TE: liver

Tissue Engineering: the liver

Funzioni del fegato

Ghiandola esocrina (bile) e endocrina (lipoproteine, ..)

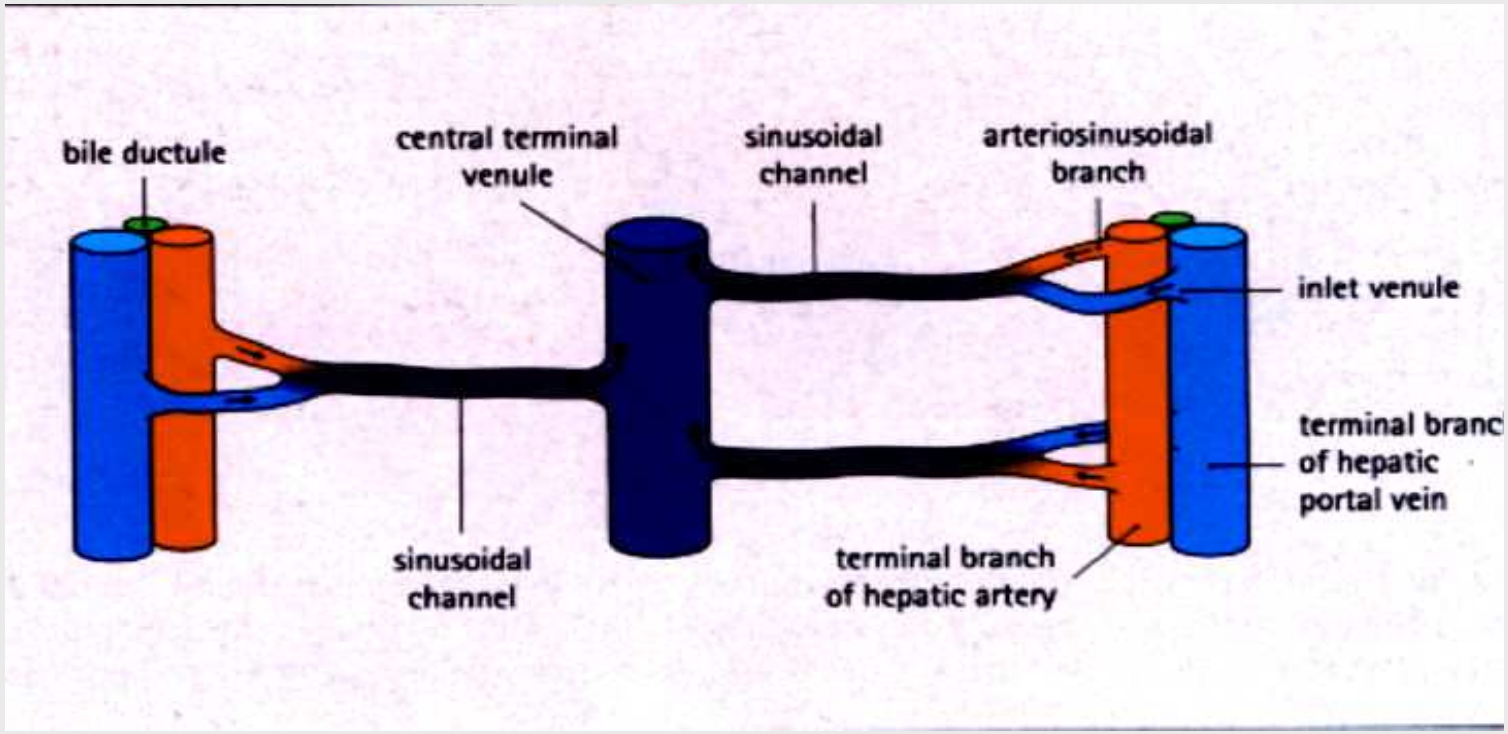
- Centralina metabolica:
  - Metabolismo dei lipidi (P-lipidi, colesterolo, …)
  - Metabolismo delle proteine (proteine plasmatiche, a.a, ..)
  - Metabolismo dei glicidi (sintesi glicogeno, …
- Detossificazione prodotti metabolici di scarto
- Detossificazione farmaci e tossine
- Distruzione eritrociti
- Sintesi e secrezione della bile

Il fegato

- STRUTTURA E COMPOSIZIONE
- Lobi, lobuli

- Epatociti et al (pochi)
- Vasi (arteria e vena epatica, vena porta, rete sinusoidale con capillari discontinui fenestrati, linfatici) con cellule del sangue
- Dotti biliari

Vascolarizzazione e microcircolazione del fegato

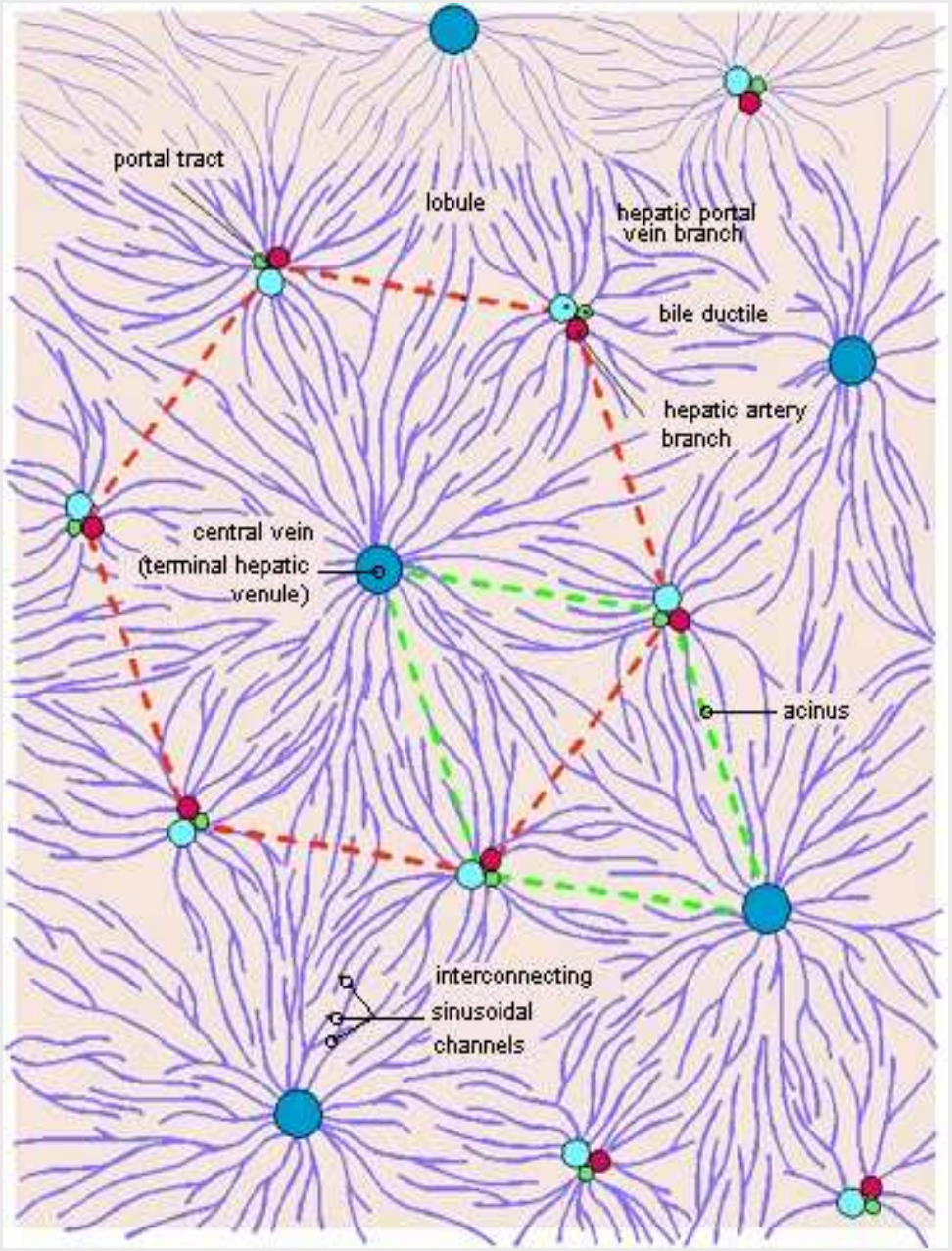


Il fegato

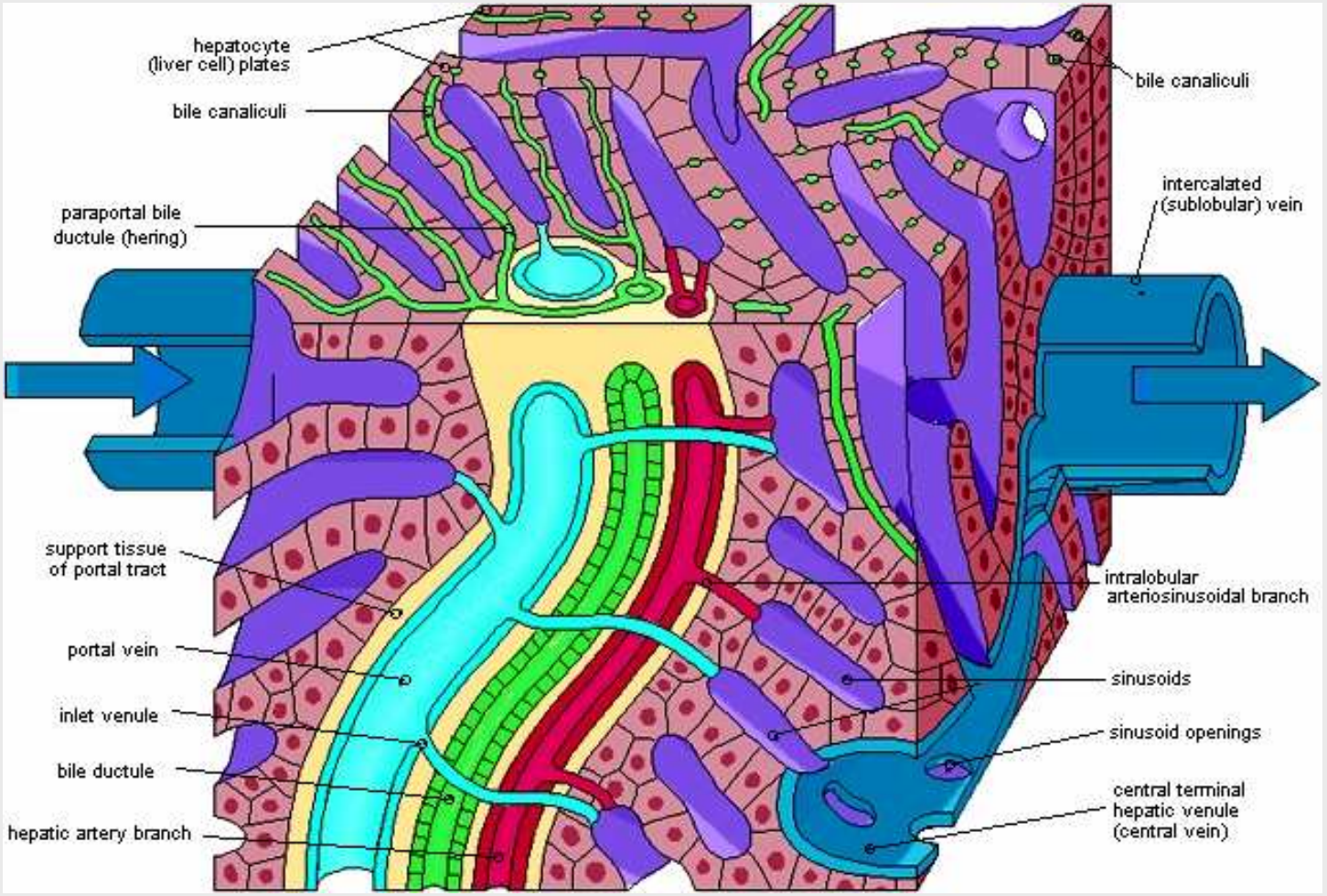
Lobulo epatico con vena centrolobulare

Definito da setti connettivali non molto evidenti nel fegato umano

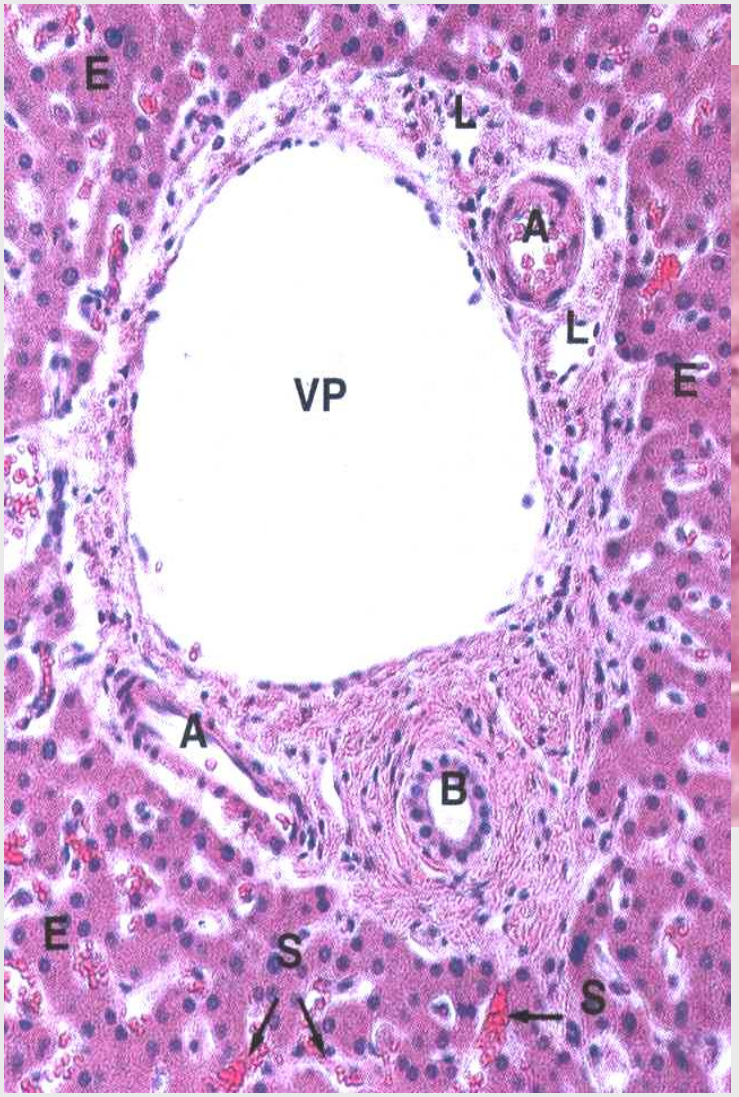
Agli apici: spazi portali

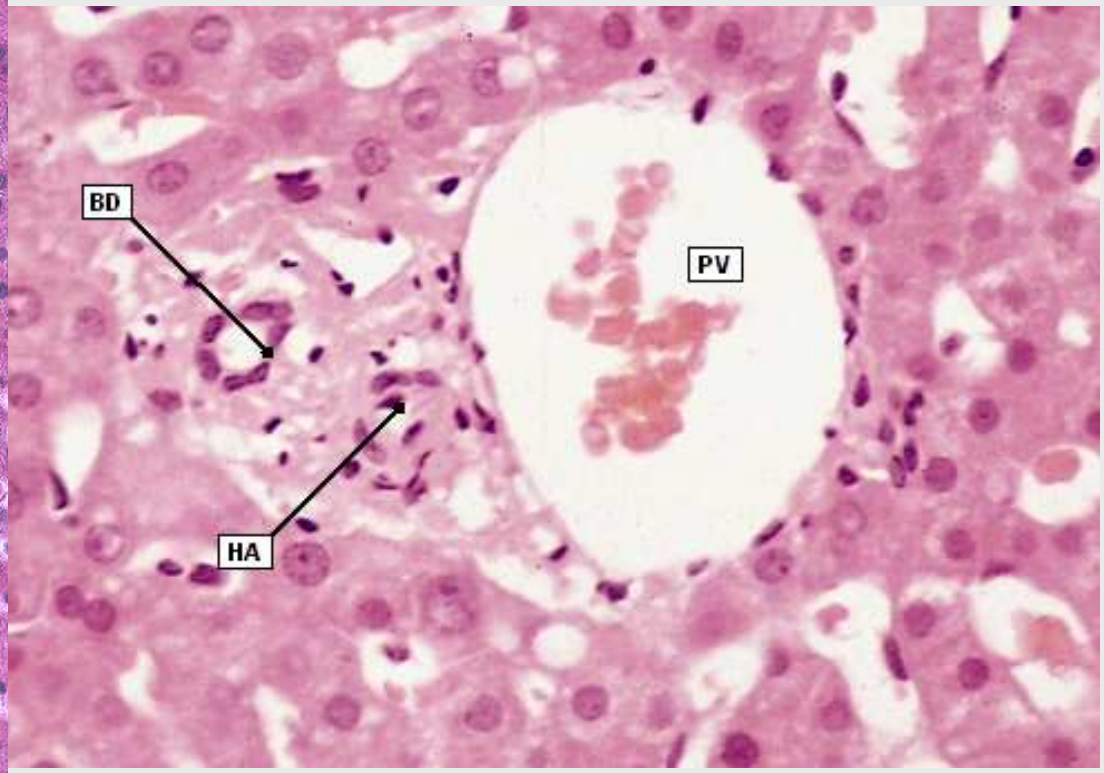


Il fegato: struttura



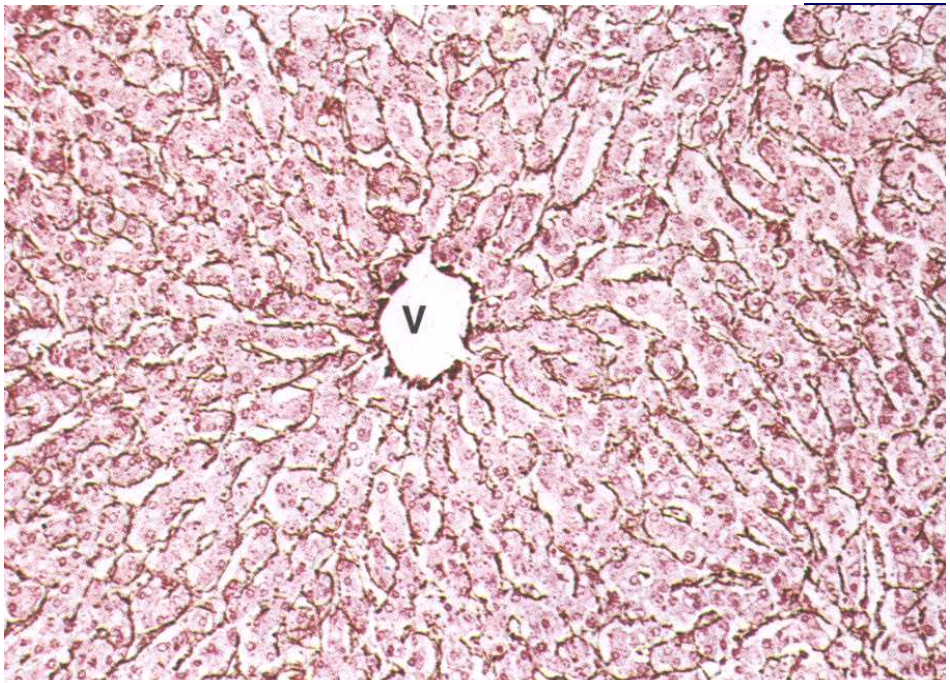
Il fegato: lo spazio portale

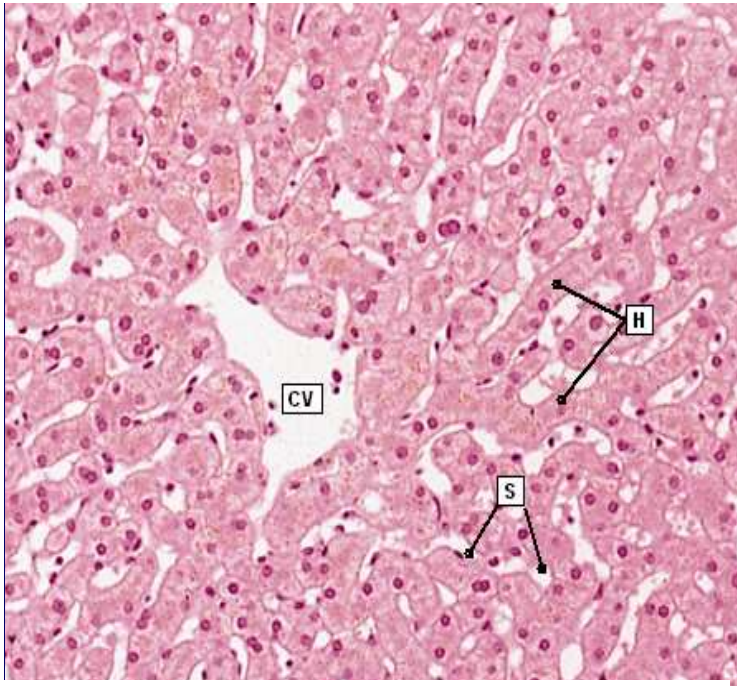




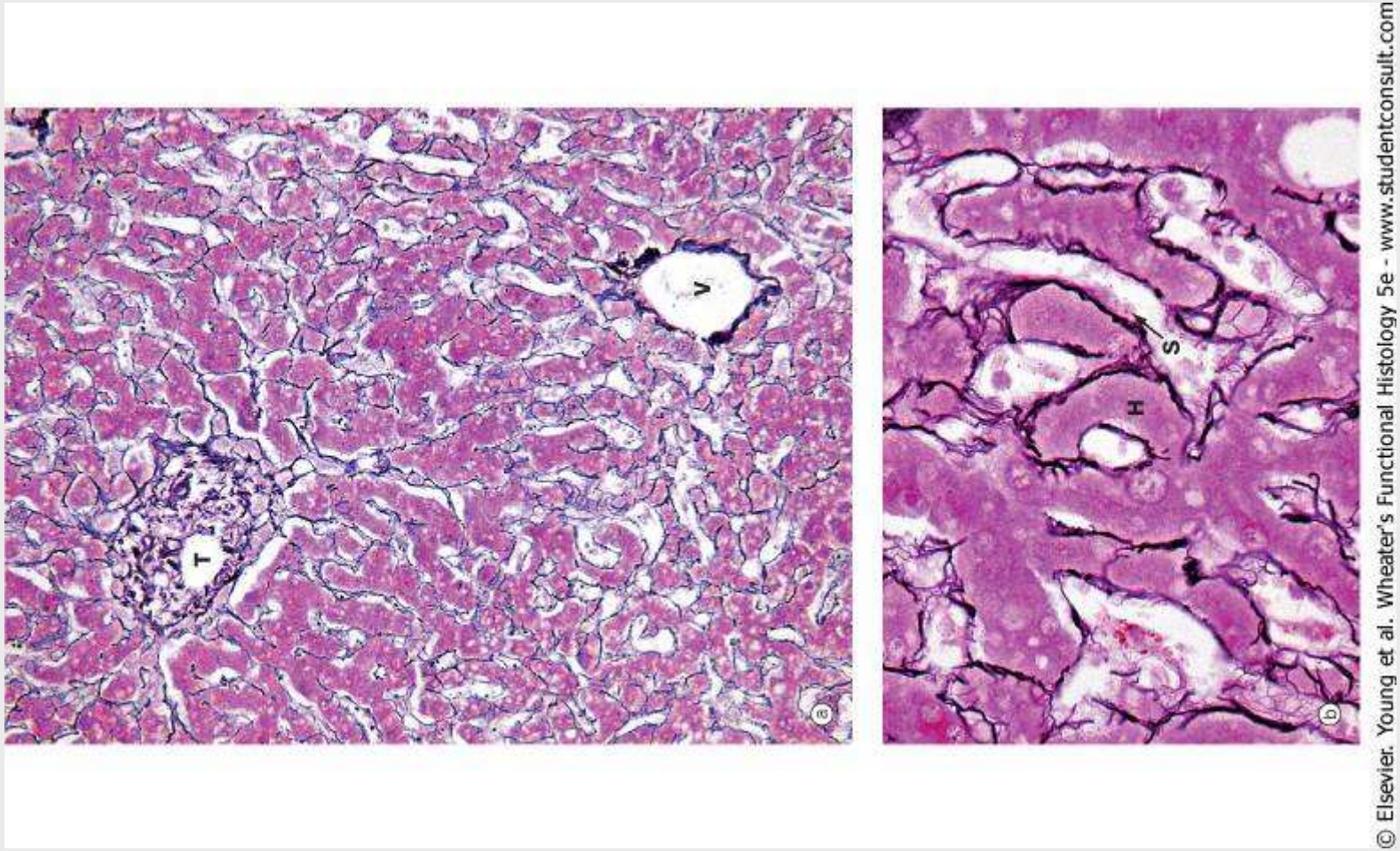
La triade:
arteriola epatica, ramo della vena porta, duttulo biliare
(+ ramo linfatico)

Il fegato: la vena centrolobulare



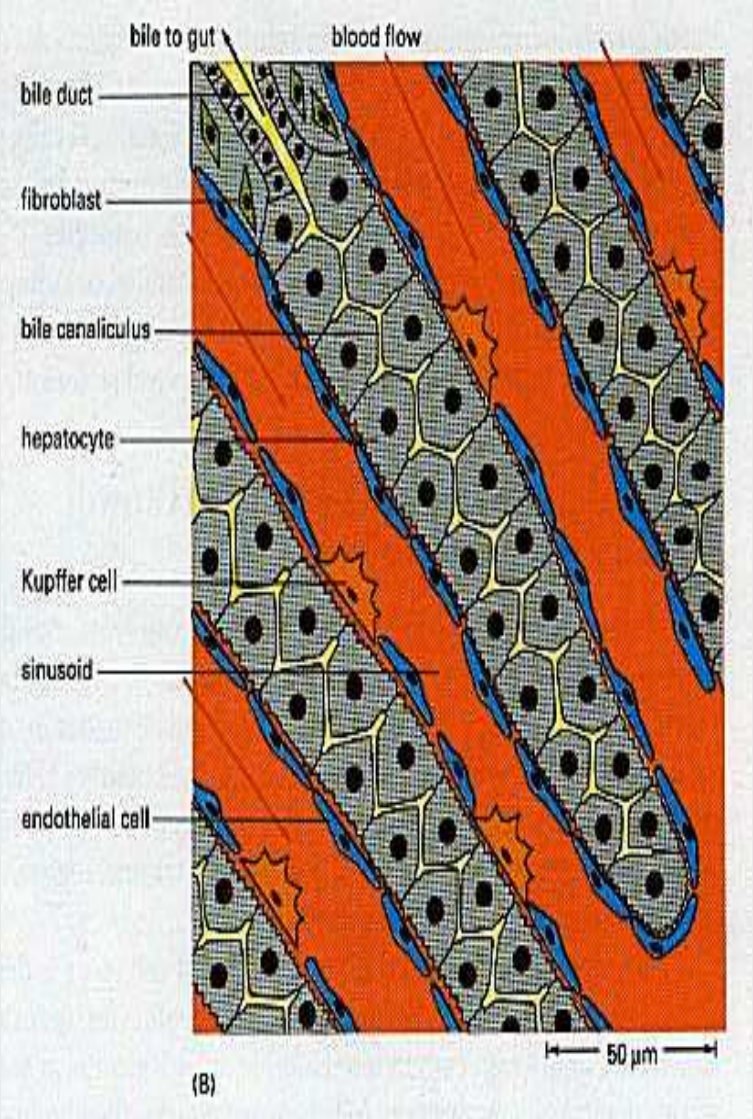


Il tessuto di supporto del fegato

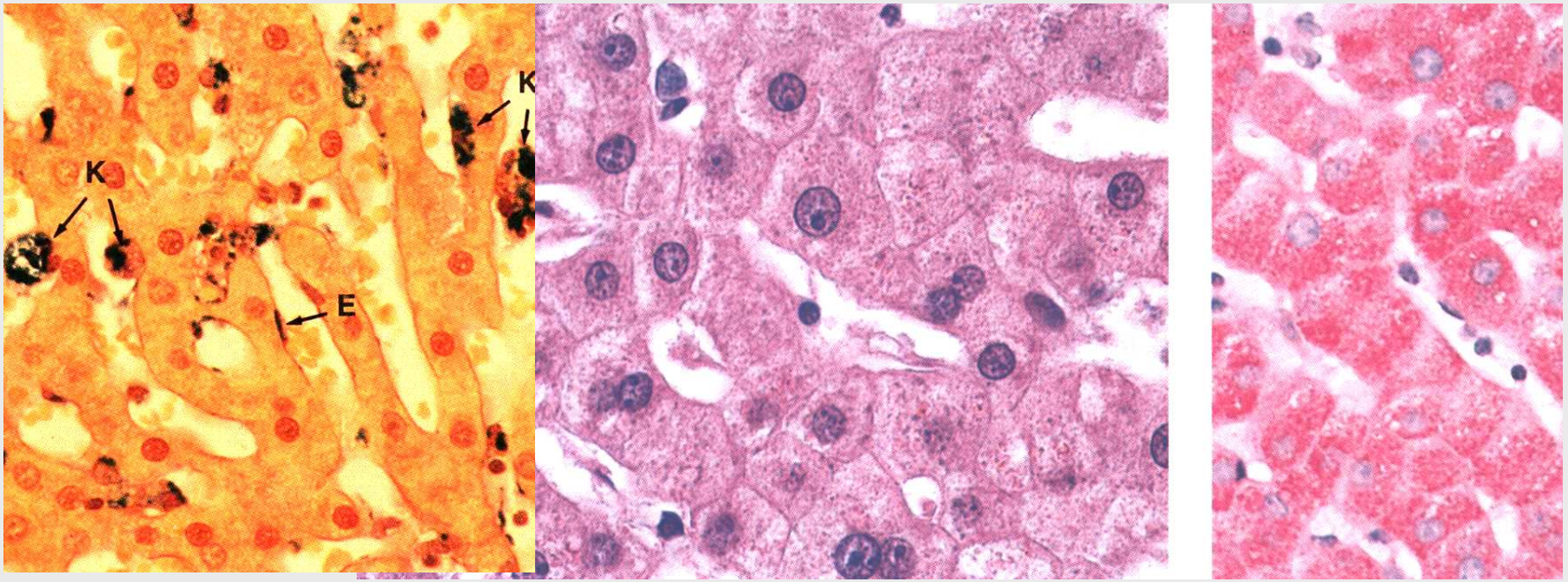


Il fegato: schema

Stretto rapporto epatociti-
rete sinusoidale

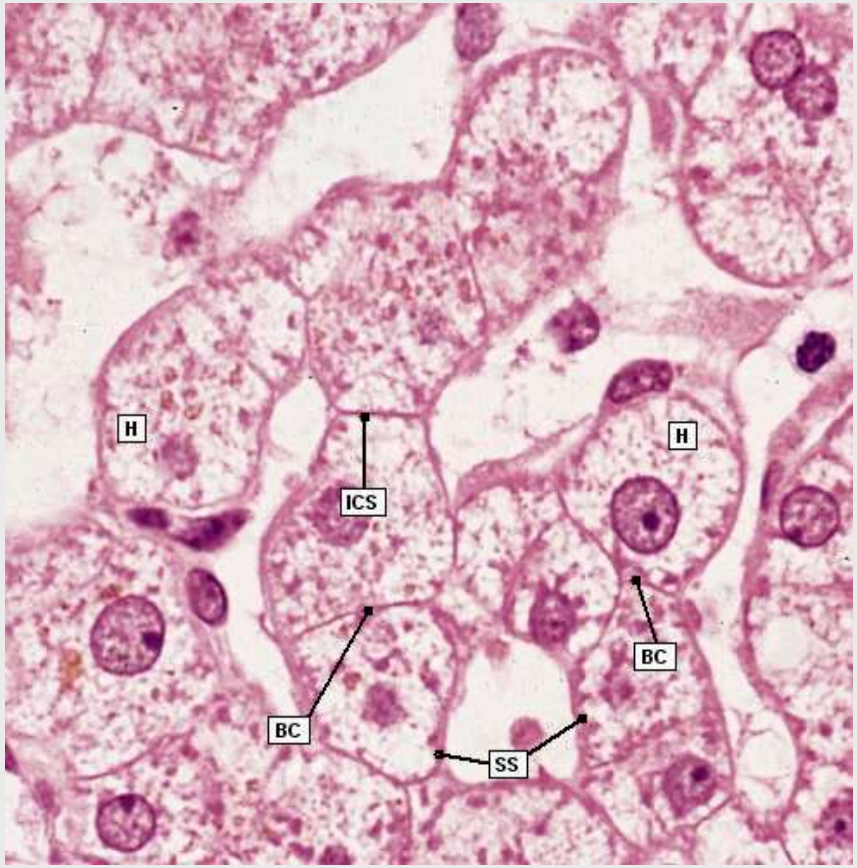


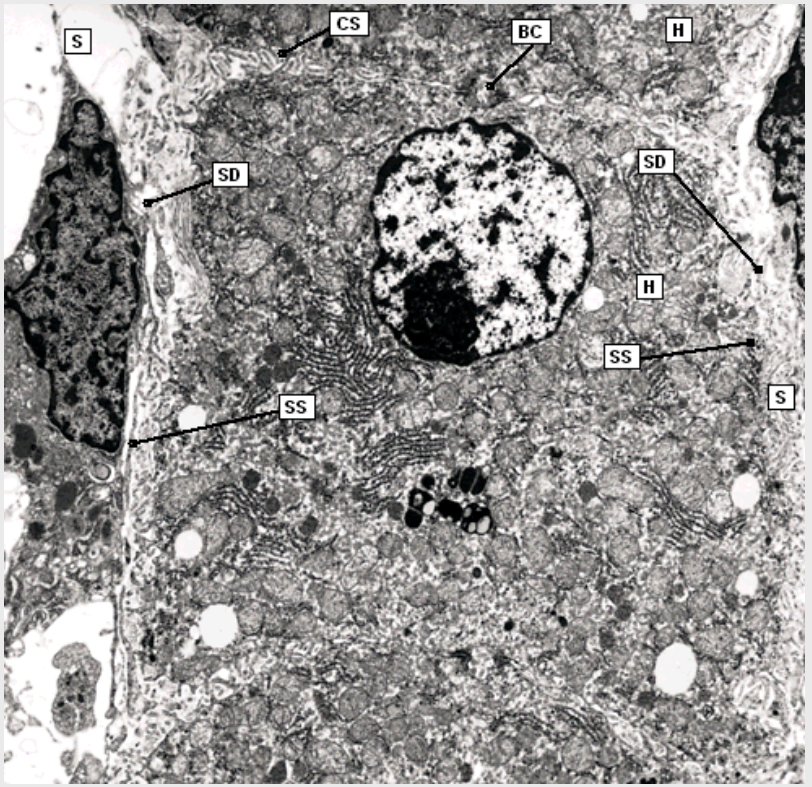
Le cellule del fegato



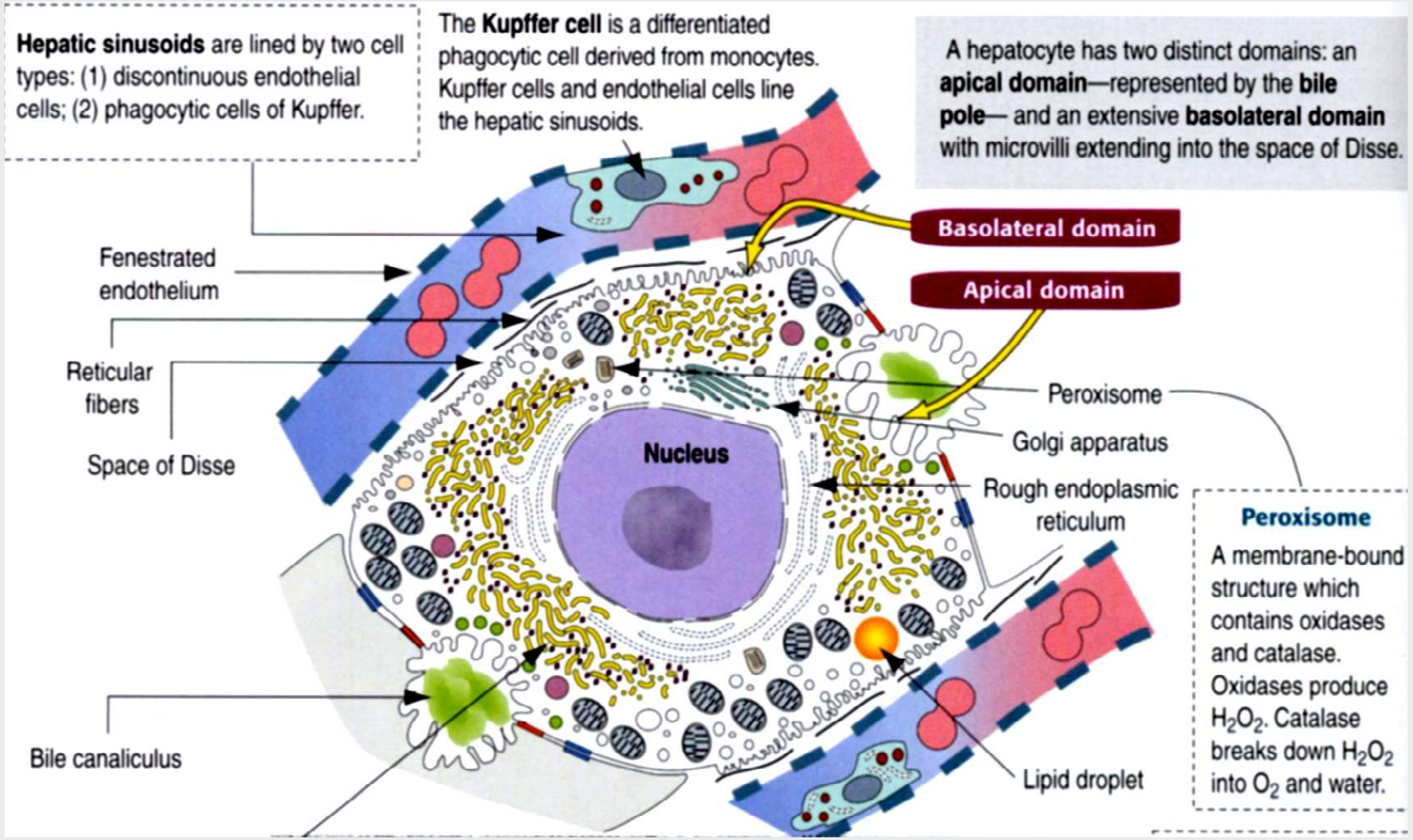
Epatociti, cellule di Kupffer, cellule di Ito, cellule ovali
Cellule endoteliali, cellule del sangue

Il fegato: gli epatociti

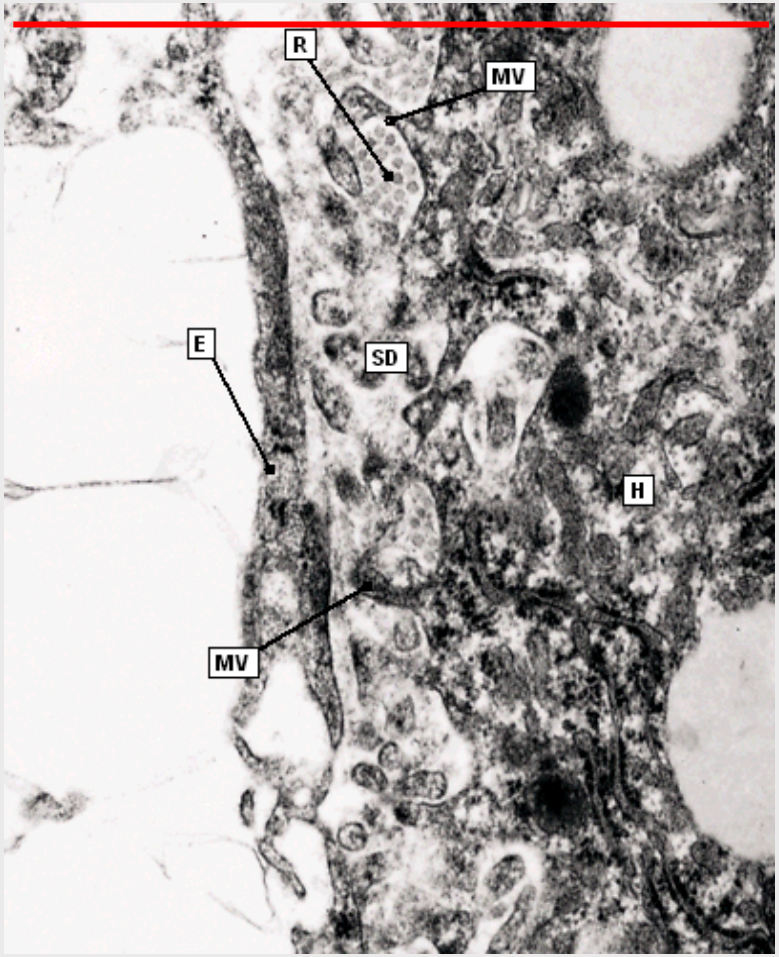


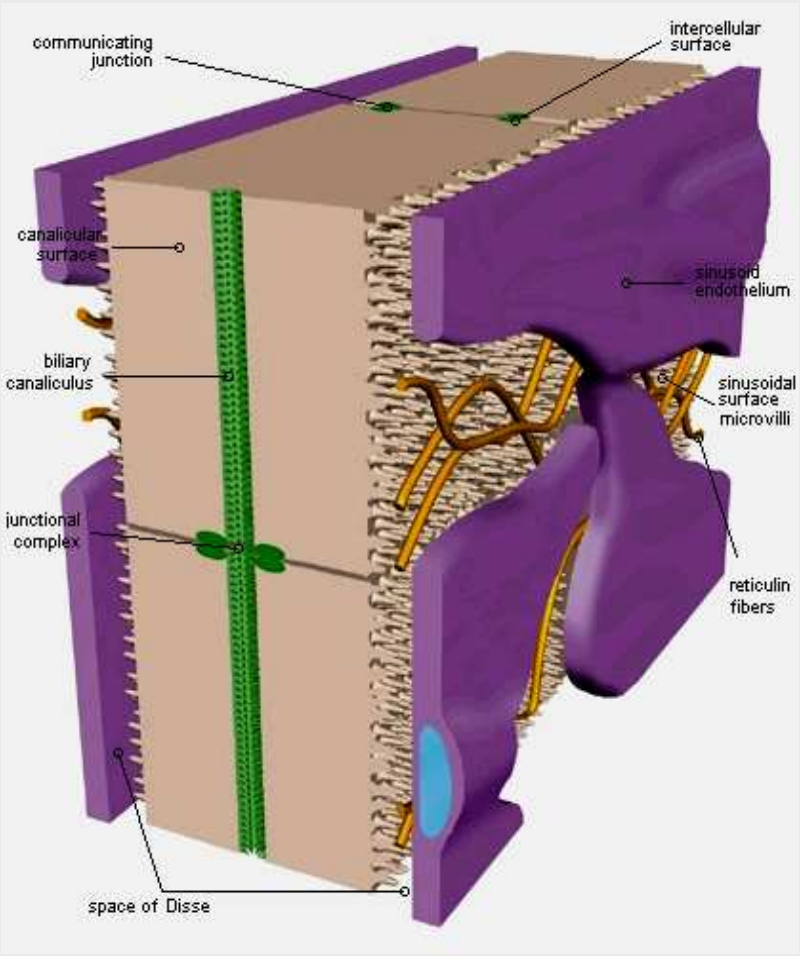


Dominii apicale e baso-laterale dell'epatocita

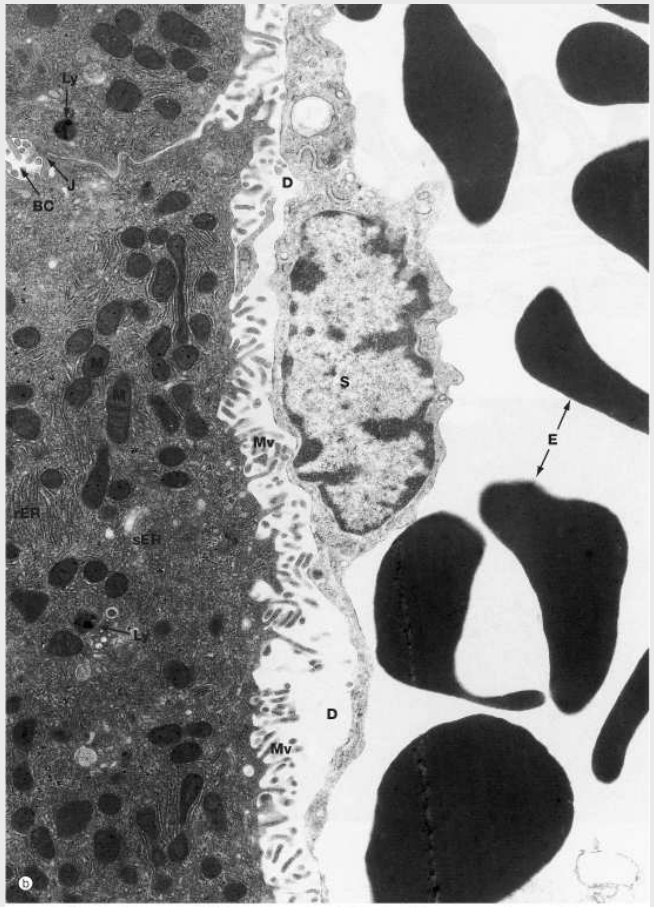


Il fegato:
interfaccia epatocita/sinusoide

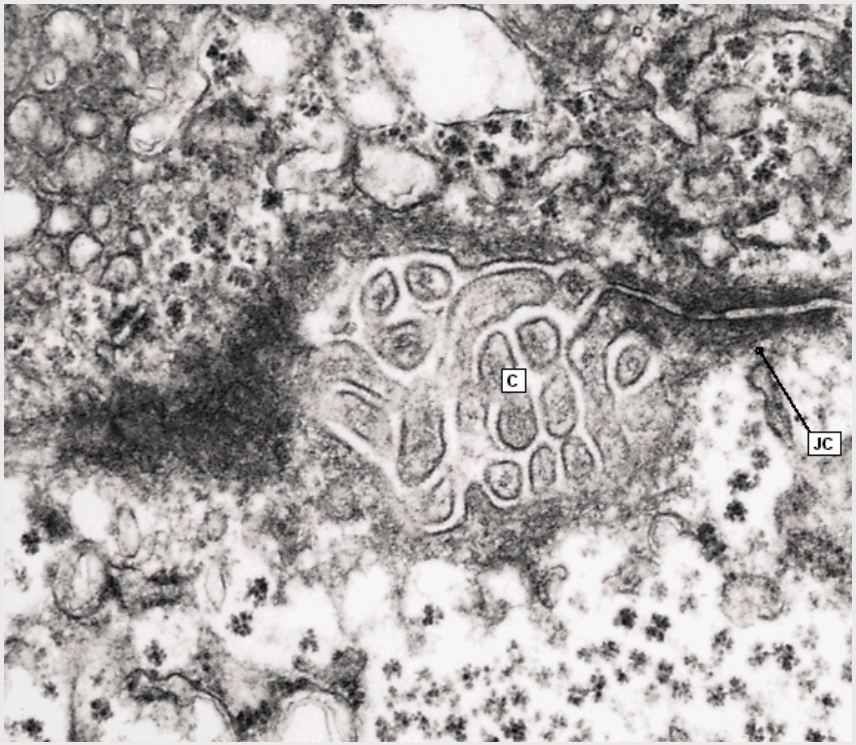


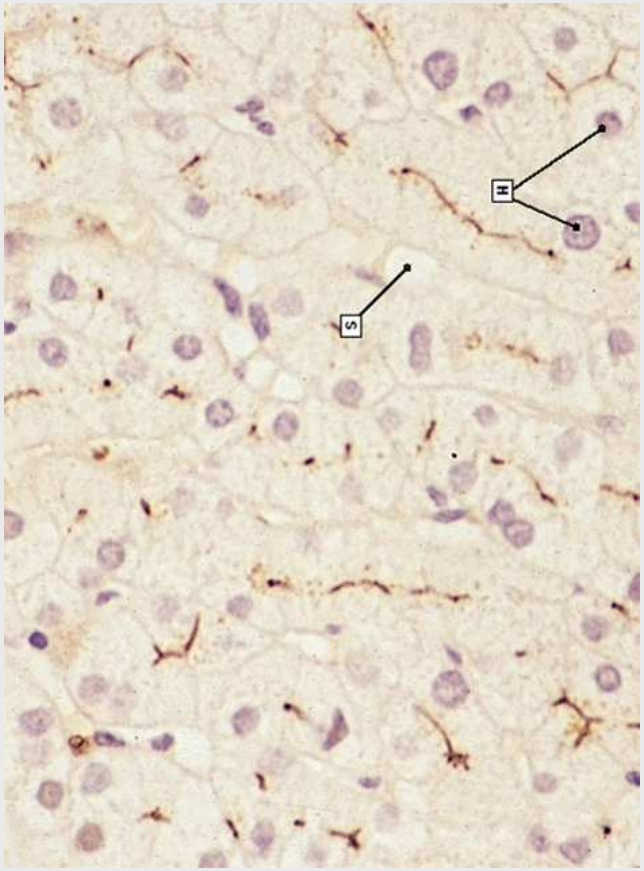


Il fegato: interfaccia epatocita/sinusoide

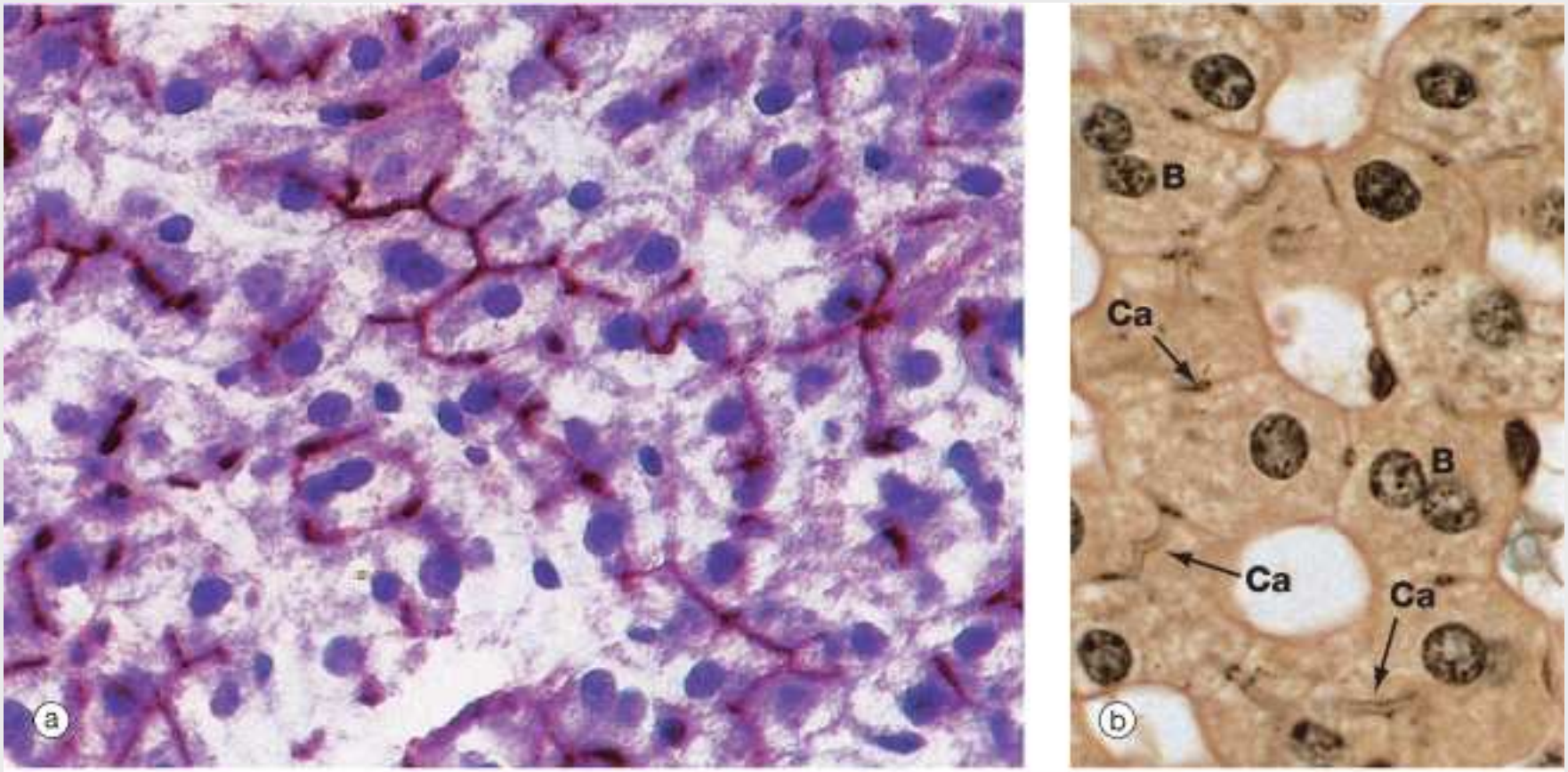


Il fegato:
secrezione della bile





Il fegato:
secrezione della bile



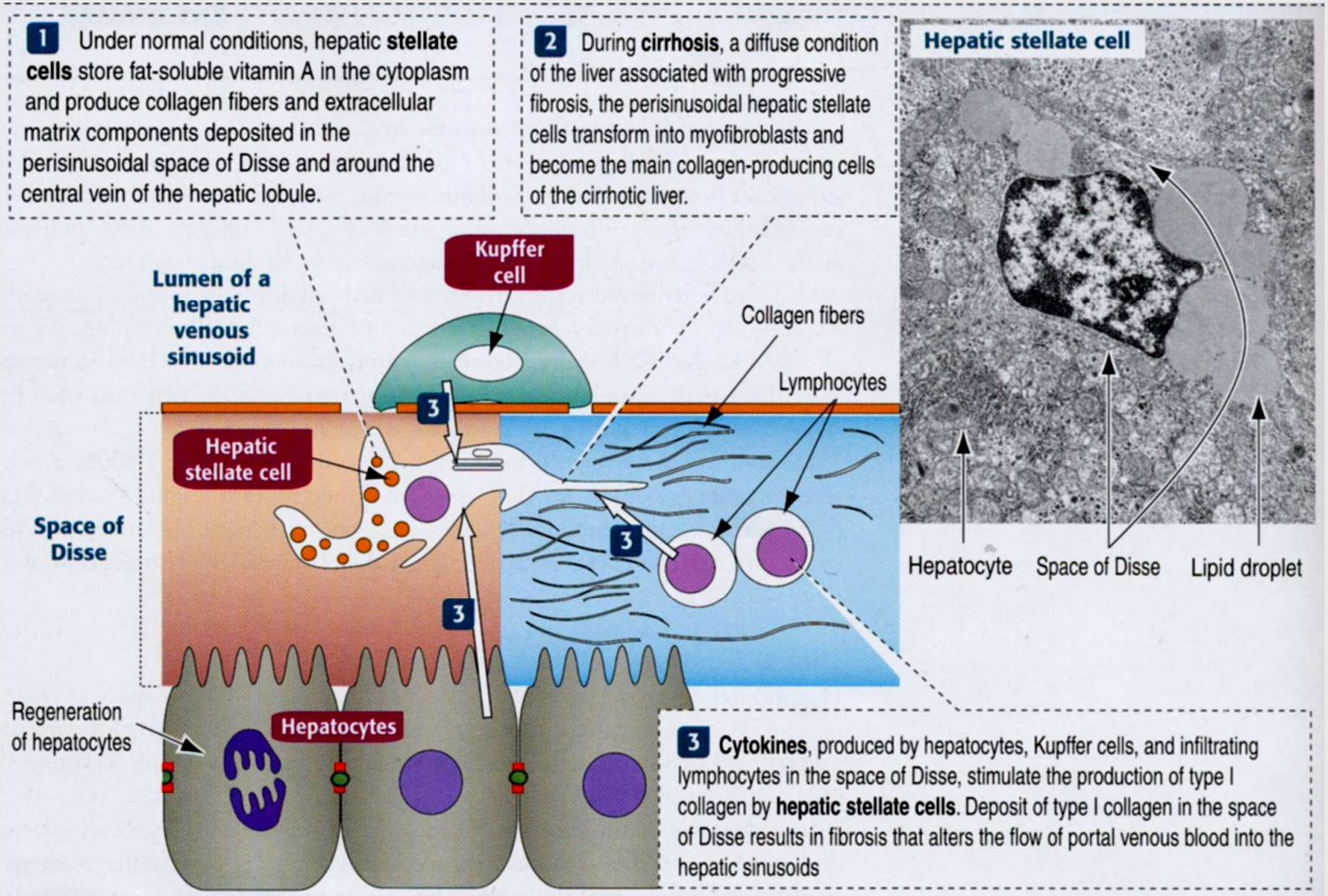
Omeostasi del fegato

- 2/3 epatectomia → rigenerazione completa in ca. 2 settimane
- (aumento di HGF nel plasma)
- Avvelenamento da barbiturici → aumento del volume del fegato che deve detossificare → ripristino della situazione normale (autolisi)
- Concetto del fattore di sopravvivenza

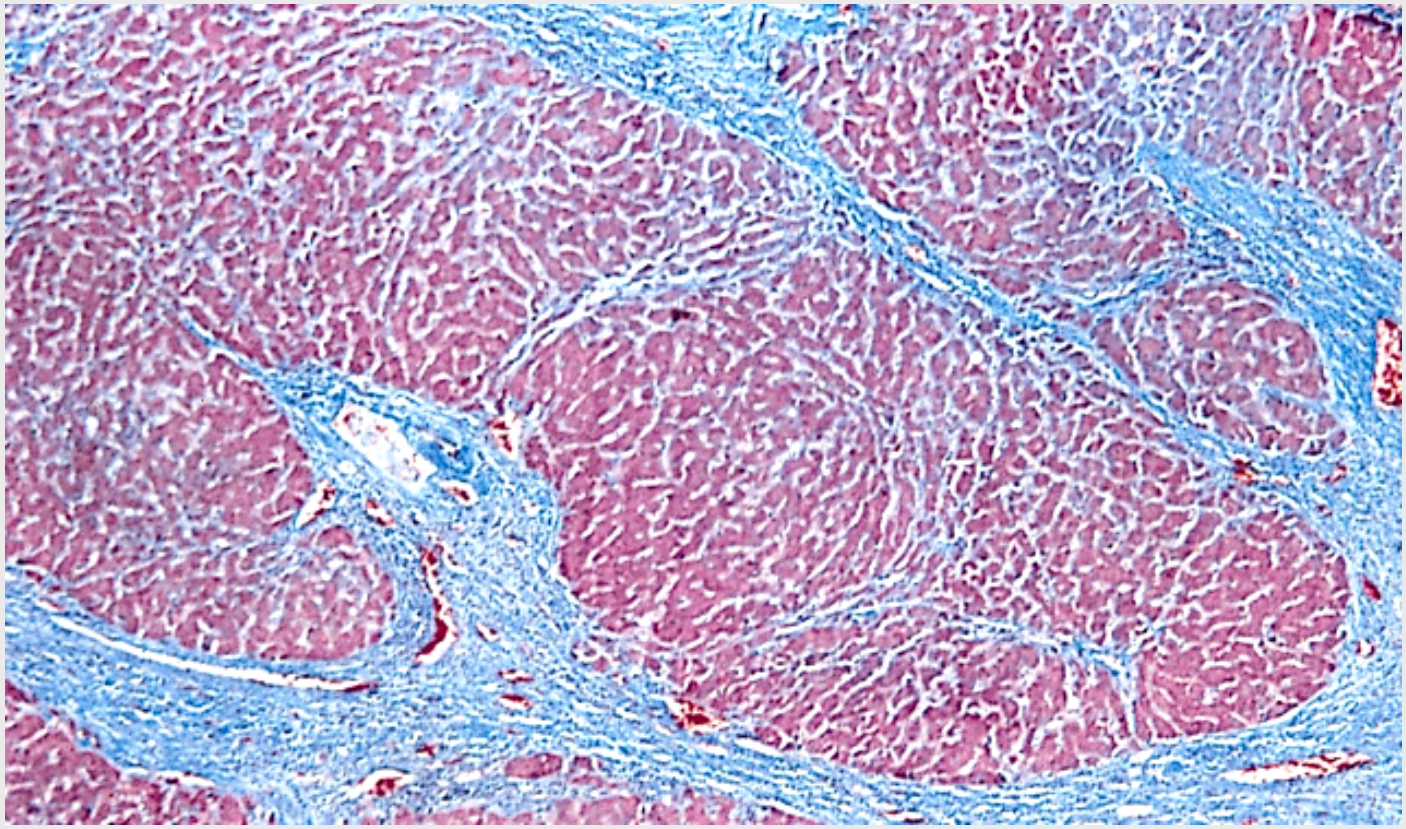
Omeostasi del fegato

- La rigenerazione deve essere bilanciata per tutti i suoi componenti
- Se danneggiato ripetutamente → cirrosi, per crescita preponderante della componente connettivale

La cellula stellata di Ito è coinvolta nella fibrosi



Cirrosi epatica



Patologie del fegato

- Cirrosi
- Insufficienza epatica acuta
- Malattie genetiche (mutazione di geni codificanti proteine/fattori della coagulazione)

BAL = bioartificial liver

- Artificial extracorporeal supportive device for an individual who is suffering from acute liver failure (ALF) or intoxication
- Bioreactor containing hepatocytes that perform the functions of a normal liver
- It processes oxygenated blood plasma, which is separated from the other blood constituents

Hollow fiber system

- Cartuccia di fibre cave semipermeabili contenenti epatociti che si attaccano al gel (ex. di collagene) che riveste le fibre
- → filtrazione del plasma
- → mantenute le funzioni metaboliche (omeostasi carboidrati, sintesi di lipoproteine, siero albumina, fattori della coagulazione, …

Transplantation of liver cells

- BAL are very expensive are require intensive care unit setting
- The transplantation of a hepatocyte mass equivalent to 10% of the patients' liver would be sufficient to normalize the metabolic situation in many enzyme deficiencies
- → higher yield, less immunogenicity, possibility to modify *in vitro* (metabolic diseases)

Transplantation of liver cells

- Hepatocyte transplantation procedure can be limited in the number of cells that can be infused at one time (portal hypertension, portal vein thrombosis and pulmonary embolism)
- Moreover, most of them do not survive
- Isolated hepatocytes engineered into biologically active tissues, which have the ability to simulate whole-organ liver function in an ectopic site for extended periods of time (> 6 months)

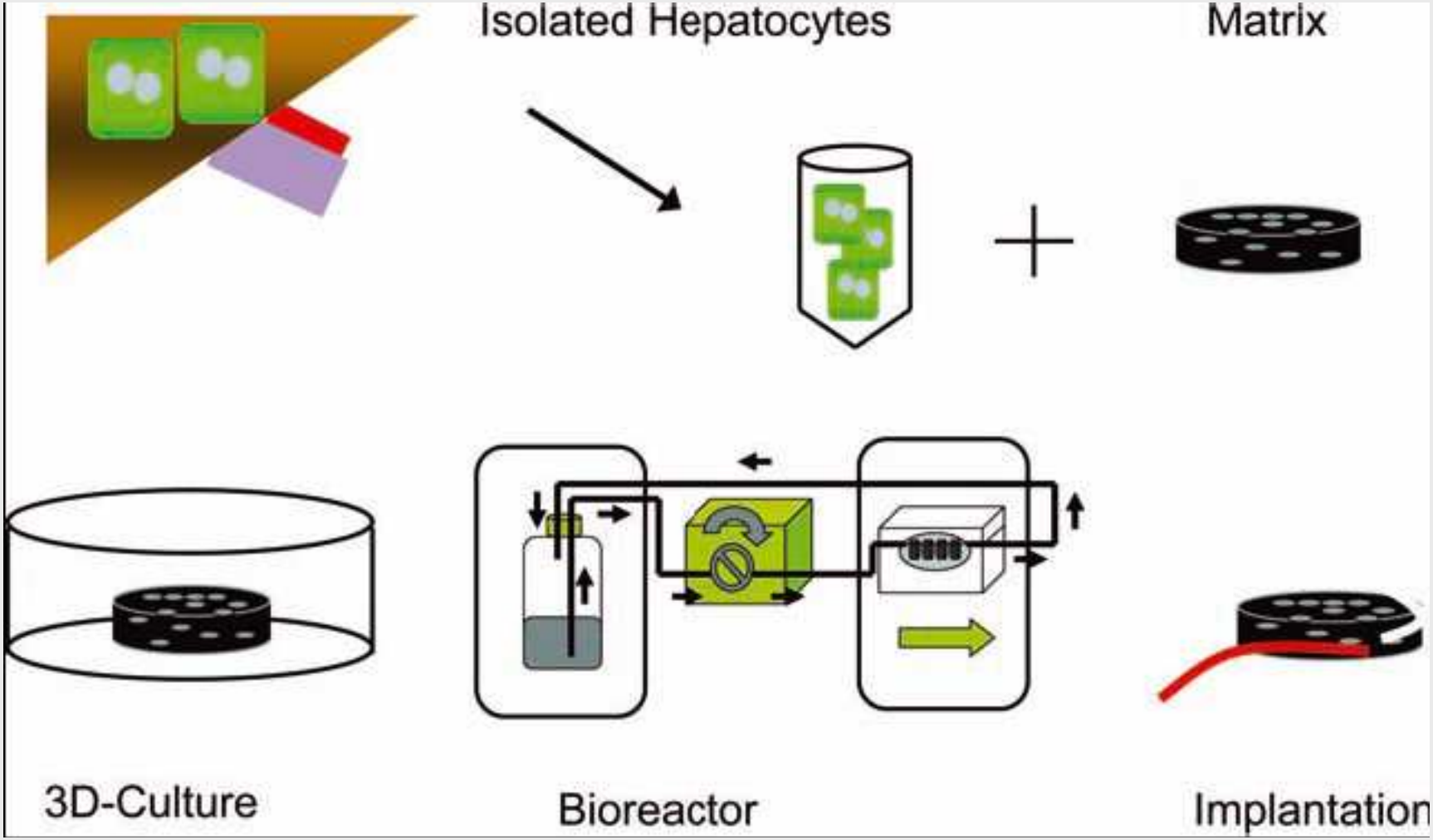
Artificial liver tissue

- recovery of hepatocytes (perfusion: 1967..)
- expansion of cells in an *in vitro* phase (Growth Factors, co-cultures, etc)
- genetic or immunological manipulation of cells for transplantation
- tissue typing and cryopreservation in a cell bank
- ex vivo genetic modification of patient's own cells prior to re-implantation

Artificial liver tissue

- Function and differentiation of liver cells are influenced by the three-dimensional organ architecture
- Polymeric 3D constructs (with 3D matrices → great surface/volume ratio) are required in a bioreactor

Tissue engineering approach for the liver



J. Cell. Mol. Med. Vol 12, No 1, 2008

Artificial liver support

- BAL = viable hepatocytes which are connected to the patient's circulation
- liver tissue homogenate could produce urea from ammonia chloride (Sorrentino, 1956)
- hepatoma cells placed in a hollowfibre cartridge could effectively conjugate bilirubin (Wolf, 1975)
- Charcoal hemoperfusion (1988)
- MARS (molecular adsorbent recirculating system
- FPAD (Fractionated Plasma Separation, Adsorption and Dialysis system, Prometheus) (1993)

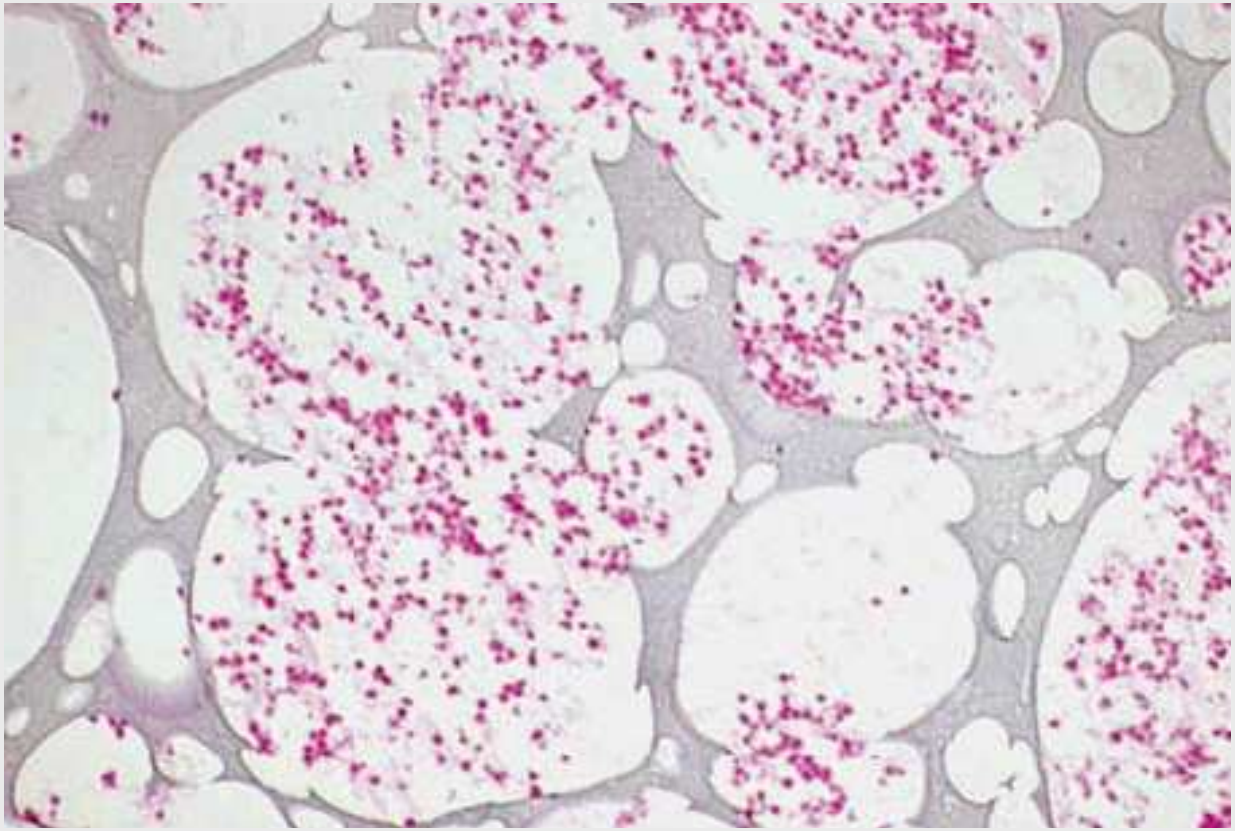
Clinical trials of BAL devices

Table 1 Clinical trials of BAL devices

Device	Cell Source	Study Type	Patients Treated	Comments
ELAD	C3A-cells (hepatoma-derived)	Phase I/II	52 [45, 46]	
HepatAssist	Porcine	Phase II/III	171 (86 patients. In control group, 85 received BAL treatment) [47]	Survival advantage in FHF
MELS	Human	Phase I	20 [49, 50]	Patients bridged to transplantation; system based on BELS (which was based on porcine hepatocytes)
AMC-BAL	Porcine	Phase I	8 with acute HF [53]	7 patients bridged to transplantation, 1 recovered
BLSS	Porcine	Phase I/II	4 [82]	Decreased bilirubin, lactate and ammonia levels

BELS, bioartificial extracorporeal liver support.

Rat hepatocytes seeded on a 3D polymeric matrix form a 3D tissue in vitro after 3 days in culture



Achievements of heterotopic hepatocyte transplantation using 3D matrices

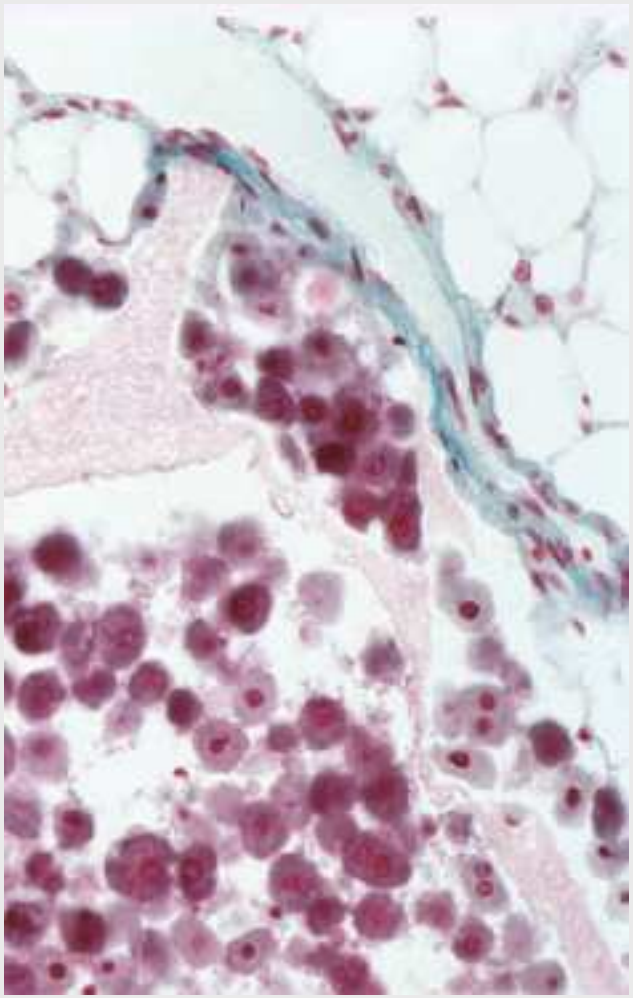
Goal	Comment
Whole liver mass transplantation	
Coating of matrices with ECM molecules or attachment of growth factors	Increased cell engraftment and function
Transplantation of genetically altered cells	Correction of metabolic defects
Co-transplantation of different cell types	Increased hepatocyte survival and proliferation
Long-term data after hepatocyte transplantation	
Correction of vitamin C deficiency	ODS rat

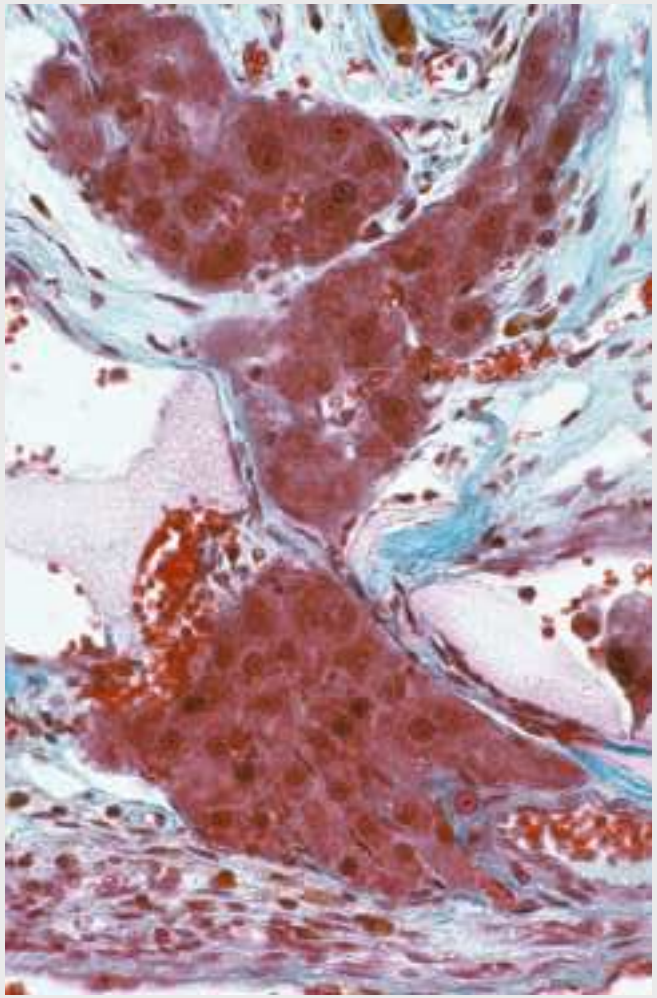
ODS, osteogenic disorder Shionogi

Cell-seeded matrix at implantation between the mesenteric leaves (peritoneal cavity)

Cell-seeded matrix is maintained in the peritoneum

d 0





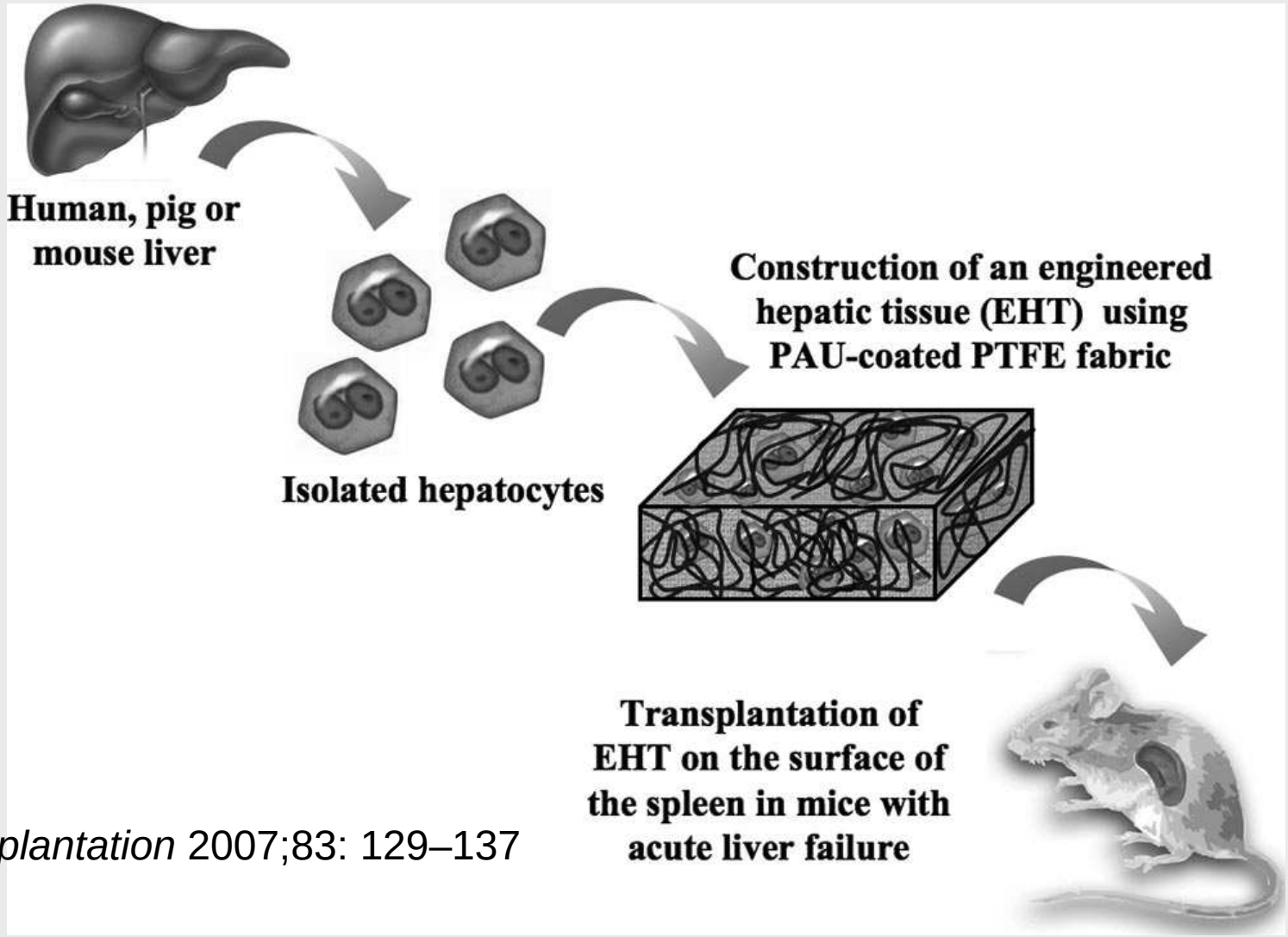
d 90

EHT (engineered hepatic tissue)

- human, pig, mouse hepatocytes
- polyaminourethane (PAU)-coated, nonwoven polytetrafluoroethylene (PTFE) fabric

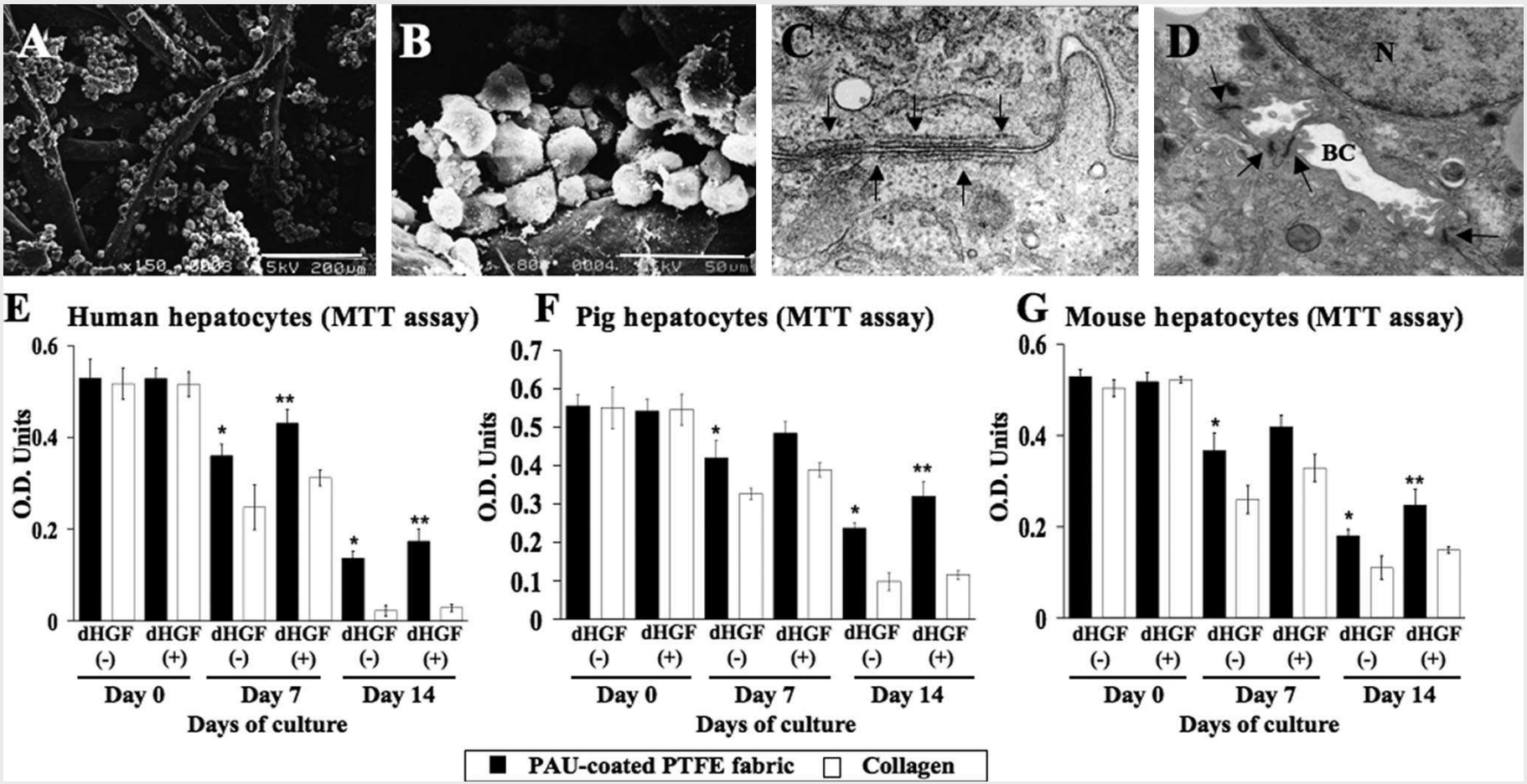
Transplantation 2007;83: 129–137

Experimental design

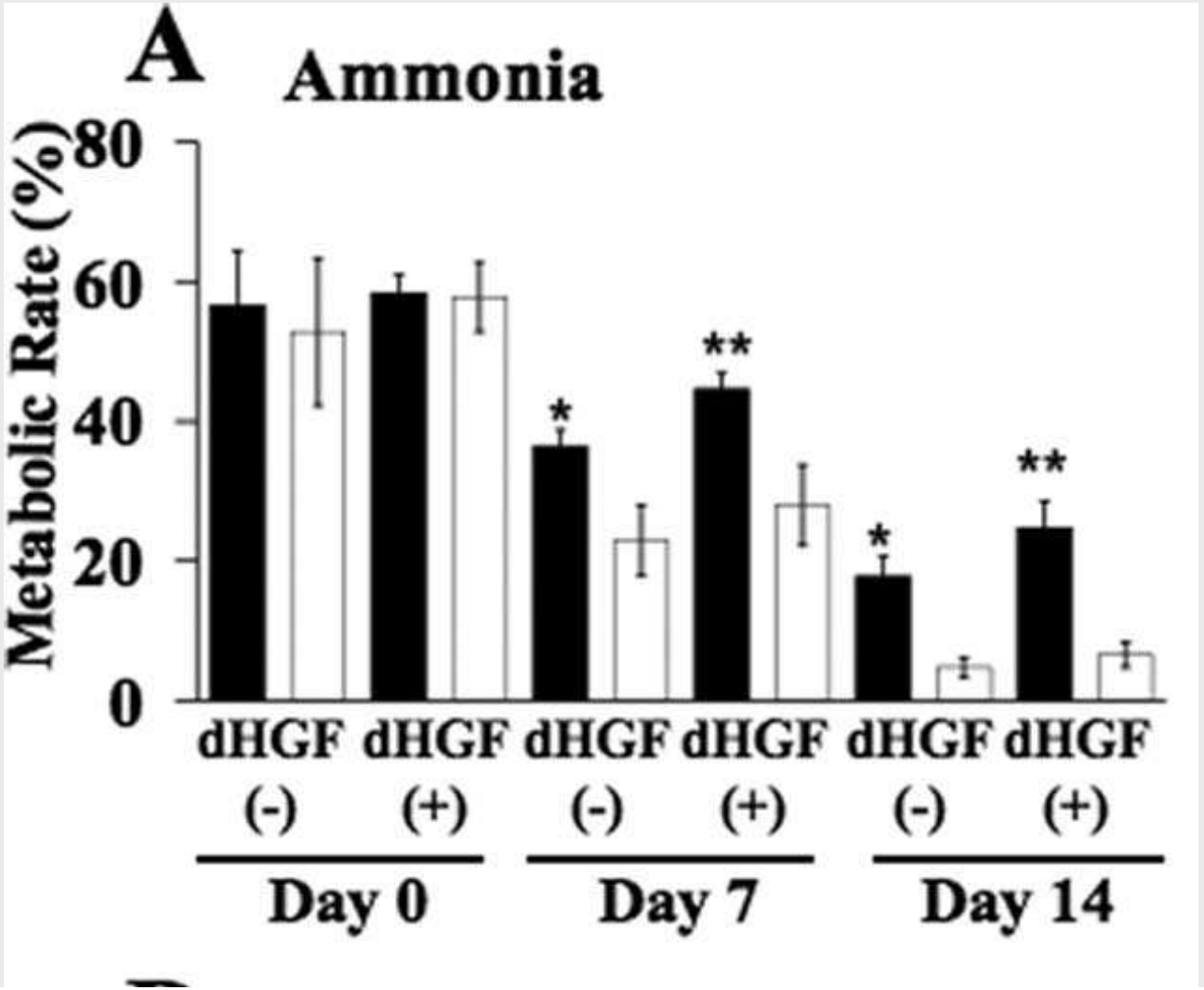


Transplantation 2007;83: 129–137

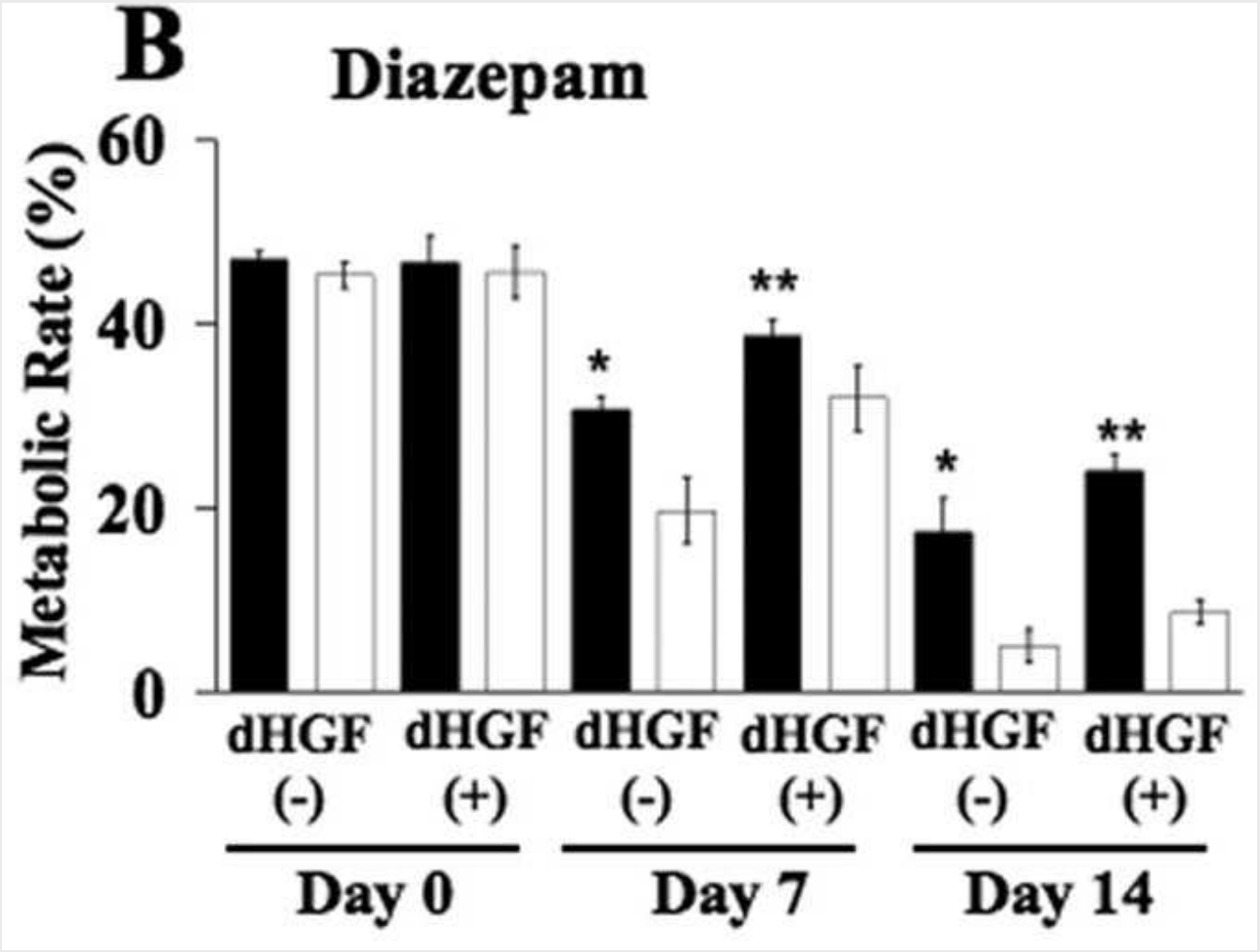
Morphology and viability of hepatocytes cultured onto PAU-coated PTFE.



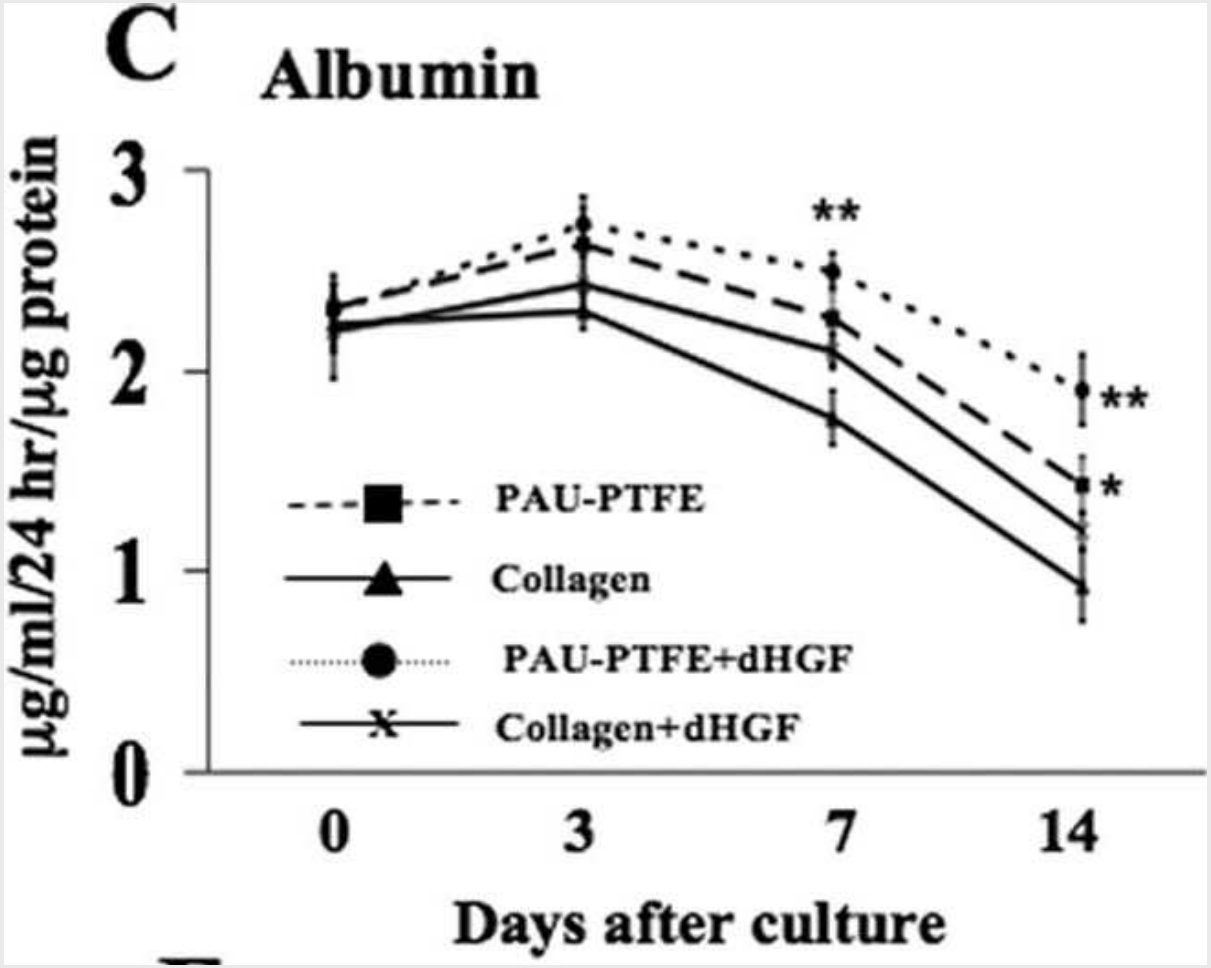
Functional capacities (hu hepatocytes)



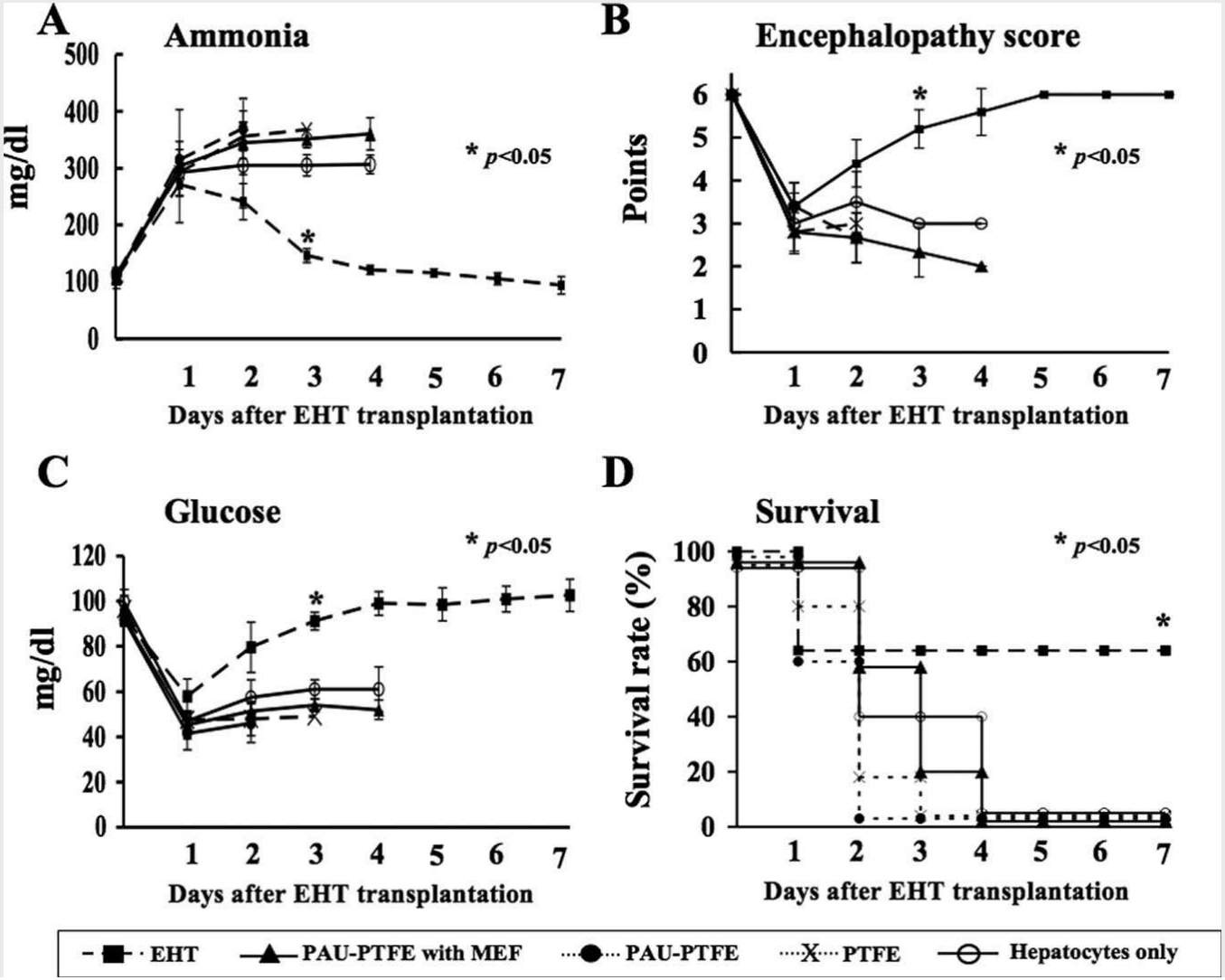
Functional capacities (hu hepatocytes)



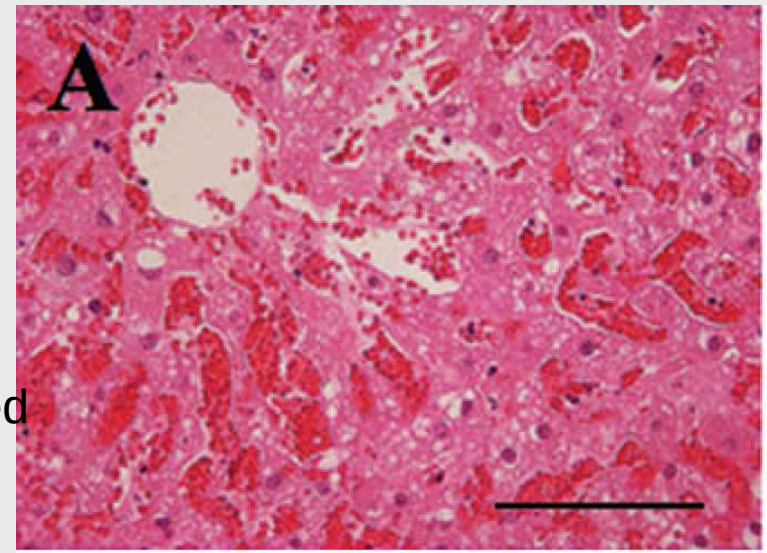
Functional capacities (hu hepatocytes)

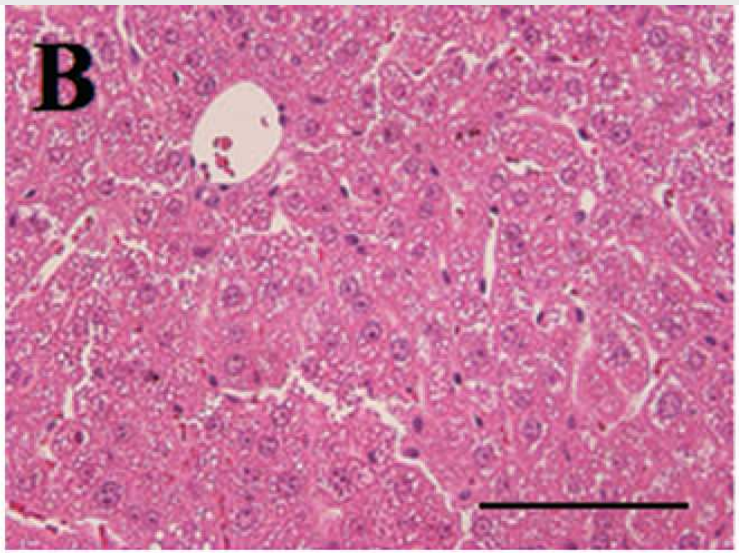


Effect of transplanted EHT on hepatectomized BALB/C mice



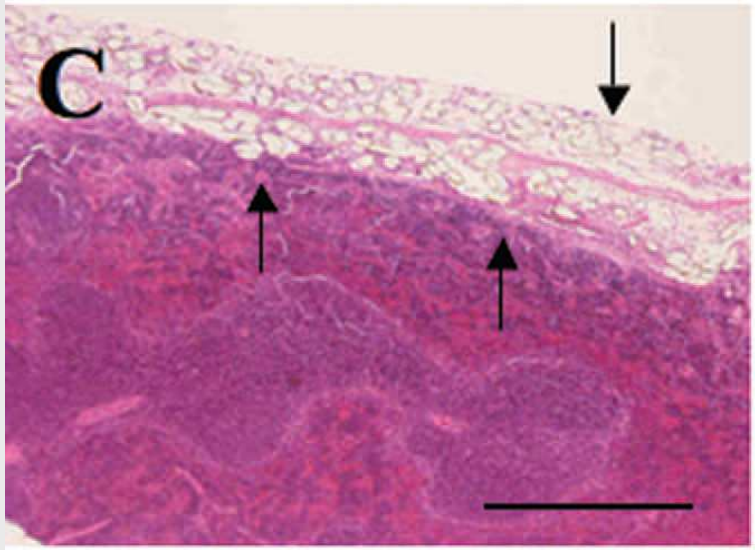
Effect of transplanted EHT

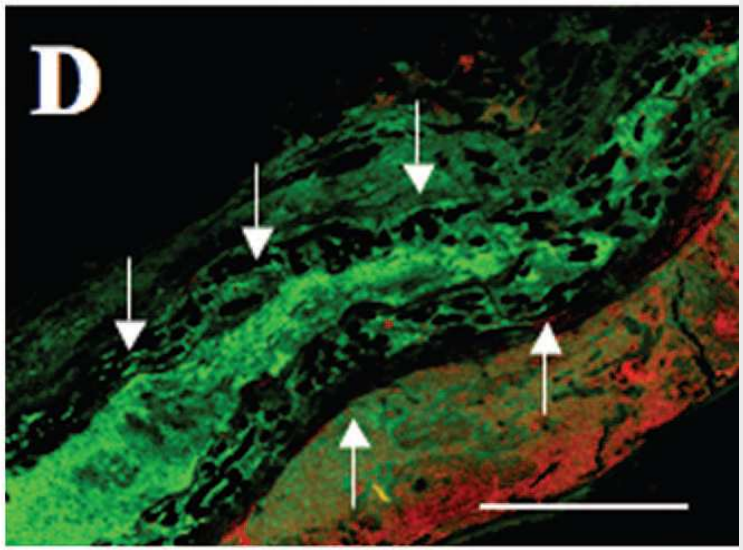




untreated control

30 d

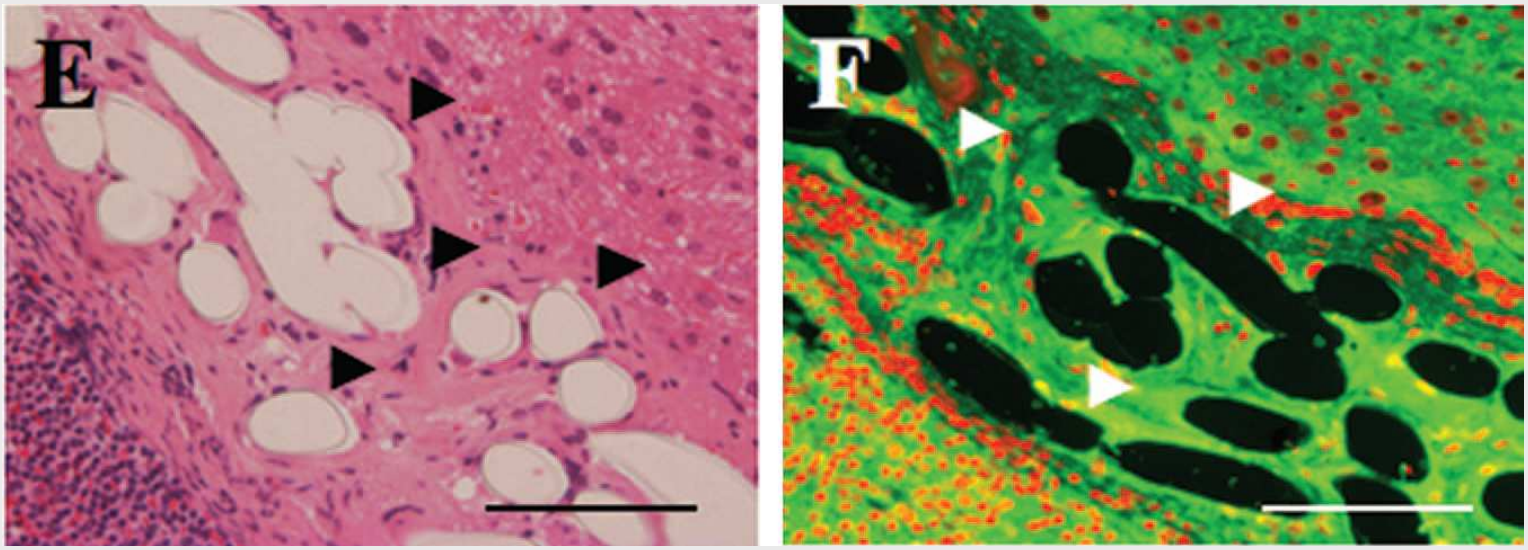




7 d

Albumin at d 7

Trabecular arrangement of hepatocytes contained into the PTFE fabric, which were positive for albumin staining



Conclusions I

- EHT (PAU-coated PTFE + human, porcine or mouse hepatocytes) is functional
- Cells adhere to material, are viable, form clusters with gap junctions and bile canaliculi
- EHT can be transplanted
- EHT rescues animals suffering ALF
- Addition of HGF (hepatocyte GF) helps in vitro

Conclusions II

- Direct transplantation of hepatocyte in the spleen not useful → most die
- When EHT is transplanted in the spleen: cells stay there → safe
- EHT is biocompatible, can be vascularized
- Easy to manipulate surgically, possibly removable (transient support)
- Possibility to genetically engineer hepatocytes

- tissue protein expressions of liver enzymes, viral infectivity, uptake of exogenous chemicals resulting in subsequent metabolism and regenerative growth potential
- medium length studies (50-200 days)

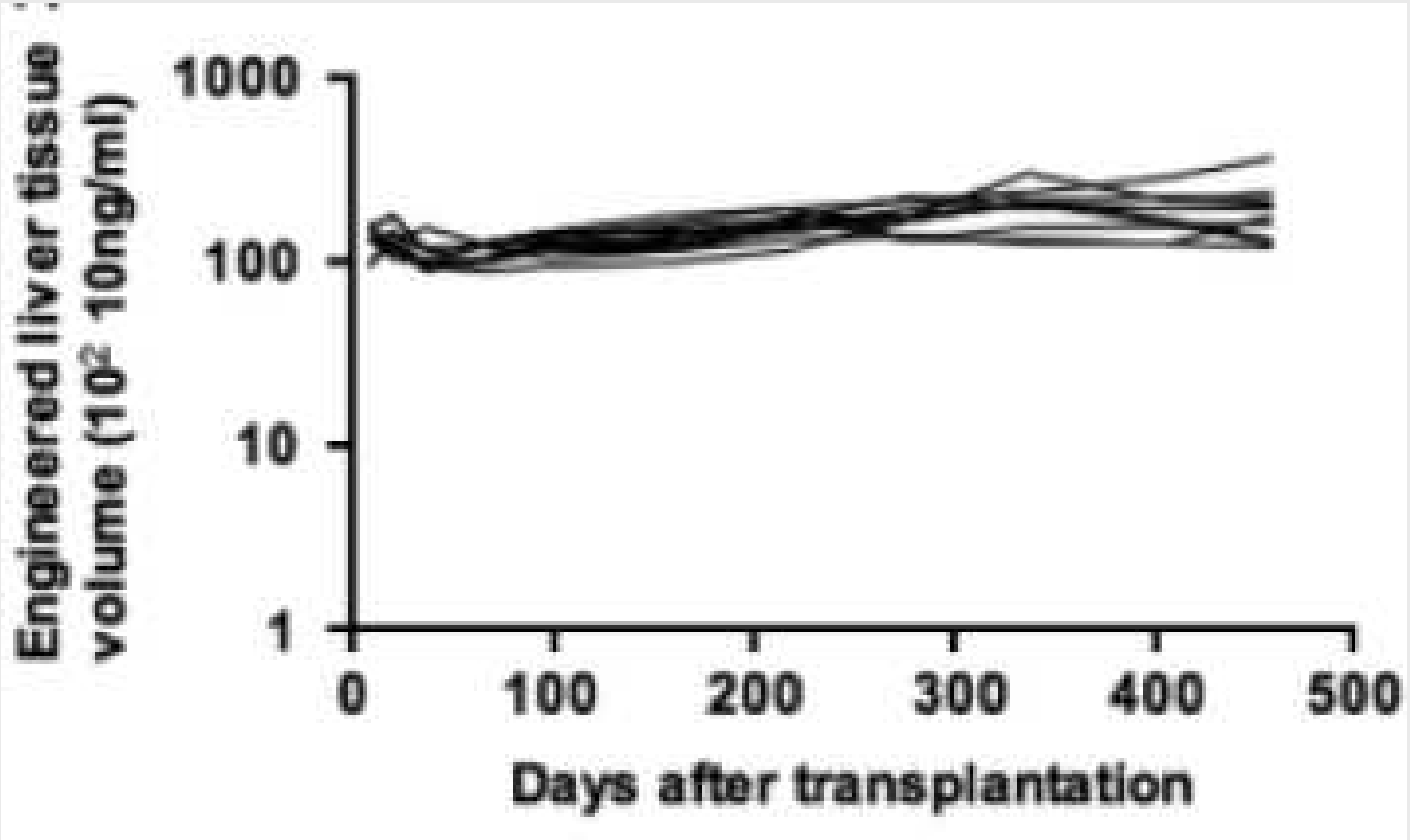
  (Ohashi *et al*., 2000, 2005a, 2005b, 2005c)

- engineered liver tissues under the kidney capsule in mice
- assessed the stability of this ectopic liver system for over 450 days, which is nearly the life-span of a normal mouse
- liver-specific functions, such as glycogen synthesis, drug metabolism and regenerative potential, within the engineered liver tissues at the end of the experiment (days 450–464)

Ohashi, J Tissue Eng Regen Med (2010).

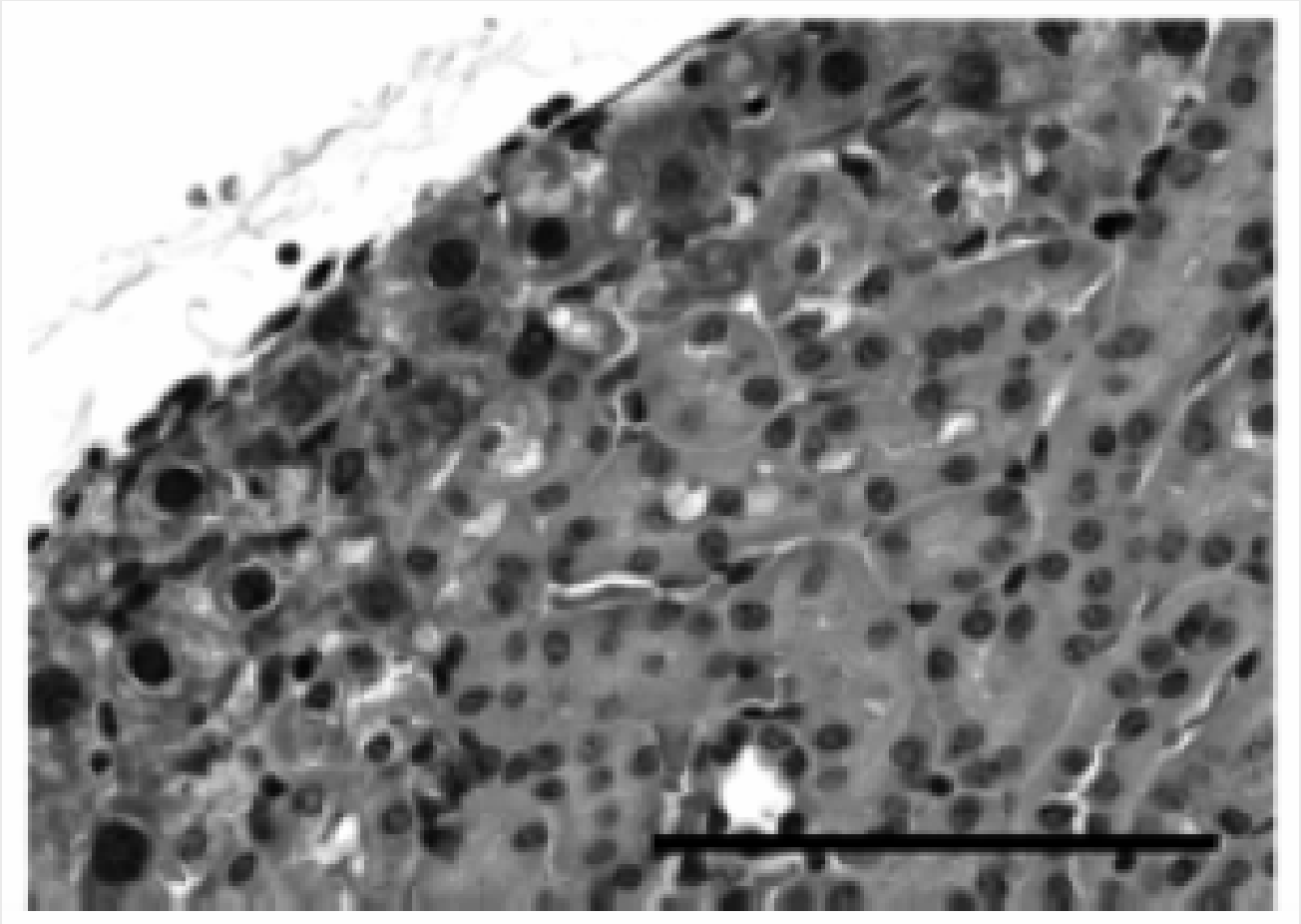
- A total of 1.5 × 10E6 hepatocytes (huA1AT+) were transplanted under the left kidney capsule space

Functional maintenance of the engineered liver tissues

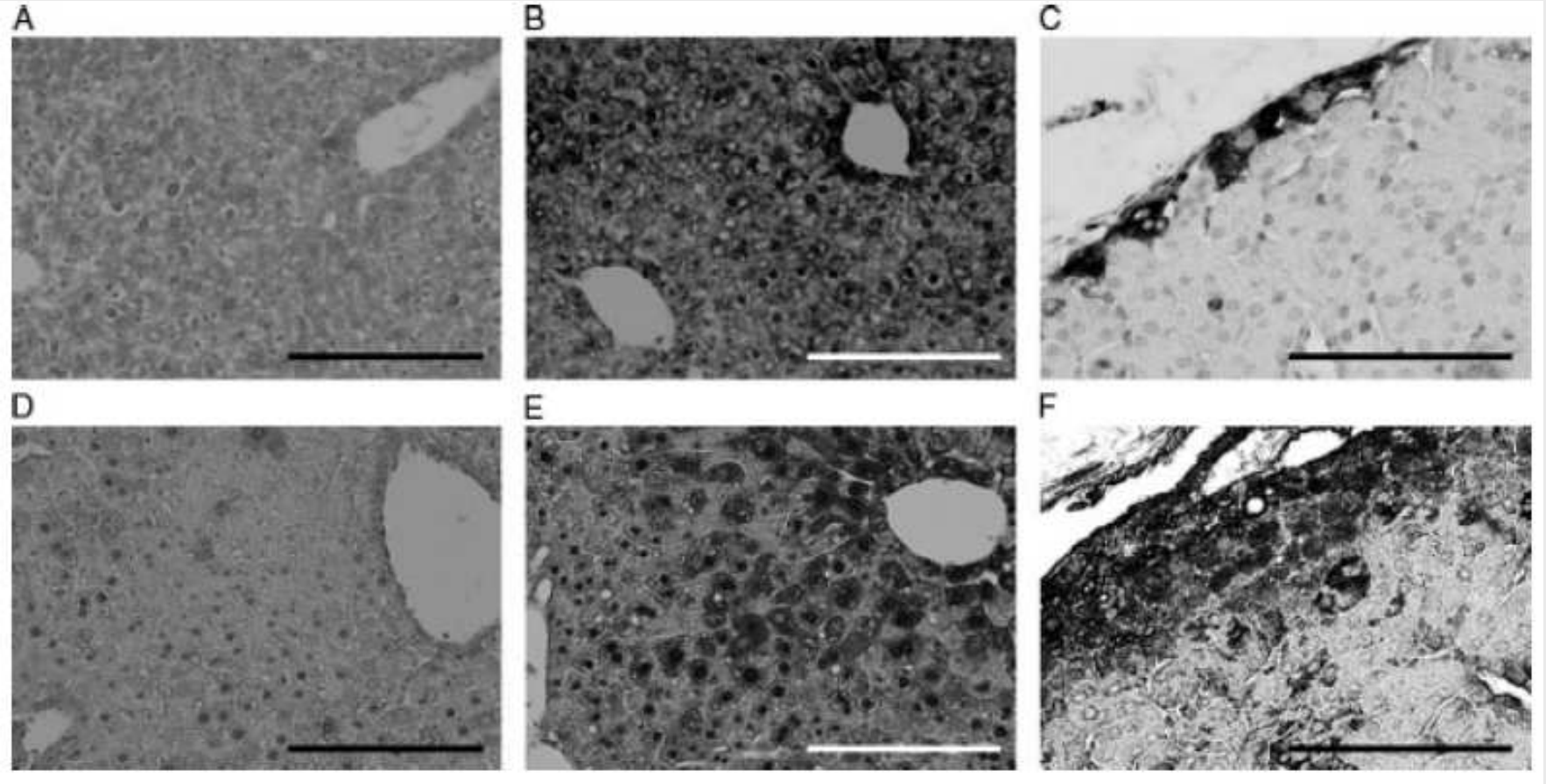


Functional volume of engineered liver tissues under the kidney capsule was determined by measuring murine serum hAAT levels

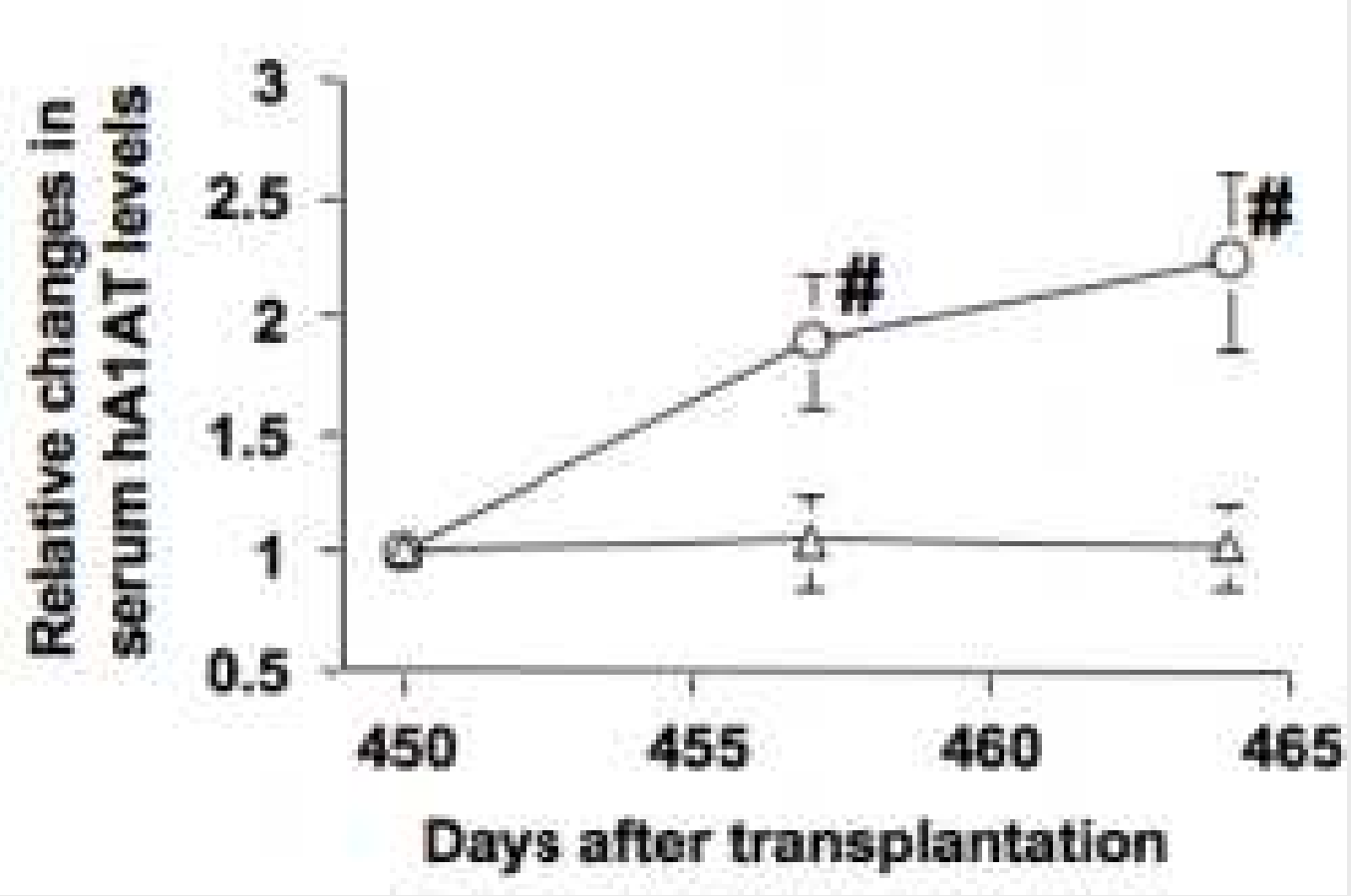
Functional maintenance of the engineered liver tissues (HE- d 453)



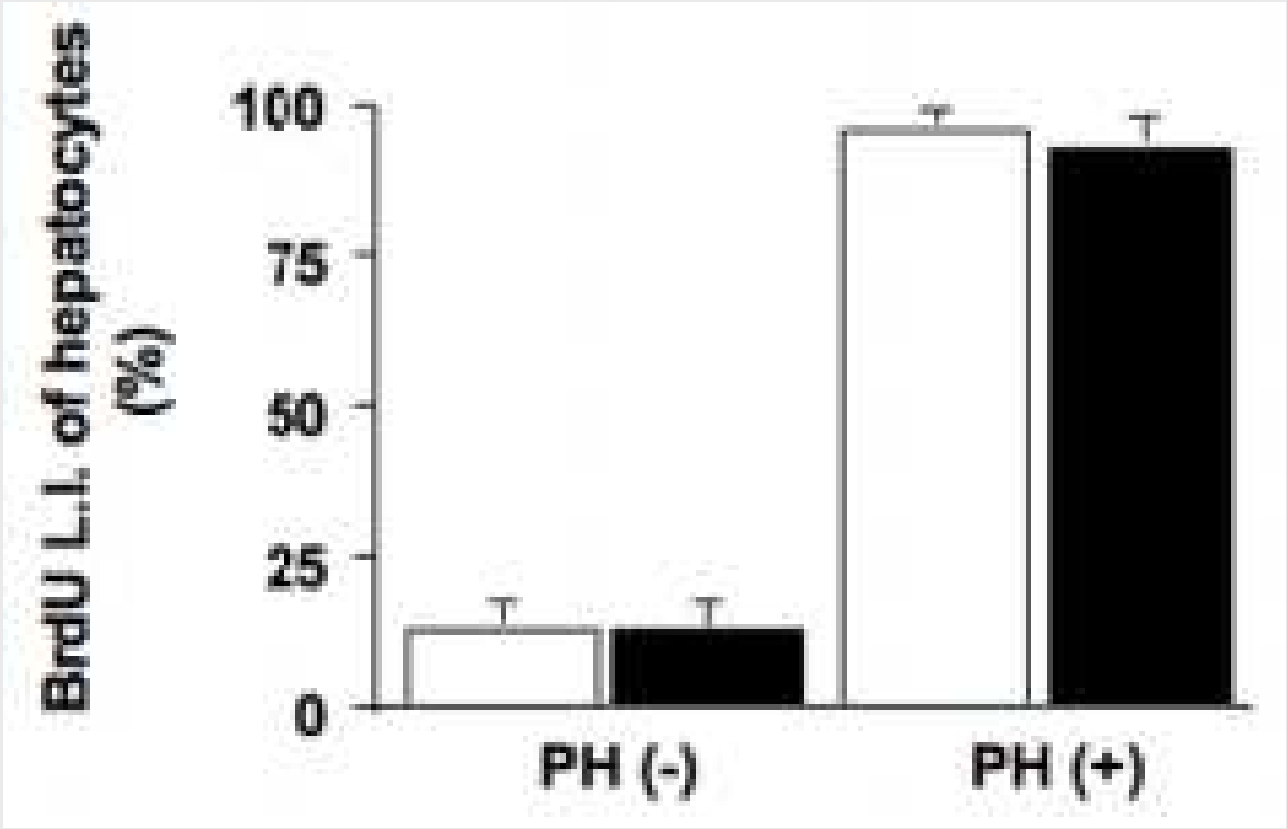
Induction of drug-metabolizing enzymes in naıve livers and engineered livers



Functional maintenance of the engineered liver tissues (huAAT)



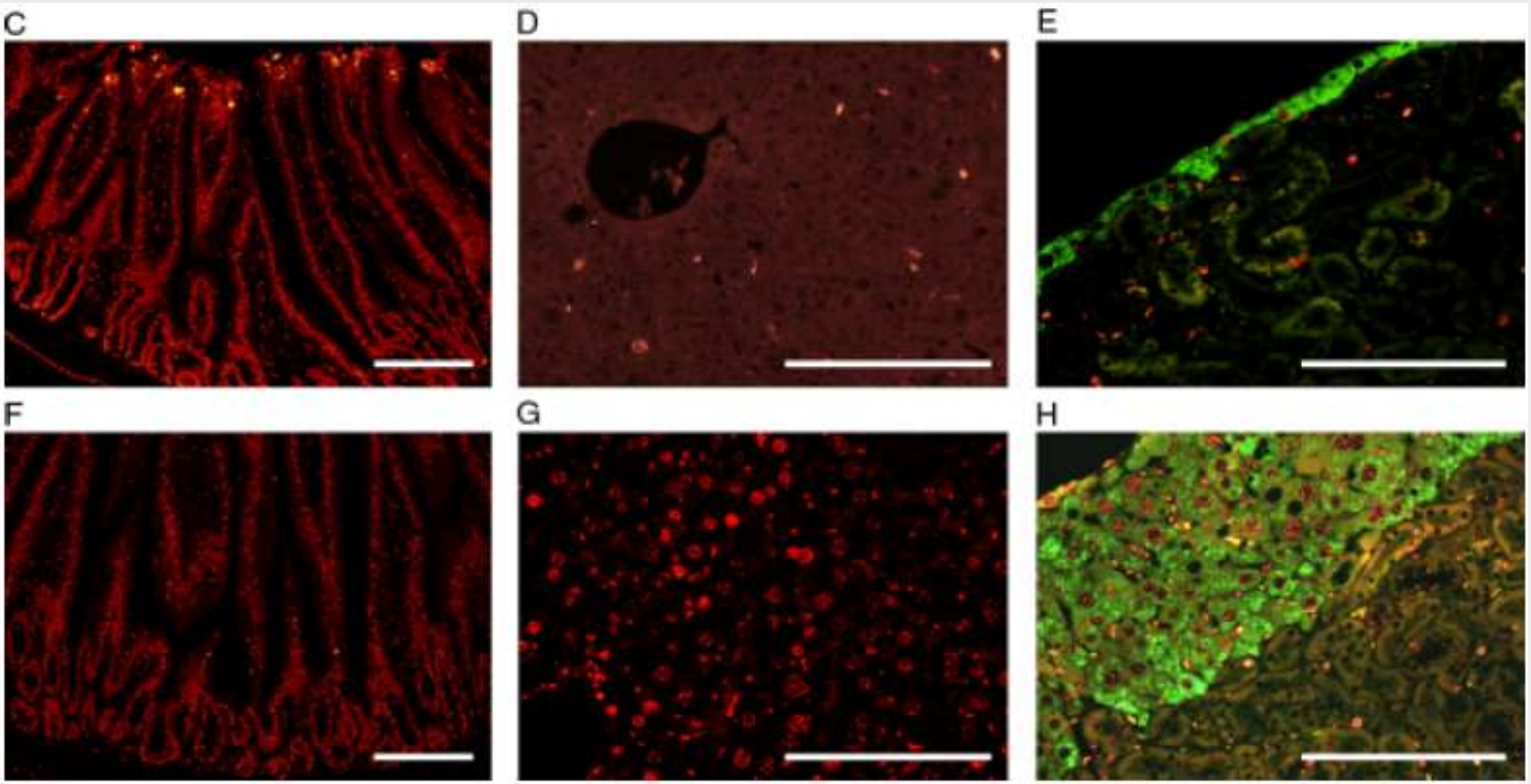
Proliferation of transplanted cells



naive liver and
enginered liver

Regenerative ability of the engineered liver tissues

duodenum | naive liver | engineered liver

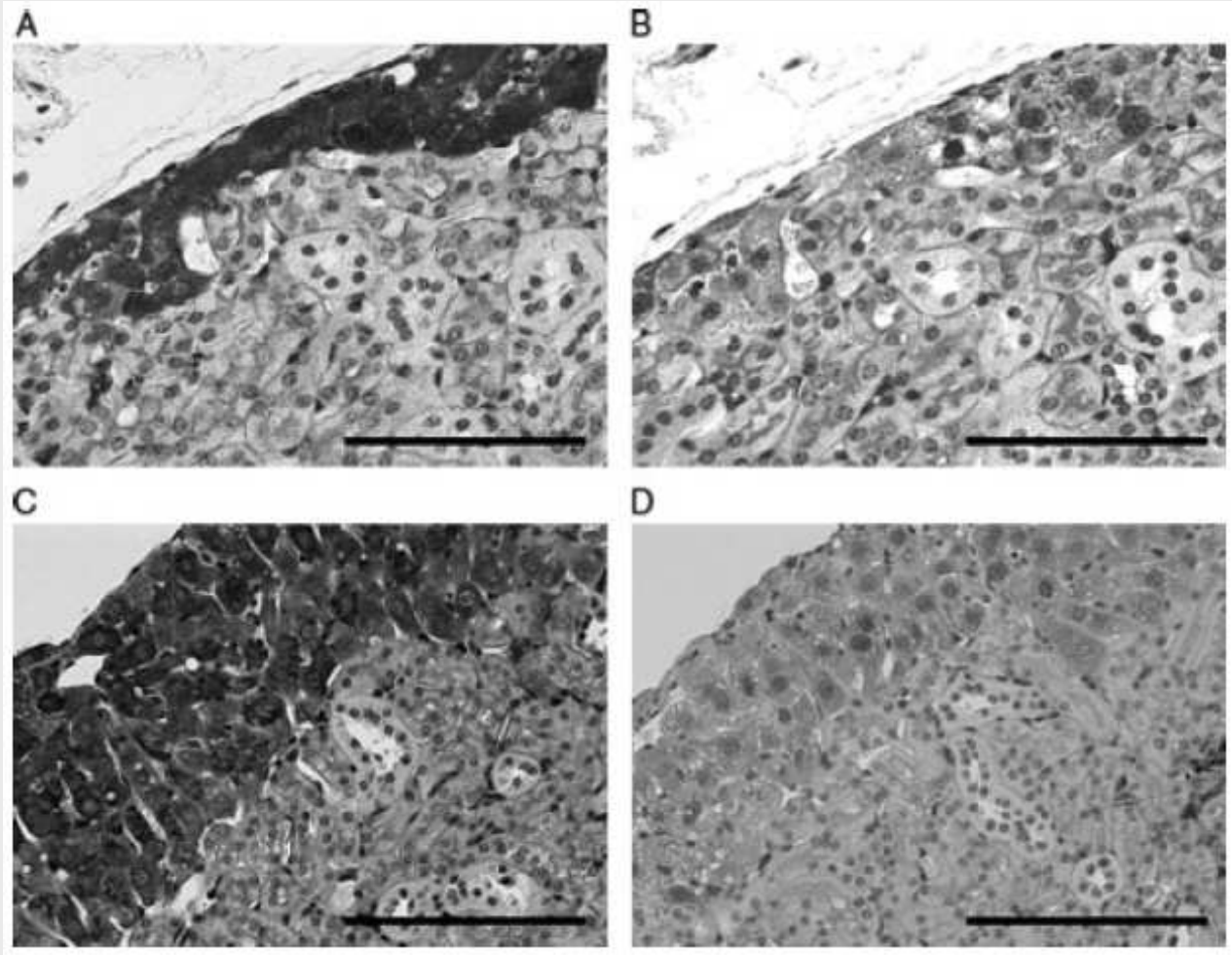


2/3 partial hepatectomy

Red = proliferation marker / green = huAAT

Engineered liver tissues synthetize determine glycogen (PAS reaction)

salivary amylase



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d 450: 2/3 partial hepatectomy
d 464: sacrifice

Conclusions

- ectopically transplanted liver tissues were functionally active for at least 450 days
- the system to mimic normal liver function
- potential to self-renew hepatocytes, also in
- the face of a surgical chemical injury
- ability to uptake exogenous drugs and subsequently activate enzymes involved in its metabolism