13º CONGRESO BARCELONA

SOCIETAT CATALANA DE TRASPLANTAMENT

Present and future of regenerative medicine

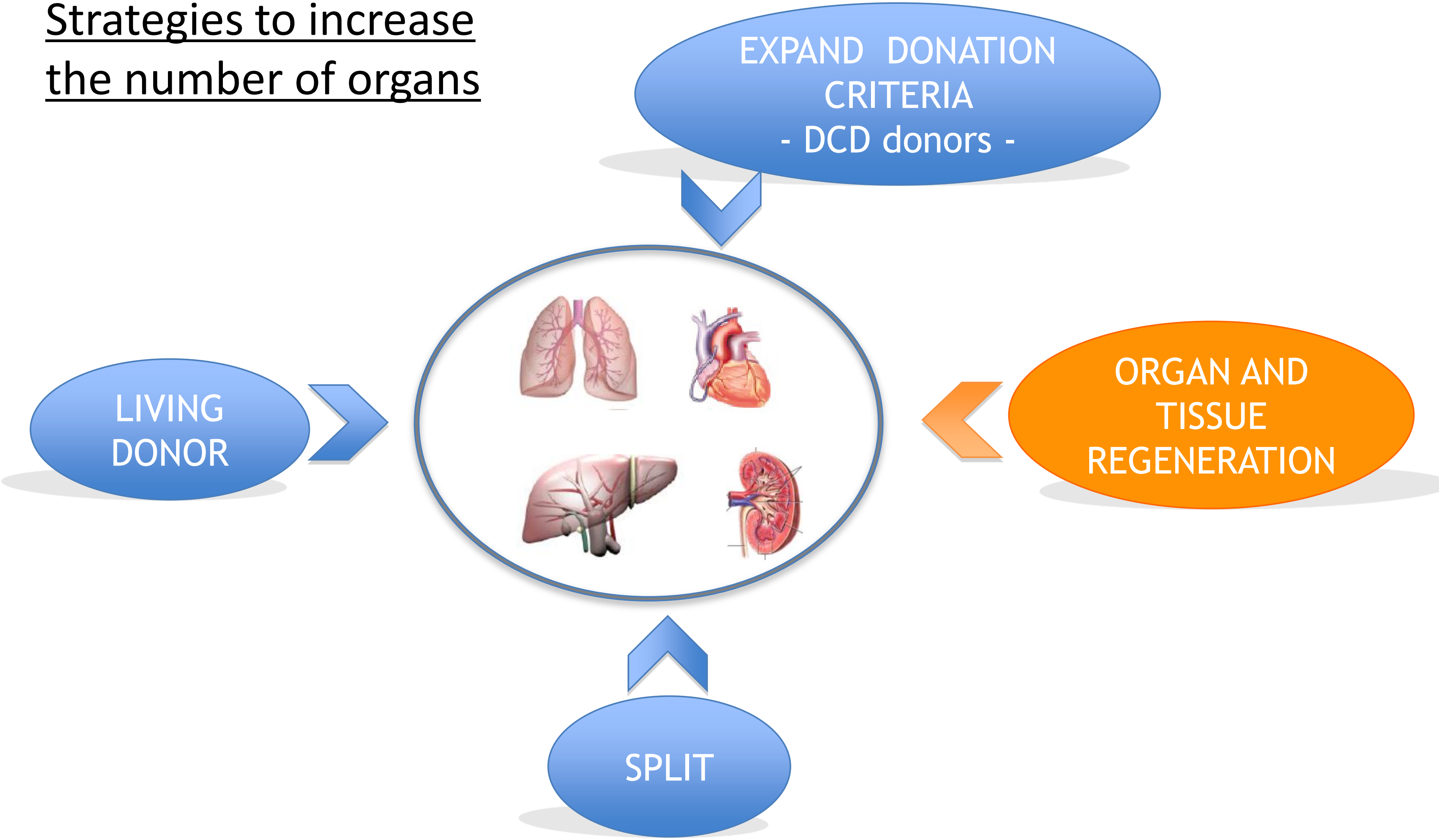
Liver Transplantation

Vall d'Hebron Hospital

Mireia Caralt, MD PhD
Servei Cirurgia HBP i Trasplantaments
March 19, 2015

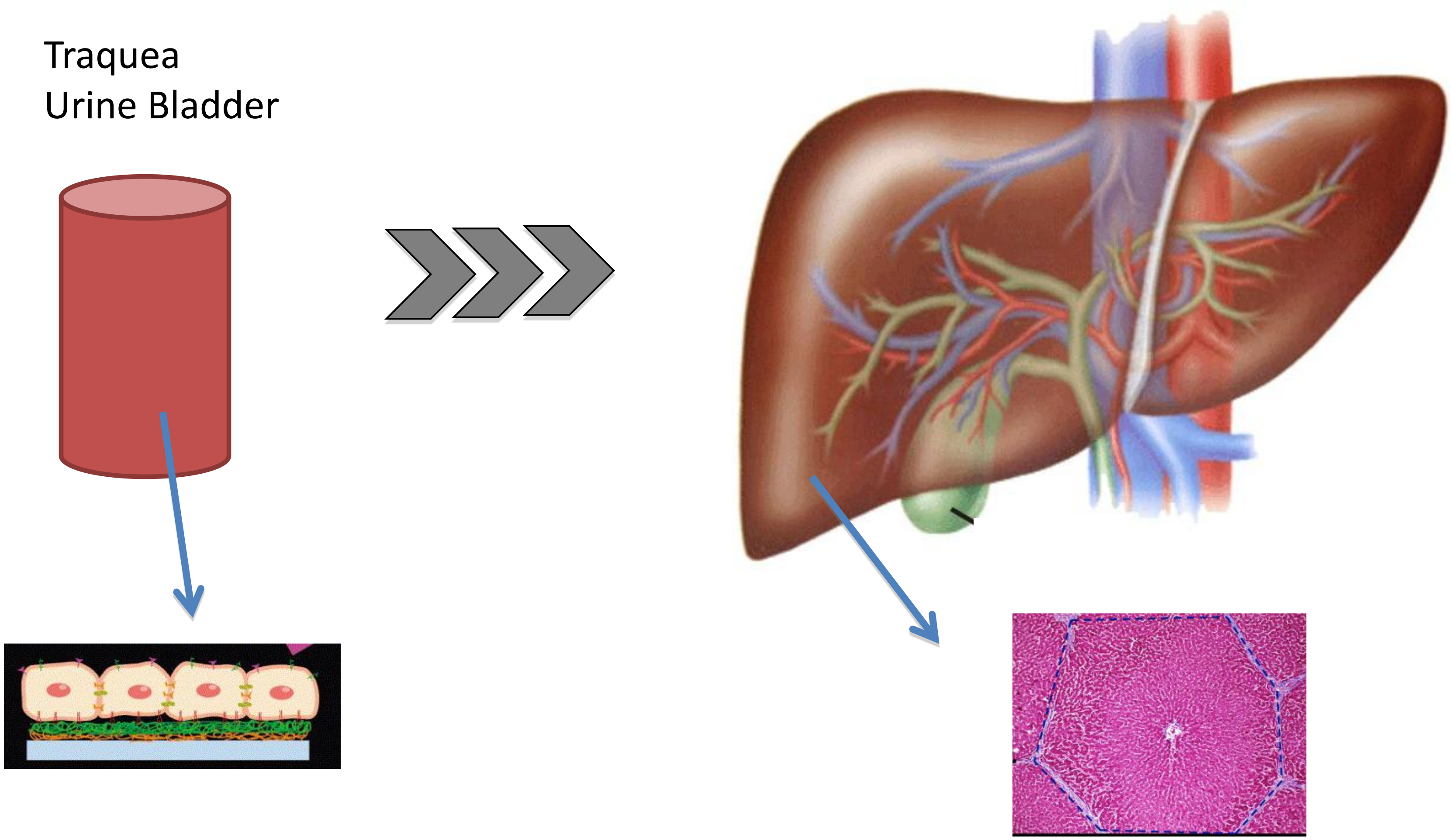
Introduction

Strategies to increase the number of organs



Solid organs: structural complexity!!

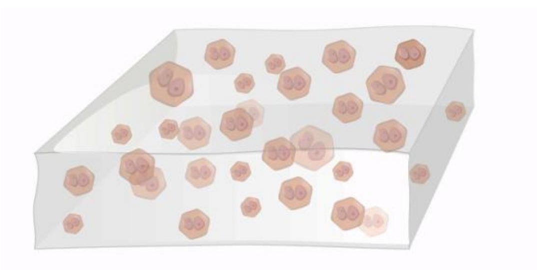
Traquea
Urine Bladder



Introduction

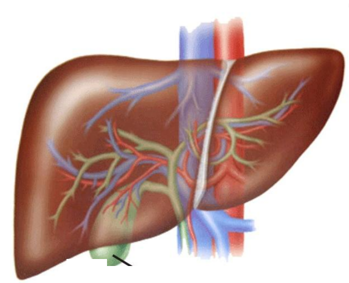
Ideal scaffold : biocompatibility, biodegradability, porosity, structural support

Artificial Scaffold

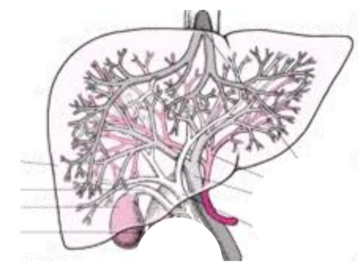


- Difficult control of size, microarchitecture and interconnectivity of pores

Natural Scaffold



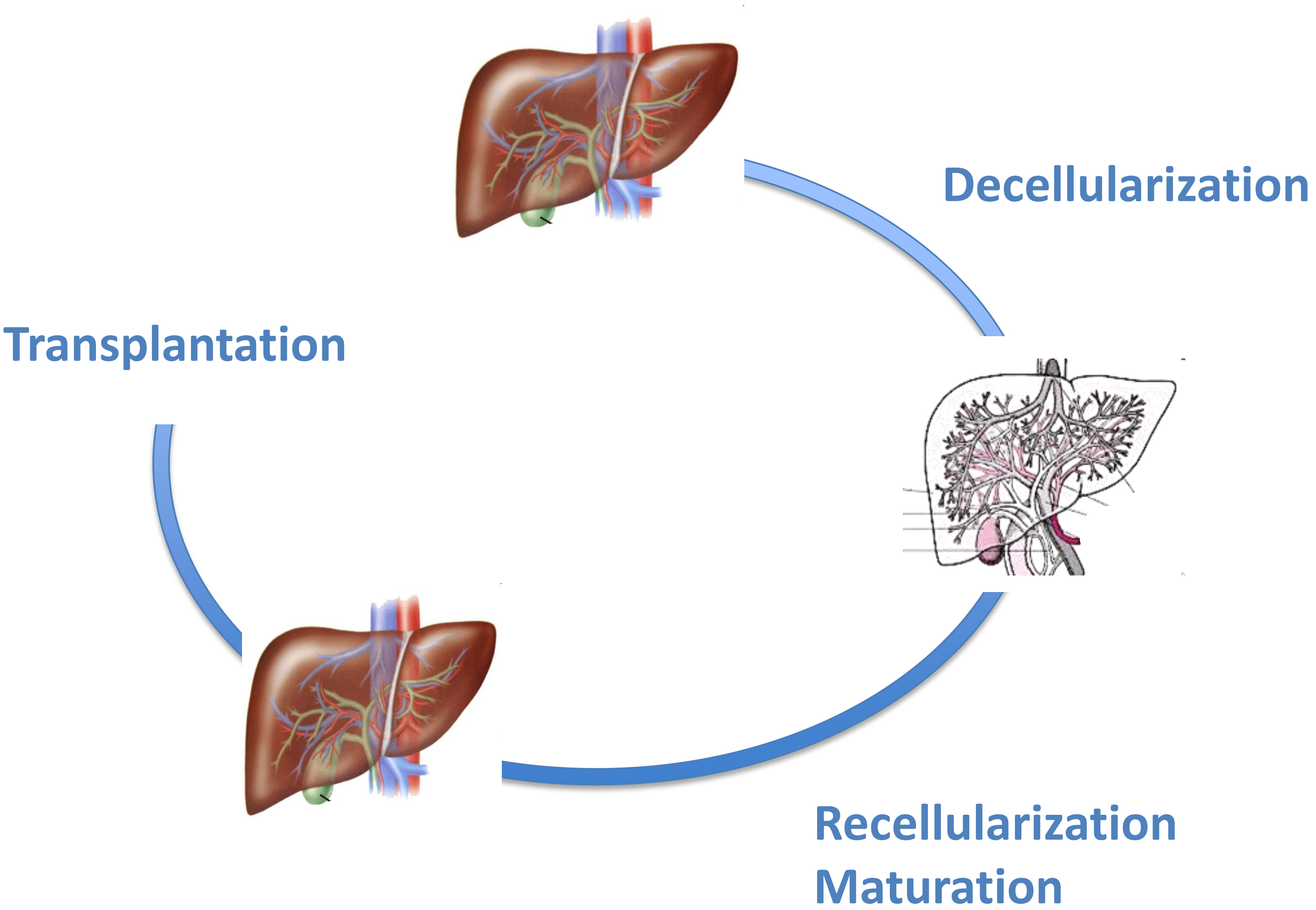
Organ



Extracellular Matrix

- Physical, chemical and molecular stimuli that enable cell engraftment
- Preservation of vascular network
- Low inmunogenicity

Introduction



Introduction

Table 1. Studies in the literature

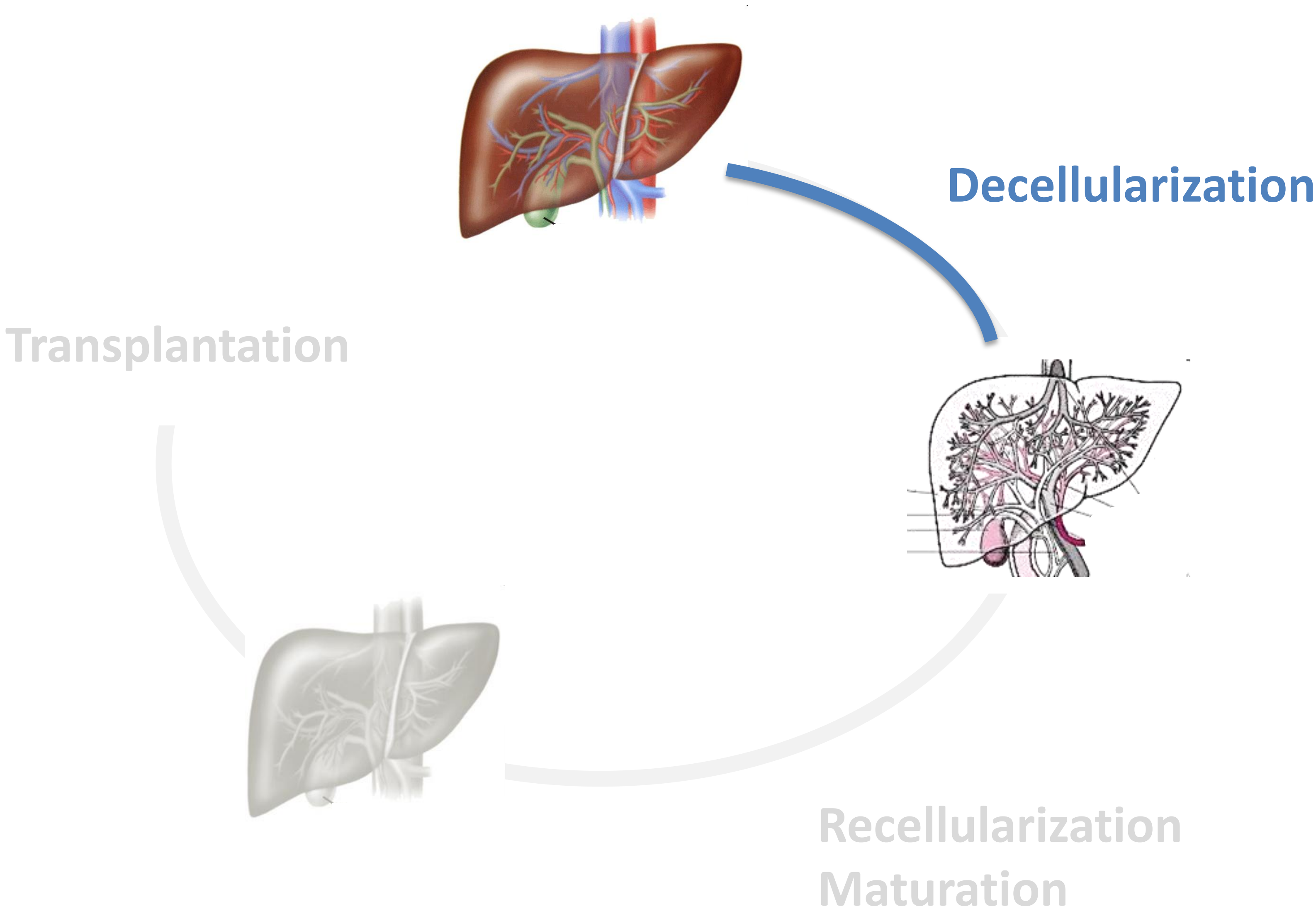
Author	Type Cells	Infusion Method	Via	Number Cells	Flow Rate	Time
Baptista 2010	hUVEC hFLC MS1	Continuous	PV IVC, PV, IVC+PV	$30x10^6$ $70 x10^6$ $100 x10^6$	3ml/min → 0.5ml/min 5ml/min	7d 3d
Uygun 2010	Rat MH Endothelial cells	Multistep	PV	$200 x10^6$	15ml/min	5d 5d
Soto 2011	Rat MH	Direct injection Continuous Multistep	PV	$10–50 x10^6$	2ml/min	7d
Yagi 2013	Pig MH	Multistep	PV	$100 x10^6$	4ml/min	7d

Table 2. Culture media used by different authors

Author	Media
Baptista 2010[1]	RPMI 1640, FBS, dexamethasone, penicillin-streptomycin, prolactin, glucagon, niacinamide, lipoic acid, triiodothyronine, hEGF, hHDL, hHGF, hGH, insulin, transferrin
Uygun 2010[3]	William's E, FBS, insulin, EGF, glucagon, hydrocortisone, penicillin-streptomycin
Soto 2011[63]	EMEM, EGF, HGF, dexamethasone, insulin, human transferring, selenous acid supplement, penicillin-streptomycin
Yagi 2013[63]	DMEM, EGF, hidrocortisone, insulin, glucagon, penicillin-streptomycin

Caralt M. Organogenesis 2014;10(2):250

Decellularization



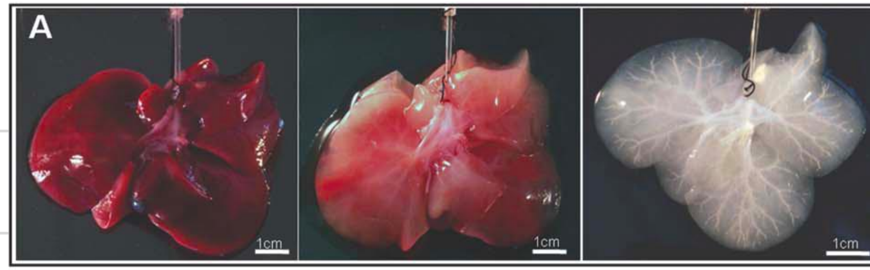
Decellularization

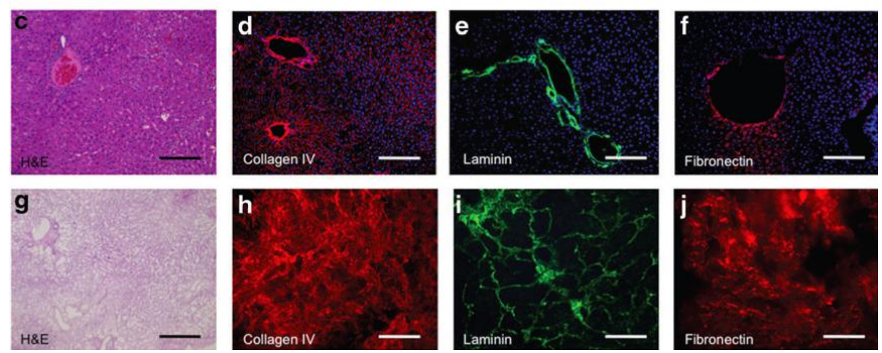
Different decellularization “recipes” in liver

Uygun	rat	SDS Triton X-100	0.01% 24h, 0.1% 24h, 1% 24h 1% 30min	1ml/min
Baptista	rat	Triton X-100 + NH4 OH	1%+3%, 3h	5ml/min
Soto	rat	Trypsin + EGTA Triton X-100 + EGTA	0.02%+0.05%, 2h 3%+0.05%, 24h	8ml/min
Bao	rat	Adenosine SDS	10mM 1% 4h, 0.5% 4h, 0.25% 4h	25ml/min
Yagi	pig	SDS Triton X-100	0.01% 24h, 0.1% 24h, 1% 48h 1% 30min	30ml/min
Ko	pig	Triton X-100 + NH4 OH	1%+3%, 3h	0.5ml/min

Different decellularization "recipes" in liver

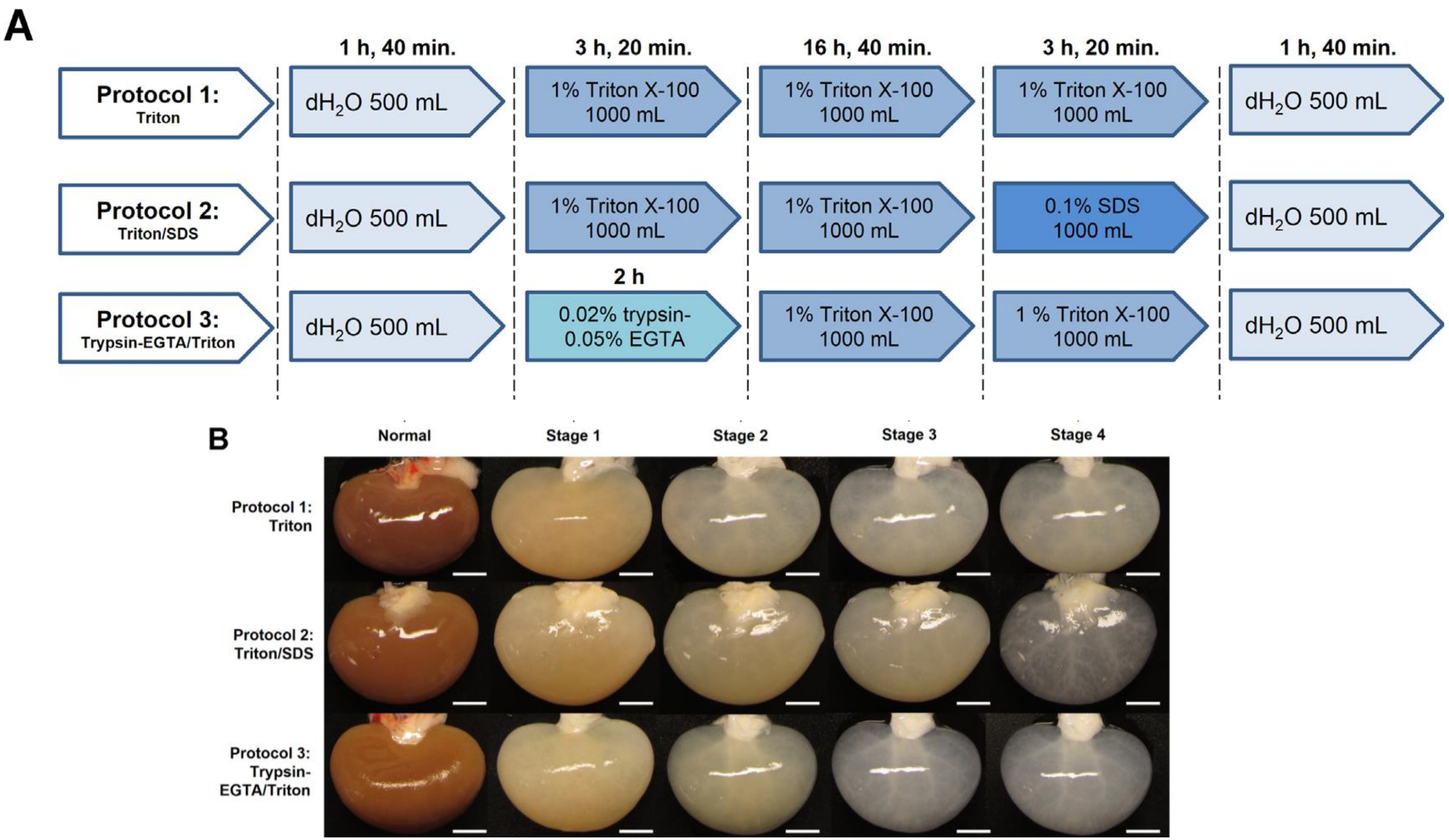
Uygun	rat	SDS Triton X-100
Baptista	rat	Triton X-100 + NH4 OH
Soto	rat	Trypsin + EGTA Triton X-100 + EGTA
Bao	rat	Adenosine SDS
Yagi	pig	SDS Triton X-100
Ko	pig	Triton X-100 + NH4 OH





Decellularization

… best decellularization protocol in kidneys



Caralt et al. Am J Transplant 2015; 15:64-75

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Decellularization

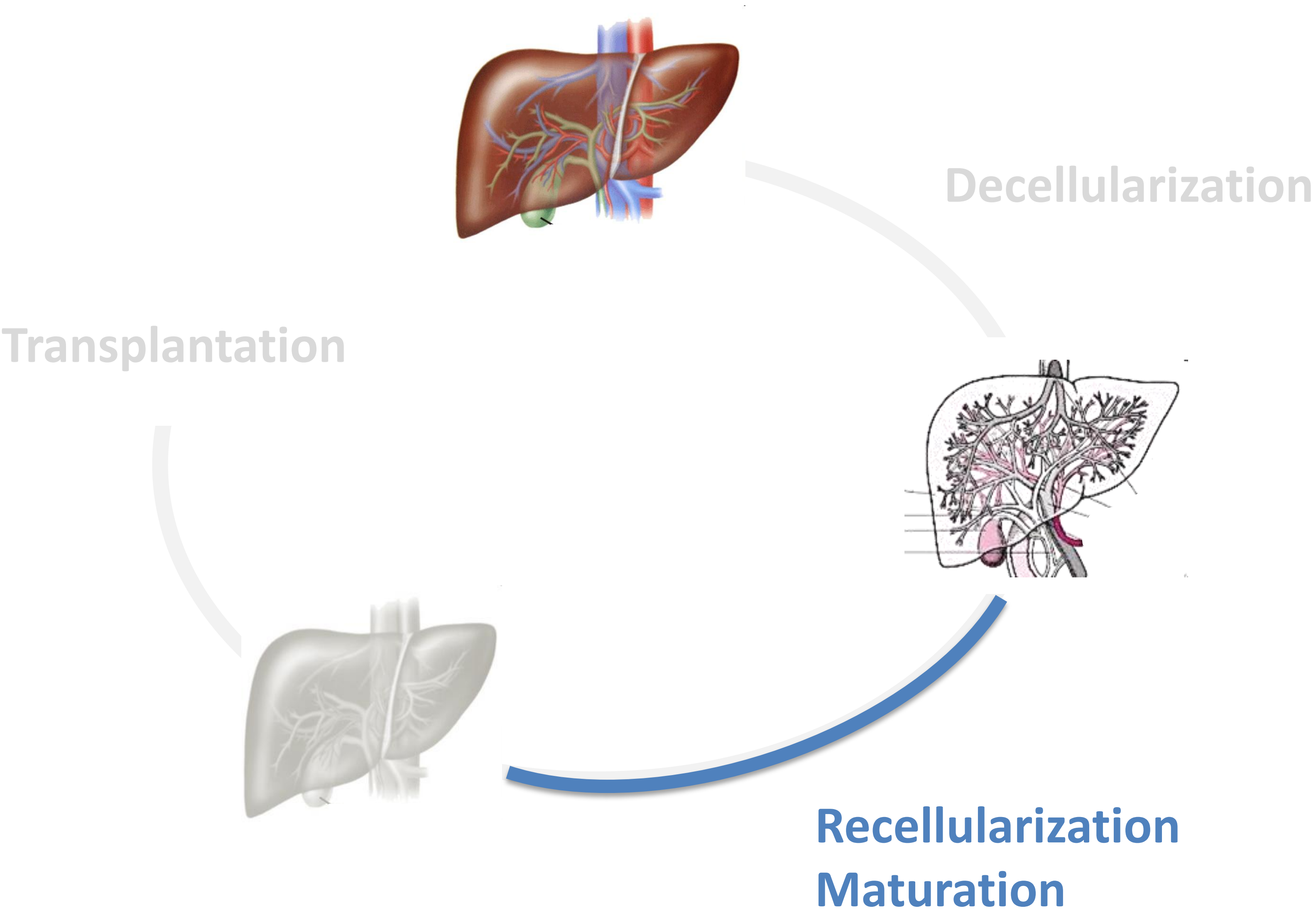
Table 1: Summary evaluation of decellularization protocols

Selection criteria	Protocol 1: Triton	Protocol 2: Triton/SDS	Protocol 3: Trypsin-EGTA/Triton
Macroscopic appearance (transparency of gross tissue is the goal)	–	+	+
Microscopic appearance (H&E, SEM, cell removal, maintenance of architecture is goal)	–	+	+/–
DNA reduction (goal: >95%)	–	+	–
Nuclear basophilia score (goal score is 1.0–2.0)	– Glomeruli: 2.1 ± 0.4; Tubules: 1.9 ± 0.9; Vessels: 3.4 ± 0.8	+ Glomeruli: 1.0 ± 0.0; Tubules: 1.0 ± 0.0; Vessels: 1.0 ± 0.0	+ Glomeruli: 1.1 ± 0.4; Tubules: 1.1 ± 0.4; Vessels: 1.3 ± 0.8
Architectural score (goal score is 4.0–5.0)	+ Glomeruli: 4.3 ± 0.5; Tubules: 4.4 ± 0.8; Vessels: 5.0 ± 0.0	+ Glomeruli: 4.0 ± 0.6; Tubules: 4.2 ± 0.8; Vessels: 5.0 ± 0.0	+ Glomeruli: 3.9 ± 0.7; Tubules: 4.1 ± 0.4; Vessels: 5.0 ± 0.0
ECM (collagen, laminin) retention (goal is retention of proteins)	+	+/–	+/–
Growth factor (bFGF, VEGF) retention (goal: >30% retention)	+	+	–

bFGF, basic fibroblast growth factor; ECM, extracellular matrix; H&E, hematoxylin & eosin; SEM, scanning electron microscopy; SDS, sodium dodecyl sulfate; VEGF, vascular endothelial growth factor.
For each criterion, protocols were evaluated and assigned one of three values: good (+), fair (+/–) or poor (–) at reaching a target goal. Each protocol was evaluated independently of the other two protocols, and is compared to normal kidneys.

Caralt et al. Am J Transplant 2015; 15:64-75

Recellularization



Recellularization

Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix

Basak E Uygun[1] NATURE MEDICINE VOLUME 16 NUMBER 7 | JULY 2010

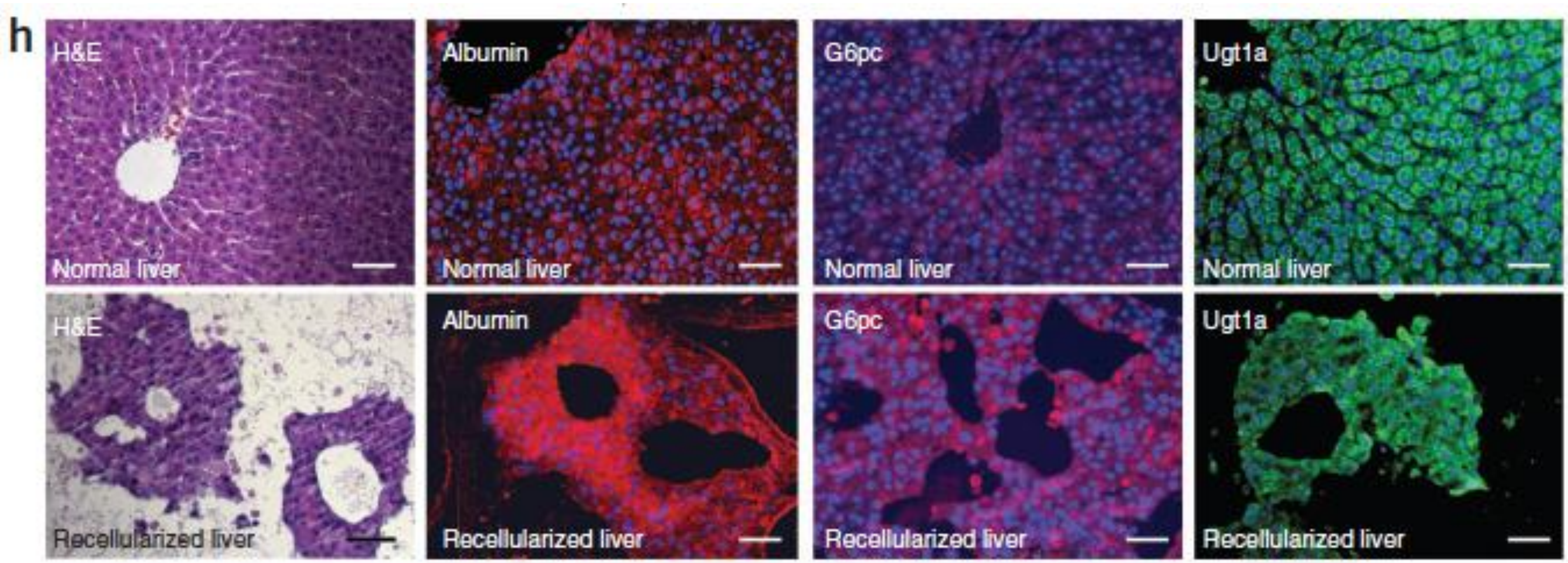
Primary rat hepatocytes

The Use of Whole Organ Decellularization for the Generation of a Vascularized Liver Organoid

BAPTISTA ET AL. HEPATOLOGY, Vol. 53, No. 2, 2011

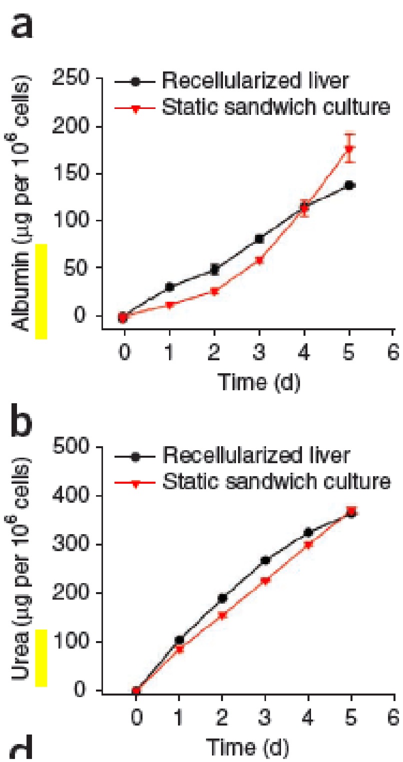
hFLCs + hUVEC

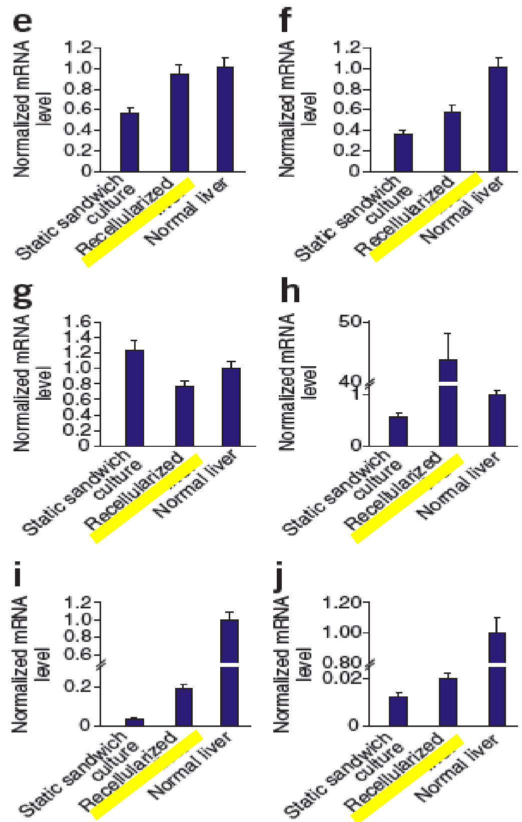
Recellularization



Uygun. Nature 2010;16(7):814-821

Recellularization





(e–j) Normalized gene expression of *Cyp2c11* (**e**), *Gstm2* (**f**), *Ugt1a1* (**g**), *Cyp1a1* (**h**), *Adh1* (**i**) and *Cyp3a18* (**j**). All error bars represent s.e.m. (*n* = 3).

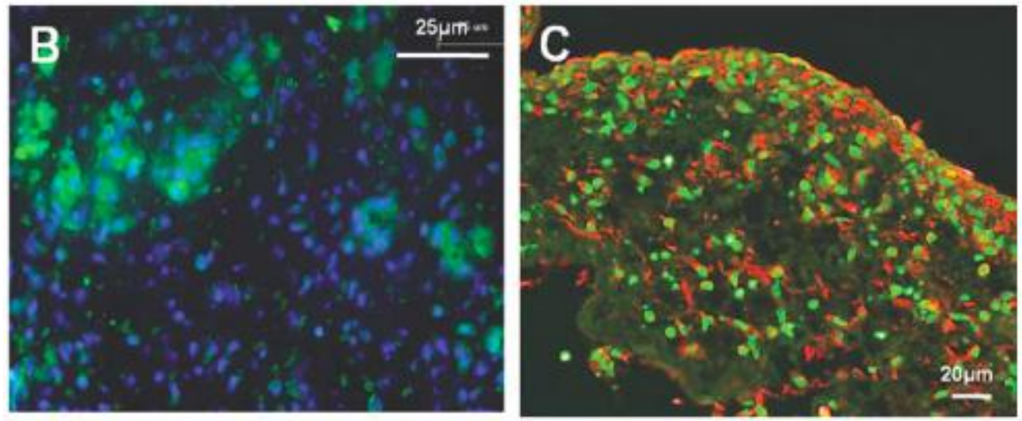
20% albumin production of *in vivo* levels

30% drug metabolism gene expression of *in vivo* levels

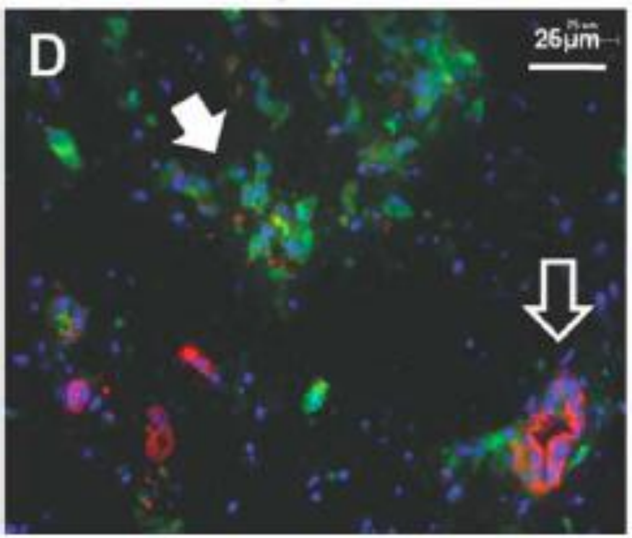
Uygun. Nature 2010;16(7):814-821

Recellularization

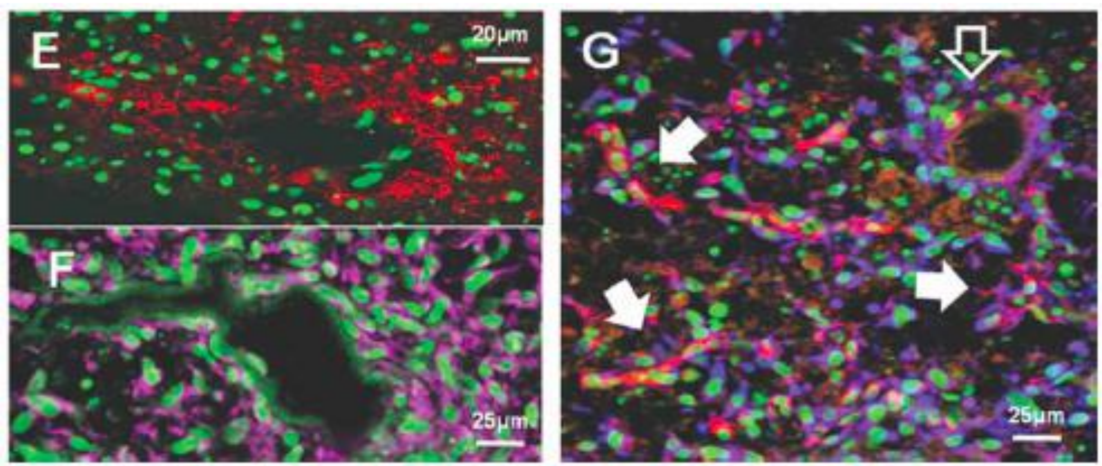
hFLC
hUVEC



p450 CYP2A (green) and p450 CYP3A (red)



CK 19 (red) and albumin (green)

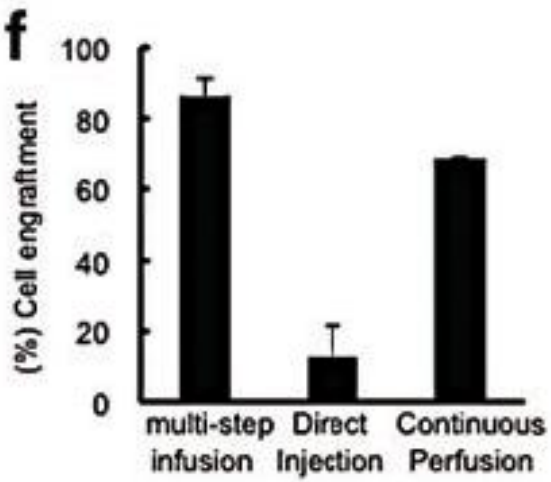


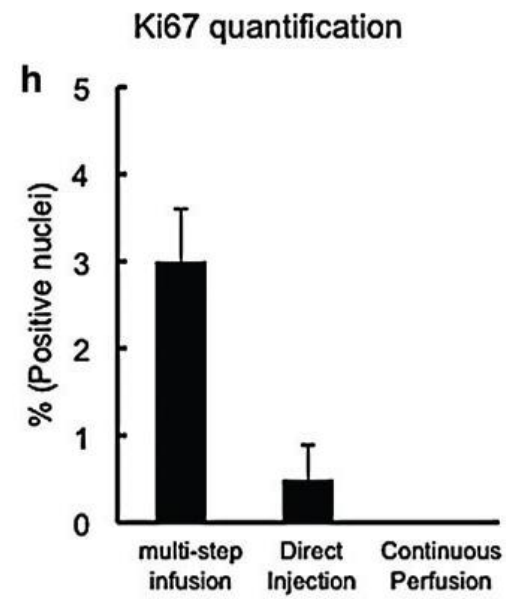
Von Willebrand (red) and endothelial oxid nitric sinthetase (pink)

Baptista et al. Hepatology 2010 Nov;12:604-617

Recellularization

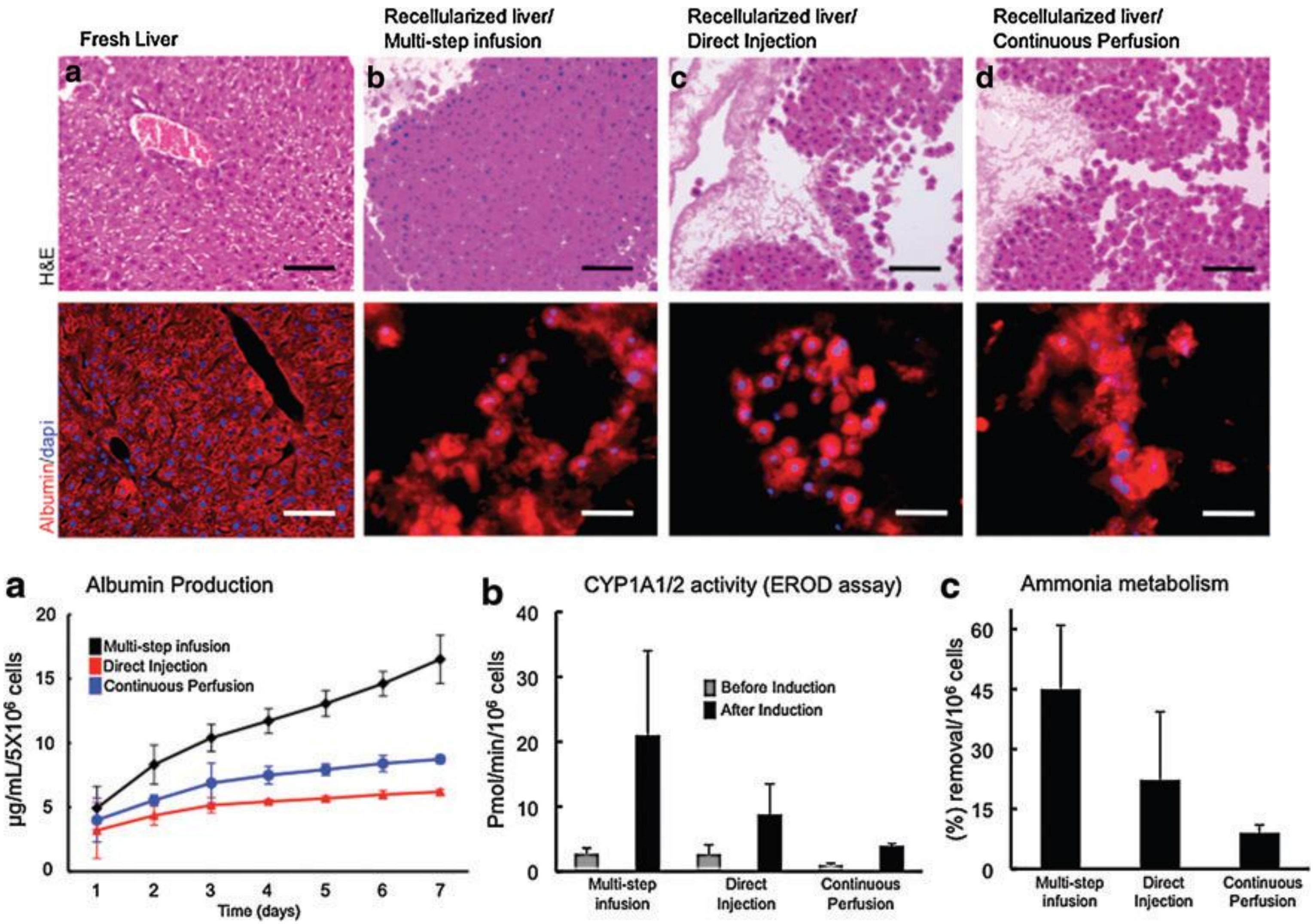
Direct parenchymal injection	Continuous perfusion	Multistep infusion
Direct injections into different lobes	Cells suspendended in culture media	Cells through Porta Vein 4 steps at 10-15min interval





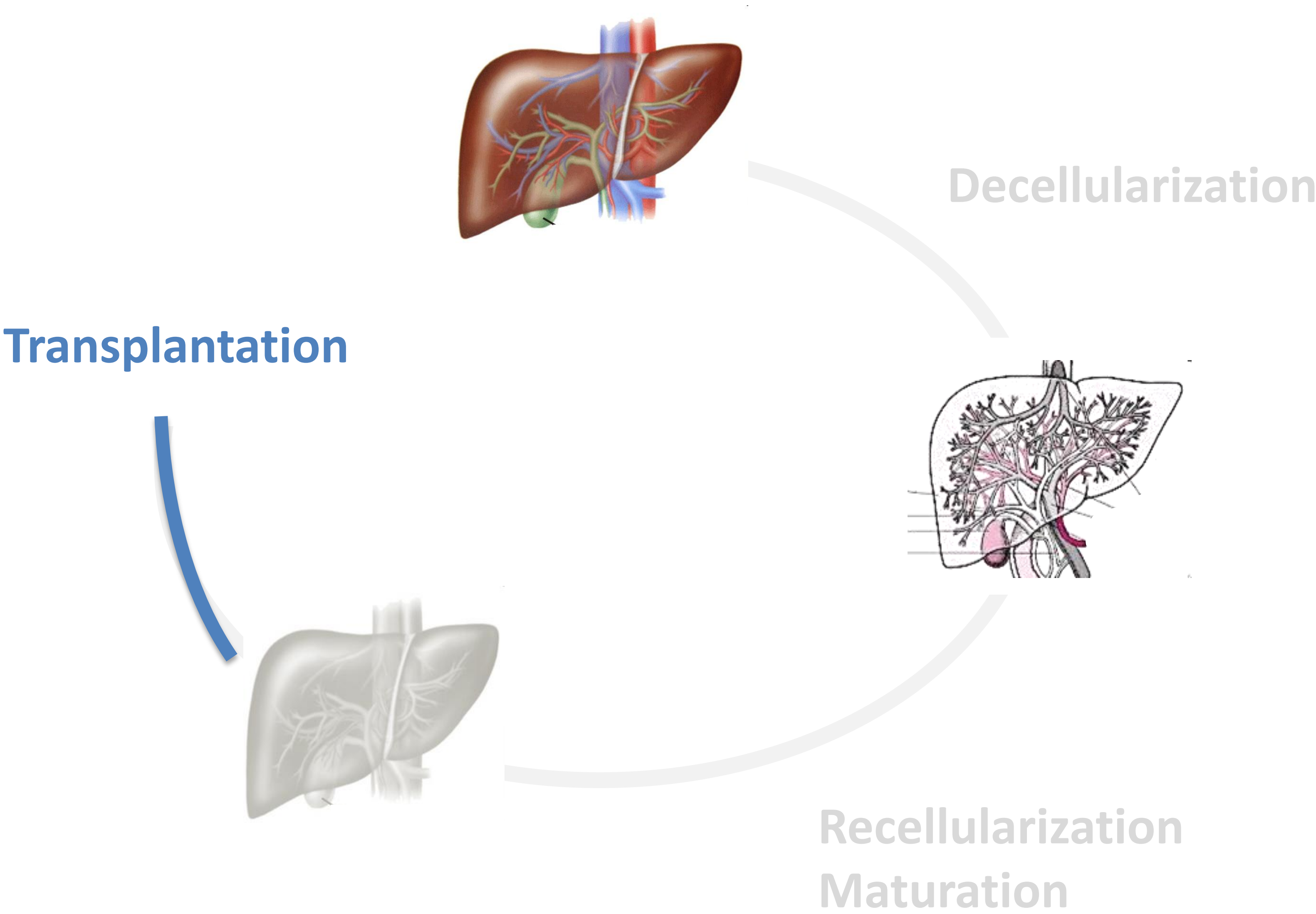
Soto A. Tissue Eng Part C Methods 2011;17(6):677-86

Recellularization

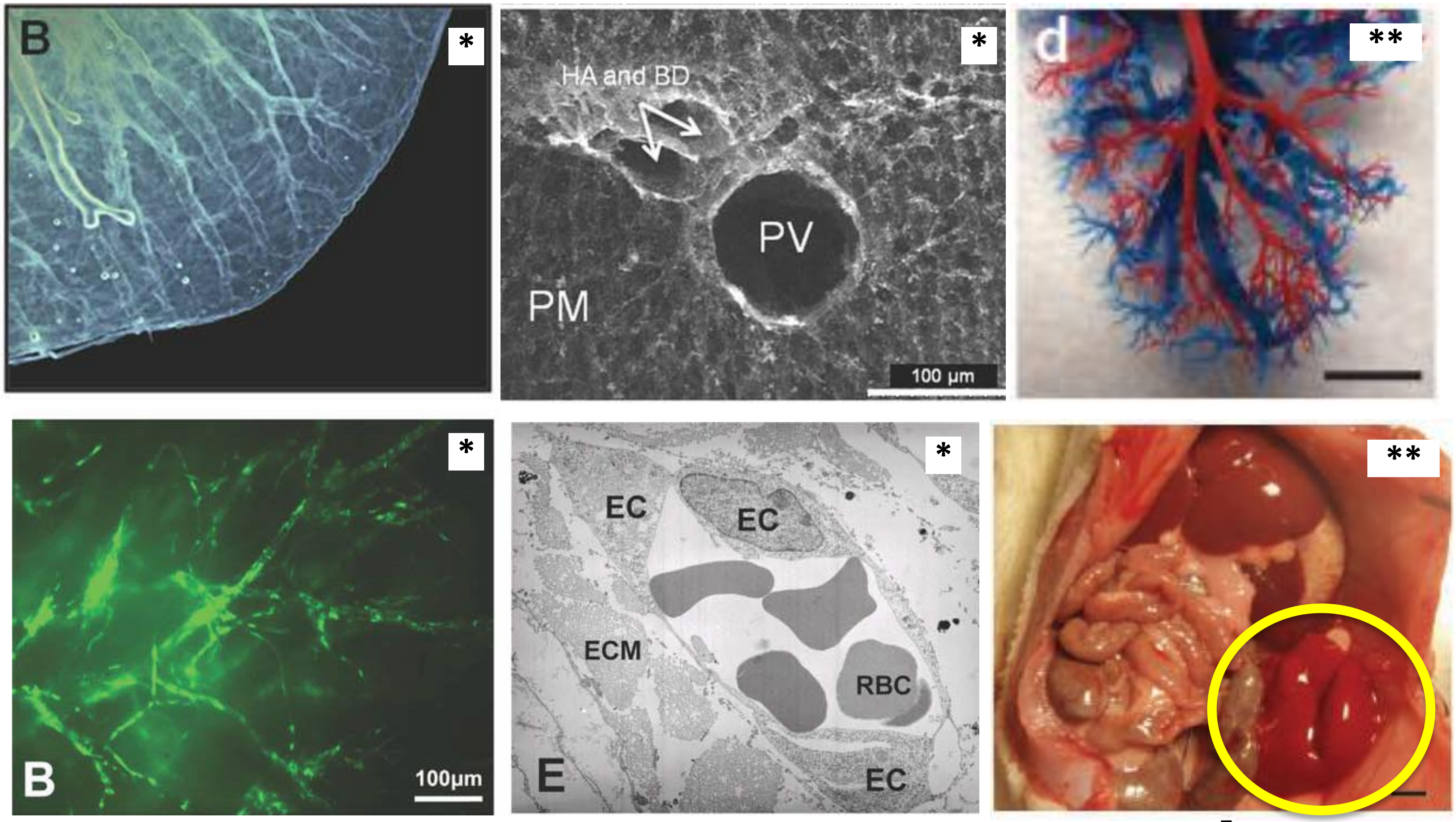


Soto A. Tissue Eng Part C Methods 2011;17(6):677-86

Background


Decellularization
Transplantation
Recellularization
Maturation

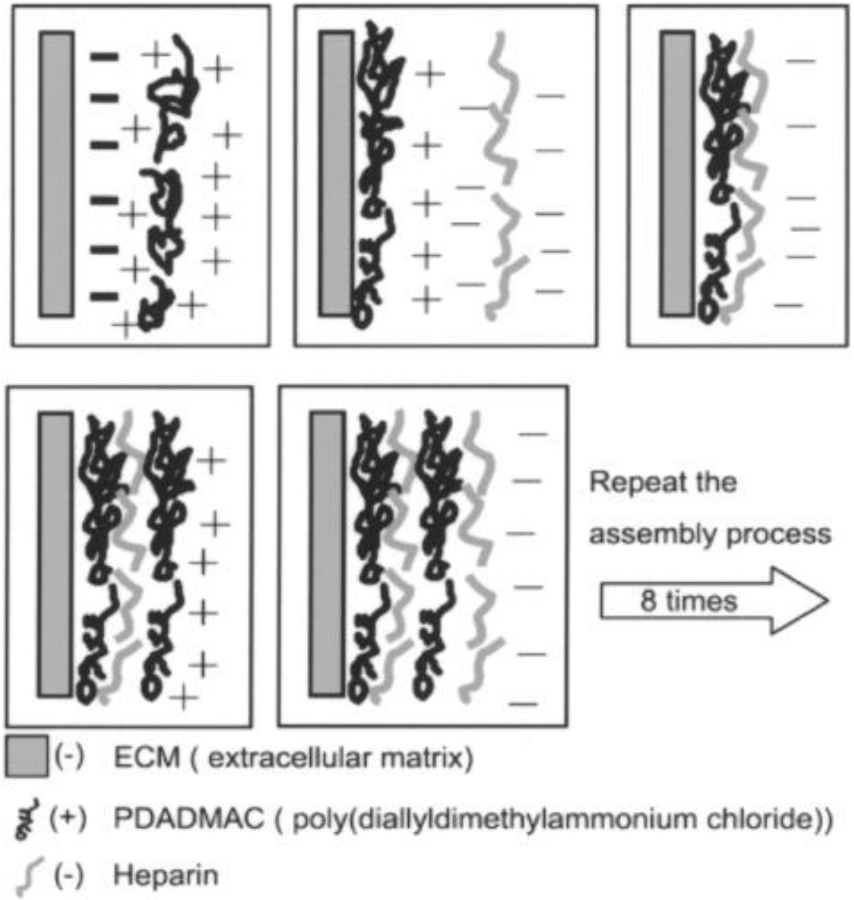
Transplantation



8h viability thrombosis

* Baptista et al. Hepatology 2010;12:604-617

** Uygun. Nature 2010;16(7):814-821



LbL self-assembly technique

Polyelectrolyte polydiallydimethylammoniuum chloride (PDADMAC) positively charged

Heparine negatively charged

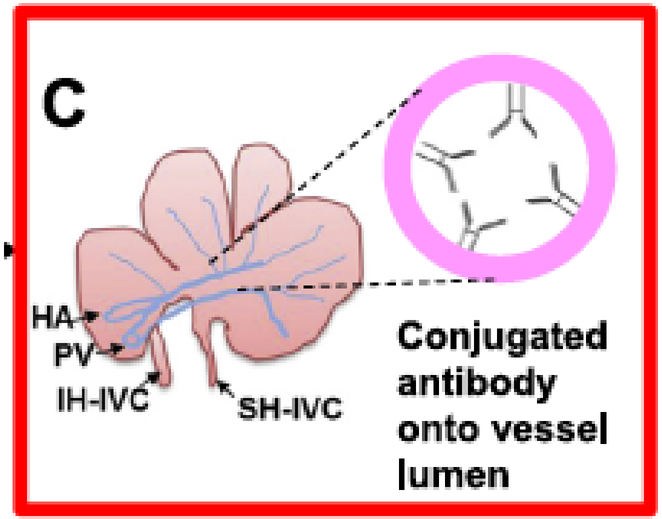
Thromboresistant after 3h of blood perfusion

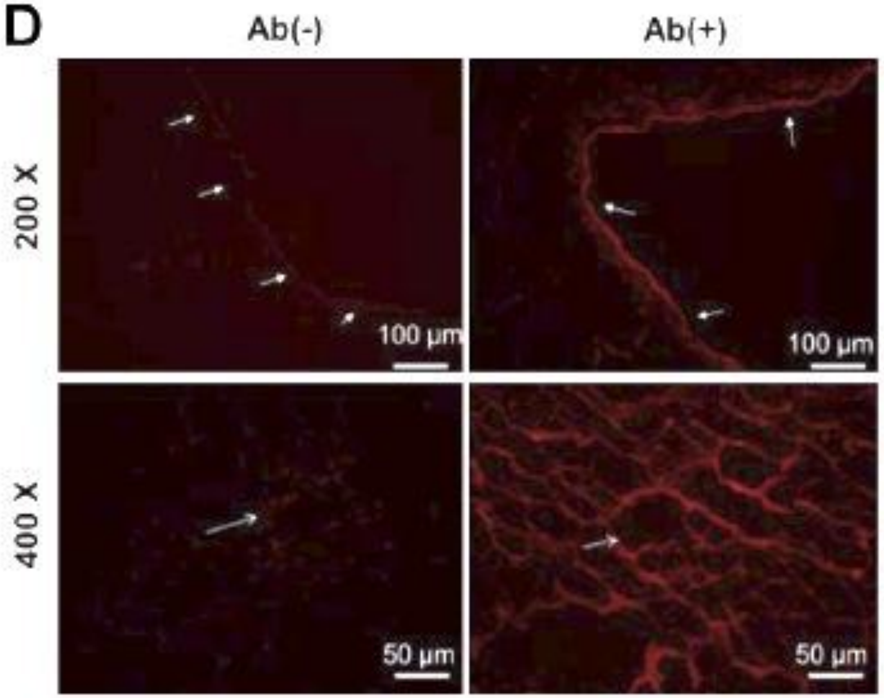
After 72h, hepatocytes maintained normal morphology

Bao et al. Cell Transplantation 2011;20:753-766

Antibody conjugation method

1. Anti endothelial cell antibodies to stabilize seeded cells on the vessel walls.
   Rat anti-mouse CD31 antibody was conjuated to the acellular liver scaffold

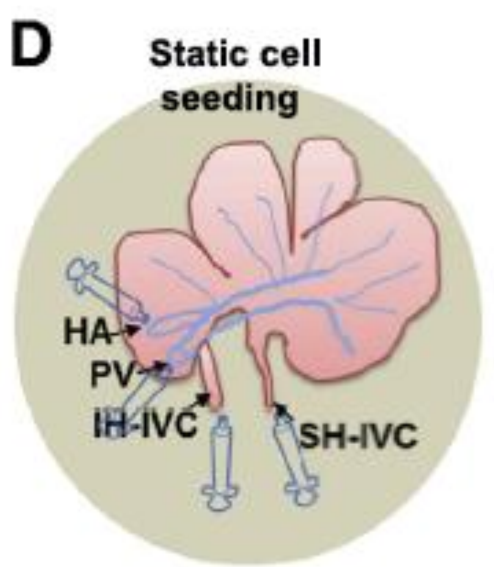


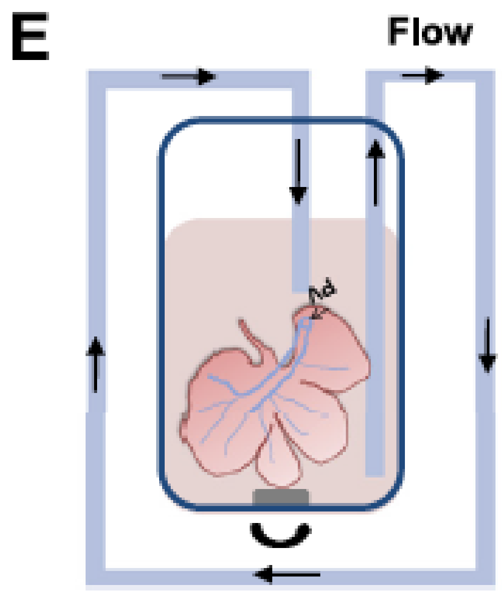


Ko et al. Biomaterials 2015;40:72-79

Antibody conjugation method

1. Anti endothelial cell antibodies to stabilize seeded cells on the vessel walls: Rat anti-mouse CD31 antibody was conjuated to the acellular liver scaffold
2. ReEndothelization with endothelial cells (MS1) expressing GFP protein

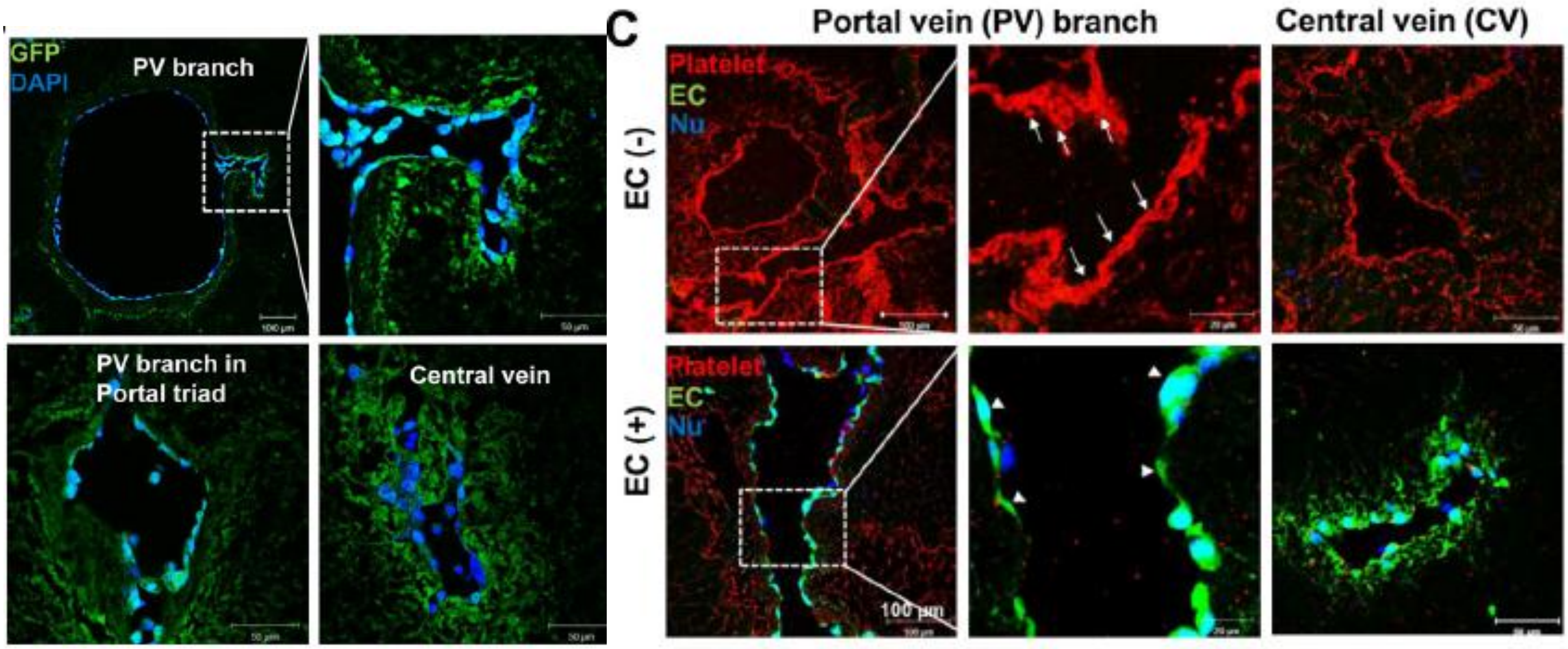




Ko et al. Biomaterials 2015;40:72-79

Transplantation

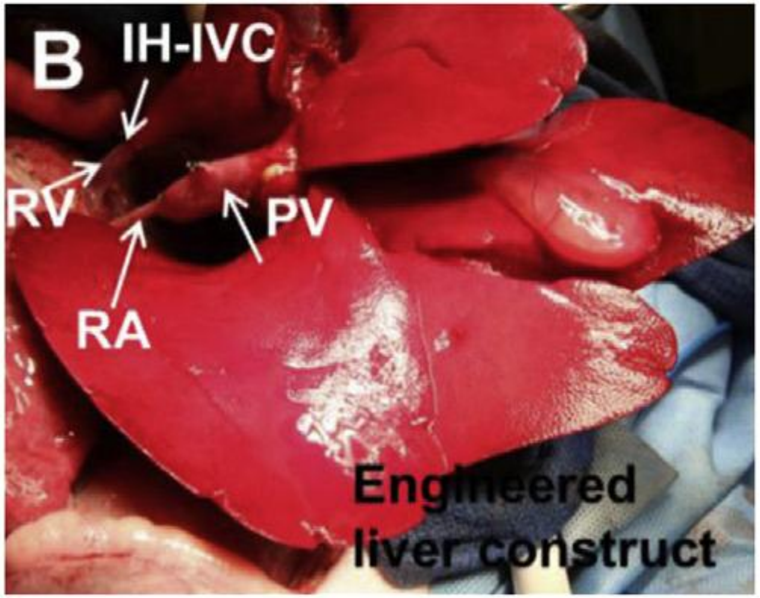
Re-endothelialization characterization



Ko et al. Biomaterials 2015;40:72-79

Antibody conjugation method

1. Anti endothelial cell antibodies to stabilize seeded cells on the vessel walls: Rat anti-mouse CD31 antibody was conjuated to the acellular liver scaffold
2. ReEndothelization with endothelial cells (MS1) expressing GFP protein
3. Implantation of engineered porcine liver construct

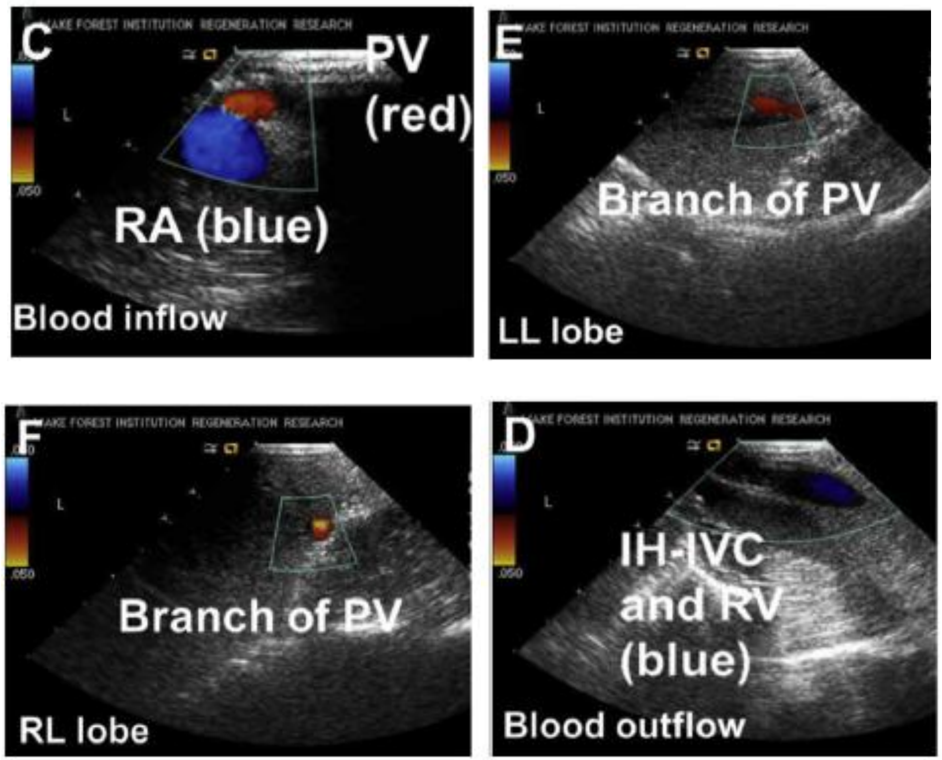


Heterotopically implantation into pig
Left renal artery – Portal vein
Left renal vein - Inferior vena cava

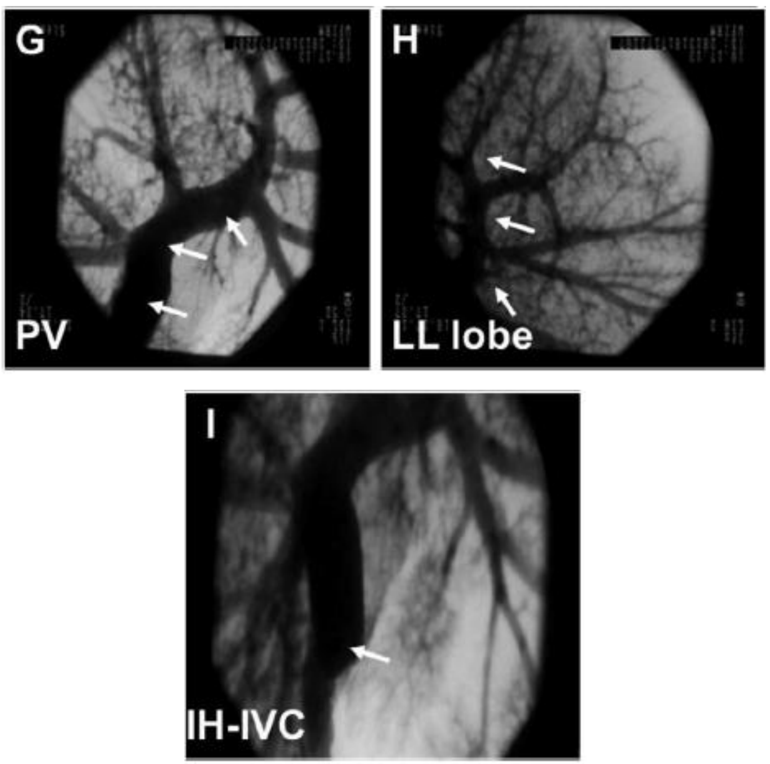
Ko et al. Biomaterials 2015;40:72-79

In vivo functional testing

Intraoperative

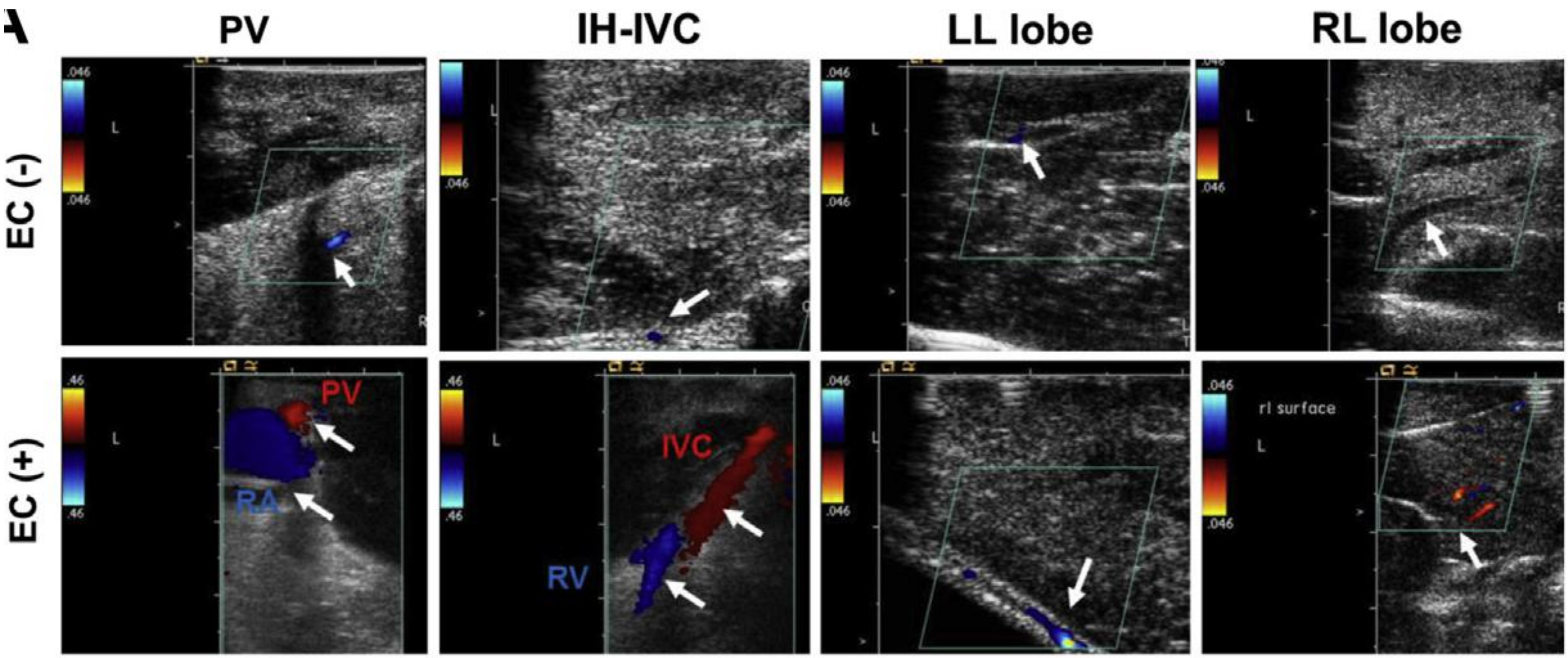


4h after implantation



Ko et al. Biomaterials 2015;40:72-79

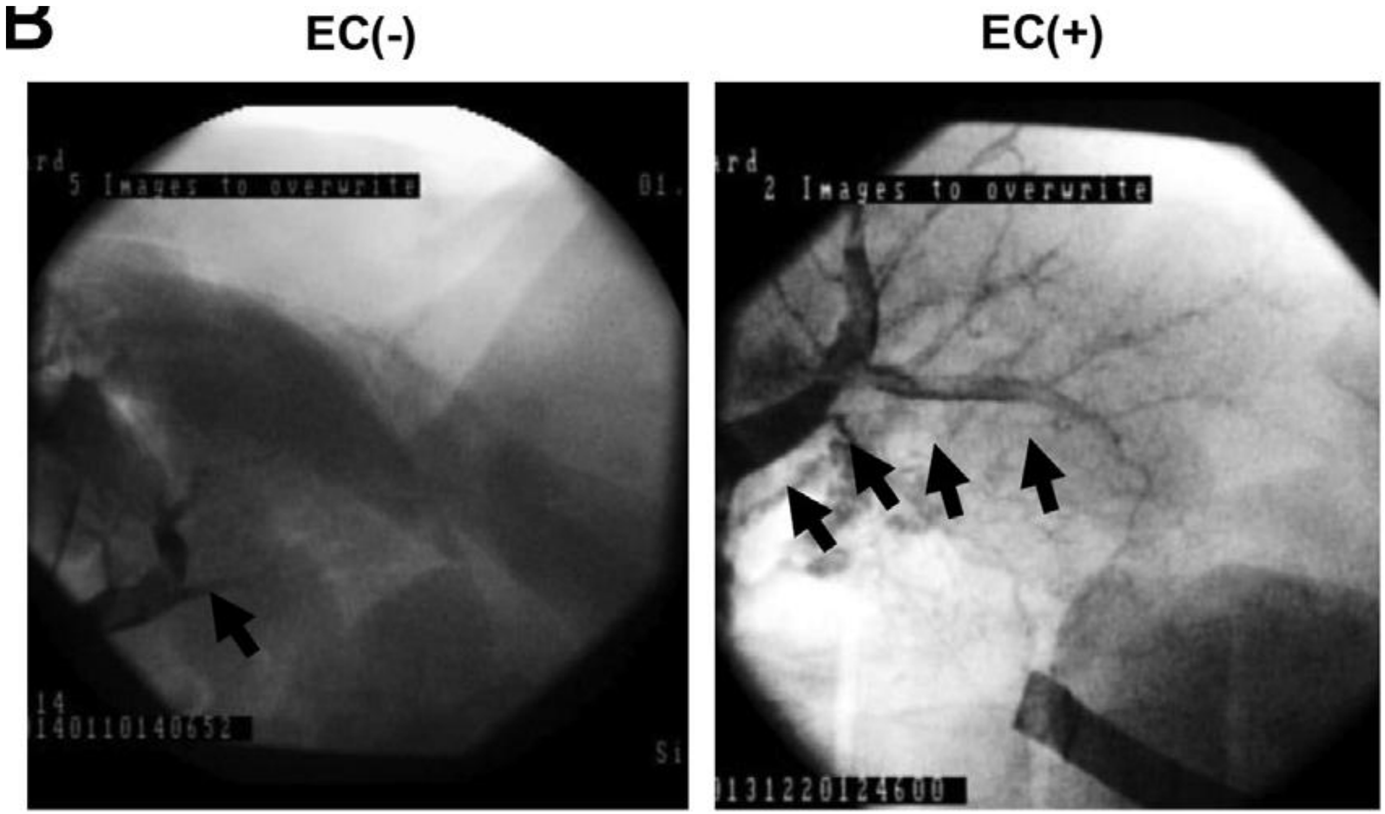
In vivo functional testing: POD 1



Ko et al. Biomaterials 2015;40:72-79

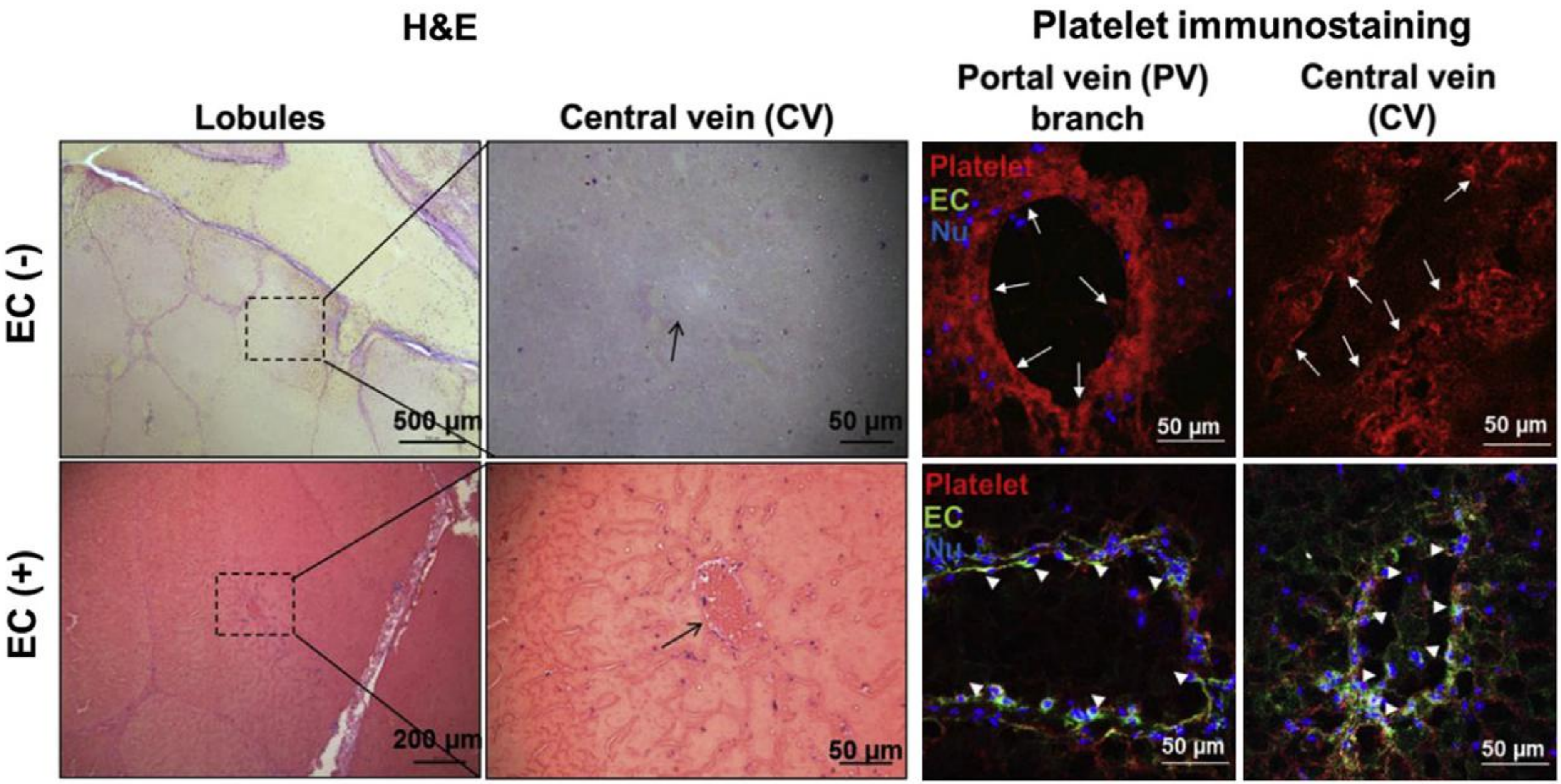
Transplantation

In vivo functional testing: POD 1



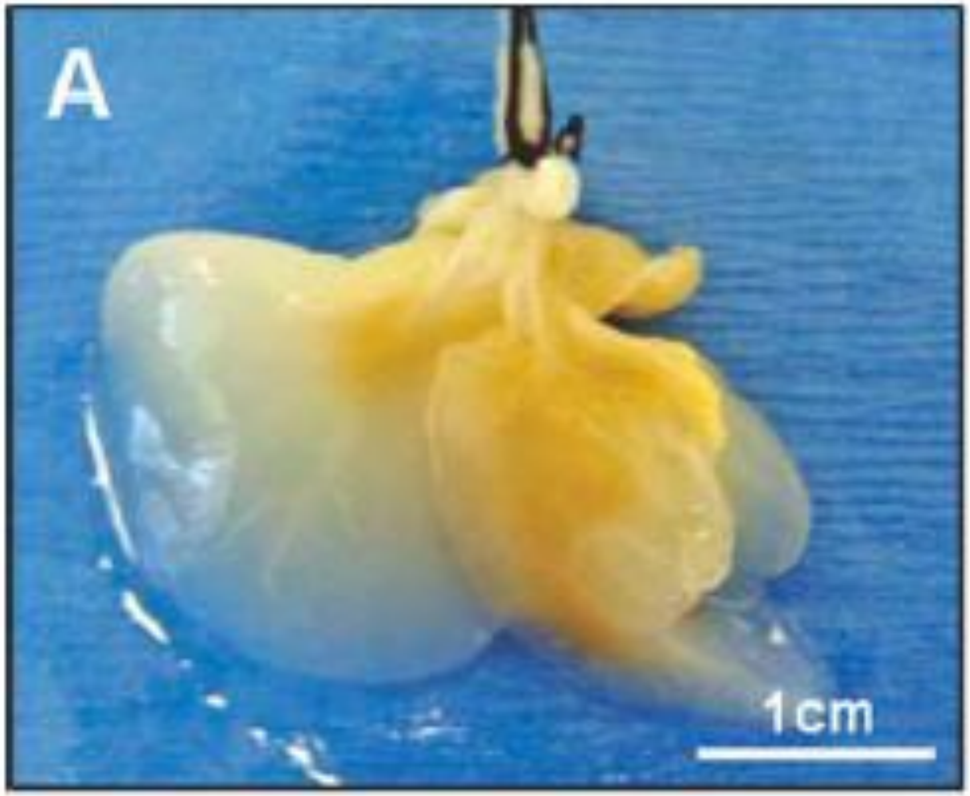
Ko et al. Biomaterials 2015;40:72-79

In vitro functional testing: POD 1



Ko et al. Biomaterials 2015;40:72-79

Main concerns



Baptista et al. Hepatology 2010 Nov;12:604-617

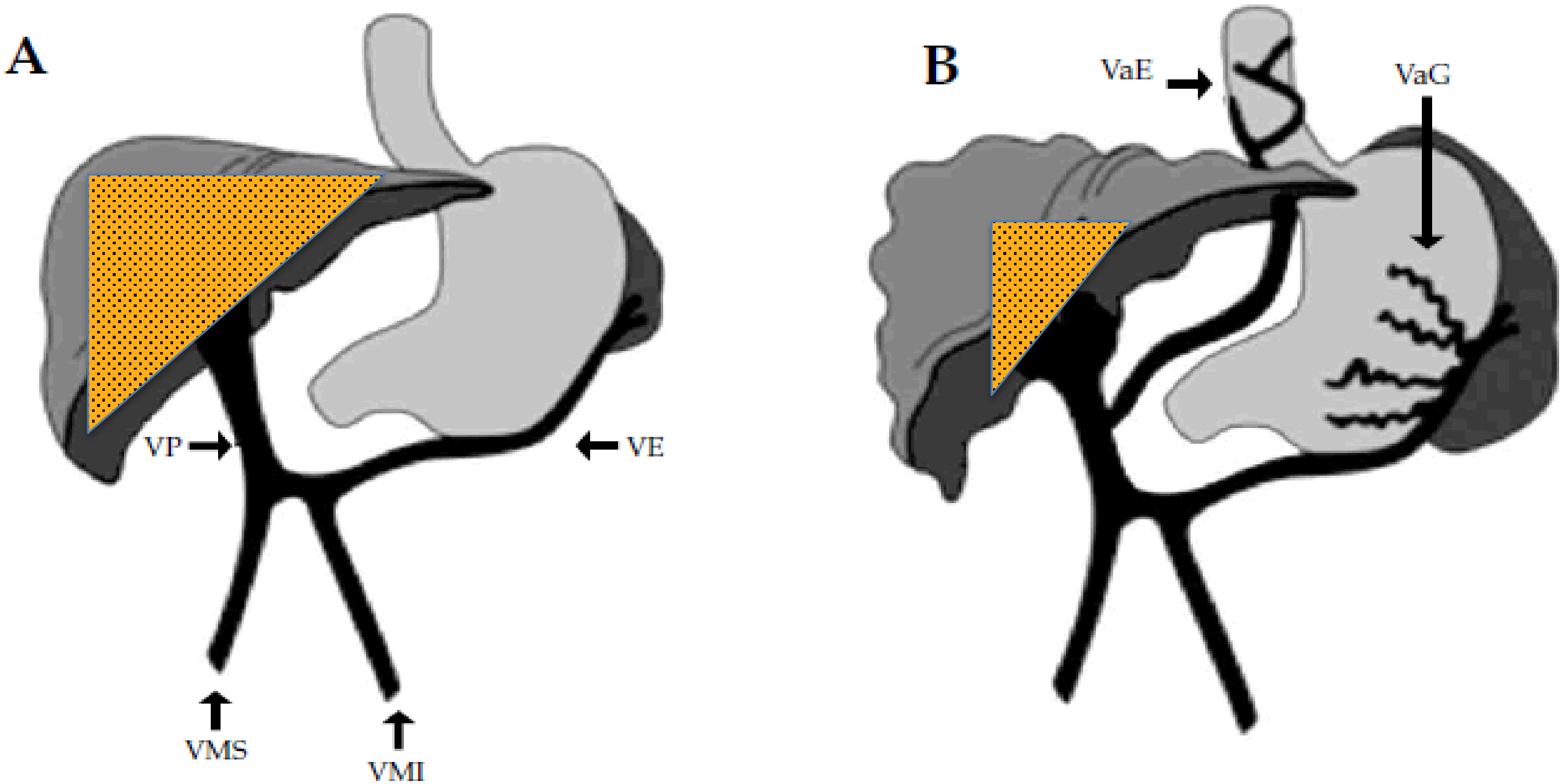
70M human fetal hepatocytes

=

4 livers
(17-21 weeks gestation)

Number is important…

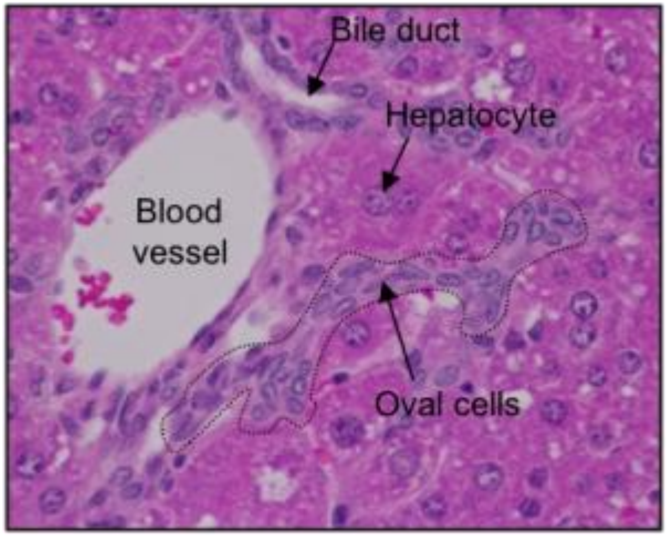
Main concerns

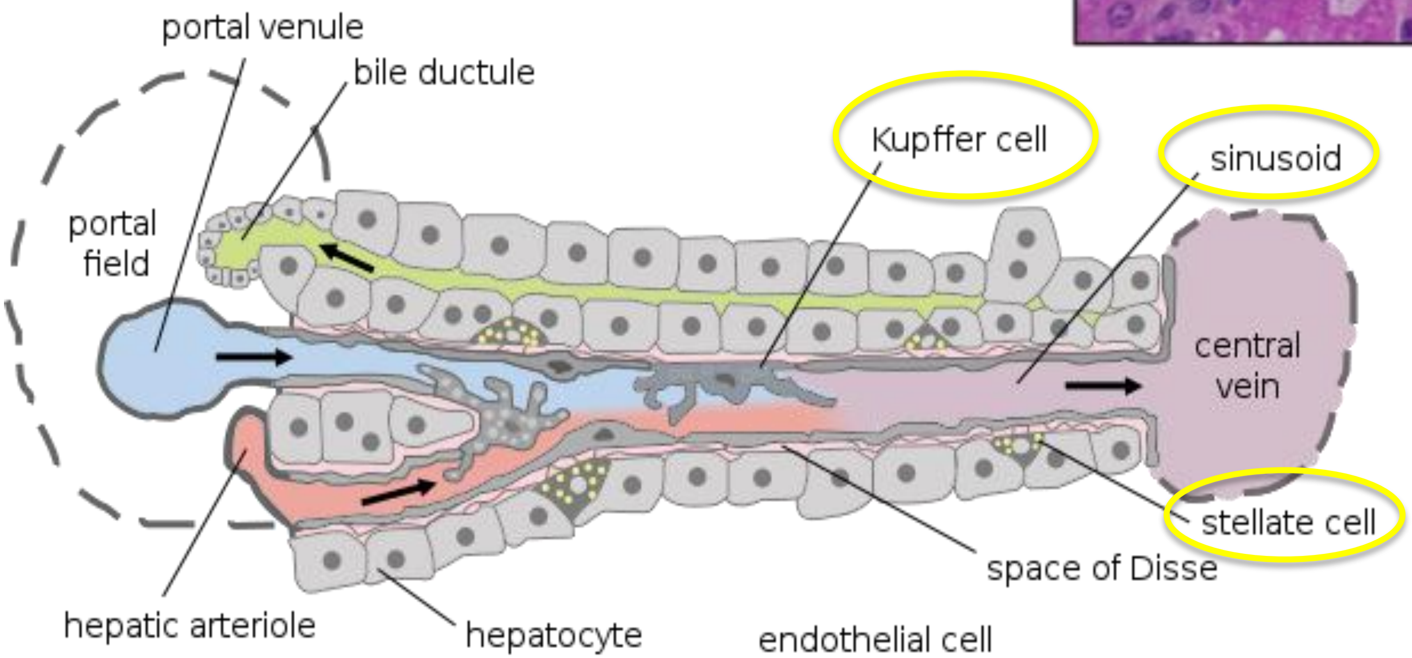


... size is important too

Future directions

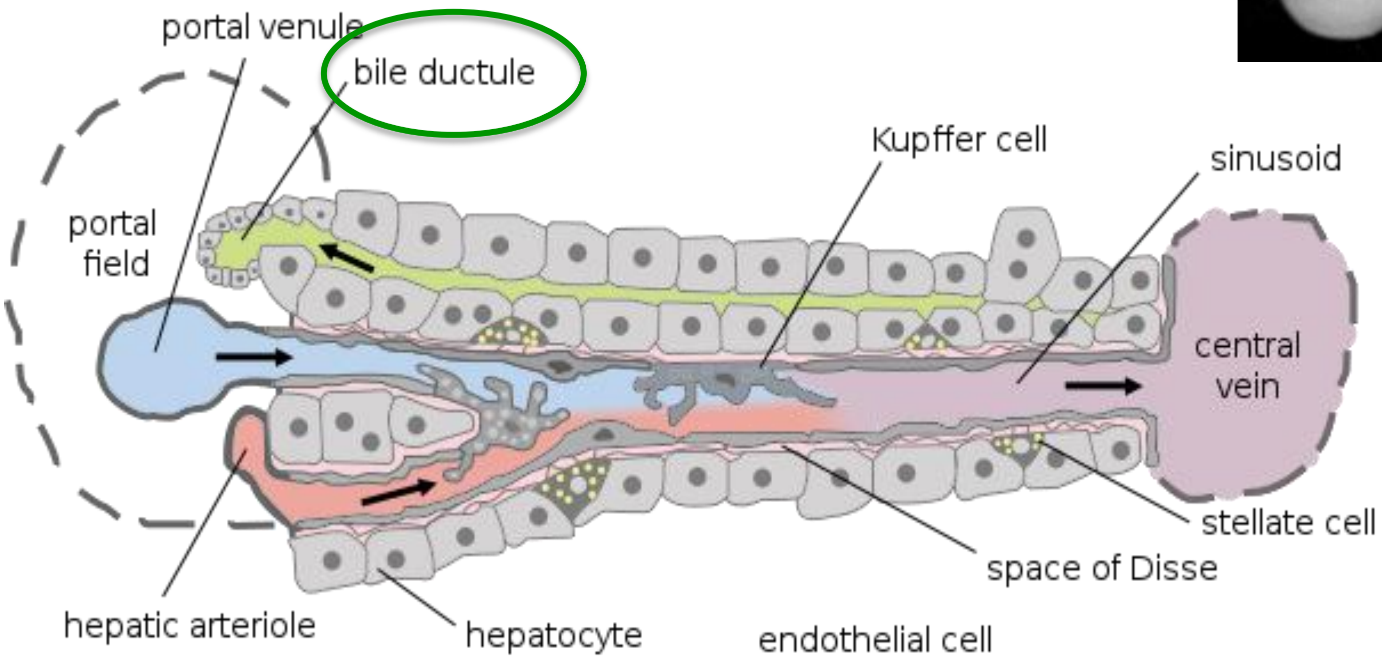
- Co-Culture with other non-parenchymal cells



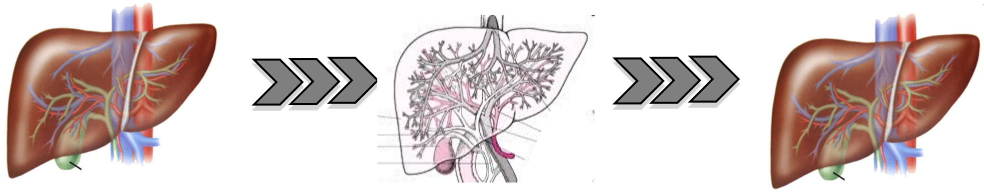


Future directions

- Co-Culture with other non-parenchymal cells
- Cholangiocytes - Biliary tree



Summary



- Decellularized livers are a good option to obtain scaffolds because architecture and vasculature are well preserved
- Cells are “happy” in the scaffolds
  - mature hepatocytes: viable and functional
  - immature cells: differentiate into cells present in the liver
- Multistep recellularization
- Endothelization needed for transplantation
- Main concerns: number of cells and size of organoid
- Future directions:
  - Coculture with other non-parenchymal cells
  - Cholangiocytes and Bile Duct

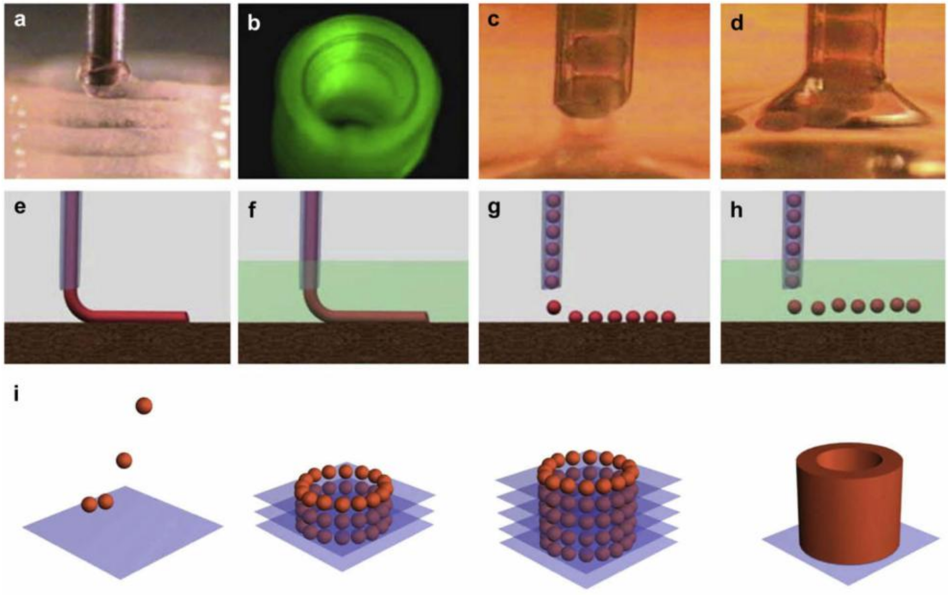
Organ Bioprinting

Organ Bioprinting

Organ Bioprinting: technology to fabricate scaffolds

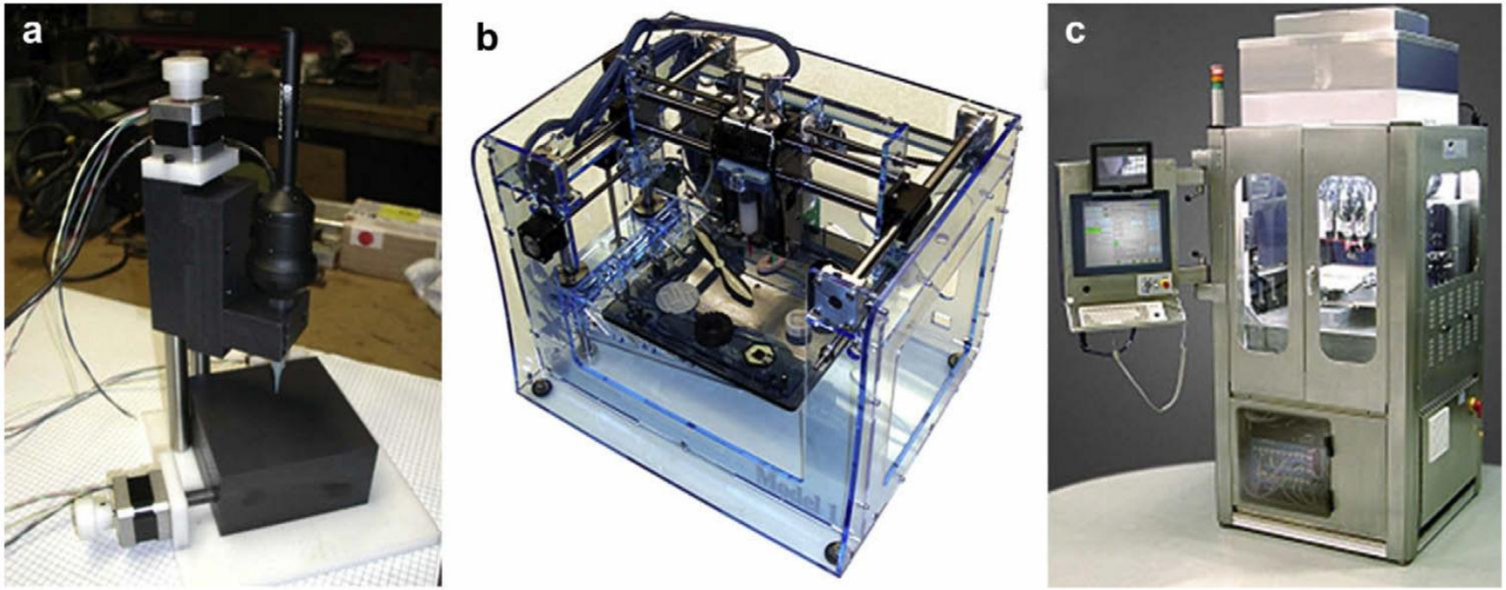
- Based on the Additive Manufacturing Technology
  Technique that produces complex 3D structures being able to control the size, shape, distribution and interconnectivity of pores of the scaffold

- Allow direct cell deposition in organotypic architecture



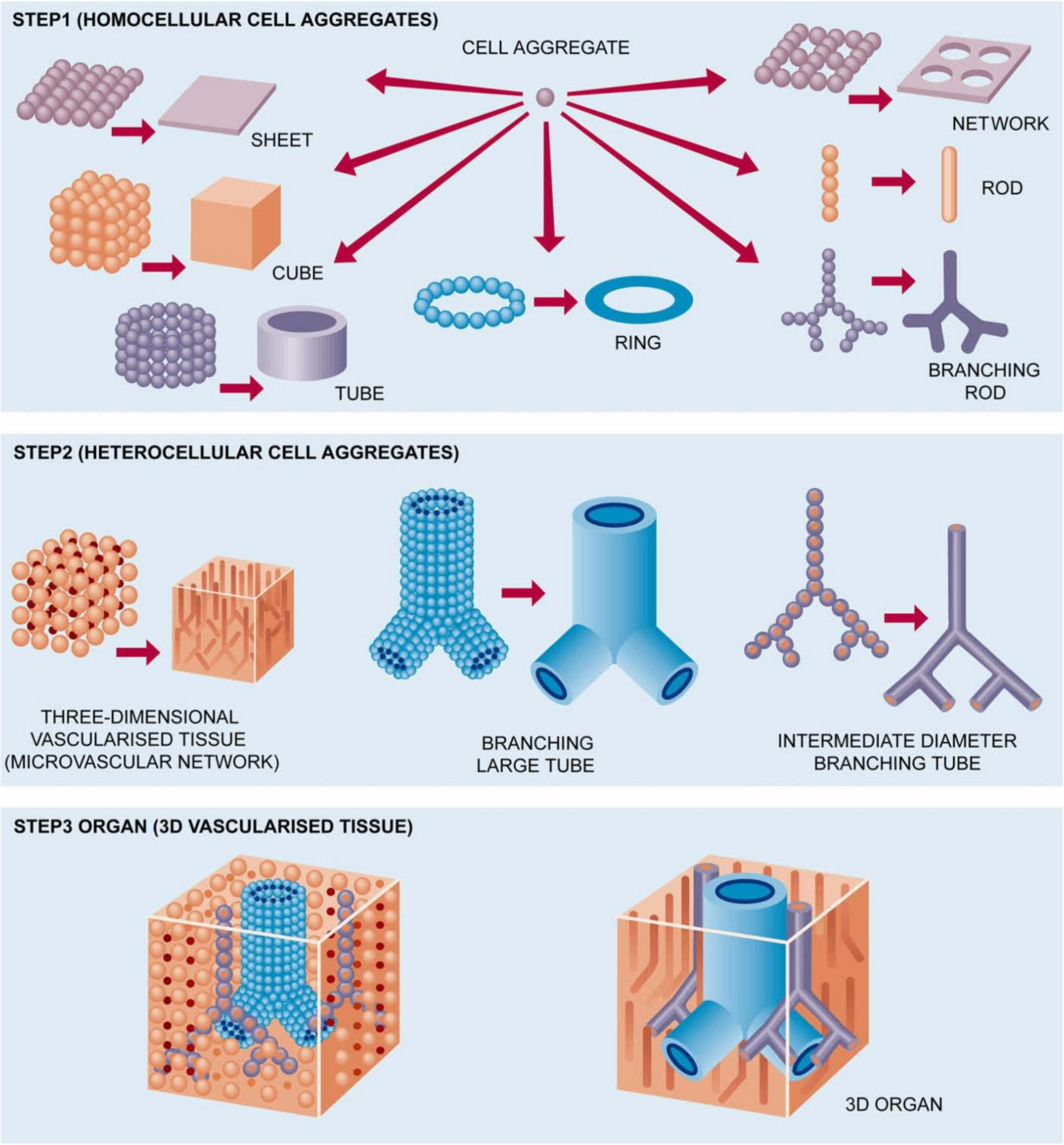
Mironov. Biomaterials 2009;30(12):2164-2174

Organ Bioprinting



Mironov. Biomaterials 2009;30(12):2164-2174

Organ Bioprinting



Mironov. Biomaterials 2009;30(12):2164-2174

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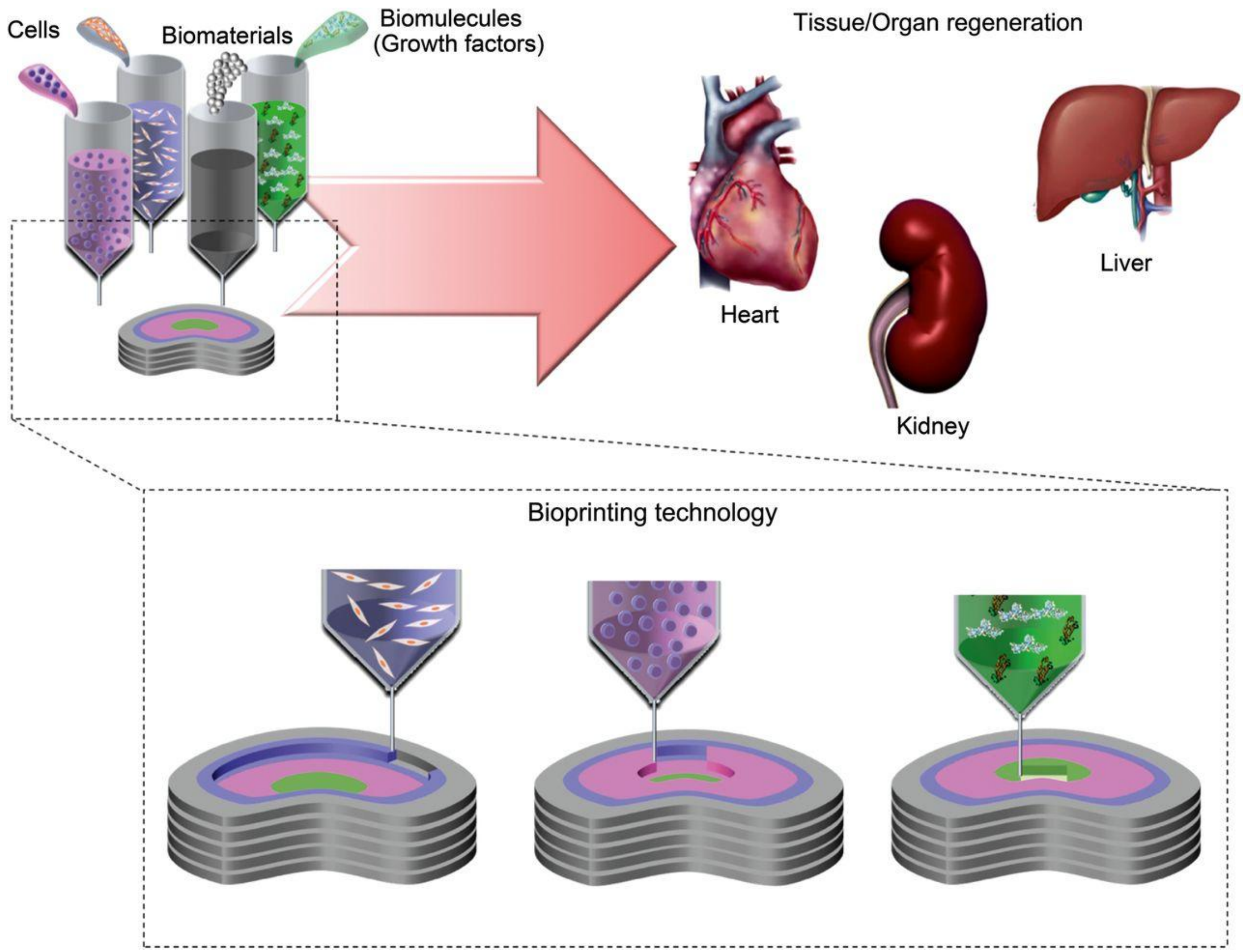
Organ Bioprinting

Table 1: Tissue engineering applications using bioprinting technology

Tissue	Techniques	Cell types	Growth factors	Materials
Heart valve	Extrusion-based bioprinting	Aortic valve interstitial cell Aortic root sinus smooth muscle cell	–	Hyaluronic acid Gelatin Alginate
Myocardial tissue	Extrusion-based bioprinting	Cardiomyocyte progenitor cell	–	Alginate
Blood vessel	Jetting-based bioprinting	Endothelial cell Smooth muscle cell Mesenchymal stem cell	–	Fibrin
	Extrusion-based bioprinting	Endothelial cell Cardiac cell Smooth muscle cell Fibroblast	–	Collagen Agarose Alginate
Musculo-skeletal tissue	Jetting-based bioprinting	Muscle-derived stem cells Myoblast Mesenchymal fibroblast	BMP-2 FGF-2	Fibrin
	Extrusion-based bioprinting	Bone marrow stromal cell Endothelial progenitor cell Endogeneous stem cell	TGF-β	Agarose Alginate Hydroxyapatite Polycaprolactone
Nerve	Jetting-based bioprinting	Embryonic motorneuron cell Hippocampal cell Cortical cell Neuronal precursor cell Neural stem cells	CNTF VEGF	Soy agar Collagen Fibrin
	Extrusion-based bioprinting	Bone marrow stem cell Schwann cells	–	Agarose
Skin	Jetting-based bioprinting	Dermal fibroblast Epidermal keratinocyte	–	Collagen

Seol et al. Eur J Cardiothorac Surg 2014;46:342-348

Organ Bioprinting



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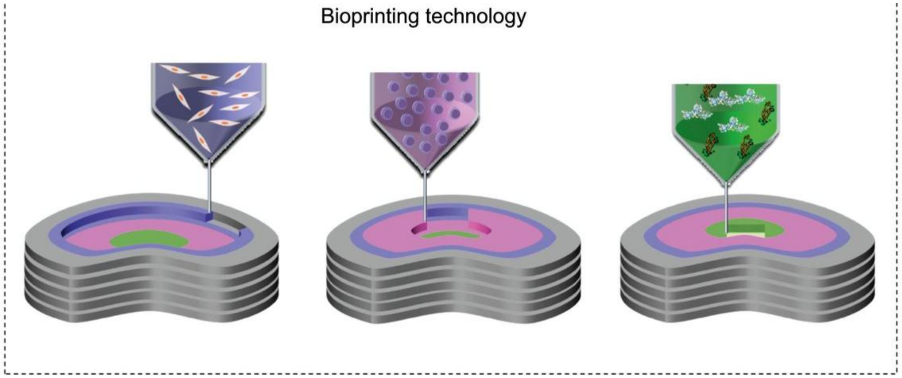
Organ Bioprinting



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