Promising Future for *Ex vivo* Tissue Fabrication

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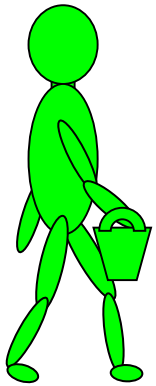
The research for fabricating tissue/organs through cultivation of stem cells gathered from the patients themselves has been accelerated in recent years.

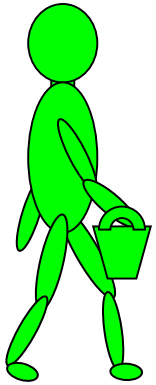
Through the usage of iPS cells and alike we develop various cellular tissues from the patients and multiply them on vascular beds to fabricate target tissues.

In the future by maximizing microsurgical technique, newly fabricated tissues derived from the patients themselves will surely be transplantable.

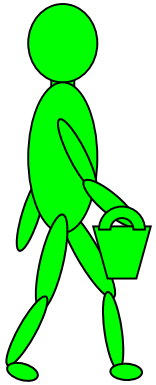
“ Translational Researches ”



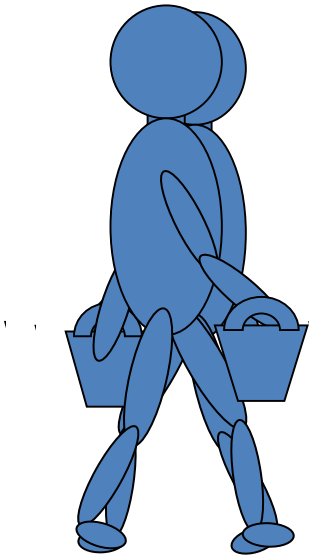
Medicine



Basic Science



Bioethics

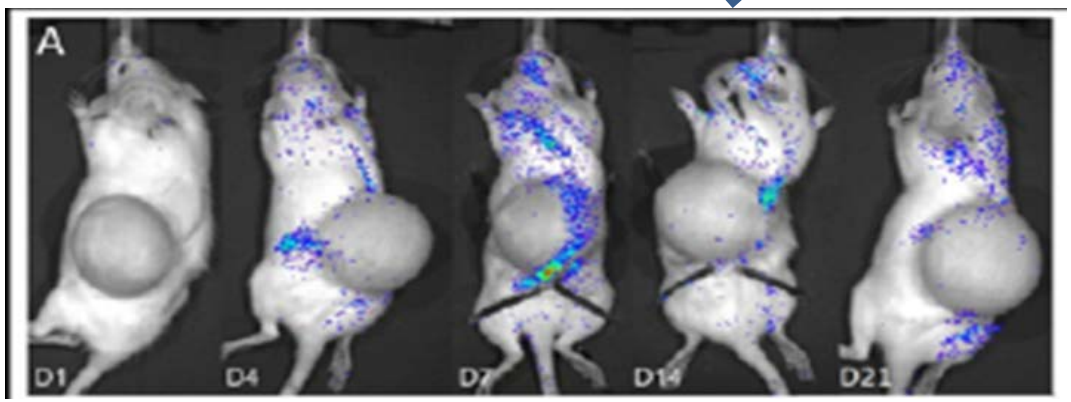


Expert Clinician

(Kobayashi E & Montero EF. Act Cri Bras 20;194,2005)

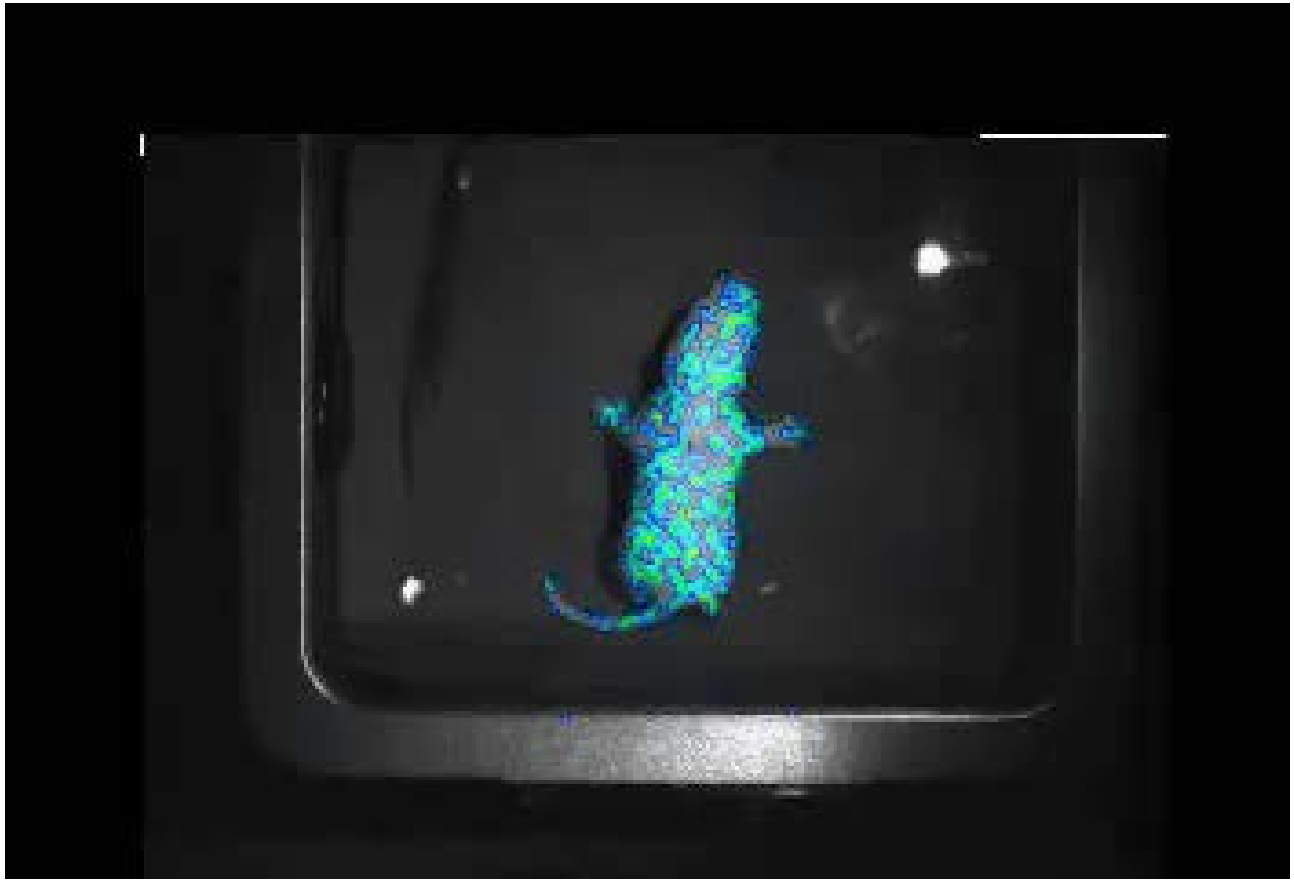
Translational Research: From rat to clinic

In vivo
Tissue Fabrication



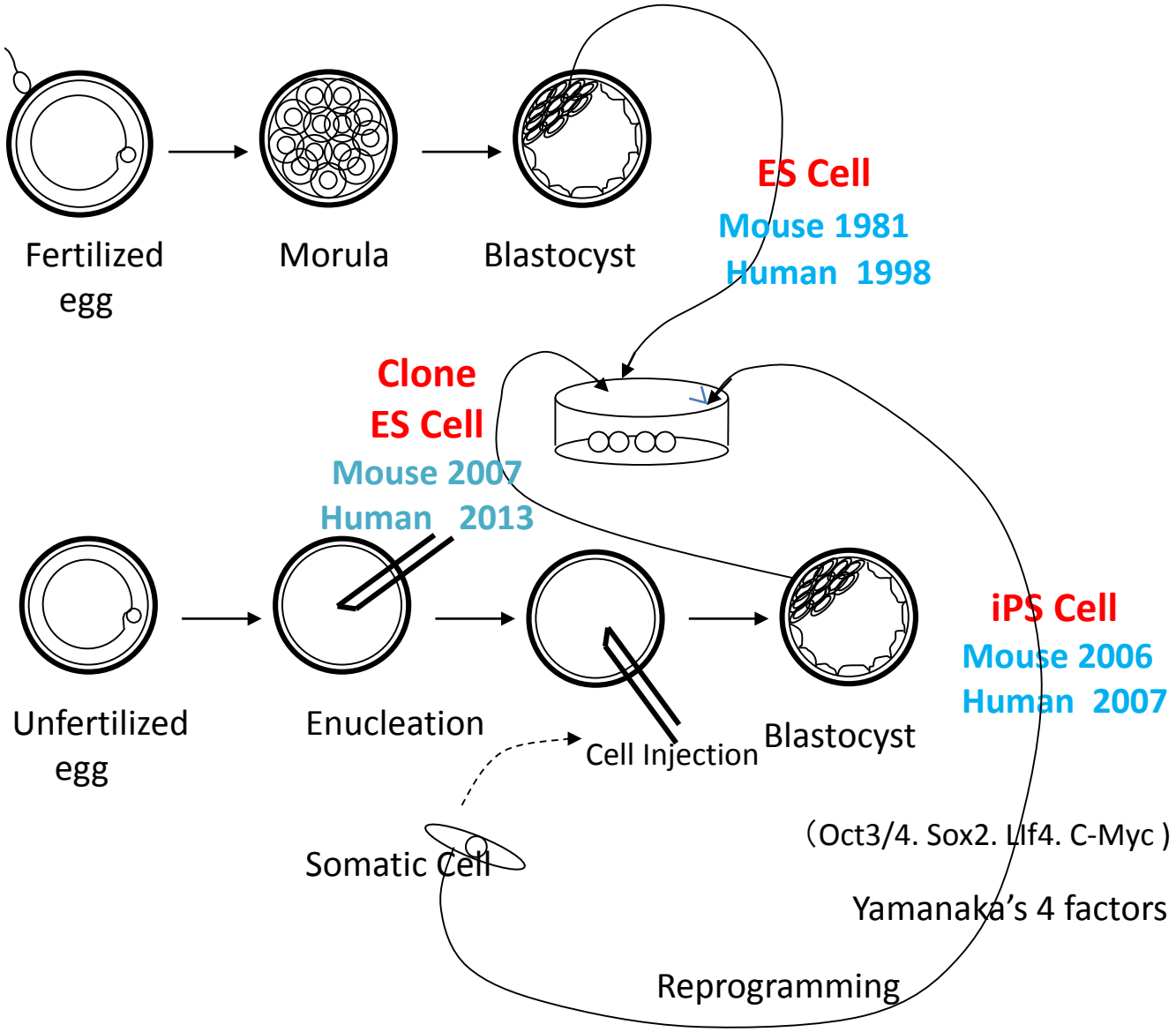
(Data from Dr. Shuangbai Zhou)

Cross-border academia collaboration between Prof. Li (China) and Prof. Kobayashi (Japan)



`The firefly Rat` ***developed world-first by Prof. Kobayashi in the early years of 2000s***

Possible Source for Human Organs



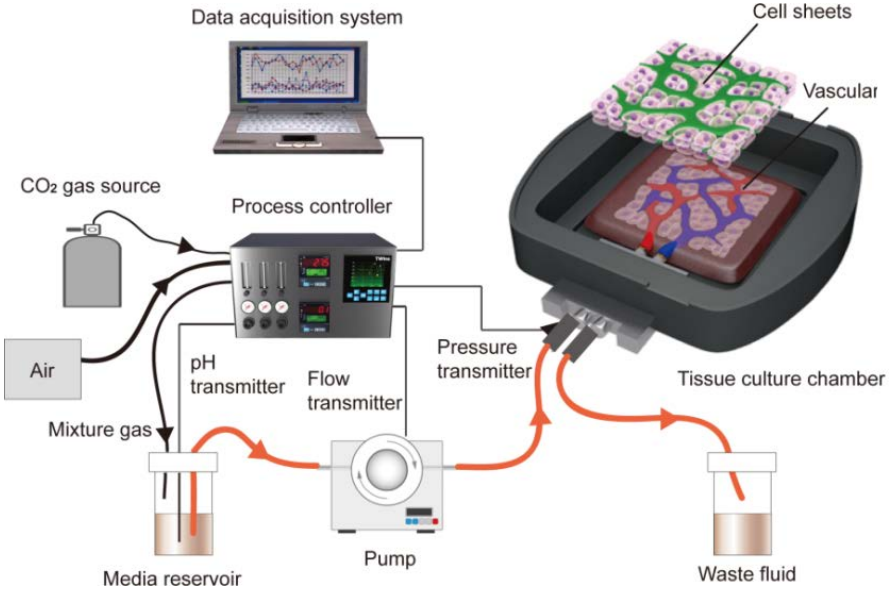
ES cell = embryonic stem cell; iPS cell = induced pluripotent stem cell

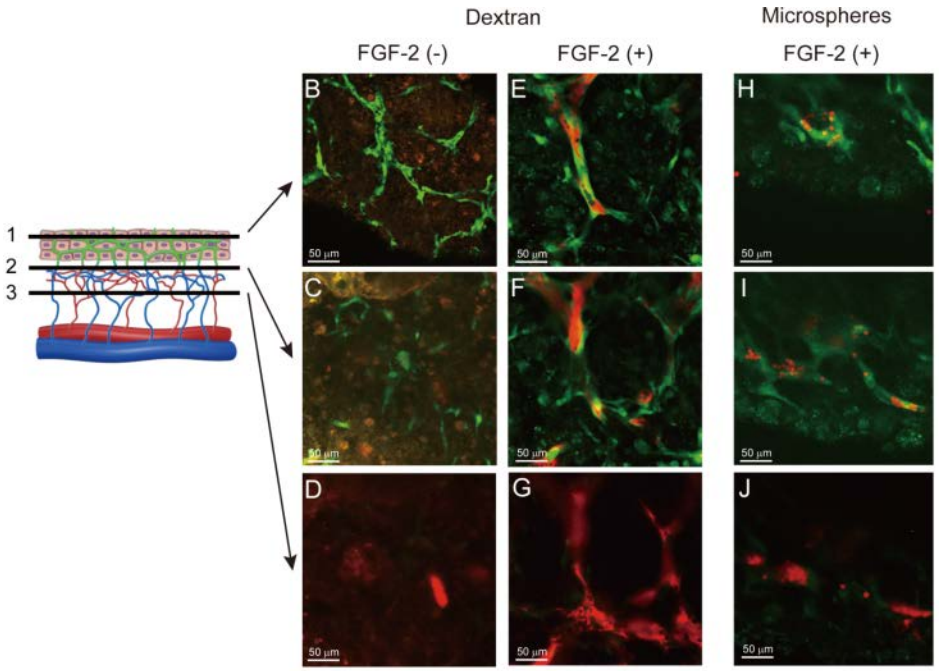
nature COMMUNICATIONS

DOI: 10.1038/ncomms2406

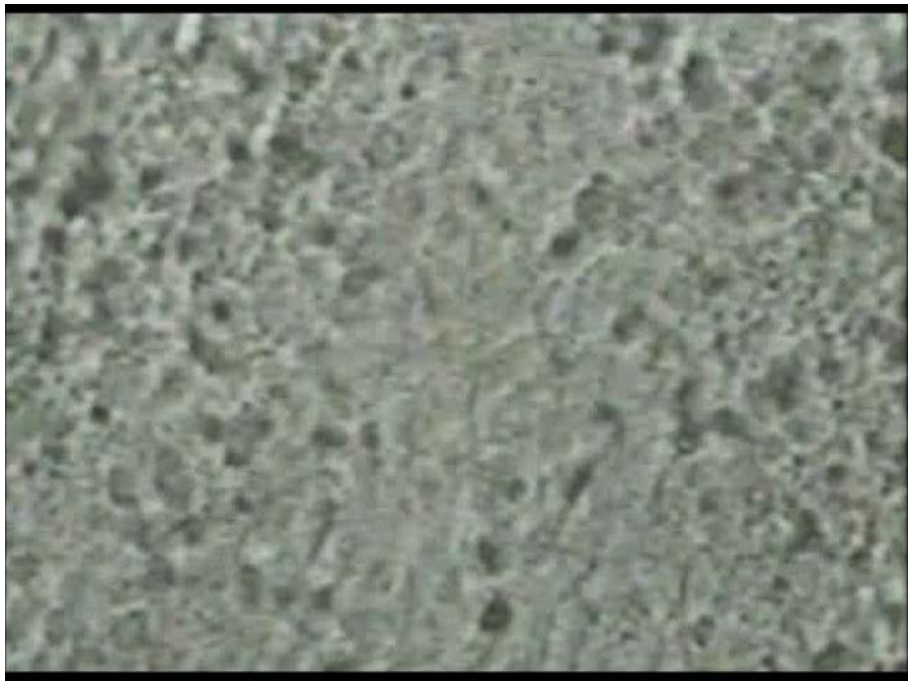
In vitro fabrication of functional three-dimensional tissues with perfusable blood vessels

Hidekazu Sekine[1,*], Tatsuya Shimizu[1,*], Katsuhisa Sakaguchi[2], Izumi Dobashi[1], Masanori Wada[3], Masayuki Yamato[1], Eiji Kobayashi[4], Mitsuo Umezu[2] & Teruo Okano[1]





`Tissue-engineered` cardiomyocytes sheet



Temperature Responsive Culture Dishes

developed by Prof. Okano

Fabrication of implantable liver tissue using bio- 3D printer and development of a novel transplantation technique for liver tissue

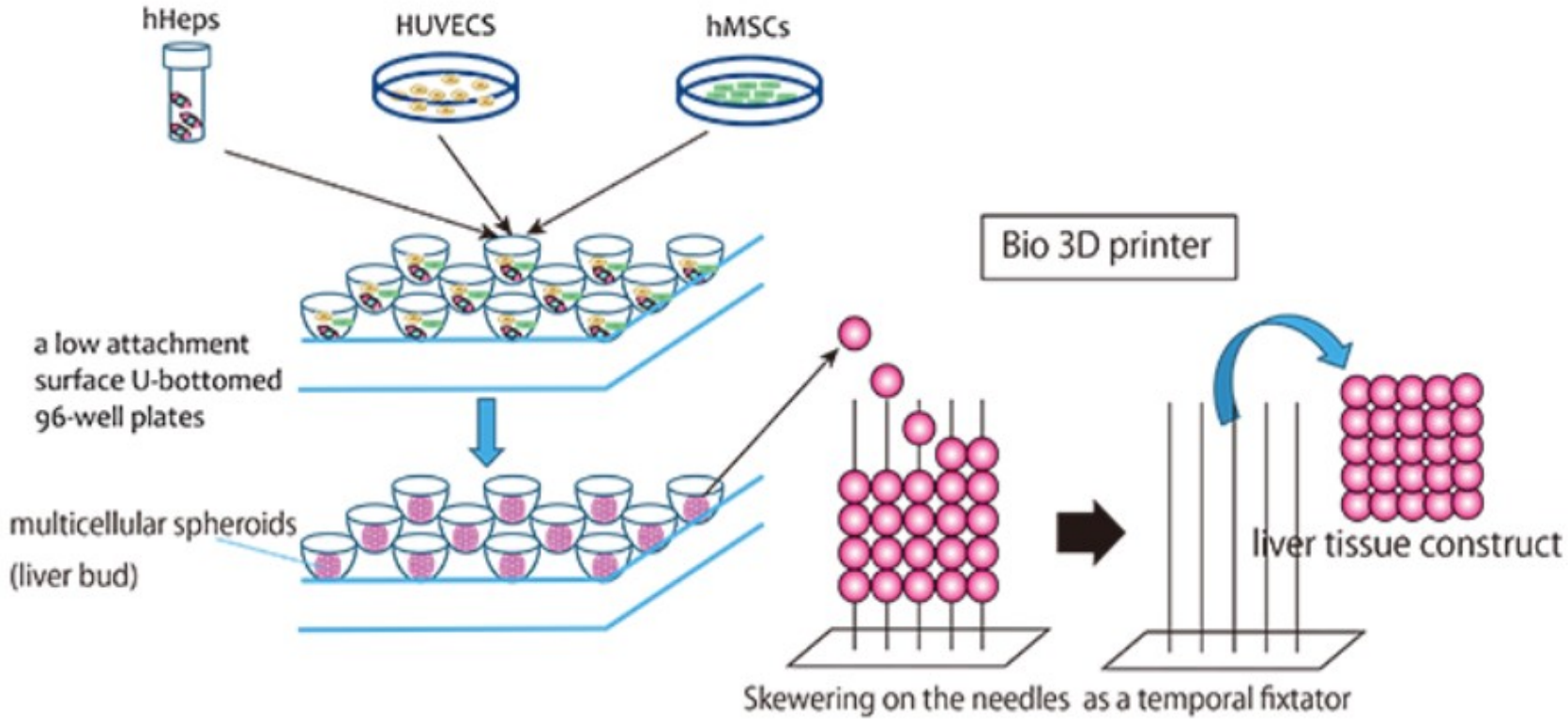
Yusuke Yanagi [1], Tomoaki Taguchi [1],Koichi Nakayama [4],Kenichi Kohashi[5], Shin Enosawa[3], Eiji Kobayashi[2]

[1] Department of Pediatric Surgery, Reproductive and Developmental Medicine, Graduate School of Medical Sciences, Kyushu University

[2] Department of Organ Fabrication, Keio University School of Medicine, Shinjuku-ku, Tokyo 160-8582, Japan

[3] National Center for Child Health and Development, 2-10-1 Okura, Setagaya-Ku, Tokyo 157-8535, Japan

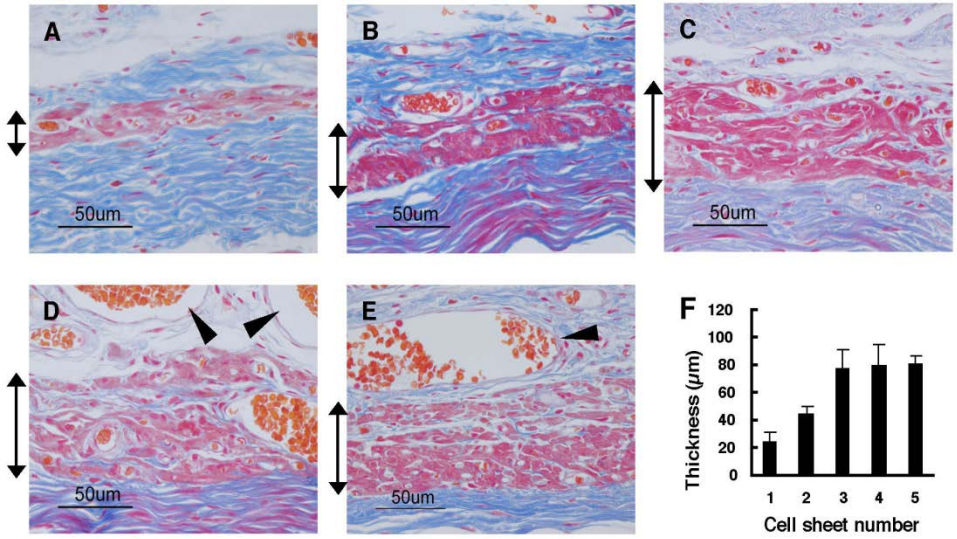
[4] Department of Advanced Technology Fusion,Advanced Technology Fusion, Graduate School of Science and Engineering, Saga University



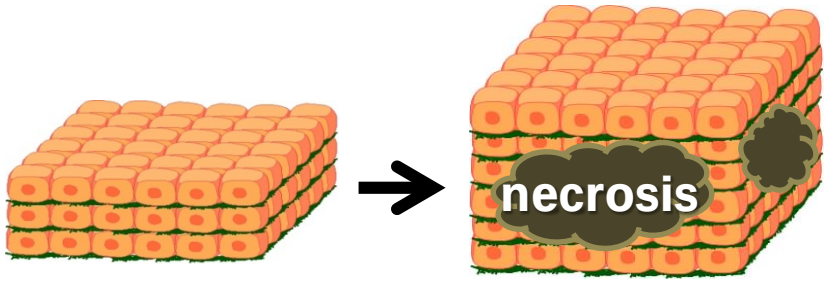
(Submitted)

Tissue Fabrication is restricted due to the limits of passive diffusion

in vivo



in vitro



Triple layered cell sheets

Tissue thickness reached a plateau at 80um

New technologies for improving the reconstruction of 3D cell-dense tissue with a well organized vasculature are required.

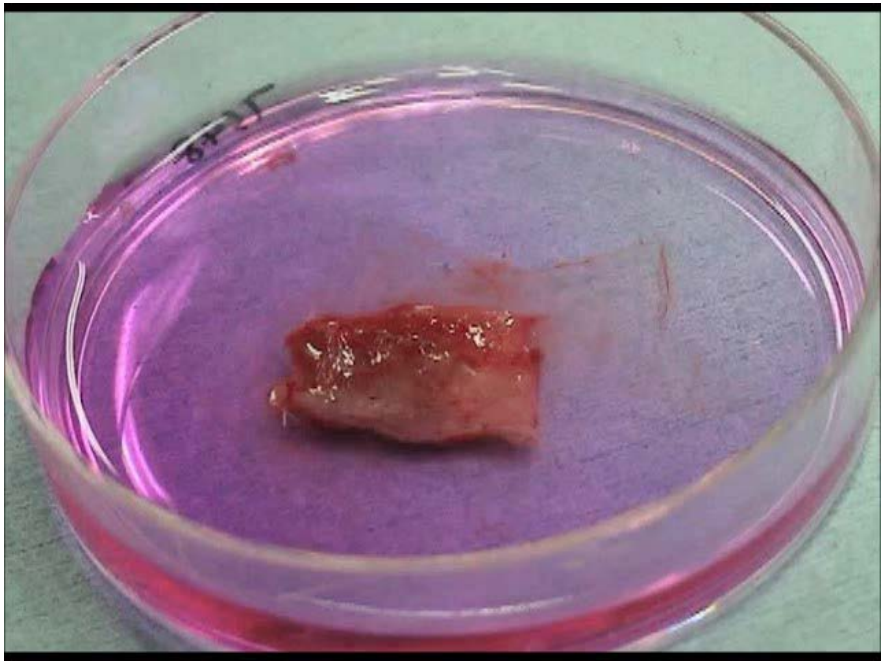
The FASEB Journal
The Journal of the Federation of American Societies for Experimental Biology
The FASEB Journal express article 10.1096/fj.05-4715fje. Published online January 26, 2006.

Polysurgery of cell sheet grafts overcomes diffusion limits to produce thick, vascularized myocardial tissues

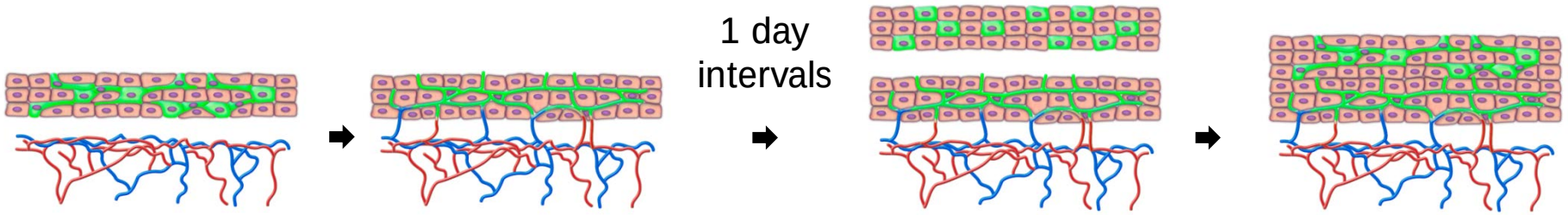
Tatsuya Shimizu,* Hidekazu Sekine,* Joseph Yang,* Yuki Isoi,* Masayuki Yamato,* Akihiko Kikuchi,* Eiji Kobayashi,† and Teruo Okano*

*Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666; and †Division of Organ Replacement Research, Center for Molecular Medicine, Jichi University Medical School, 3311-1 Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498 Japan

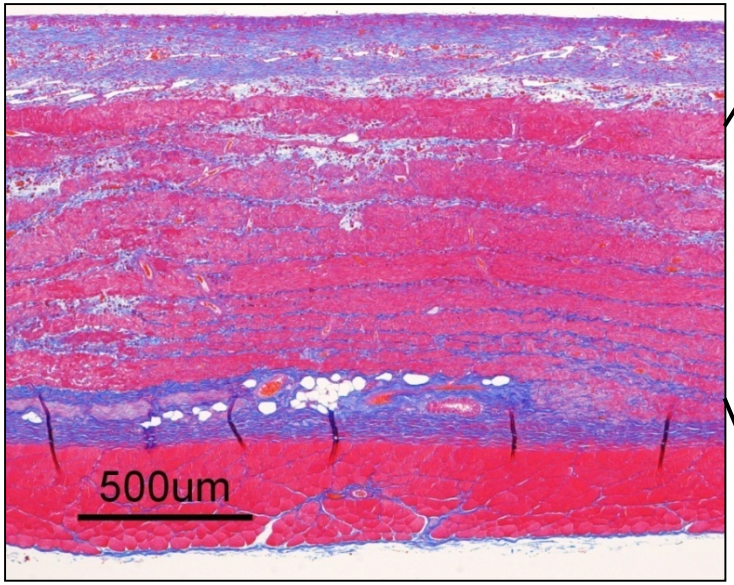
Tatsuya Shimizu and Hidekazu Sekine contributed equally to this work.

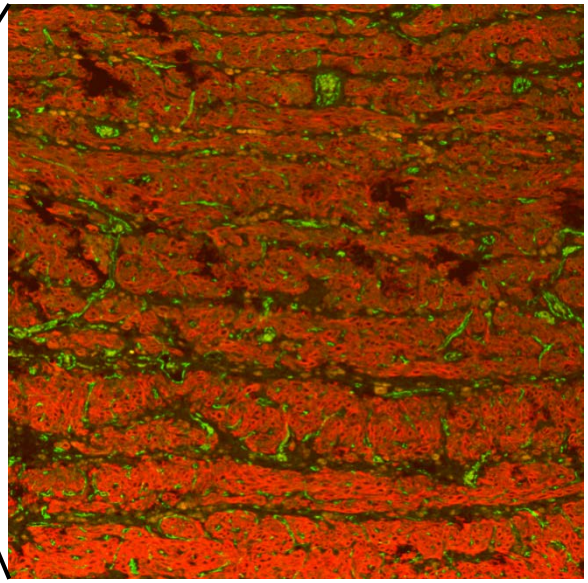


Overcome diffusion limits by multi-step transplantation



Allowed for sufficient blood-vessel formation





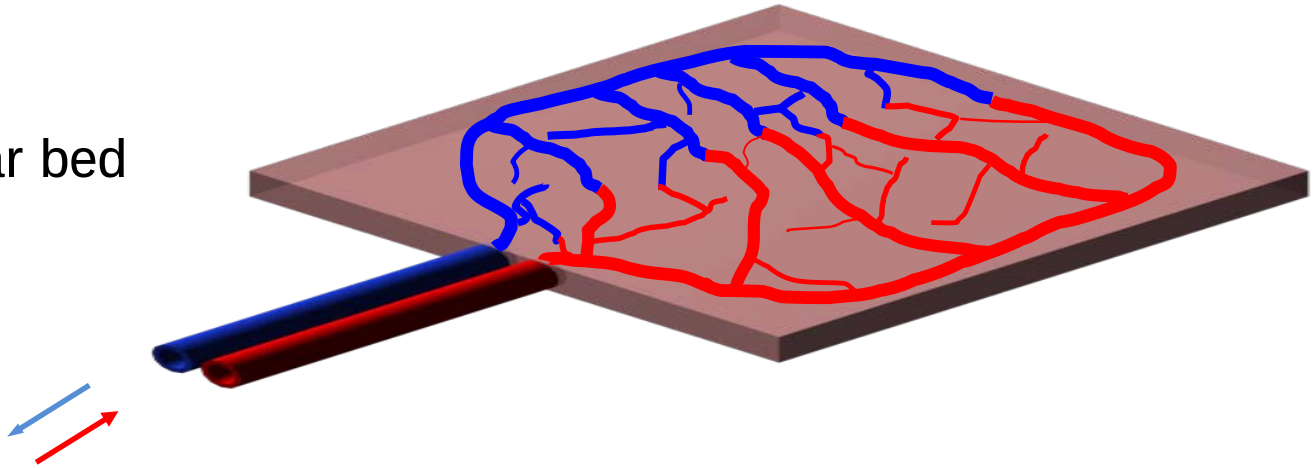
cTnT/CD31

Objective

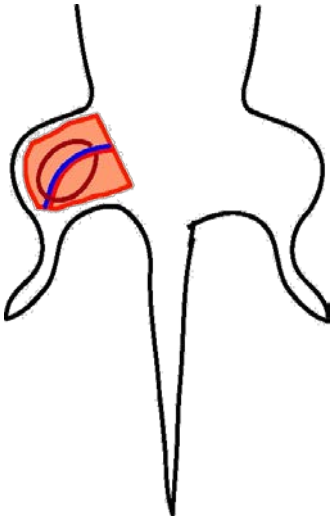
Followed by in vivo study, we hypothesized that it may be possible to overcome these diffusion limits in vitro by employing vascular bed and bioreactor system that allowed for sufficient vascular network in the engineered cardiac tissues.

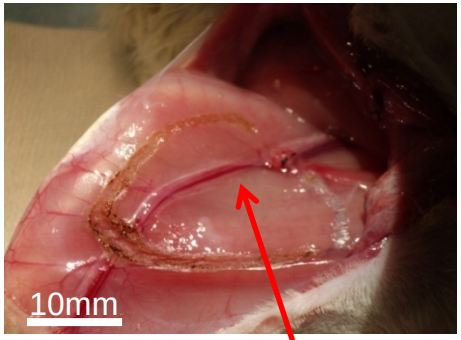
Imitating in vivo condition

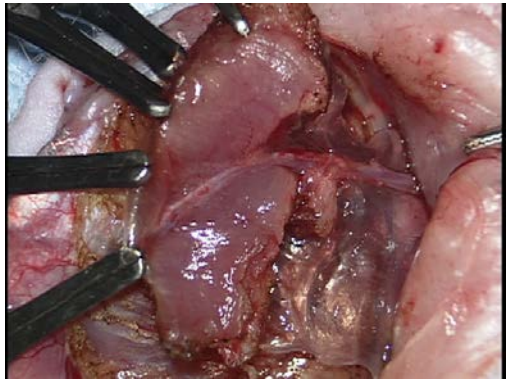
Vascular bed



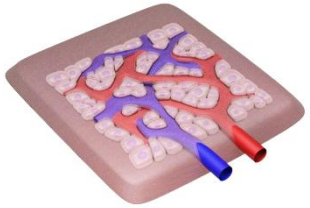
Ex vivo vascular bed

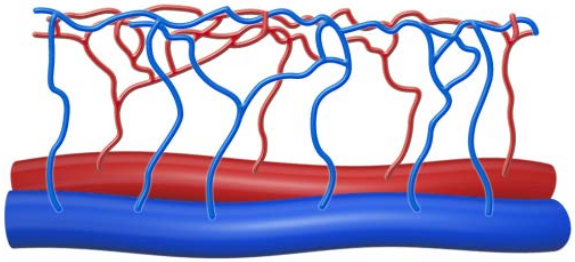


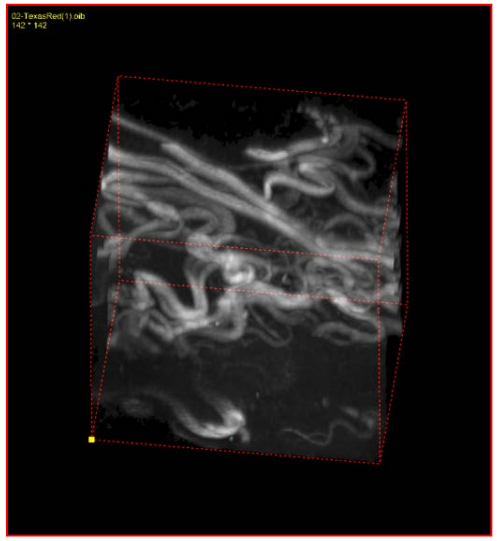




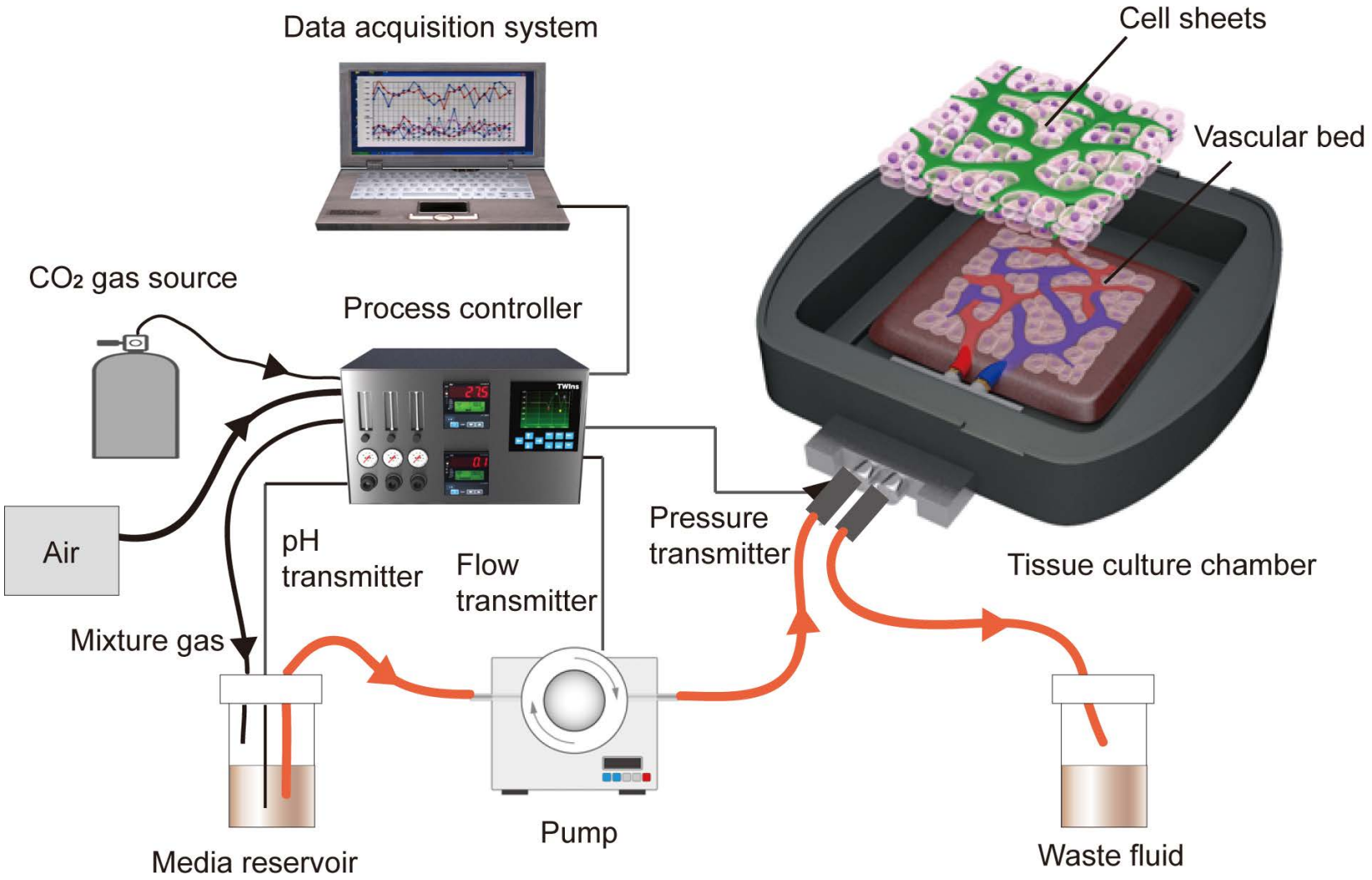
Muscle with femoral artery and vein



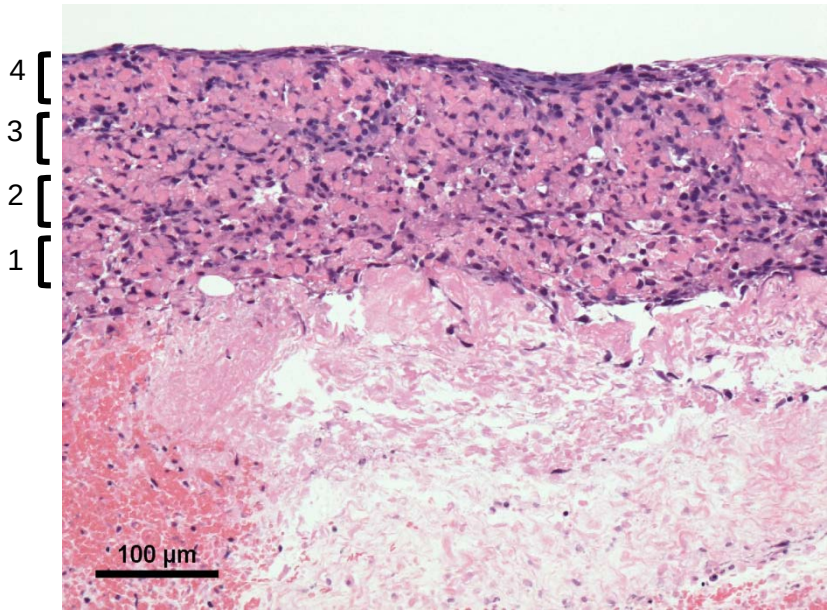


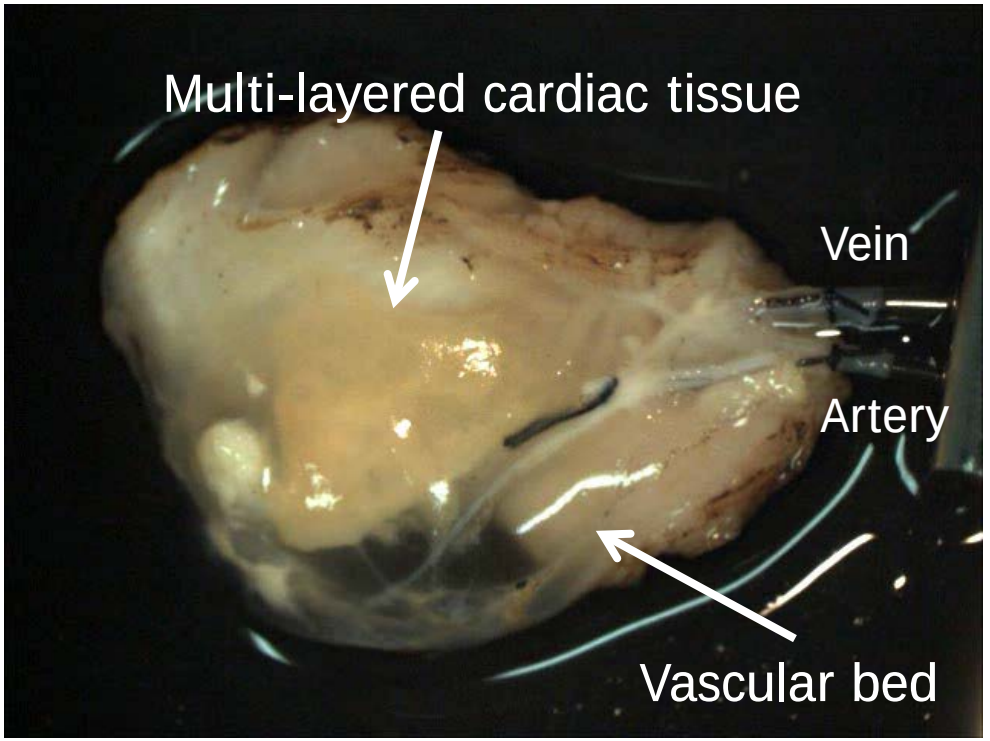


Schematic illustration of the concept used for in virto engineering of 3-D tissue with perfusable blood vessels



Multi-step overlaying of triple-layer cardiac cell sheets for scale up

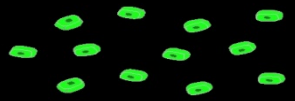




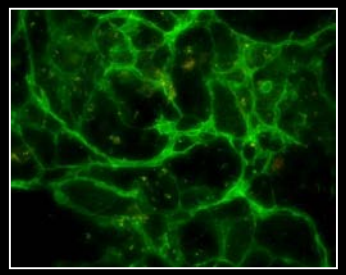
12 days after perfusion culture

Methods

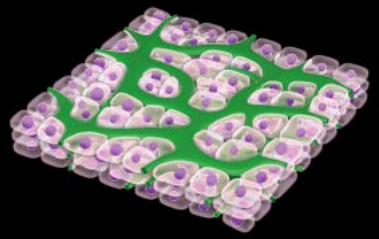
GFP-positive endothelial cells(ECs)



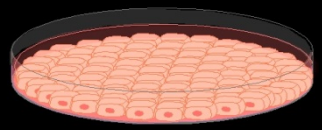
(+) or (-)



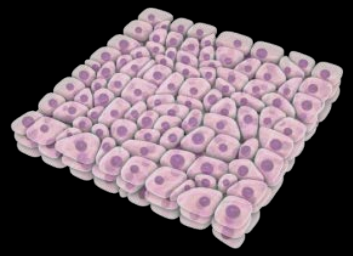
4 days culture

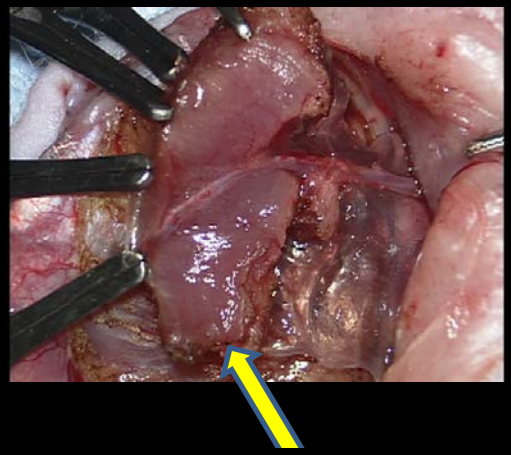


Layered

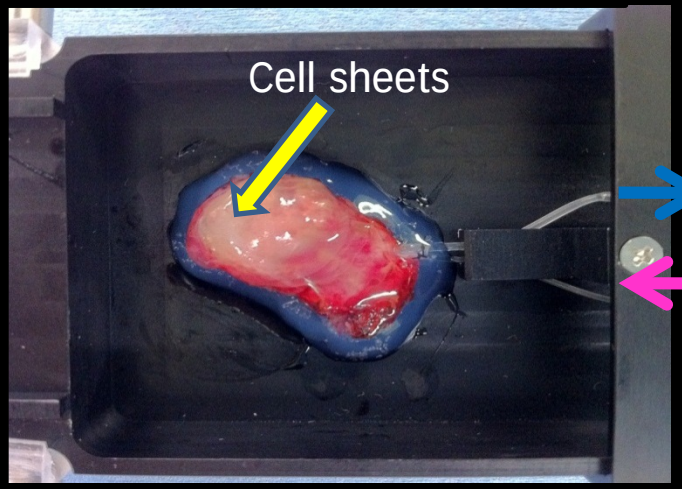


Cardiomyocytes



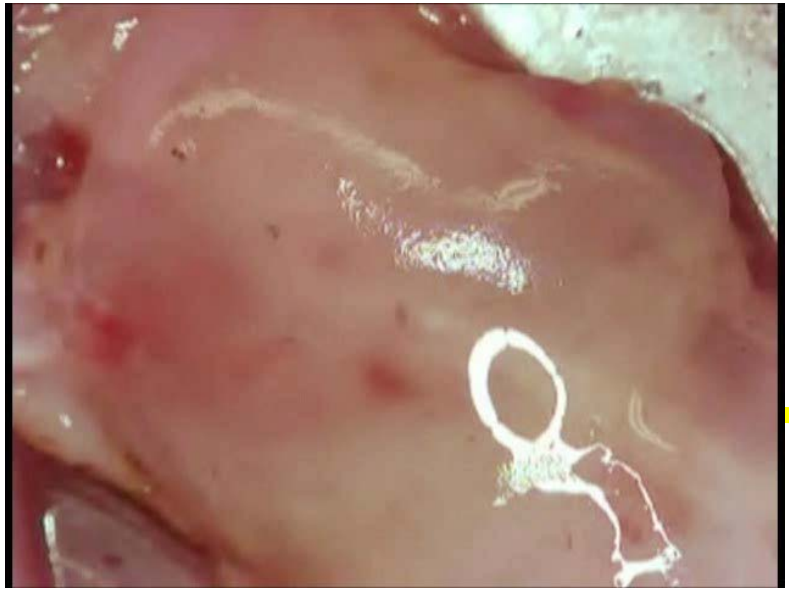


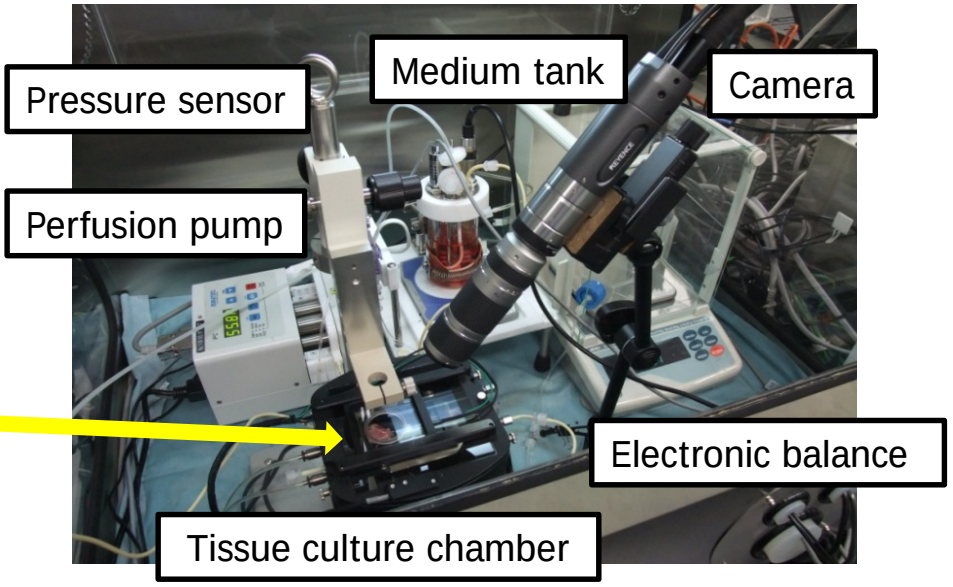
Femoral muscle with an artery and a vein

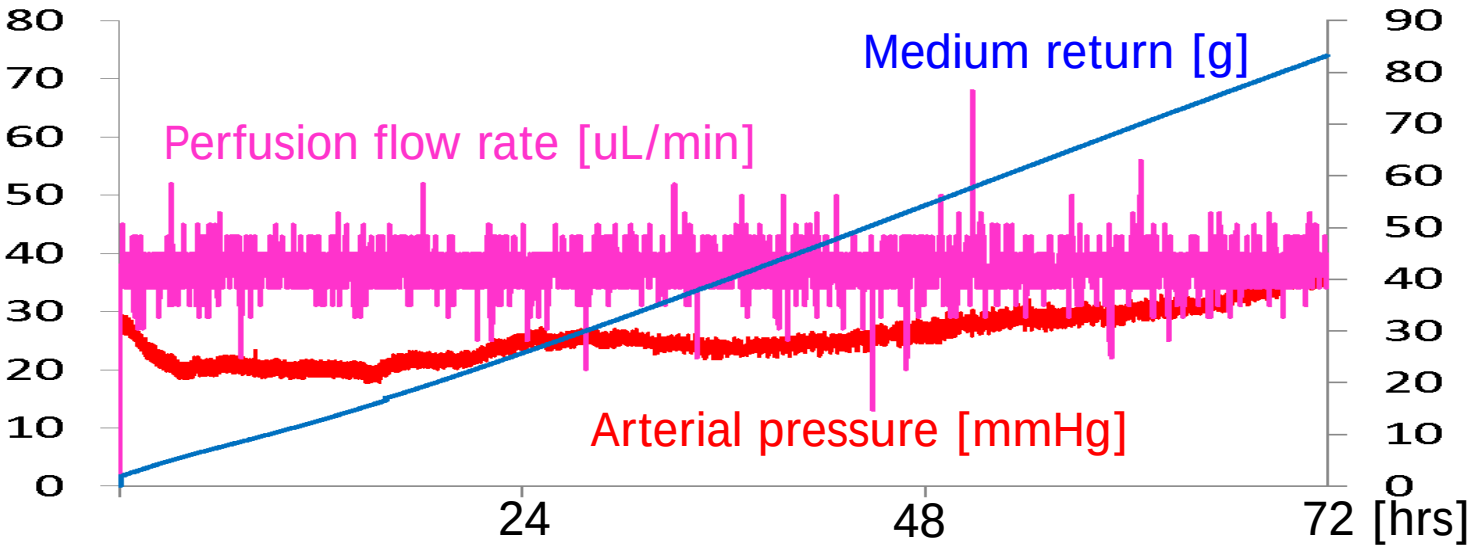


Tissue perfusion bioreactor

Bioreactor set up and tissue perfusion culture

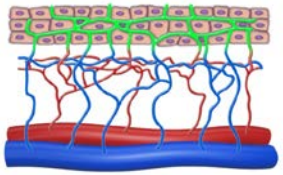






Contribution of co-cultured endothelial cells within the engineered construct

Black ink

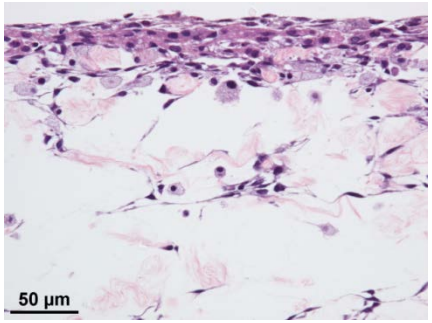


3 days after perfusion culture

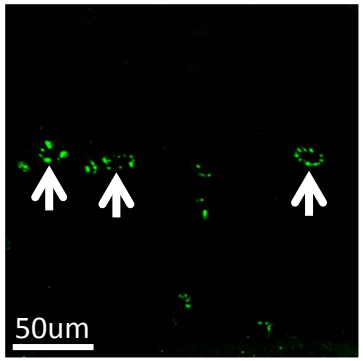
FGF-2 (-)

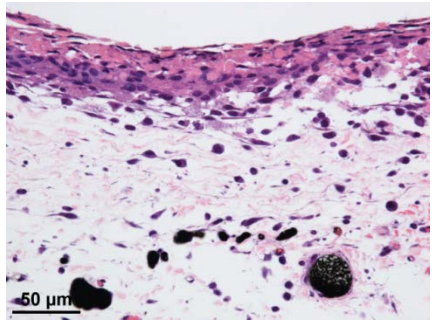
FGF-2 (+)

ECs (-)

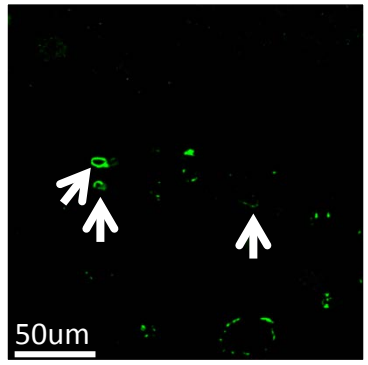


G

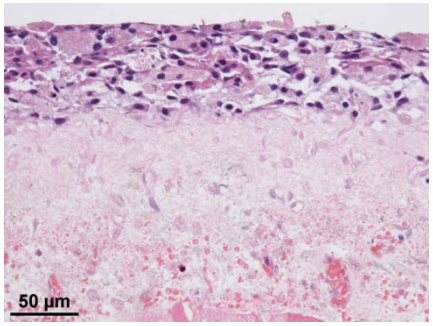




G



ECs (+)



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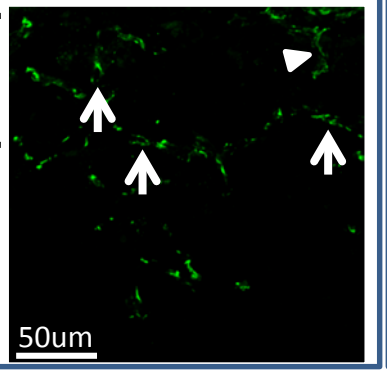

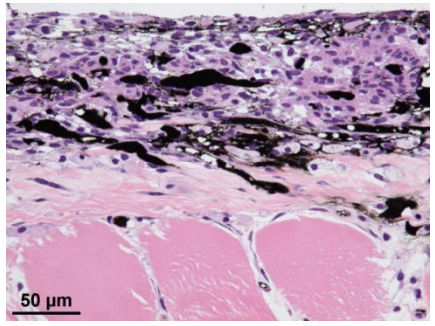

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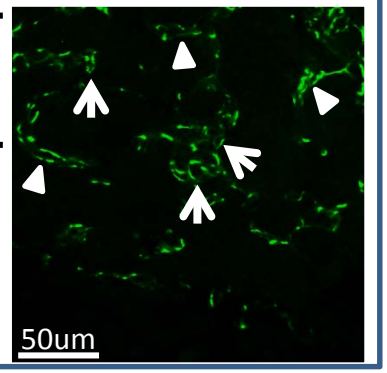
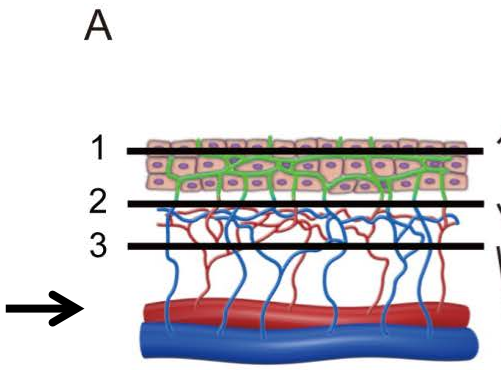
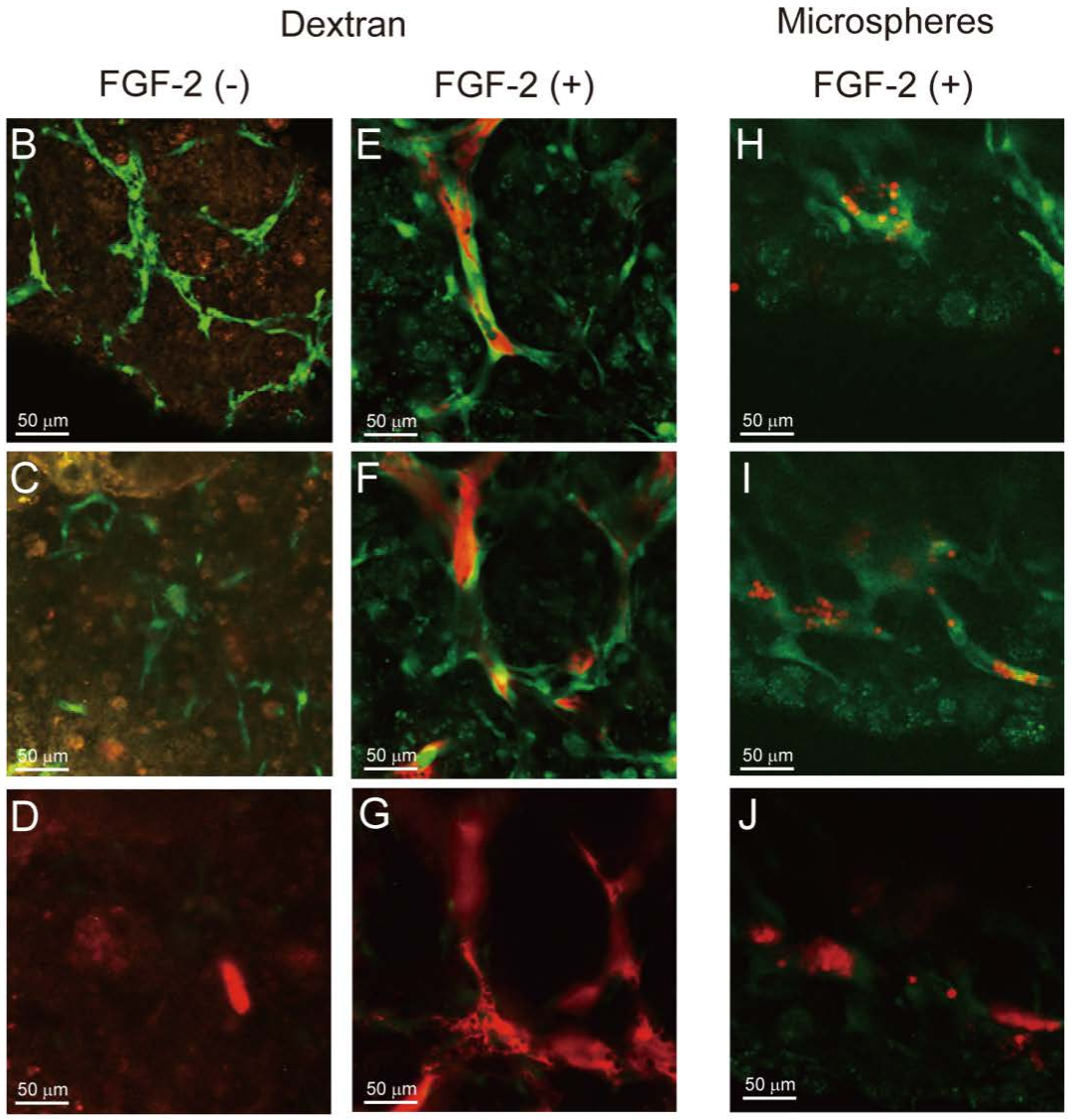


Contribution of co-cultured endothelial cells within the engineered construct

Two-photon microscope images

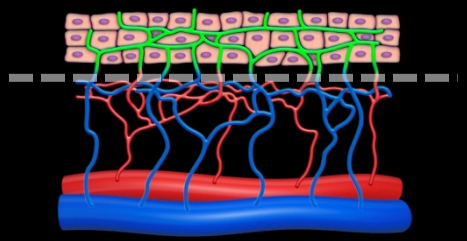


Perfused with
Red fluorescent dextran
or
Red fluorescent 4 um spheres



Contribution of co-cultured endothelial cells within the engineered construct

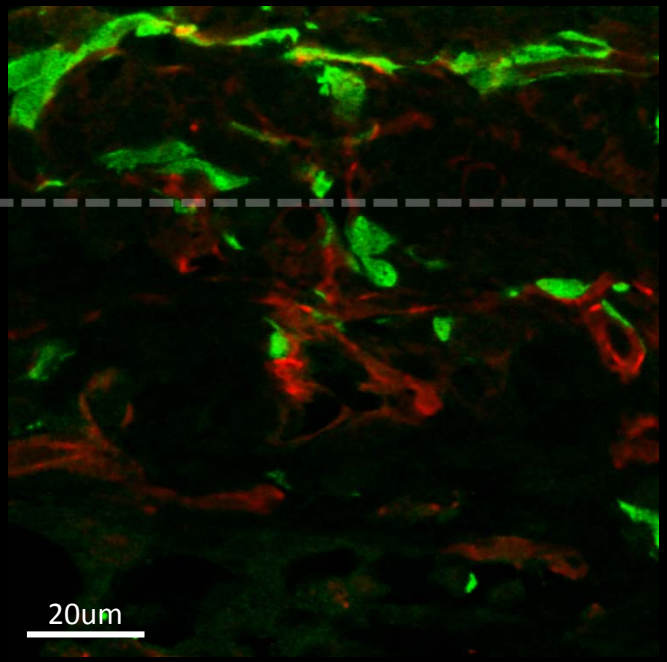
ECs (+) FGF-2 (+)

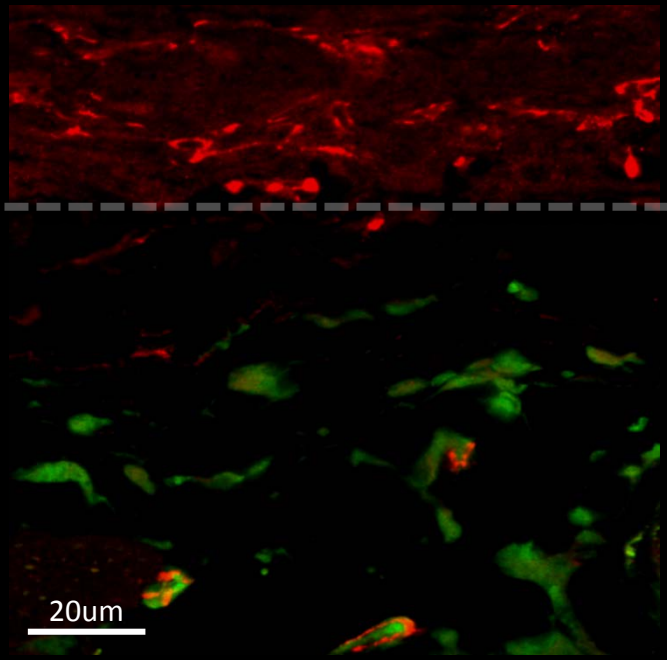


GFP positive grafted endothelial cells

GFP positive vascular bed

Border zone

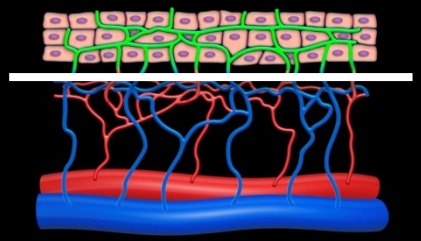


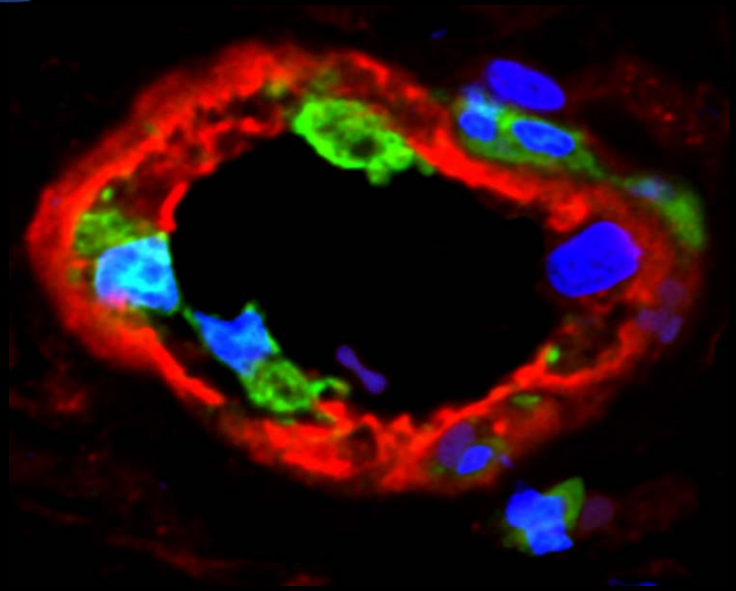


CD31 / GFP

Contribution of co-cultured endothelial cells within the engineered construct

ECs (+) FGF-2 (+)





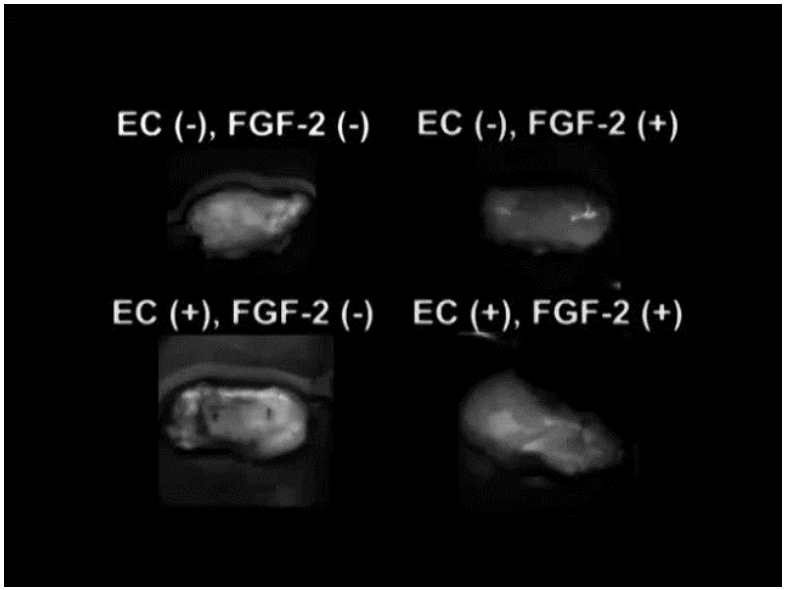
20 um

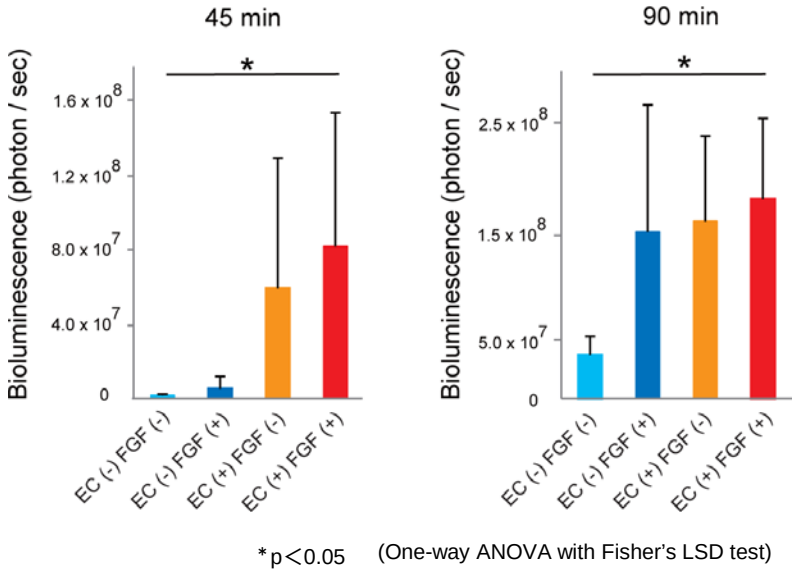
GFP ECs

Total ECs

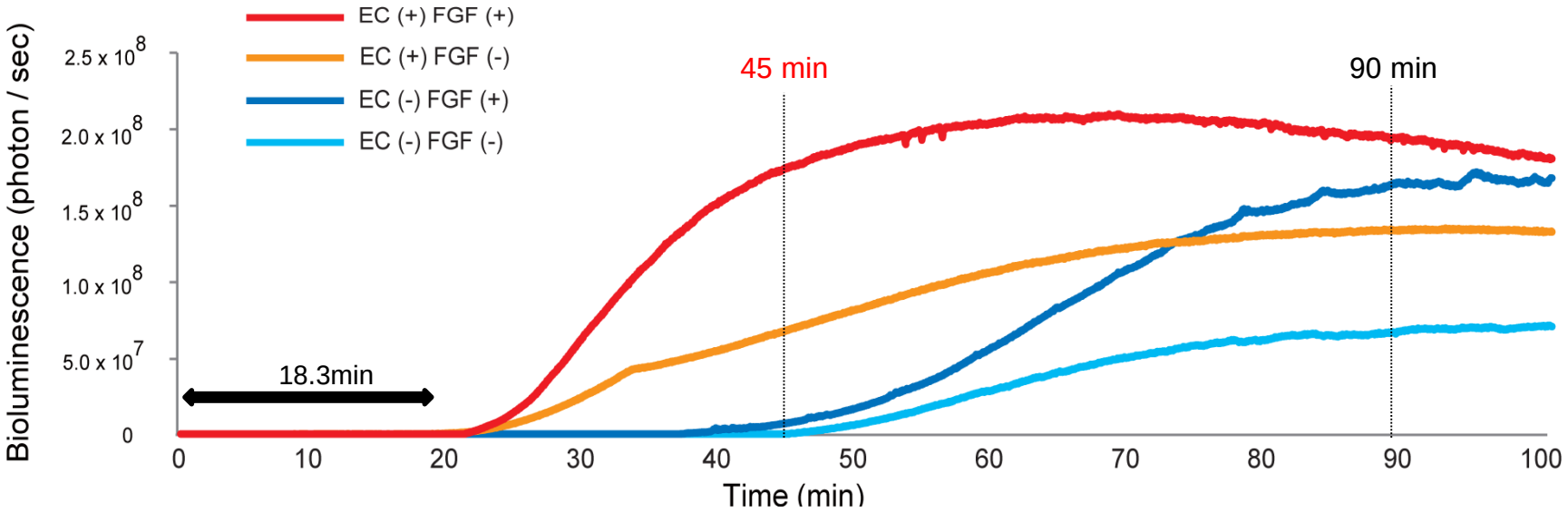
CD31 / GFP / DAPI

In vitro perfusable blood vessel formation and viable cardiac tissue fabrication

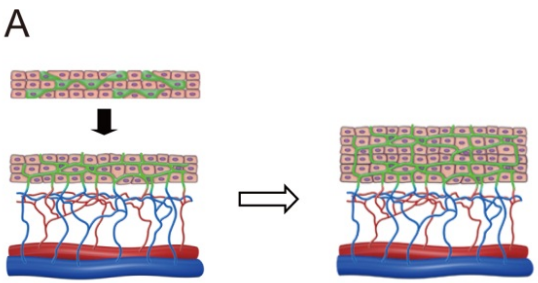


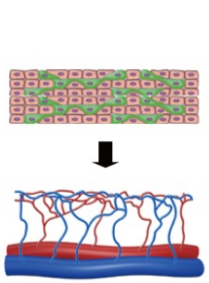


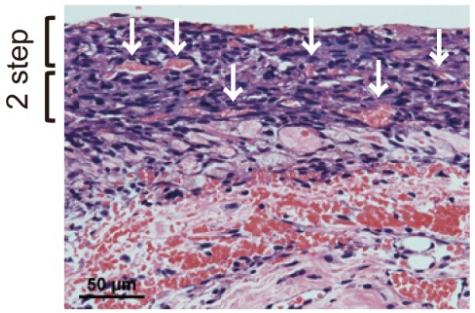
*p＜0.05 (One-way ANOVA with Fisher's LSD test)

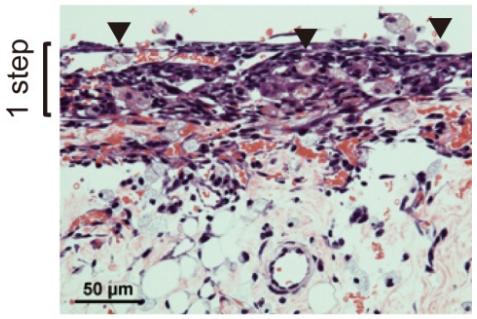


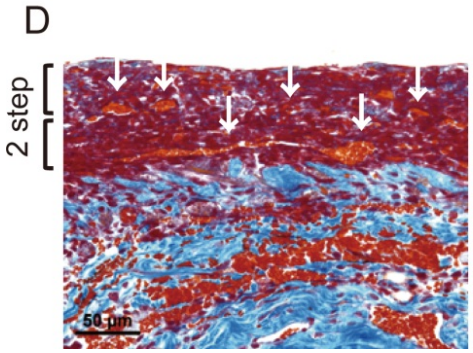
Double-step overlaying of triple-layer cell sheets for thick tissue formation *in vitro* conditions and their viability assay

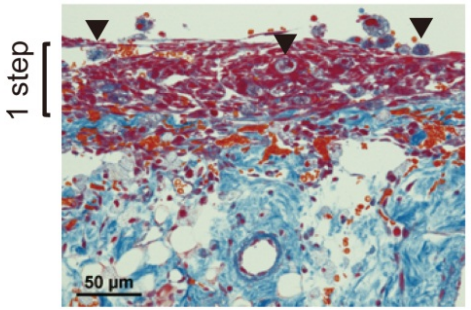












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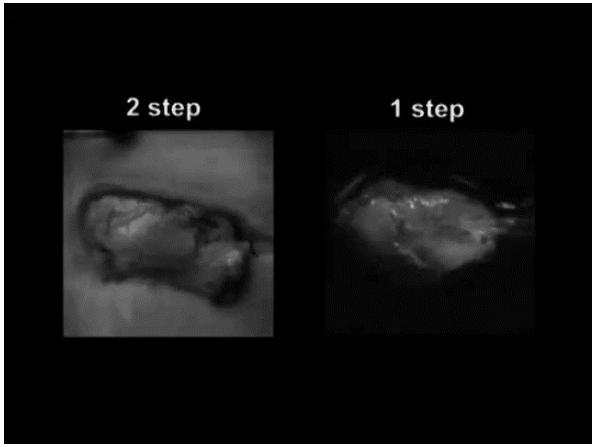
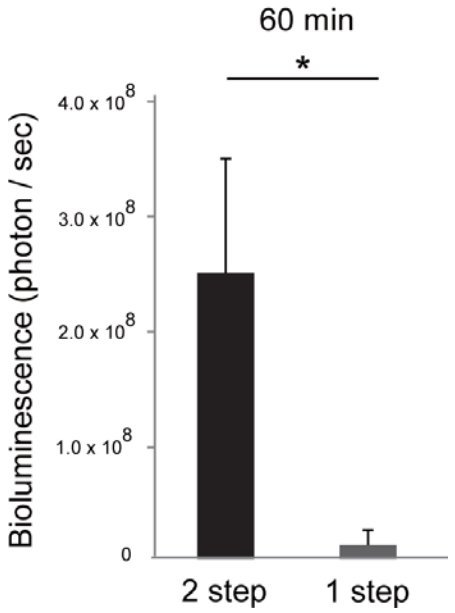
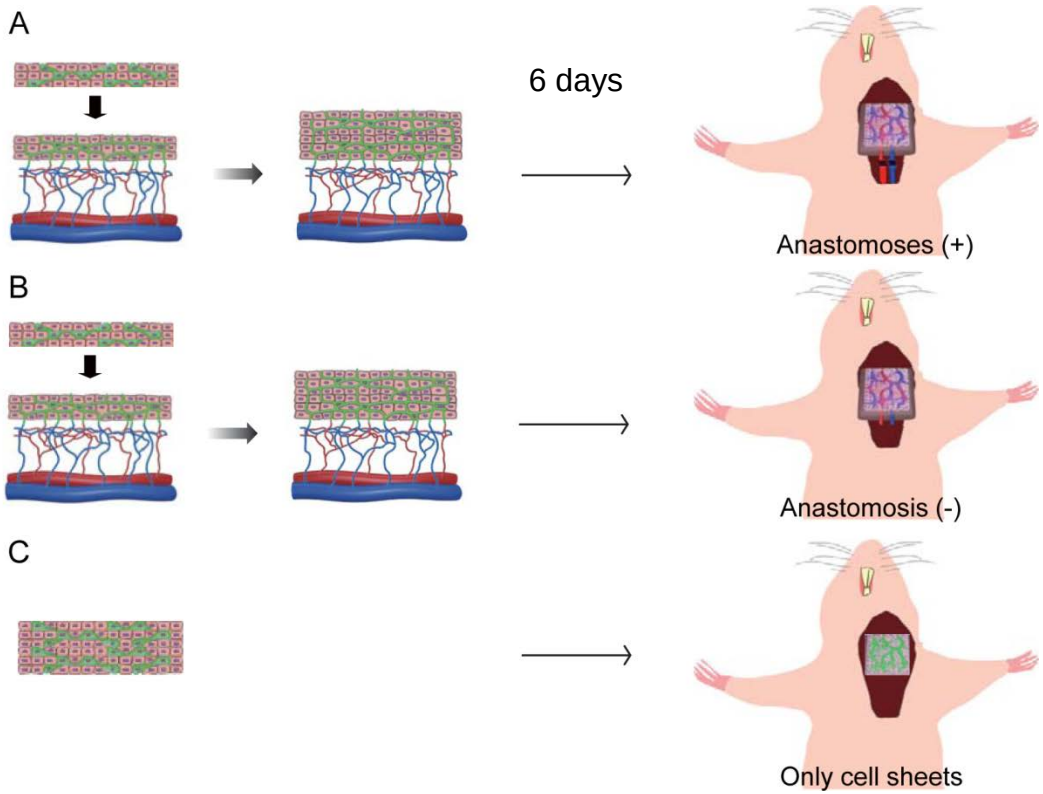
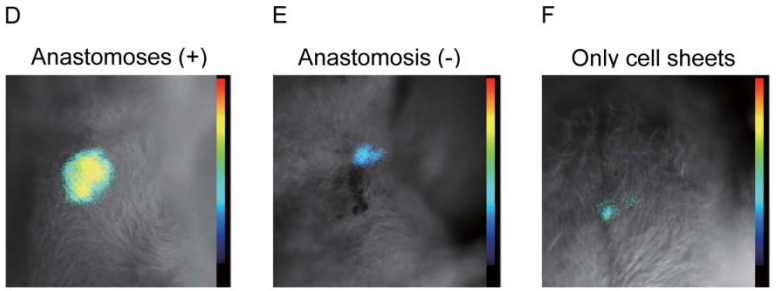


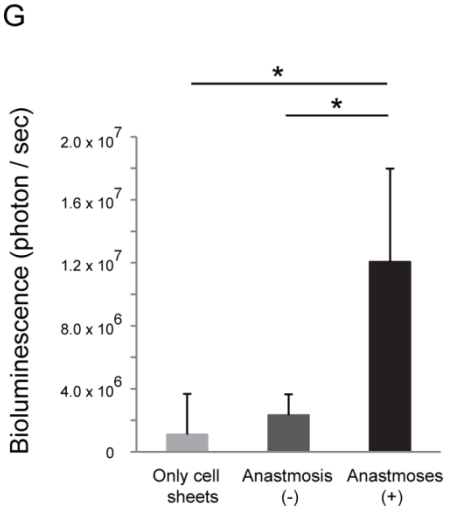


* $p < 0.05$ (Unpaired Student *t* test)

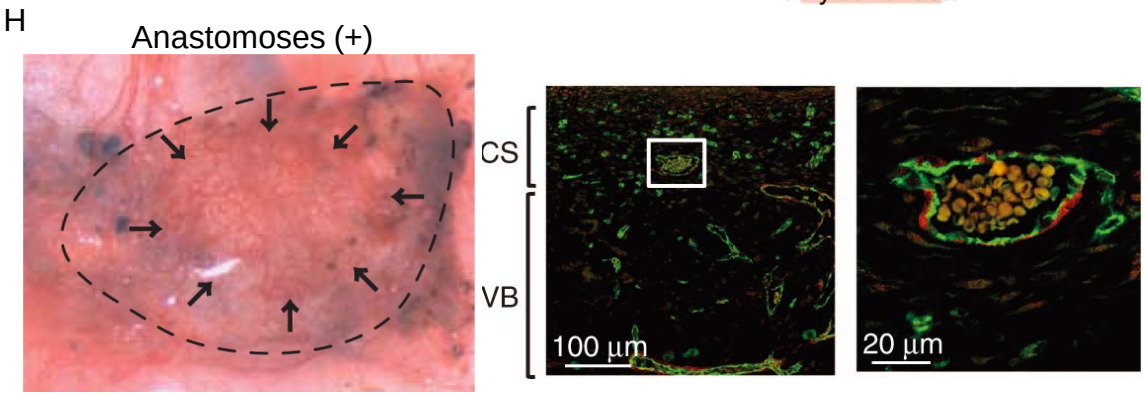
Transplantation of *in vitro* vascularized cardiac tissues





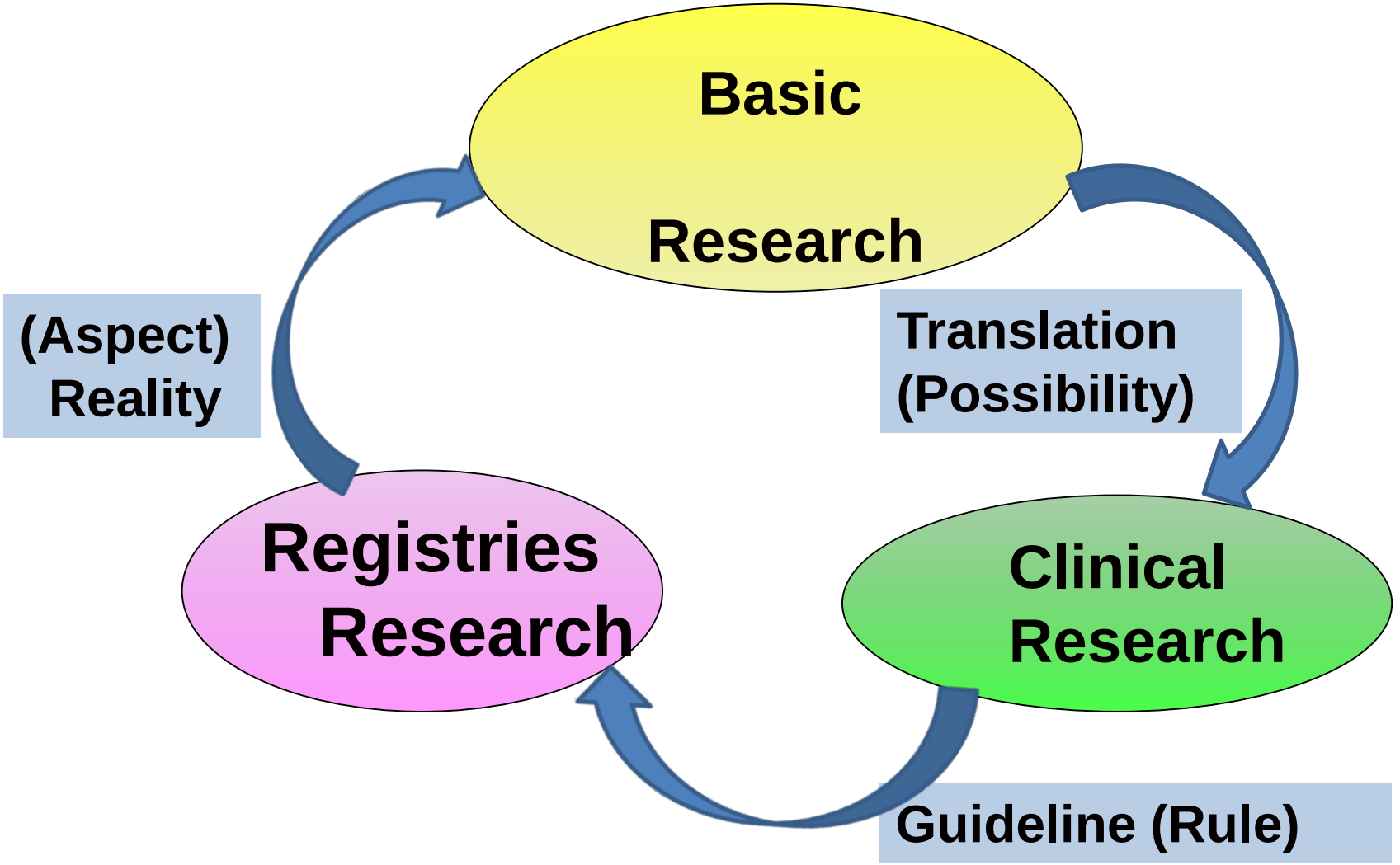


* $p < 0.05$ (One-way ANOVA with Fisher's LSD test)



2 weeks after transplantation

Why doctors are in need of pushing forward researchers ?




Shall we do our best for the suffering patients
Eiji Kobayashi
2011/06/10 21:53