XXV CONGRESO SETH

Málaga 2015
Sociedad Española de Trasplante Hepático

Nuevas Fuentes de Órganos

Regeneración hepática y cronobiología

Vall d'Hebron Hospital

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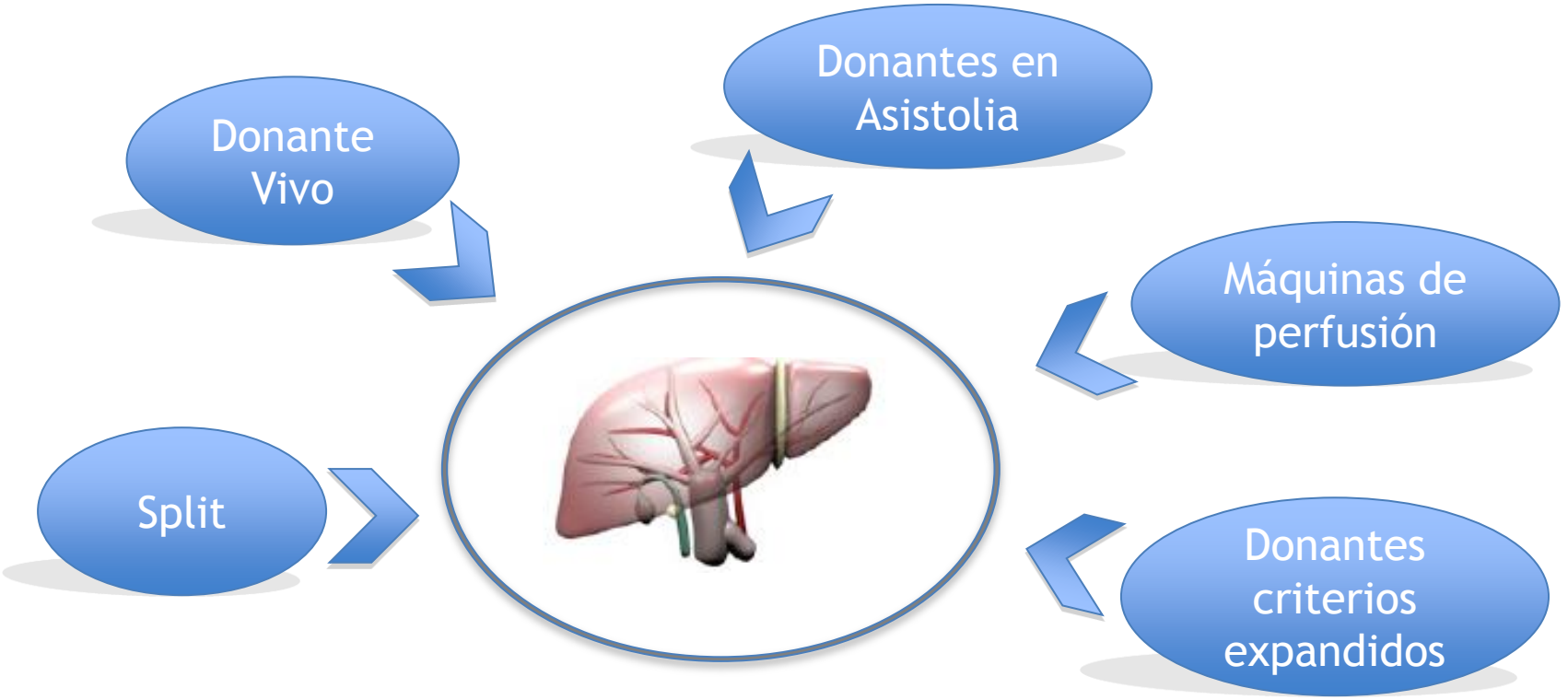
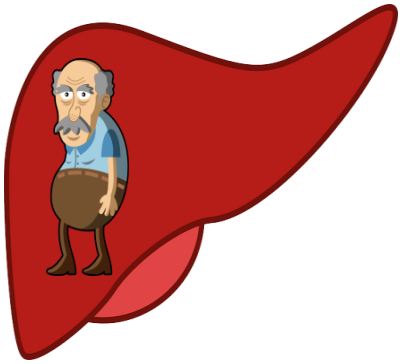
Servicio Cirugía HBP y Trasplantes
Octubre 2015

Estrategias para aumentar el número de órganos



Donantes añosos - Cronobiología

Introducción



Mínima isquemia
VHC negativo (?)
No PCR
No esteatosis
No fibrosis

Table 2 Series of orthotopic liver transplantation with liver grafts older than 70 years

Ref.	Cases (*n*)	Donor mean age (yr)	Cold ischemic time (h)	Recipient mean age (yr)	Primary non-function	Patient survival (yr)	Graft survival (yr)
Emre *et al*[13]	36	73.5	9	55	5.5%	1-yr: 91%	1-yr: 85%
Kim *et al*[54]	25	74	7.6	49	8%	1-yr: 95.4%	1-yr: 82.7%
						3-yr: 89.8%	3-yr: 71.7%
Gastaca *et al*[23]	55	-	5	-	0%	1-yr: 93.8%	1-yr: 92.6%
						3-yr: 90.6%	3-yr: 89.4%
Borchert *et al*[99]	41	73.4	8.9	50.9	2.4%		
Segev *et al*[25] (UNOS)	1043	74.8					
Cescon *et al*[26]	111	-			7%		
Fouzas *et al*[65]	17	73	7.2	57	11.8%		
						5-yr: 46.2%	
Lai *et al*[61]	28	74	6.4	57	3.6%	5-yr: 47%	5-yr: 40.7%
Sampedro *et al*[112]	24	78.3	3.7	53.9	0%	1-yr: 78%	
						5-yr: 63%	
Darius *et al*[58]	58	77	8	61	0%	1-yr: 90%	1-yr: 88%
						5-yr: 84%	5-yr: 79%
Jiménez-Romero *et al*[55]	50	75.7	6.1	51	0%	1-yr: 76%	1-yr: 73.9%
						5-yr: 62.9%	3-yr: 64.6%
							5-yr: 58.3%

1a 69.7-95.4%
3a 57.5-90.6%
5a 46.2-84%

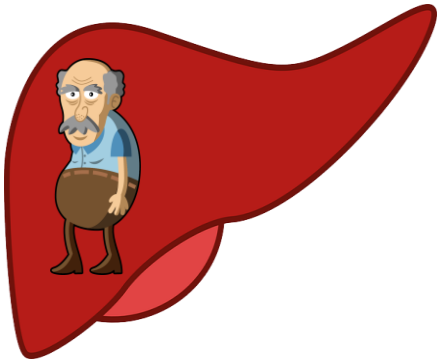
Table 3 Series of orthotopic liver transplantation with liver grafts older than 80 years

Ref.	Cases (*n*)	Donor mean age (yr)	Cold ischemic time (h)	Recipient mean age (yr)	Primary non-function	Patient survival (yr)	Graft survival (yr)
Jiménez-Romero *et al*[55]	4	85.7	5.5	50.2	0%	1-yr: 75%	1-yr: 75%
Nardo *et al*[53]	30	82.3	7.5	52.5	0%		
Zapletal *et al*[24]	5		9.5	52	0%		
Cescon *et al*[26]	41			52.5	0%		
Petridis *et al*[79]	10	83.5	5	57.4	10%		
Singhal *et al*[80] (UNOS)	197			58.5			
						3-yr: 69.1%	3-yr: 61.2%

1a 75-100%
3a 40-86%
5a 86%

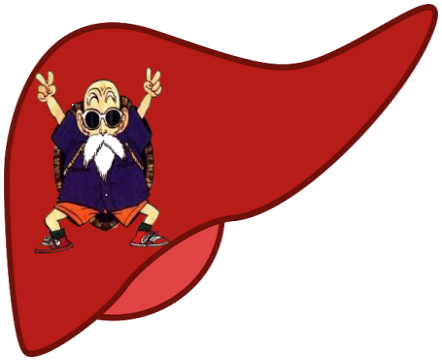
Jiménez-Romero C. World J Gastroenterol 2014;21(31):10691

Introducción



¿ Cuánto tiempo puede vivir un hígado?

¿ Los hígados envejecen al mismo ritmo que el donante o pueden rejuvenecer en un receptor joven?



Rejuvenation of aged progenitor cells by exposure to a young systemic environment

NATURE | VOL 433 | 17 FEBRUARY 2005

"Aumento de la proliferación de hepatocitos ancianos en un ratón joven"

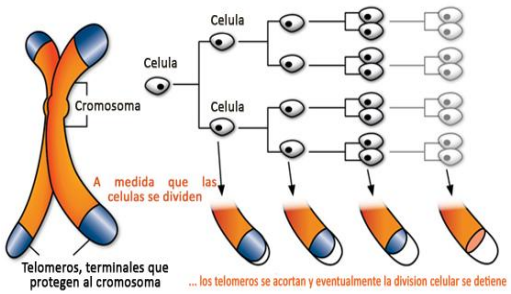
El entorno sistémico joven

- recuperación mecanismo señalización molecular joven de células progenitoras ancianas e incrementa su proliferación.
- facilita la regeneración, mientras que un entorno anciano no permite una regeneración tisular optima

Introducción

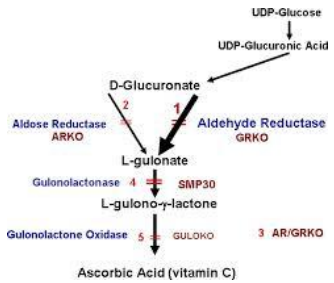
Marcadores de longevidad de los trasplantes hepáticos (tx infantil donante vivo)

Telómeros



- Longitud disminuye con la edad[1] y el daño celular[2].
- Longitud en los injertos es menor que en la población de la misma edad que el donante; injertos envejecen más rápidamente que la población normal[1].
- Quimerismo escaso: <1% hepatocitos del receptor[1].

Sensescence marker protein-30 (SMP-30)

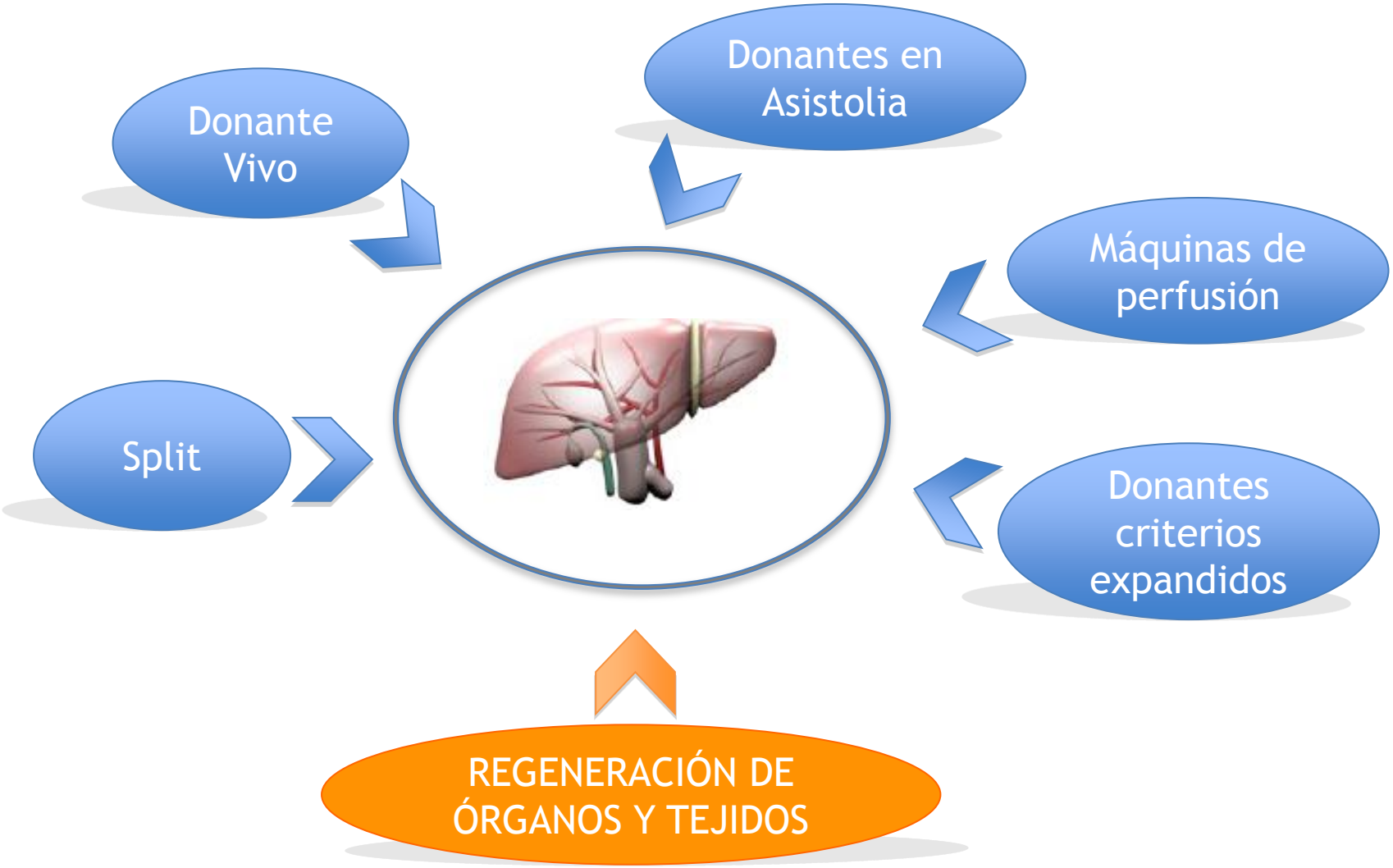


- SMP-30 presente en los hígados en edad pediátrica
- SMP-30 no se expresó en el injerto tras el trasplante de donante vivo

Hígados no rejuvenecen tras el trasplante

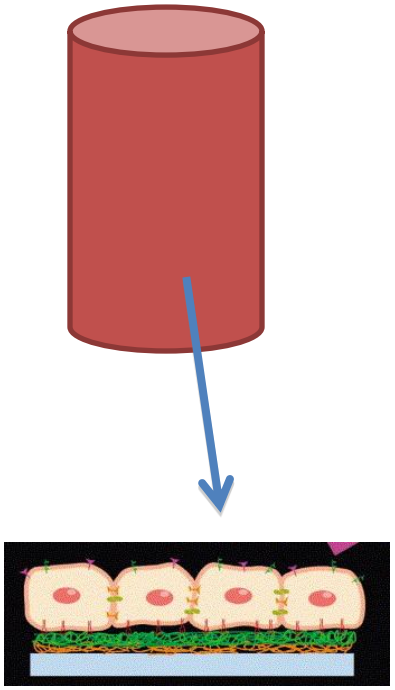
1. Aini. Transplant Int 2012;25:956. 2. Aikata.Exp Cell Res 2000;256:578-582 3. Eguchi. Dig Dis Sci 2011;56;1542-1547

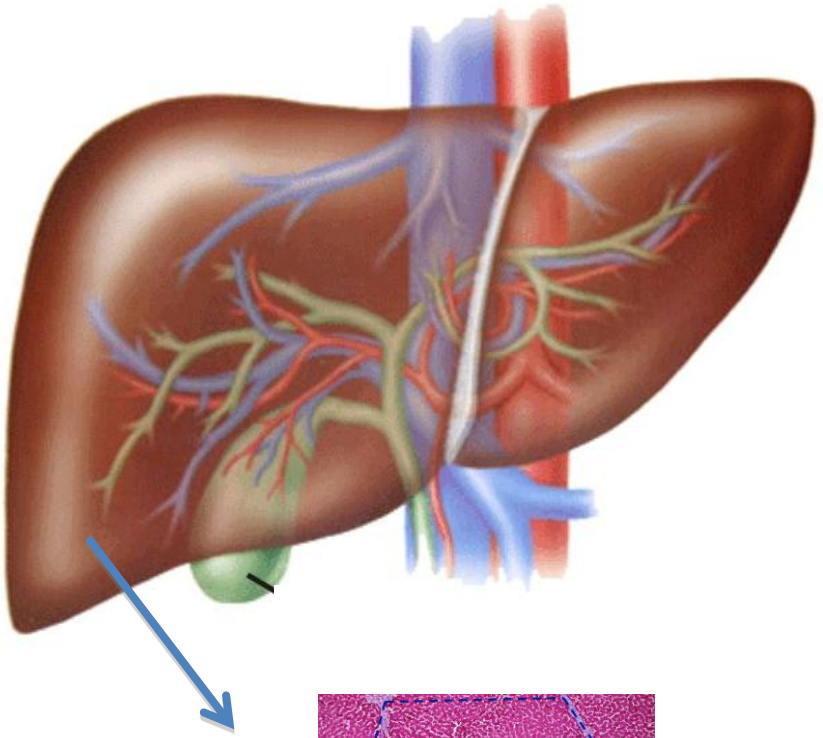
Estrategias para aumentar el número de órganos

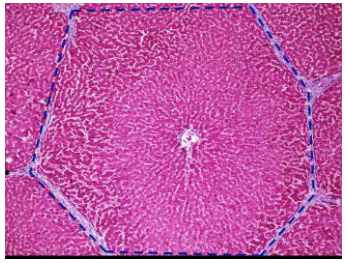


Órganos sólidos: estructura compleja!!

Tráquea
Vejiga urinaria



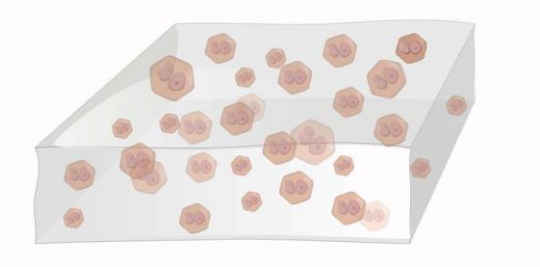




Introducción

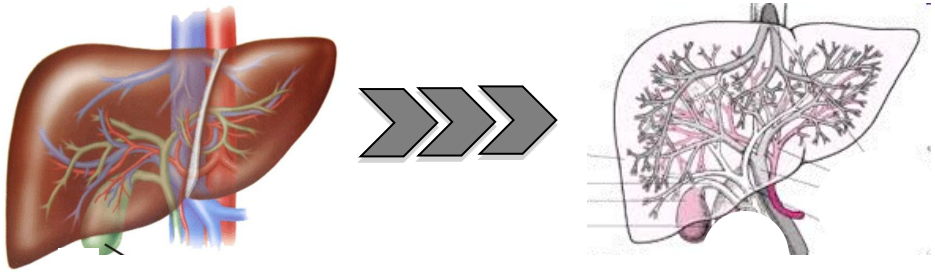
Scaffold ideal : biocompatible, biodegradable, porosidad, soporte estructural

Scaffold Artificial



- Difícil control del tamaño, microarquitectura y interconectividad de los poros

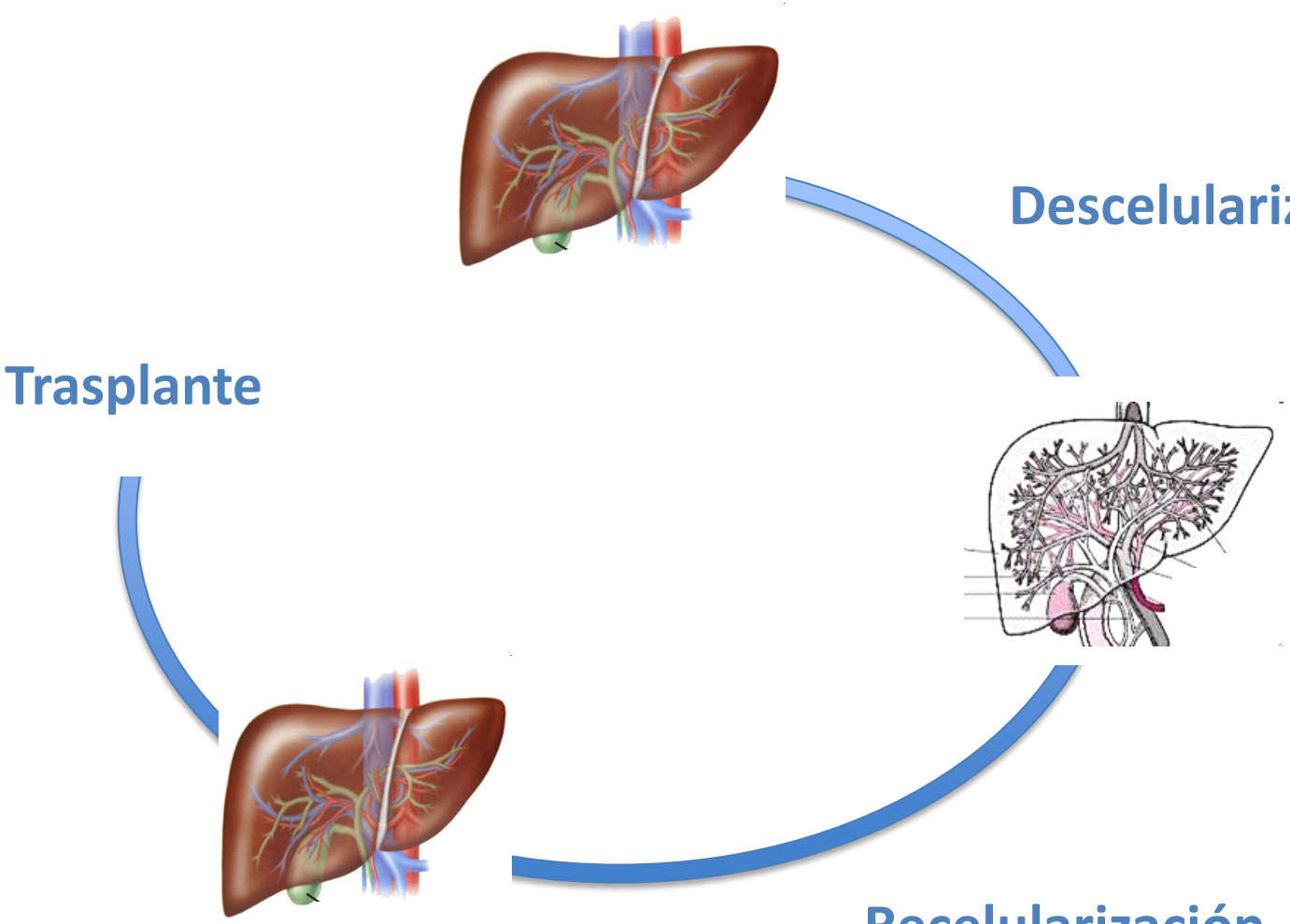
Scaffold Natural



Órgano → Matriz extracelular

- Estímulos físicos, químicos y moleculares que permiten el engraftment celular
- Preservación de la trama vascular
- Escasa inmunogenicidad

Introducción



Introducción

Table 1. Studies in the literature

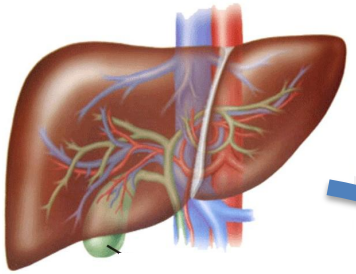
Author	Type Cells	Infusion Method	Via	Number Cells	Flow Rate	Time
Baptista 2010	hUVEC hFLC MS1	Continuous	PV IVC, PV, IVC+PV	$30x10^6$ $70\ x10^6$ $100\ x10^6$	3ml/min → 0.5ml/ min 5ml/min	7d 3d
Uygun 2010	Rat MH Endothelial cells	Multistep	PV	$200\ x10^6$	15ml/min	5d 5d
Soto 2011	Rat MH	Direct injection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

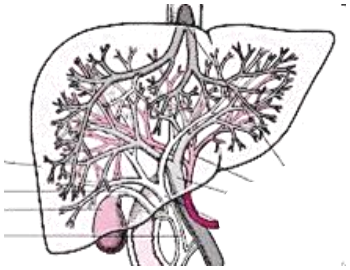
Author	Media
Baptista 2010[1]	RPMI 1640, FBS, dexamethasone, penicillin-streptomycin, prolactin, glucagon, niacinamide, lipoic acid, triiodothyronine, hEGF, hHDL, hHGF, hGH, insulin, transferrin
Uygun 2010[3]	William's E, FBS, insulin, EGF, glucagon, hydrocortisone, penicillin-streptomycin
Soto 2011[63]	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

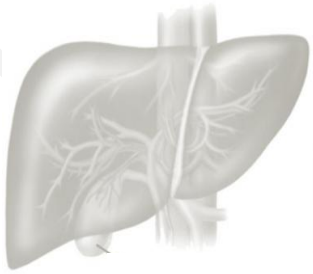
Descelularización



Descelularización



Recelularización
Maduración



Transplante

Descelularización

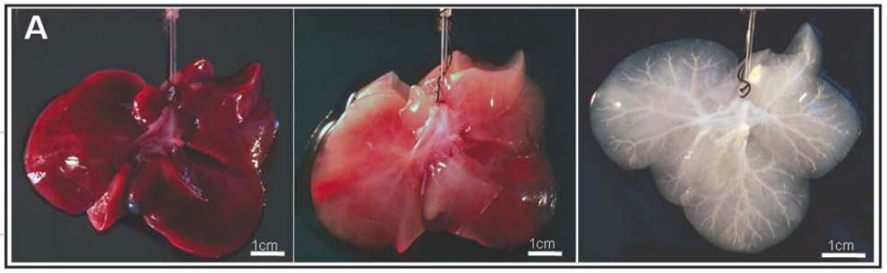
Diferentes tipos de “recetas” para la descelularización de hígados

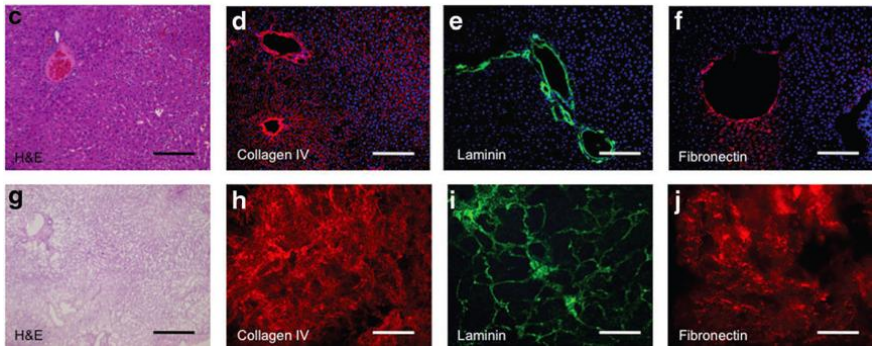
Uygun	rata	SDS Tritón X-100	0.01% 24h, 0.1% 24h, 1% 24h 1% 30min	1ml/min
Baptista	rata	Tritón X-100 + NH4 OH	1%+3%, 3h	5ml/min
Soto	rata	Tripsina + EGTA Tritón X-100 + EGTA	0.02%+0.05%, 2h 3%+0.05%, 24h	8ml/min
Bao	rata	Adenosina SDS	10mM 1% 4h, 0.5% 4h, 0.25% 4h	25ml/min
Yagi	cerdo	SDS Tritón X-100	0.01% 24h, 0.1% 24h, 1% 48h 1% 30min	30ml/min
Ko	cerdo	Tritón X-100 + NH4 OH	1%+3%, 3h	0.5ml/min

Descelularización

Diferentes tipos de “recetas” para la descelularización de hígados

Uygun	rata	SDS Tritón X-100
Baptista	rata	Tritón X-100 + NH4 OH
Soto	rata	Tripsina + EGTA Tritón X-100 + EGTA
Bao	rata	Adenosine SDS
Yagi	cerdo	SDS Tritón X-100
Ko	cerdo	Tritón X-100 + NH4 OH





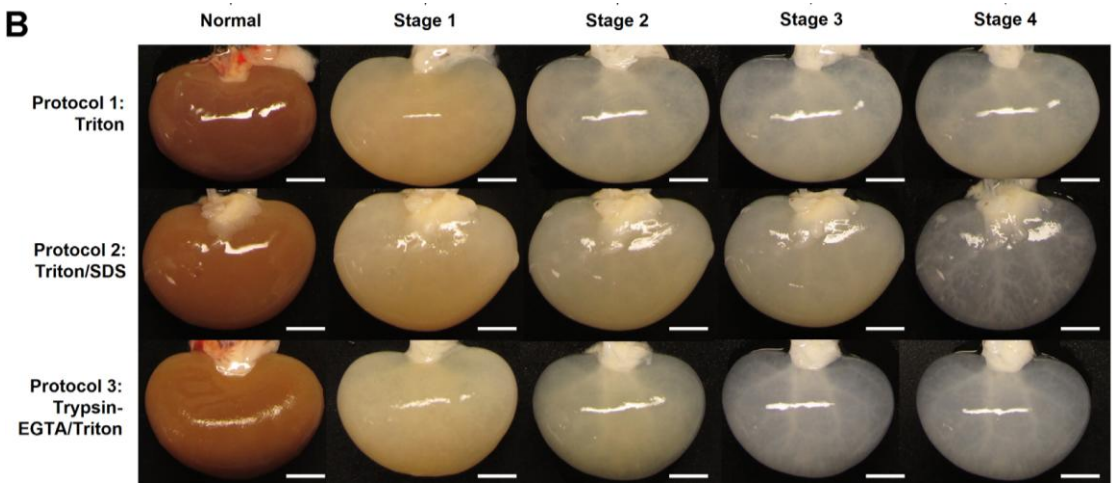
Descelularización

… el mejor protocolo de descelularización en riñones

A

	1 h, 40 min.	3 h, 20 min.	16 h, 40 min.	3 h, 20 min.	1 h, 40 min.
Protocol 1: Triton	dH_2O 500 mL	1% Triton X-100 1000 mL	1% Triton X-100 1000 mL	1% Triton X-100 1000 mL	dH_2O 500 mL
Protocol 2: Triton/SDS	dH_2O 500 mL	1% Triton X-100 1000 mL	1% Triton X-100 1000 mL	0.1% SDS 1000 mL	dH_2O 500 mL
Protocol 3: Trypsin-EGTA/Triton	dH_2O 500 mL	2 h 0.02% trypsin-0.05% EGTA	1% Triton X-100 1000 mL	1 % Triton X-100 1000 mL	dH_2O 500 mL

B



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

Descelularización

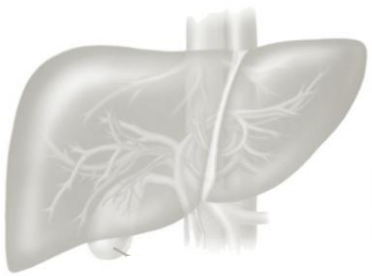
Table 1: Summary evaluation of decellularization protocols

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)	+	+	–

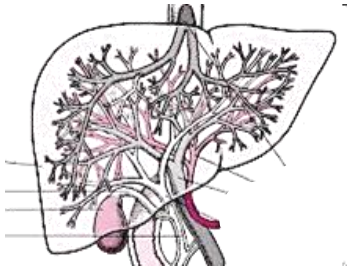
bFGF, basic fibroblast growth factor; ECM, extracellular matrix; H&E, hematoxylin & eosin; SEM, scanning electron microscopy; SDS, sodium dodecyl sulfate; VEGF, vascular endothelial growth factor.
For each criterion, protocols were evaluated and assigned one of three values: good (+), fair (+/–) or poor (–) 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

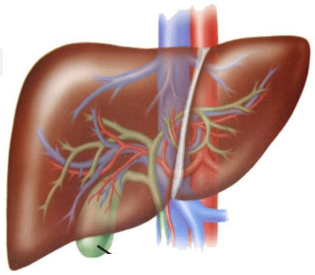
Recelularización



Descelularización



Recelularización
Maduración



Trasplante

Recelularización

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

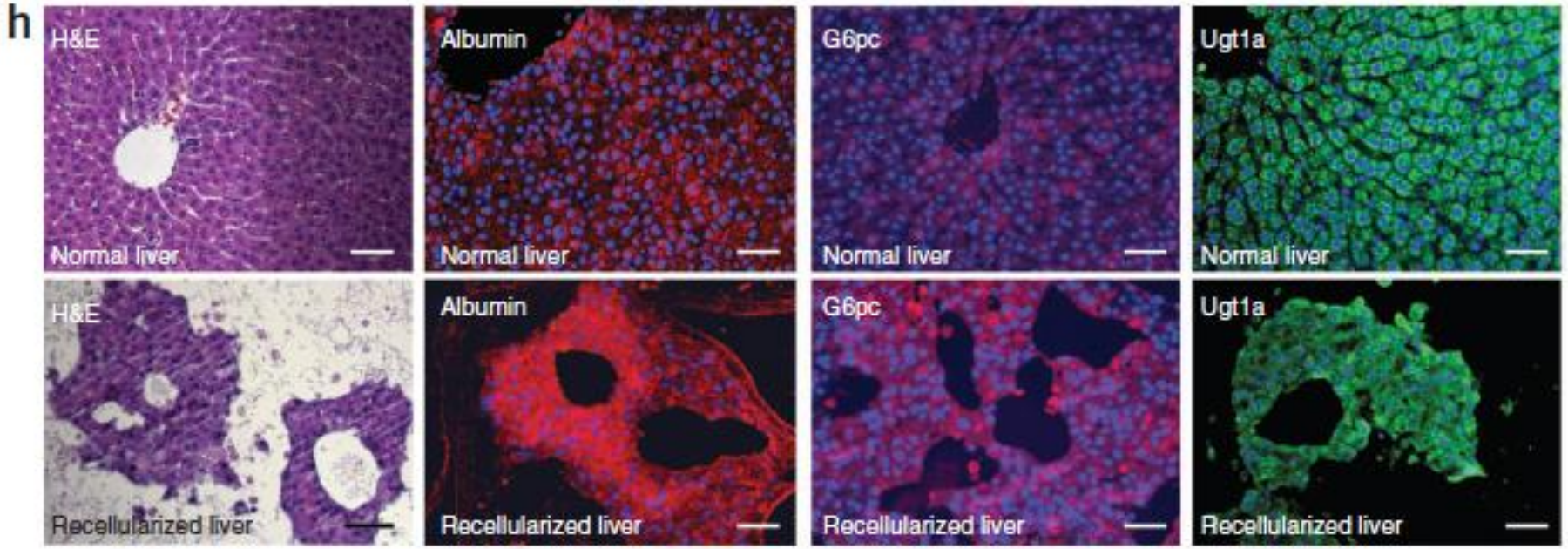
Heptocitos adultos de rata

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

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

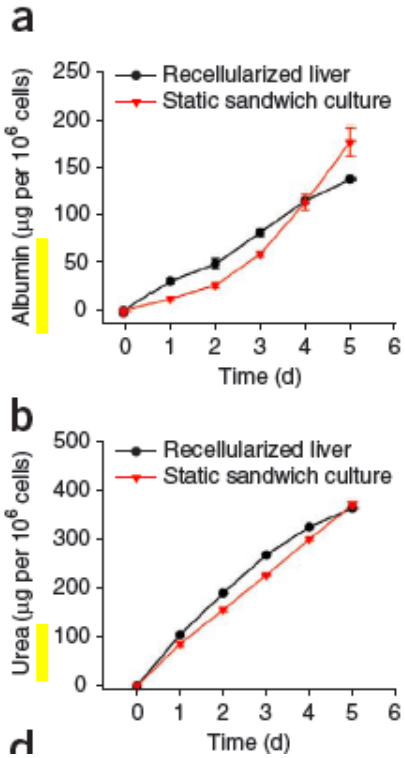
hFLCs + hUVEC

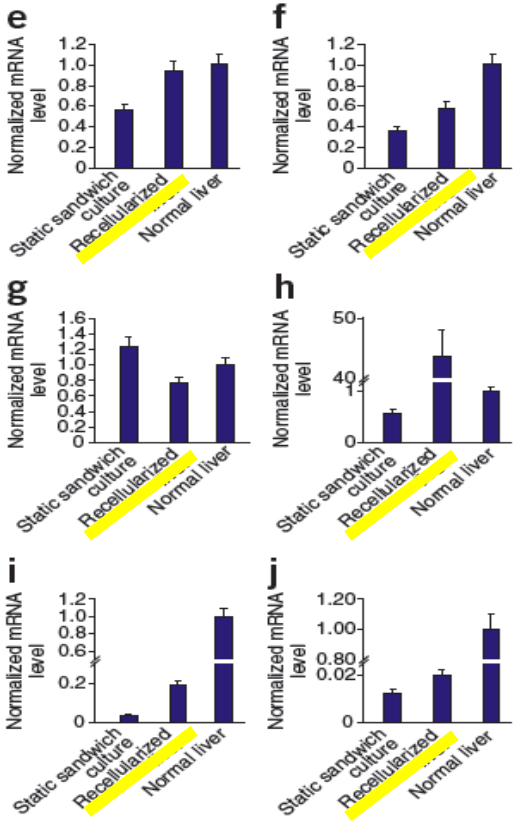
Recelularización



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

Recelularización





(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. ($n = 3$).

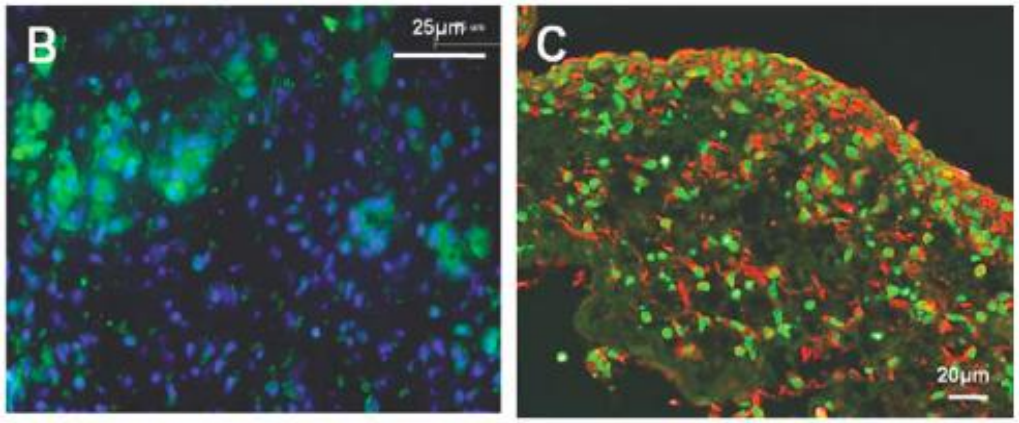
20% albumin production of *in vivo* levels

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

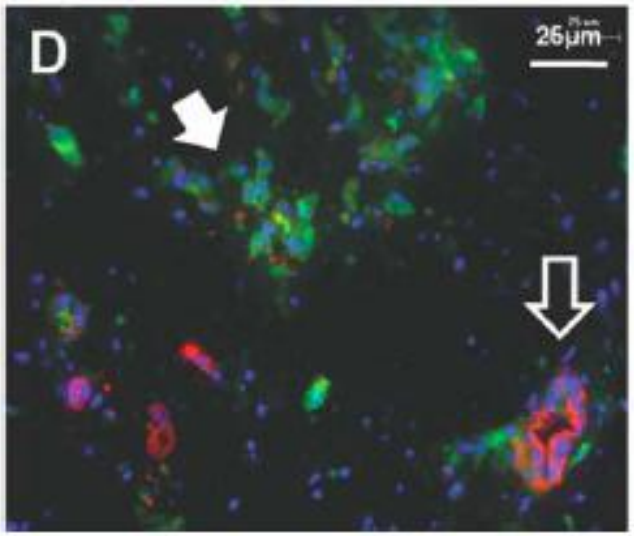
Uygun. Nature 2010;16(7):814-821

Recelularización

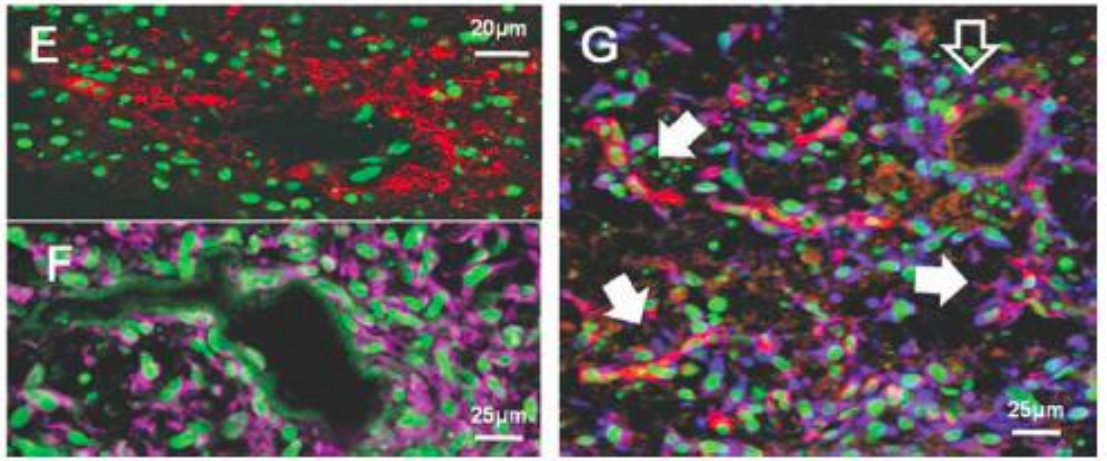
hFLC
hUVEC



p450 CYP2A (verde) y p450 CYP3A (rojo)



CK 19 (rojo) y albúmina (verde)

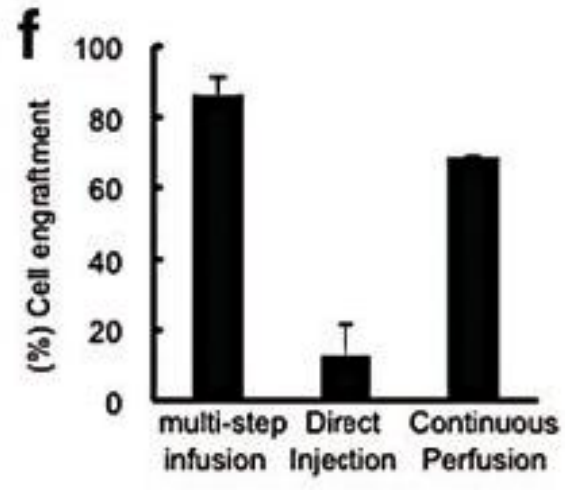


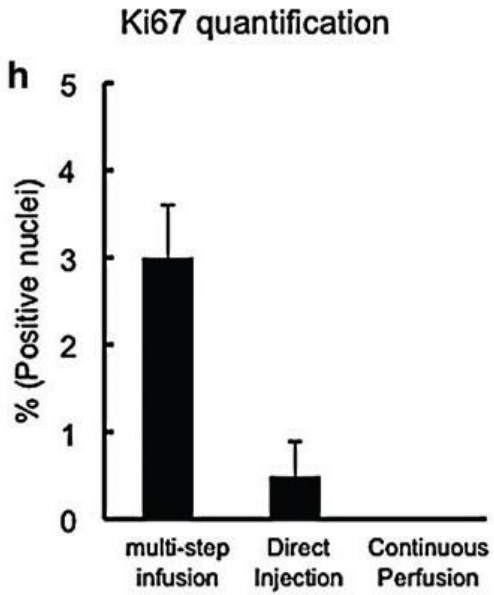
Von Willebrand (rojo) y óxido niítrico sintetasa endotelial (rosa)

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

Recelularización

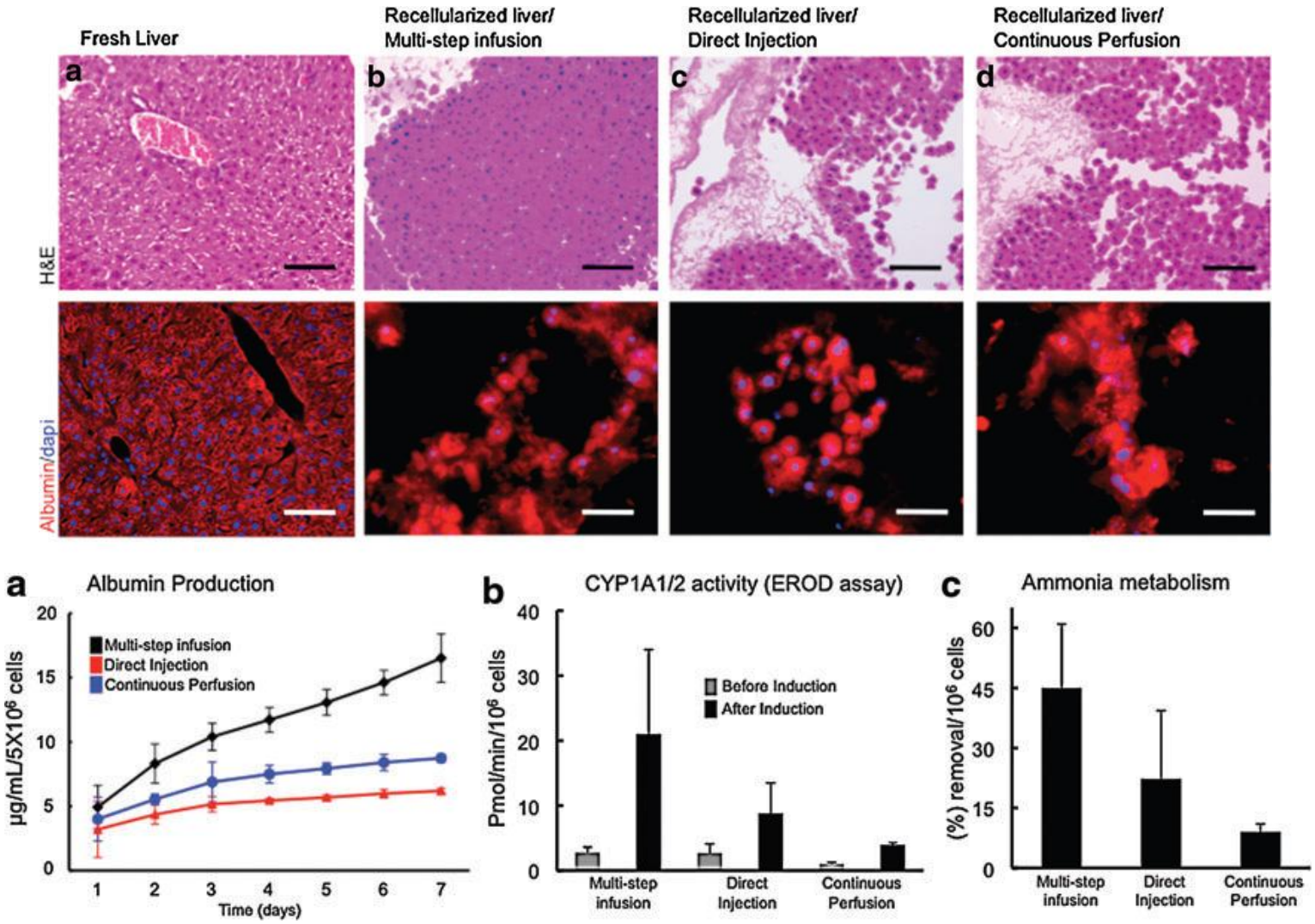
Inyección directa parénquima	Perfusión continua	Infusión multistep
Inyección directa en diferentes lóbulos	Células suspendidas en el medio de cultivo	Células a través vena Porta en 4 steps cada 10-15min





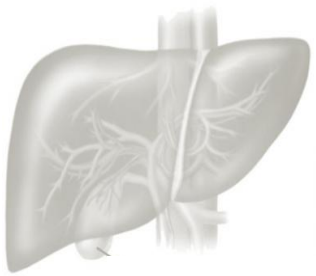
Soto A. Tissue Eng Part C Methods 2011;17(6):677-86

Recelularización

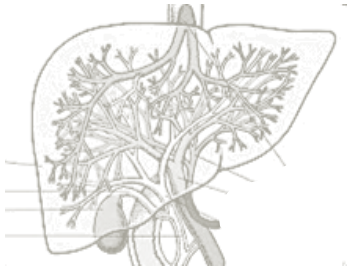


Soto A. Tissue Eng Part C Methods 2011;17(6):677-86

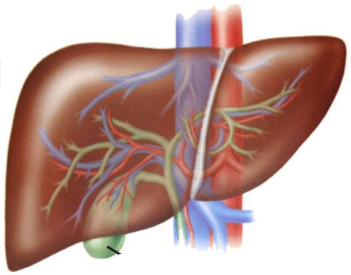
Trasplante



Descelularización

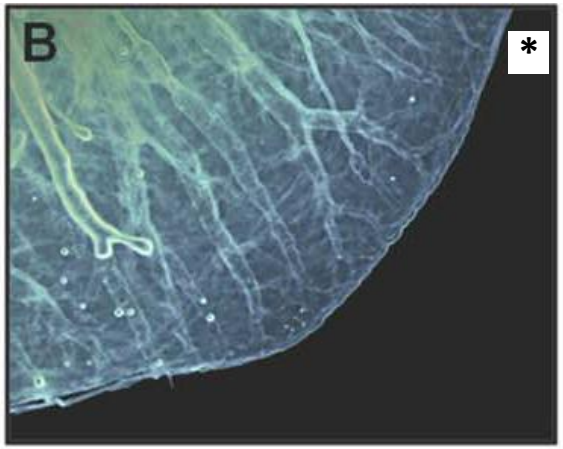


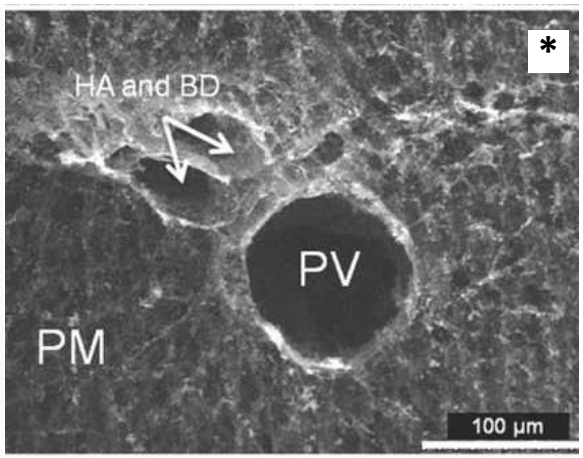
Recelularización
Maduración

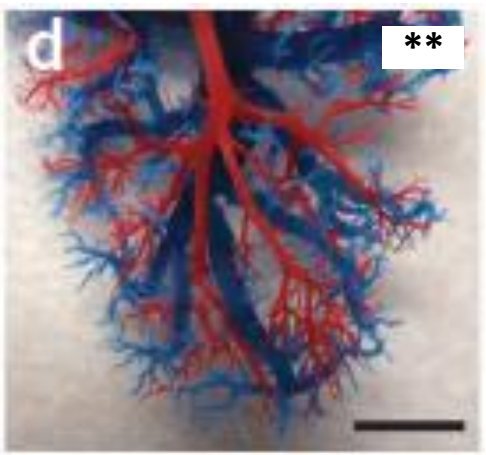


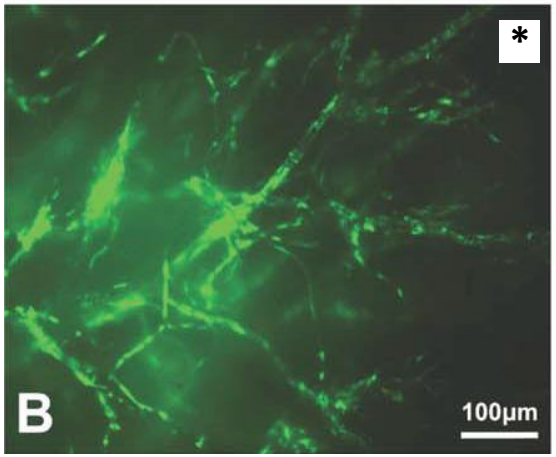
Trasplante

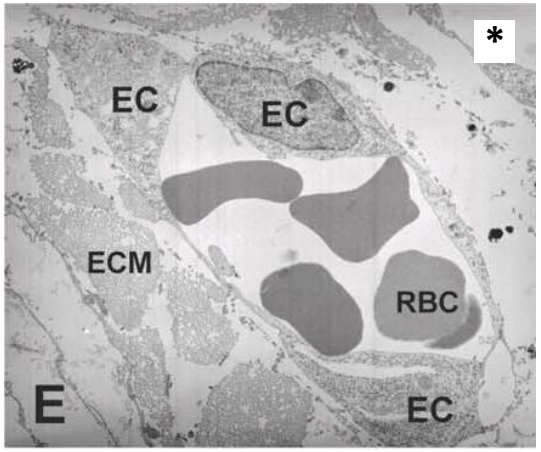
Trasplante

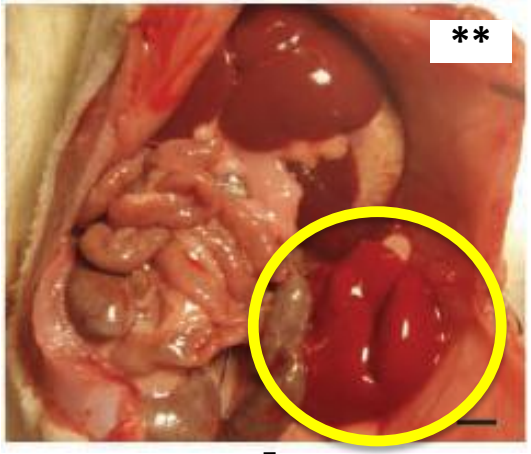








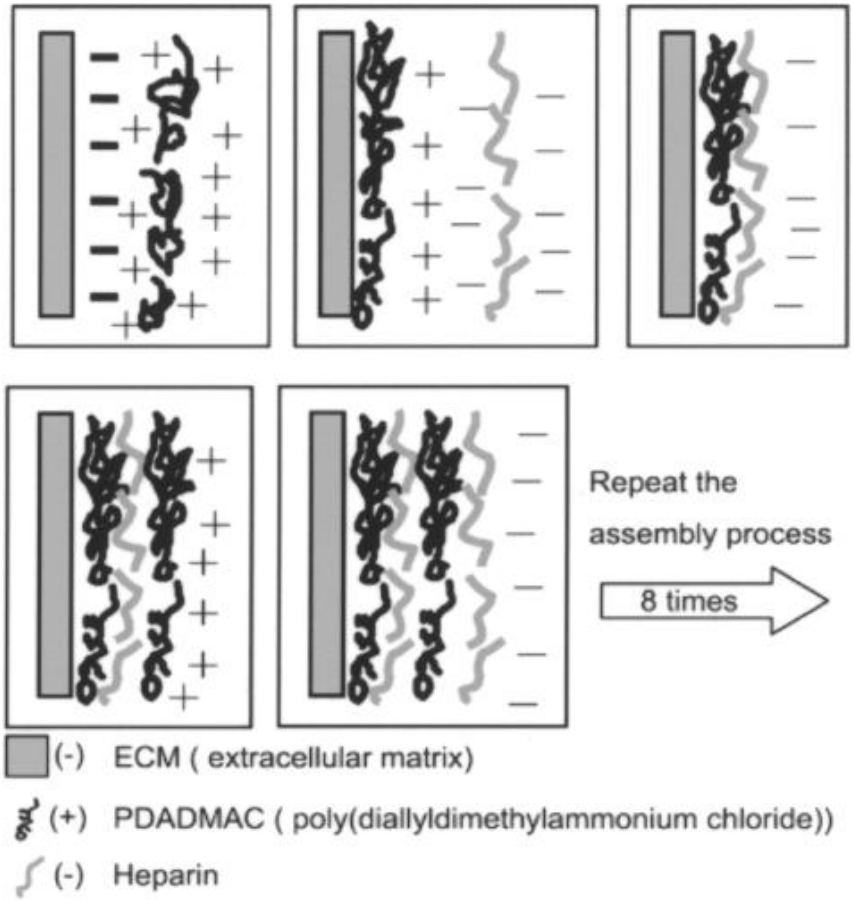




Viabilidad 8h trombosis

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

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



Técnica LbL self-assembly

Polielectrolito polidialidimetilamonio clorido (PDADMAC) con carga positiva

Heparina con carga negativa

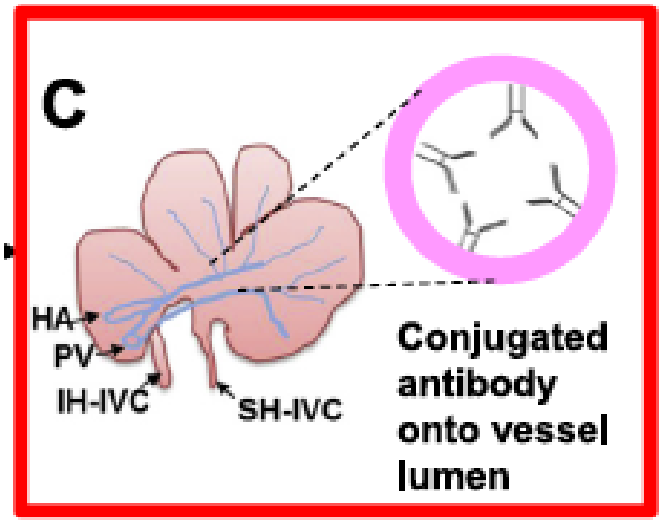
Resistencia trombótica después de 3h de perfusión sanguínea

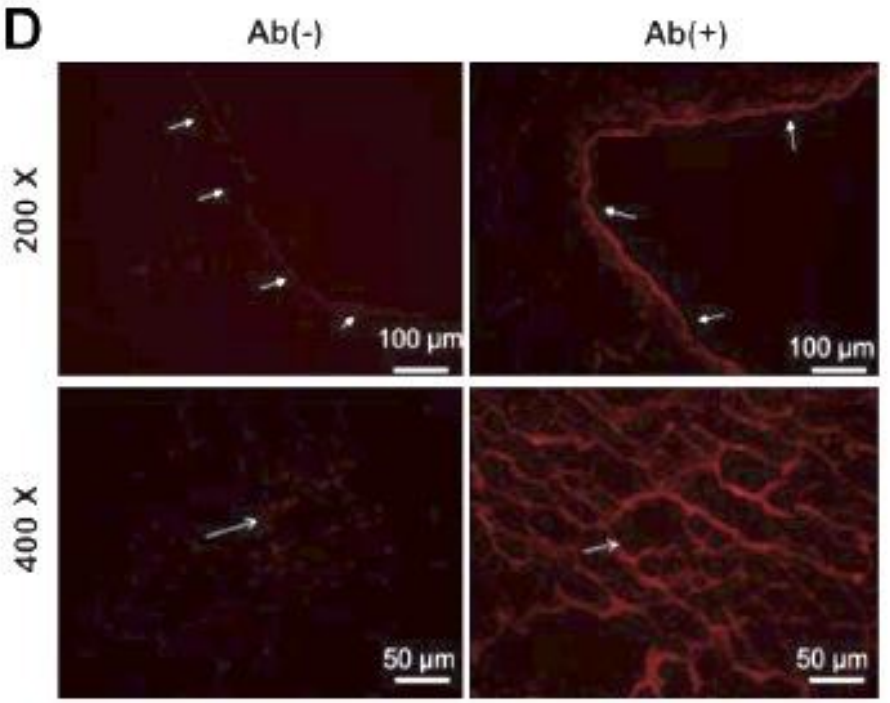
Tras 72h, hepatocitos mantienen morfología normal

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

Método de conjugación de anticuerpos

1. Anticuerpo anti célula endotelial en la pared vascular para estabilizar las células perfundidas: anticuerpo CD31 de rata anti-ratón se adhiere a la pared vascular acelular del scaffold

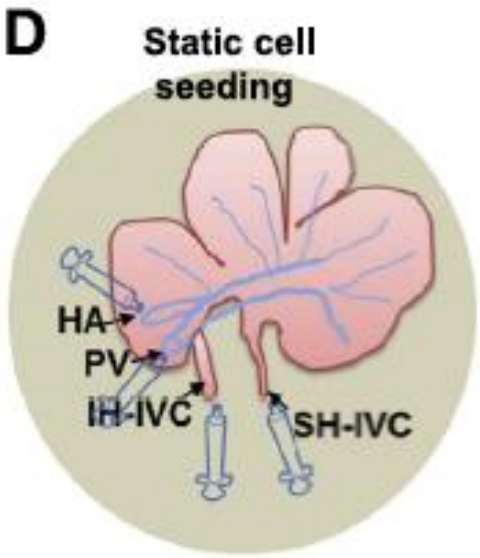


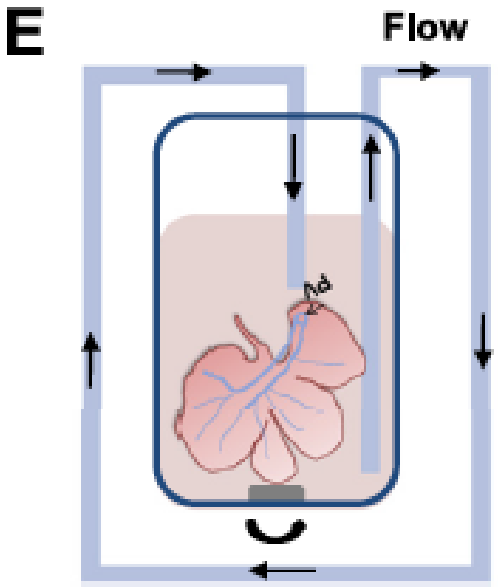


Ko et al. Biomaterials 2015;40:72-79

Método de conjugación de anticuerpos

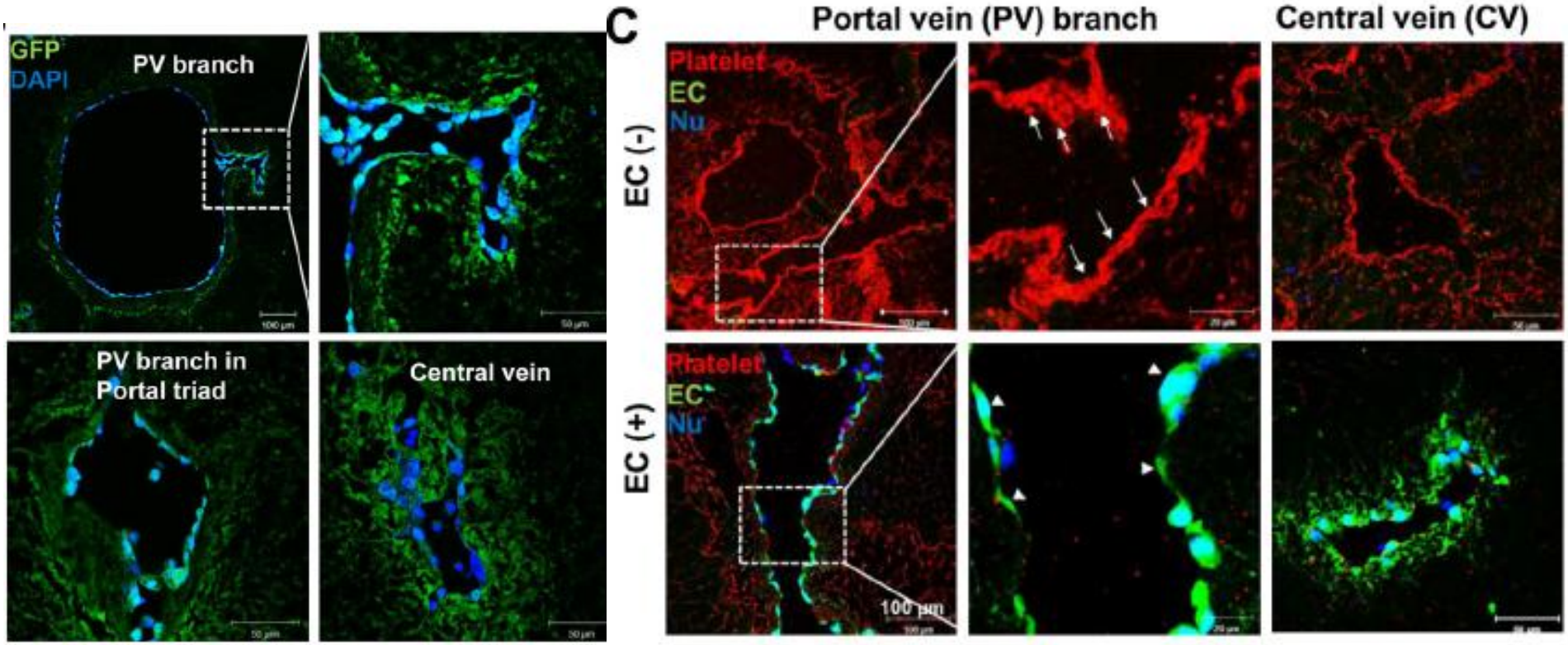
1. Anticuerpo anti célula endotelial en la pared vascular para estabilizar las células perfundidas: anticuerpo CD31 de rata anti-ratón se adhiere a la pared vascular acelular del scaffold
2. ReEndotelización con células endoteliales (MS1) que expresan proteína GFP





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

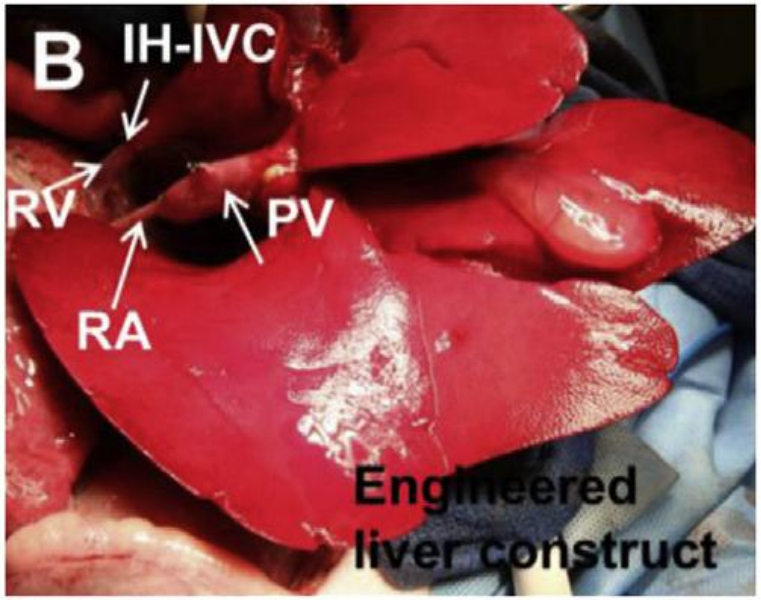
Caracterización de la Re-endotelización



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

Método de conjugación de anticuerpos

1. Anticuerpo anti célula endotelial en la pared vascular para estabilizar las células perfundidas: anticuerpo CD31 de rata anti-ratón se adhiere a la pared vascular acelular del scaffold
2. ReEndotelización con células endoteliales (MS1) que expresan proteína GFP
3. Implante del organoide hepático



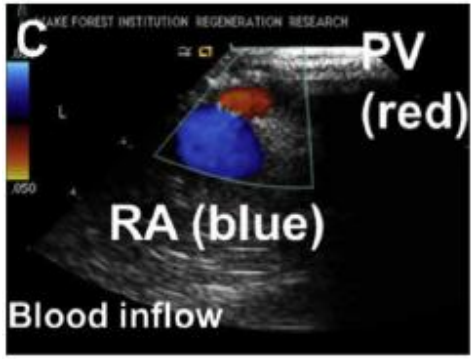
Trasplante heterotópico en cerdo:

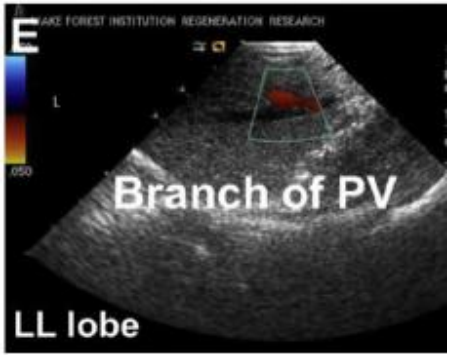
Vena Porta (I) – Arteria renal izquierda (R)
VCI (I) – Vena renal izquierda (R)

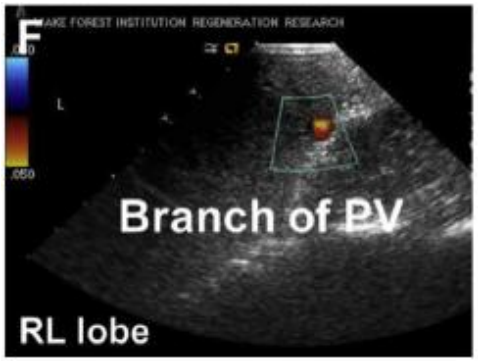
Ko et al. Biomaterials 2015;40:72-79

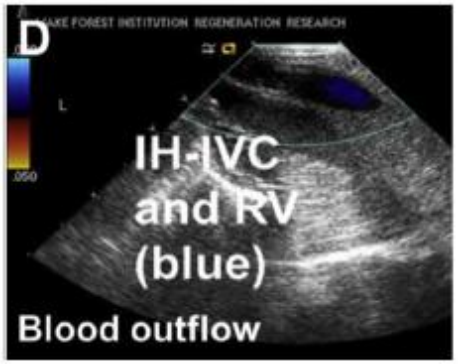
Estudios funcionales *In vivo*

Intraoperatorio

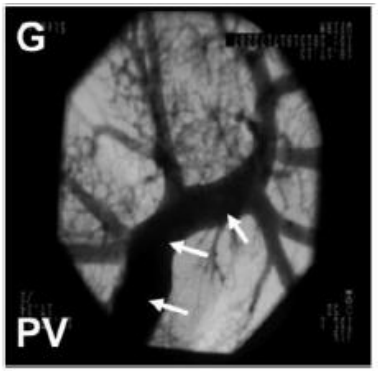


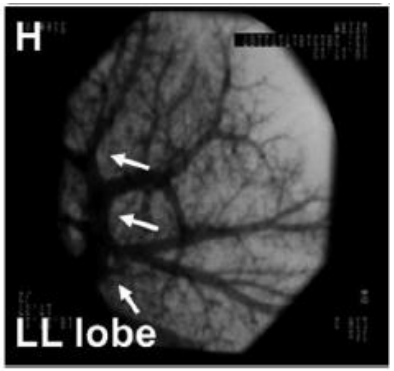


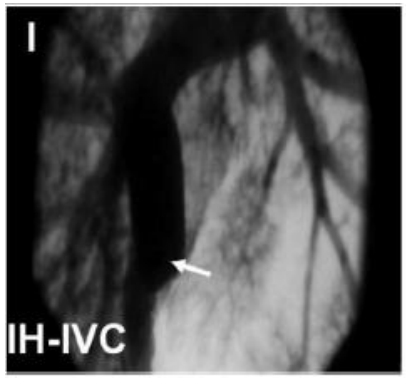




4h post implante

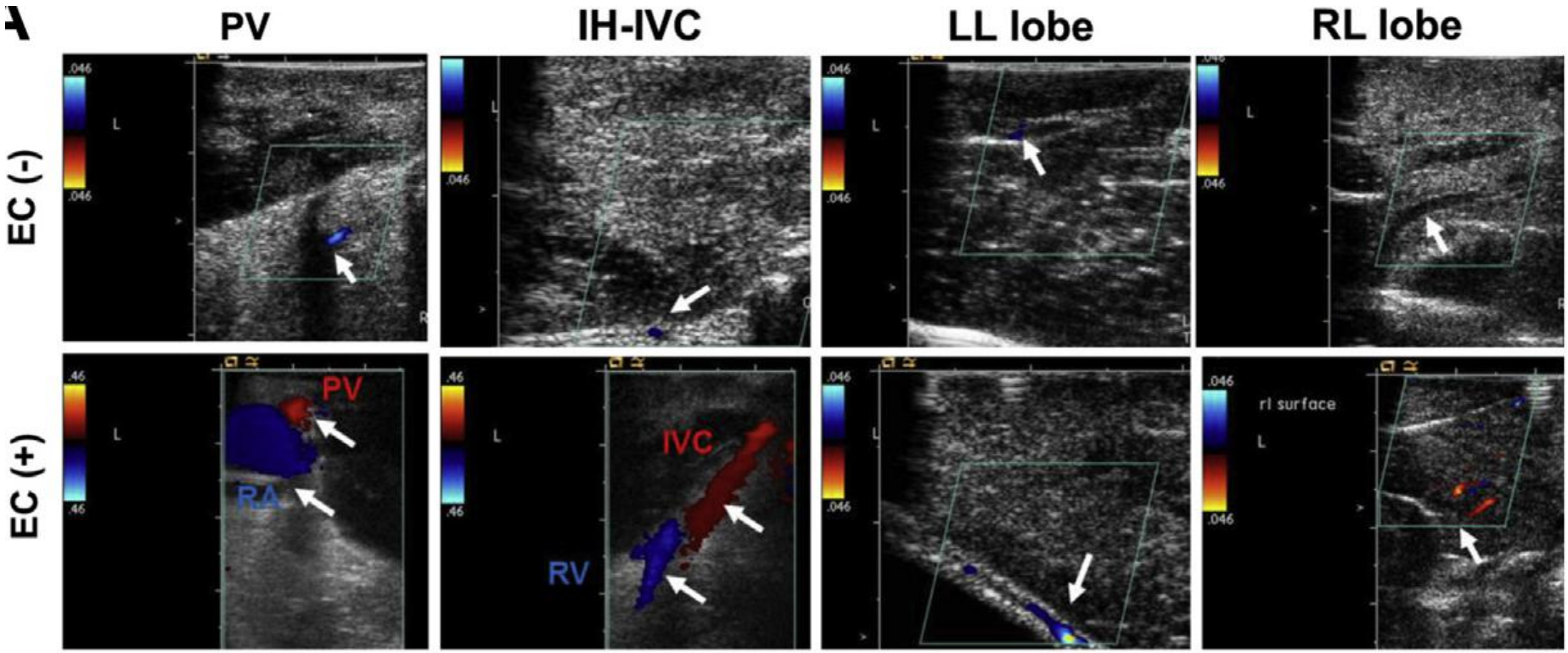






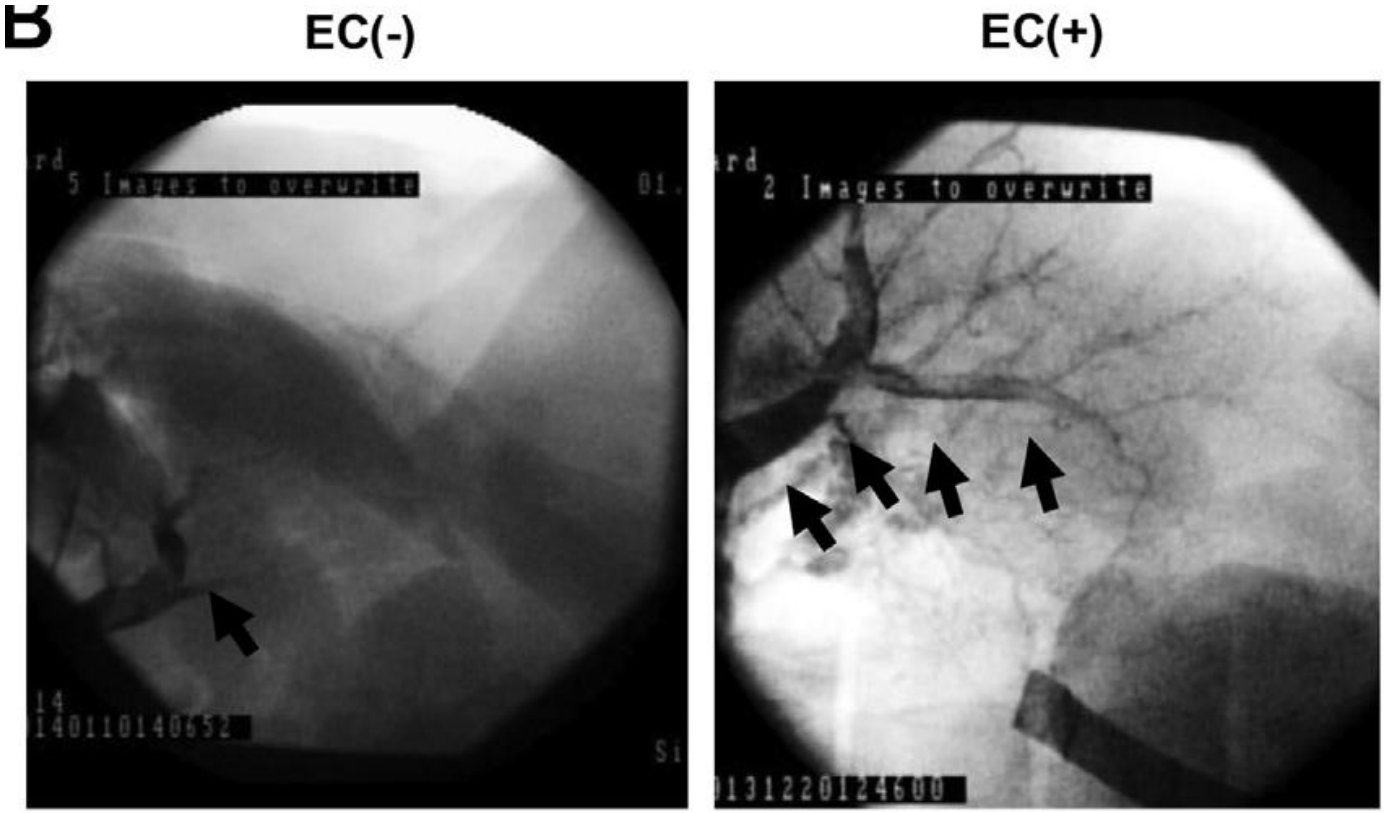
Ko et al. Biomaterials 2015;40:72-79

Estudios funcionales *In vivo*: 1er día PostTx



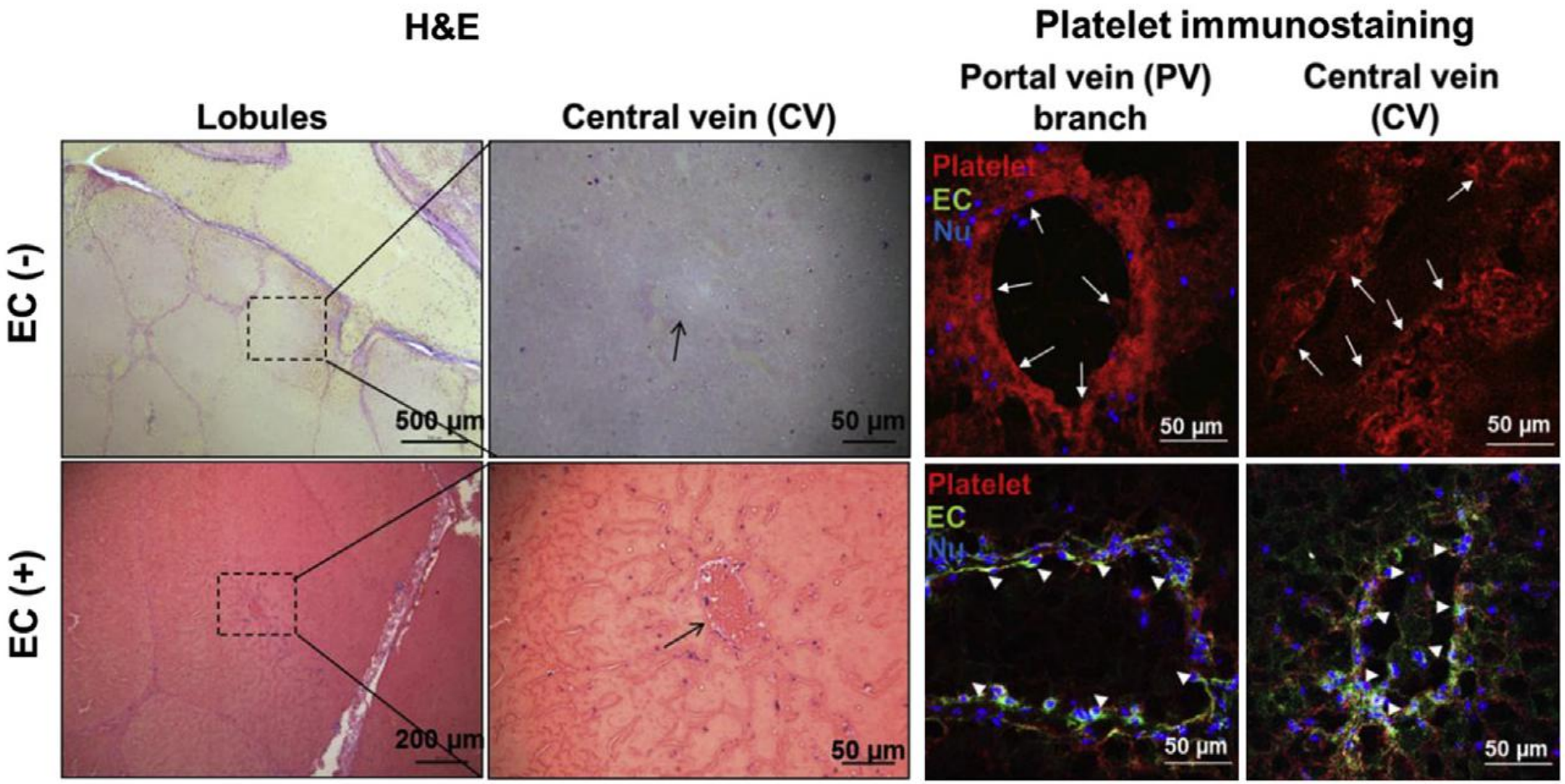
Ko et al. Biomaterials 2015;40:72-79

Estudios funcionales *In vivo*: 1er día PostTx



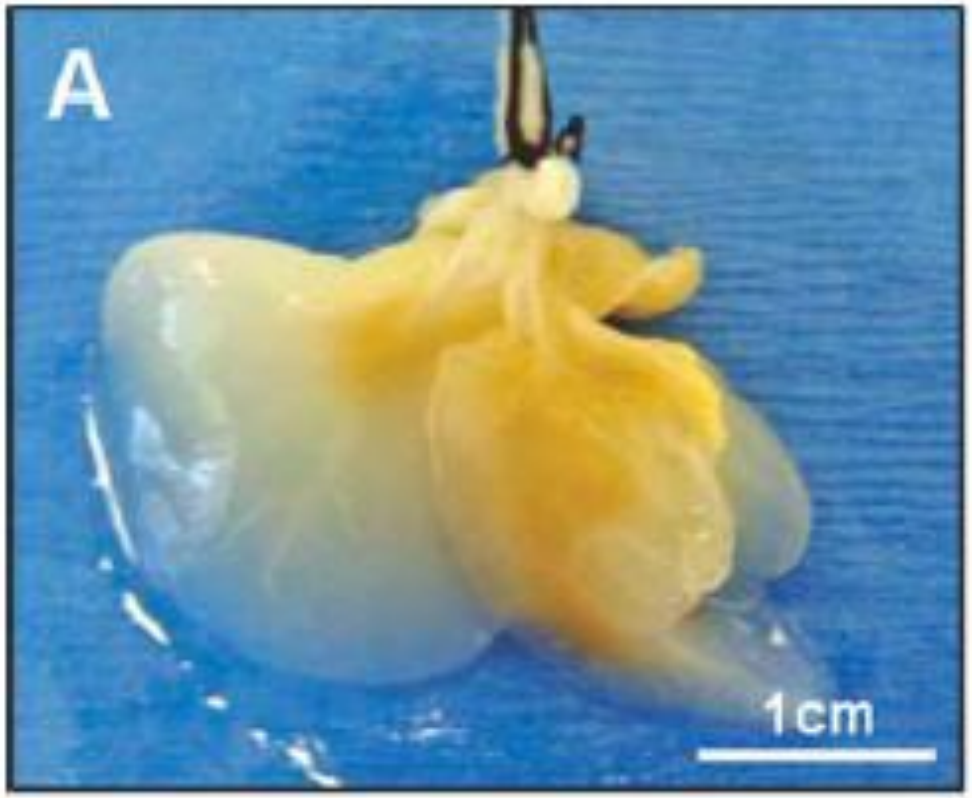
Ko et al. Biomaterials 2015;40:72-79

Estudios funcionales *In vivo*: 1er día PostTx



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

Dificultades



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

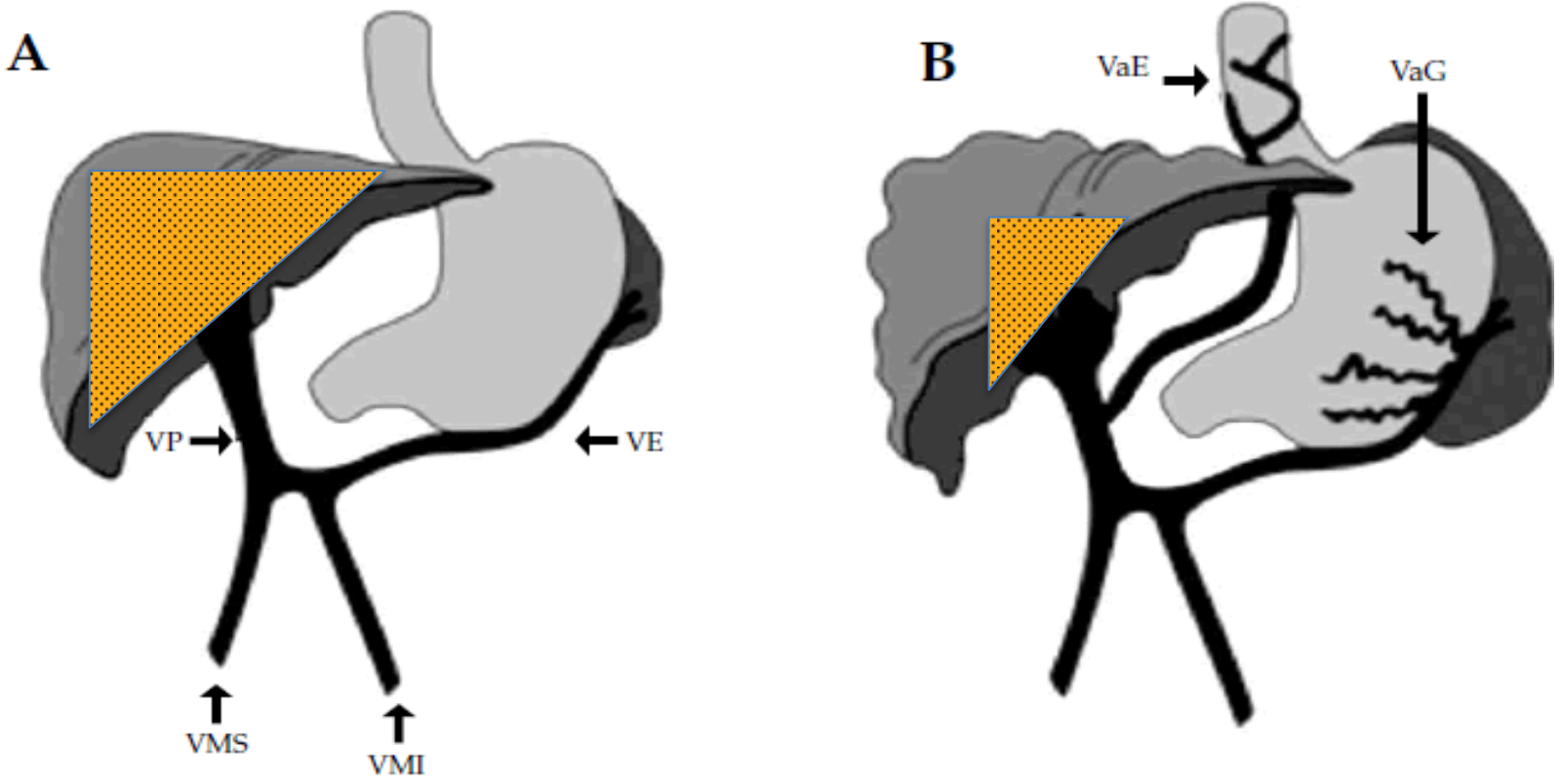
70M hepatocitos humanos fetales

=

4 hígados
(17-21 semanas gestación)

Número es importante…

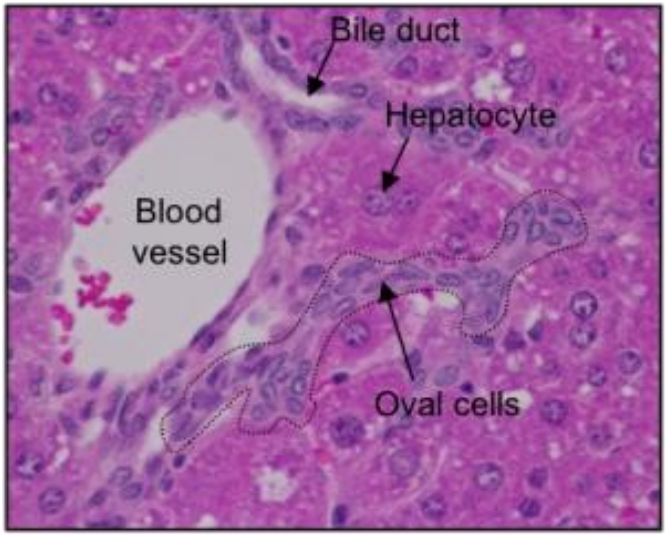
Dificultades

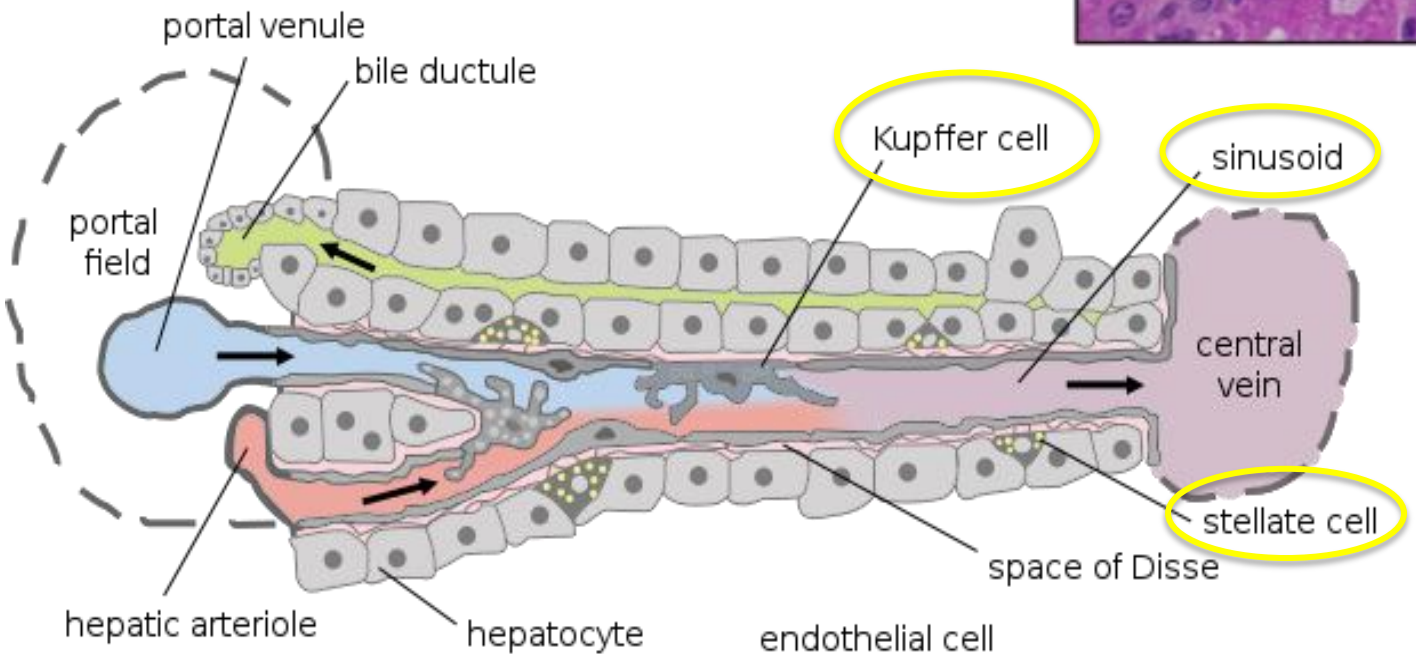


… tamaño también es importante

Líneas futuras

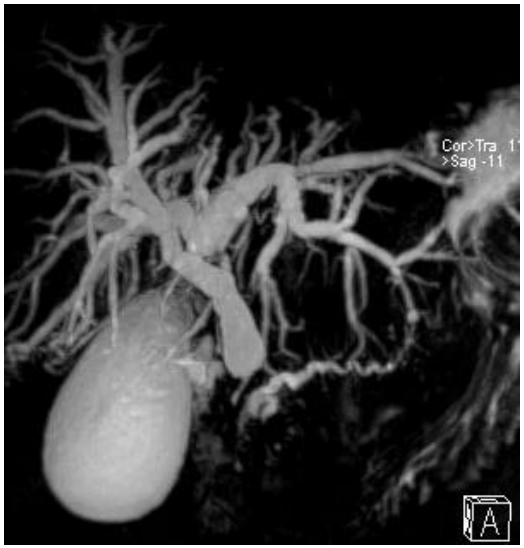
- Co-Cultivo con otras células no-parenquimatosas

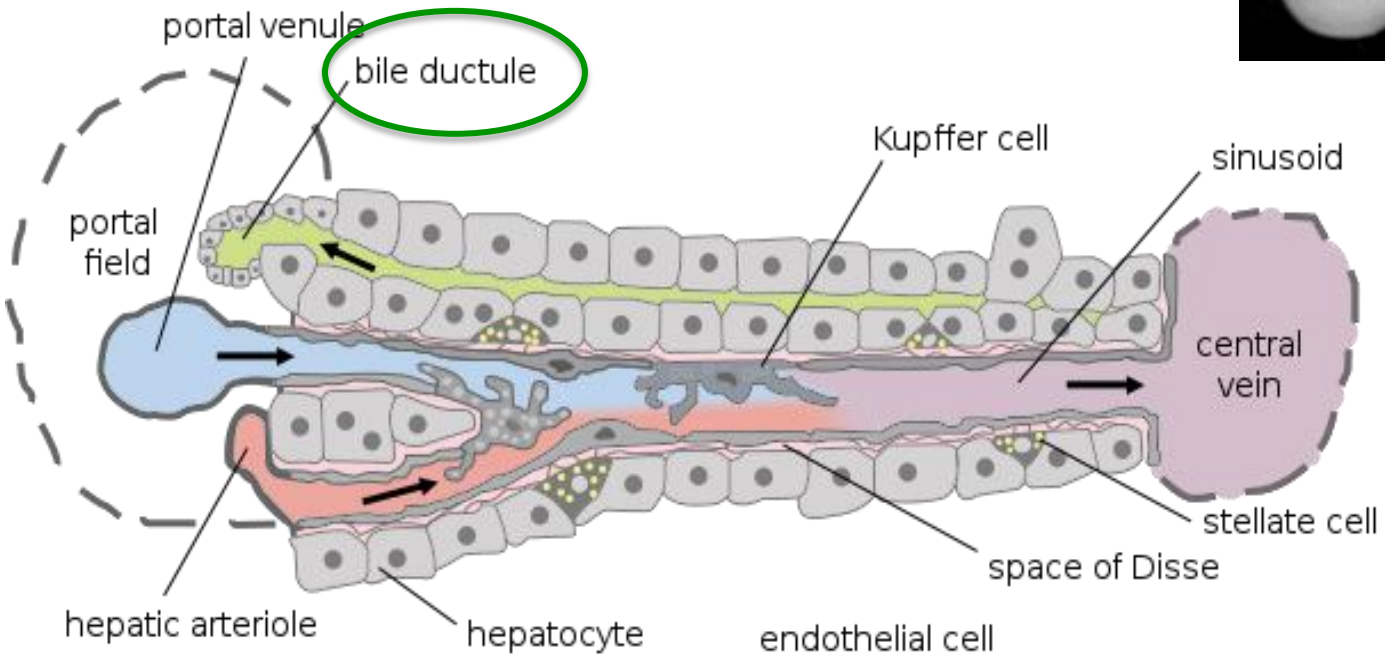




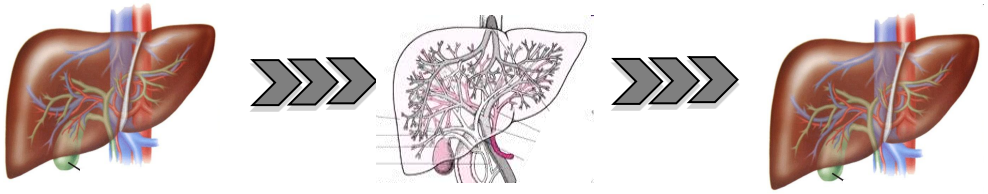
Líneas futuras

- Co-Cultivo con otras células no-parenquimatosas
- Colangiocitos y Vía Biliar





Resumen



- Hígados descelularizados son una buena opción para obtener scaffolds porque la arquitectura y vascularización están conservadas
- Células están “contentas” en el scaffold
  - hepatocitos maduros: viables y funcionales
  - células inmaduras: diferenciación a células adultas hepáticas
- Recelularización multistep
- Correcta endotelización es fundametnal para el trasplante
- Dificultades: número de células y tamaño del organoide
- Líneas futuras:
  - Co-cultivo con otras células no parenquimatosas
  - Colangiocitos y vía biliar

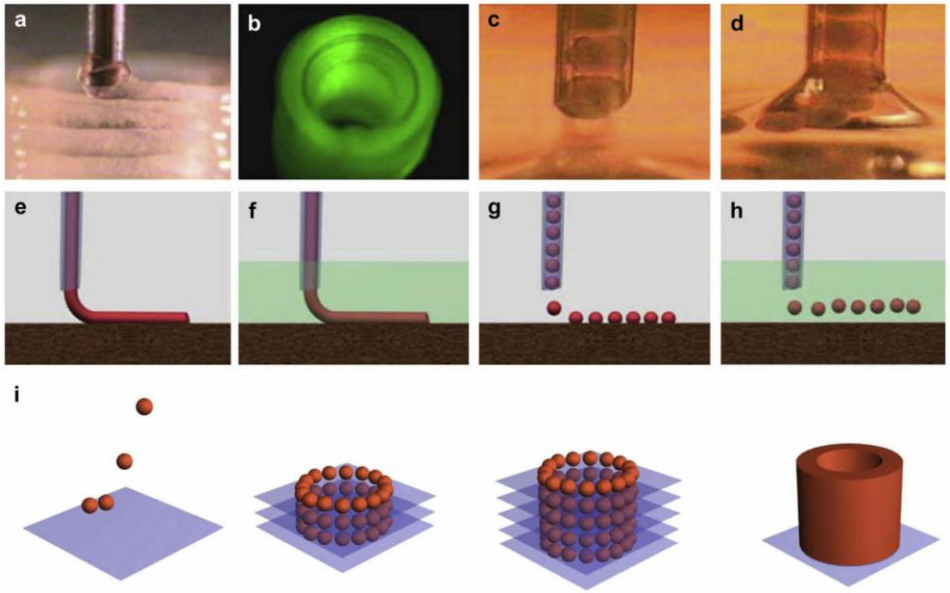
BioImpresión de Órganos

BioImpresión de Órganos

BioImpresión de órganos: tecnología para construir scaffolds

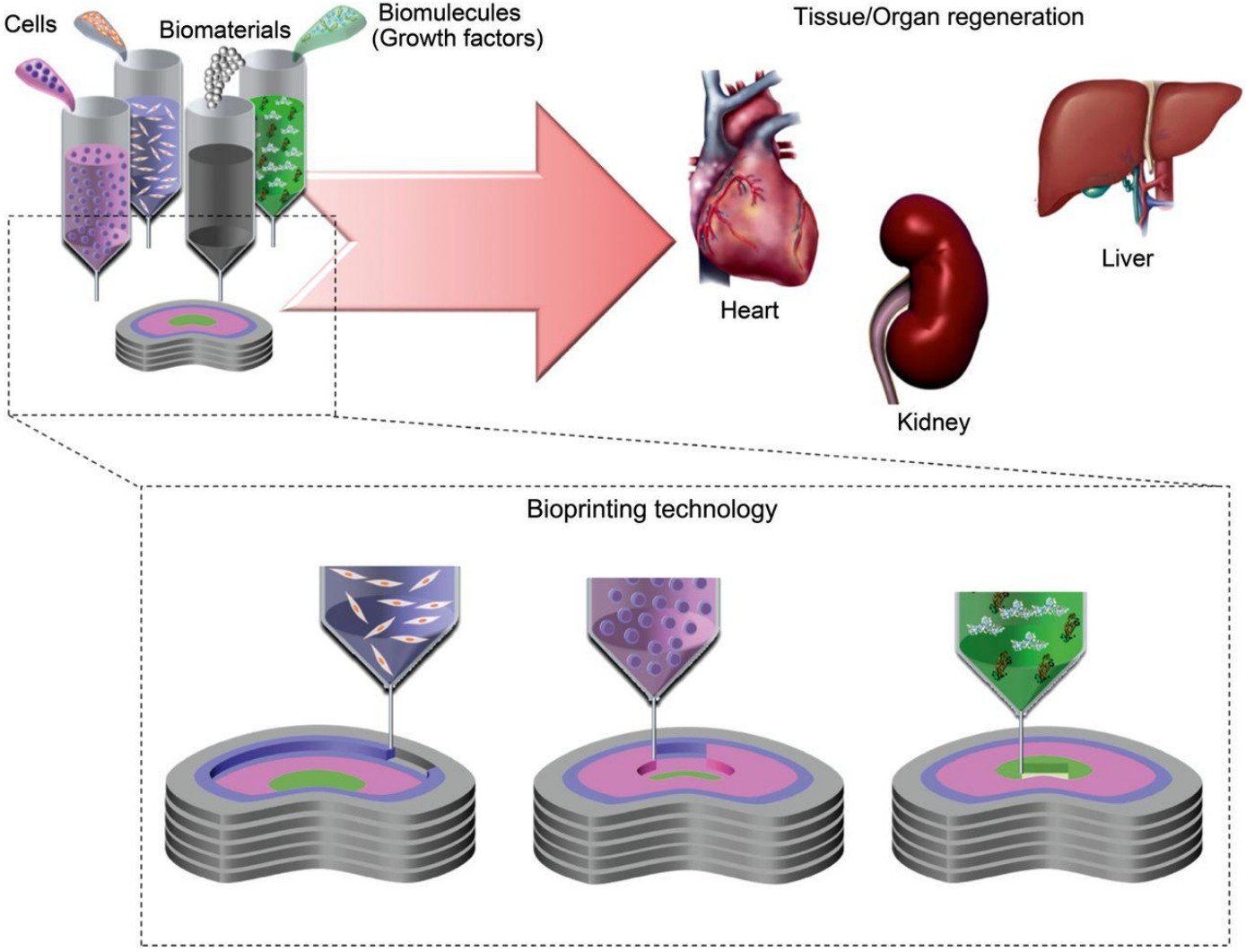
- Técnica que produce estructuras 3D complejas bajo control del tamaño, forma, distribución e interconectividad de los poros de los scaffolds

- Permite una distribución directa de las células siguiendo la arquitectura de los órganos



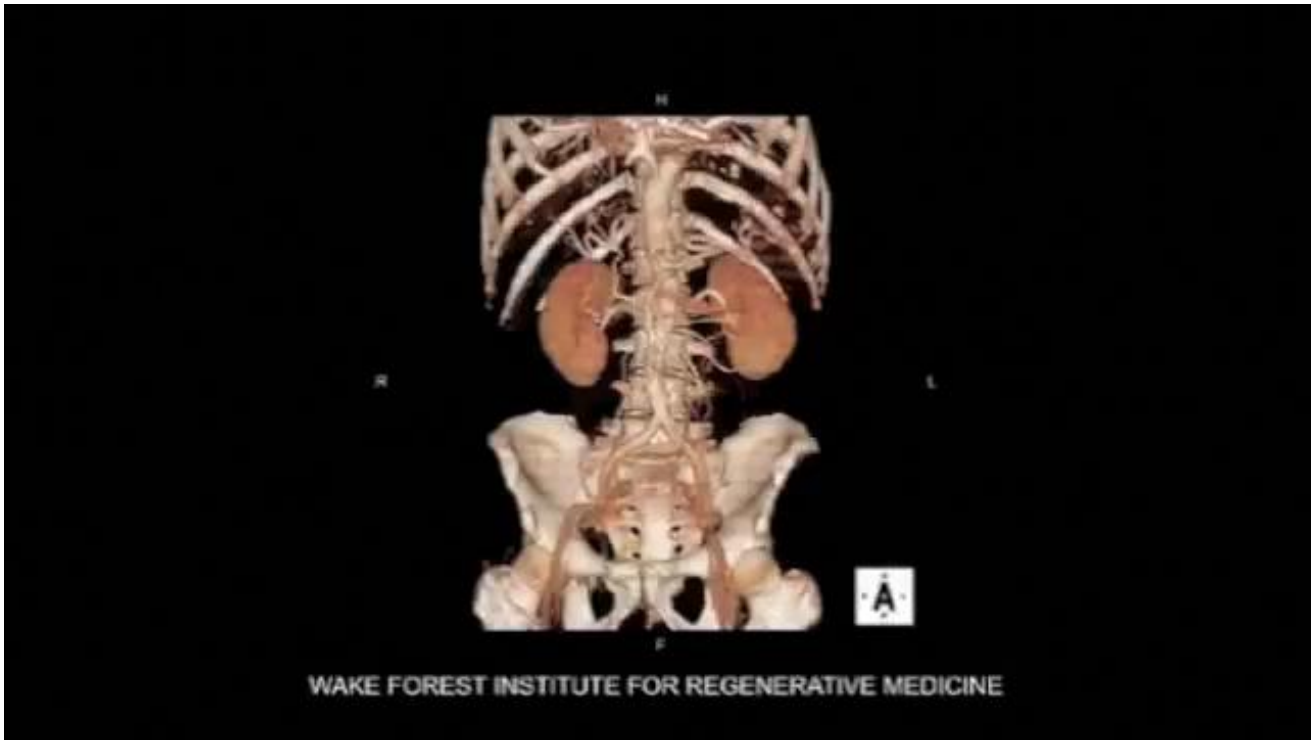
Mironov. Biomaterials 2009;30(12):2164-2174

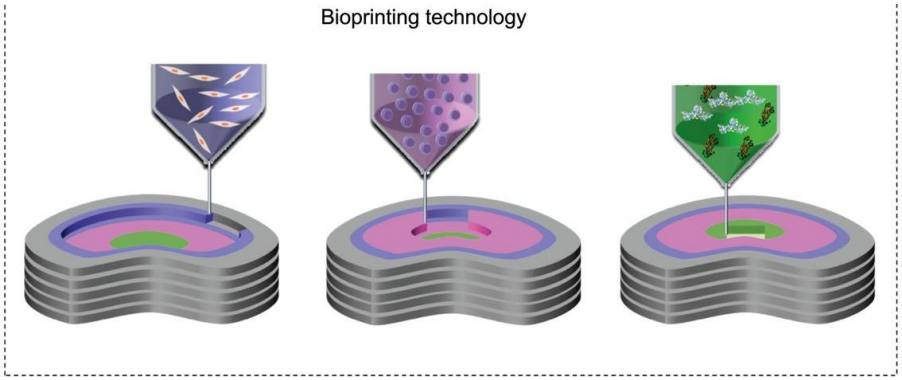
BioImpresión de Órganos



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

BioImpresión de Órganos





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

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