第30回　神奈川移植医学会

（２０１９年６月８日、神奈川）

「ブタの体内でヒトの臓器を発育させる」

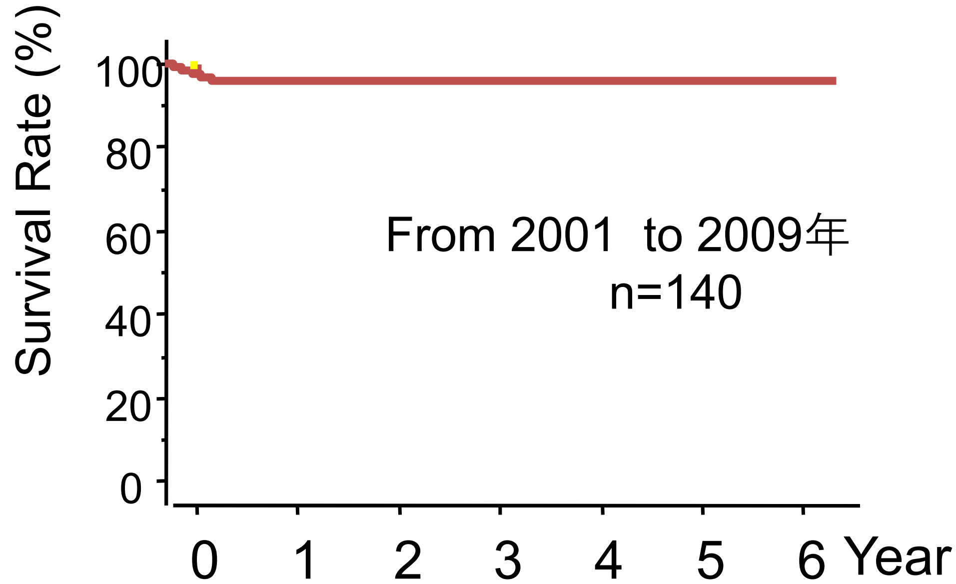
Human Cell-Organogenesis in the Pig

Keio University

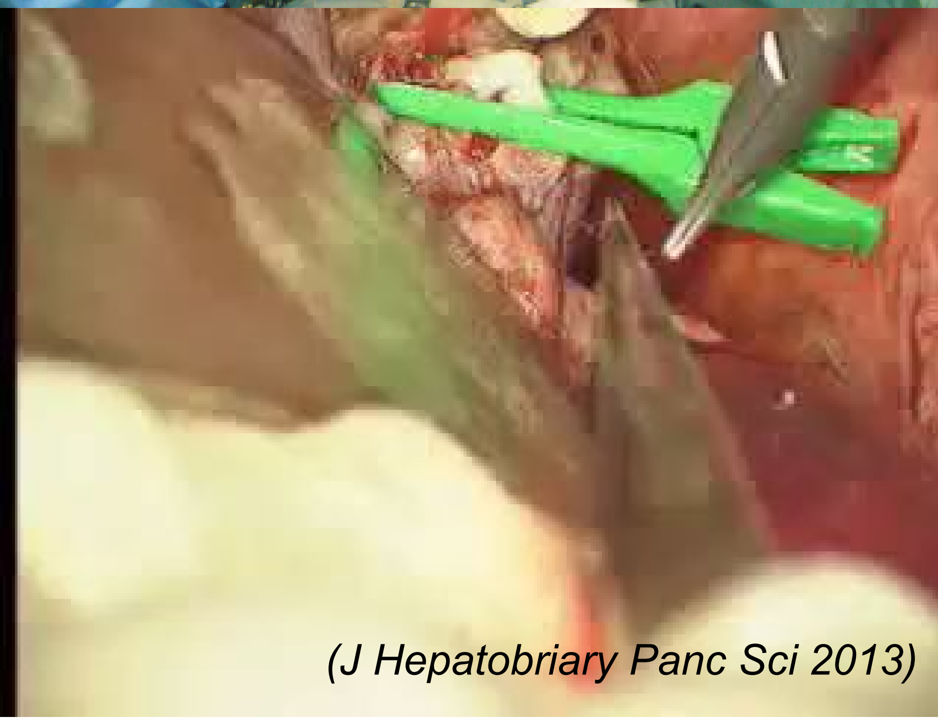
Eiji Kobayashi, MD, PhD
Department of Organ Fabrication,
Keio University School of Medicine, Japan

COI:シスメックス、スクリーン、バイオス
アステラス(2009-2011)

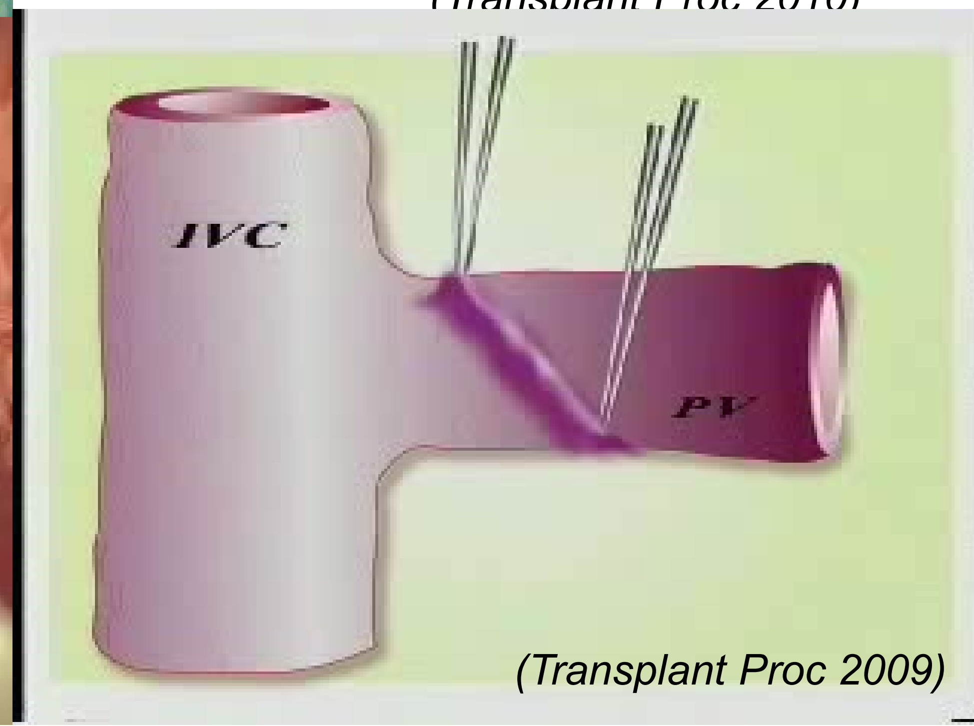
Clinical Microsurgery in JMU



(Transplant Proc 2010)



(J Hepatobriary Panc Sci 2013)

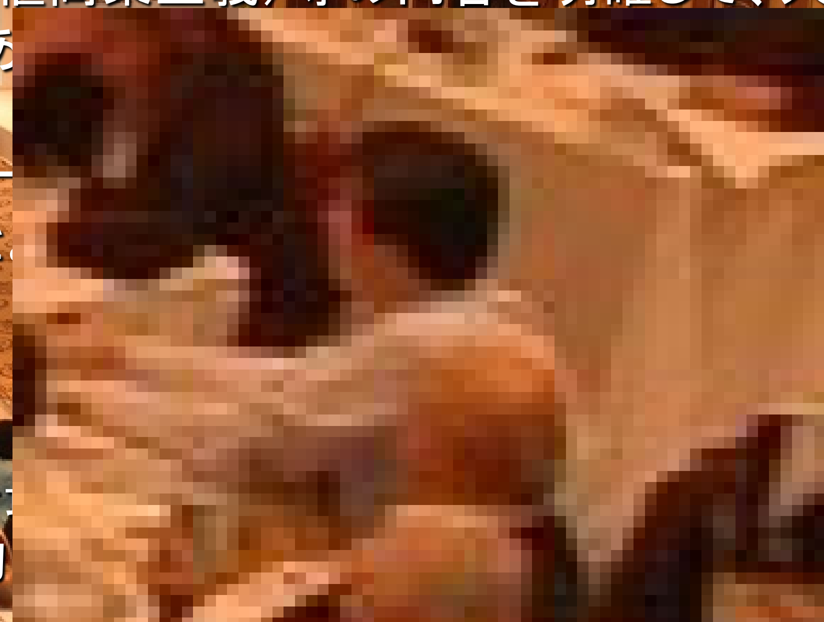


(Transplant Proc 2009)

イスタンブール宣言

(Istanbul, 30th April – 3rd May 2008)

1. Organ trafficking（臓器取引）、Transplant tourism（移植ツーリズム）、Transplant commercialism（移植商業主義）等の内容を明確して、人道的、社会的、国際的に問題があ



2. 死体（脳死、心停止）ドナー　を増やすよう呼びかけることと

3. 生体ドナーは、ドナー保護を　的な保障等の制度を国家的

152 professionals from 78 countries

（Ｌａｎｃｅｔ　２００８年７月６日）

The Transplantation Society


The National Diet
（6 Sep ,2009）
参考人
自治医科大学先端医療技術開発センター
先端治療開発部門客員教授
小林 英司

NHKスペシャル　2009

人体“製造”

—再生医療の衝撃—

「移植可能な臓器作り」

戦略分類	小動物による プルーフ･オフ･コンセプト実験	大型動物でのPOC
（1） **丸ごと使う** **（動物工場）**	膵（*Kobayashi T, et al. Cell 2010*） 膵（*Yamaguchi T, et al. Nature 2017*） 腎（*Usui J, et al. Am J Path 2012*） 肝（*Hata T, et al. Ann Surg 2013*）*	膵（*Matsunari H, et al. PNAS 2013*） 肝（*Fisher JE, et al. Liver Transplant 2013*） 肝（*Hsu H, et al Transplant Proc 2017*）* 豚ヒトキメラ（*Wu J, et al Cell 2017*） **ヒト血管**（*Ito M, et al. Nature Comm 2019*）*
（2） **発生原器を使う** **（胎仔原器グラフト）**	腎（*Matsumoto K, et al. Stem Cells 2012*）* 腎（*Mae S, et al Nature Communi 2013*） 腎（*Taguchi A, et al . Cell Stem Cell 2014*） 肝（*Takebe T, et al. Nature 2013*））	膵（*Hammerman M, et al. Organogenesis 2012*） 腎（*Yokote S, et al. PNAS 2015*）*
（3）**Vitroでの再構築** **（脱細胞充填）** **（組織再構築）** **（Ex vivo再構築）**	心（*Ott HC, et al. Nature Med 2008*） 腎（*Ross EA, et al. JASN 2009*） 肝（*Uygun BE, et al. Nature Med 2010*） 肺（*Ott HC, et al. Nature Med 2010*） 心（*Sekine H, et al. Nature Com 2013*）*	腎（*Orland G, et al. Ann Surg 2012*） 肝（*Yagi H, et al. Cell Transplant 2012*） 心（*Kitahara H, et al. Interact Cardio Thrac Surg 2016*） **脾臓、骨髄を含め多種臓器** （*Kobayashi E, et al. unpublished*）*

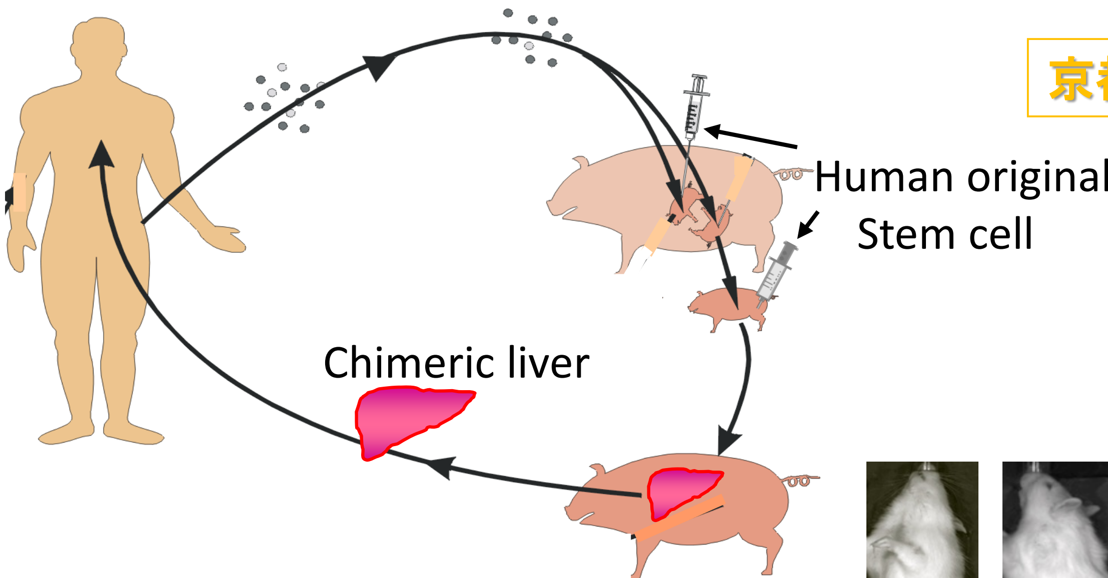
＊著者ら

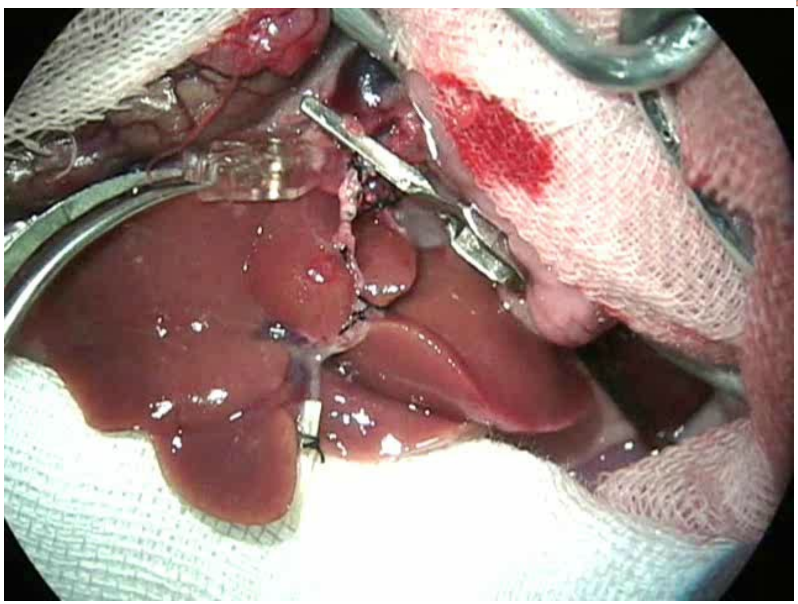
（2013年内閣府生命倫理委員会資料　小林英司 より改編）

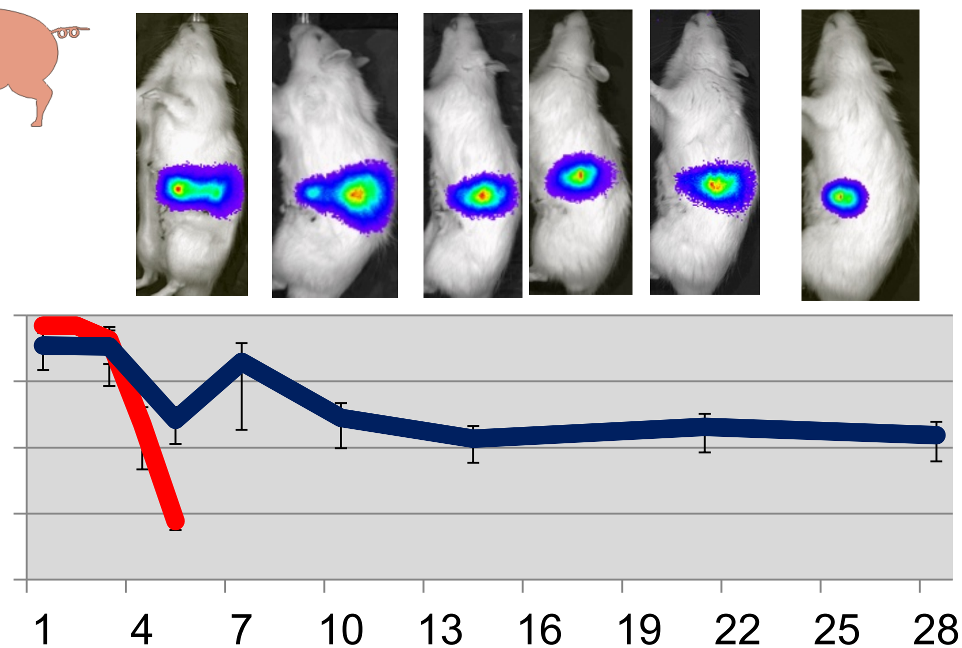
戦略(1)

Creating the human liver in Pigs
動物の体内でヒトの臓器を作る

京都大学　畑先生、上本教授

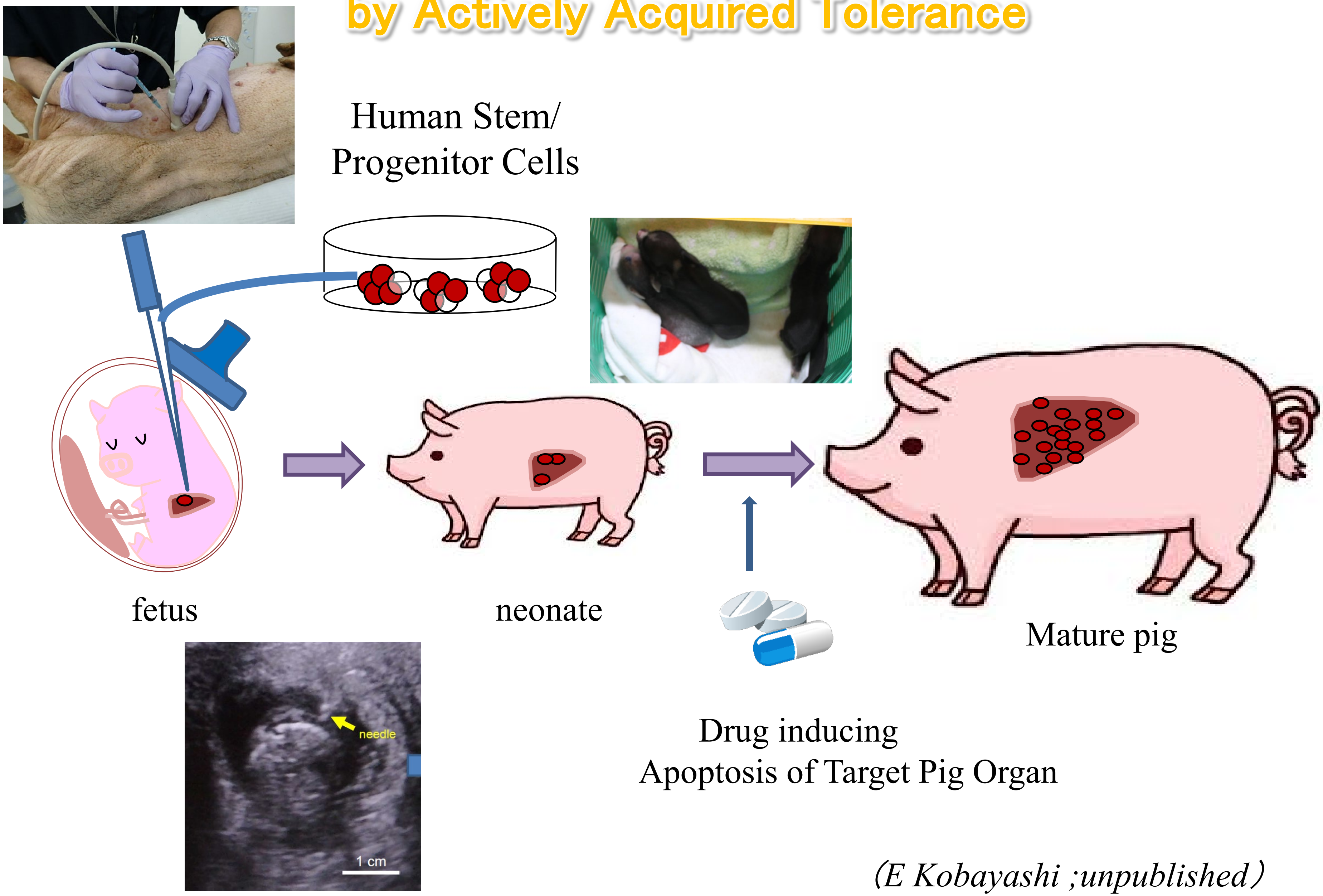




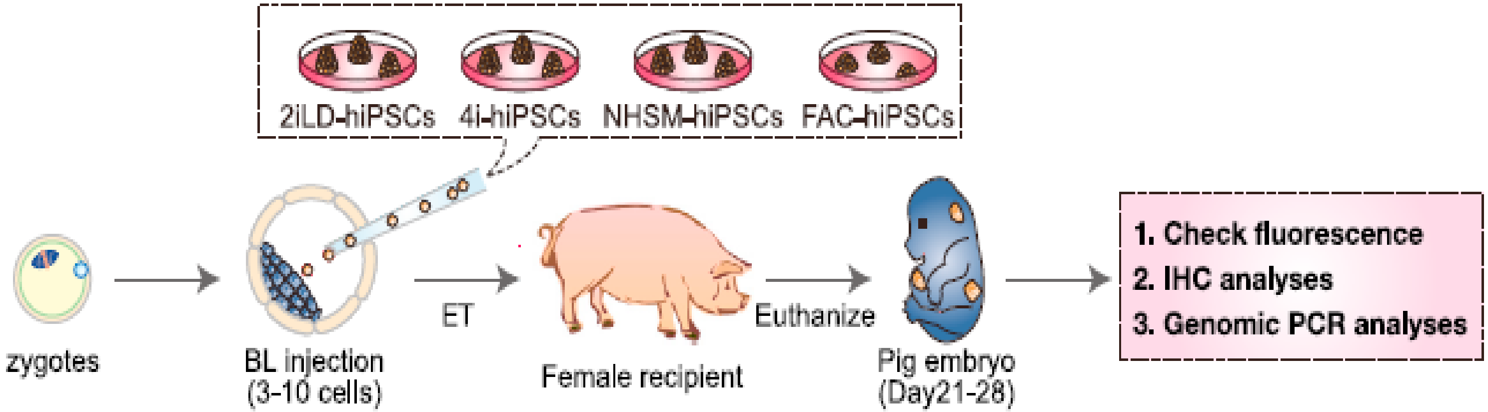


(Hata T, et al. Ann Surg 2013)

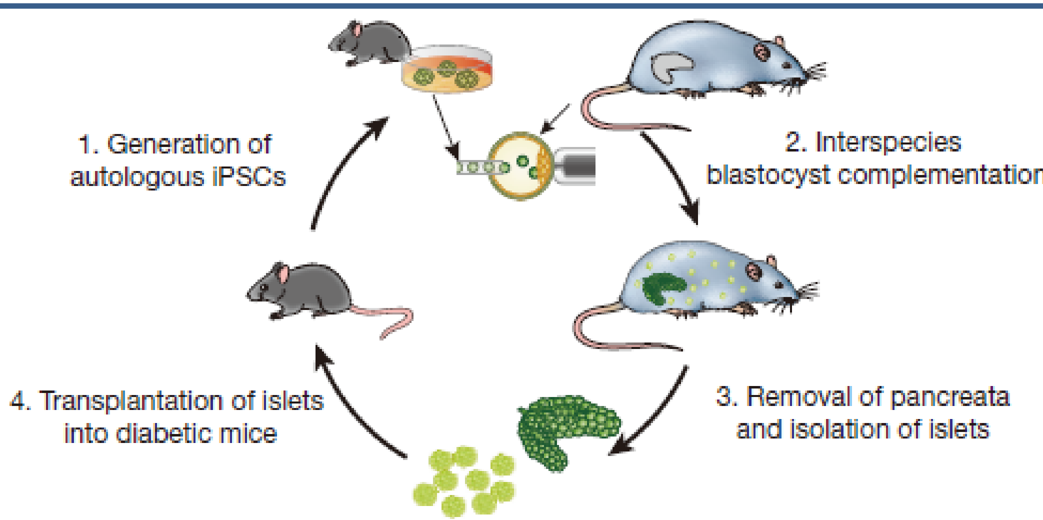
Project of Production of Pigs with Human Organ by Actively Acquired Tolerance



In the heat of argument, Pig as In vivo bioreactor for human organs

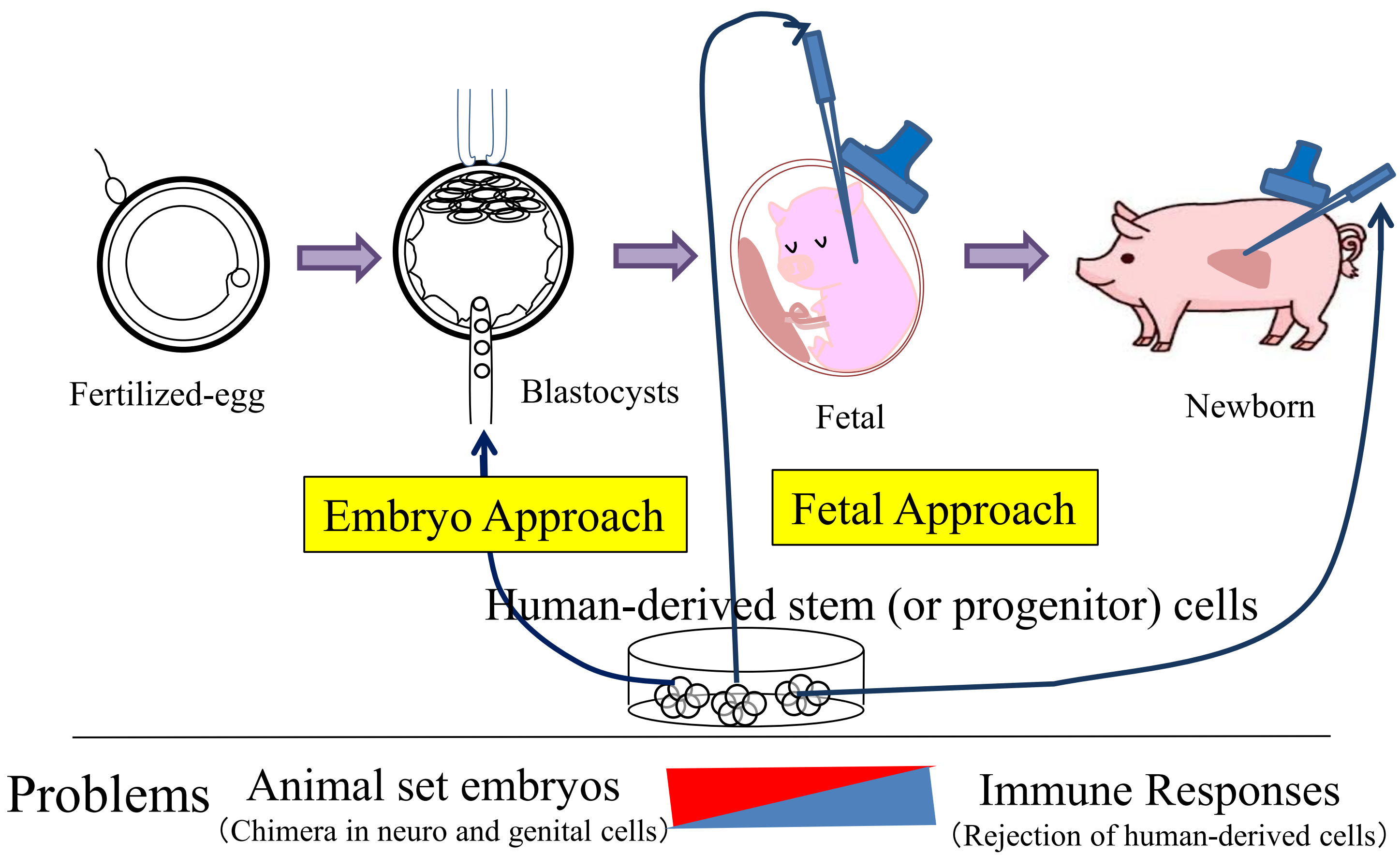


(Wu J, et al. Cell 2017)



(Yamaguchi T, et al. Nature2017)

Strategies for human organ development in Pigs



(Kobayashi E, et al. Keio J Med in press)

朝日新聞 DIGITAL

2019年5月22日14時00分

「人間」を拒絶しないブタ　再生医療で移植臓器に期待

　免疫に関わる細胞を作る臓器を取り除き、体外からの異物を拒絶しない状態にしたブタを、慶応大の小林英司・特任教授らが生み出した。体内で人間の臓器を作る再生医療の研究に役立つという。

　21日、英科学誌ネイチャー・コミュニケーションズで発表した。

Development of an immunodeficient pig model allowing long-term accommodation of artificial human vascular tubes

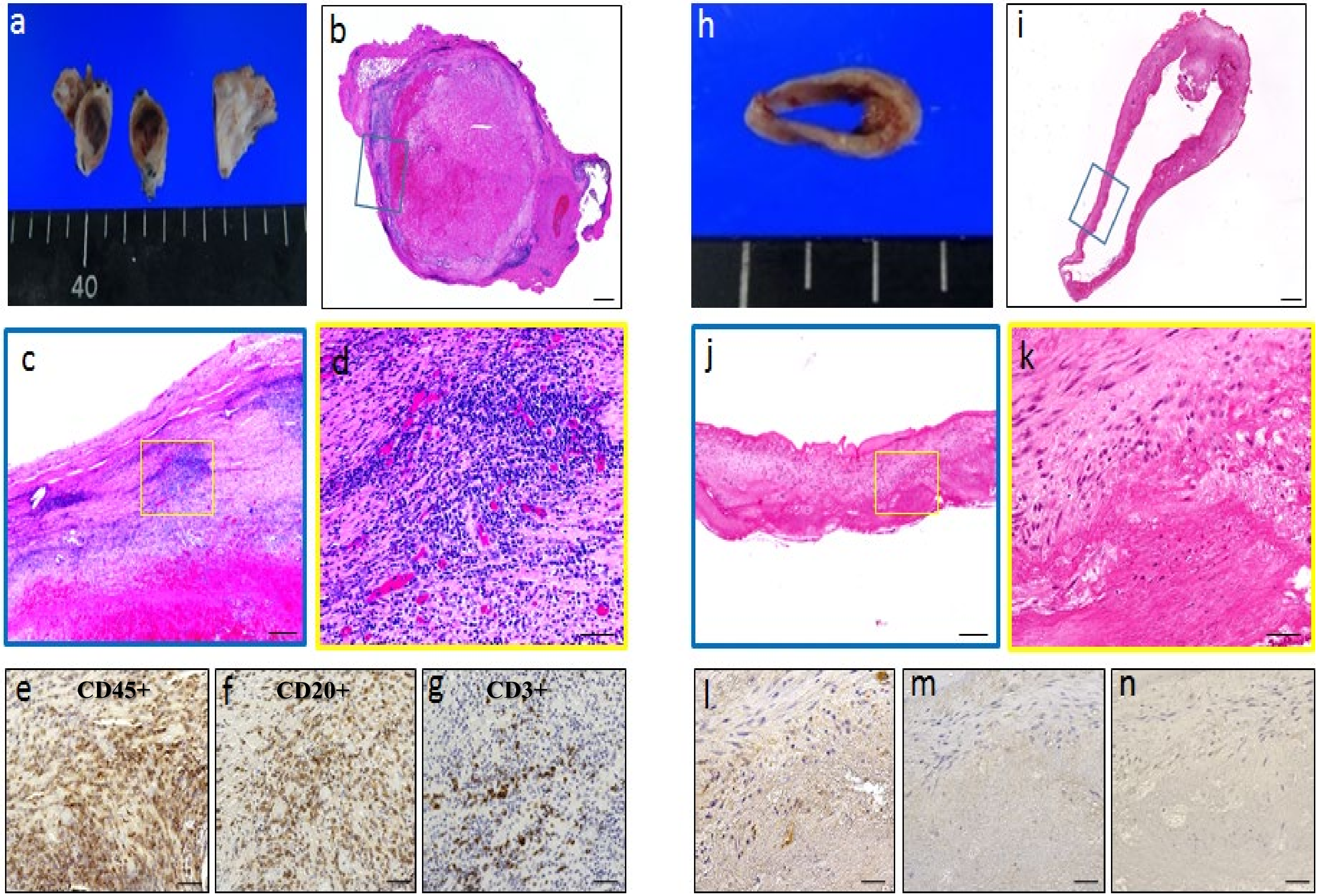


Figure 3. A histological analysis of the patent or rejected HOBPTs at two weeks after transplantation.

(Nat Commun.2019 May 21;10(1):2244.)

Table 1 | Profile of pigs and post-operative immunosuppressant regimens in the various laboratories

Institutes	Nihon Bioresearch Inc. (Reference 4)	National Center for Child Health and Development Axcelead Drug Discovery Partners, Inc	Tokyo Women's Medical University	Kyoto University
Kinds of Pigs	Göttingen mini-pigs Male, 6-7 months, >15 kg	Micro mini-pigs Male and Female, >12 months	Farm animals Female, 3-4 months, 20-25 kg	Clawn mini-pigs Male, 30–35 kg
Tacrolimus (mg/kg/day)	0.5	0.5 - 0.6	1.0	0.5
MMF (mg/kg/day)	60	40–60	60-62	50
Prednisolone (mg/animal/day)	20	20	20	20

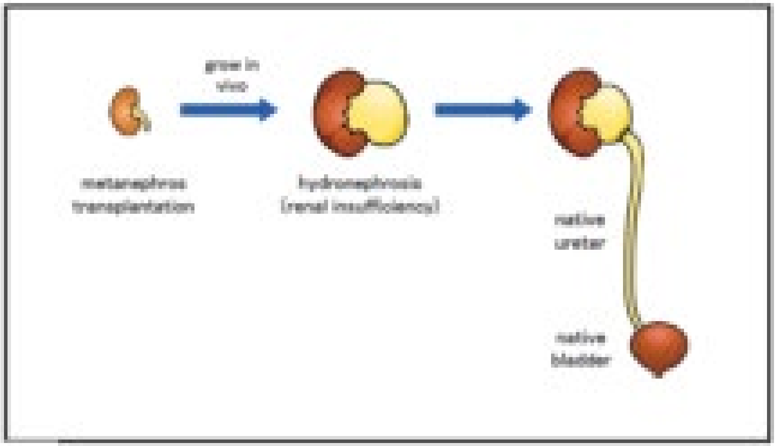
MMF: mycophenolate mofetil

(Kobayashi E, et al. Submitted)

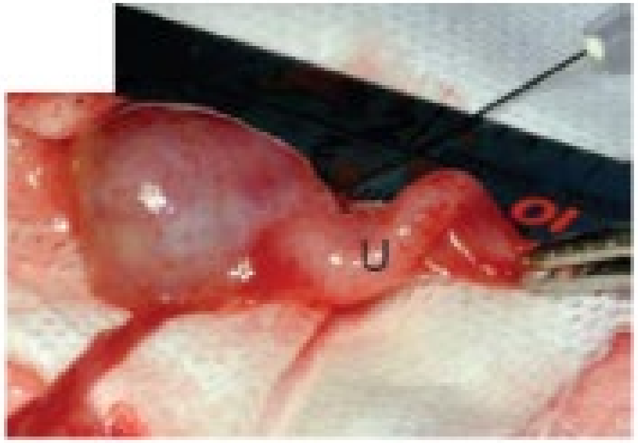
戦略(2)

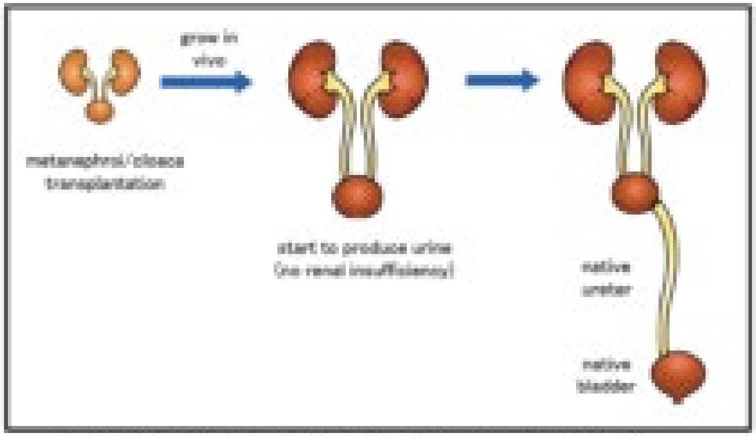
臓器の芽を患者体内で育てる

慈恵医大　横尾教授、 明治大学　長嶋教授

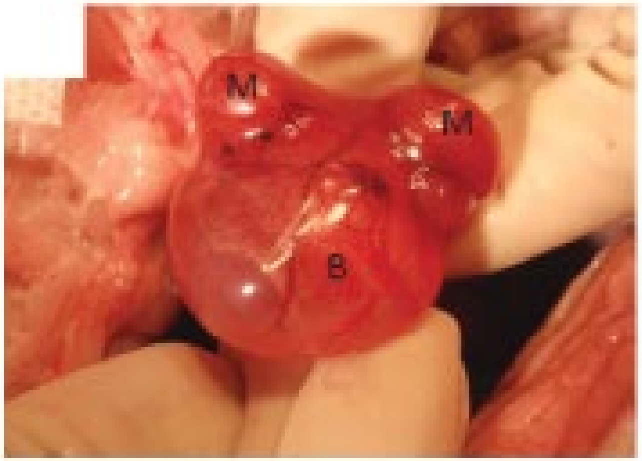


Conventional method





Step-wise peristaltic ureter (SWPU) system

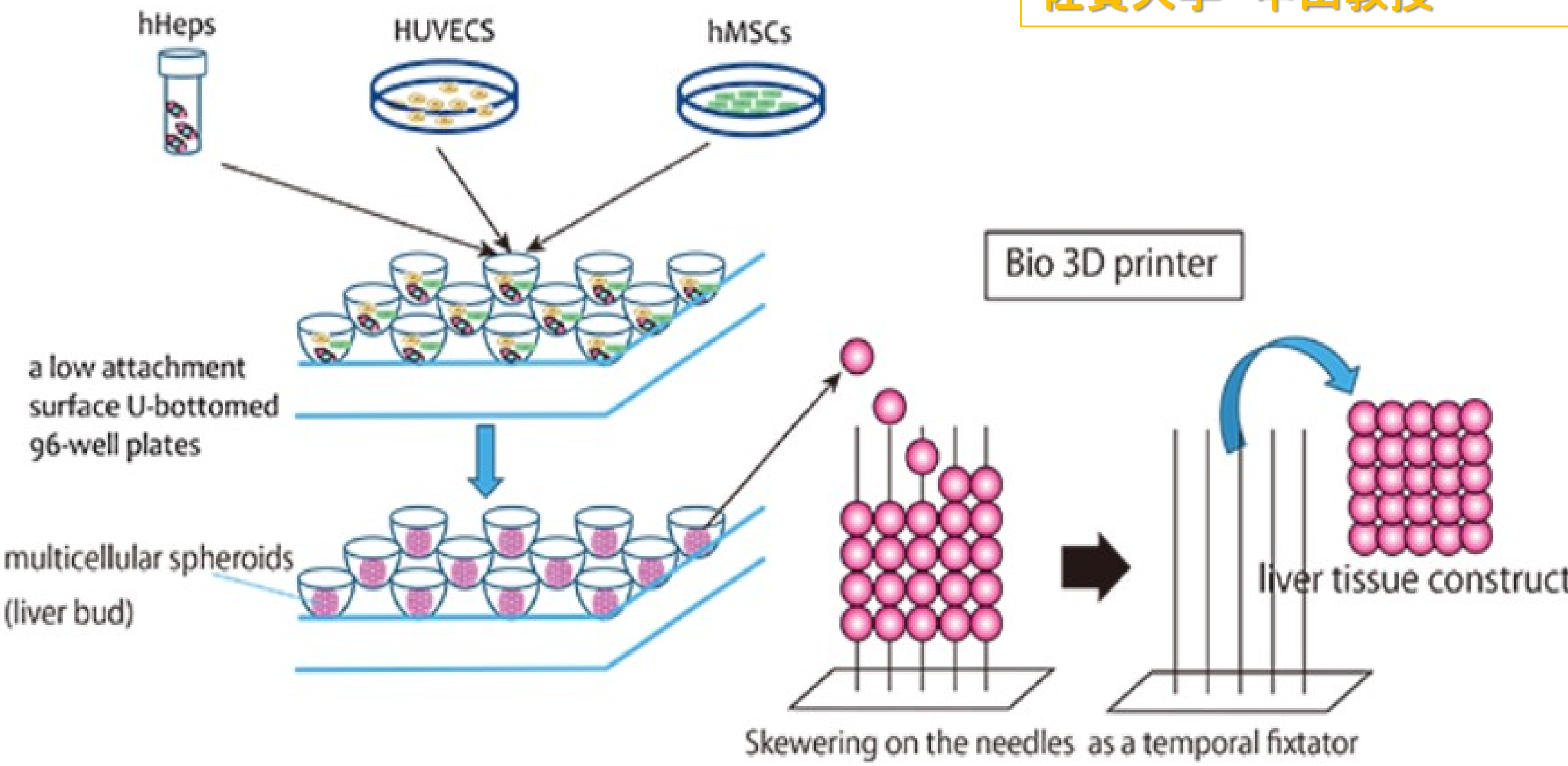


(Yokote T, et al. PNAS 2015　より改変)

戦略(2)

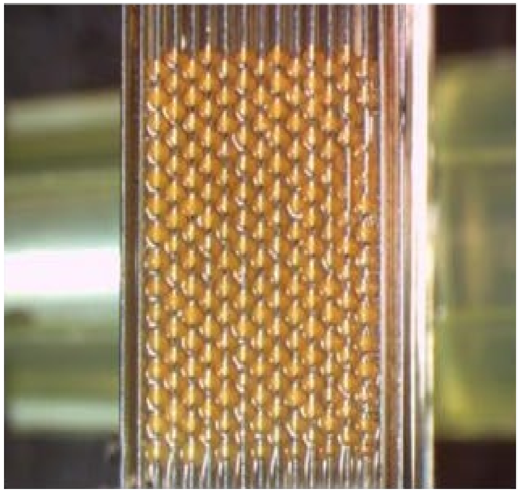
‘3Dプリンターで作った 肝臓の芽’を患者に移植する

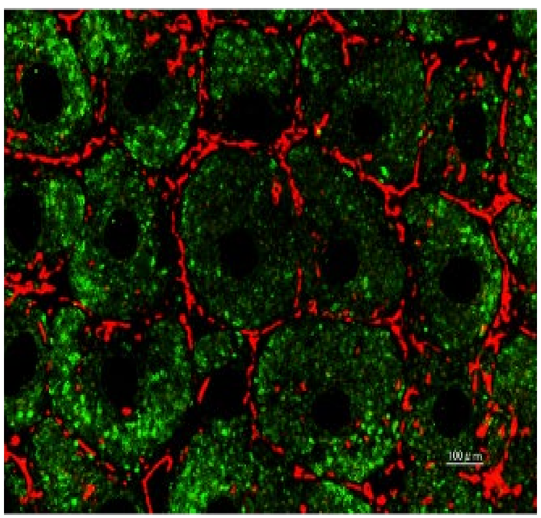
九州大学　柳先生、田口教授
佐賀大学　中山教授



(Yanagi Y, et al. Scientific Report 2017)

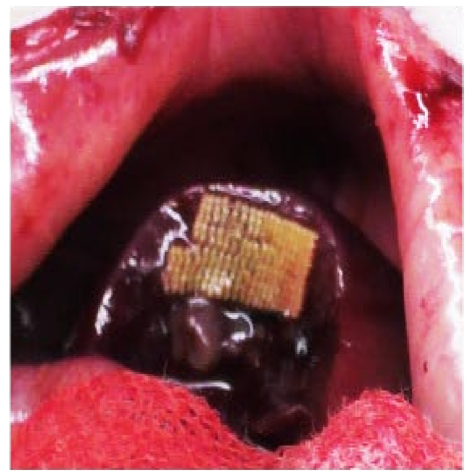
バイオ３Dプリンターで作製したヒト肝臓組織

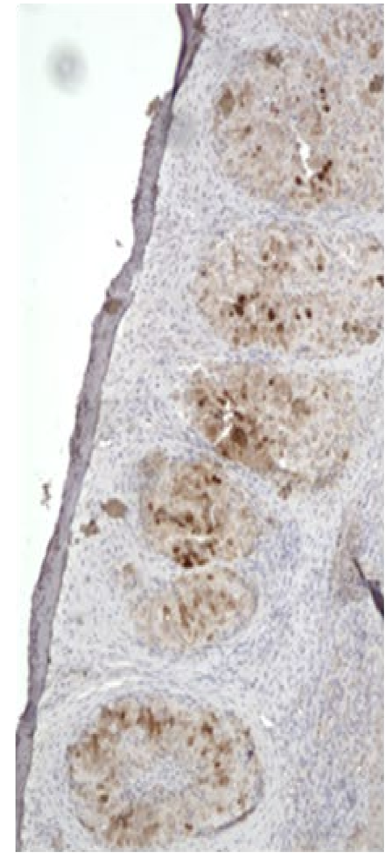




緑ー肝細胞
赤ー血管内皮細胞

ラットの肝臓断端に移植したヒト肝臓組織がヒトアルブミンを産生

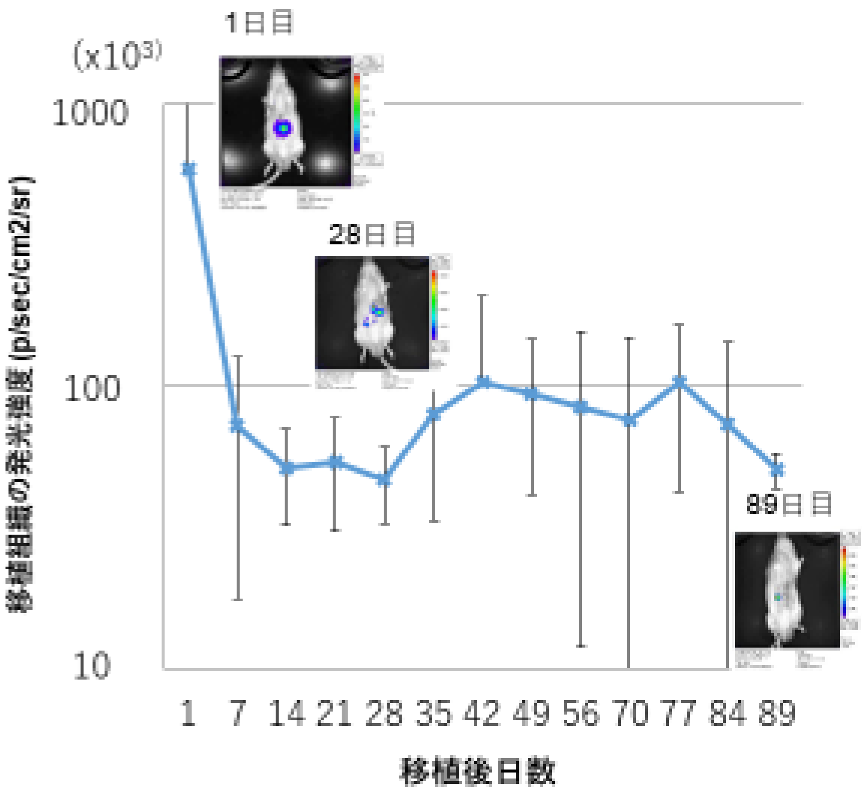




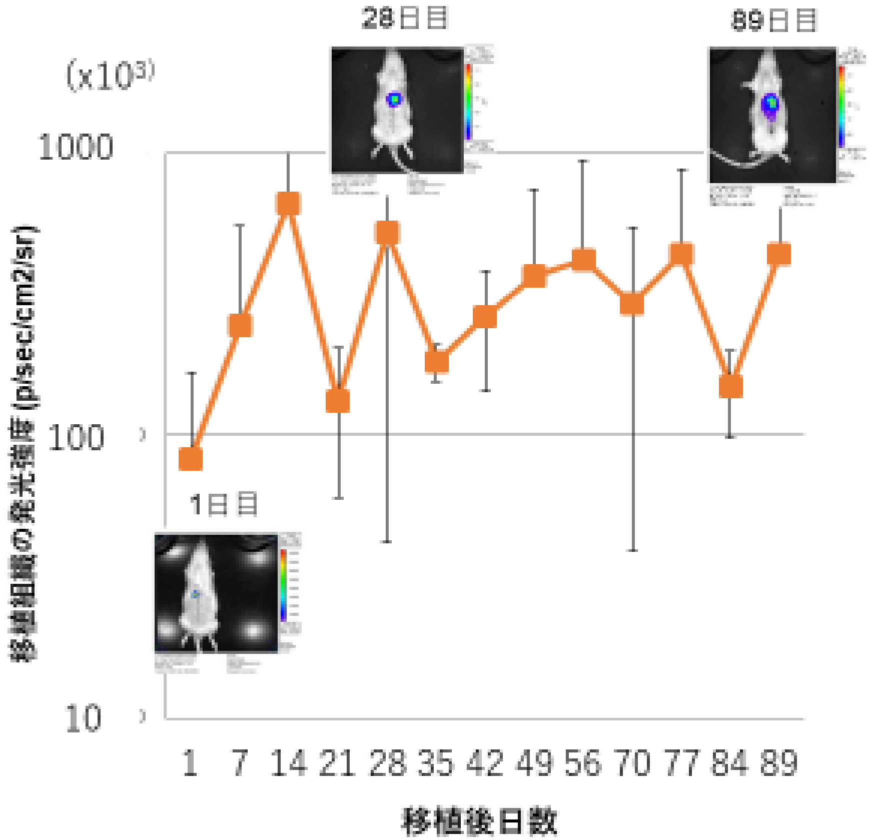
茶－ヒトアルブミンを産生する肝細胞

肝芽は、異所性では育たない

A．腸管膜内移植（異所性移植）



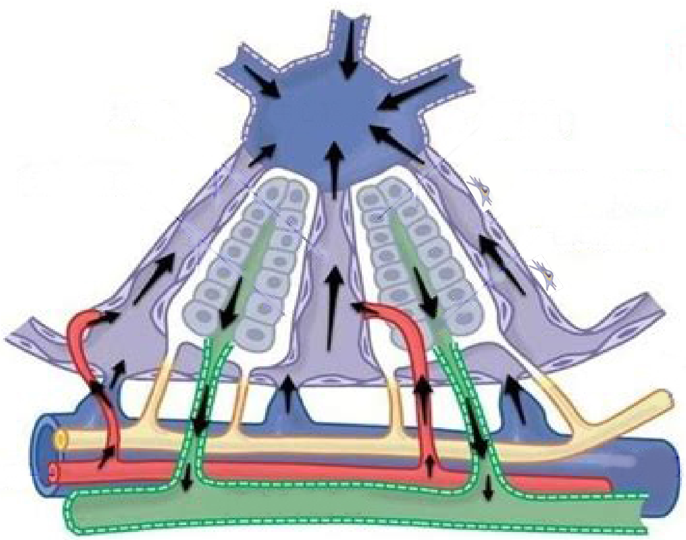
B．断端移植（同所性移植）



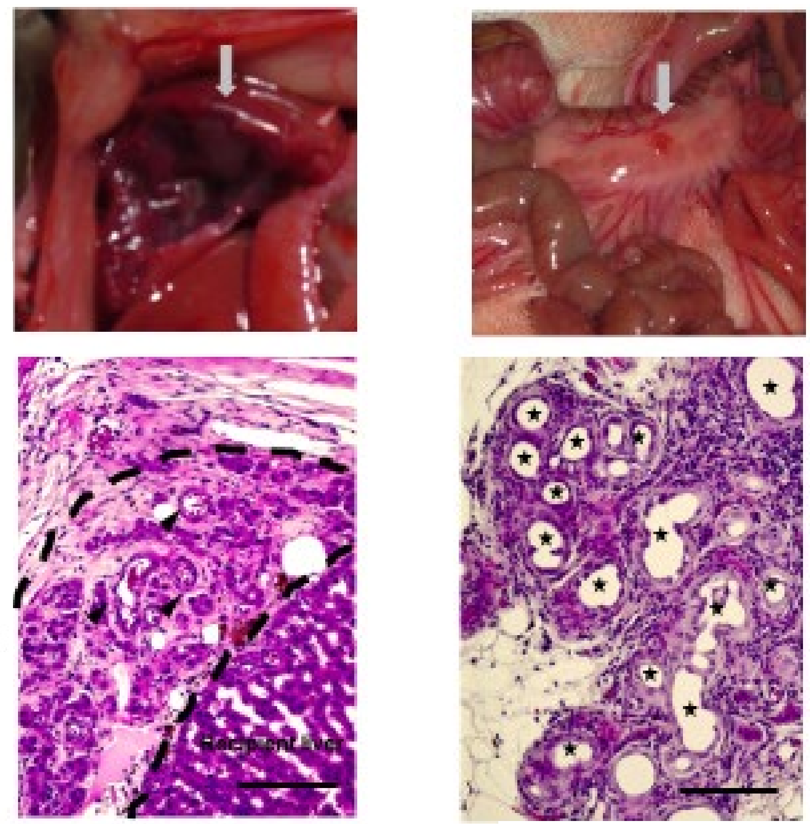
*(Yanagi Y, et al. Scientific Reports 2017*より改変)

なぜ肝芽が異所では育たないか？

肝臓維持に必要な解剖学



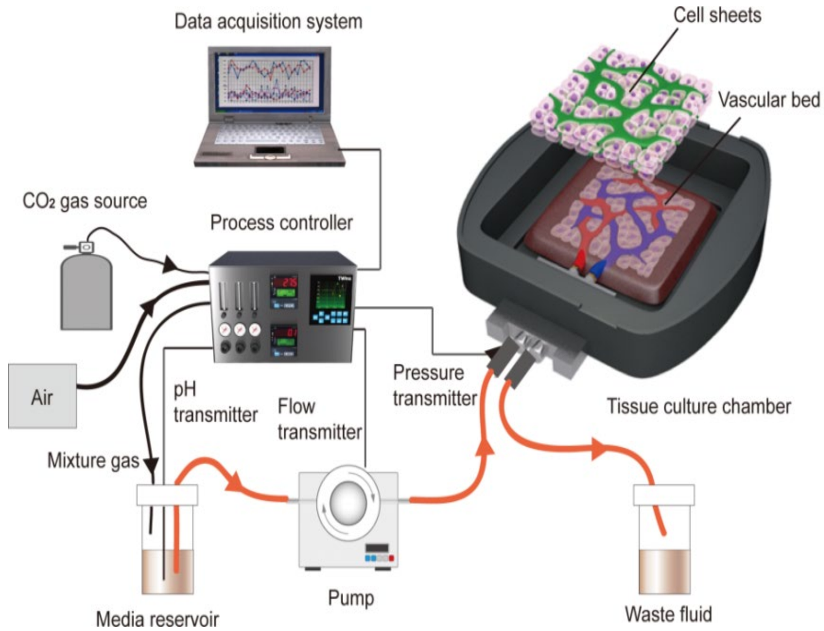
断端移植（同所性移植）　腸管膜内移植（異所性移植）

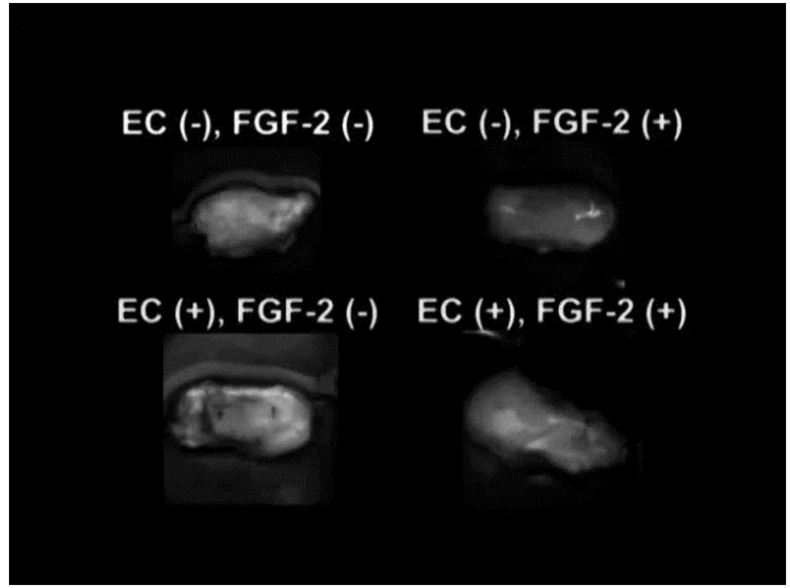


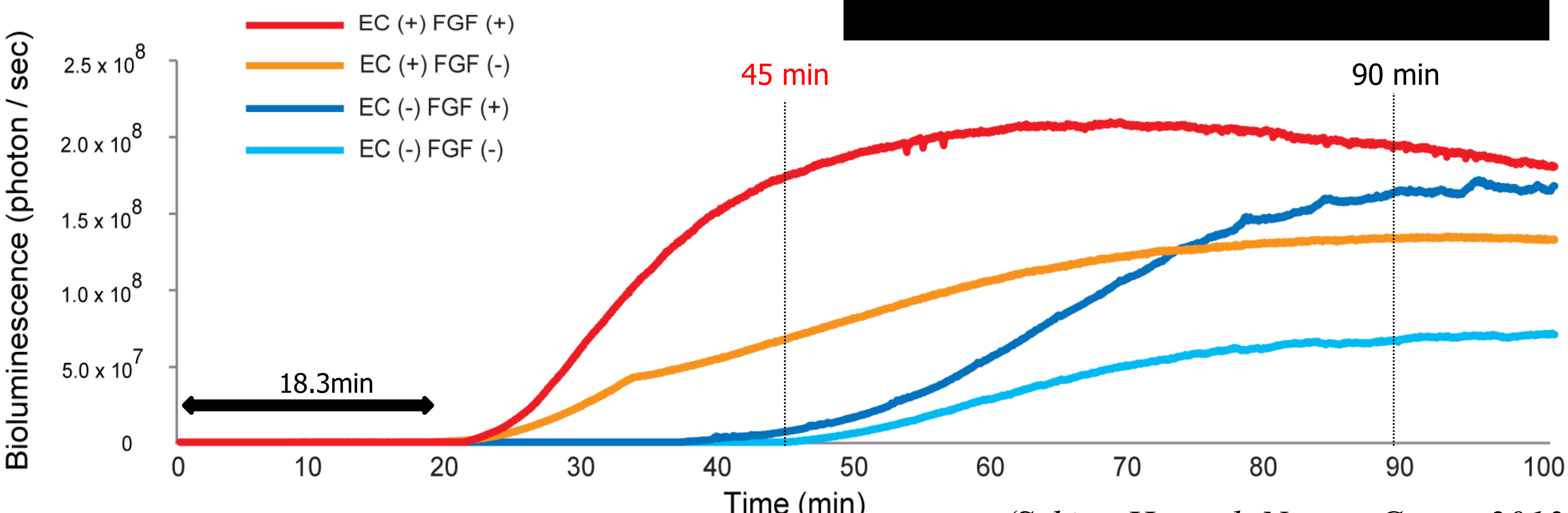
戦略 (3)

Ex vivoの還流で組織を積み上げる

東京女子医大　関根先生、清水教授



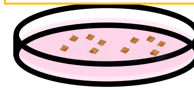
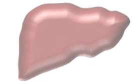




(Sekine H, et al. Nature Comm 2013)

ブタ臓器灌流からヒトの細胞を分化させる

シスメックスとの共同研究

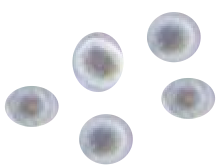




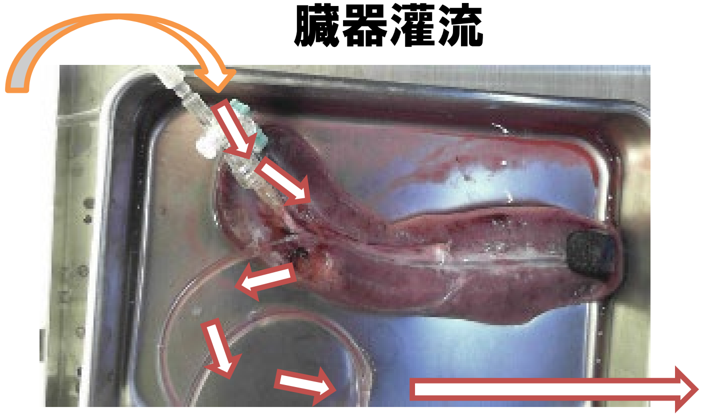
1000～5000血小板/巨核球　10～100血小板/巨核球　？血小板/巨核球

要素	In vivo	In vitro	Ex vivo
足場環境	○	X	○
細胞間相互作用	○	△	△
シェアストレス	○	X	○
液性因子	○	△	△

臓器灌流

未分化細胞



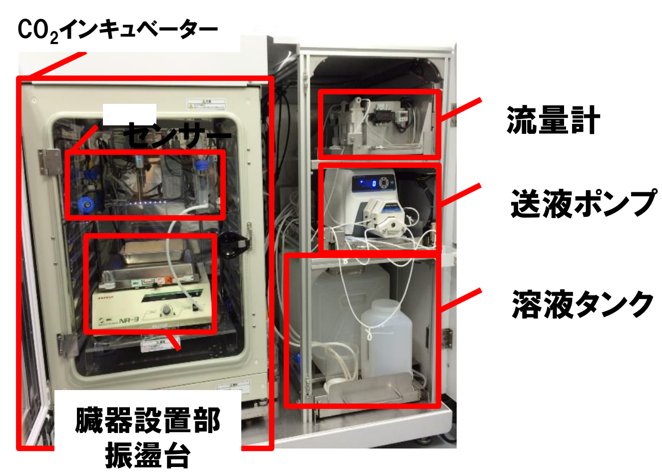


成熟細胞

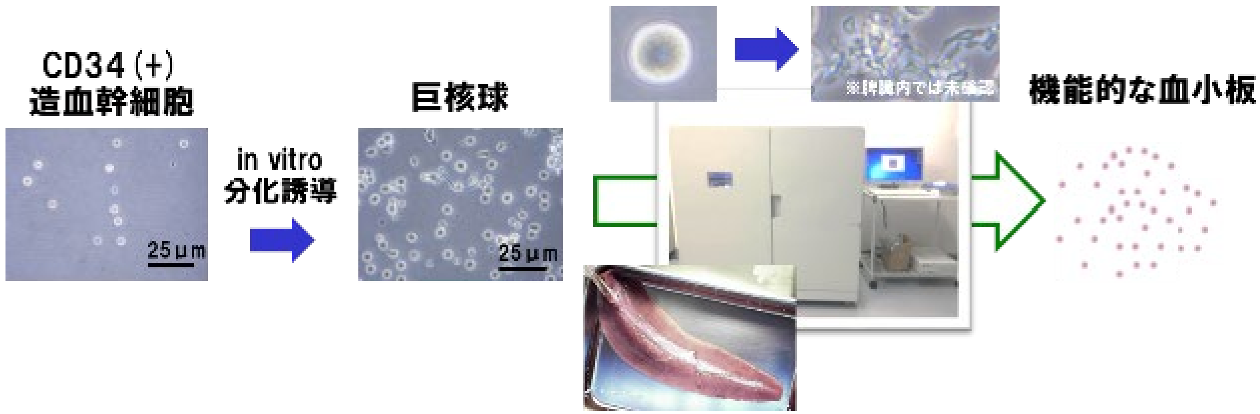
実験用ブタ

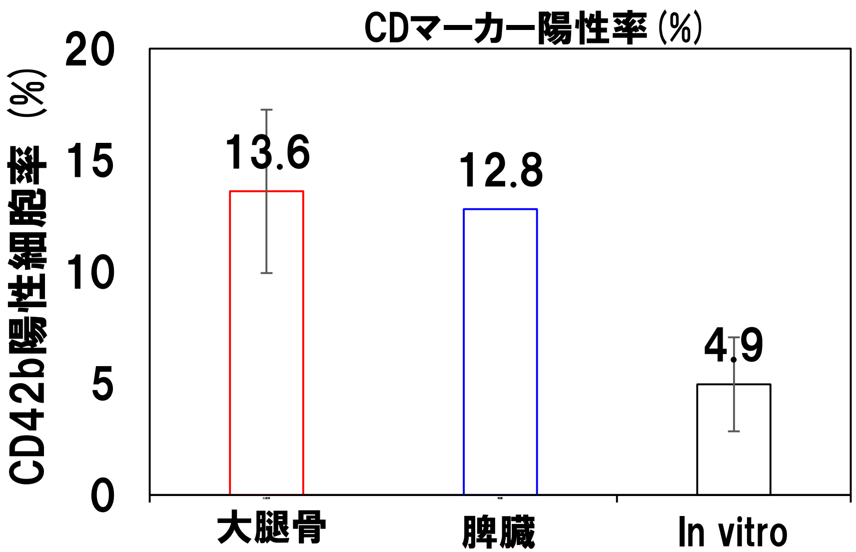
臓器摘出

臓器灌流培養システム

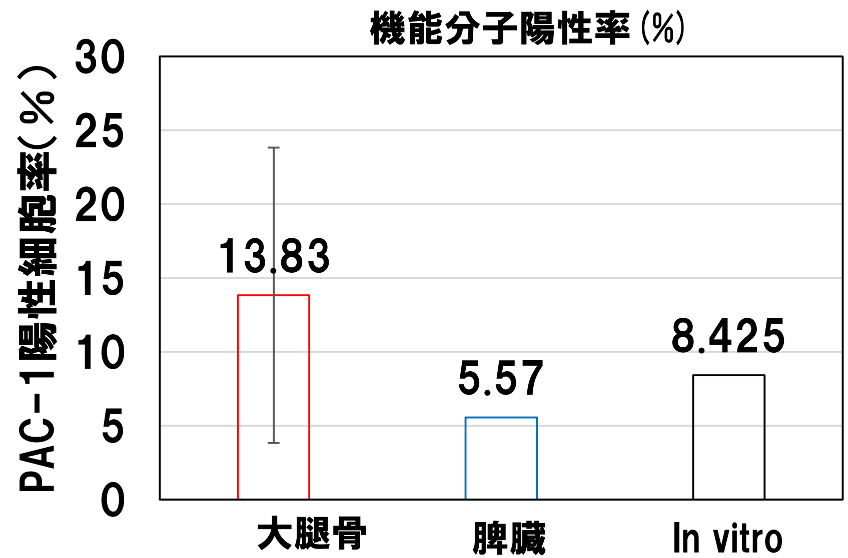


これまでの成果：Ex vivo臓器培養灌流システム





大腿骨n=4脾臓n=2,In vitro n=5
エラーバーは標準偏差を示す



大腿骨n=3,脾臓n=1,In vitro n=2
エラーバーは標準偏差を示す

大腿骨を使用することで、CDマーカー陽性率が高く機能的な血小板を作製することが出来た

(Fujiyama S, et al. Submitted)

Don Quixote Project for Organ Resurrection

(Kobayashi E with Screen Ltd. from 2015)

The Culture of Whole Organs

Author(s): Alexis Carrel and Charles A. Lindbergh
Source: *Science*, New Series, Vol. 81, No. 2112 (Jun. 21, 1935), pp. 621-623
Published by: American Association for the Advancement of Science
Stable URL: http://www.jstor.org/stable/1660192
Accessed: 13-11-2017 02:50 UTC

Background

1812
Le Gallois Proposal for idea

1866
de Cyron Frog heart
Beating for 48 hours
Liver Urea production

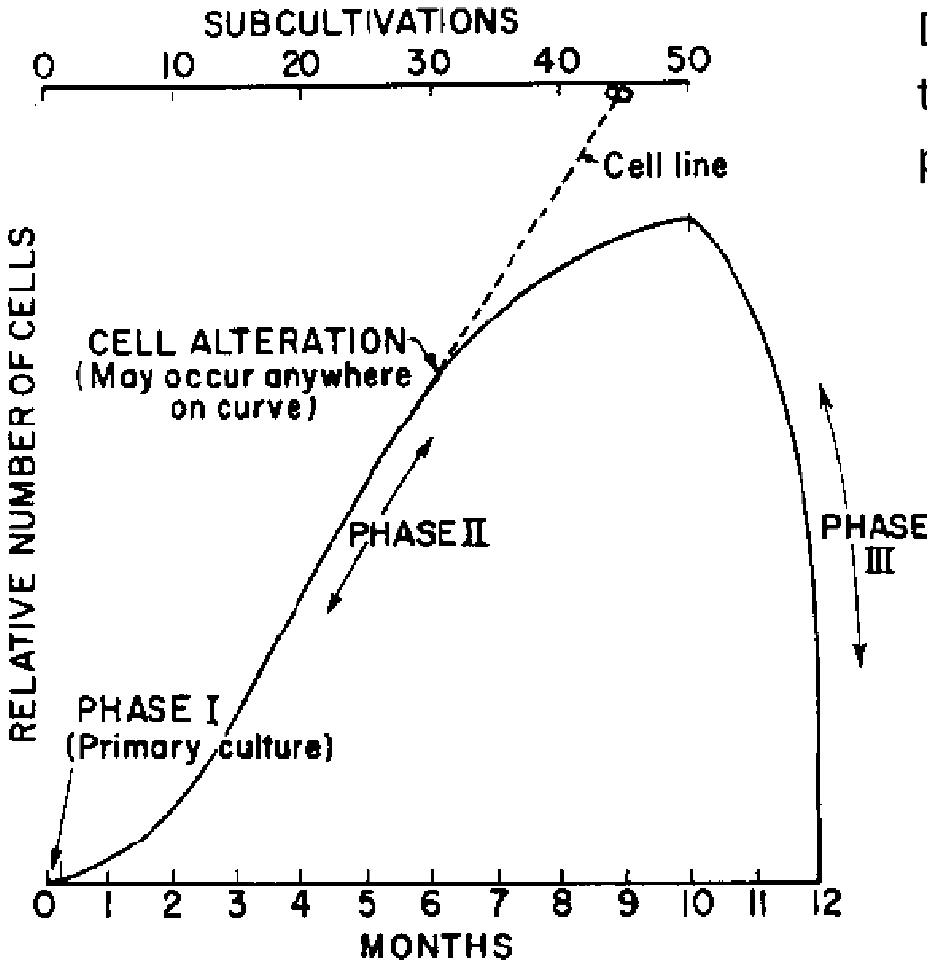
Brown-Sequard Brain circulation

A cat's thyroid gland, varying in weight from 85 to 110 mgs, demands about 230 cc of nutrient fluid.

The solutions contain protein split-products, hemin, cysteine, insulin, thyroxine, glutathione, vitamin A, ascorbic acid, blood serum, et. The apparatus is kept in an incubator at a temperature of 37-38C.

Thyroid glands were kept more than 20 days with pulsating arteries and active circulation.

THE SERIAL CULTIVATION OF HUMAN DIPLOID CELL STRAINS

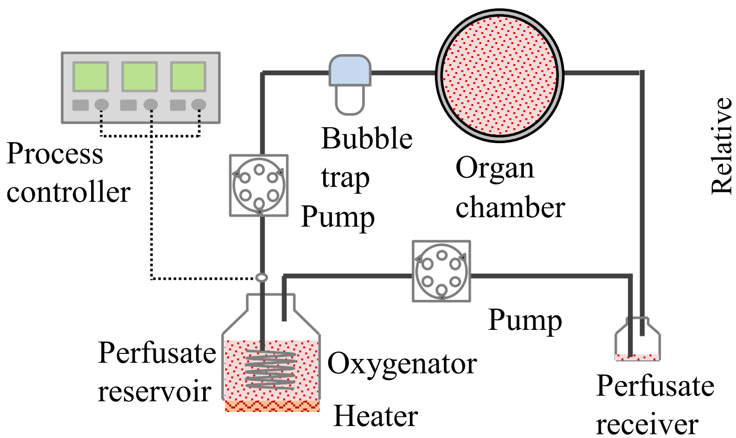


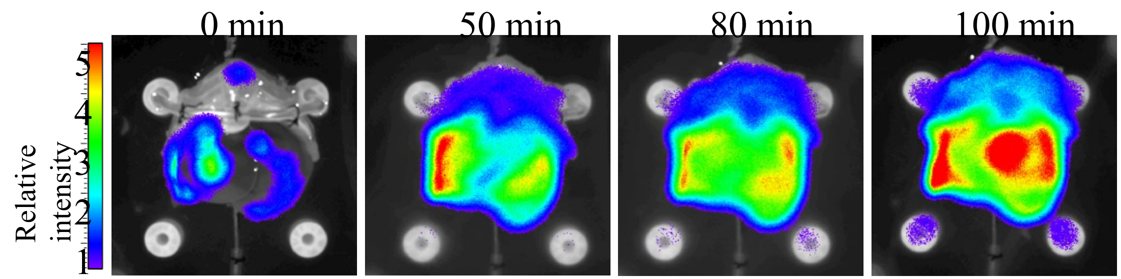
Dagrammatic representation of the history of cell strains and the phenomenon of cell alteration.

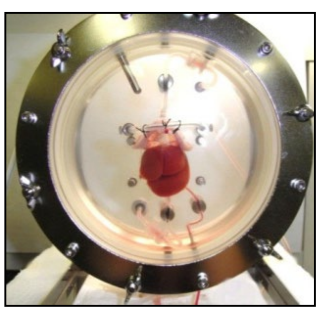
Phase I, or the primary culture, terminates with the formation of the first confluent sheet. **Phase II** is characterized by luxuriant growth necessitating many subcultivations. Cells in this phase are termed "cell strains". An alteration may occur at any time giving rise to a "cell line" whose potential life is infinite. Conversely, cell strains characteristically enter **Phase III** and are lost after a finite period of time.

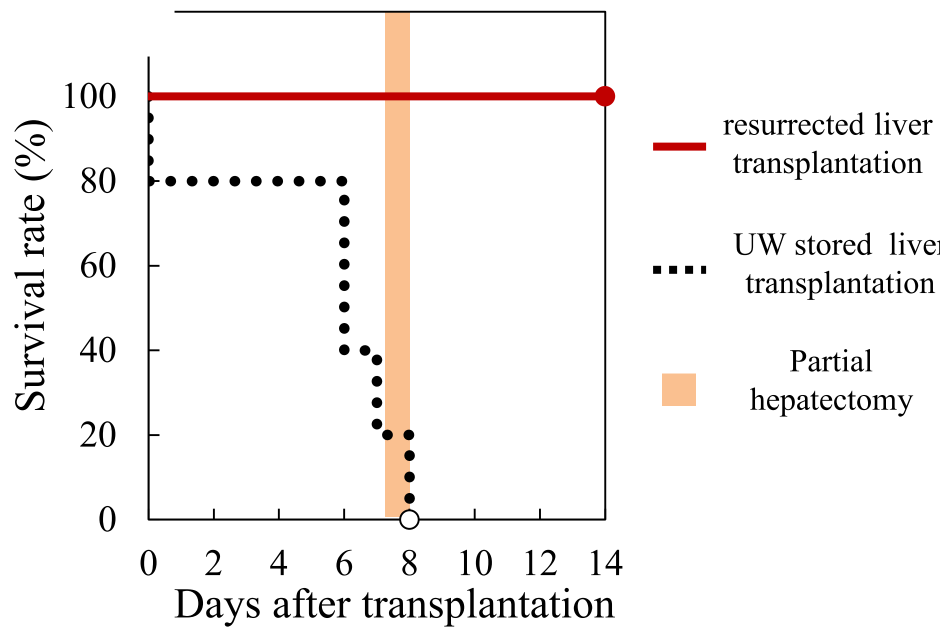
(L. Hayflick and PS Moorhead. Experimental Cell Research 1961)

Hypothermic temperature effects on organ survival and restoration









(Ishikawa J, et al. Scientific Reports 2015)

循環停止した臓器の蘇生研究の加速

修正マーストリヒト分類と実施される主な部署

カテゴリー	内容	DCDのタイプ	実施される部署
I	来院時心停止	uncontrlled	移植センター救急部
II	蘇生不成功	uncontrlled	移植センター救急部
III	予測される心停止	controlled	ICU、救急部
IV	脳死ドナーの心停止	controlled	ICU、救急部
V	ICU患者で予測されていなかった心停止	uncontrlled	移植センターICU

（心停止後の臓器提供（DCD）| 標準医療情報センター*ebm.jp/topics/dcd.htmlより*）

Uncontrolled donation after circulatory death: A cohort study of data from a long-standing deceased-donor kidney transplantation program

Despite good long term outcomes of kidney transplants from controlled donation after circulatory death (DCD) donors, there are few uncontrolled DCD (uDCD) programs.

This longitudinal study compares outcomes for all uDCD (N=774) and all donation after brain death (DBD) (N=613) kidney transplants performed from 1996 to 2015 at our center. DBD transplants were divided into those from standard-criteria (SCD) (N=366) and expanded-criteria (N=247) brain dead donors (ECD).

One-, five- and ten year graft survival rates were: 91.7%, 85.7% and 80.6% for SCD; 86.0%, 75.8% and 61.4% for ECD; and 85.1%, 78.1% and 72.2% for uDCD respectively. Graft survival was worse in recipients of uDCD kidneys than of SCD ($p=0.004$) but better than in transplants from ECD ($p=0.021$). The main cause of graft loss in the uDCD transplants was primary non-function. Through logistic regression, donor death due to pulmonary embolism (OR 4.31, 95% CI 1.65-11.23), extrahospital CPR time ≥75 min (OR1.94, 95%CI 1.18-3.22), and in-hospital CPR time ≥50 min (OR 1.79, 95% CI 1.09-2.93) emerged as predictive factors of primary non function.

According to the outcomes of our long-standing kidney transplantation program, uDCD could help expand the kidney donor pool.

(Sánchez-Fructuoso AI, et al. Am J Transplant. 2018 Dec 27.)

Kidney transplant from uncontrolled donation after circulatory death donors maintained by nECMO has long-term outcomes comparable to standard criteria donation after brain death.

Uncontrolled donation after circulatory death (uDCD) increases organ availability for kidney transplant (KT) with short-term outcomes similar to those obtained from donation after brain death (DBD) donors. However, heterogeneous results in the long term have been reported.

We compared 10-year outcomes between 237 KT recipients from uDCD donors maintained by normothermic extracorporeal membrane oxygenation (nECMO) and 237 patients undergoing KT from standard criteria DBD donors during the same period at our institution. We further analyzed risk factors for death-censored graft survival in the uDCD group.

Delayed graft function (DGF) was more common in the uDCD group (73.4% vs 46.4%; P < .01), although glomerular filtration rates at the end of follow-up were similar in the 2 groups. uDCD and DBD groups had similar rates for 10-year death-censored graft (82.1% vs 80.4%; P = .623) and recipient survival (86.2% vs 87.6%; P = .454). Donor age >50 years was associated with graft loss in the uDCD group (hazard ratio: 1.91; P = .058), whereas the occurrence of DGF showed no significant effect.

uDCD KT under nECMO support resulted in similar graft function and long-term outcomes compared with KT from standard criteria DBD donors. Increased donor age could negatively affect graft survival after uDCD donation.

(Molina M, et al. Am J Transplant. 2018 Jun 27.)

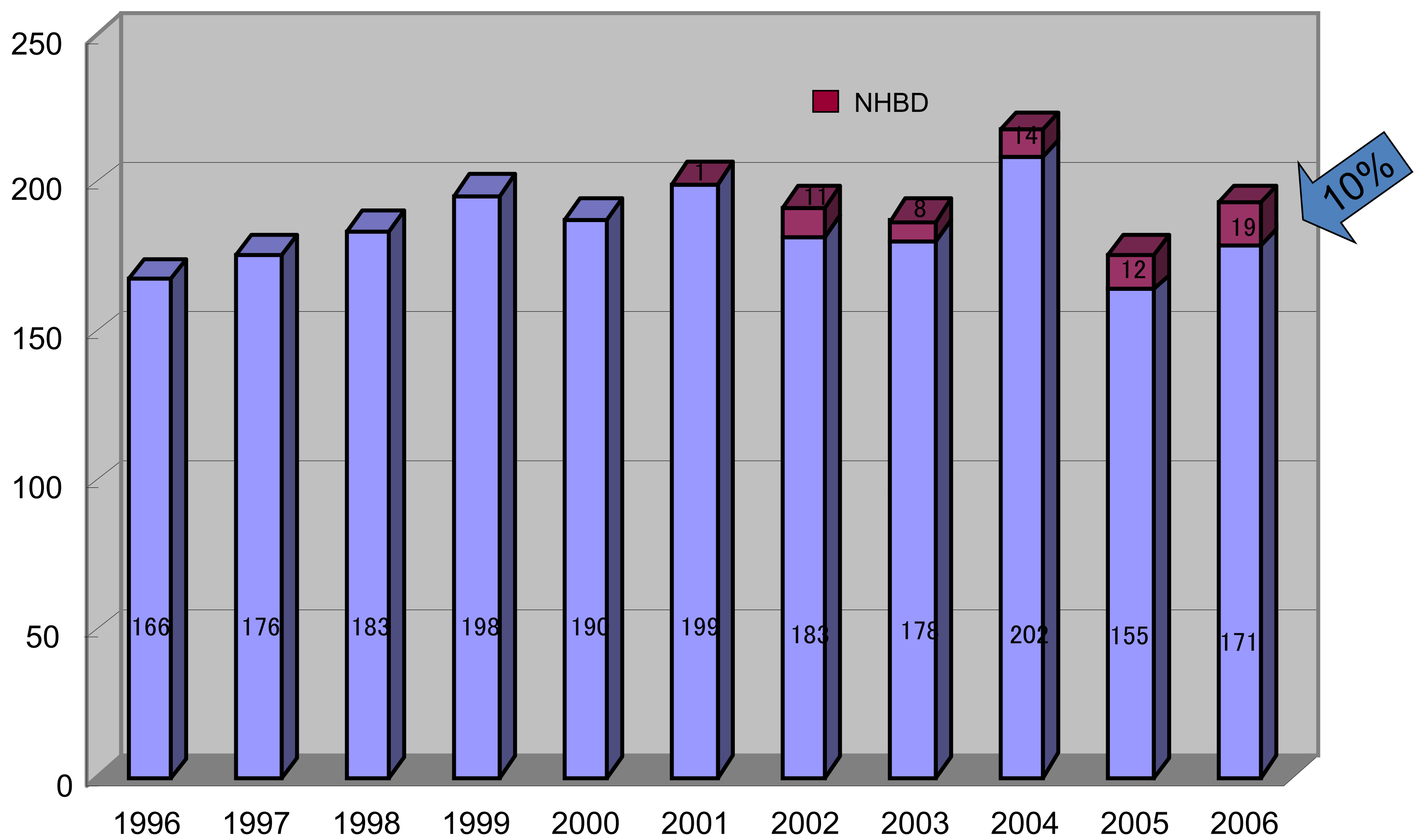
Donor acceptance criteria for uDCD donor

1. Donor age between 18 and 60 y
2. Known time of CA
3. Time between CA and the initiation of aCPR <15 min
4. Cause of death known or easily ascertainable
5. No evidence of bleeding lesions in the thoracic or abdominal cavities that may interfere with organ perfusion measures
6. No suspicion (as per external appearance) of intravenous drug abuse posing a potential risk for infection due to human immunodeficiency virus, hepatitis C virus, or hepatitis B virus
7. Total warm ischemia time <150 min

aCPR, advanced cardiopulmonary resuscitation;
CA, cardiac arrest;
uDCD, uncontrolled donation after circulatory death.

(Molina M, et al. Am J Transplant. 2018 Jun 27.)

Impact of NHBD liver transplantation in King's college Hospital



(Muiesan P. 2008)

A randomized trial of normothermic preservation in liver transplantation

NMP device and circuit.
OrganOx metra (generation 1)

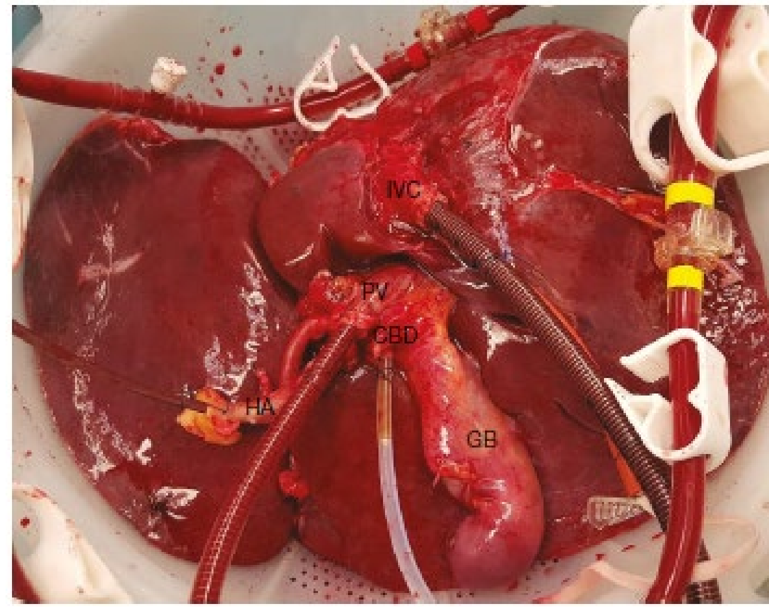
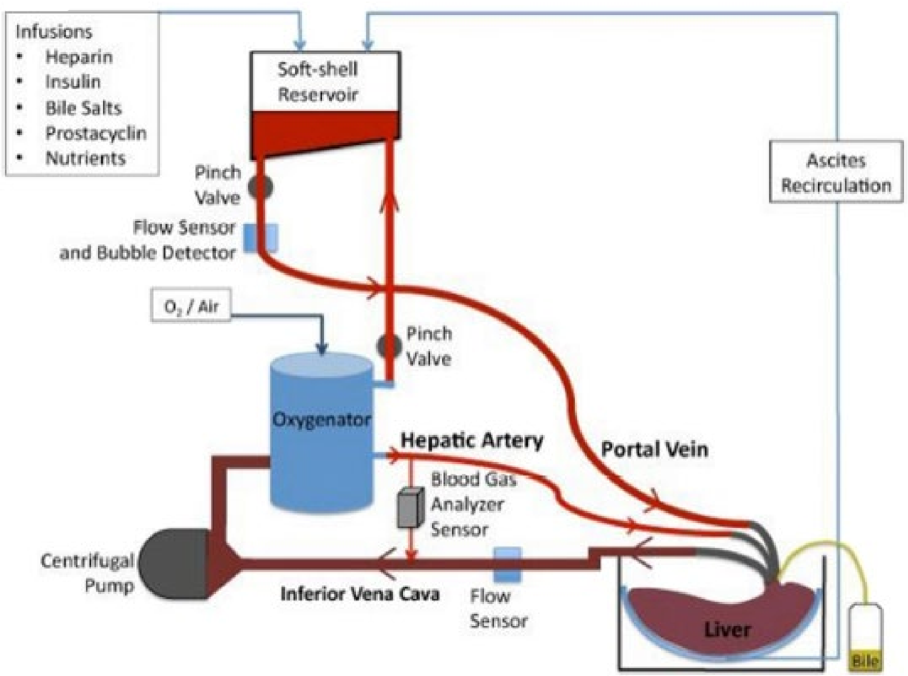
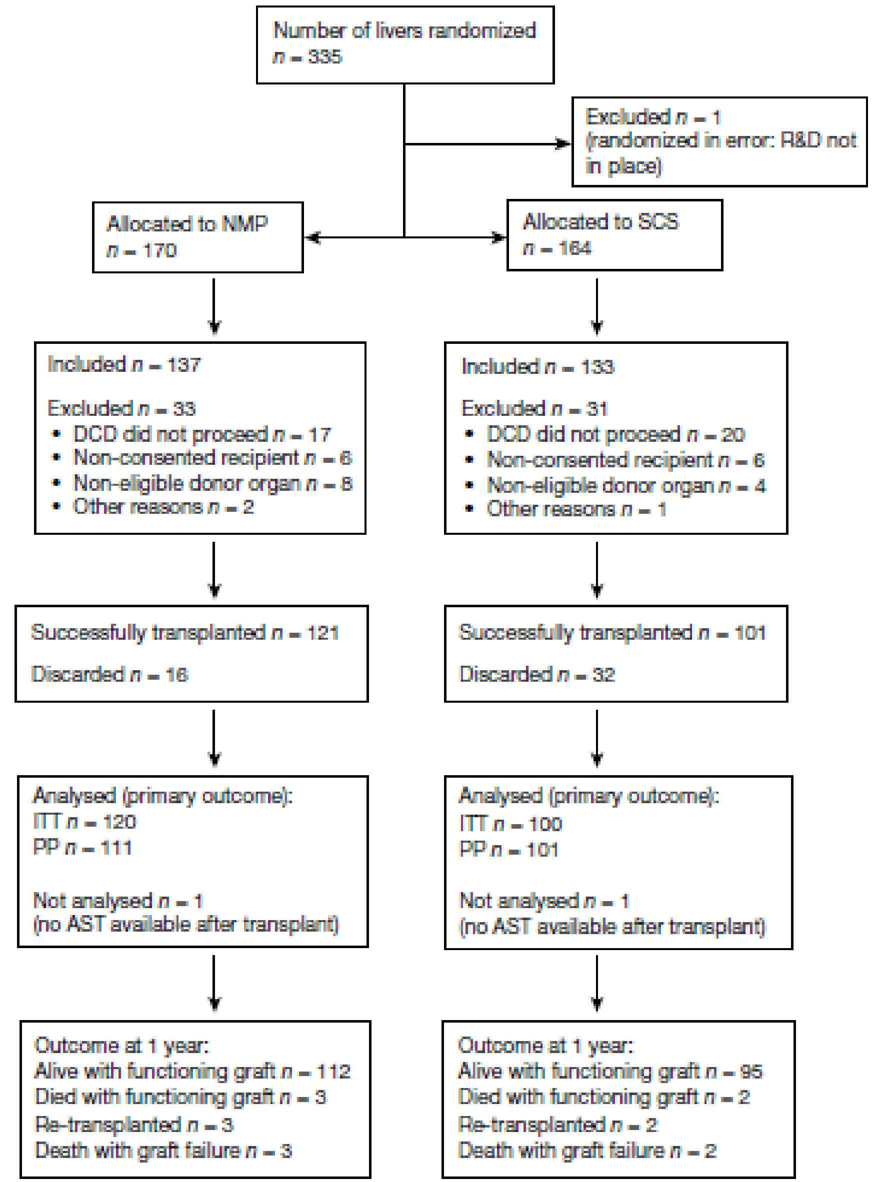


Image of liver during normothermic machine perfusion

A randomized trial of normothermic preservation in liver transplantation



NMP device and circuit ; OrganOx



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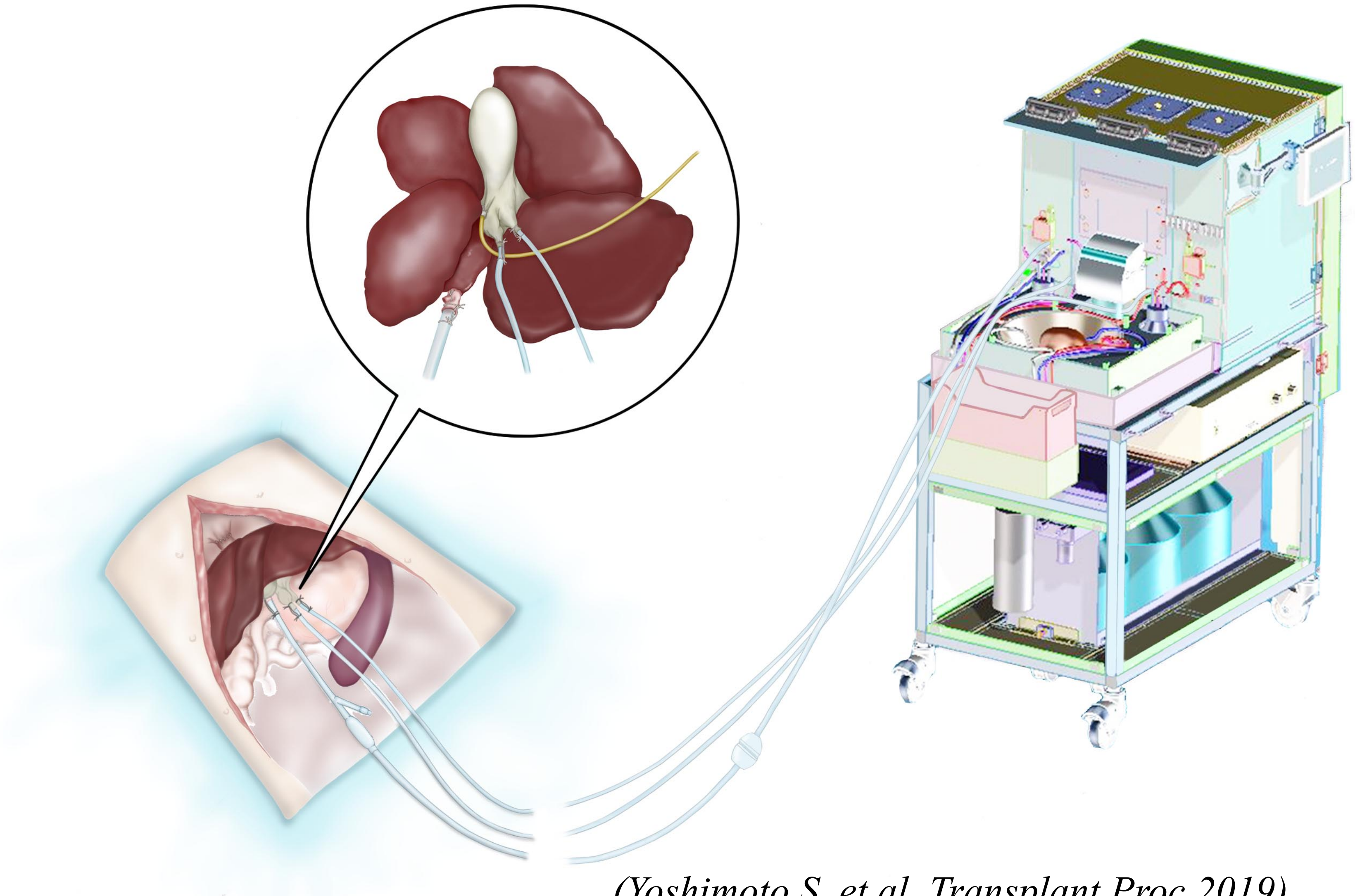
Ischemia Free Transplantation in Pigs



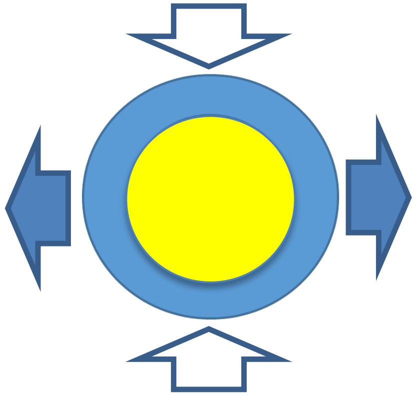
(Yoshimoto S, et al. Transplant Proc 2019)

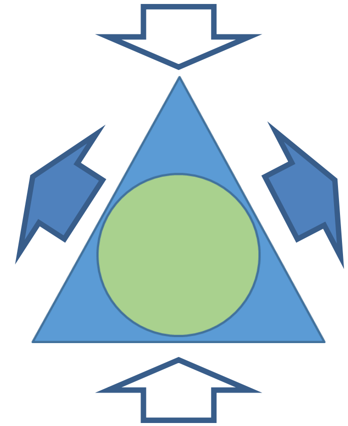
特殊チューブの開発

The cylindrical tube	The triangular tube

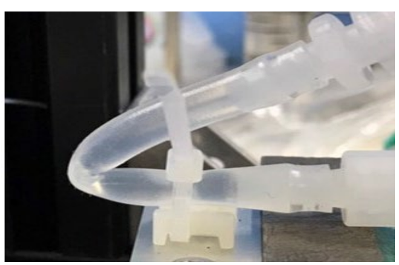
Applied stress

Stress diffusion

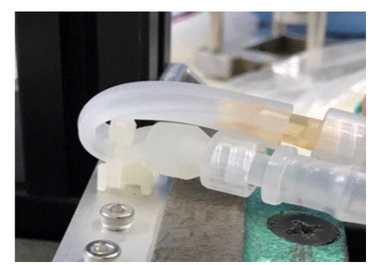




Collapsed shapes image



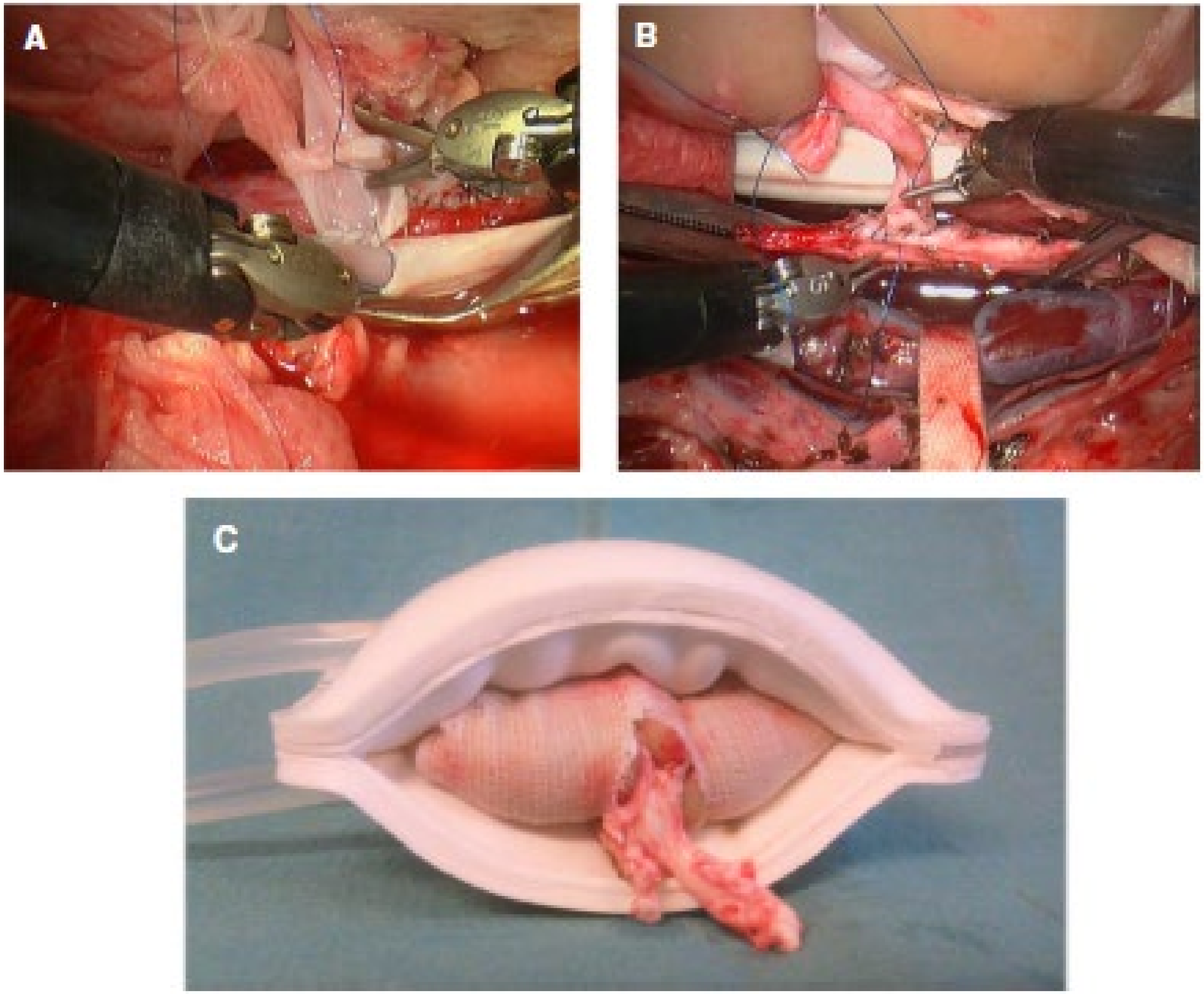
The flow was stopped at 150°



Non-stop

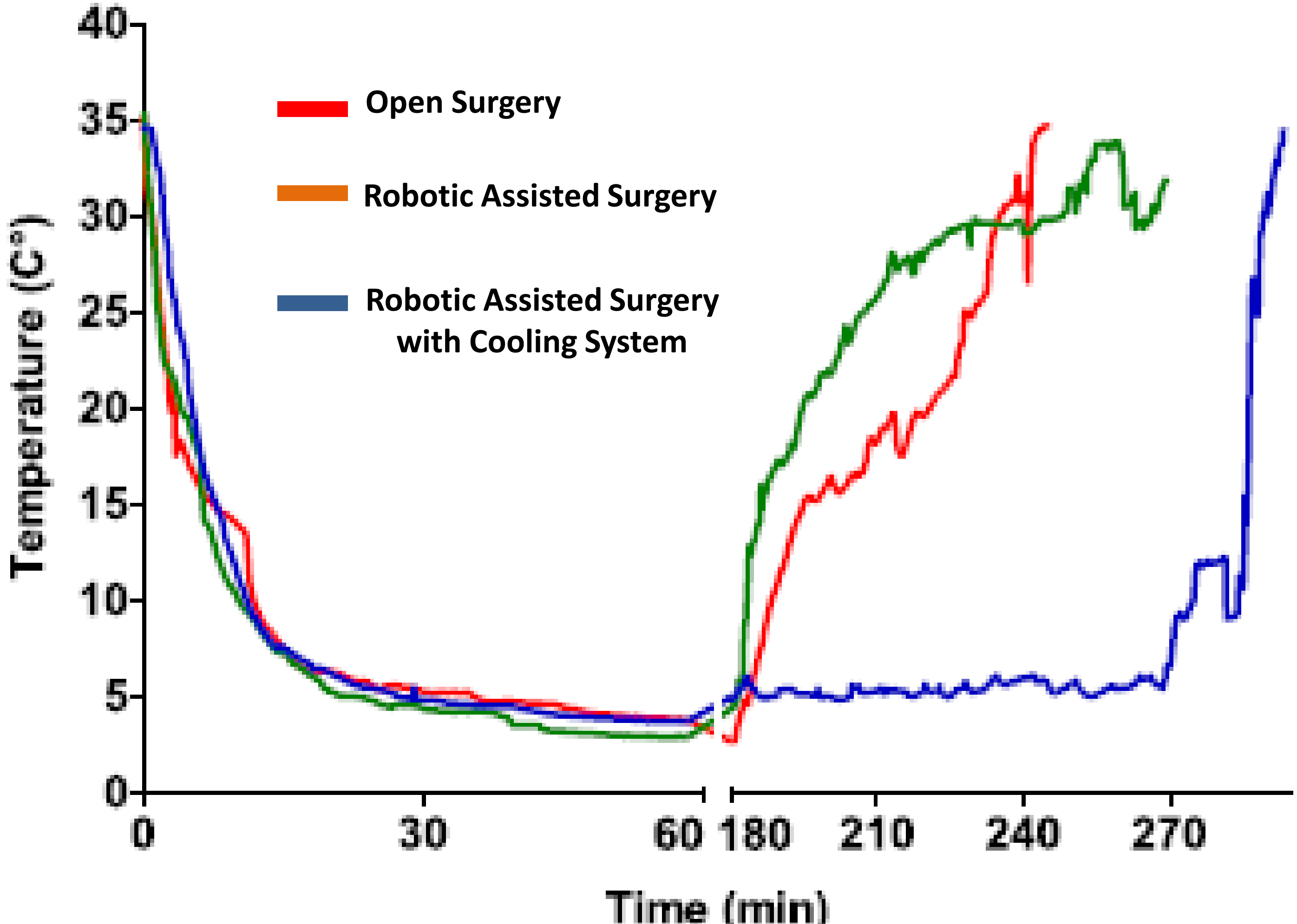
(Kobayashi E, et al. Transplant Direct 2019)

Intra-Abdominal Cooling System Limits Ischemia–Reperfusion Injury During Robot-Assisted Renal Transplantation



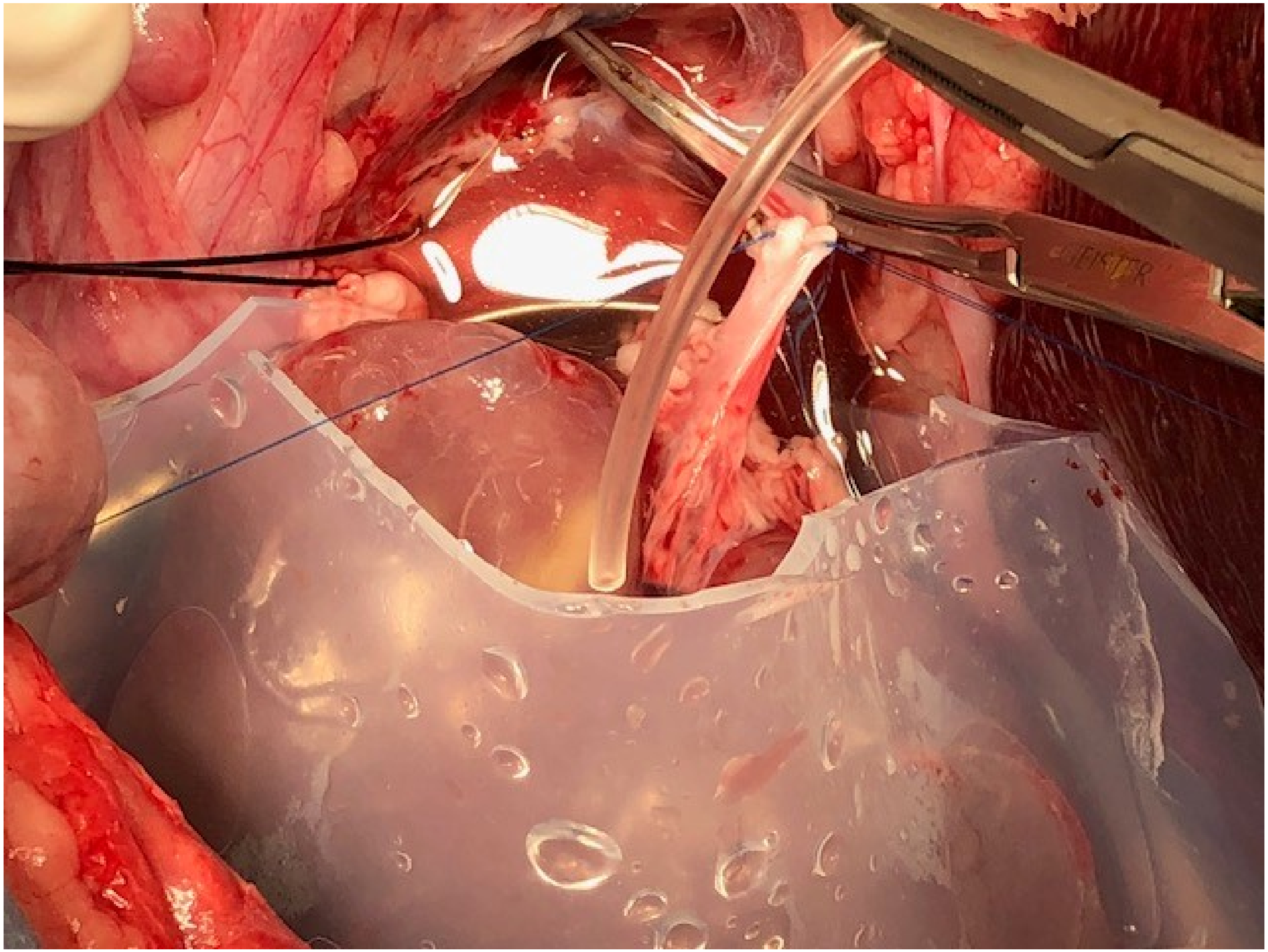
(R. P. H. Meier et al. AJT 2018)

Temperature curves from kidney explantation to kidney revascularization



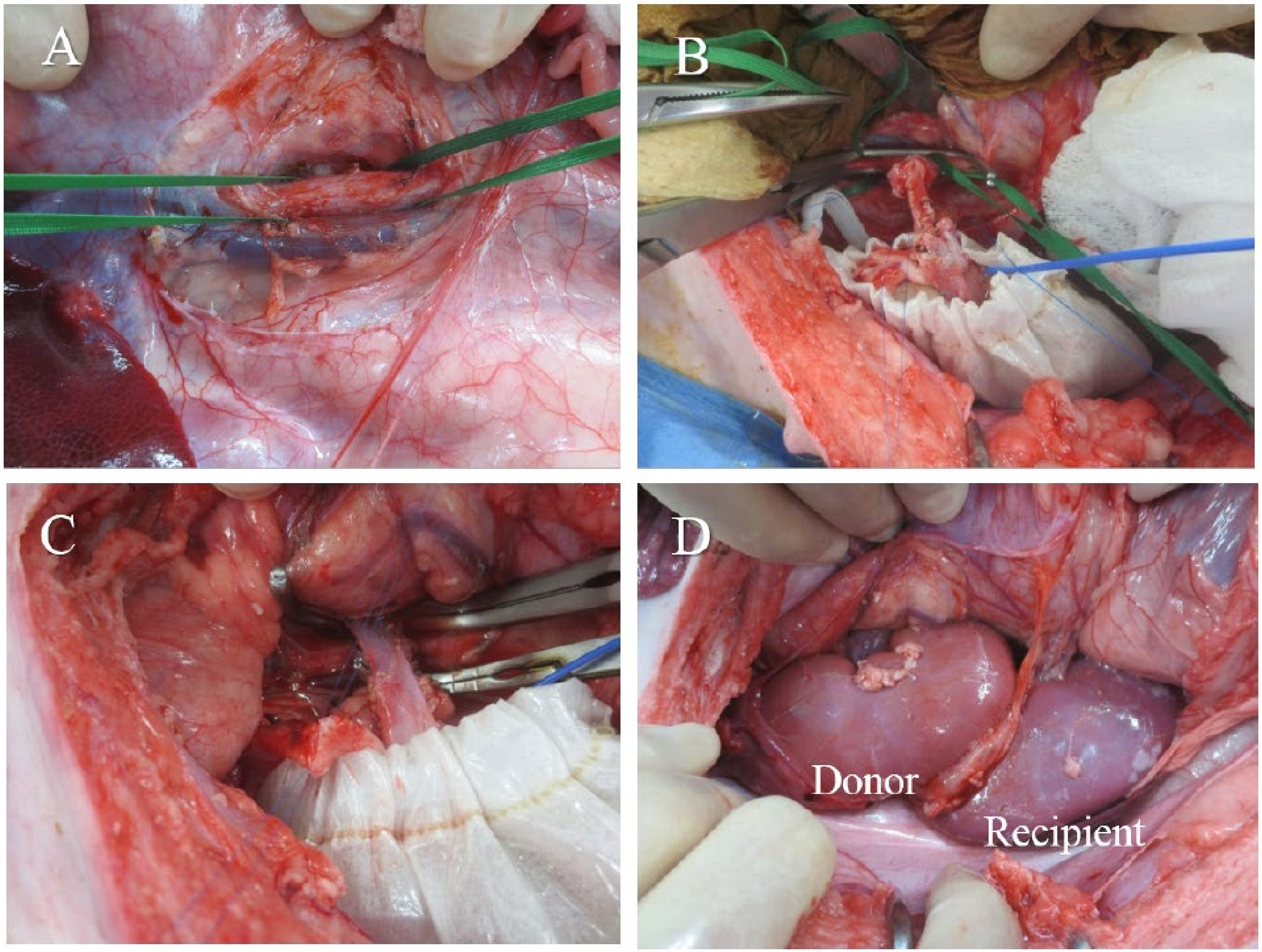
(R. P. H. Meier et al. AJT 2018)

Intra-abdominal cooling by the TBB made of medical grade silicone



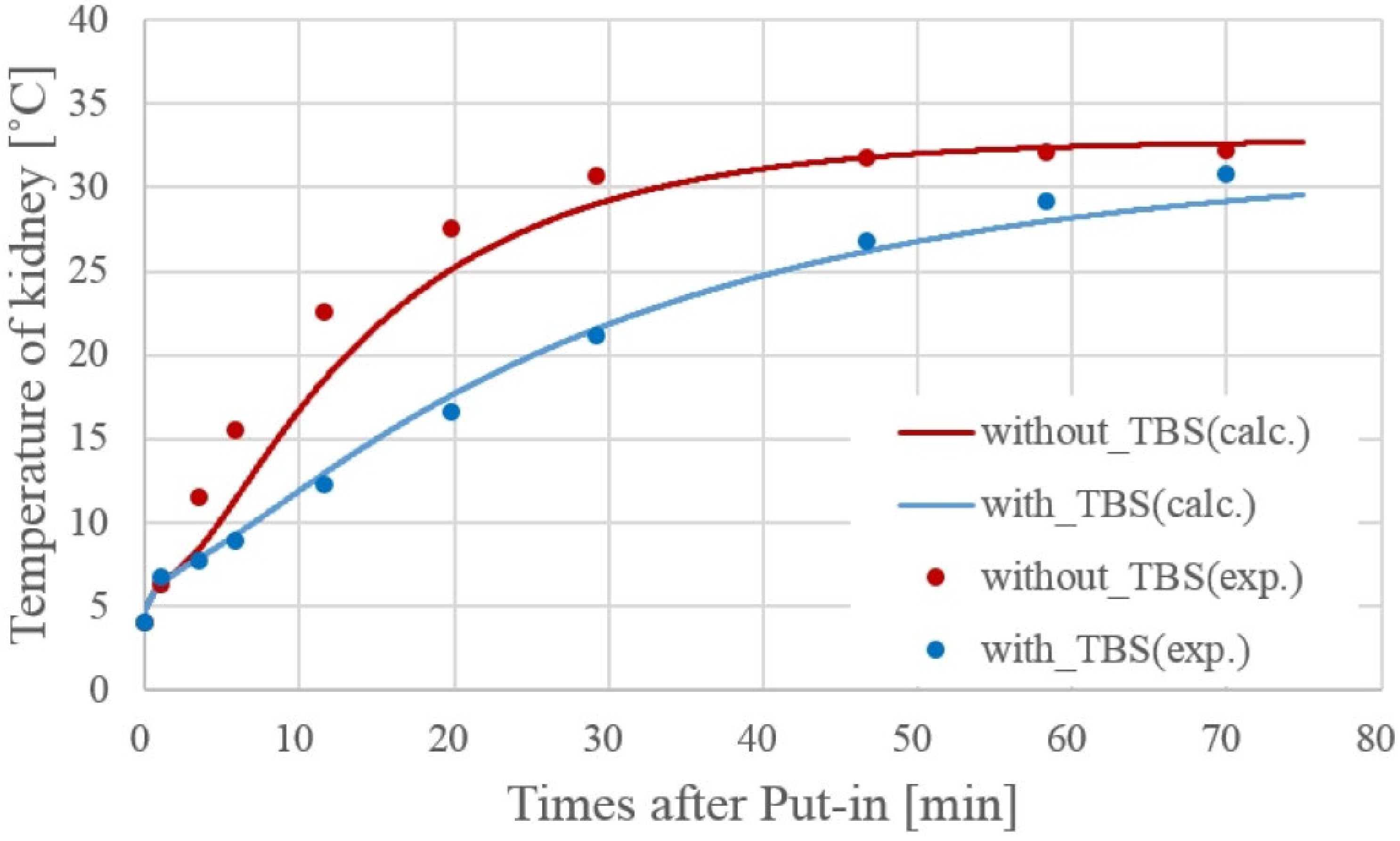
(Kobayashi E, et al. Transplant Direct 2019)

Reduction of warm ischemia using a Thermal Barrier Bag in kidney transplantation: a pig study



(Torai S, et al. Transplant Proc 2019)

Comparison of kidney temperature rise trends in ex vivo experiment and simulation



(Torai S, et al. Transplant Proc 2019)

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