Título: Bioingeniería aplicada al trasplante hepático

Nombre y apellidos: Pablo Royo Dachary

Centro de Trabajo: Hospital Clínico “Lozano Blesa” de Zaragoza

Imaginemos…


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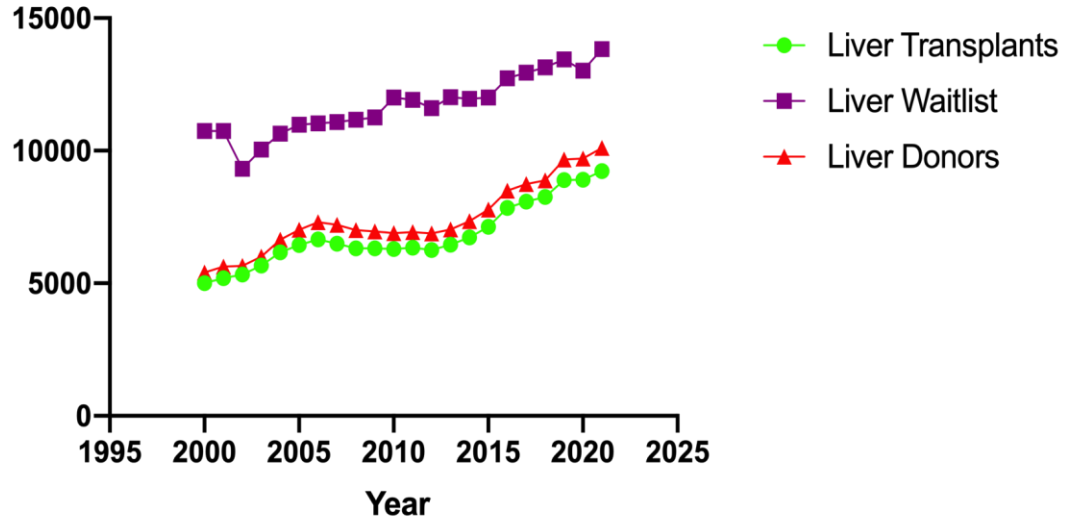
061
salud

INTRODUCCIÓN

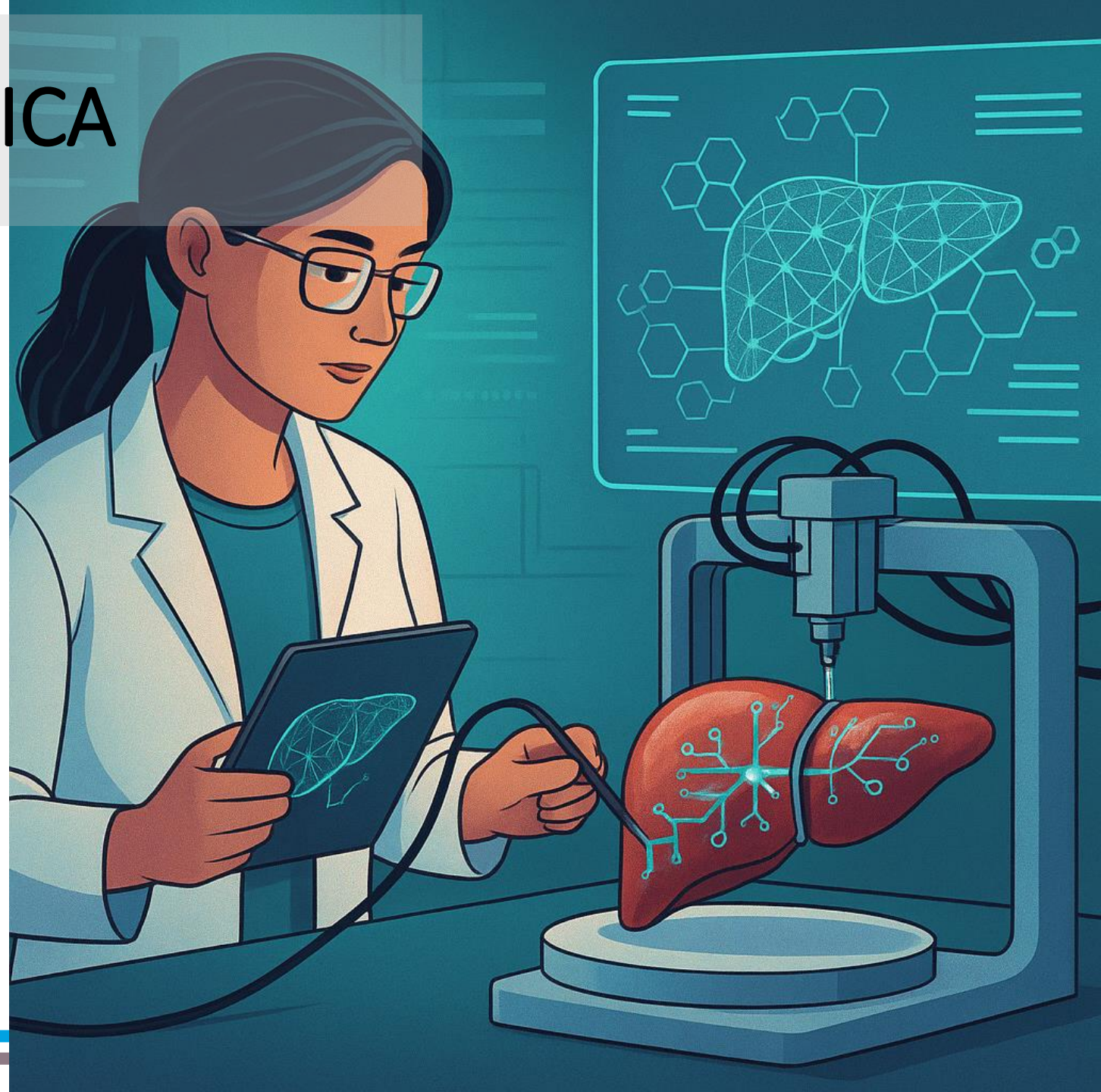


- *Retos actuales:*
  - *Escasez de órganos*
  - *Morbilidad y mortalidad en LEQ*
  - *Rechazo*
  - *Complicaciones de la inmunosupresión*

- *¿Puede la bioingeniería cambiar este paradigma?*

BIOINGENIERÍA HEPÁTICA

- Biología, ingeniería y medicina regenerativa
- Estudio, reparación y sustitución del hígado.
- Estrategias:
  - Scaffolds:
    - Impresión 3D
    - Descelularización.
  - Ingeniería genética (Xenotrasplante)
  - Terapias celulares
  - Soporte artificial



IMPRESIÓN 3D

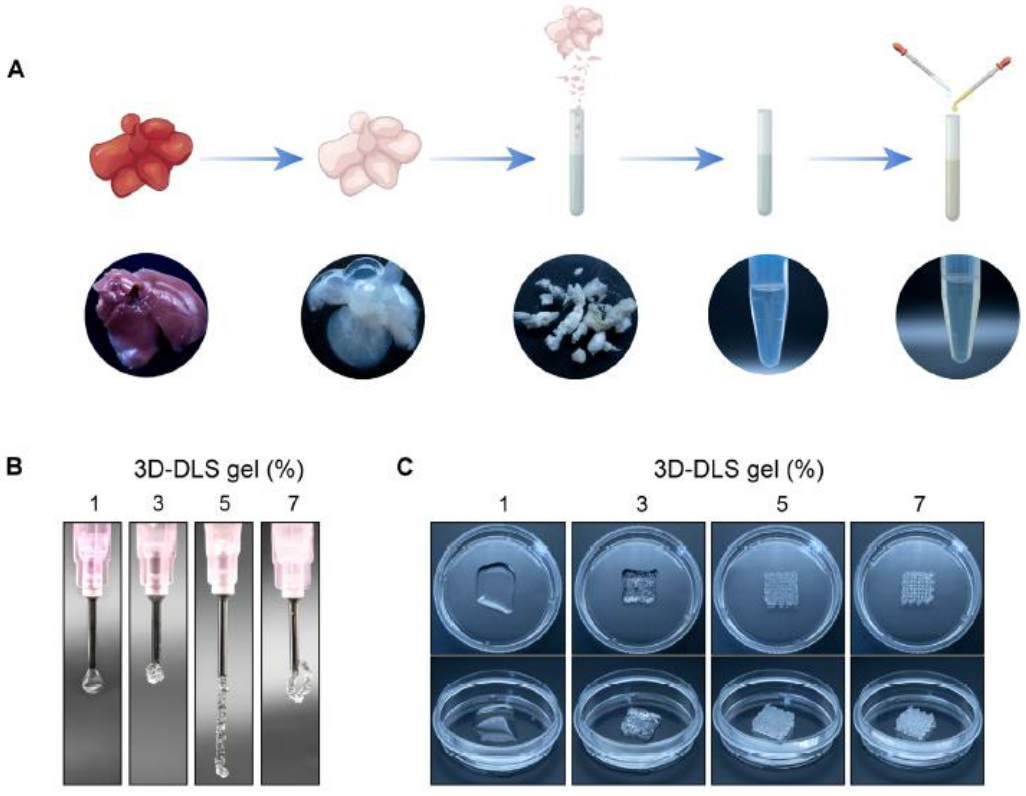
SCIENCE ADVANCES | RESEARCH ARTICLE

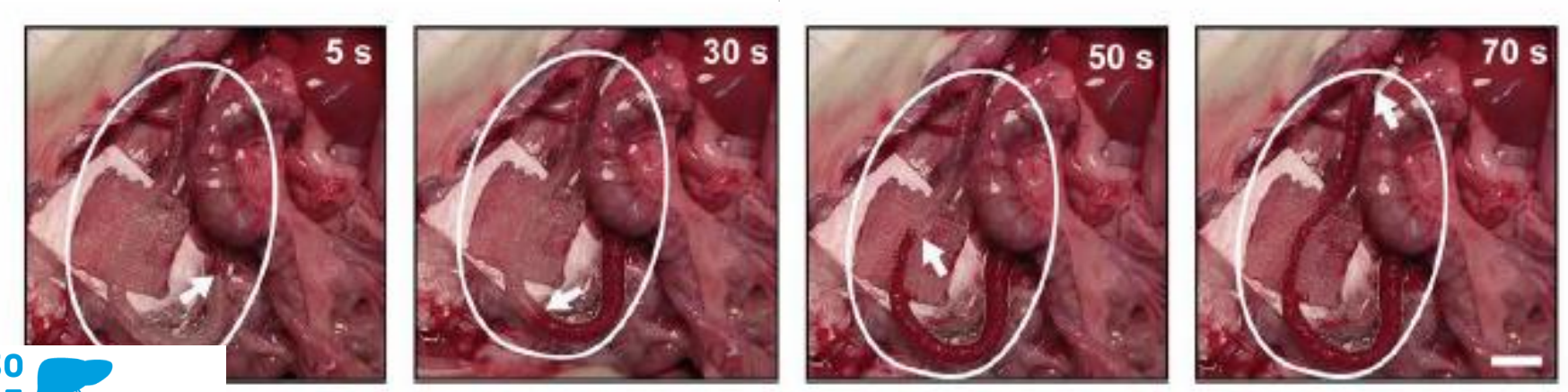
CELL BIOLOGY

Revitalizing liver function in mice with liver failure through transplantation of 3D-bioprinted liver with expanded primary hepatocytes

Bo Deng[1]†, Yue Ma[1]†, Jialyu Huang[2]†, Runbang He[1], Miaomiao Luo[1], Lina Mao[1], Enhua Zhang[1], Yuanyuan Zhao[1], Xiaoli Wang[1], Qiangsong Wang[1], Mingchang Pang[3], Yilei Mao[3], Huayu Yang[3]*, Lanxia Liu[1]*, Pengyu Huang[1]*

The utilization of three-dimensional (3D) bioprinting technology to create a transplantable bioartificial liver emerges as a promising remedy for the scarcity of liver donors. This study outlines our strategy for constructing a 3D-bioprinted liver, using in vitro–expanded primary hepatocytes recognized for their safety and enhanced functional robustness as hepatic cell sources for bioartificial liver construction. In addition, we have developed bioink biomaterials with mechanical and rheological properties, as well as printing capabilities, tailored for 3D bioprinting. Upon heterotopic transplantation into the mesentery of tyrosinemia or 90% hepatectomy mice, our 3D-bioprinted liver effectively restored lost liver functions, consequently extending the life span of mice afflicted with liver injuries. Notably, the inclusion of an artificial blood vessel in our 3D-bioprinted liver allowed for biomolecule exchange with host blood vessels, demonstrating, in principle, the rapid integration of the bioartificial liver into the host vascular system. This model underscores the therapeutic potential of transplantation for the treatment of liver failure diseases.

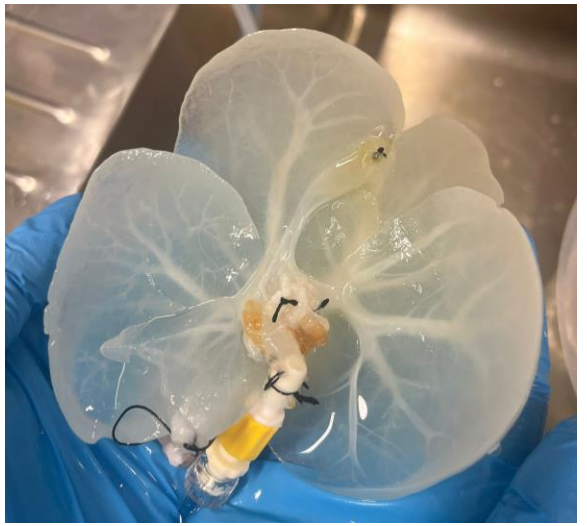


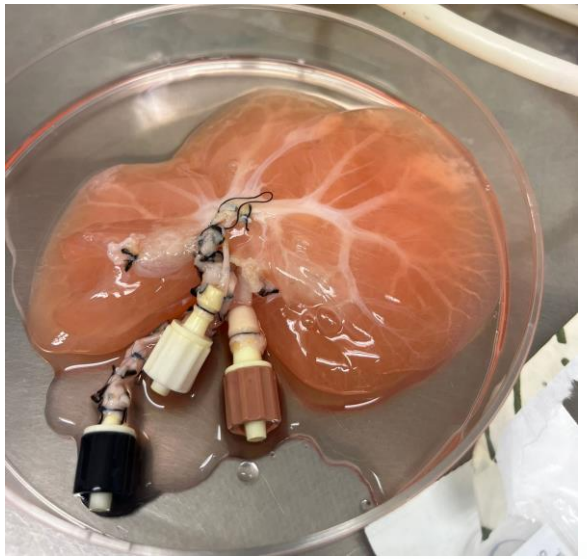


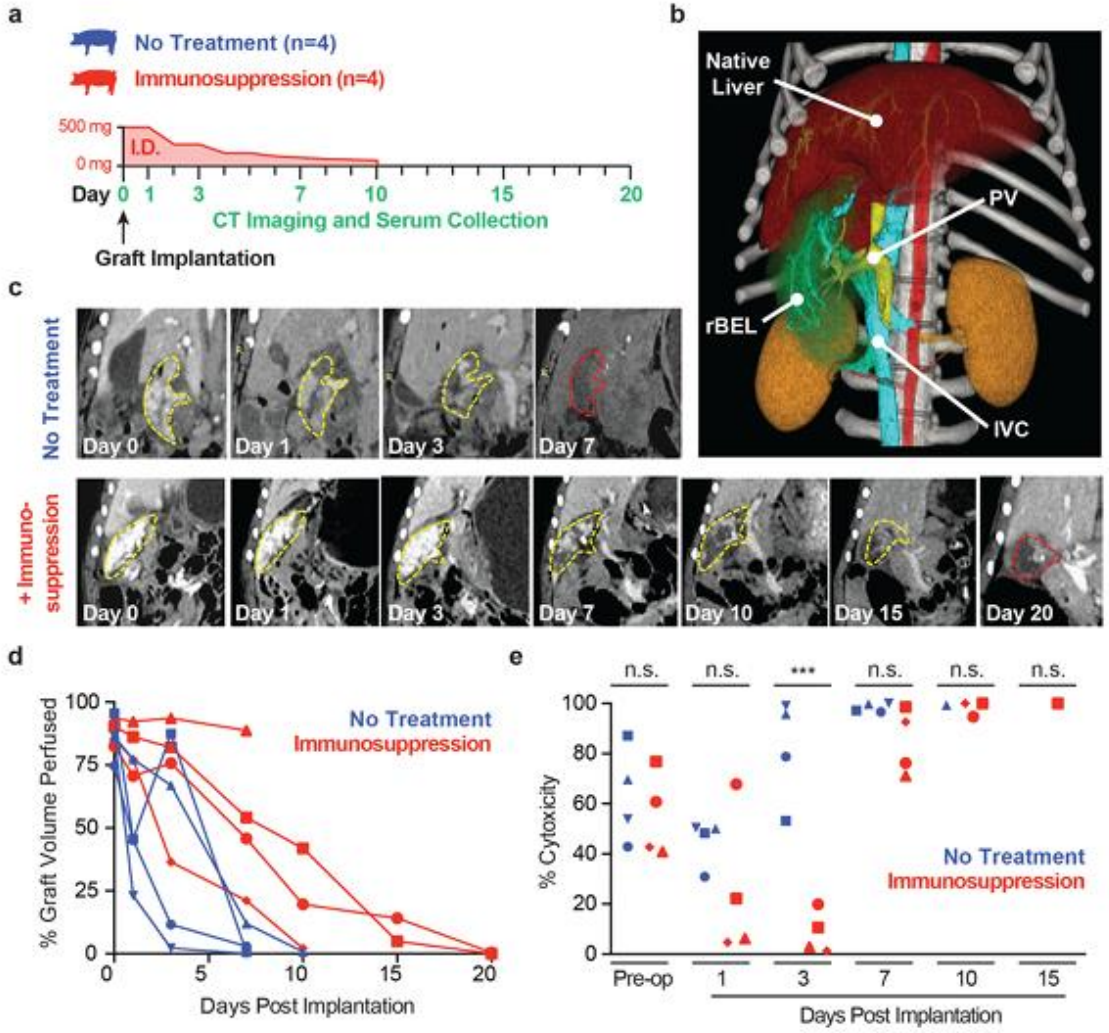
Junio 2024

RECELULARIZACIÓN SCAFFOLDS

- **Scaffold:** esqueleto de tejido acelular
- **Descelularización:** Eliminar componentes celulares manteniendo arquitectura y matriz.
- **Recelularización:** Reposición de células funcionales en el scaffold



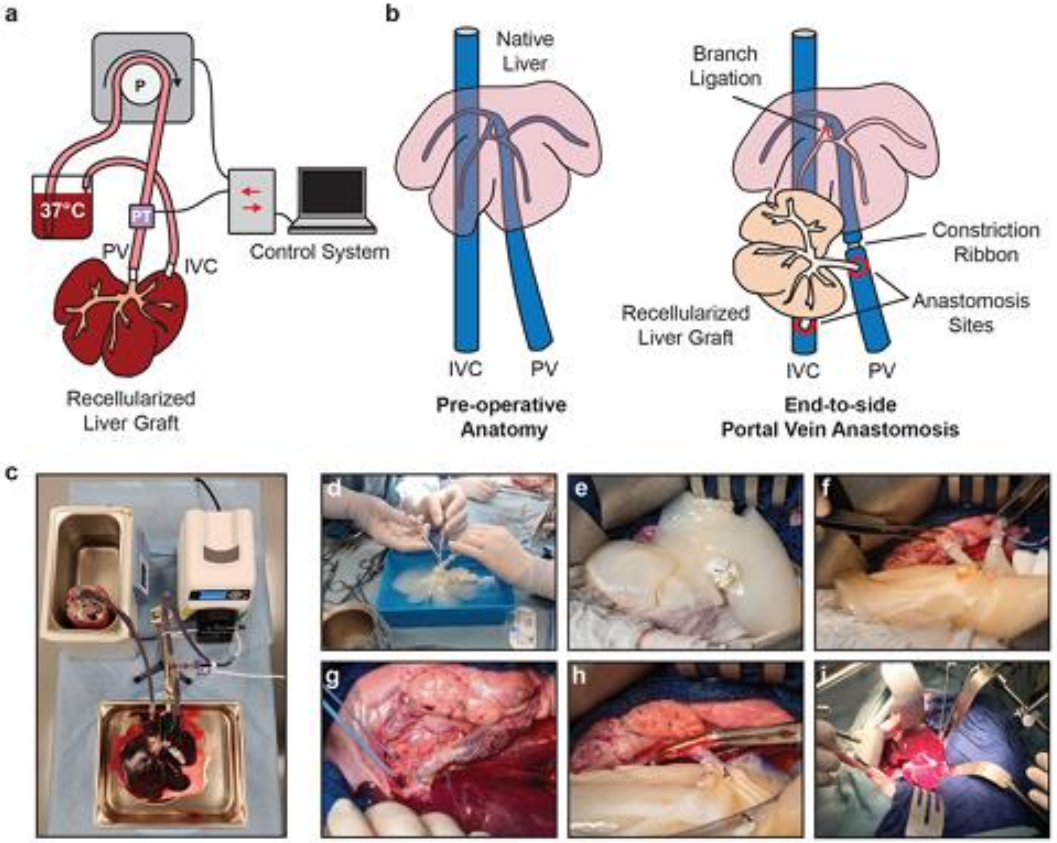




Published in final edited form as:
Nat Biomed Eng. 2020 April ; 4(4): 437–445. doi:10.1038/s41551-019-0460-x.

Sustained perfusion of revascularized bioengineered livers heterotopically transplanted into immunosuppressed pigs

Mohammed F. Shaheen[1,2], **DongJin Joo**[1,3], **Jeffrey J. Ross**[4,*], **Brett D. Anderson**[4], **Harvey S.**



28 días de perfusión…

- Perfusión continua de heparina

Published in final edited form as:
Am J Transplant. 2022 March ; 22(3): 731–744. doi:10.1111/ajt.16928.

Transplantation of bioengineered liver capable of extended function in a preclinical liver failure model

Hisanobu Higashi[1], Hiroshi Yagi[1], Kohei Kuroda[1], Kazuki Tajima[1,2], Hideaki Kojima[1], Kotaro Nishi[1], Toshinori Morisaku[1], Kazuya Hirukawa[1], Kazumasa Fukuda[1], Kentaro Matsubara[1], Minoru Kitago[1], Masahiro Shinoda[1], Hideaki Obara[1], Shungo Adachi[3], Kumiko Nishimura[3], Tohru Natsume[3], Masatoshi Tomi[4], Alejandro Soto-Gutierrez[5,6,7], Yuko Kitagawa[1]

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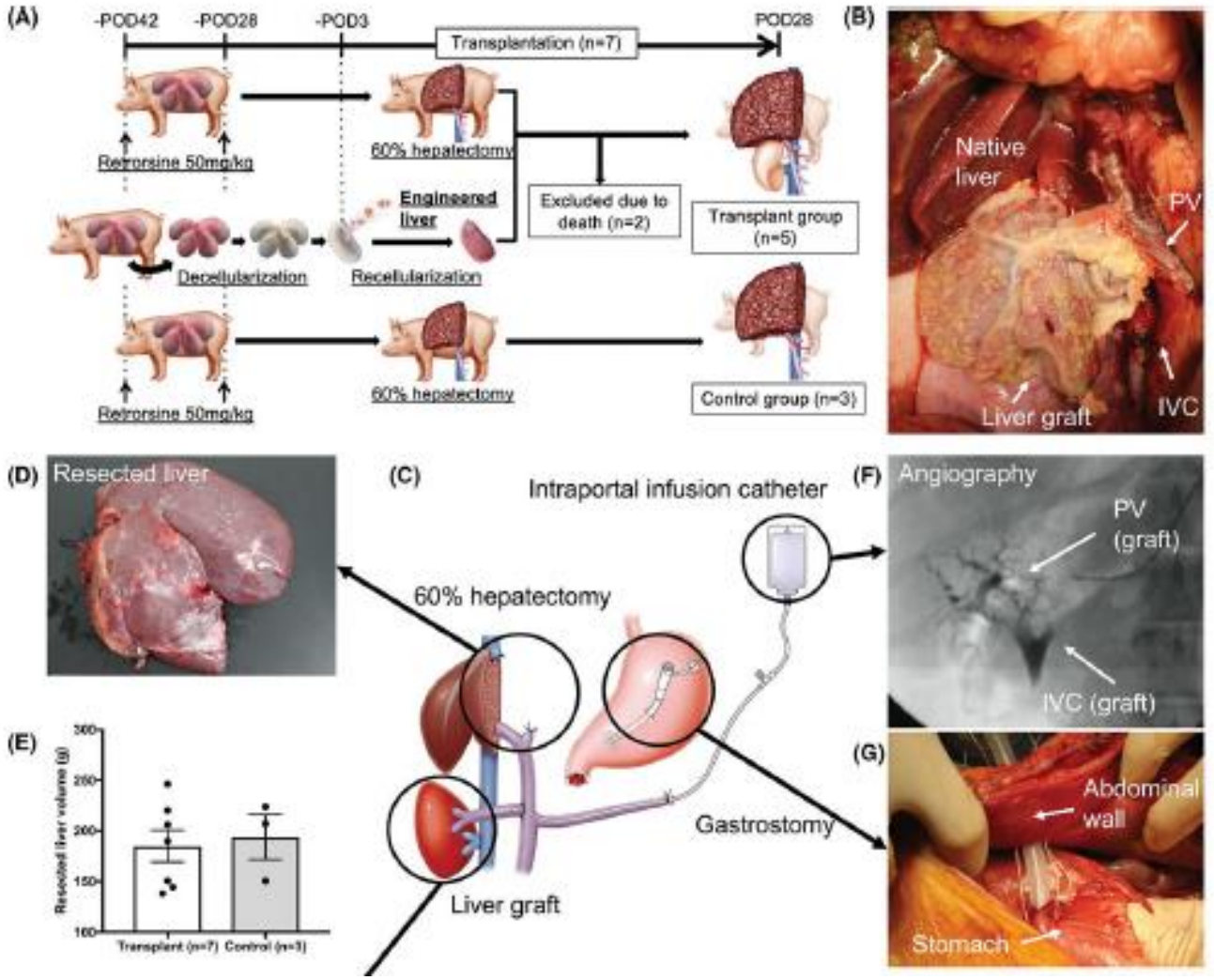
[2]Department of Small Animal Internal Medicine, Kitasato University School of Veterinary Medicine, Towada, Aomori, Japan

[3]Molecular Profiling Research Center for Drug Discovery, National Institute of Advanced Industrial Science and Technology, Koto, Tokyo, Japan



XENOTRASPLANTE

Article

Gene-modified pig-to-human liver xenotransplantation

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Received: 5 June 2024
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Open access
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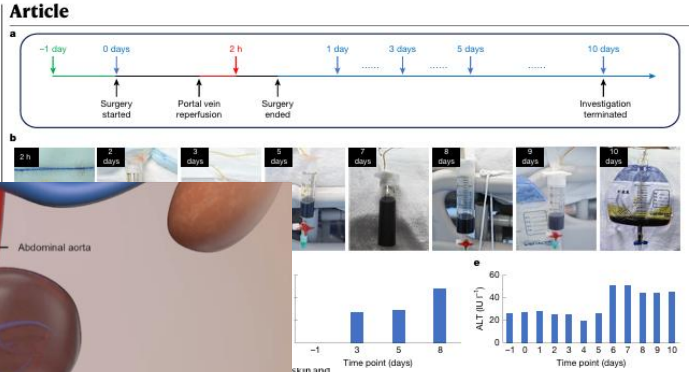
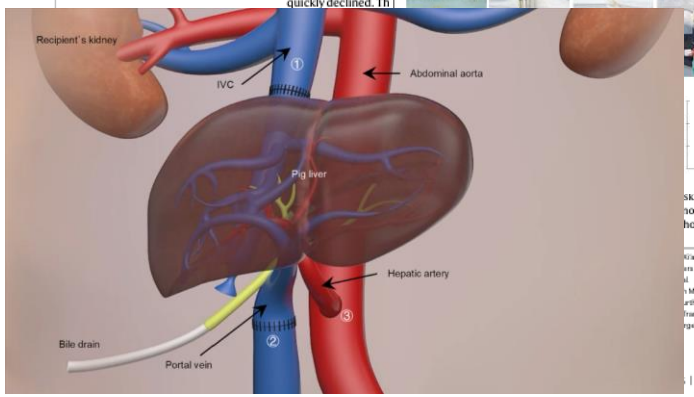
Kai-Shan Tao[1,16], Zhao-Xu Yang[1,16], Xuan Zhang[1,16], Hong-Tao Zhang[1,16], Shu-Qiang Yue[1], Yan-Ling Yang[1], Wen-Jie Song[2], De-Sheng Wang[1], Zheng-Cai Liu[1], Hai-Min Li[1], Yong Chen[1], Rui Ding[1], Shi-Ren Sun[3], Ming Yu[3], Ji-Peng Li[4], Wei-Xun Duan[5], Zhe Wang[6], Jing-Wen Wang[7], Jia-Yun Liu[8], Min-Wen Zheng[9], Xi-Jing Zhang[10,11], Wen Yin[12], Wei-Jun Qin[13], Dong-Mei Bian[14], Lin Li[2], Min Li[2], Zhi-Bin Lin[2], Hao Xu[1], Dan Wei[2], Hong Zhang[1], Juan-Li Duan[1], Deng-Ke Pan[15], Hai-Long Dong[11]✉, Lin Wang[1]✉ & Ke-Feng Dou[1]✉

The shortage of donors is a major challenge for transplantation; however, organs from genetically modified pigs can serve as ideal supplements[1,2]. Until now, porcine hearts and kidneys have been successively transplanted into humans[3–7]. In this study, heterotopic auxiliary transplantation was used to donate a six-gene-edited pig liver to a brain-dead recipient. The graft function, haemodynamics, and immune and inflammatory responses of the recipient were monitored over the subsequent 10 days. Two hours after portal vein reperfusion of the xenograft, goldish bile was produced, increasin… albumin also increa… normal range, while … day 1 and then rapid… portal and hepatic v… decreased early afte… analyses showed tha… T cell activity was inl… activation increased… were no significant … M levels. C-reactive … quickly declined. Th…





frontiers in Immunology
REVIEW
published: 23 February 2022
doi: 10.3389/fimmu.2022.827535

Current Barriers to Clinical Liver Xenotransplantation

Arthur A. Cross-Najafi[1†], Kevin Lopez[1†], Abdulkadir Isidan[1†], Yujin Park[1†], Wenjun Zhang[1†], Ping Li[1†], Sezai Yilmaz[2†], Sami Akbulut[2†] and Burcin Ekser[1*†]

[1] Transplant Division, Department of Surgery, Indiana University School of Medicine, Indianapolis, IN, United States, [2] Department of Surgery and Liver Transplant Institute, Inonu University Faculty of Medicine, Malatya, Turkey

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Preclinical trials of pig-to-nonhuman primate liver xenotransplantation have recently achieved longer survival times. However, life-threatening thrombocytopenia and coagulation dysregulation continue to limit preclinical liver xenograft survival times to less than one month despite various genetic modifications in pigs and intensive pharmacological support. Transfusion of human coagulation factors and complex immunosuppressive regimens have resulted in substantial improvements in recipient survival. The fu… dysregulation r… von Willebranc… increased O-li… Porcine liver si… in an asialogly… respectively. … incompatible … constitutive ac… factor pathwa… thrombomodul… leading to a hy… may mitigate … dysregulation, … xenotransplant…

Keywords: liver xer…

INTRODUC…

End-stage organ… definitive treatm… demand for orga… inevitable reality … organ. In order t… from one species … ideal organ dono…

TABLE 1 | Genetically-engineered pig-to-nonhuman primate liver xenotransplantation.

Year	Genetic Modifications	Recipient	Transplant	N	Pharmacologic Regimen	Survival	Reference
2000	hCD55	Baboon	Orthotopic	2	CyP CsA Cs	96, 192 h	(16)
2005	hCD55.CD59.HT	Baboon	Orthotopic	5	CyP CsA Cs Daclizumab Rituximab MMF	13, 18, 20, 21, 24 h	(17)
2010	GTKO	Baboon	Orthotopic	2	Cs MMF ATG Tacrolimus	3, 144 h	(10)
2010	GTKO.hCD46	Baboon	Orthotopic	5	Cs MMF ATG Tacrolimus	3, 20, 24, 96, 120 h	(10)
2010	GTKO.hCD46	Baboon	Orthotopic	3	CyP Cs MMF Tacrolimus	144, 144, 168 h	(10)
2012	GTKO	Baboon	Orthotopic	2	Cs ATG Tacrolimus CVF AZA anti-CD154 LoCD2b (one case)	144, 216 h	(19)
2012	MGH MS, GTKO	Baboon	Orthotopic	3	Cs ATG Tacrolimus CVF AZA anti-CD154 LoCD2b	6, 8, 9 days	(19)
2014	MGH MS, GTKO	Baboon	Heterotopic	3	Cs ATG Tacrolimus CVF	6, 9, 15 days	(20)
2015	WZ MS, GTKO	Tibetan monkey	Heterotopic	3	Cs MMF ATG Tacrolimus CVF anti-CD154 salvias miltiorrhizae	2, 5, 14 days	(21)
2016	MGH MS, GTKO	Baboon	Orthotopic	6	Cs ATG Tacrolimus CVF hPCC	1, 3, 4, 4, 6, 7 days	(22)
2016	MGH MS, GTKO	Baboon	Orthotopic	1	Cs ATG Tacrolimus CVF hPCC Belatacept	25 days	(6)
2017	MGH MS, GTKO	Baboon	Orthotopic	4	Cs ATG Tacrolimus CVF hPCC anti-CD40mAb	25, 5, 8, 29 days	(7)
2020	GTKO, CMAH-KO, B4GALNT2-KO, PERV-KO, hCD46, hCD55, hCD59, hTHBD, hTFPI, hCD39, hB2M, HLA-E, hCD47-TG	Rhesus monkey	Heterotopic	1	ATG anti-CD40mAb	26 days	Dou, personal communication

ATG, anti-thymocyte globulin; AZA, azathioprine; B4GALNT2, Beta-1,4-N-Acetyl Galactosaminyltransferase 2; CMAH, Cytidine monophospho-N-acetylneuraminic acid hydroxylase; Cs, corticosteroids; CsA, cyclosporine; Cyp, cyclophosphamide; GTKO, galactosyltransferase knockout; h, humanized; hPCC, human prothrombin complex concentrate; LoCD2b, rat anti-primate CD2 IgG2b; MGH MS, Massachusetts General Hospital miniature swine; MMF, mycophenolate mofetil; PERV, porcine endogenous retrovirus; WZ MS, Wu Zhanshan miniature swine.

Journal Pre-proof

Genetically engineered pig-to-human liver xenotransplantation

Wenjie Zhang, Qingxiang Xu, Kaixiang Xu, Runqiu Jiang, Shouyu Wang, Meijuan Zheng, Nian Liu, Deling Jiao, Zhangding Wang, Jian Ge, Xianfu Lu, Guoqiang Li, Fan Huang, Lei Liu, Yin Yin, Yang Liu, Jianxiong Guo, Kai Liu, Hong-Jiang Wei, Beicheng Sun

PII: S0168-8278(25)02497-3

DOI: https://doi.org/10.1016/j.jhep.2025.08.044

Reference: JHEPAT 10284

To appear in: *Journal of Hepatology*

Received Date: 19 July 2025

Revised Date: 25 August 2025

Accepted Date: 31 August 2025

Highlights

1.First successful auxiliary porcine liver xenotransplantation from a 10-gene edited pig to a living recipient, distinct from prior brain-dead recipient cases.

2. Porcine albumin and coagulation factors were bioactive, sustaining hepatic metabolic function without allergic reactions or other adverse effects.

3. Early postoperative course showed no hyperacute or acute rejection, supporting the effectiveness of donor gene editing and immunosuppression.

4.Xenotransplantation-associated thrombotic microangiopathy (xTMA) was successfully controlled by graft removal, followed by eculizumab and plasma exchange.

5.Establishes auxiliary liver xenotransplantation as a life-saving bridge for unresectable liver cancer or liver failure, and provides a clinical paradigm for future liver xenotransplantation trials.

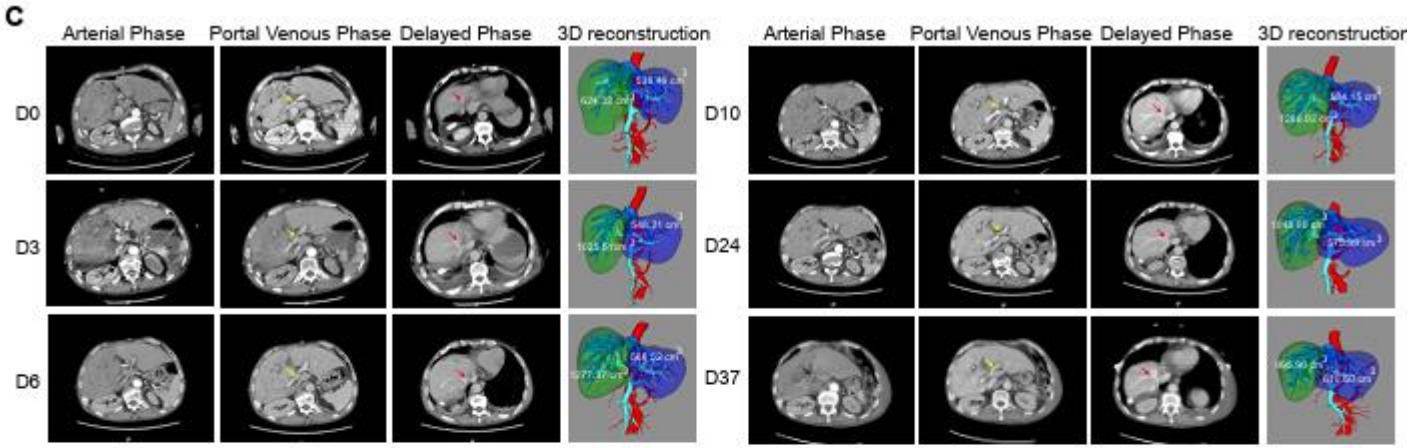
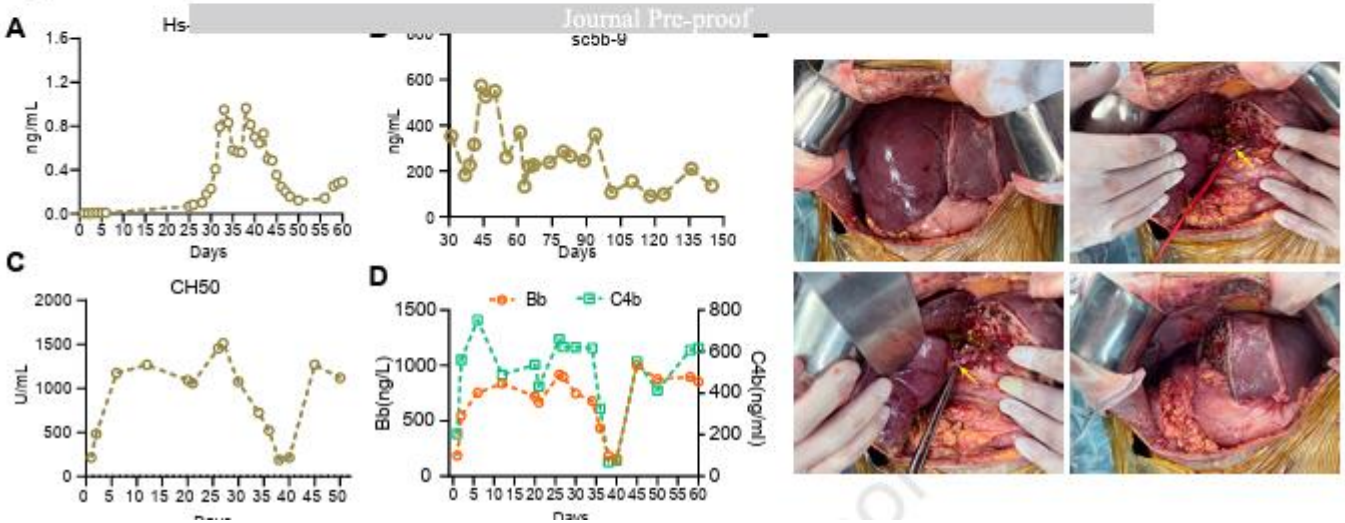


Figure 6



TERAPIAS CELULARES / GÉNICAS

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Thinking Out Loud

Biofabrication of Autologous Human Hepatocytes for Transplantation: How Do We Get There?

Nandini Agarwal,*† Branimir Popovic,† Nicole J. Martucci,†
Nicolas A. Fraunhoffer,†‡§ and Alejandro Soto-Gutierrez†

*School of Bioscience and Technology, Vellore Institute of Technology, Vellore,
†Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh,
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Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina
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Directed differentiation of hepatocytes from induced pluripotent stem cells (iPSCs) holds g
material for treating some liver disorders. The unlimited availability of perfectly differentia
hepatocytes will dramatically facilitate cell therapies. While systems to manufacture large q
derived cells have been developed, we have been unable to generate and maintain stable
liver cells ex vivo. This short review highlights important challenges and possible solutions t
of hepatocyte biofabrication for cellular therapies to treat liver diseases. Successful cell tra
require optimizing the best cell function, overcoming limitations to cell numbers and safety,
ber of other challenges. Collaboration among scientists, clinicians, and industry is critical fo
autologous stem cell-based therapies to treat liver diseases.

Key words: Hepatocyte proliferation; Liver regeneration

INTRODUCTION

The demand for transplantable livers has increased on the organ donor pool[1]. Nev
to investigating the liver organ

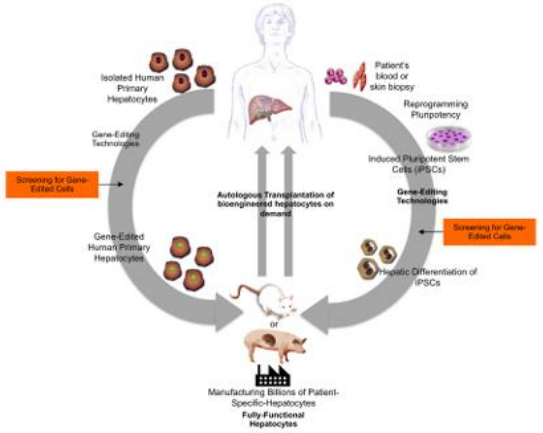


Figure 1. Schematic representation of manufacturing approach of autologous hepatocytes for transplantation. Primary hepatocytes are isolated from the patient and edited with CRISPR/Cas9 to correct the pathogenic genomic alteration ex vivo. Then the edited primary hepatocytes are select and multiplied in a "bioreactor" to be transplanted in patients. Alternatively, fibroblasts/blood cells are isolated from the patient and edited for pathogenic mutations using CRISPR/Cas9 ex vivo. The cells are then screened and reprogrammed to produce induced pluripotent stem cells (iPSCs) in vitro. These corrected iPSCs are differentiated to generate hepatocytes, which are transplanted into a "bioreactor" to produce functional hepatocytes that can be used for transplantation in patients.

Original Article

Procurement and Evaluation of Hepatocytes for Transplantation From Neonatal Donors After Circulatory Death

Cell Transplantation
1–11

DOI: 10.1177/09636897211069900
journals.sagepub.com/home/cll
SAGE

Emil Bluhme[1,2*], Ewa Henckel[1,3*], Roberto Gramignoli[4], Therese Kjellin[3], Christina Hammarstedt[4], Greg Nowak[1,2], Ahmad Karadagi[1,2], Helene Johansson[1], Öystein Jynge[5], Maria Söderström[5], Björn Fischler[1,6], Stephen Strom[4], Ewa Ellis[1], Boubou Hallberg[1*], and Carl Jorns[1,2*]

Abstract

Hepatocyte transplantation is a promising treatment for liver failure and inborn metabolic liver diseases, but progress has been hampered by a scarcity of available organs. Here, hepatocytes isolated from livers procured for a neonatal hepatocyte donation program within a research setting were assessed for metabolic function and suitability for transplantation. Organ donation was considered for infants who died in neonatal intensive care in the Stockholm region during 2015–2021. Inclusion was assessed when a decision to discontinue life-sustaining treatment had been made and hepatectomy performed after declaration of death. Hepatocyte isolation was performed by three-step collagenase perfusion. Hepatocyte viability, yield, and function were assessed using fresh and cryopreserved cells. Engraftment and maturation of cryopreserved neonatal hepatocytes were assessed by transplantation into an immunodeficient mouse model and analysis of the gene expression of phase I, phase II, and liver-specific enzymes and proteins. Twelve livers were procured. Median warm ischemia time (WIT) was 190 [interquartile range (IQR): 80–210] minutes. Median viability was 86% (IQR: 71%–91%). Median yield was 6.9 (IQR: 3.4–12.8) x10^6 viable hepatocytes/g. Transplantation into immunodeficient mice resulted in good engraftment and maturation of hepatocyte-specific proteins and enzymes. A neonatal organ donation program including preterm born infants was found to be feasible. Hepatocytes isolated from neonatal donors had good viability, function, and engraftment despite prolonged WIT. Therefore, neonatal livers should be considered as a donor source for clinical hepatocyte transplantation, even in cases with extended WIT.

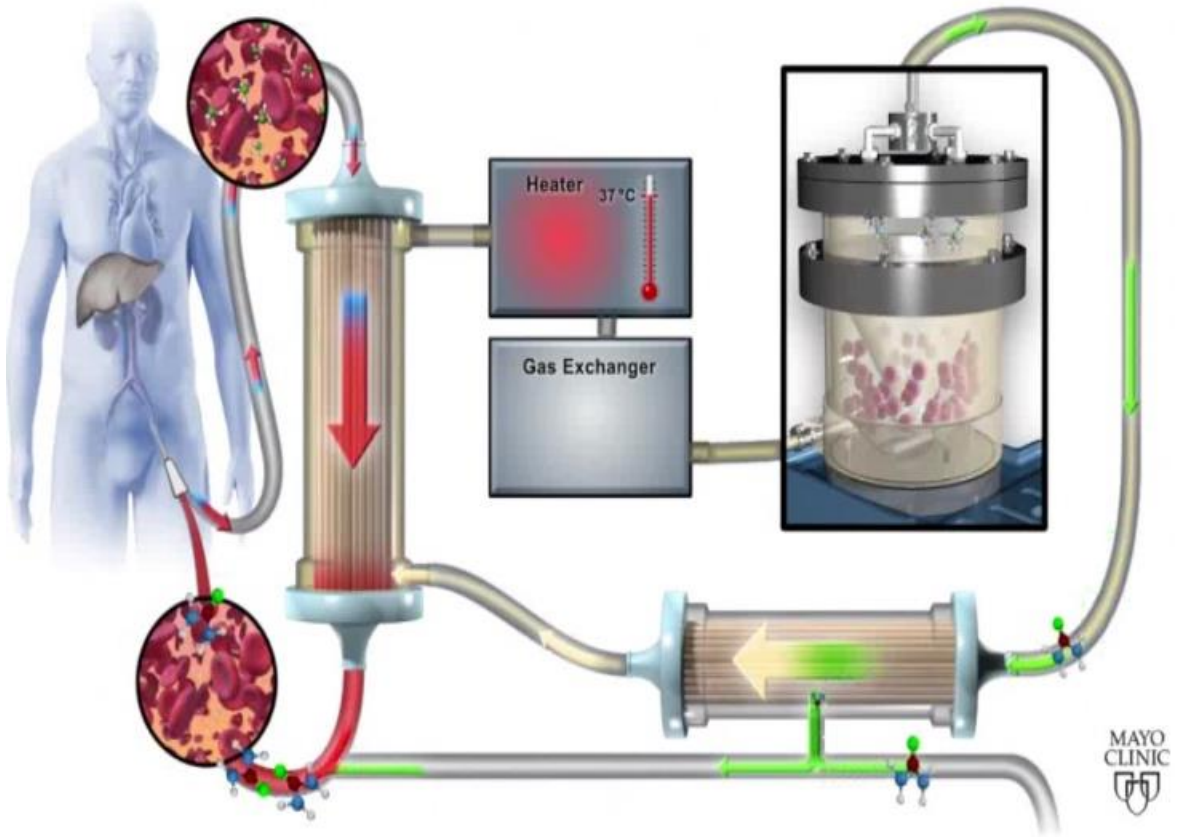
Keywords

neonatal organ donation, warm ischemia time, FRGN mice

Introduction

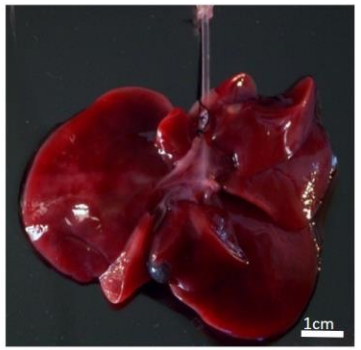
Progress in hepatocyte transplantation has been hampered by access to good-quality donor tissue, as most donor organs

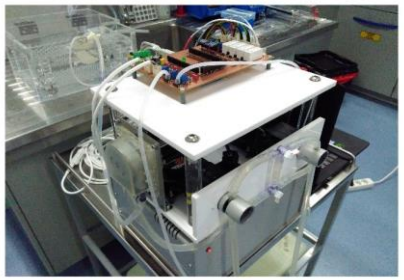
SOPORTE ARTIFICIAL

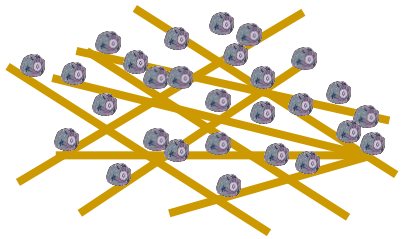


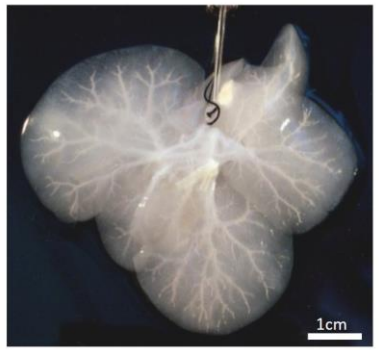
Bioingeniería de órganos de Zaragoza

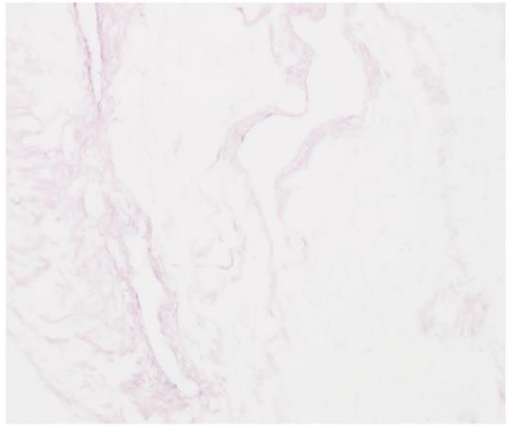
DESCELULARIZACIÓN DE HÍGADO

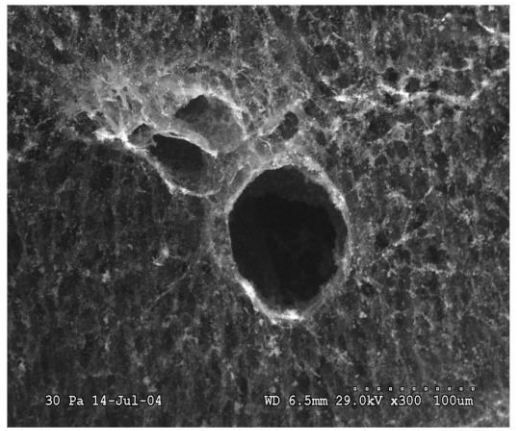






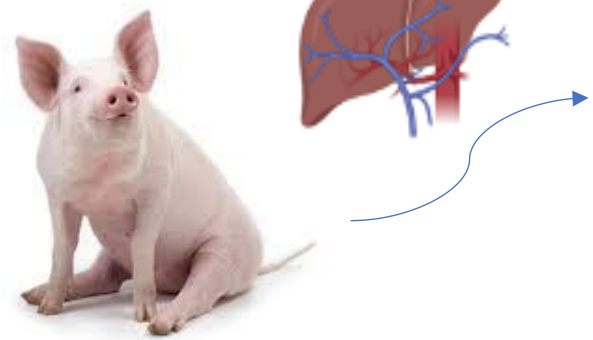


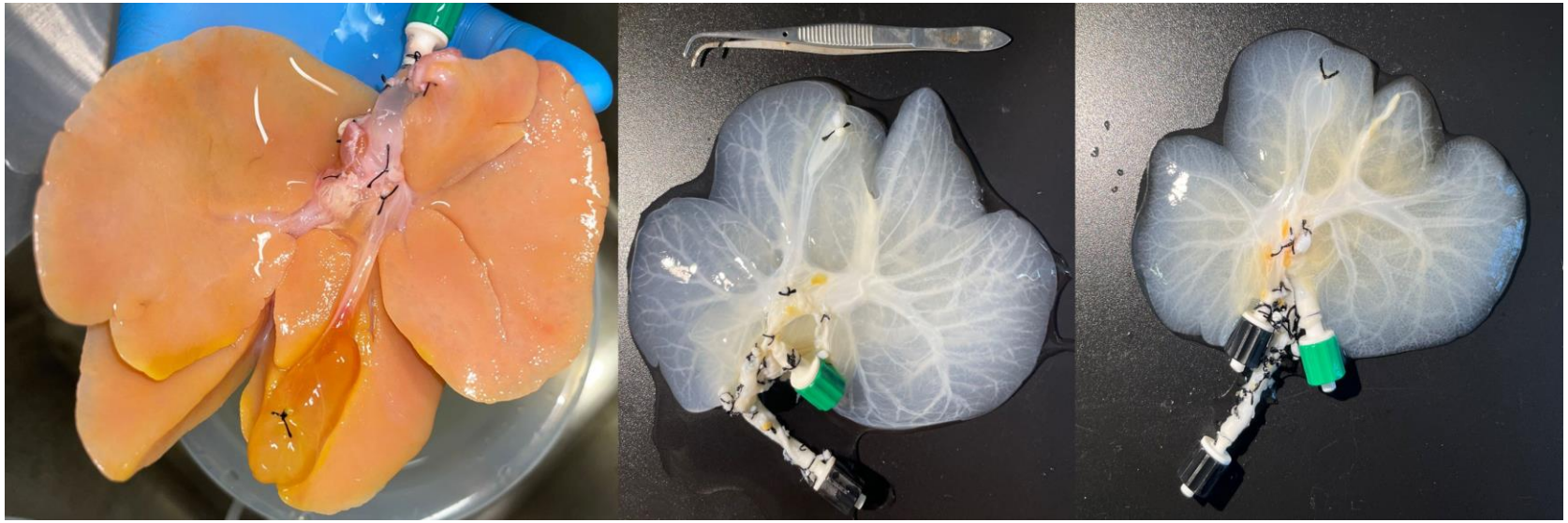




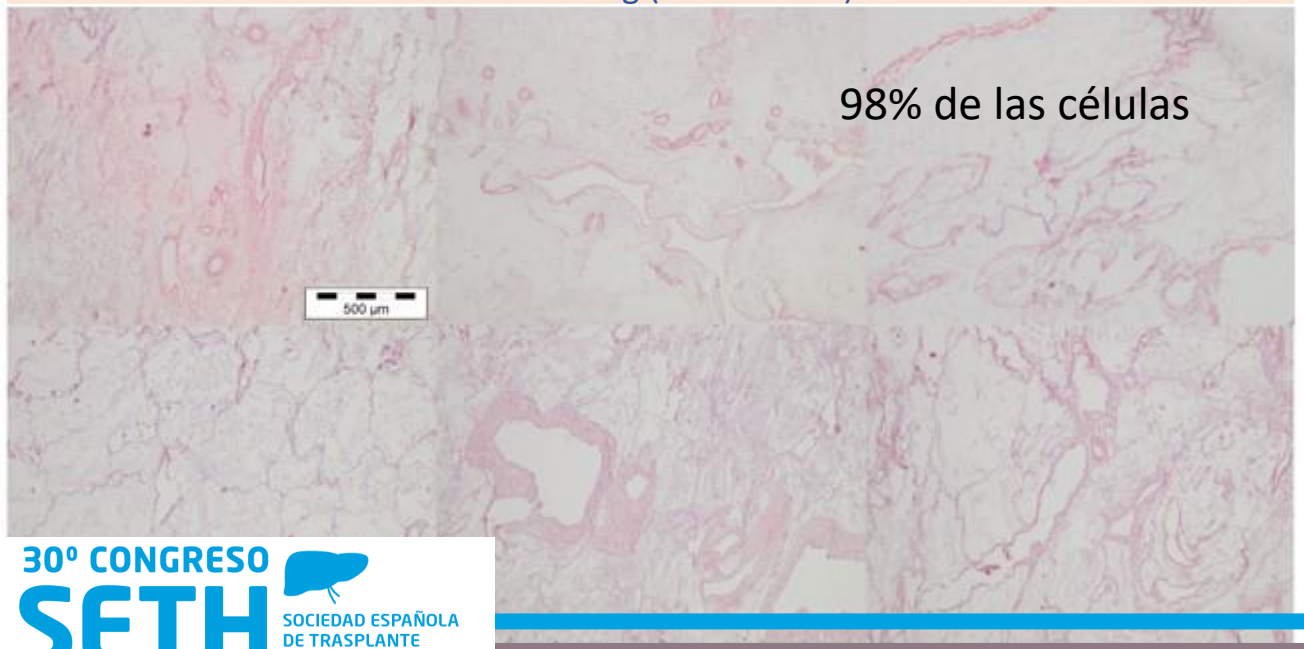
Baptista *et al.* Conf Proc IEEE Eng Med Biol Soc. 2009; Baptista *et al.* Hepatology, 2010.

TOWARD WHOLE REVASCULARIZATION OF A BIOENGINEERED PIG LIVER



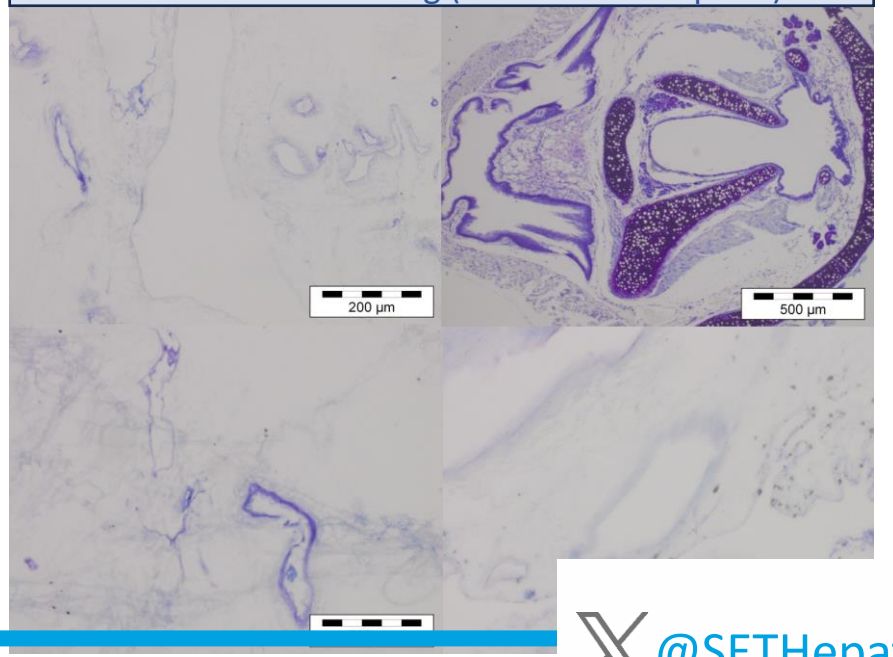


H&E staining (cell removal)

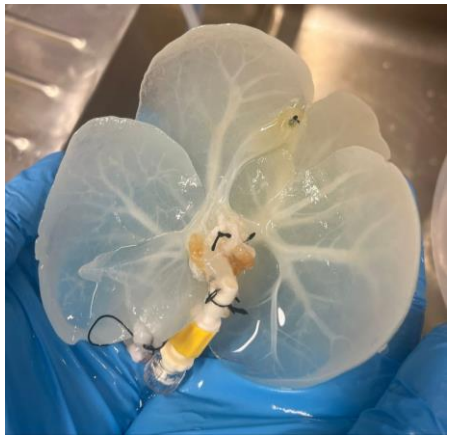


98% de las células

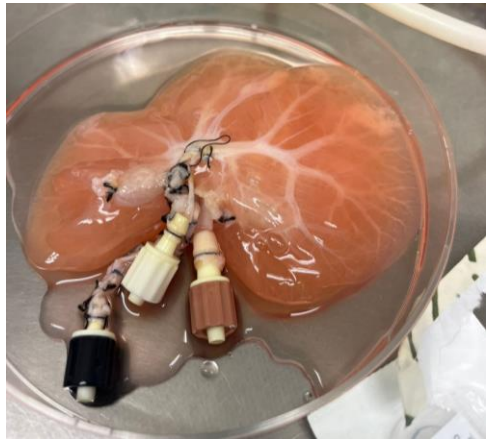
Toluidine Blue staining (Immobilized heparin)



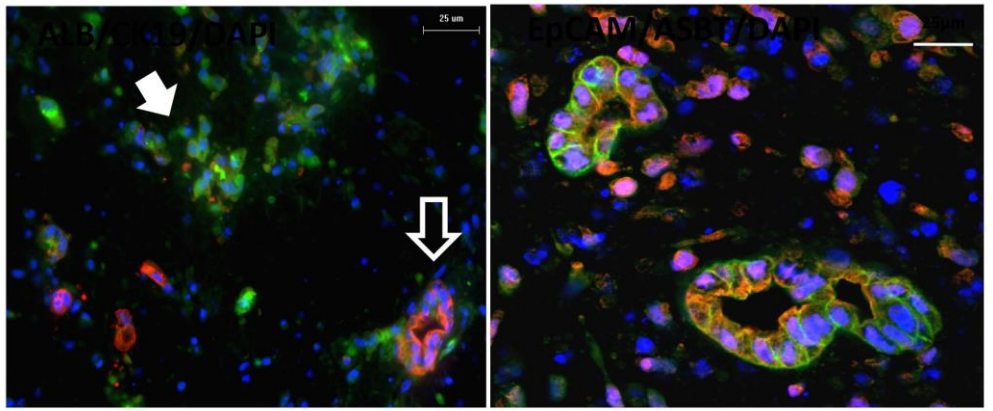
RECELULARIZACIÓN DE HÍGADO



Células vasculares

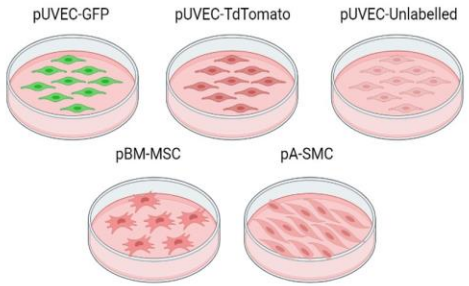


- >10^9 hepatocitos.
- 2,5% de masa hepática funcionante



Baptista *et al.* Hepatology, 2010.

VASCULARIZACIÓN DE SCAFFOLDS HEPÁTICOS

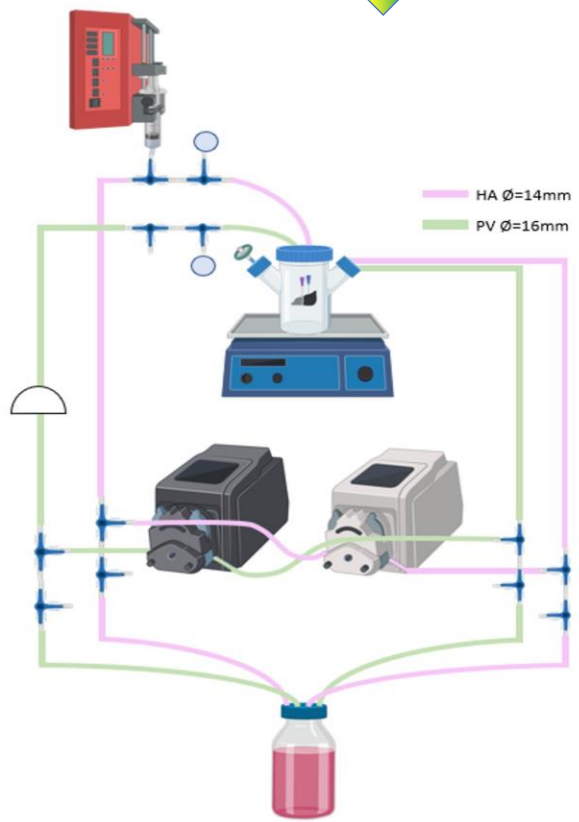


pUVECs
Porcine umbilical vein endothelial cells

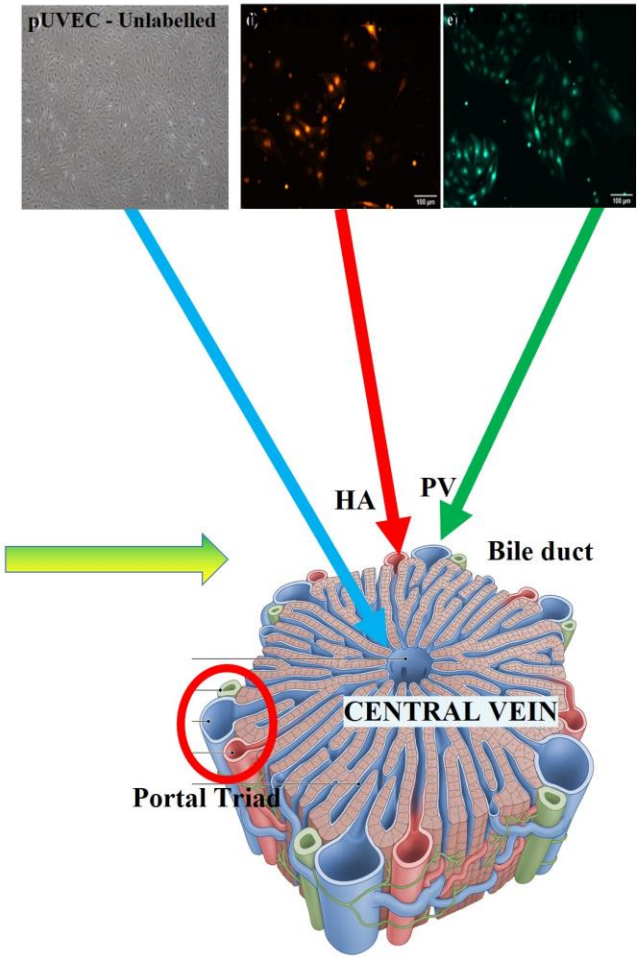
pA-SMC:
Porcine aortic vascular smooth muscle cells

pBM-MSC
Porcine Bone marrow mesenchymal stem cells

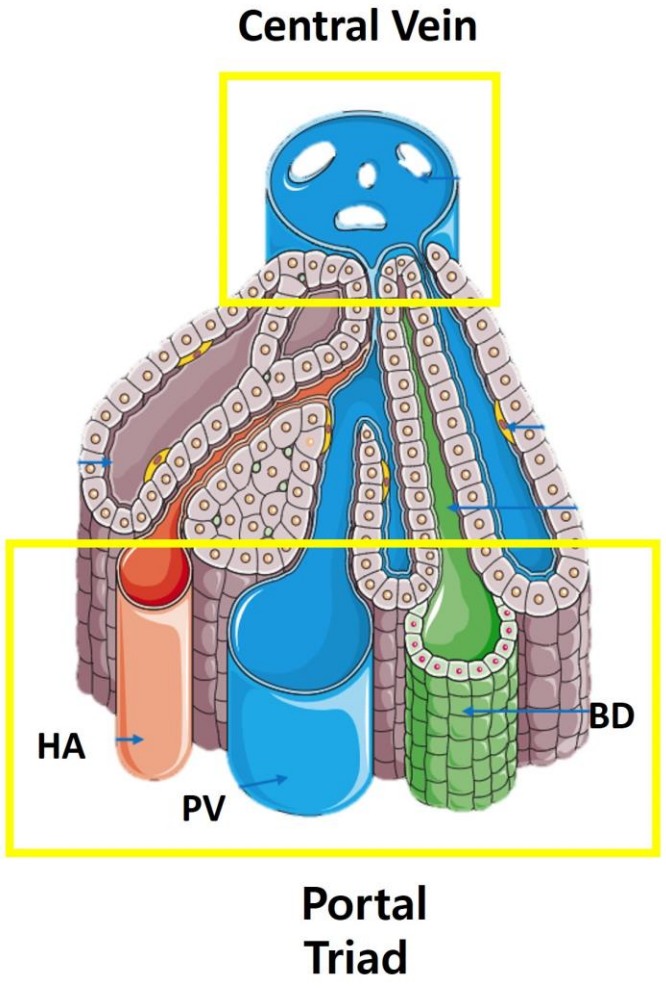
pOECs
Porcine umbilical cord blood outgrowth endothelial cells

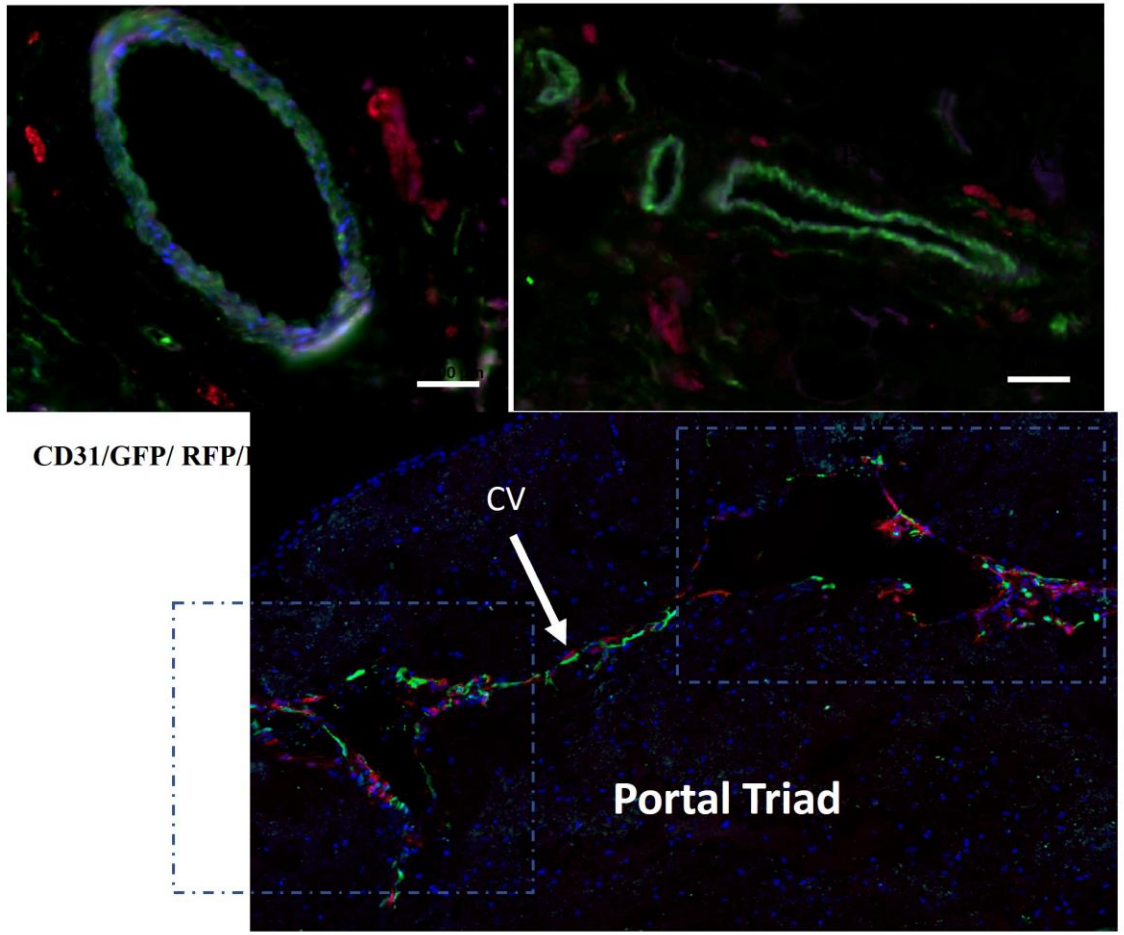


Differential seeding

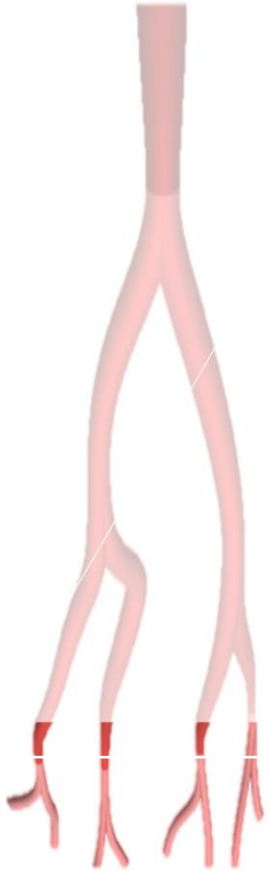


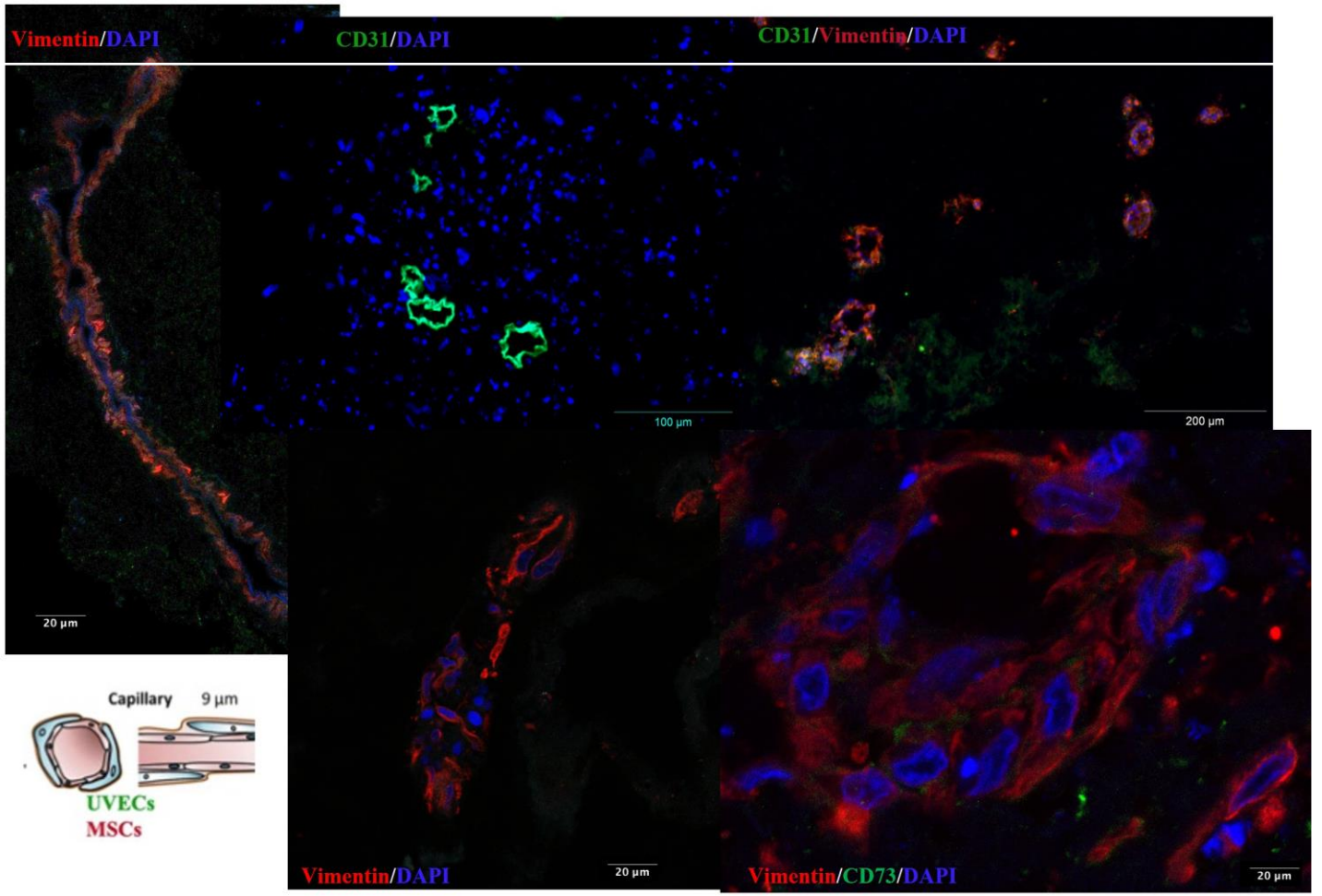
SIEMBRA DE CÉLULAS VASCULARES EN VENAS Y ARTERIAS



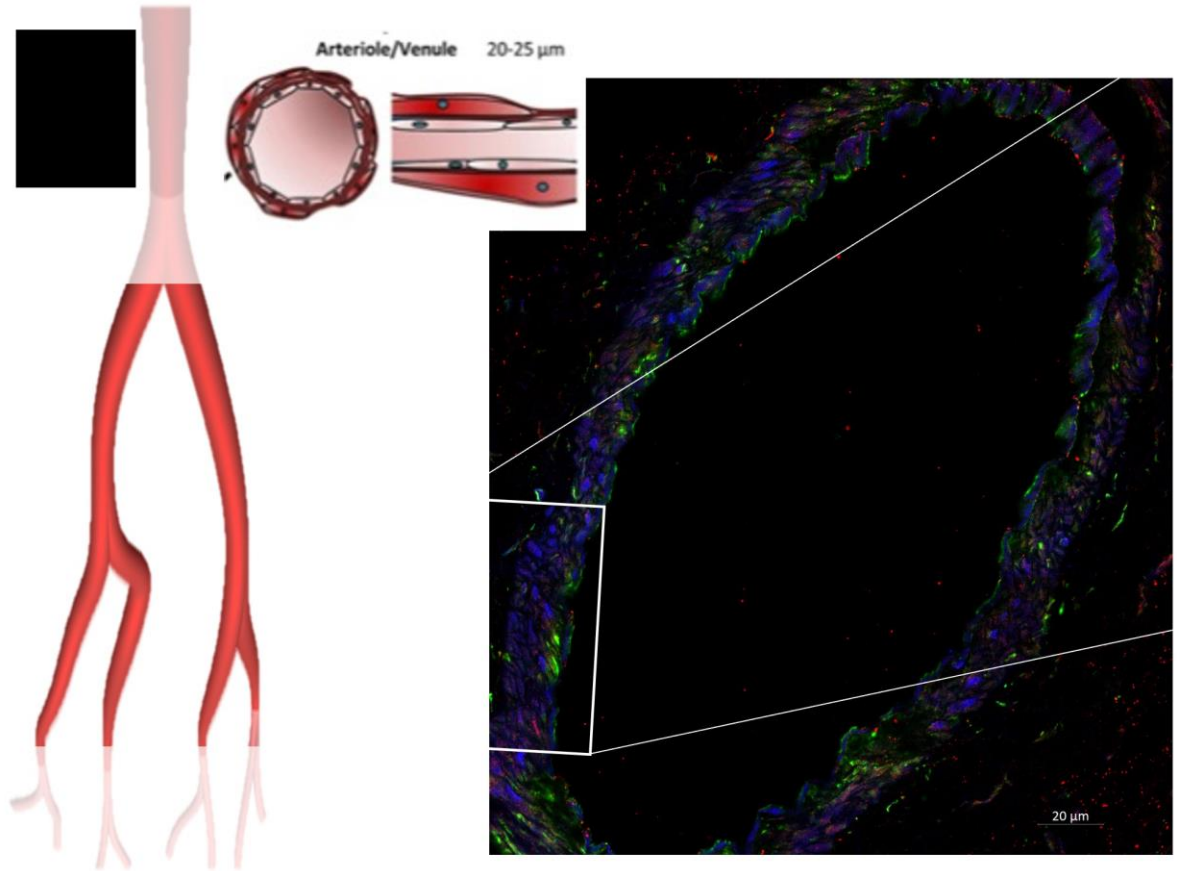


GENERACIÓN DE ESTRUCTURAS VASCULARES (EC, SMC Y MSC)

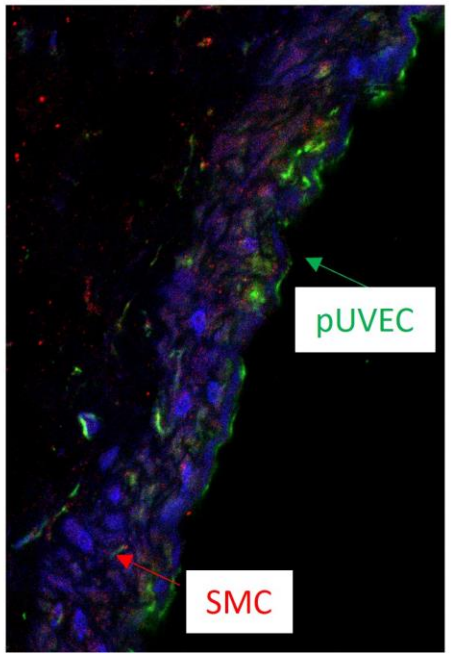




GENERACIÓN DE ESTRUCTURAS VASCULARES (EC, SMC Y MSC)



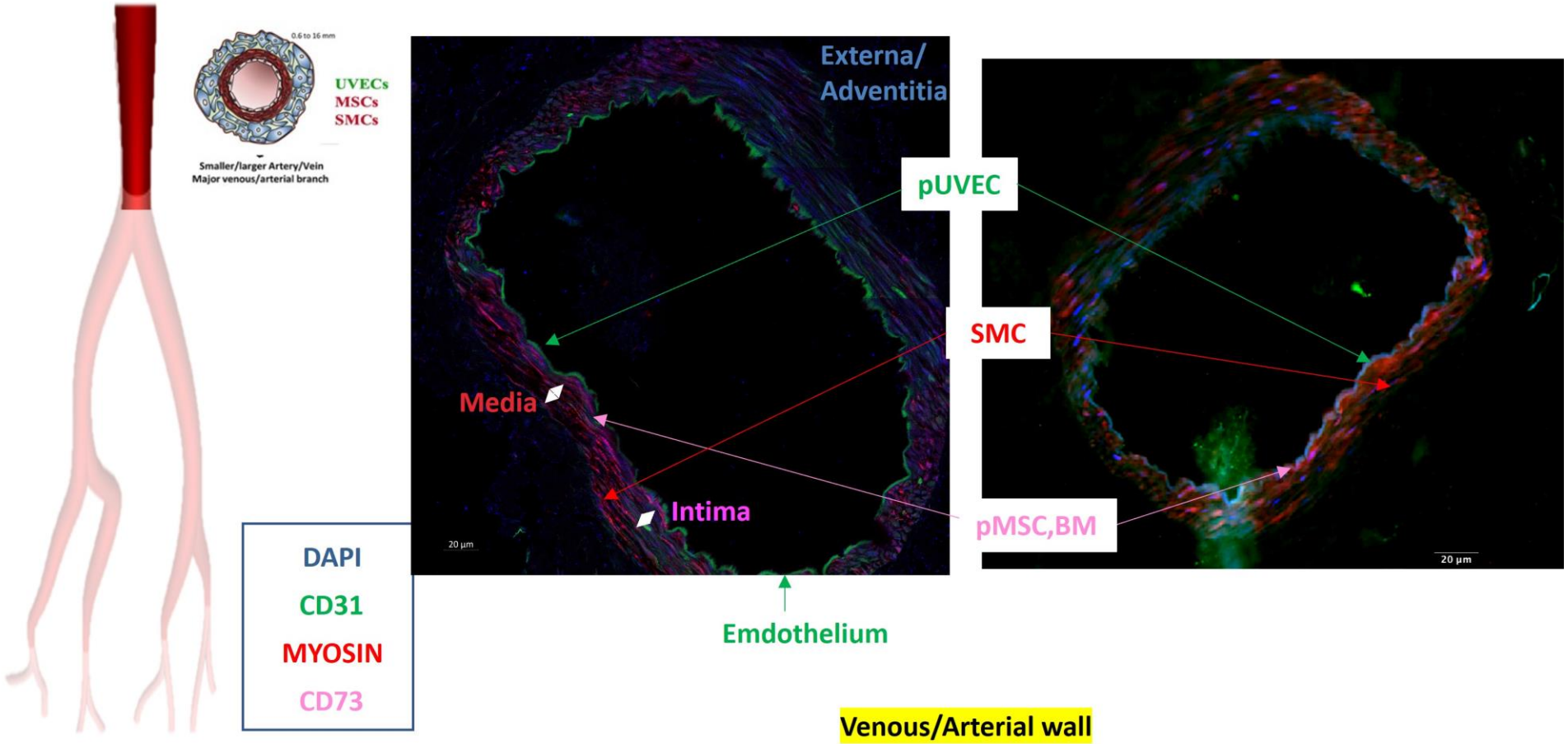
Confocal Zeiss LSM 880



DAPI
CD31
MYOSIN

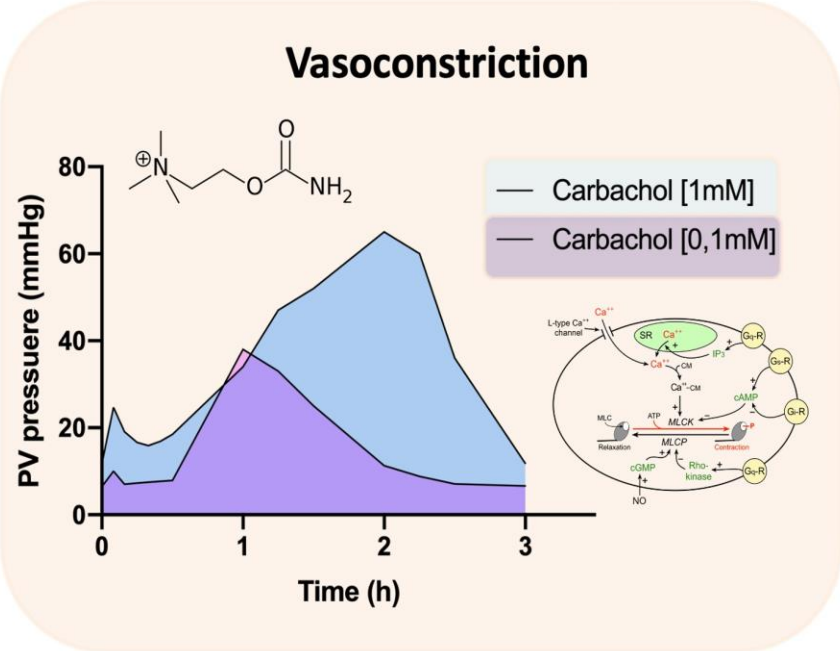
GENERACIÓN DE ESTRUCTURAS VASCULARES (EC, SMC Y MSC)

Results

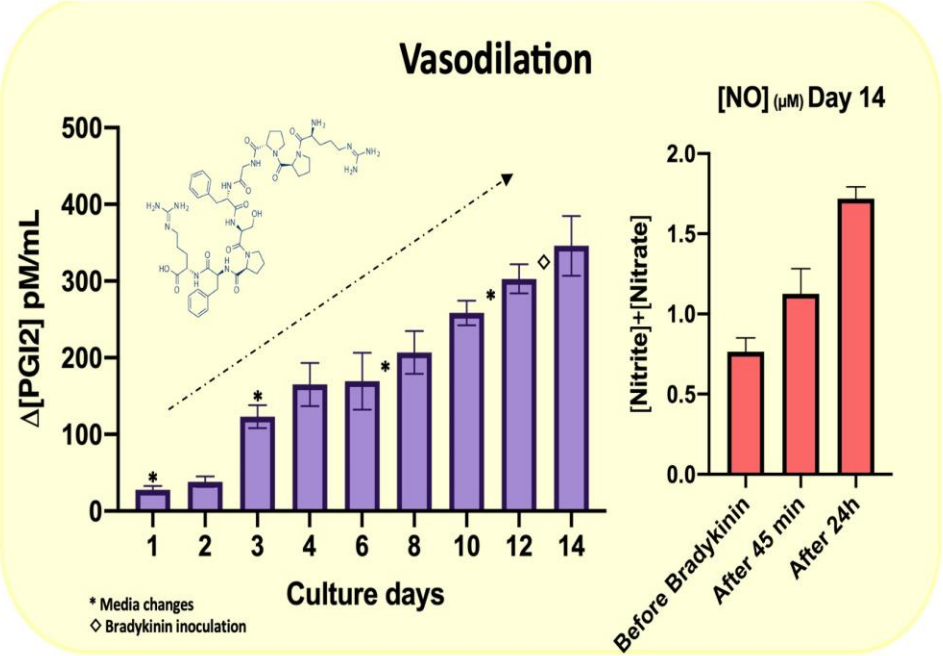


Las estructuras vasculares creadas son capaces de contraerse y secretar NO y prostaciclinas.

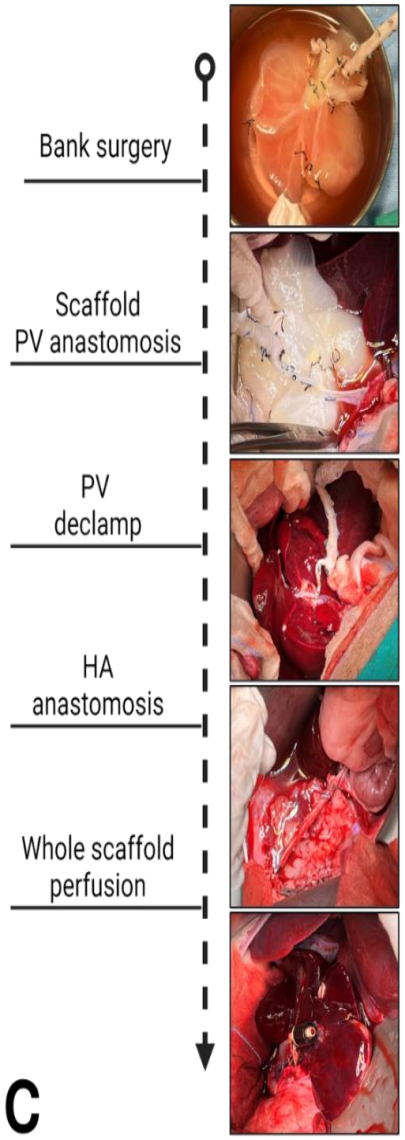
Upon Carbachol Exposure



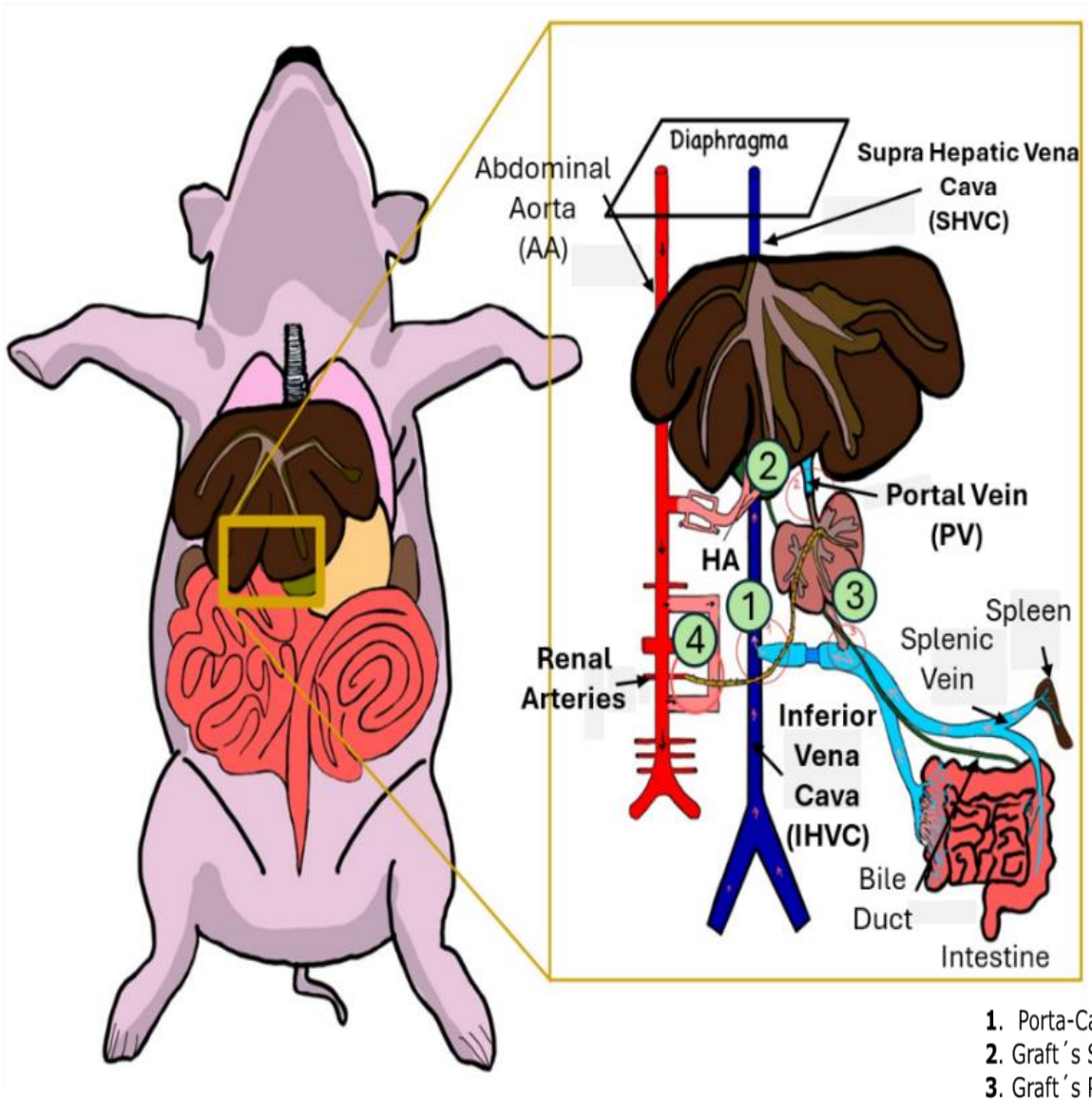
Upon Bradikinin Challenge



MODELO DE TRASPLANTE HEPÁTICO AUXILIAR CON SHUNT PORTO CAVA.

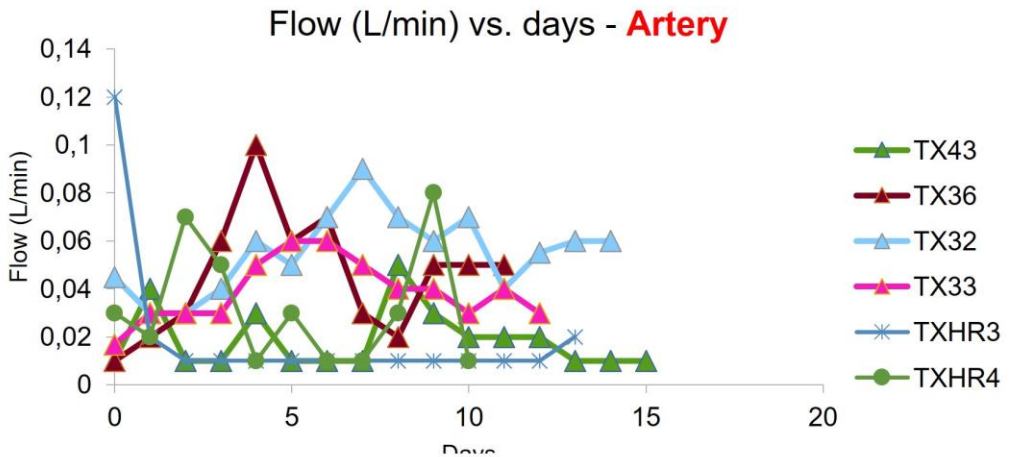


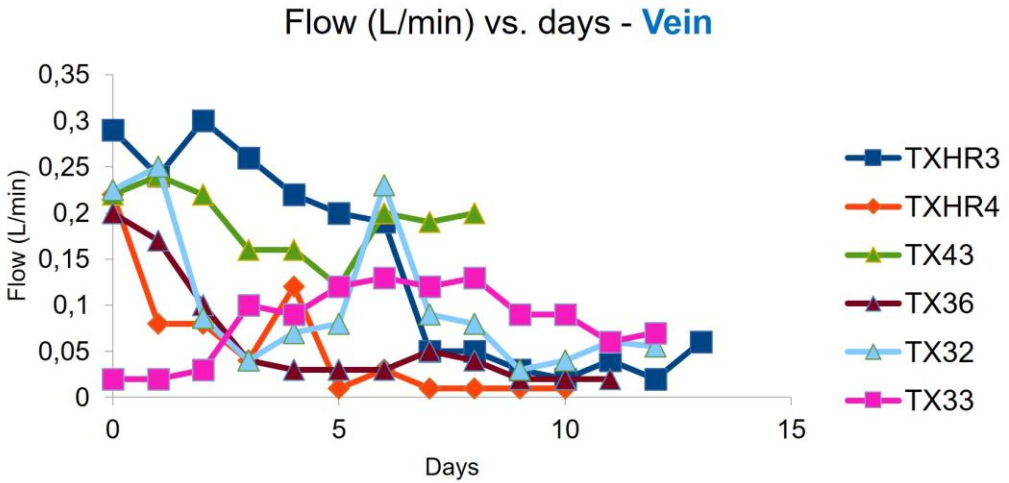
C

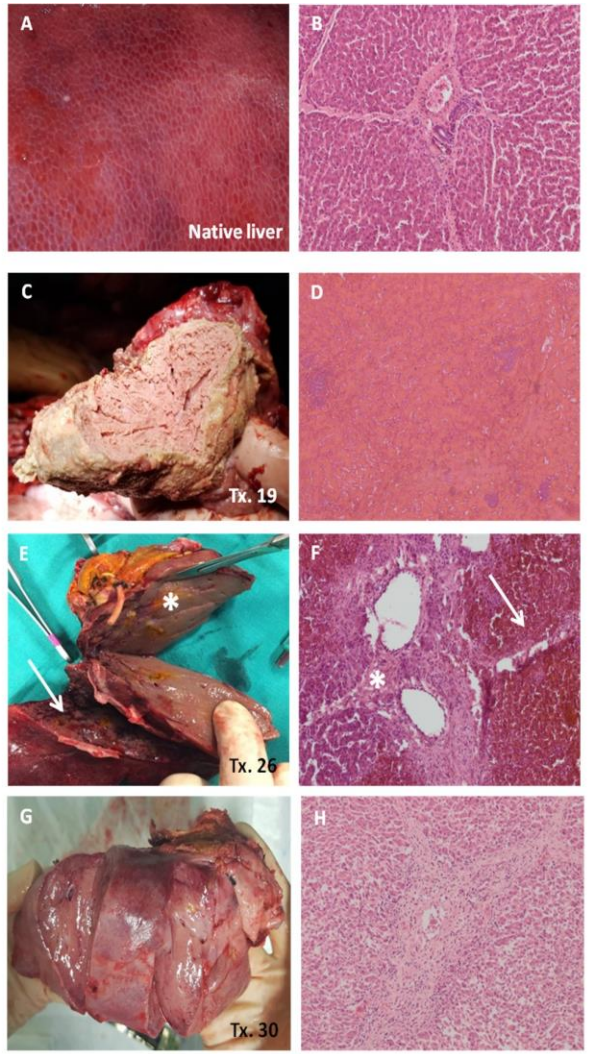


1. Porta-Caval Shunt
2. Graft´s SVC-Native PV Anastomosis
3. Graft´s PV-Native PV Anastomosis
4. Graft´s Aorta-Native Right Kidney Artery

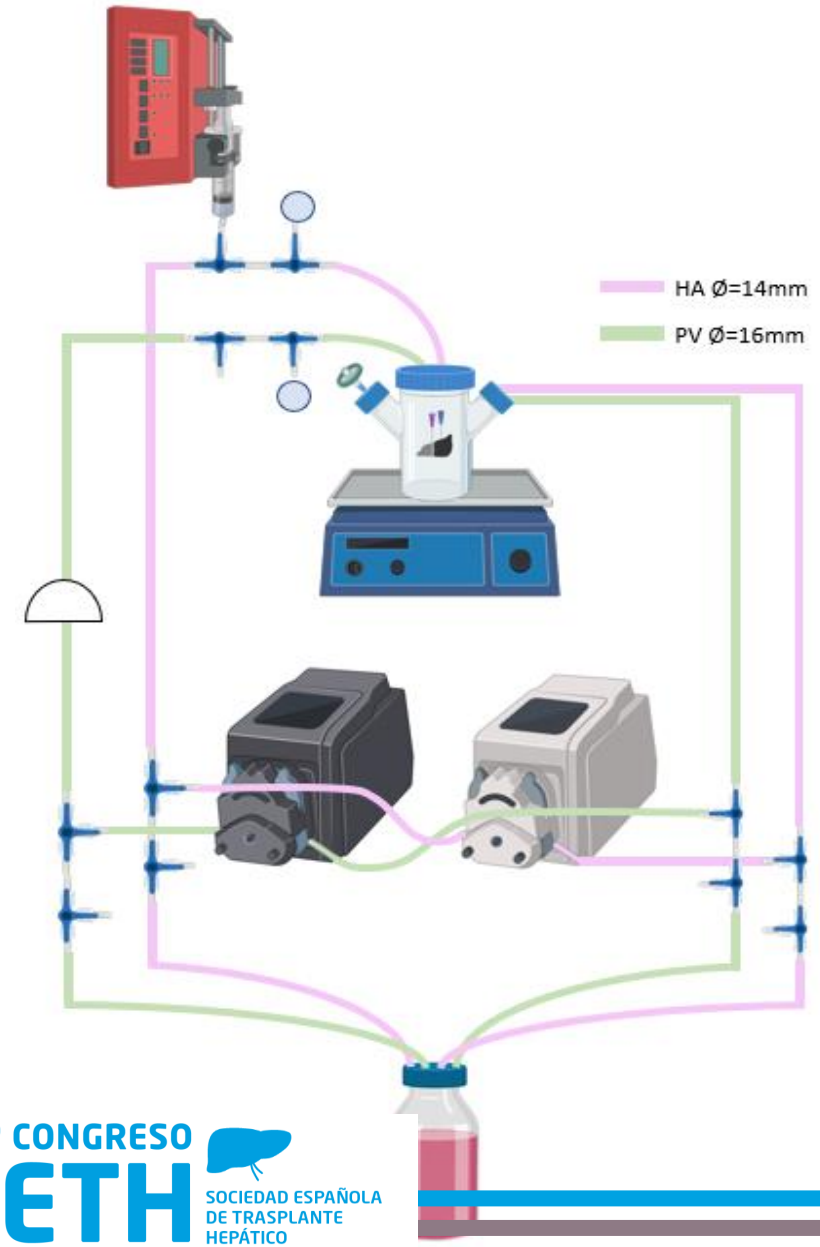
MODELO PORCINO DE REGENERACIÓN HEPATICA



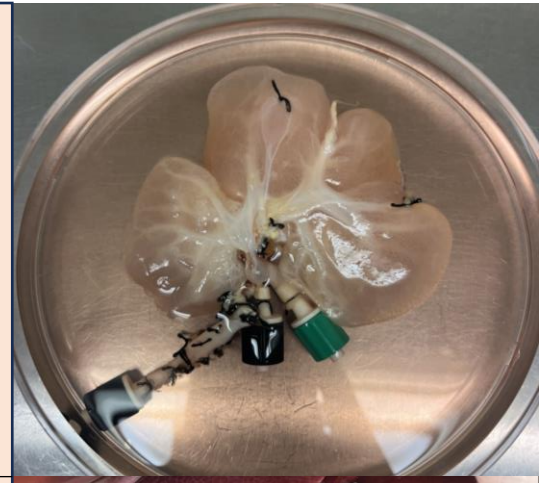




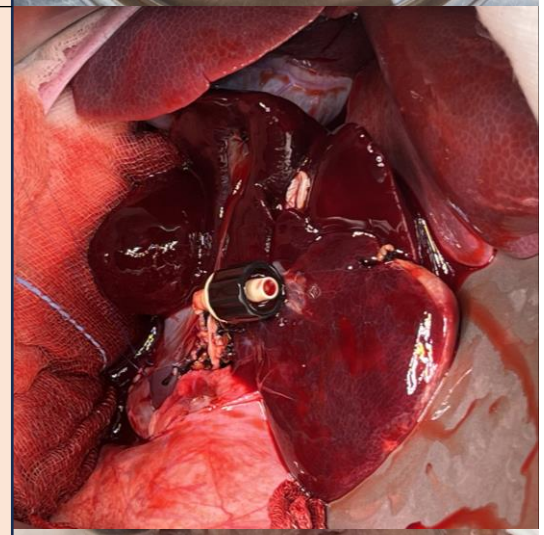
Trasplantes TXHR7 y TXHR8



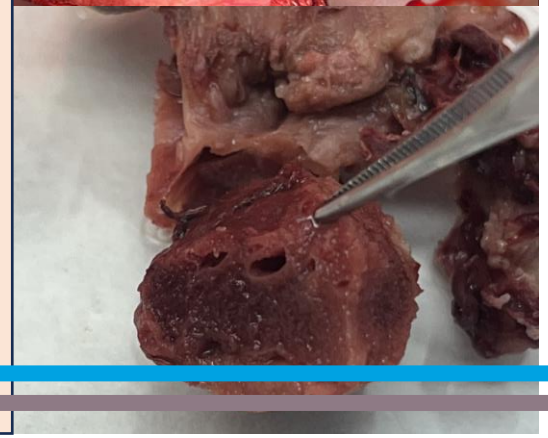
After 14 days BR

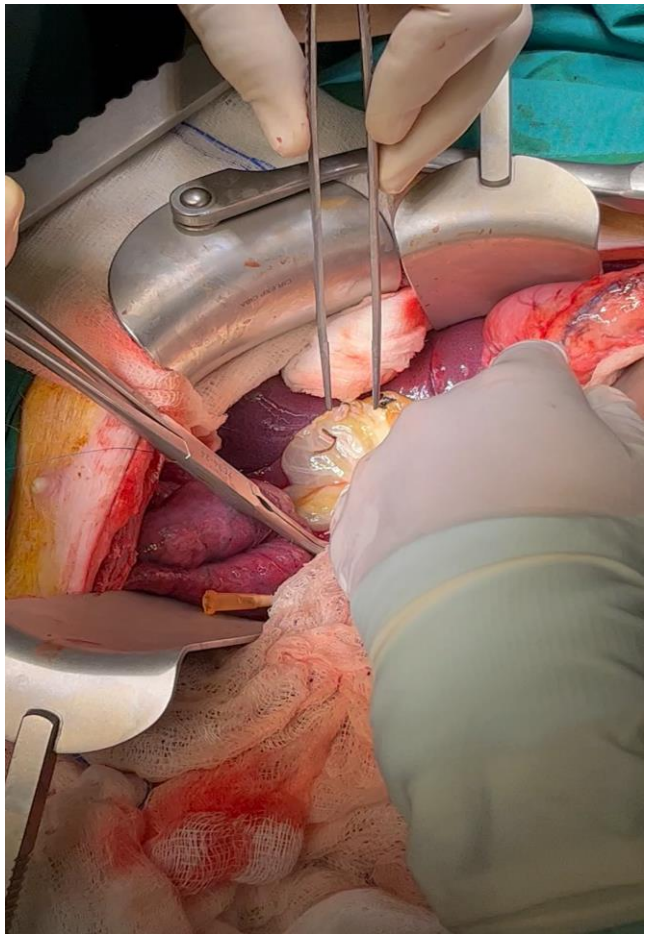


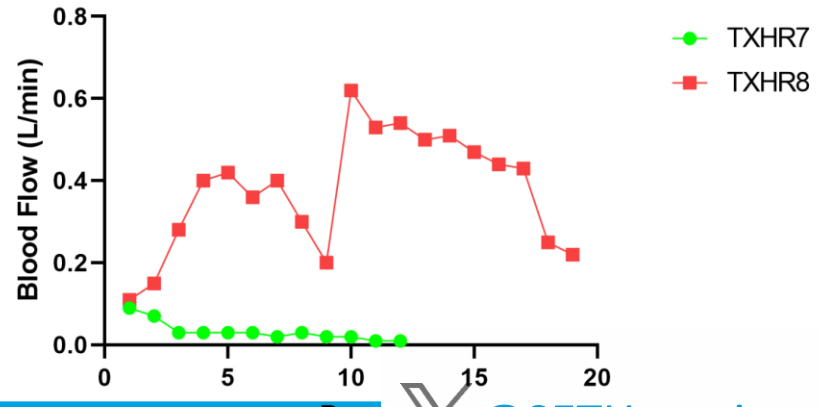
After PV/HA anastomosis



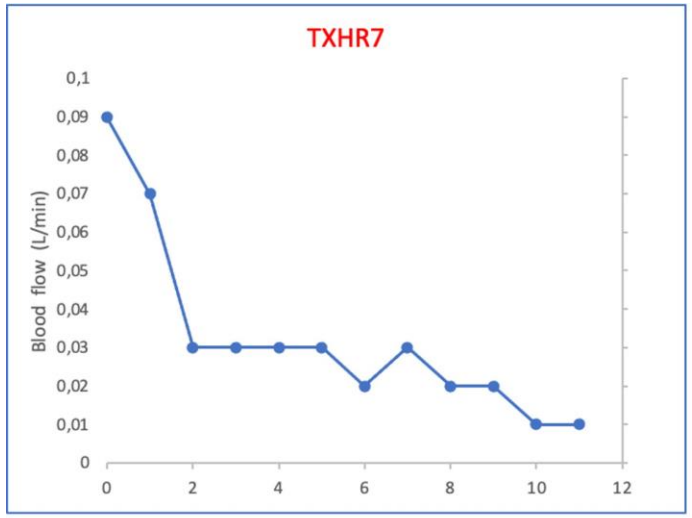
After 12 days in vivo



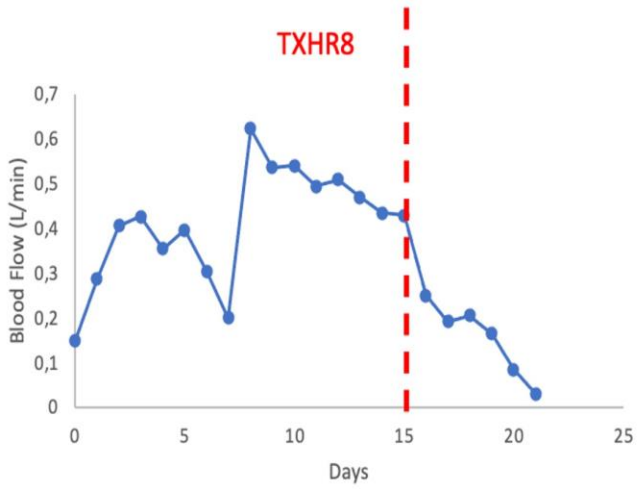


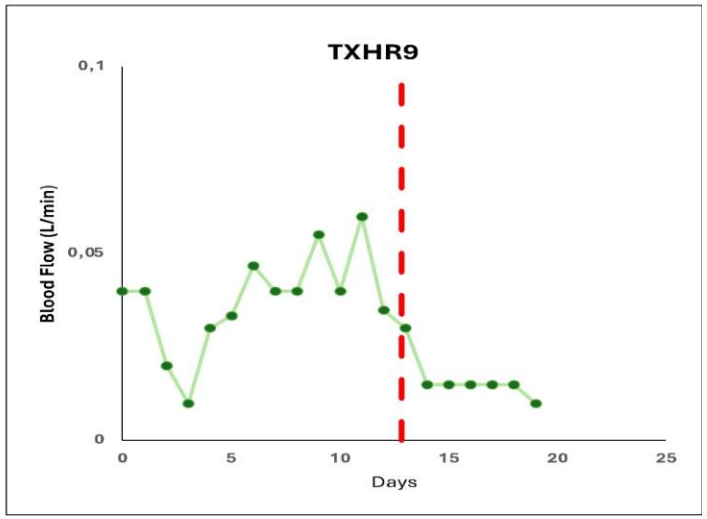


HÍGADOS DE BIOINGENIERÍA TRASPLANTADOS EN CERDOS

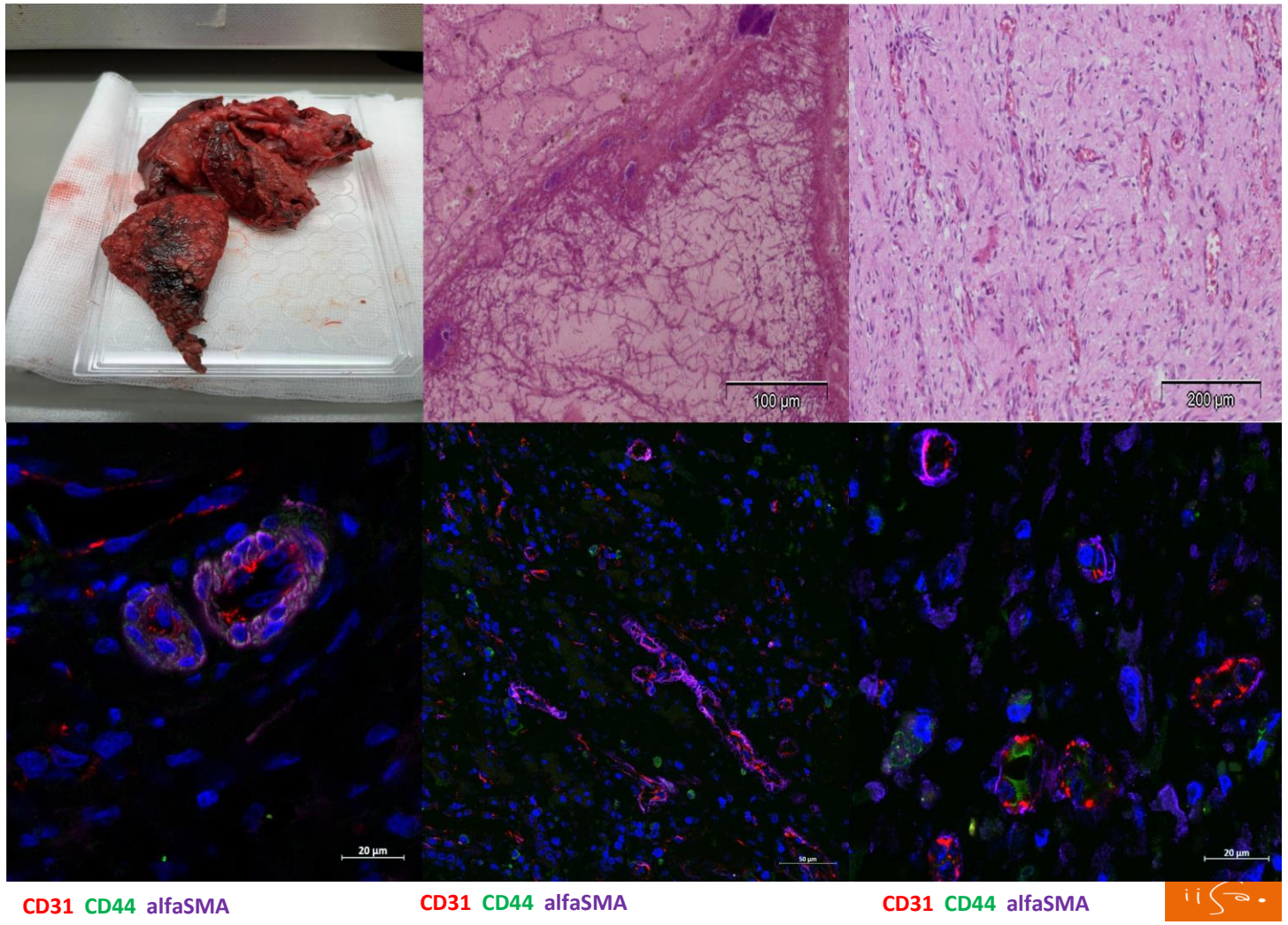


Caída de flujo a las dos semanas…



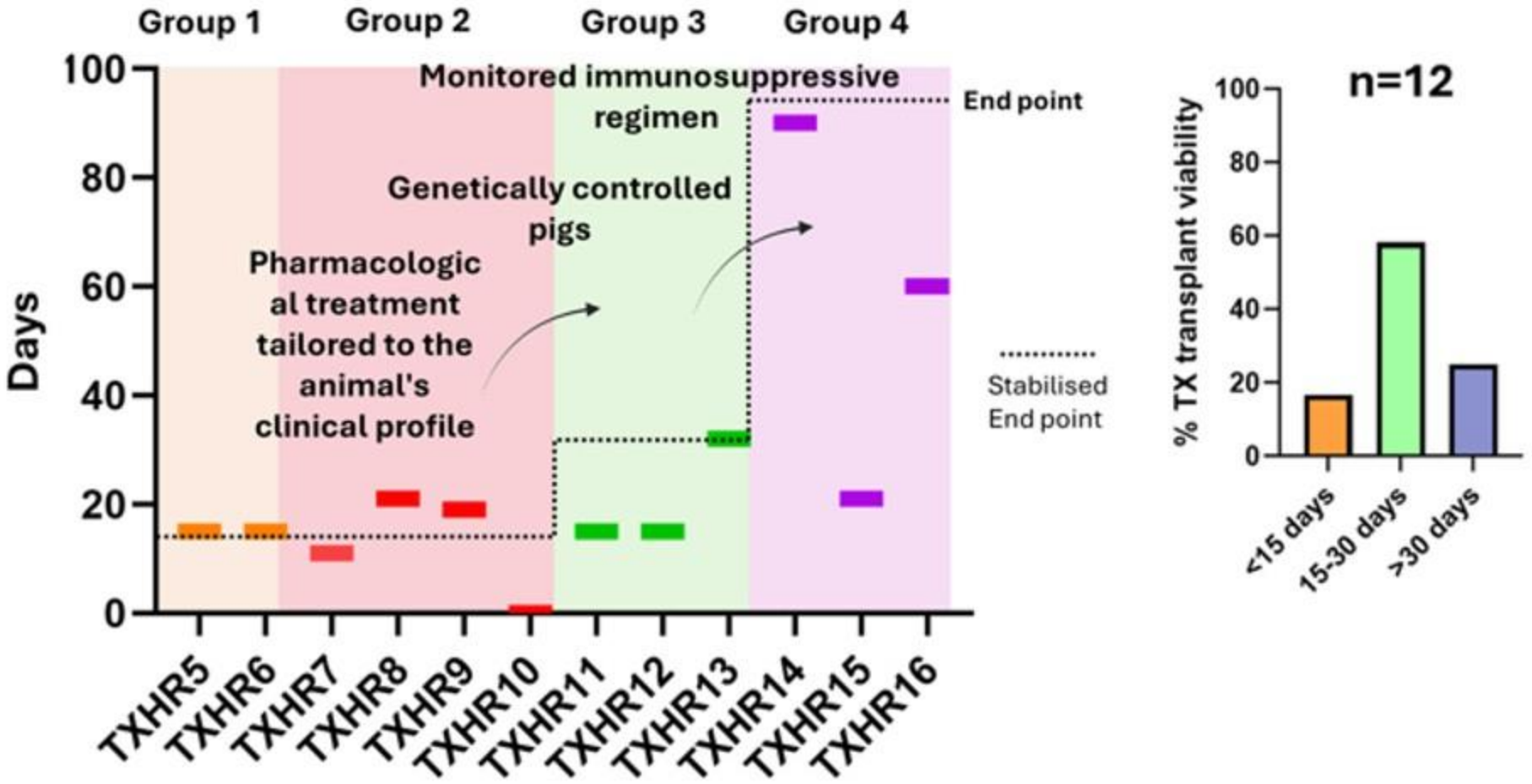


HÍGADOS DE BIOINGENIERÍA TRASPLANTADOS EN CERDOS



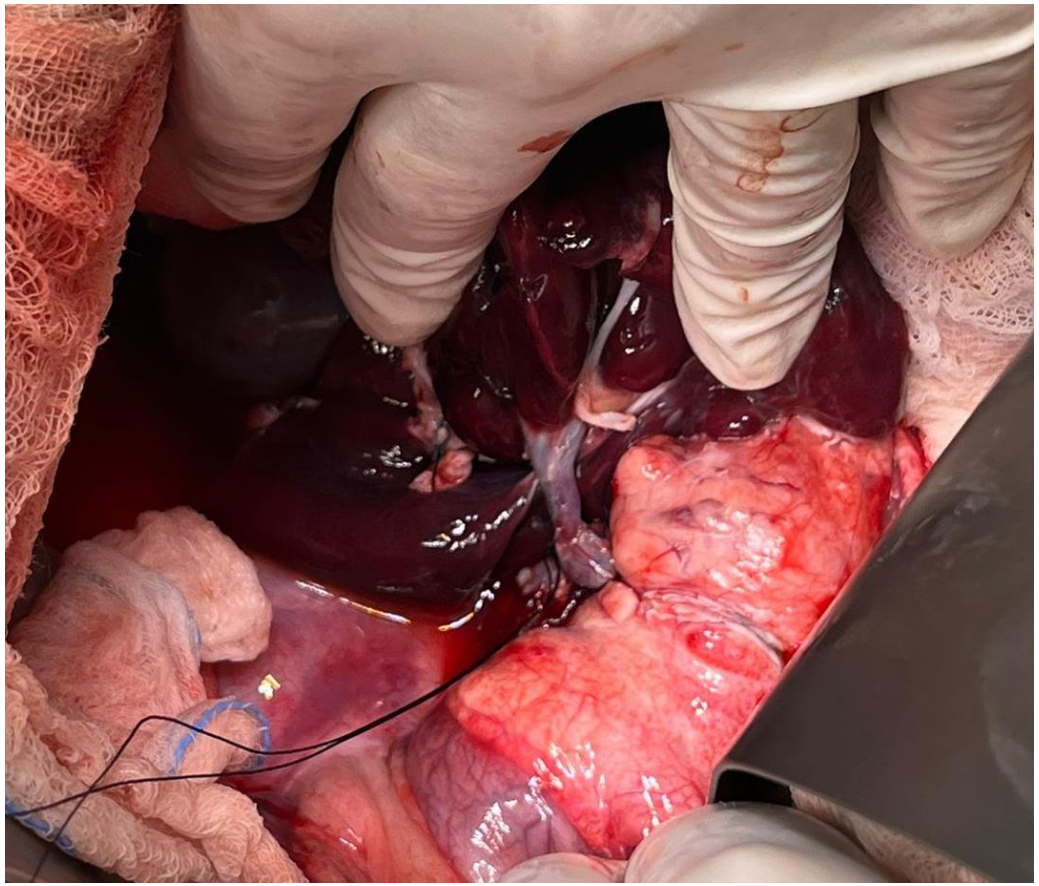
- Necrosis
- Hemorragia
- Infiltrado inflamatorio (Rechazo)

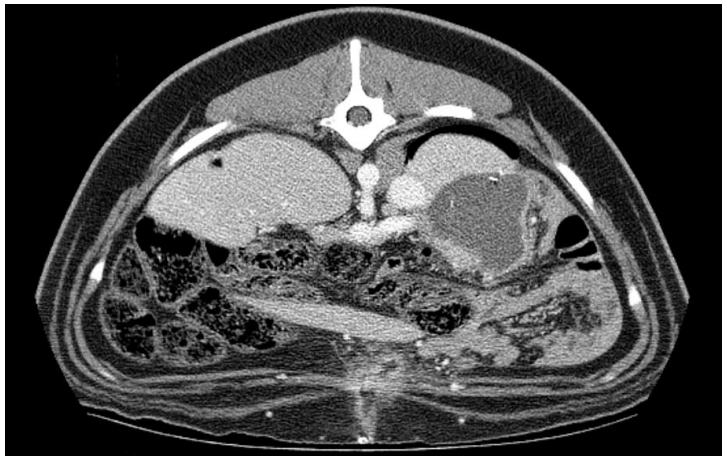
- Persistencia de estructuras vasculares


Group 1
Group 2
Group 3
Group 4
Monitored immunosuppressive regimen
End point
Genetically controlled pigs
Pharmacologic al treatment tailored to the animal's clinical profile
Stabilised End point
Days
100
80
60
40
20
0
TXHR5
TXHR6
TXHR7
TXHR8
TXHR9
TXHR10
TXHR11
TXHR12
TXHR13
TXHR14
TXHR15
TXHR16

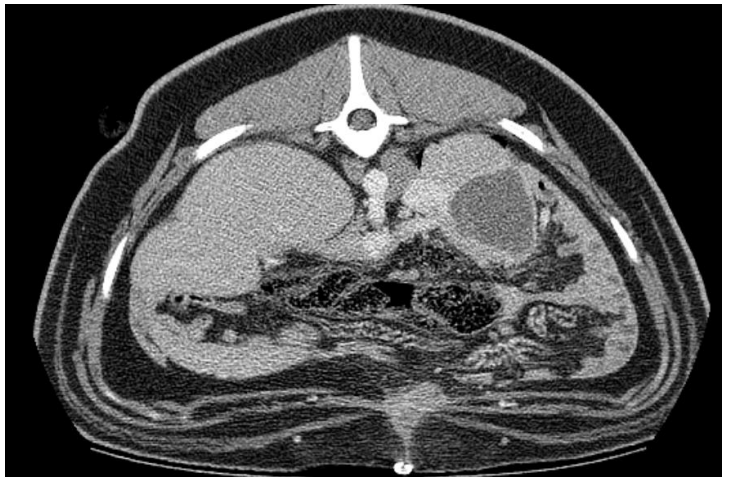
n=12
% TX transplant viability
100
80
60
40
20
0
<15 days
15-30 days
>30 days

TXHR17 (22/09/2025)



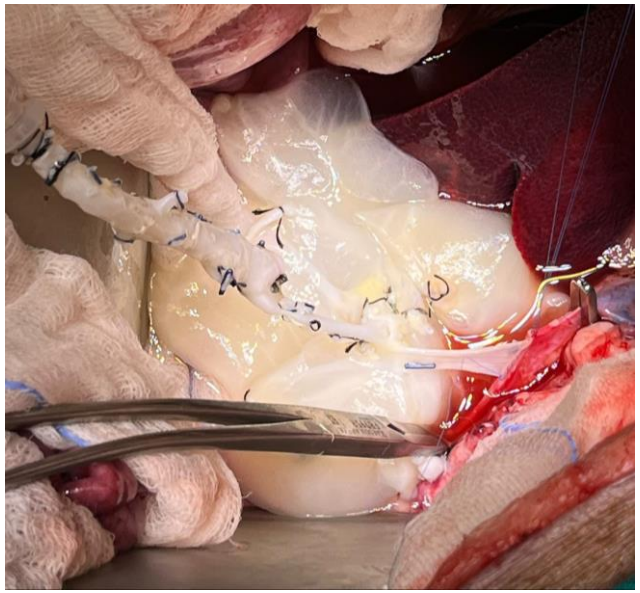


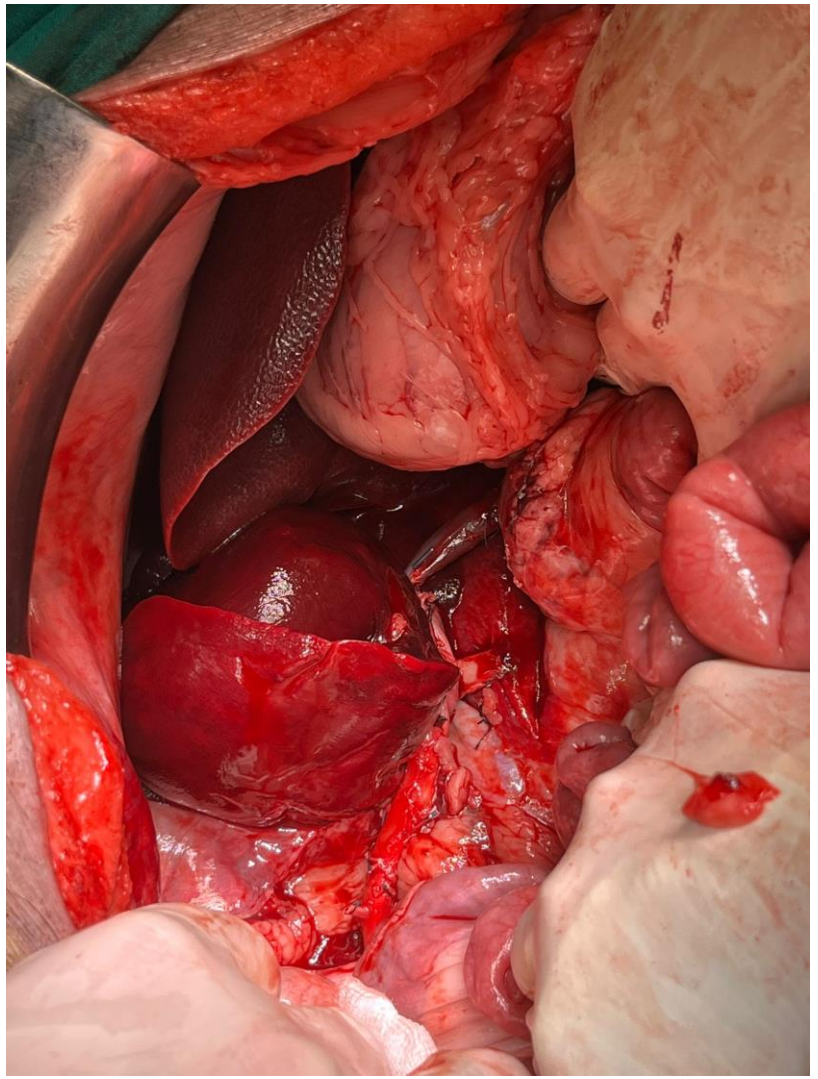
8 DPO (30/9/2025)

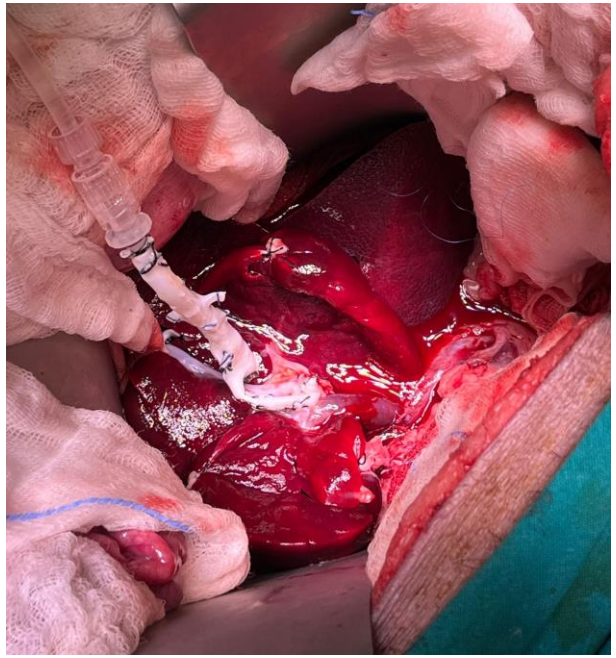


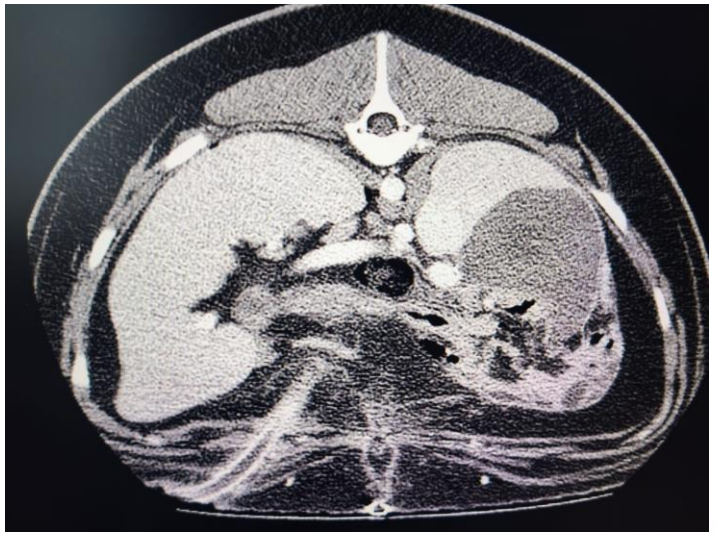
16 DPO (8/10/2025)

MANEJO QUIRÚRGICO



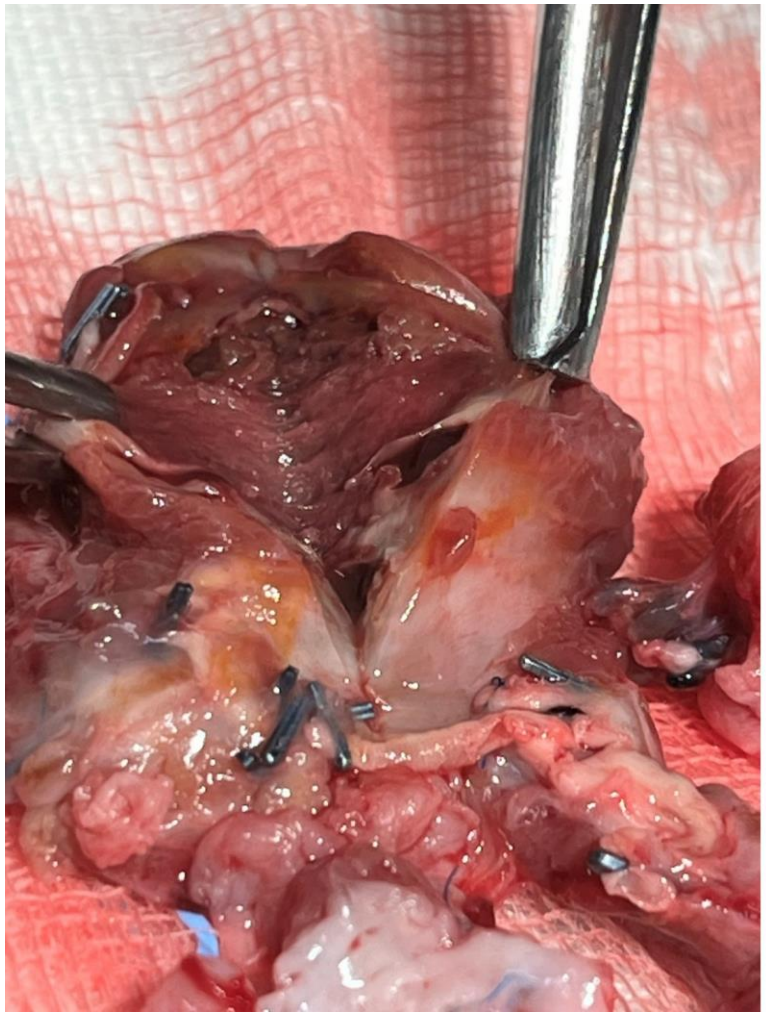


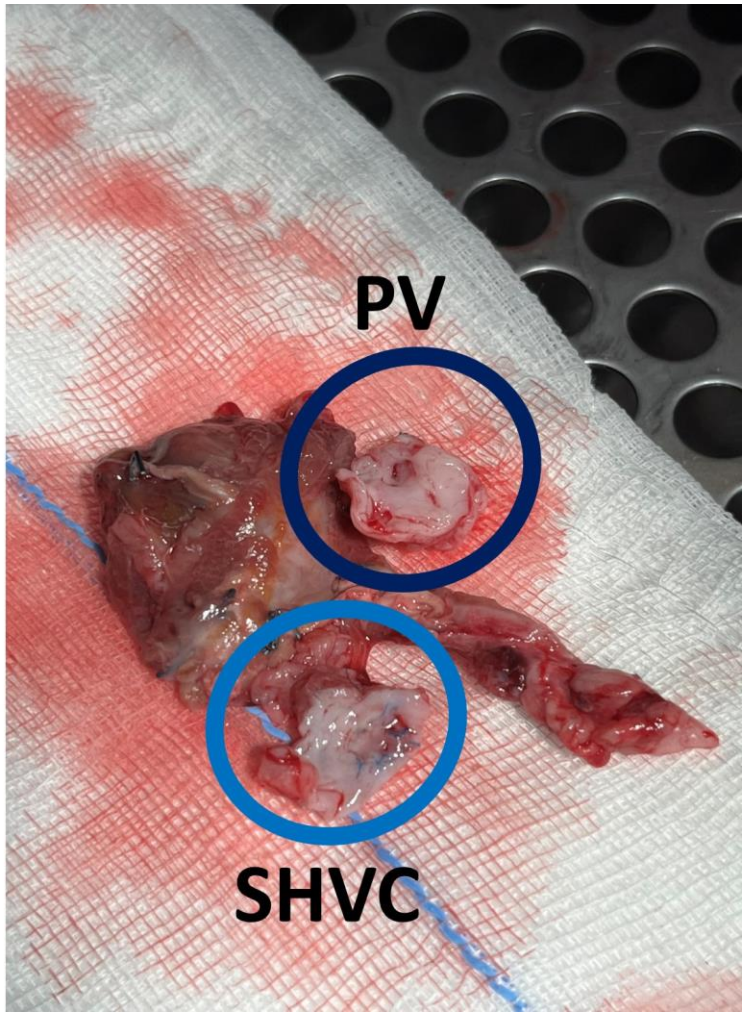




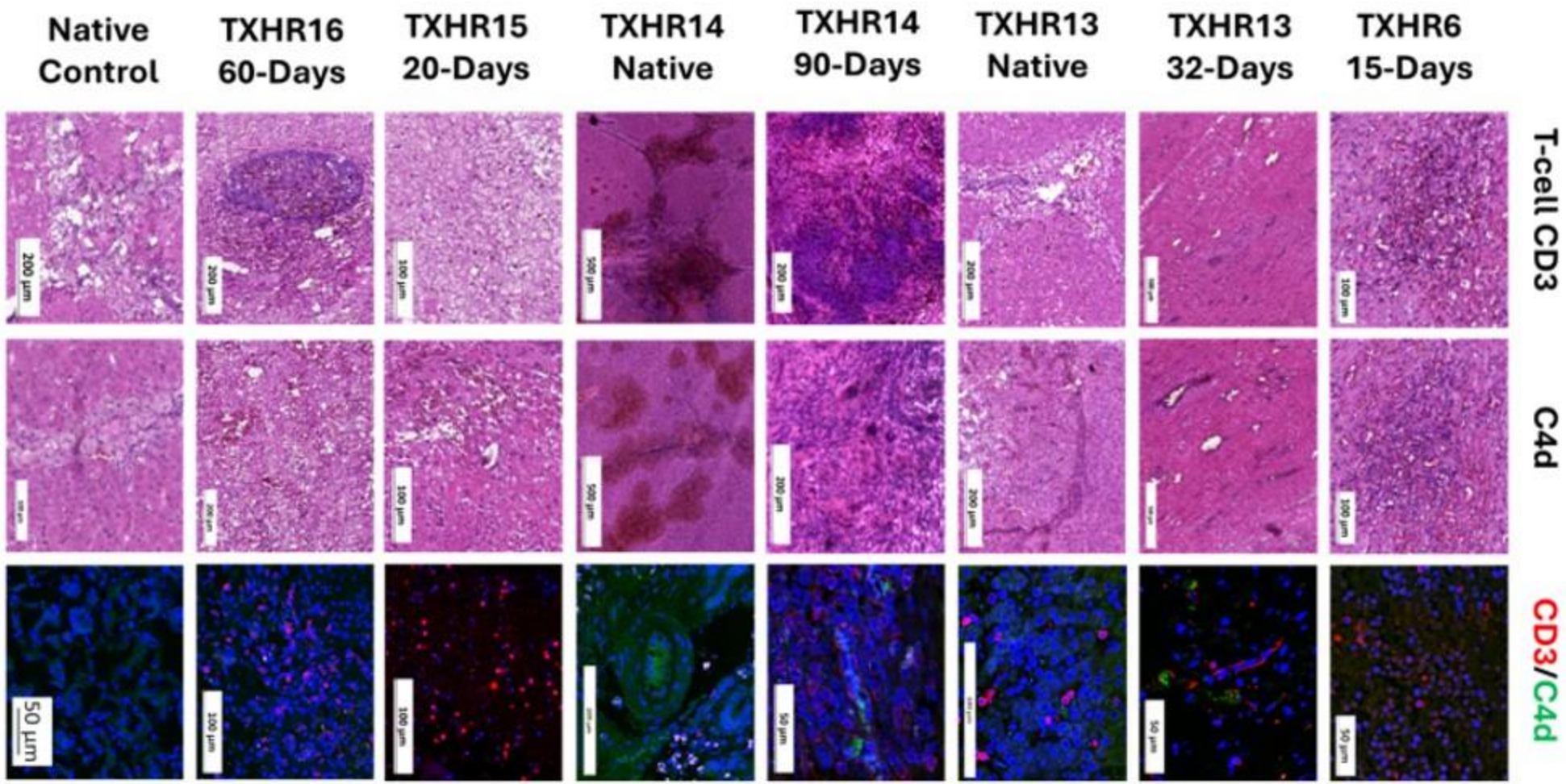
HÍGADOS DE BIOINGENIERÍA TRASPLANTADOS EN CERDOS

TXHR13: Después de 32 día “in vivo” (el punto final fue por una neumonía)

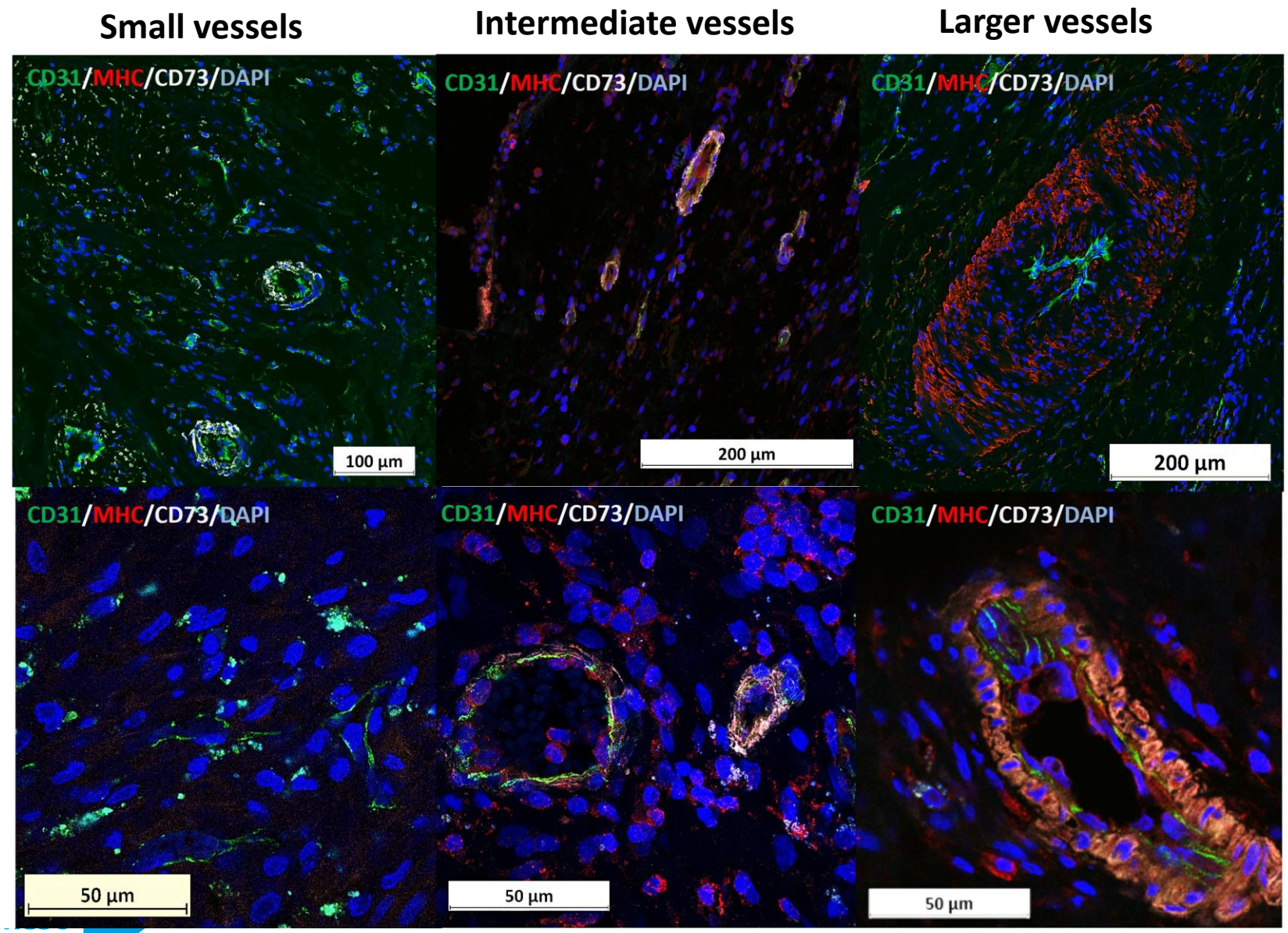




Seguimos viendo signos de rechazo….



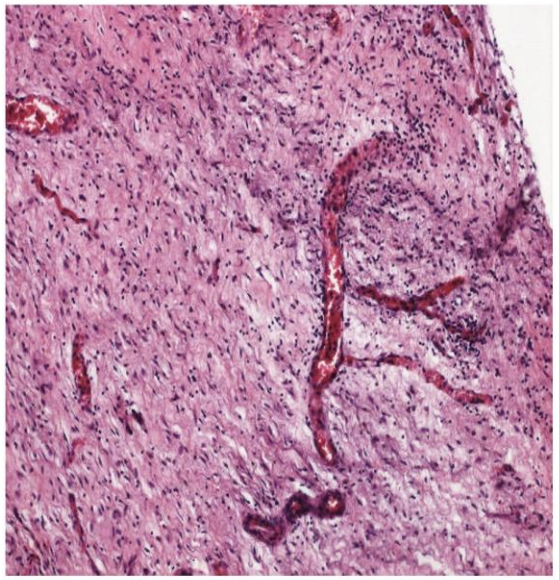
HÍGADOS DE BIOINGENIERÍA TRASPLANTADOS EN CERDOS



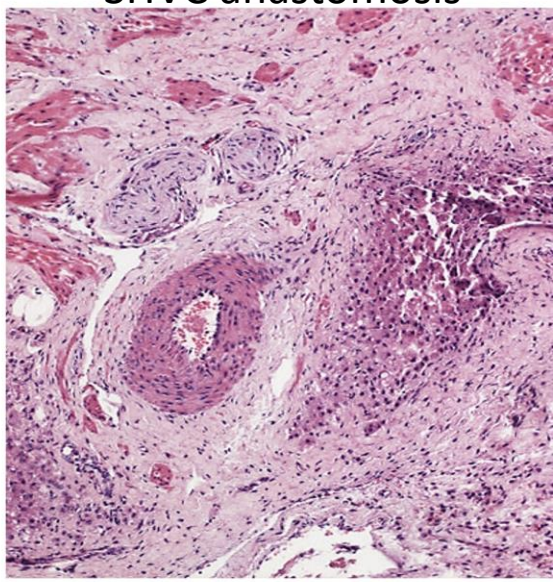
- Persistencia de estructuras vasculares

HÍGADOS DE BIOINGENIERÍA TRASPLANTADOS EN CERDOS

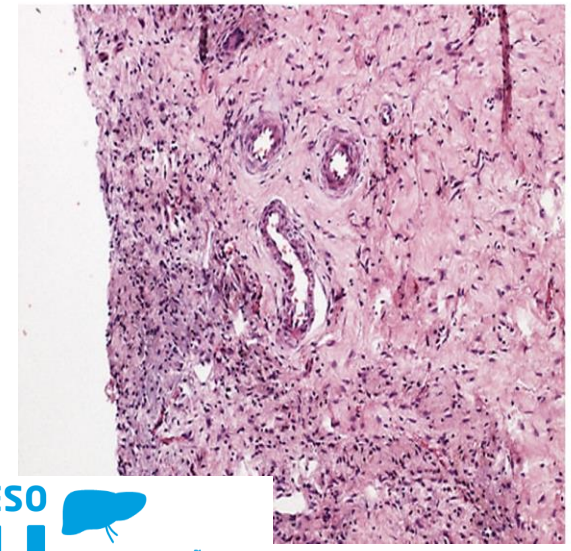
HA Scaffold



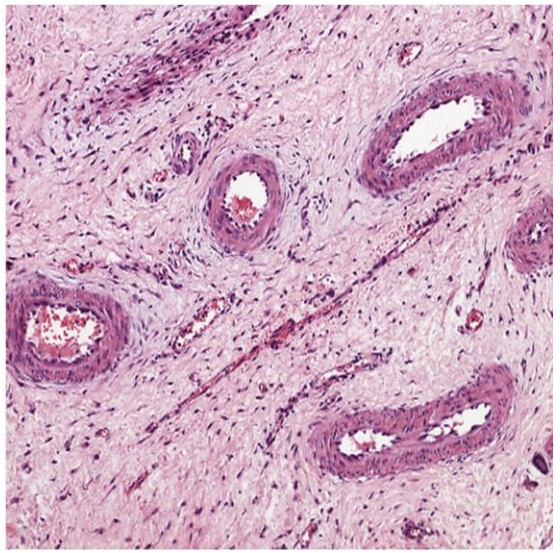
SHVC anastomosis



Parenchyma



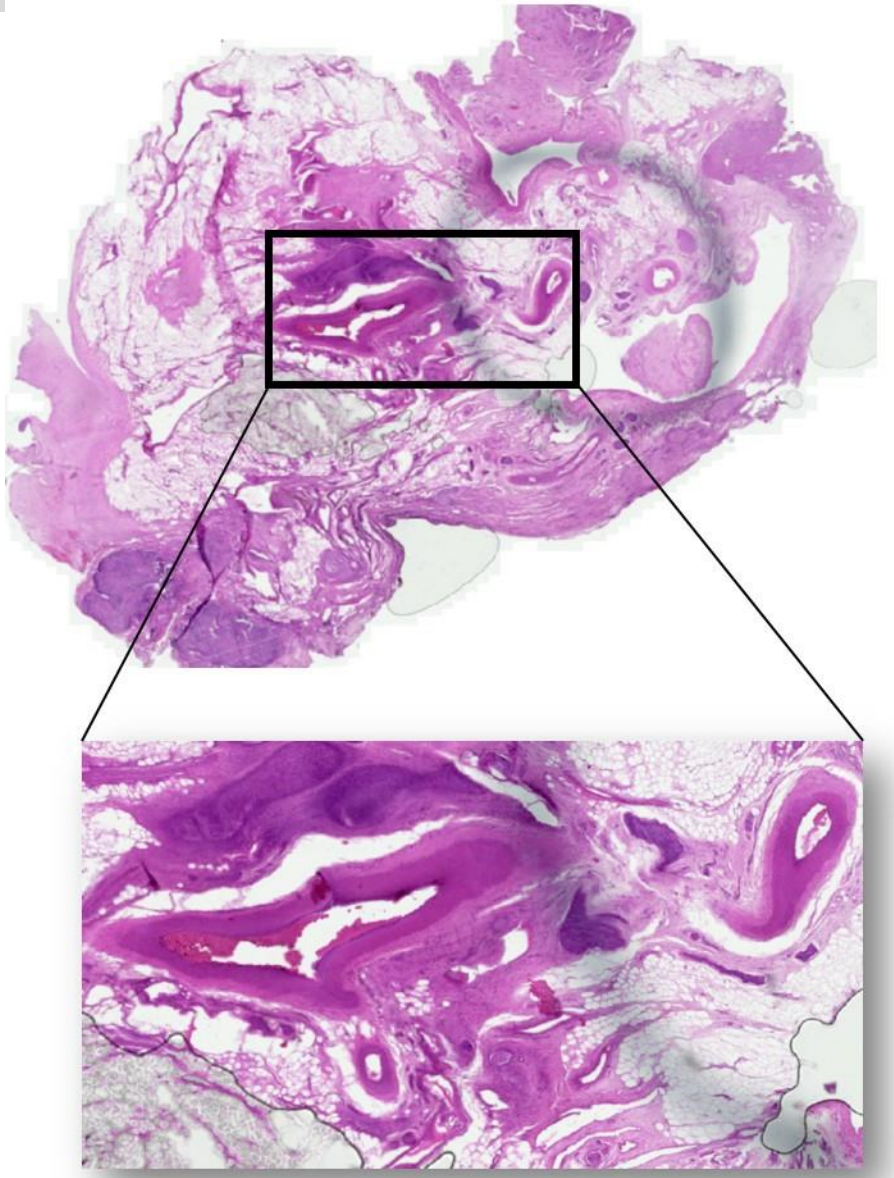
PV anastomosis

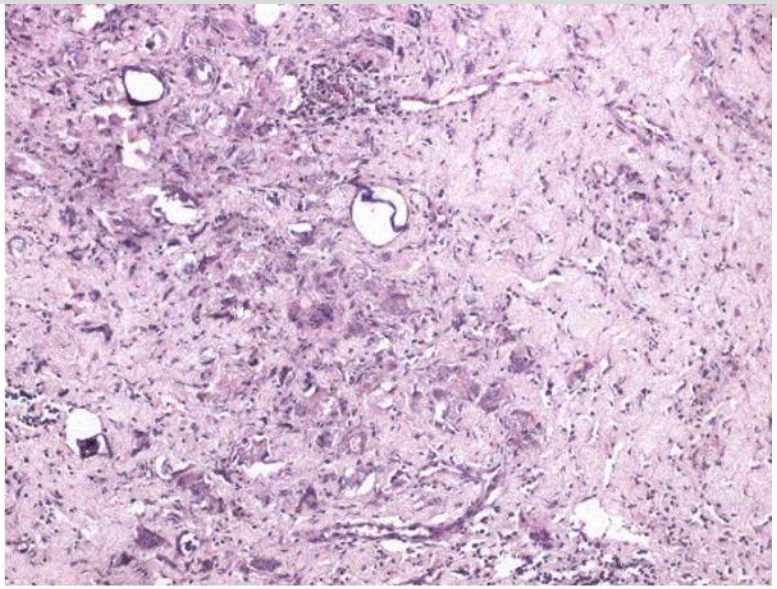


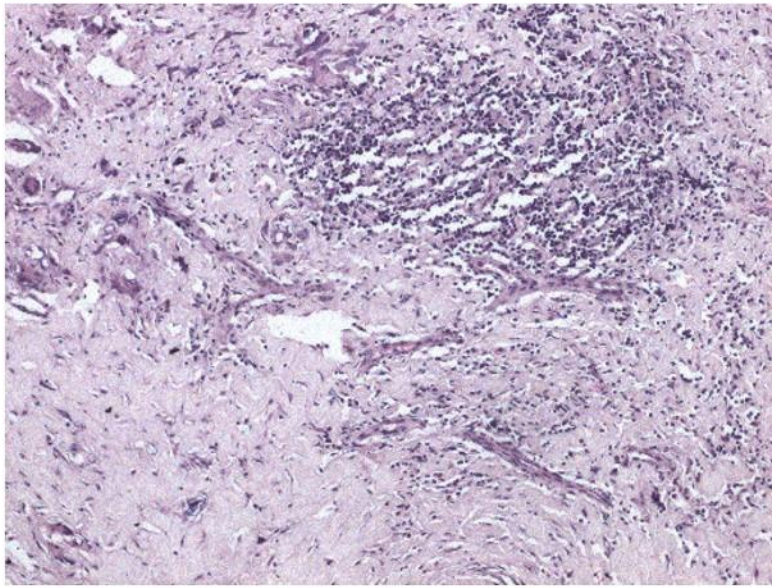
Tacrolimus
Micofenolato
Prednisona

Ciclosporina 7mg/Kg cada 12 horas
(monitorizar en torno a 300ng/ml)
Micofenolato 500mg / 24h
Prednisona 2mg/Kg/24h

HÍGADOS DE BIOINGENIERÍA TRASPLANTADOS EN CERDOS







RESUMEN Y PUNTOS CLAVE ACTUALES

- **Control de la inmunosupresión y el rechazo.**
- **Generación de una gran cantidad de progenitores hepáticos y otras células.**
- **Manejo clínico del postoperatorio a largo plazo (control de infecciones, alergias y medicación)**
- **Mejora de los biorreactores para la maduración hepatica.**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