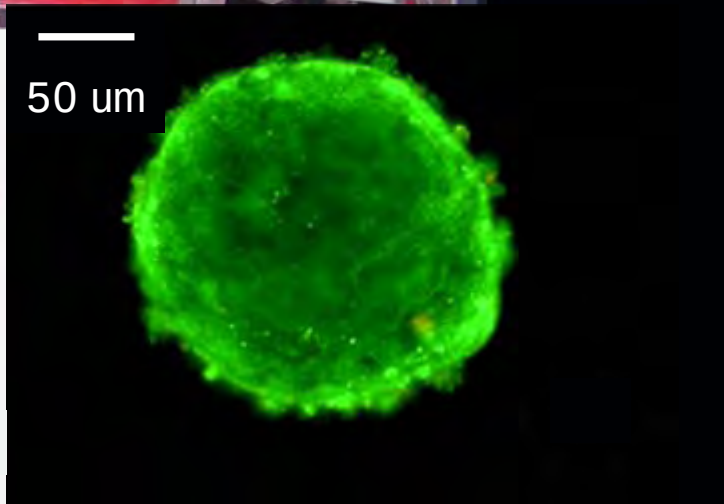
- Over 30 different Ex Vivo liver systems reported since 1987
- Over 14 systems reported in Clinical Trials
- None of these BAL systems have FDA approval

Next generation "hybrid" systems



Prototype Ex Vivo Liver Construct: Spheroid Reservoir



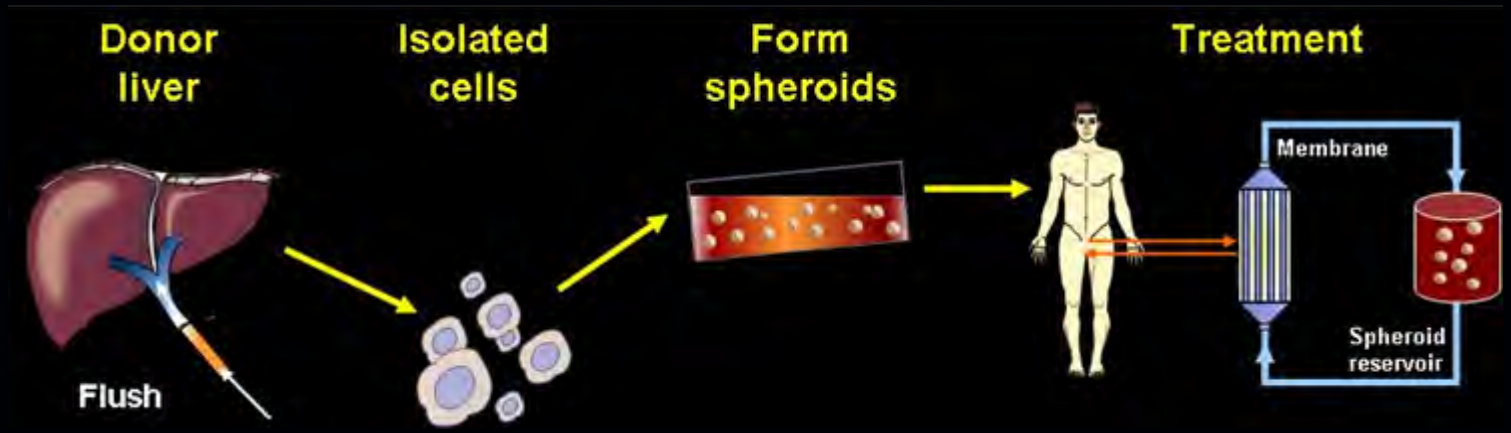


Standards to Evaluate Ex Vivo Liver Constructs

(Ex Vivo devices to treat liver failure)

Safety	(to patient)
Purity	(vs. impurity)
Consistency	(batch-to-batch deviation)
Potency	(minimum criteria)
Efficacy	(to patient)

Step-by-Step Summary of Release Criteria for Cell-Based Liver Support



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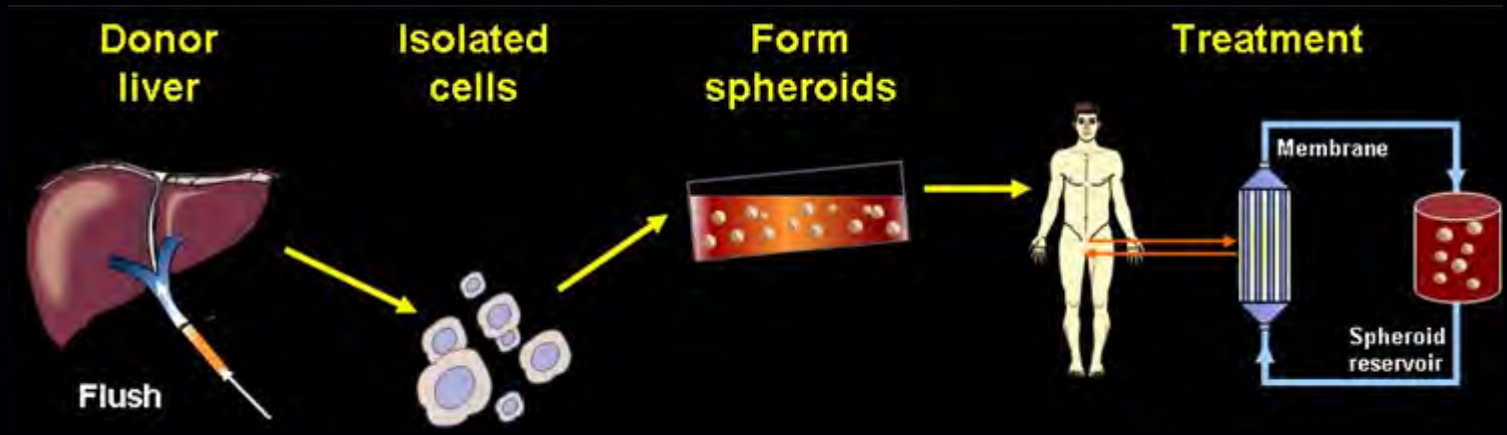


Release criteria	Donor liver	Isolated cells	Form spheroids	Treatment
Safety	✓		✓	✓
Purity		✓		
Potency			✓	
Consistency	✓	✓	✓	
Efficacy				✓

Safety – Cells

(standard to evaluate ex vivo liver constructs)

Code of Federal Regulations (21CFR610)

- Sterility (bacteria, virus, mycoplasma)
- Pyrogenicity (LAL test)

Tumorigenicity (cell loss from BAL)

(risk: tumor > primary)

Safety – Patient

(to evaluate ex vivo liver construct)

Zoonosis: PERV (serology, DNA)

Morbidity: bleeding, clot, renal, lung, other

Purity – Cells

(standard to evaluate ex vivo liver construct)

	Markers (flow cytometry)
Hepatocytes	Albumin, HNF4α
Bile duct cells	CK19
Kupffer cells	F4/80 antigen
Stellate (Ito) cells	SMA (active), GFAP (inactive)
Endothelial cells	PECAM (platelet/endothelial cell)

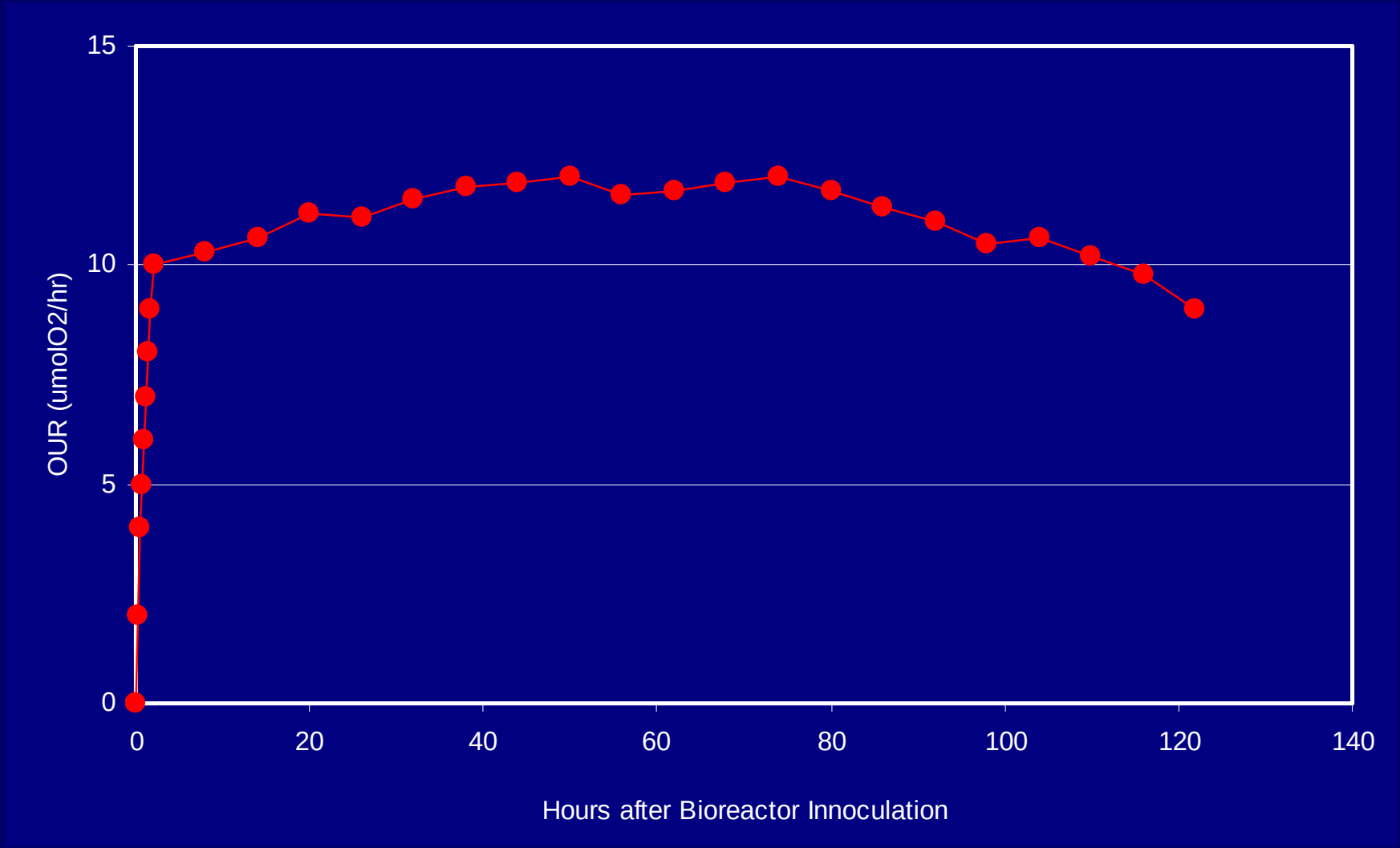
Potency – Cell

(standard to evaluate ex vivo liver construct)

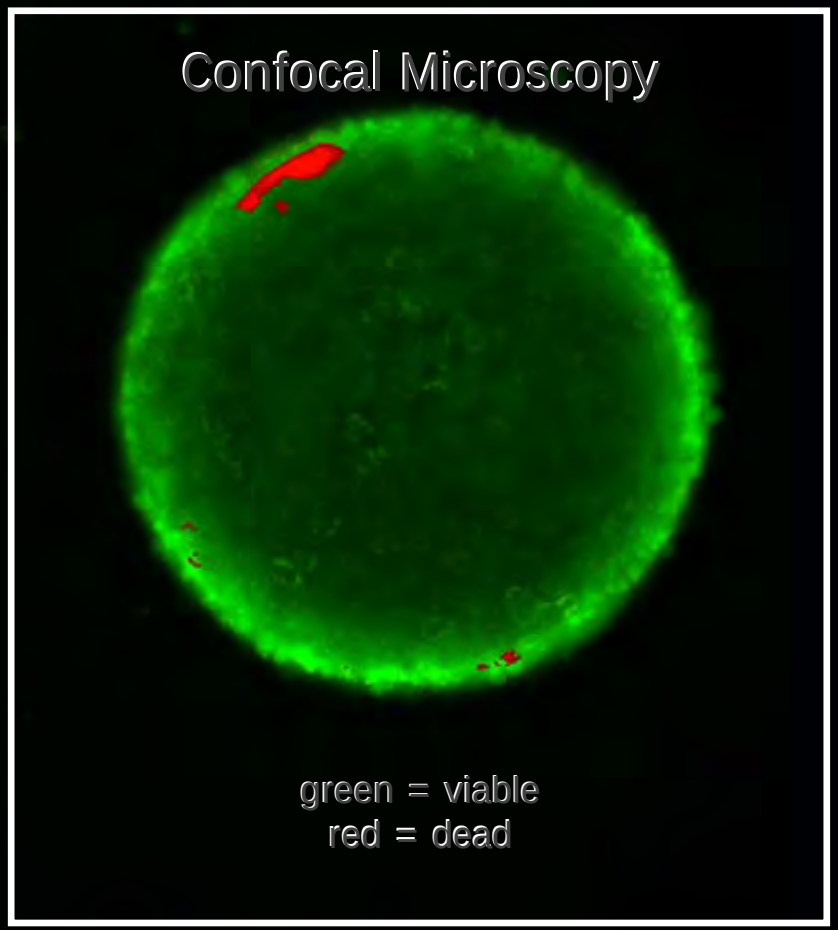
- Oxygen consumption (OUR)
- Viability (vital stain)
- Albumin production (ELISA)
- P450 assay (fluorogenic)
- Urea cycle (mass spec)
- **Microarray & proteomics (custom data)

** need rapid turnaround and high throughput

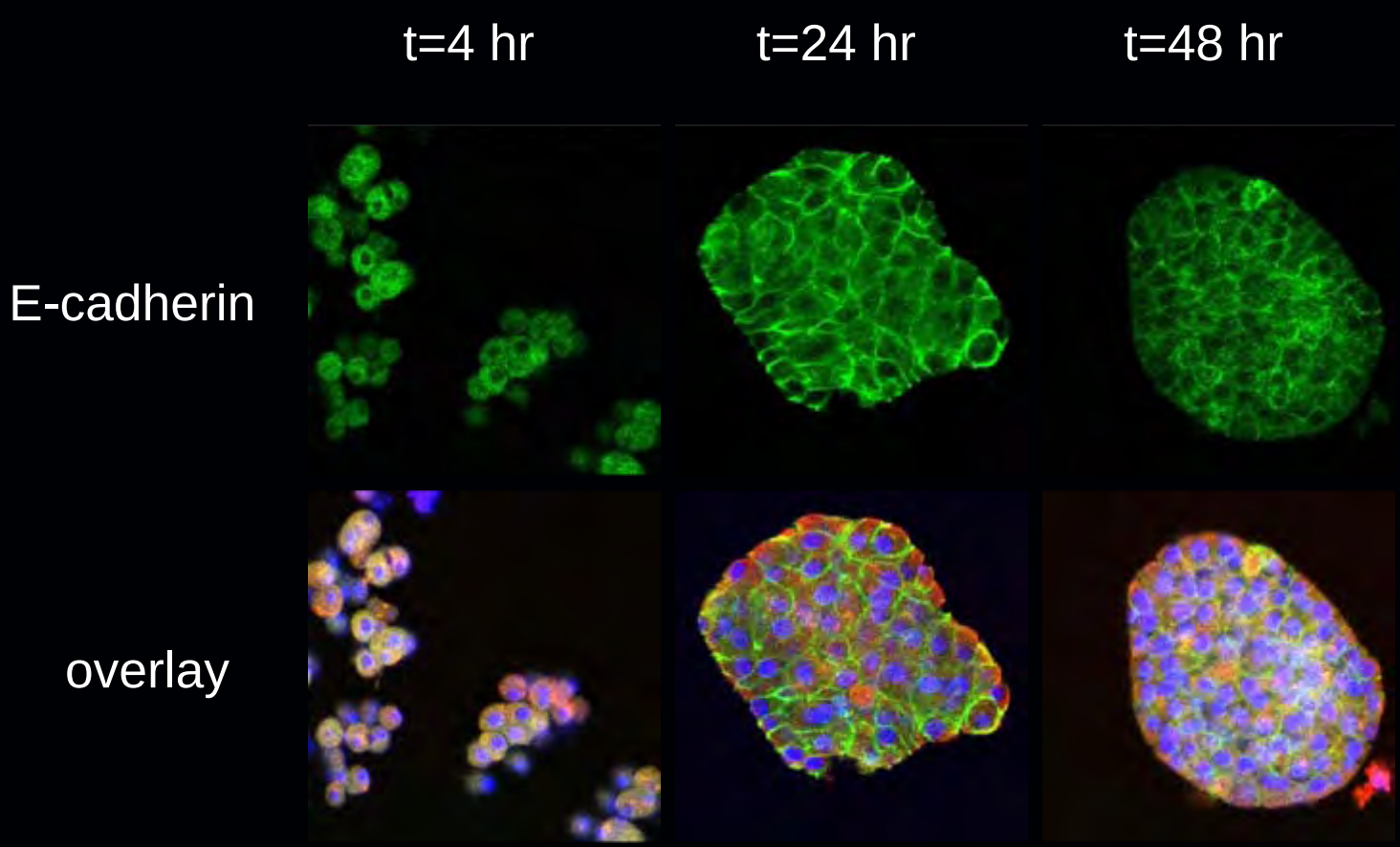
Example of Oxygen Uptake Rate (OUR)
Ex Vivo Porcine Hepatocyte Bioreactor



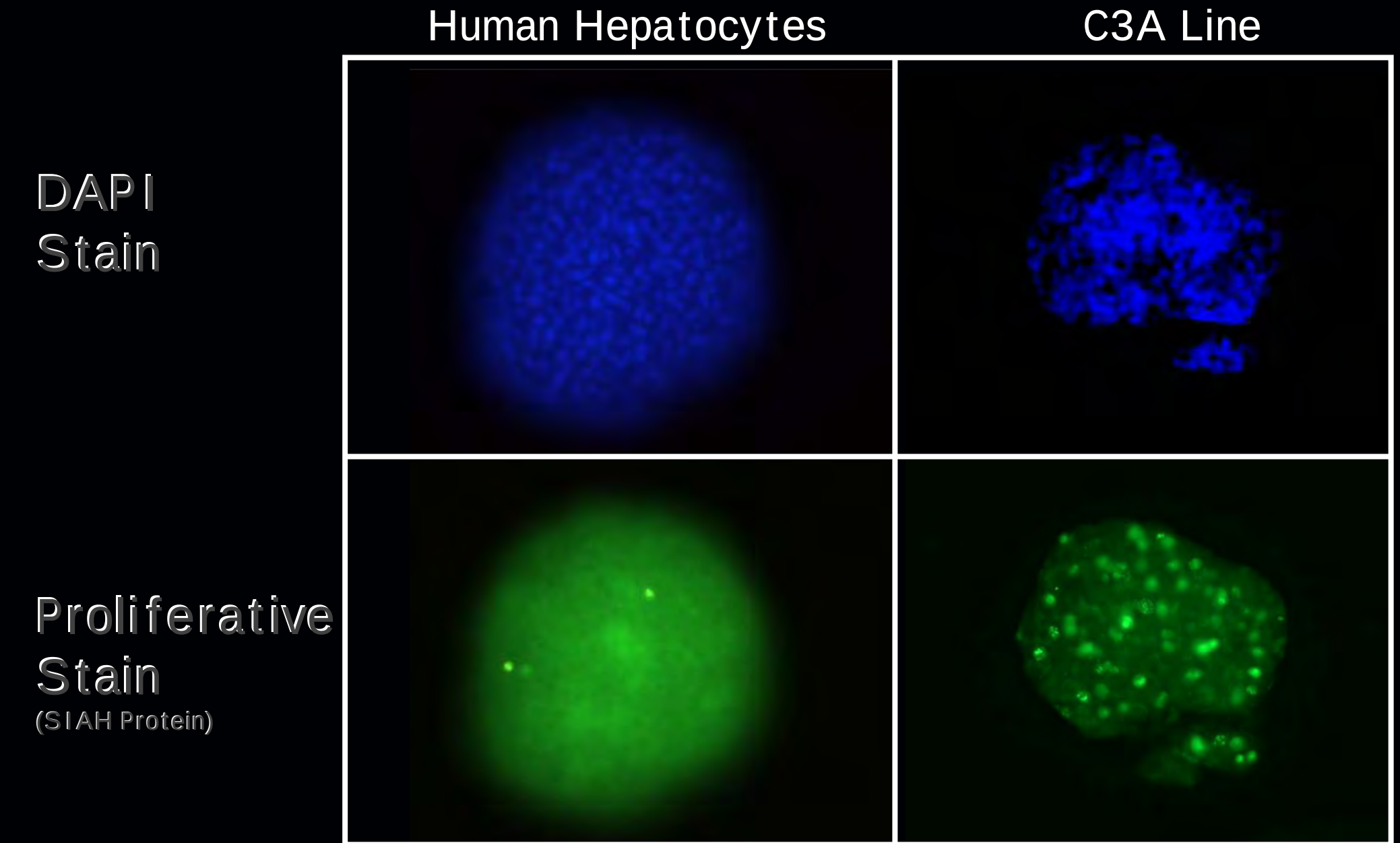
Stable Viability of Hepatocyte Spheroids x 28 days



Cadherin E Staining during Spheroid Formation by Confocal Microscopy

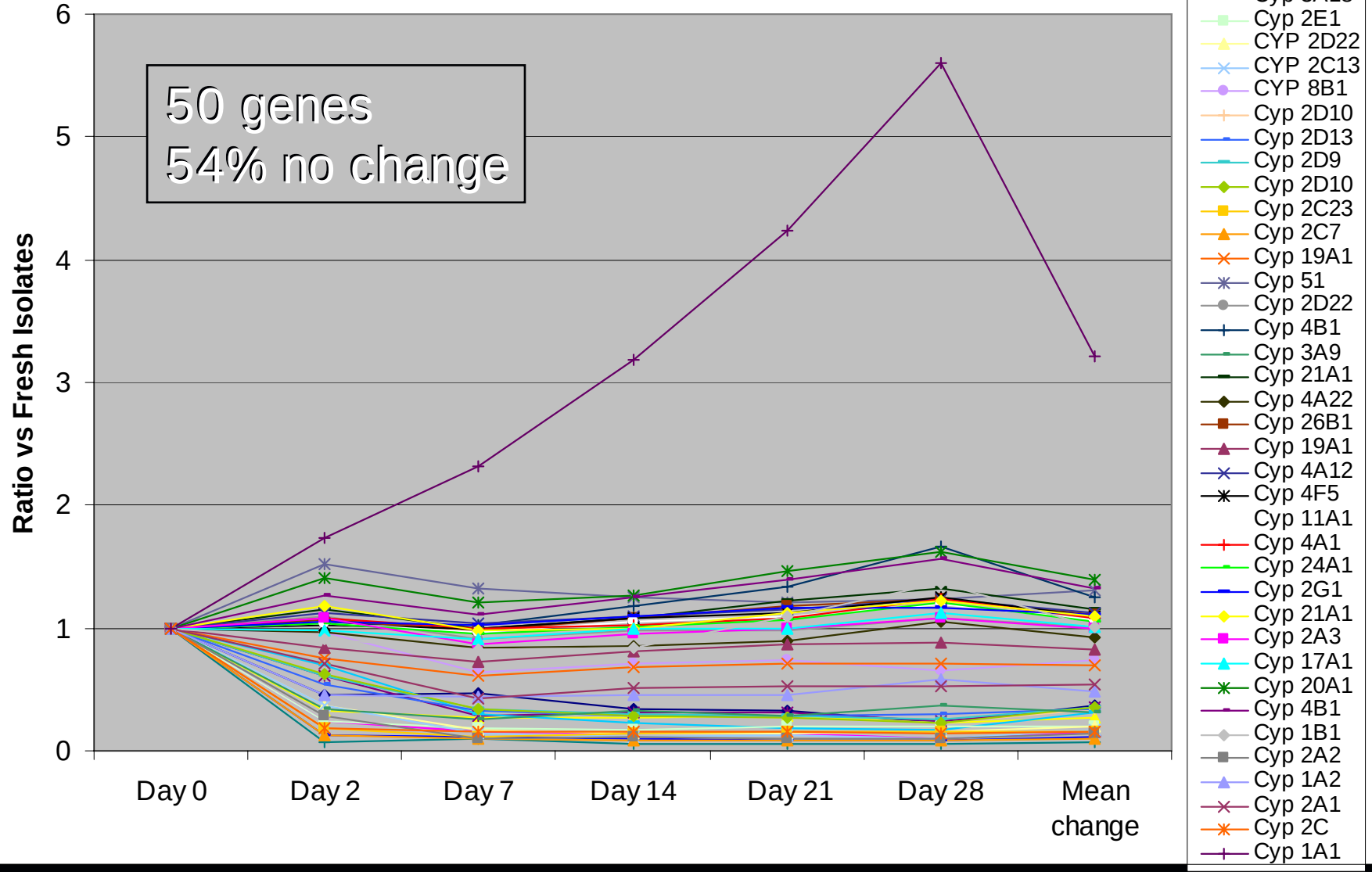


Confocal microscopy of Human Spheroids - Day 14

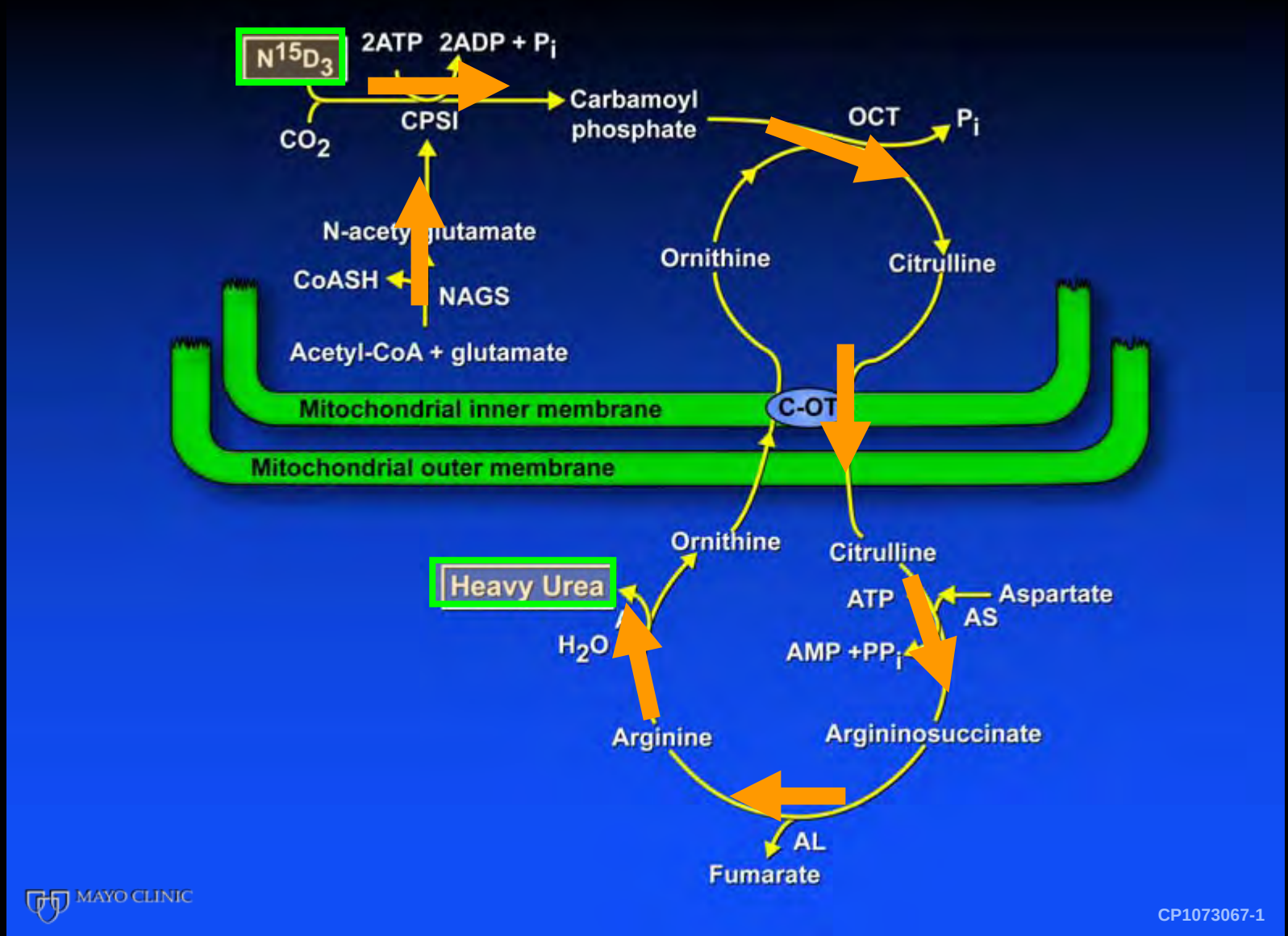


Custom Microarray - Cyp450 Gene Expression

Ex Vivo Liver Construct - Rat Hepatocyte Spheroids

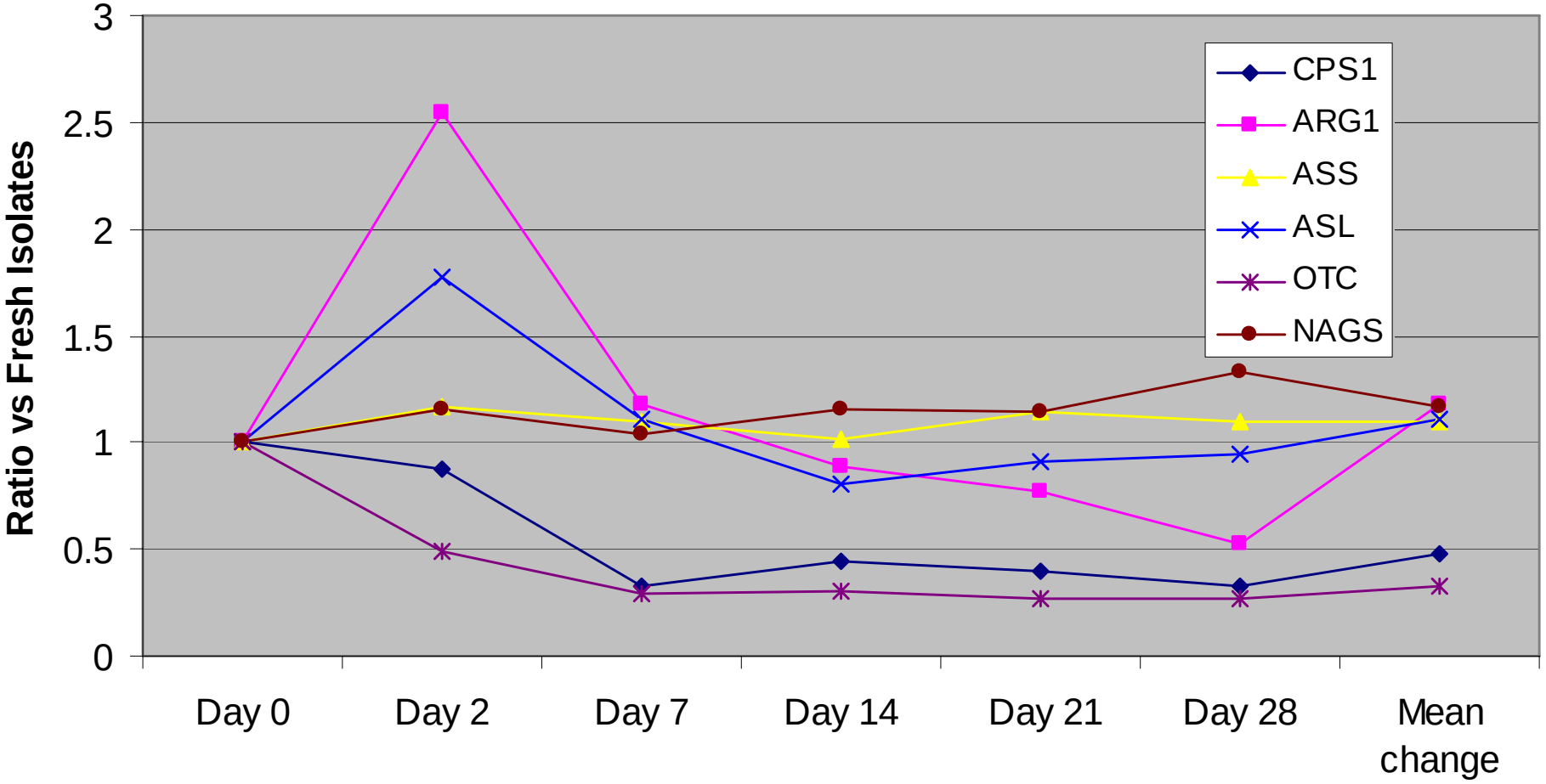


Ureagenesis Assay: Heavy ammonia to heavy urea



Custom Microarray - Urea Cycle Gene Expression

Ex Vivo Liver Construct - Rat Hepatocyte Spheroids



Consistency – Batch-to-Batch

(standard to evaluate ex vivo liver construct)

Cells	BAL
Cell marker profiles	Viability (initial, final)
Microarray data	Oxygen consumption
Proteomics data	Other functions (?detox)

Efficacy – Patient

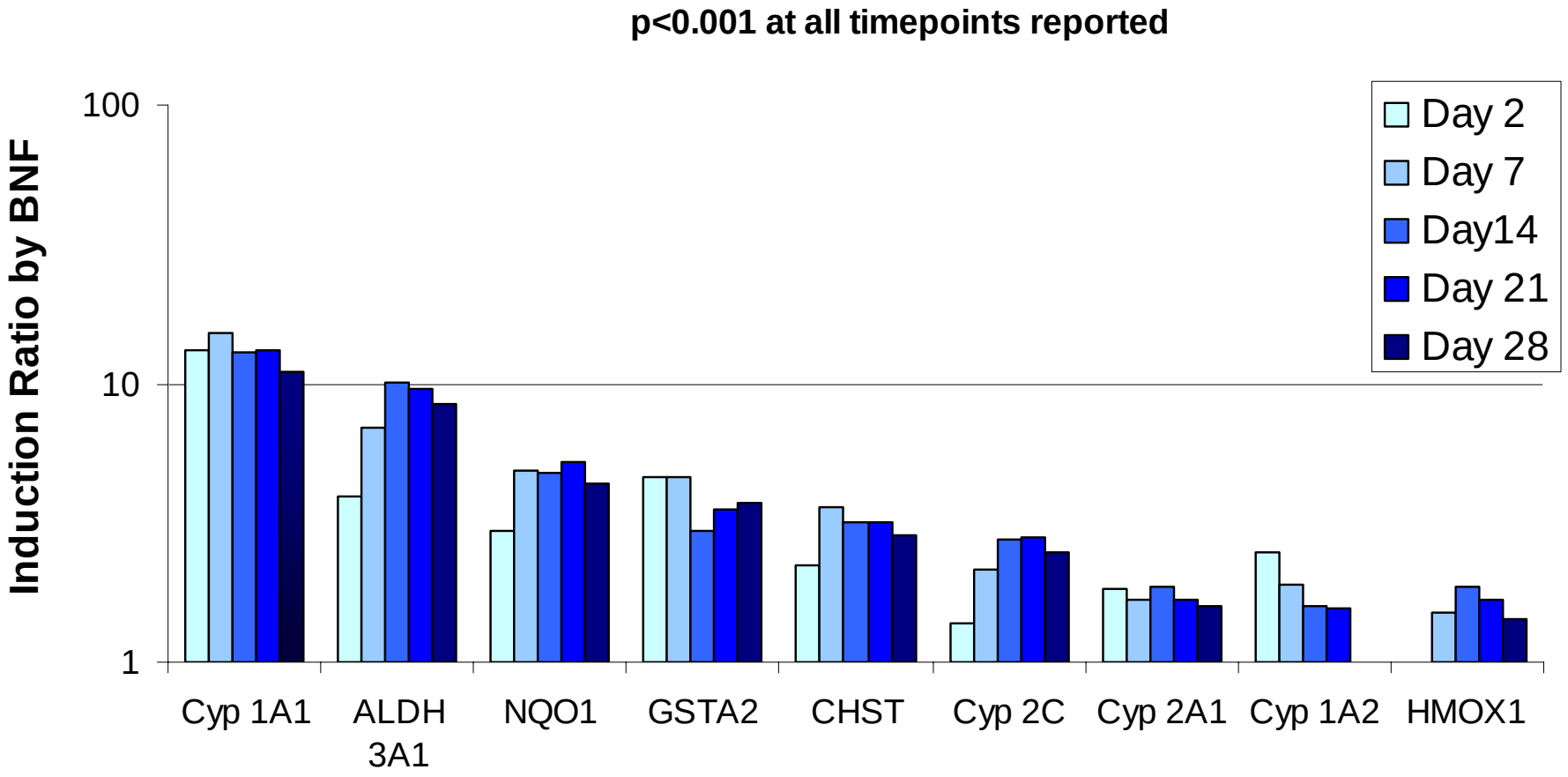
(standard to evaluate ex vivo liver construct)

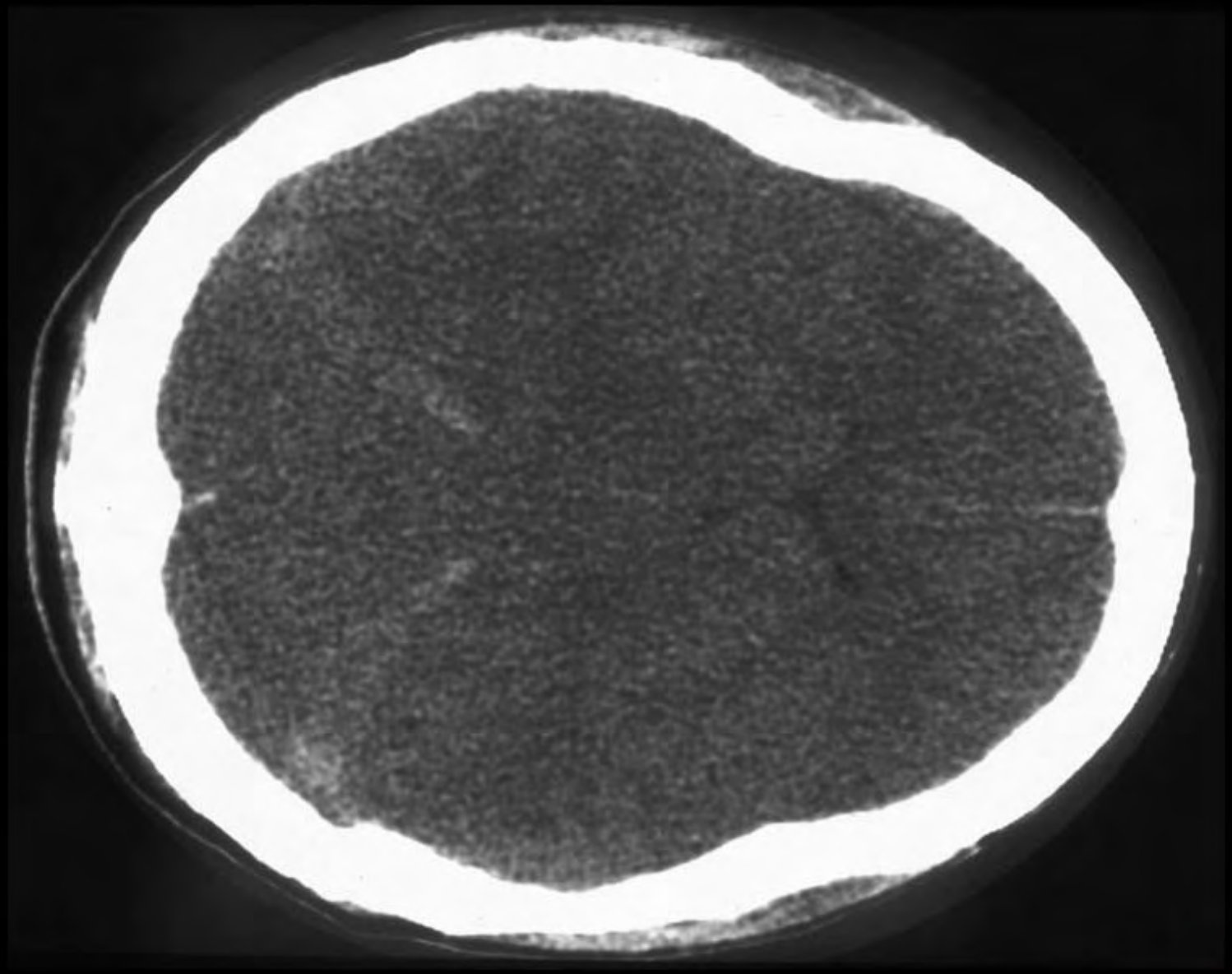
- Survival (30 day, 1 year)
  - spontaneous (without transplant)
  - with liver transplant
- Time to Recovery (ICU, hospital)
- Extrahepatic manifestations of liver failure
  - brain edema (ICP)
  - lung dysfunction (PO_2 /FIO_2)
  - kidney dysfunction (Cr, dialysis)

Summary

- What Questions Should be Asked to assess *ex vivo* liver constructs ?
  Is it safe and reliable? Does it work?
- What Methods are available to assess *ex vivo* liver constructs ?
  Many - Microscopic, biochemical, clinical
- What Methods should be developed to assess *ex vivo* liver constructs ?
  Custom microarrays, proteomics, microscopy, liver specific function (ureagenesis, albumin)

Inducibility of Hepatic Genes is measure of Potency and Stable Differentiated Phenotype





mayo

CP1007639-10

UPSHOT

Liver Failure:

→ brain swelling

→ increased intracranial pressure

→ brain death

UPSHOT

Liver Failure:

→ brain swelling

→ increased intracranial pressure

→ brain death

Ex Vivo Liver Therapy:

provides hepatic function

↓ brain swelling

improved mental status

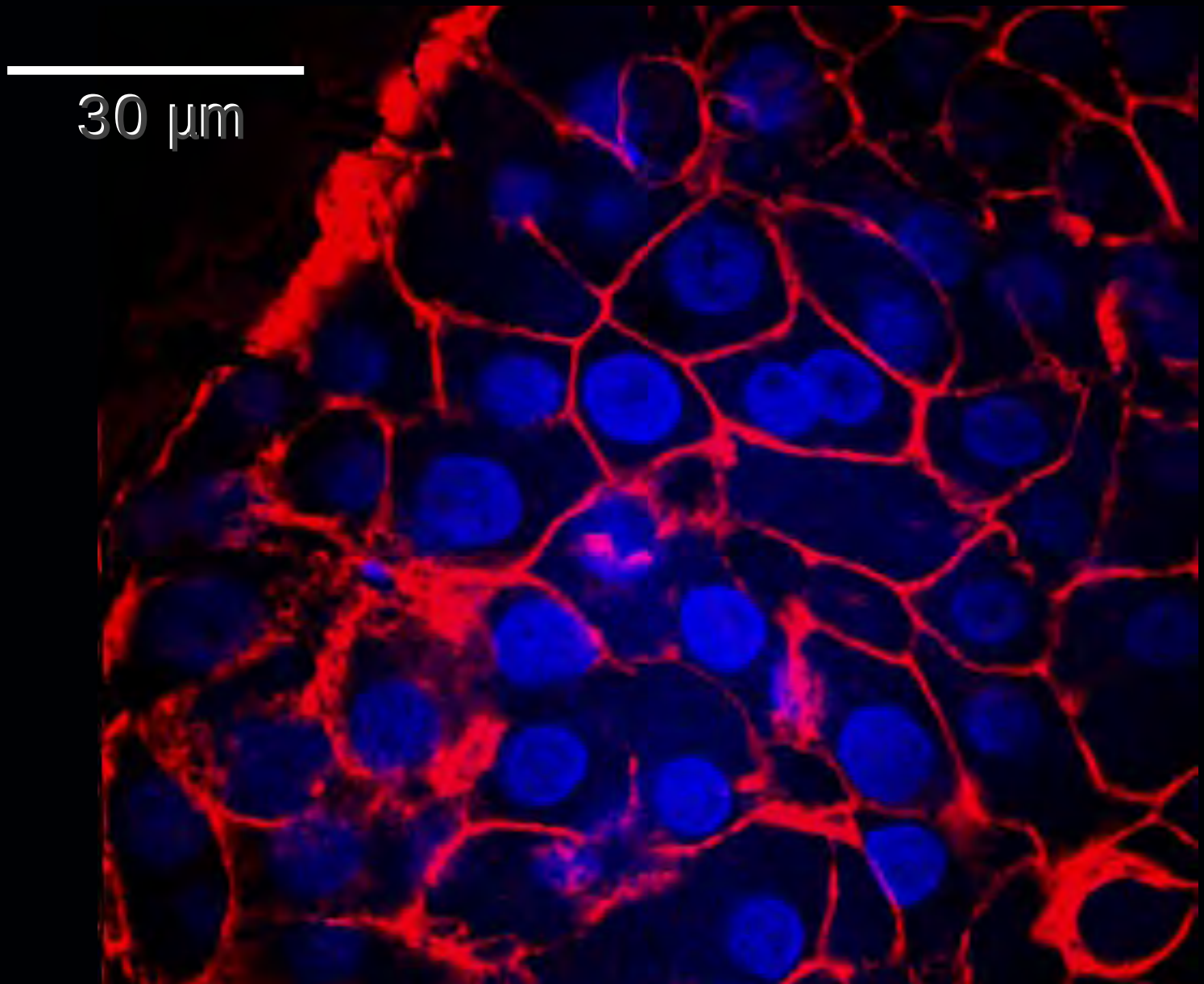
avoidance of brain death

Characterization of Ex Vivo Liver Construct by Custom Micro Array

Gene Expression in Rat Hepatocyte Spheroids

Group	# of genes	2 fold ↑	2 fold ↓	No change
Totals	250	5%	13%	82%

Confocal microscopy of Human Spheroids - Day 7



Influence of Spheroid Culture x 28 days on Hepatic Gene Expression - 1

Group	# of genes	2 fold ↑	2 fold ↓	No change
Anti-oxidants	24	9%	0%	91%
Coag. Factors	7	0%	14%	86%
Caspases	9	0%	0%	100%

Clinical Reports of Ex Vivo Liver Therapy

• HepatAssist (Arbios/Circe)	≥ 100 pts.
• ELAD System (Vital Therapies)	≥ 40 pts.
• Margulis et al. 1989	≥ 35 pts.
• MELS (Berlin)	≥ 10 pts.
• Various others (≤ 2 pts each)	≥ 8 pts
	~ 200 pts.

Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
The International Journal of Biochemistry & Cell Biology 39 (2007) 555–564
IJBCB
www.elsevier.com/locate/biocel

Ornithine transcarbamylase and arginase I deficiency are responsible for diminished urea cycle function in the human hepatoblastoma cell line HepG2

Demetra Mavri-Damelin [a,*], Simon Eaton [b], Leonard H. Damelin [a], Myrddin Rees [c], Humphrey J.F. Hodgson [a], Clare Selden [a]

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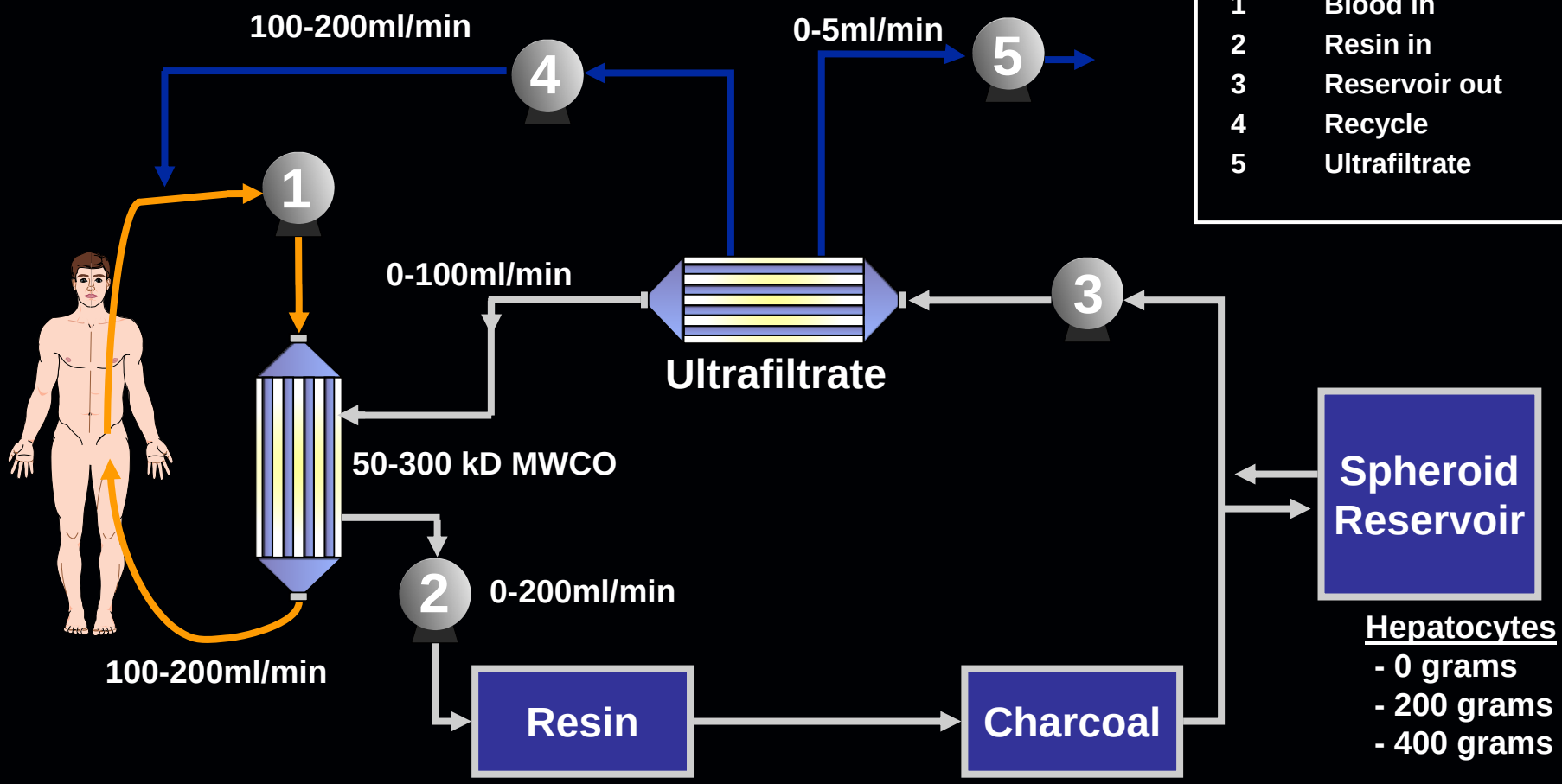
Received 1 August 2006; received in revised form 2 October 2006; accepted 11 October 2006
Available online 21 October 2006

Extracorporeal Bioartificial Liver System
Five Pumps

Pumps

1	Blood in
2	Resin in
3	Reservoir out
4	Recycle
5	Ultrafiltrate



CP1287284-2

HepatAssist™ (Circe) Phase II/III Trial

Largest Clinical Experience with BAL

- Dates: August 1,1998 - February 16, 2001
- Randomized, prospective, controlled
- Enrollment 171 (Total , 20 sites)
  147 (FHF)
  10 (Mayo)

Need Exists for Liver Support Device

- Acute failure: short-term support
  bridge to transplantation
- End-stage failure: chronic supportive therapy ?

Annals of Surgery, May 2004

Prospective, Randomized, Multicenter, Controlled Trial of a Bioartificial Liver in Treating Acute Liver Failure

Achilles A. Demetriou, MD, PhD, Robert S. Brown, Jr, MD, MPH,† Ronald W. Busuttil, MD, PhD,‡ Jeffrey Fair, MD,§ Brendan M. McGuire, MD,¶ Philip Rosenthal, MD,‖ Jan Schulte Am Esch, II, MD,** Jan Lerut, MD,†† Scott L. Nyberg, MD, PhD,‡‡ Mauro Salizzoni, MD,§§ Elizabeth A. Fagan, MD¶¶ Bernard de Hemptinne, MD,‖‖ Christoph E. Broelsch, MD, PhD,*** Maurizio Muraca, MD, PhD,††† Joan Manuel Salmeron, MD,‡‡‡ John M. Rabkin, MD,§§§ Herold J. Metselaar, MD,¶¶¶ Daniel Pratt, MD,‖‖‖ Manuel De La Mata, MD,**** Lawrence P. McChesney, MD,†††† Gregory T. Everson, MD,‡‡‡‡ Philip T. Lavin, PhD,§§§§ Anthony C. Stevens, MD,¶¶¶¶ Zorina Pitkin, PhD,¶¶¶¶ and Barry A. Solomon, PhD¶¶¶¶*

Objective: The HepatAssist liver support system is an extracorporeal porcine hepatocyte-based bioartificial liver (BAL). The safety and efficacy of the BAL were evaluated in a prospective, randomized, controlled, multicenter trial in patients with severe acute liver failure.

Summary Background Data: In experimental animals with acute liver failure, we demonstrated beneficial effects of the BAL. Similarly, Phase I trials of the BAL in acute liver failure patients yielded promising results.

From the *Liver Support Unit, Department of Surgery, Cedars-Sinai Medical

Circe Study Patient #163
after Liver Transplant and
7 BAL treatments

One year follow-up

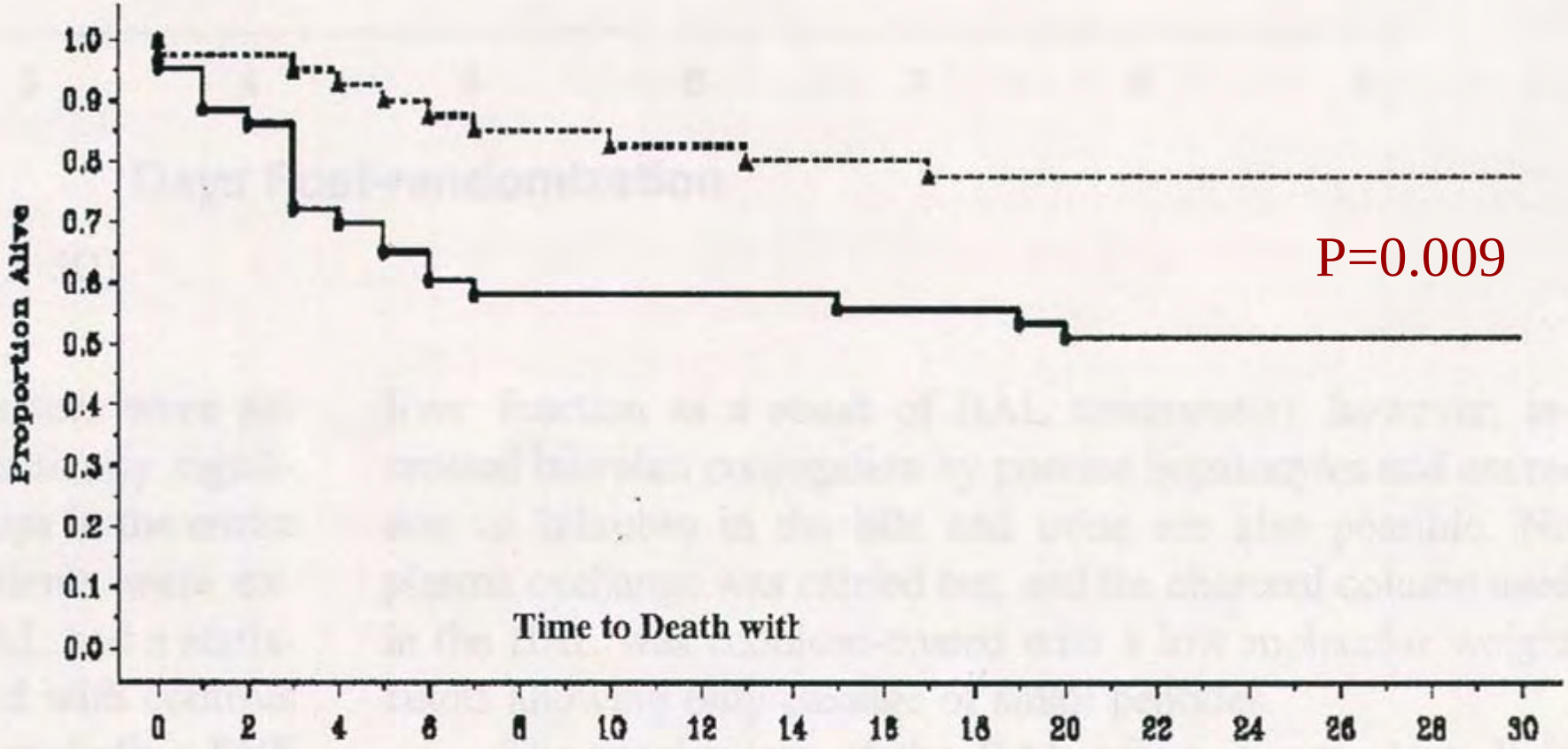
Patient with husband and newborn son
14 months after BAL therapy and OLTx for FHF

Summary of Causes of Death (n=58)

	LAS (n)	C (n)	Total (n)	% total deaths
Neuro causes	8	14	22	37
Sepsis	7	9	16	27
Multiorgan fail	5	8	13	23
Bleeding	4	3	7	12

Mortality: 28% vs 40% p > 0.10

Time to Death: Known Cause of FHF (n=87)



Next generation BAL systems