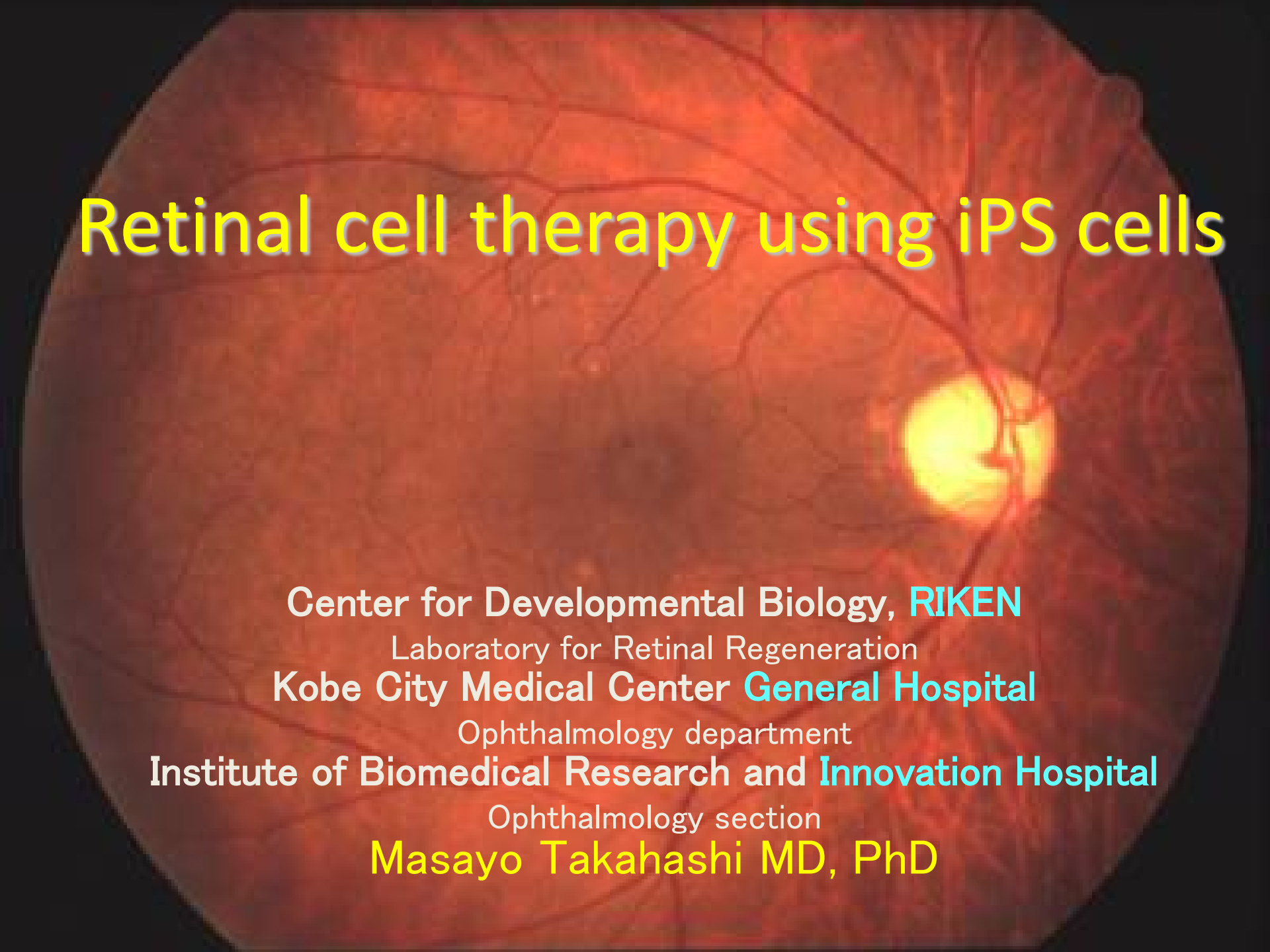


Retinal cell therapy using iPS cells

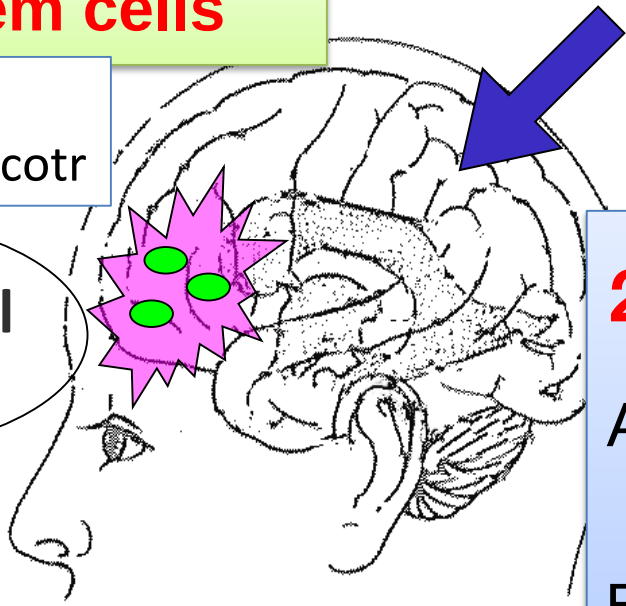
Center for Developmental Biology, RIKEN
Laboratory for Retinal Regeneration
Kobe City Medical Center General Hospital
Ophthalmology department
Institute of Biomedical Research and Innovation Hospital
Ophthalmology section
Masayo Takahashi MD, PhD

Regeneration of central nervous system using stem cells

1. . Regeneration from intrinsic stem cells

Mitotic factor
Differentiation facotr

intrinsic neural stem cells



embryonic tissue neural stem cell

2. Transpla

Functional cell
Mature cell
Enough amount

A **Replacement therapy**

B **Trophic effect**

Trophic factor
Immature cell
Small amount

Regulation for cell therapy in Japan

Enforcement 2015.11.25~

Preclinical research

Clinical research
(Act on the safety of regenerative medicine) forced on Nov 25, 2014

Clinical trial
(revised Pharmaceutical affairs act)
forced on Nov 25, 2014

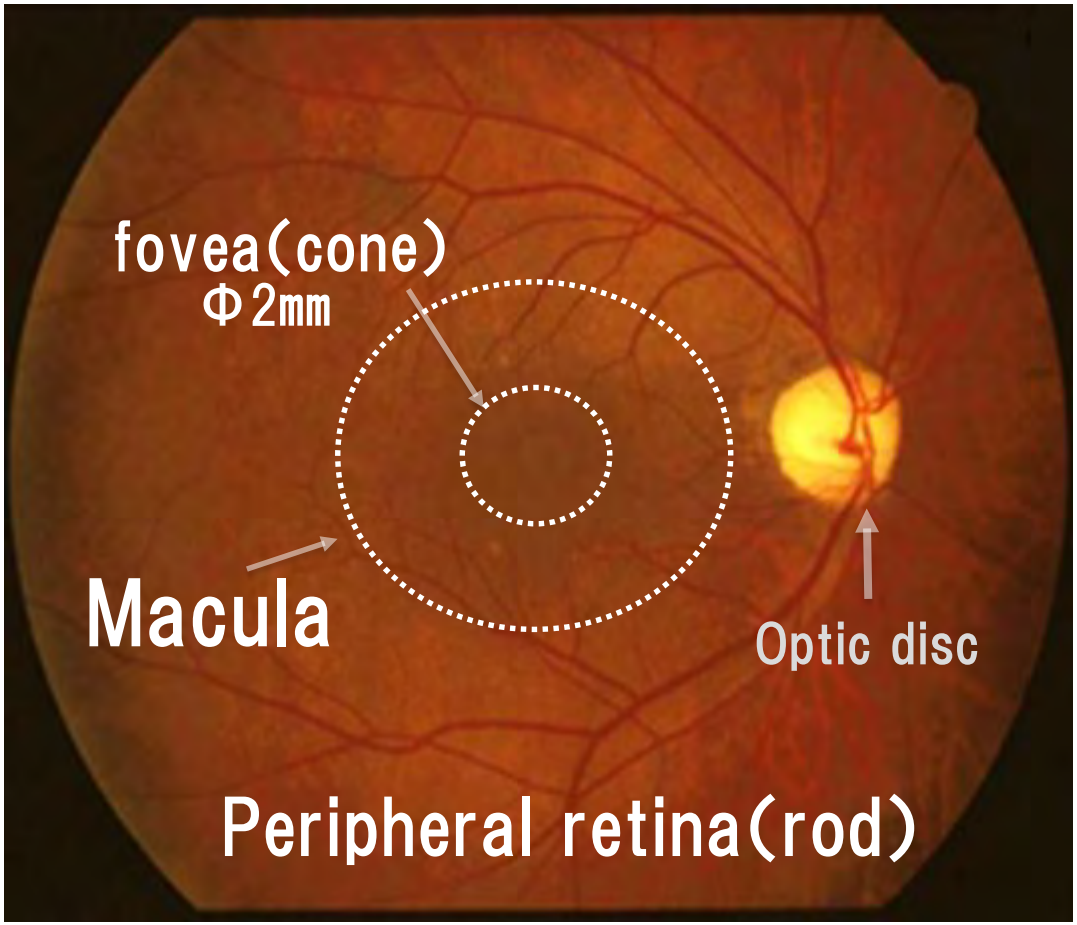
【Regenerative medicine 2014.11.25~】

※Earlier Patient Access !

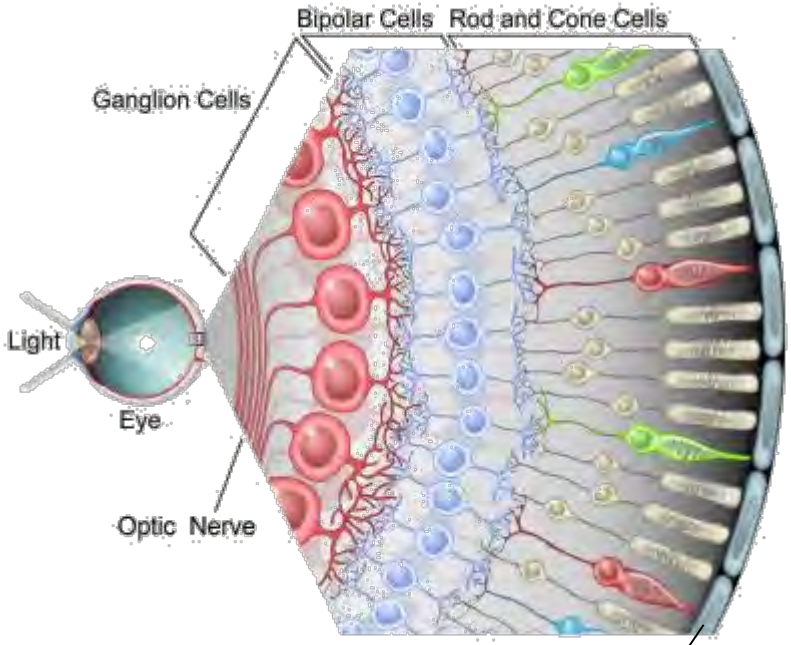
Informed Consent and Post Market Safety Measures

* Probable benefit: Confirmation of efficacy with small patient population.
** Safety: Earlier detection and evaluation of adverse events.

Retina

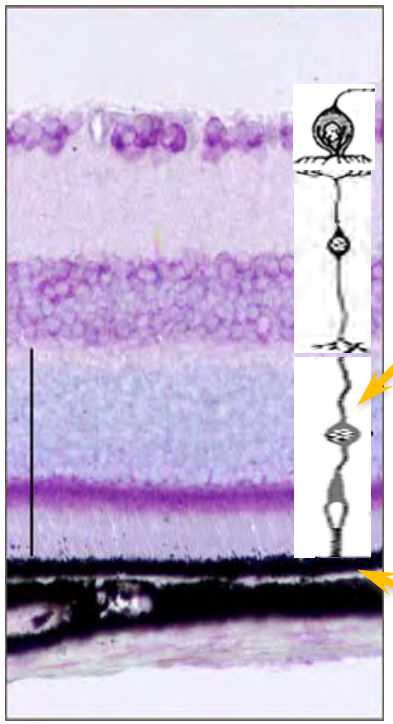


Fundus



Retinal pigment epithelium (RPE)

Retinal cell transplantation



Photoreceptor (for Retinitis pigmentosa)

Issue

Structure of photoreceptor cells

Purification

Functional connection with the host's neurons

RPE cells (for AMD)

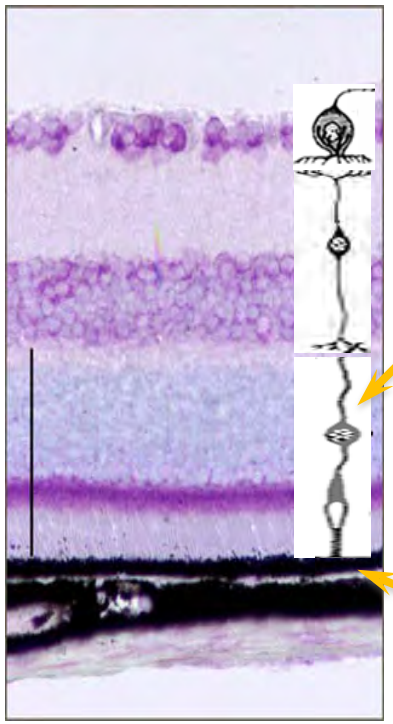
Started clinical study (Safaty)

Issue

Variety of cell forms

Efficacy

Retinal cell transplantation



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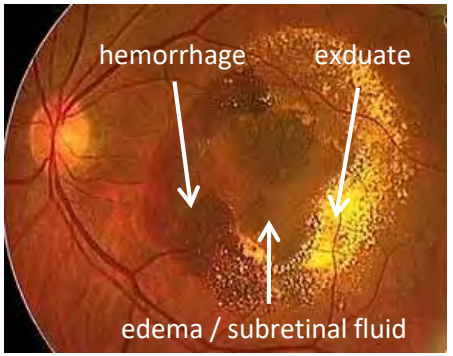
Issue

Variety of cell forms

Efficacy

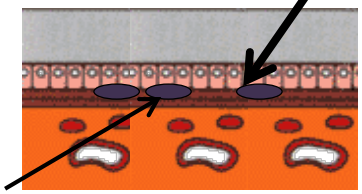
Age-related macular degeneration （AMD)

Current treatment
（anti-VEGF drug injection）



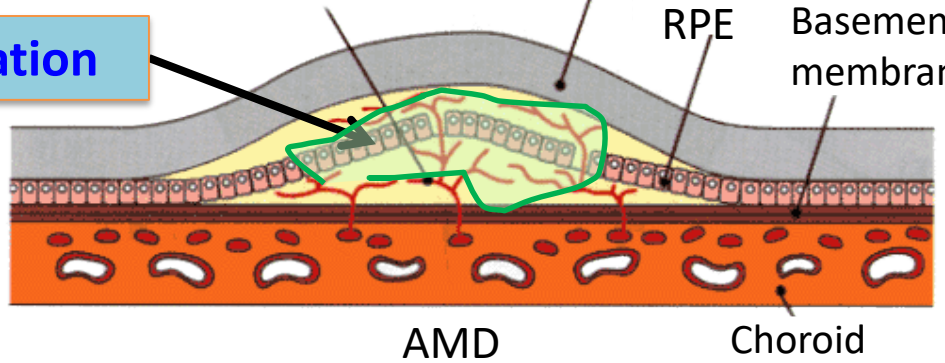
Neovascularization

RPE transplantation



Drusen

RPE atrophy



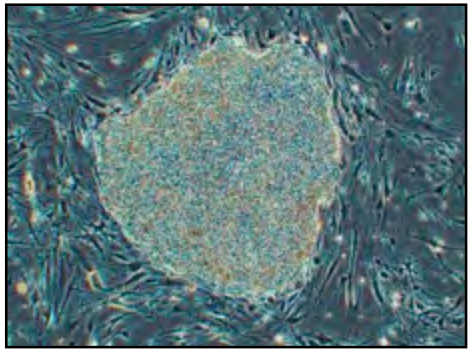
Dry-type
Chronic

Wet –type
Subacute

Treatment for age-related macular degeneration (AMD) with hiPS-RPE cell sheets

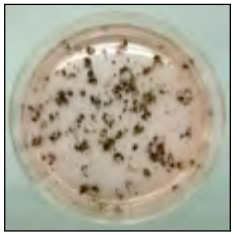
Plasmid（Glis1）
（Reprogramming）

iPS cells 24 line → 6 line



Differentiation

4 Mo



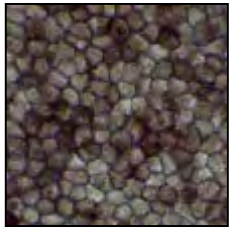
4 Mo

Patients with AMD

Skin fibrobrasts

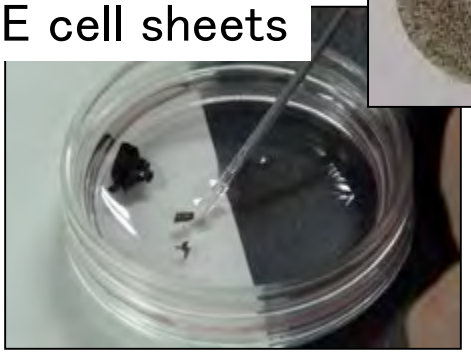
Pure RPE cells

2 Mo



3 line

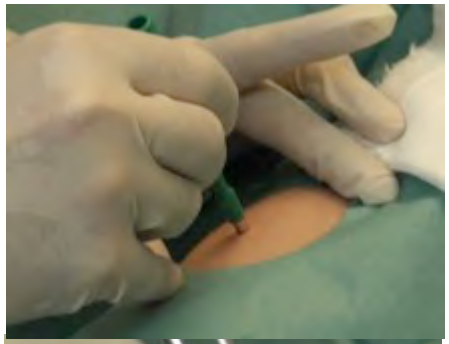
RPE cell sheets



Transplantation

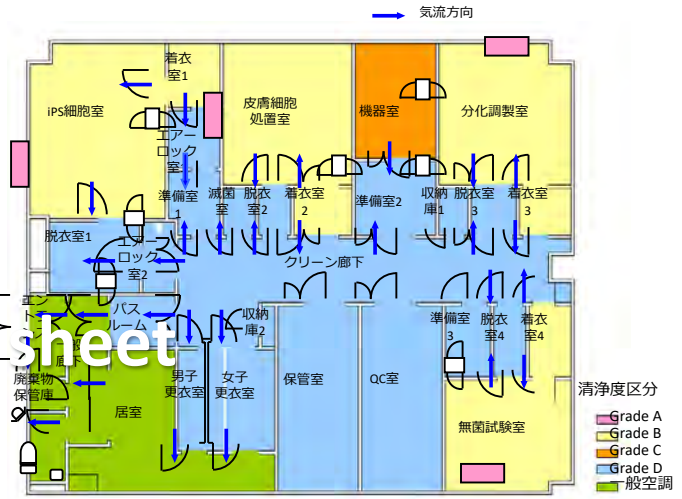
Cell Processing Facility (CPF)

Skin harvesting from the patient



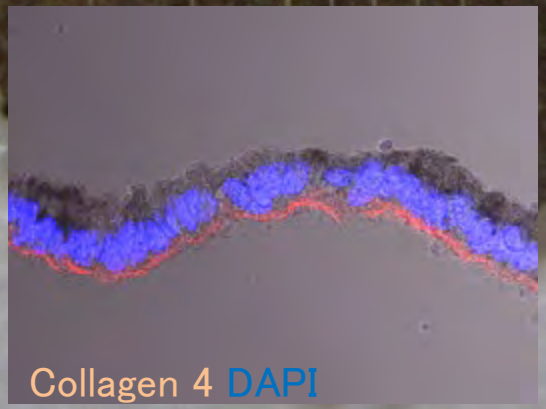
Skin trephine

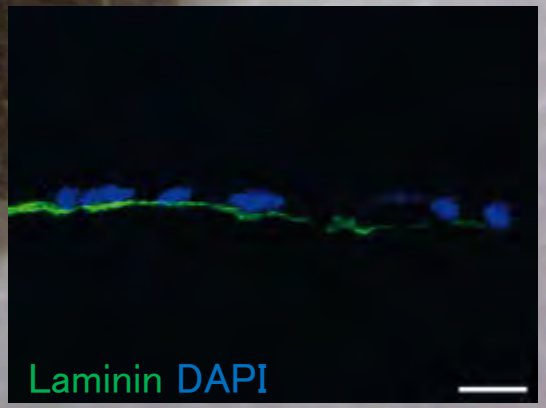
Cell culture in the cell processing facility

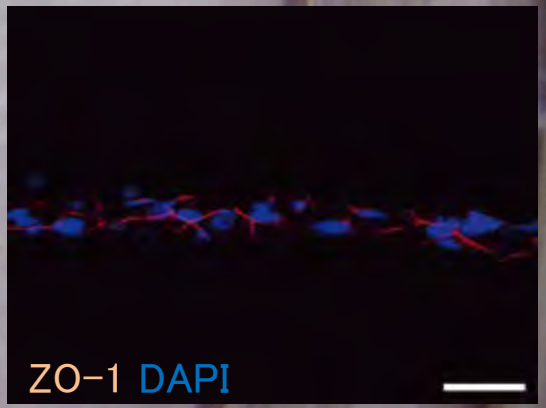


Quality & quantity of hiPSC-RPE cell-sheets

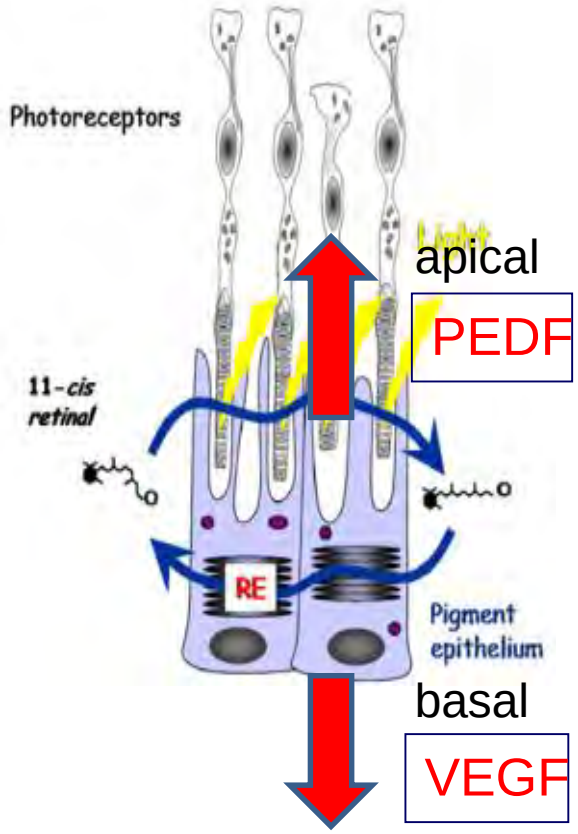
Hiroyuki Kamao

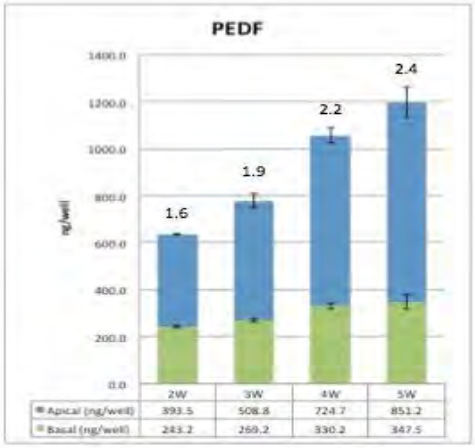


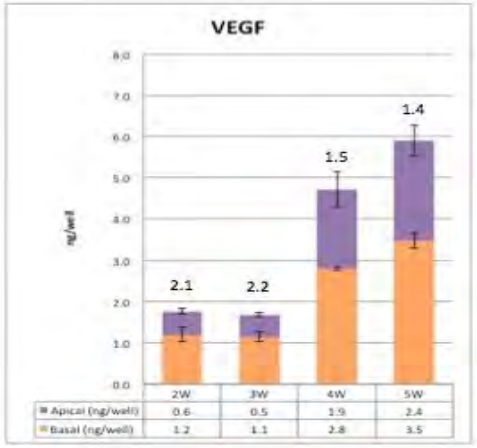




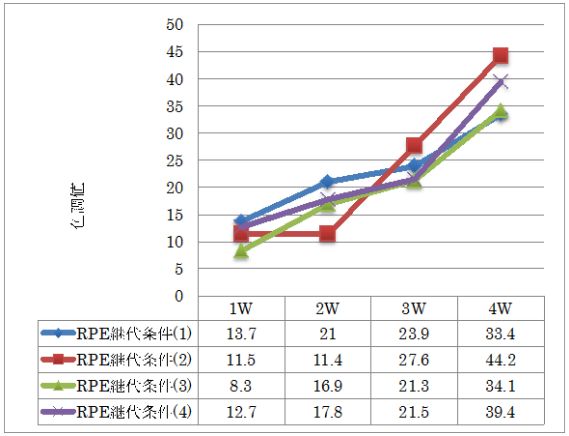
Monitoring points : RPE function



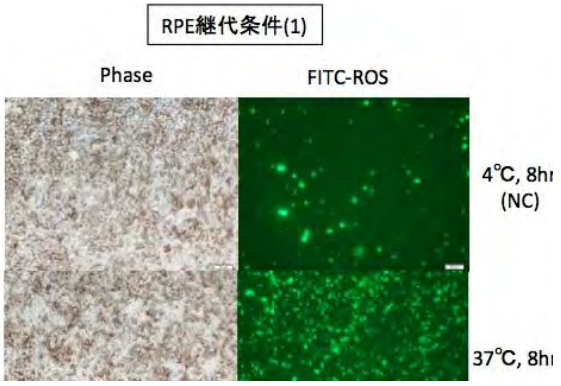




PEDF Growth factors VEGF

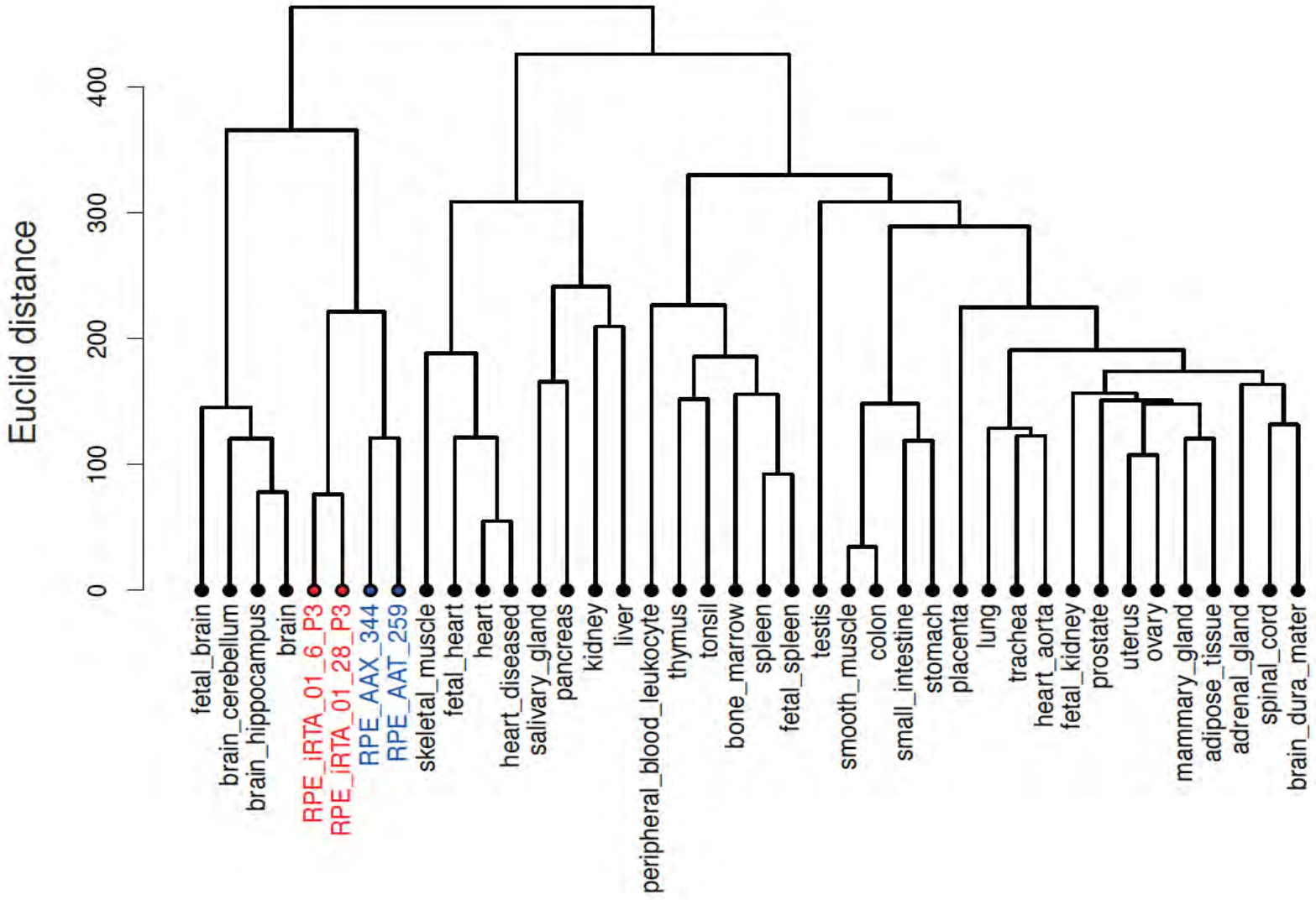


Color



Phagocytosis

Cluster analysis of gene expression profiles



RPE & Tumors

- No report of metastatic tumor ever in the history
  - Even in the familial tumor patients

=various cancer formation with hereditary oncogene (ex. p53) mutations

only hyperplasia of RPE occurs

- PEDF (pigment epithelial derived factor)

= strong antitumor factor

Cells

- Eye ball is full of Retinoic Acid = antitumor factor

Environment

Summary of tumorigenicity test

（Kanemura et al.2014 PlosONE)

NOD-SCID mice, subcutaneous, 1x10^5 cells in Matrigel

Purified hiPS-RPE did not generate tumors

	donor	Vector	# of animal	tumor
1st 2010. 11～2011. 9	RPE cell	Retrovirus Sendai virus Plasmid A	57	**0**
2nd 2011. 2～2012 4	RPE cell	Plasmid A	11	**0**
	RPE cell : OCT3/4 remnant+	Plasmid A	7	**0**
3rd 2012. 3～10	RPE cell	Plasmid B	27	**0**
4th 2012. 12～2013 7	RPE sheet （subcutaneous・subretinal）	Plasmid B	16	**0**

The most important thing is ….

the robust protocol that will not make tumor
(even with genetic changes)

Safety issue (risk size) will be different depending on

1. Final cell types
2. Treatment procedure (i.v. or surgery)
3. Host environment (diseases)

Hazard Level

1. The protocol that sometimes makes tumor in vivo
2. Purity: contamination of tumor cells

↑ Actual hazard — difficult to prove negative

↓ Possible hazard — easy to find

3. Genetic change: SNV, Indel, CNV、Plasmid remnant
4. Epigenetic change …..

Regulatory Science vs Basic Science

Requirements for replacement therapy

Quality 1: function

Quality 2: Purity

Kuroda et al. 2012 PlosONE

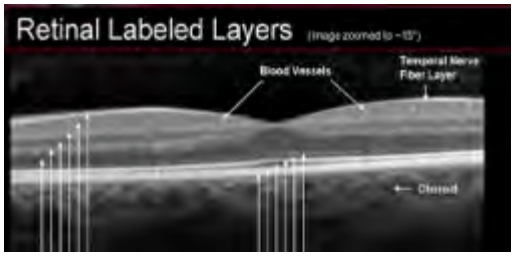
Quantity: Small amount of cells

Safety: no tumor formation

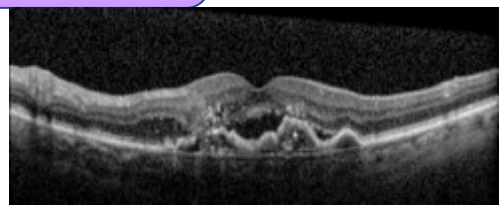
Kanemura et al. 2013 Scientific report

OCT ; fine examination

OCT



OCT

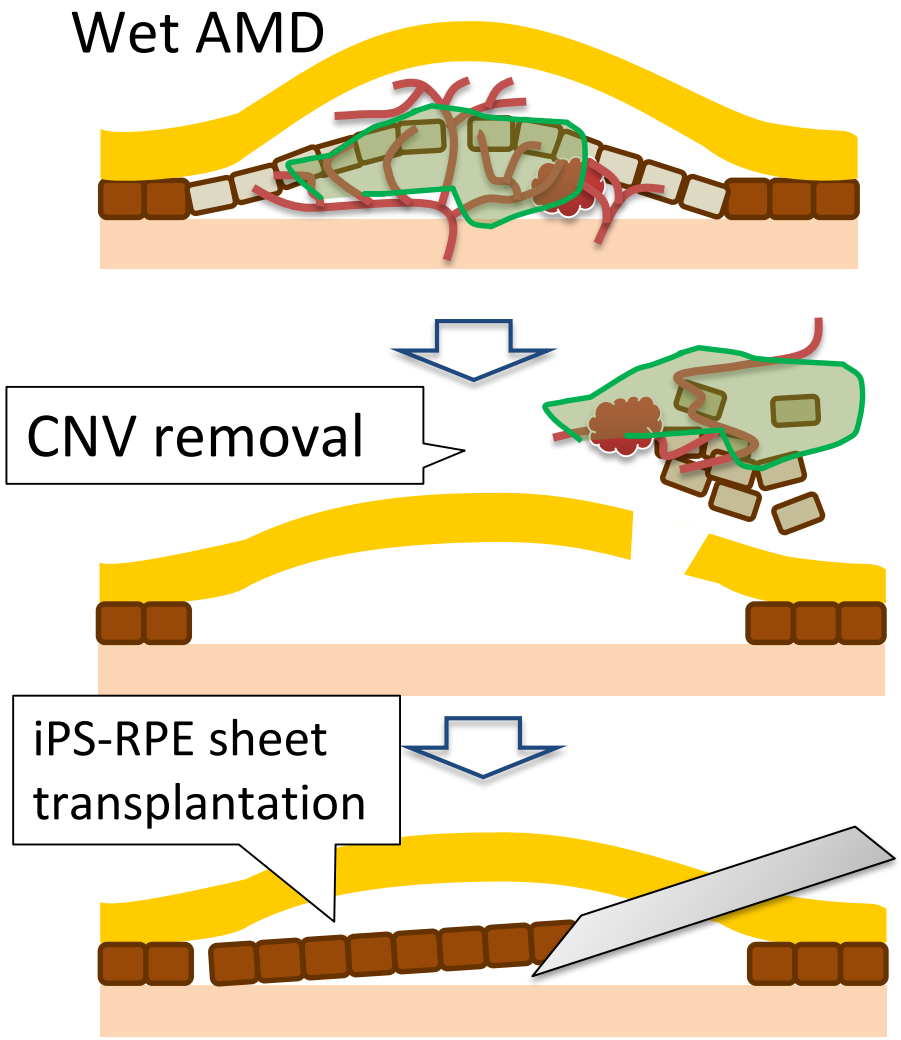


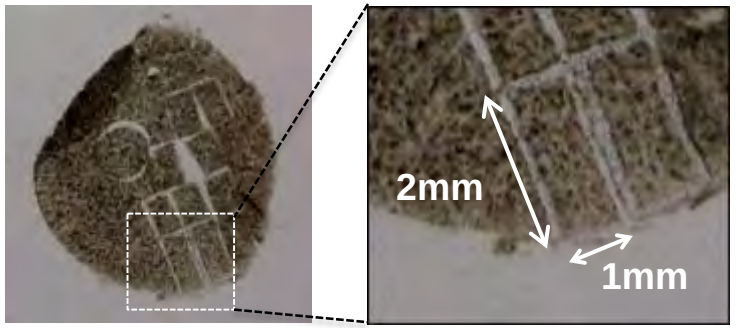
Clinical application

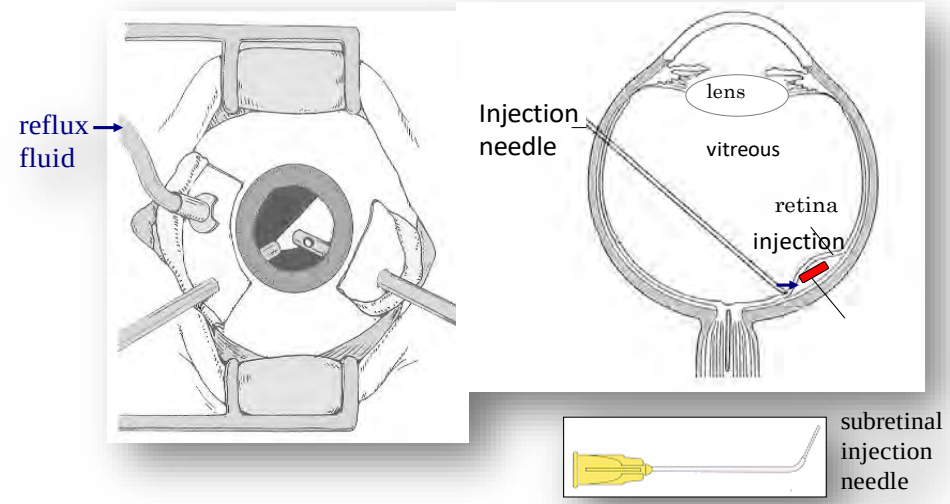
SURGERY ROOM

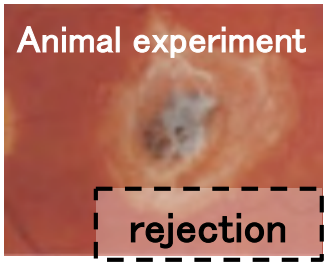
(SEP. 12. 2014)

iPS-RPE sheet transplantation

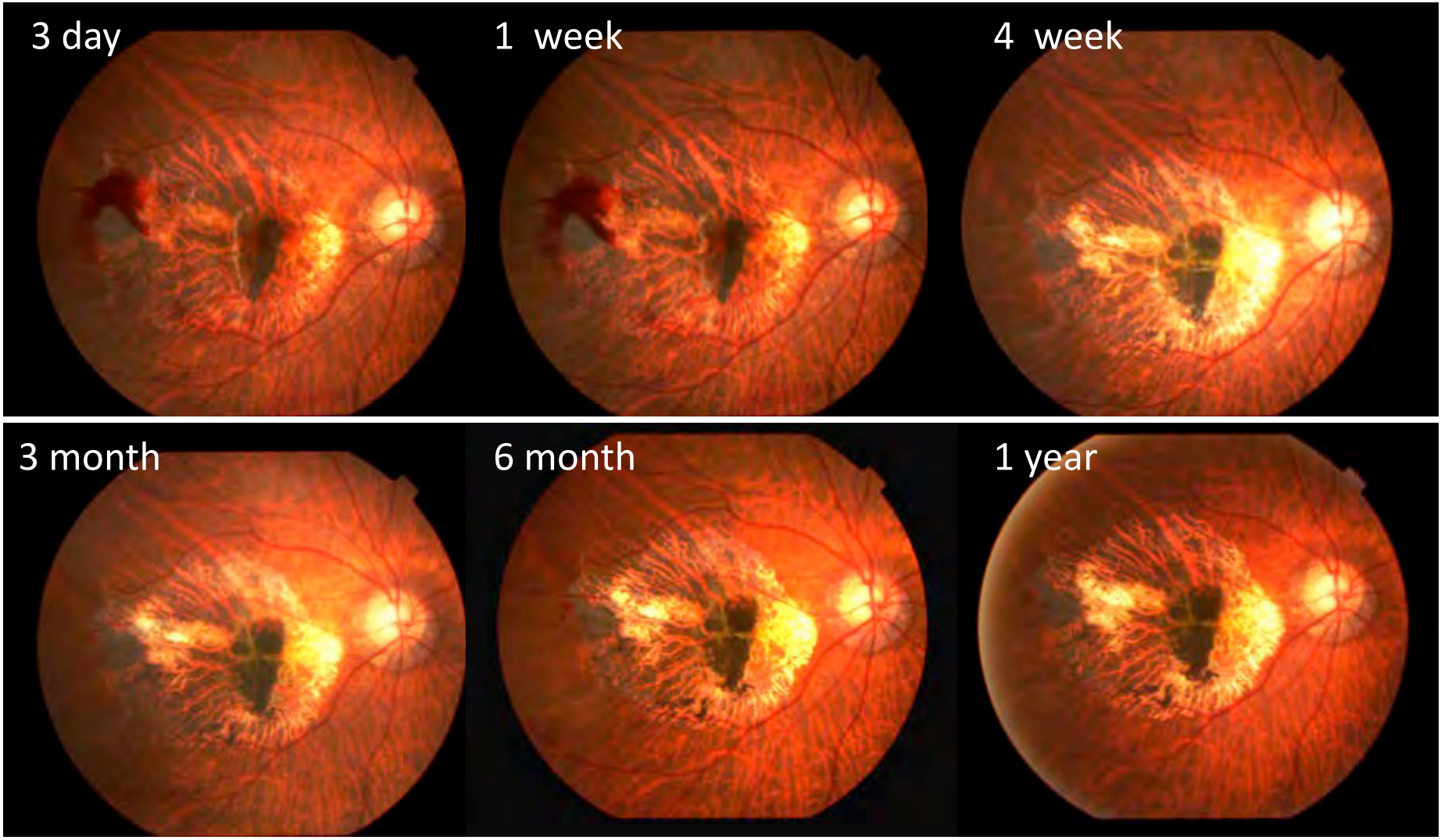




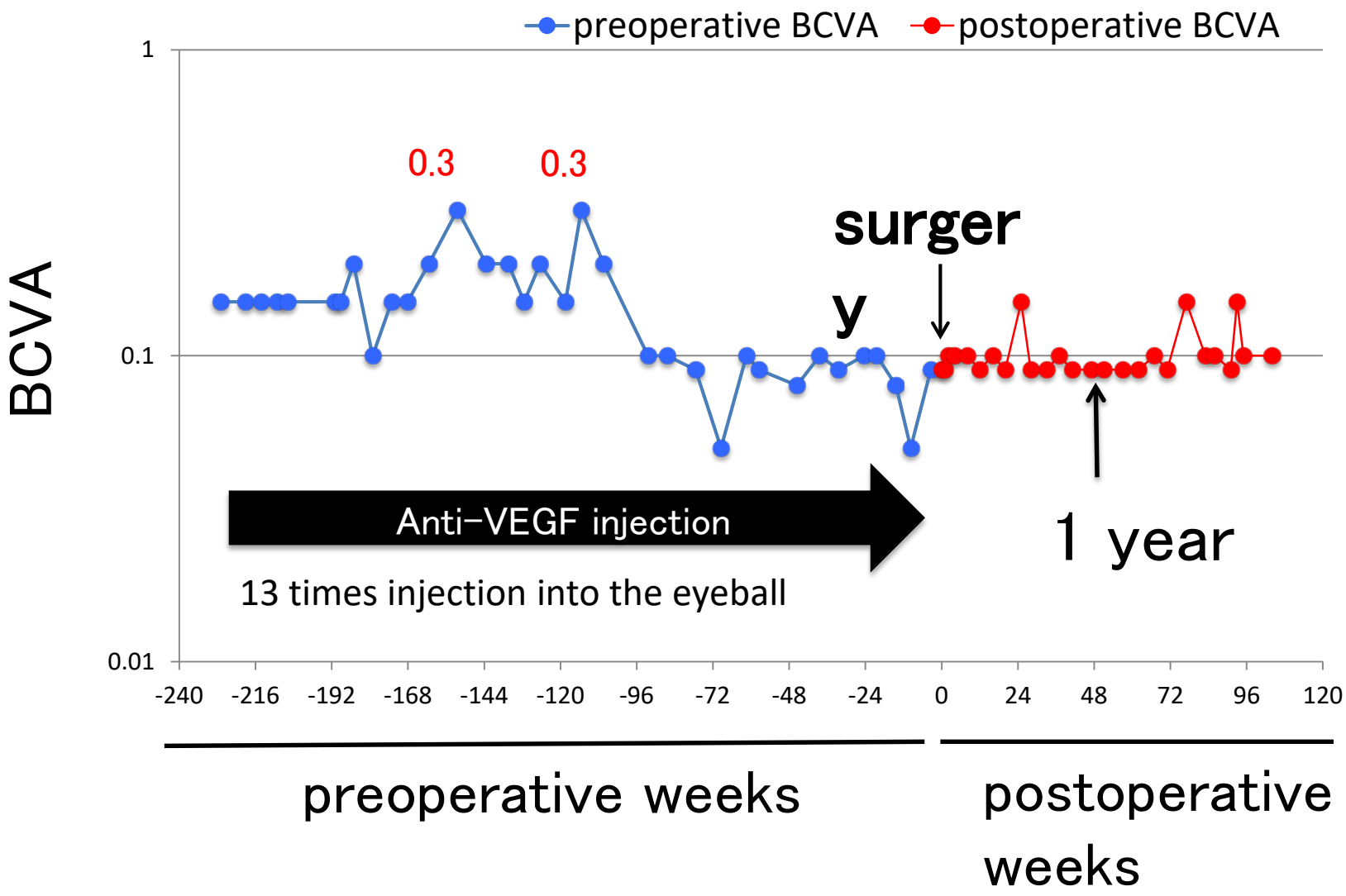




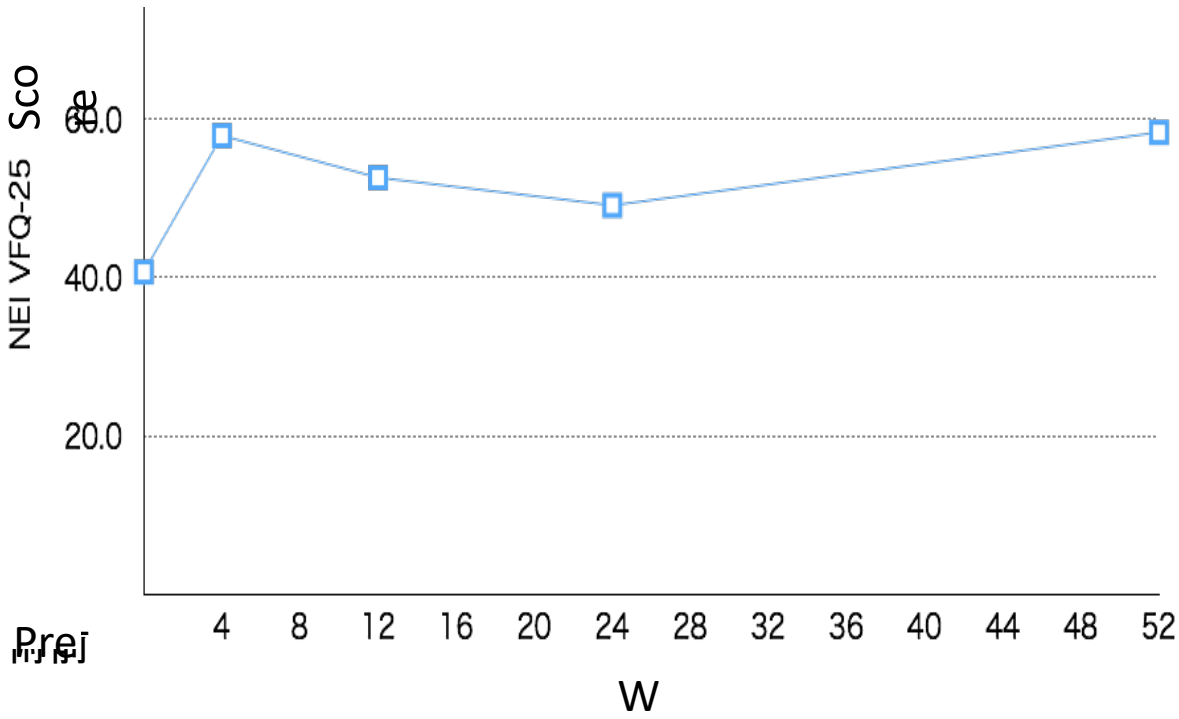
Fundus photograph after the surgery



Visual acuity before & after the surgery



NEI VFQ-25 (QOL)



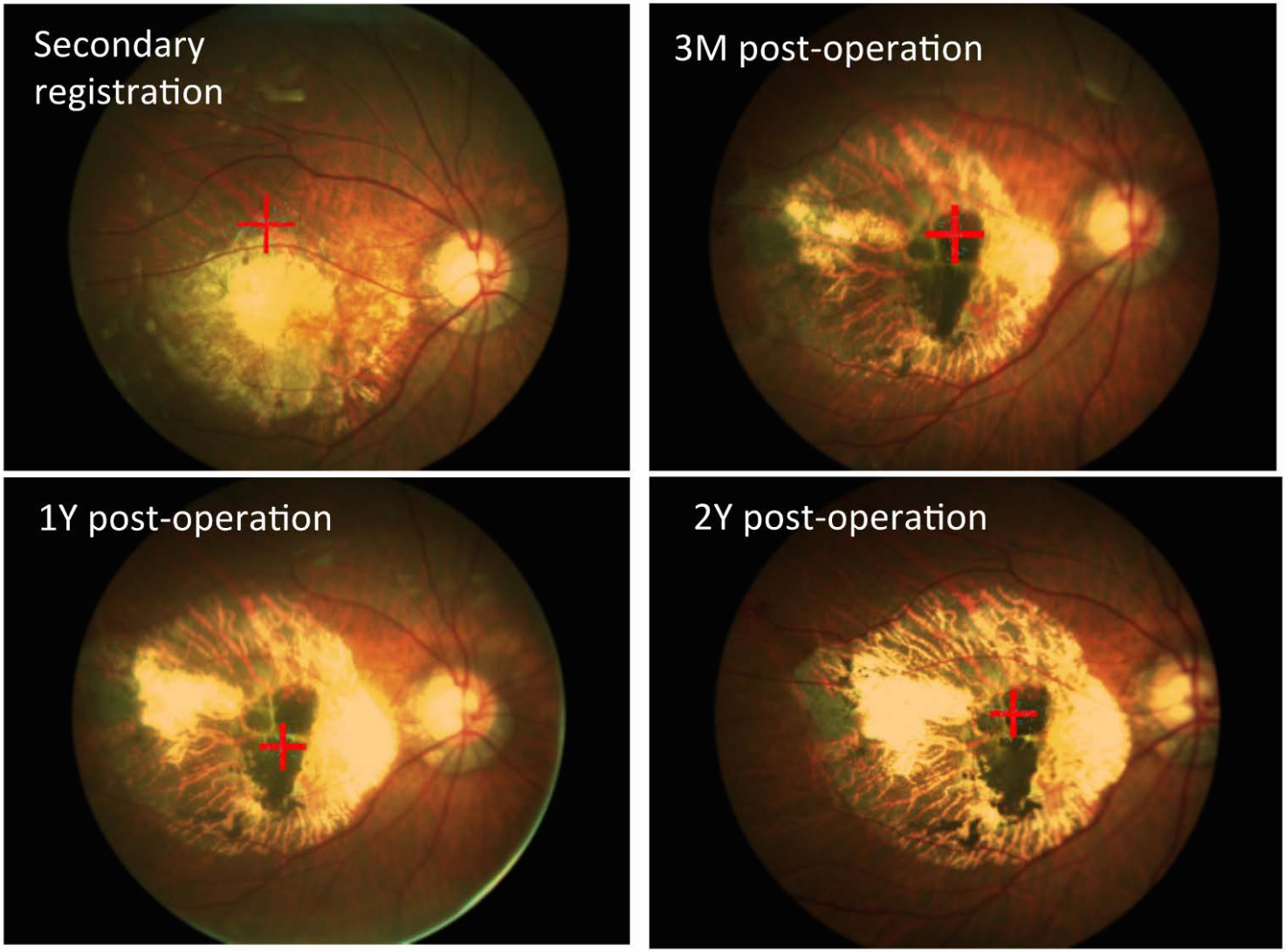
The patient, who has remained anonymous, told **The Japan Times:**

‘I am glad I received the treatment.

I feel my eyesight has brightened and widened.’

1 year results： OCT (transvers section)

Fixation tests using MP-1



March, 2017

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

BRIEF REPORT

Autologous Induced Stem-Cell–Derived Retinal Cells for Macular Degeneration

Michiko Mandai, M.D., Ph.D., Akira Watanabe, Ph.D., Yasuo Kurimoto, M.D., Ph.D., Yasuhiko Hirami, M.D., Ph.D., Chikako Morinaga, Ph.D., Takashi Daimon, Ph.D., Masashi Fujihara, M.D., Ph.D., Hiroshi Akimaru, Ph.D., Noriko Sakai, B.S., Yumiko Shibata, M.S., Motoki Terada, Yui Nomiya, M.S., Shigeki Tanishima, B.S., Masahiro Nakamura, M.D., Ph.D., Hiroyuki Kamao, M.D., Ph.D., Sunao Sugita, M.D., Ph.D., Akishi Onishi, Ph.D., Tomoko Ito, Kanako Fujita, Shin Kawamata, M.D., Ph.D., Masahiro J. Go, Ph.D., Chikara Shinohara, Ph.D., Ken-ichiro Hata, D.D.S., Ph.D., Masanori Sawada, M.D., Ph.D., Midori Yamamoto, Sachiko Ohta, Yasuo Ohara, B.S., Kenichi Yoshida, M.D., Ph.D., Junko Kuwahara, Yuko Kitano, M.S., Naoki Amano, M.S., Masafumi Umekage, M.S., Fumiyo Kitaoka, Ph.D., Azusa Tanaka, Ph.D., Chihiro Okada, M.S., Naoko Takasu, M.S., Seishi Ogawa, M.D., Ph.D., Shinya Yamanaka, M.D., Ph.D., and Masayo Takahashi, M.D., Ph.D.

N Engl J Med 2017; 376:1038-1046 | March 16, 2017 | DOI: 10.1056/NEJMoa1608368

EDITORIAL

Polar Extremes in the Clinical Use of Stem Cells

George Q. Daley, M.D., Ph.D.

N Engl J Med 2017; 376:1075-1077
March 16, 2017

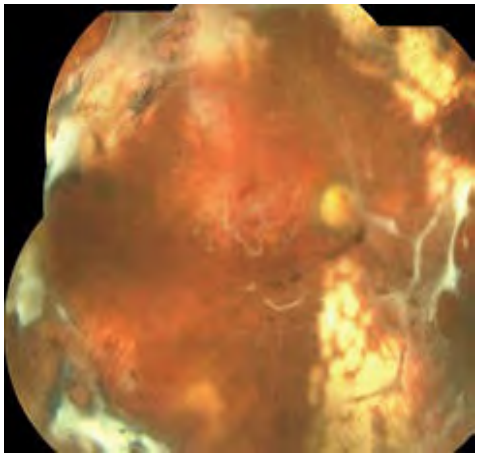
ORIGINAL ARTICLE

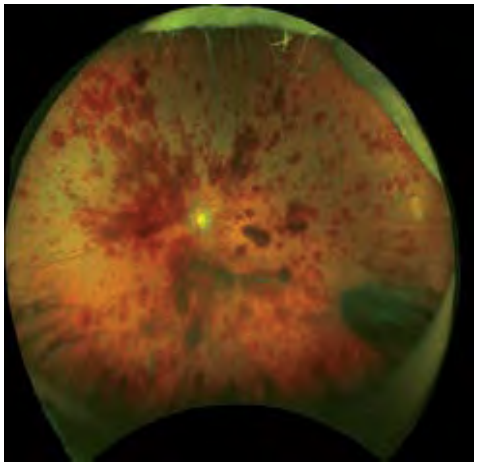
BRIEF REPORT

Vision Loss after Intravitreal Injection of Autologous "Stem Cells" for AMD

Ajay E. Kuriyan, M.D., Thomas A. Albini, M.D., Justin H. Townsend, M.D., Marianeli Rodriguez, M.D., Ph.D., Hemang K. Pandya, M.D., Robert E. Leonard, II, M.D., M. Brandon Parrott, M.D., Ph.D., Philip J. Rosenfeld, M.D., Ph.D., Harry W. Flynn, Jr., M.D., and Jeffrey L. Goldberg, M.D., Ph.D.

N Engl J Med 2017; 376:1047-1053 | March 16, 2017 | DOI: 10.1056/NEJMoa1609583





Regulation for cell therapy in Japan

Enforcement 2015.11.25~

Preclinical research

Clinical research
(Act on the safety of regenerative medicine) forced on Nov 25, 2014

Clinical trial
(revised Pharmaceutical affairs act)
forced on Nov 25, 2014

【Regenerative medicine 2014.11.25~】

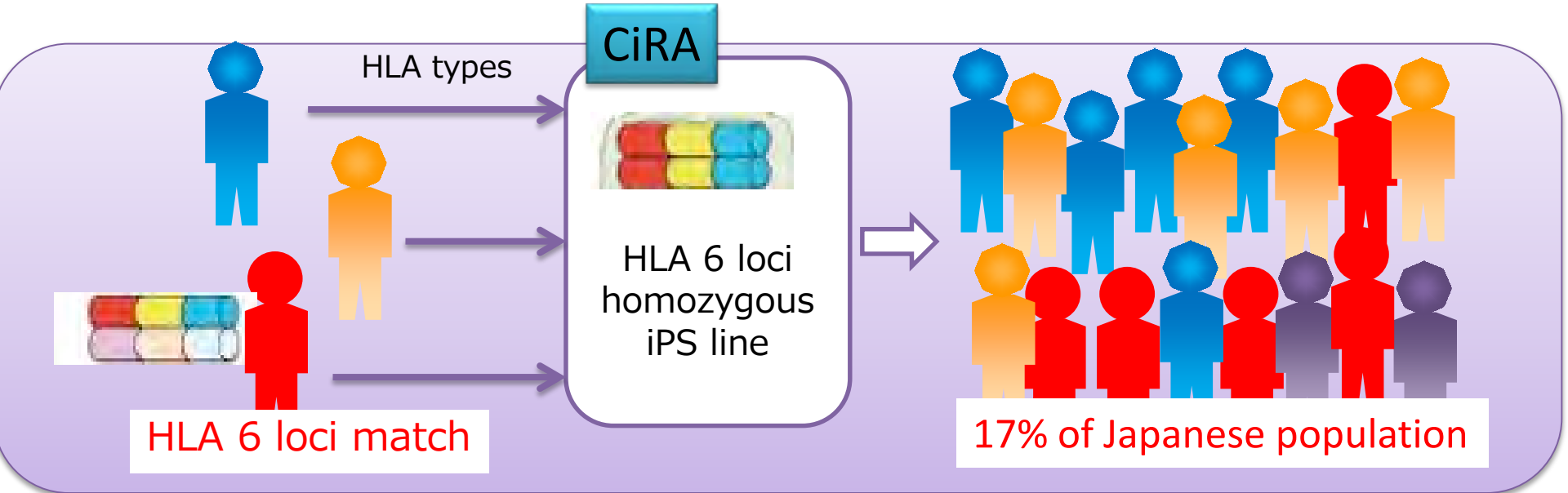
※Earlier Patient Access !

Informed Consent and Post Market Safety Measures

* Probable benefit: Confirmation of efficacy with small patient population.
** Safety: Earlier detection and evaluation of adverse events.

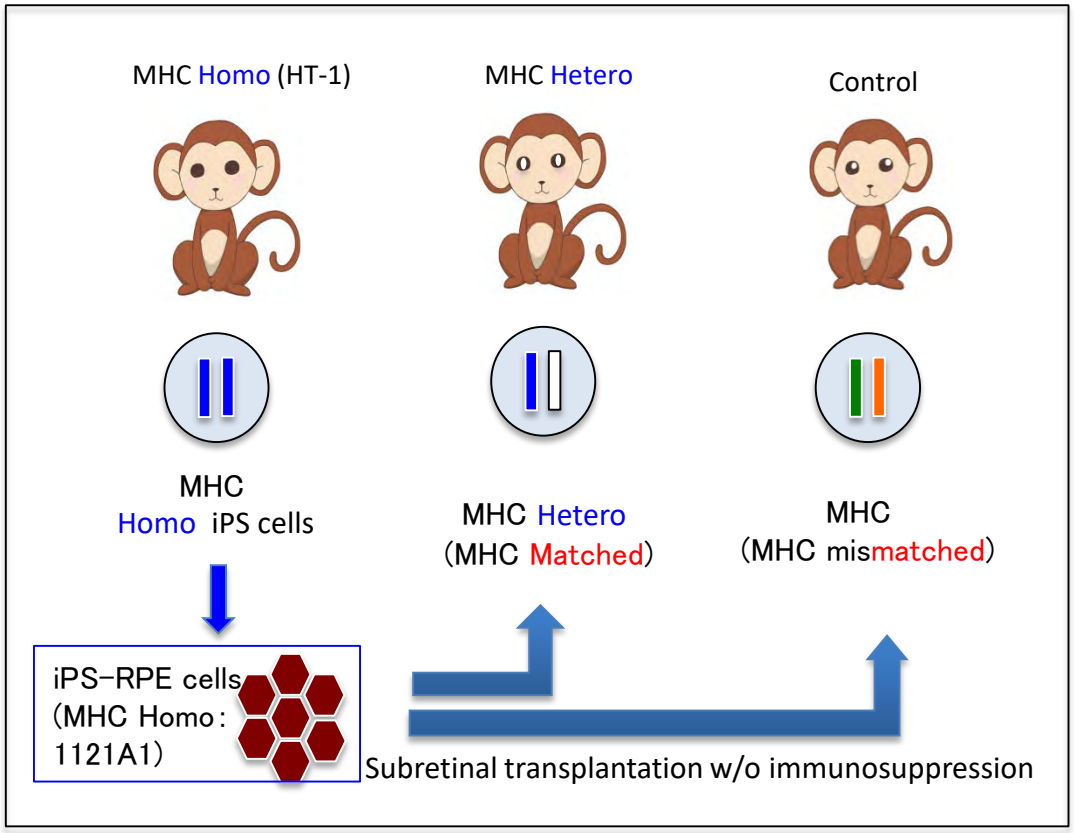
Types of hiPS-RPE cell transplantation

	Sheet Surgery risk: Big	Suspension Surgery risk: Small
Auto Cost: Big	Auto/Sheet 8.2013～（Clinical research）	Auto/Suspension Application in preparation
Allo Cost : Small	Allo/Sheet Application in preparation	Allo/Suspension Approved by the ministry 2.3.2017

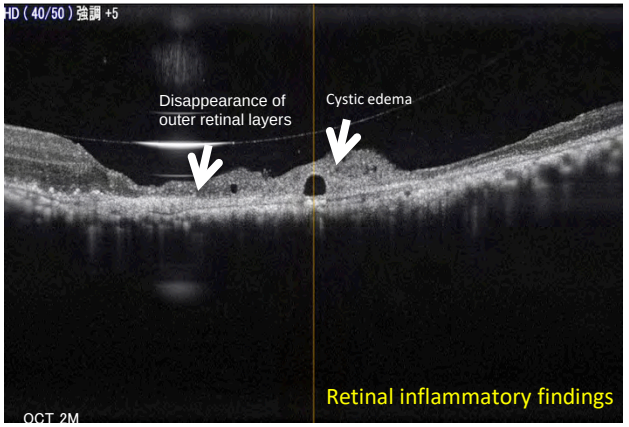


Immune reaction to the MHC matched or unmatched iPS-RPE (in normal monkey)

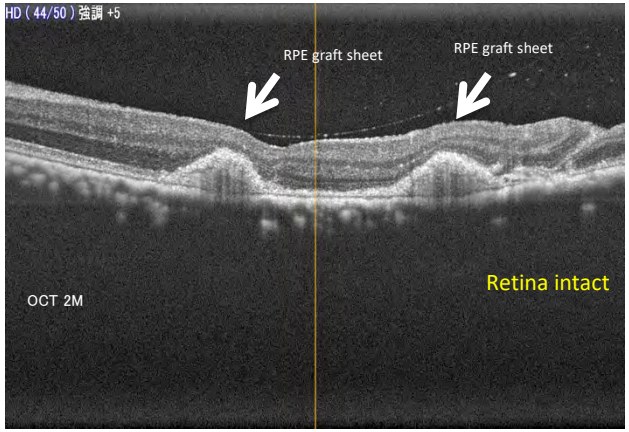
MHC homo iPS-RPE transplantation into hetero matched monkey eye: no immune rejection



MHC mismatched monkey → rejection



MHC matched monkey → no rejection



The scheme of new clinical study using allograft

Superdonor blood cells

Center for iPS Cell Research and Application, Kyoto University
CiRA

iPSCs
HLA homozygous

A*24:02-B*52:01-C*12:02-DRB1*15:02-DQB1*06:01-DPB1*09:01

RIKEN

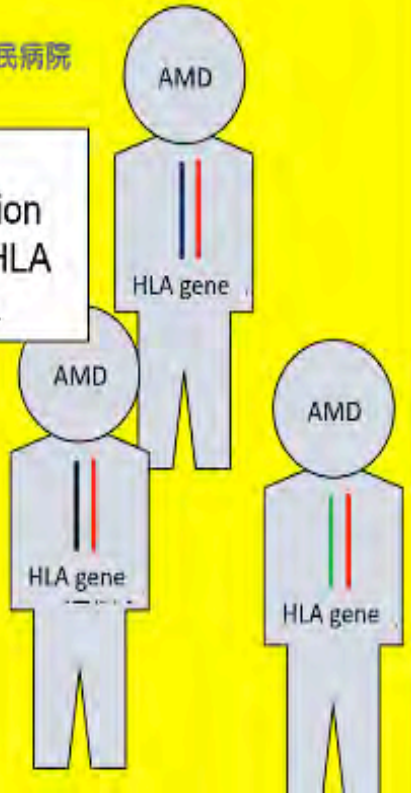
Induction of differentiation into PRE

地方独立行政法人 神戸市民病院機構
神戸市立医療センター中央市民病院
Kobe City Medical Center General Hospital

Transplantation
- ✓ w/o immunosuppressive medication
- ✓ AMD patients who has matched HLA haplotype in at least one gene.

Frozen → culture → graft

Cell suspension

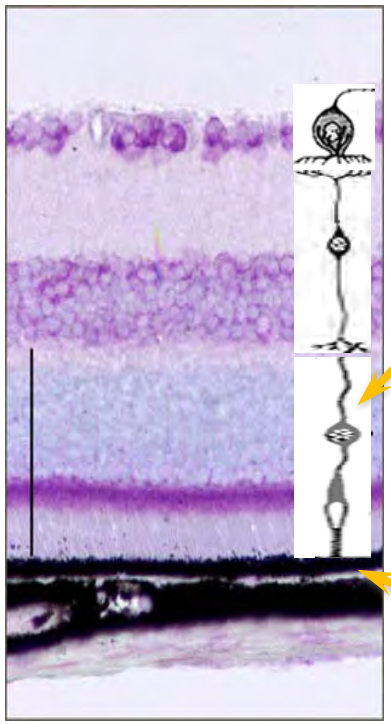


Japanese man is first to receive 'reprogrammed' stem cells from another person

World-first transplant, used to treat macular degeneration, represents a major step forward in movement to create banks of ready-made stem cells.

David Cyranoski 3/28/2017

Retinal cell transplantation



Photoreceptor (for Retinitis pigmentosa)

Issue

Structure of photoreceptor cells

Purification

Functional connection with the host's neurons

RPE cells (for AMD)

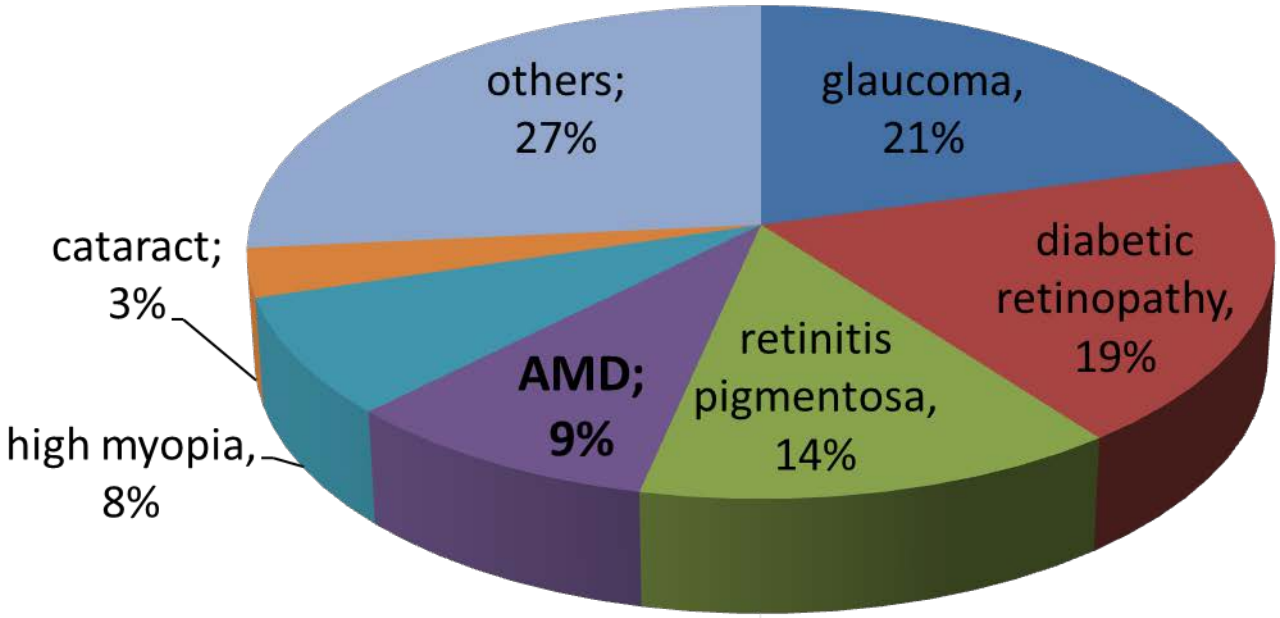
Started clinical study (Safaty)

Issue

Variety of cell forms

Efficacy

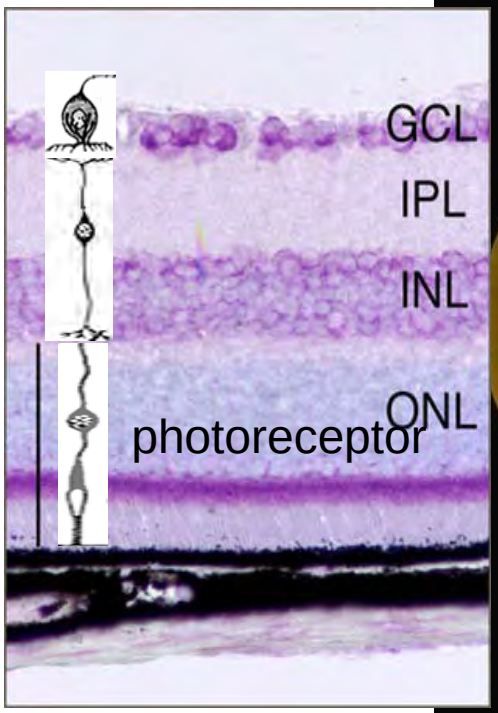
Causative diseases of visual impairment in Japan (2005)

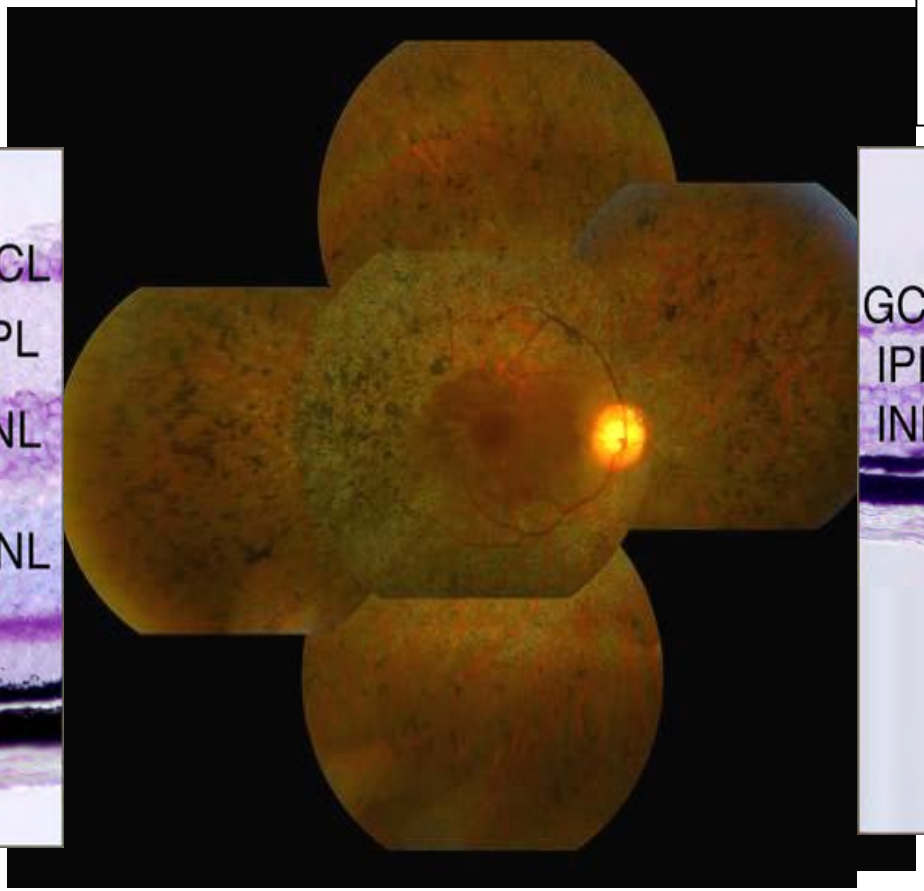


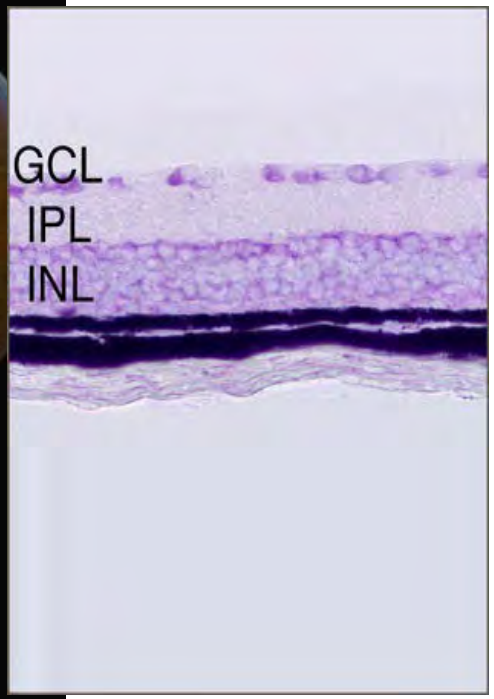
Health Labour Sciences Research Grant for Research on Measures for Intractable Diseases
網脈絡膜・視神経萎縮症に関する研究　平成17年度総括・分担研究報告書　p.p.263-267 (2006)

Retinitis pigmentosa

- More than 30000 patients in Japan
- No treatment







After birth（normal）

Photoreceptor degeneration

Self-organizing optic-cup morphogenesis in three-dimensional culture

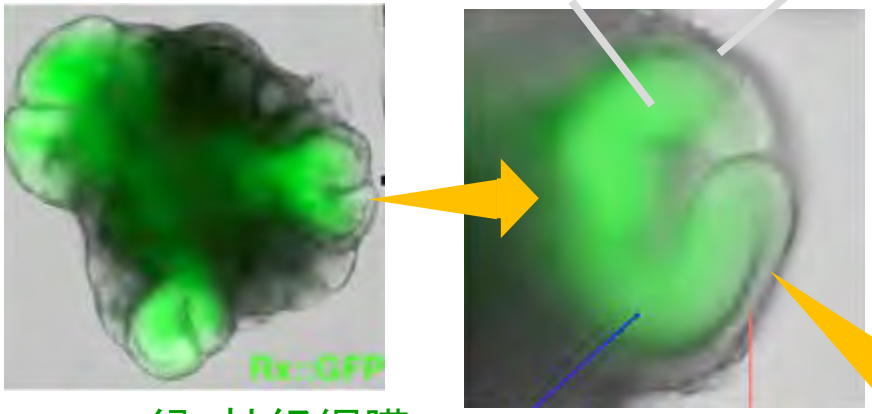
April 7, 2011

Mototsugu Eiraku,
Nozomu Takata,
Hiroki Ishibashi,
Masako Kawada,
Eriko Sakakura,
Satoru Okuda,
Kiyotoshi Sekiguchi,
Taiji Adachi,
Yoshiki Sasai (CDB, RIKEN)

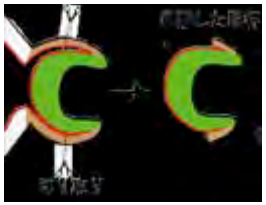
Retinal tissue derived from mouse ES cells in vitro

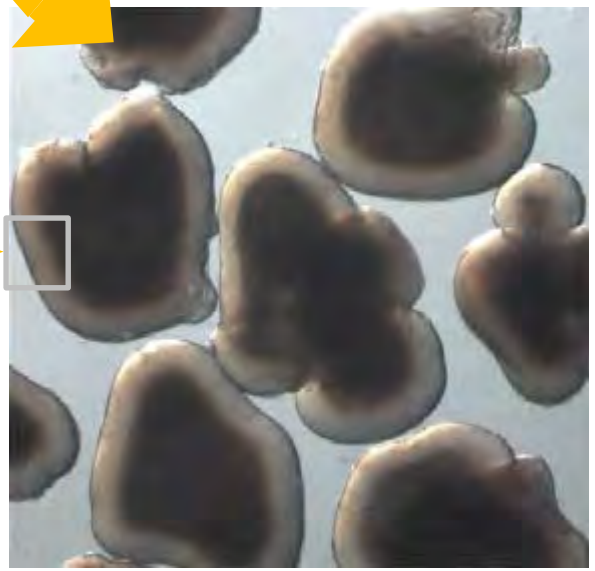
Dr. Eiraku

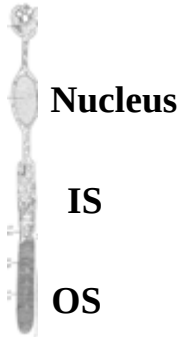
Sensory retina RPE

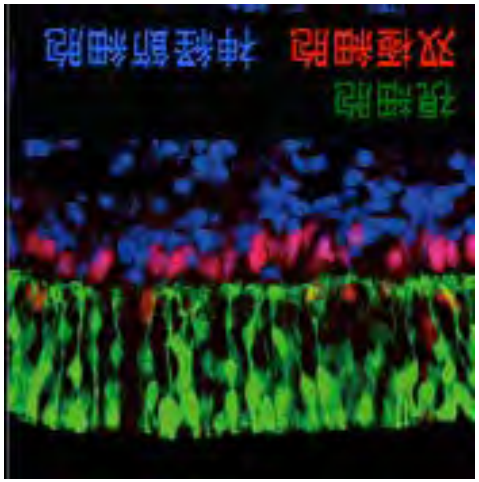


緑：神経網膜



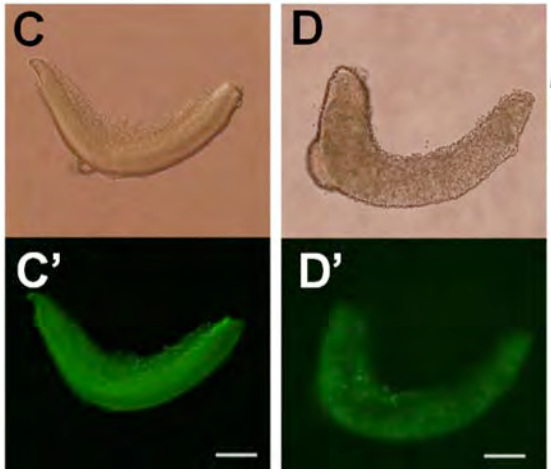


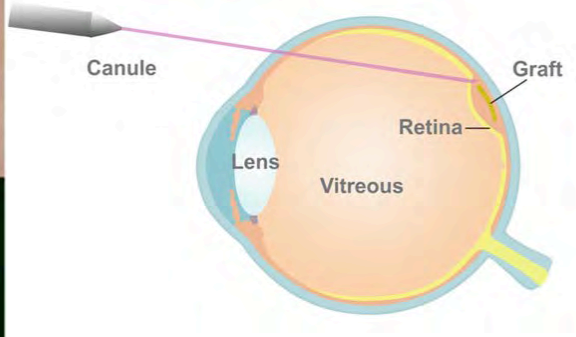




Green: photoreceptor

Mouse retinal sheet transplantation

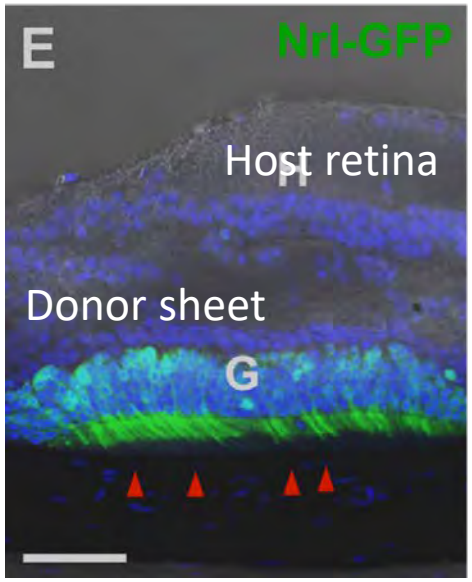


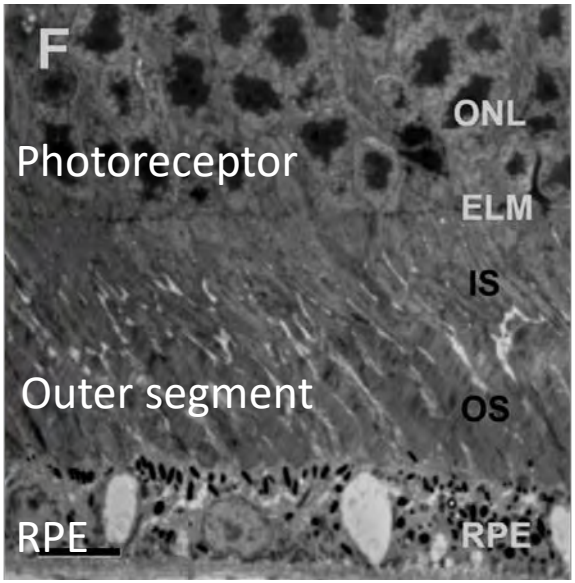


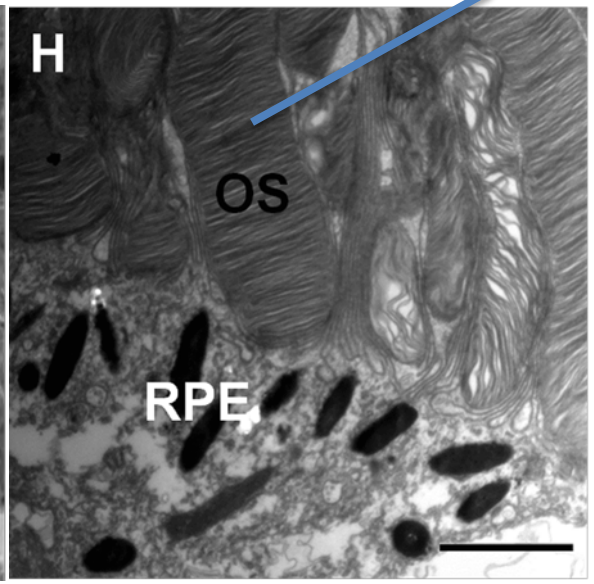
Assawachananont
MD, Phd

Michiko Mandai
MD, Phd

Outer segment



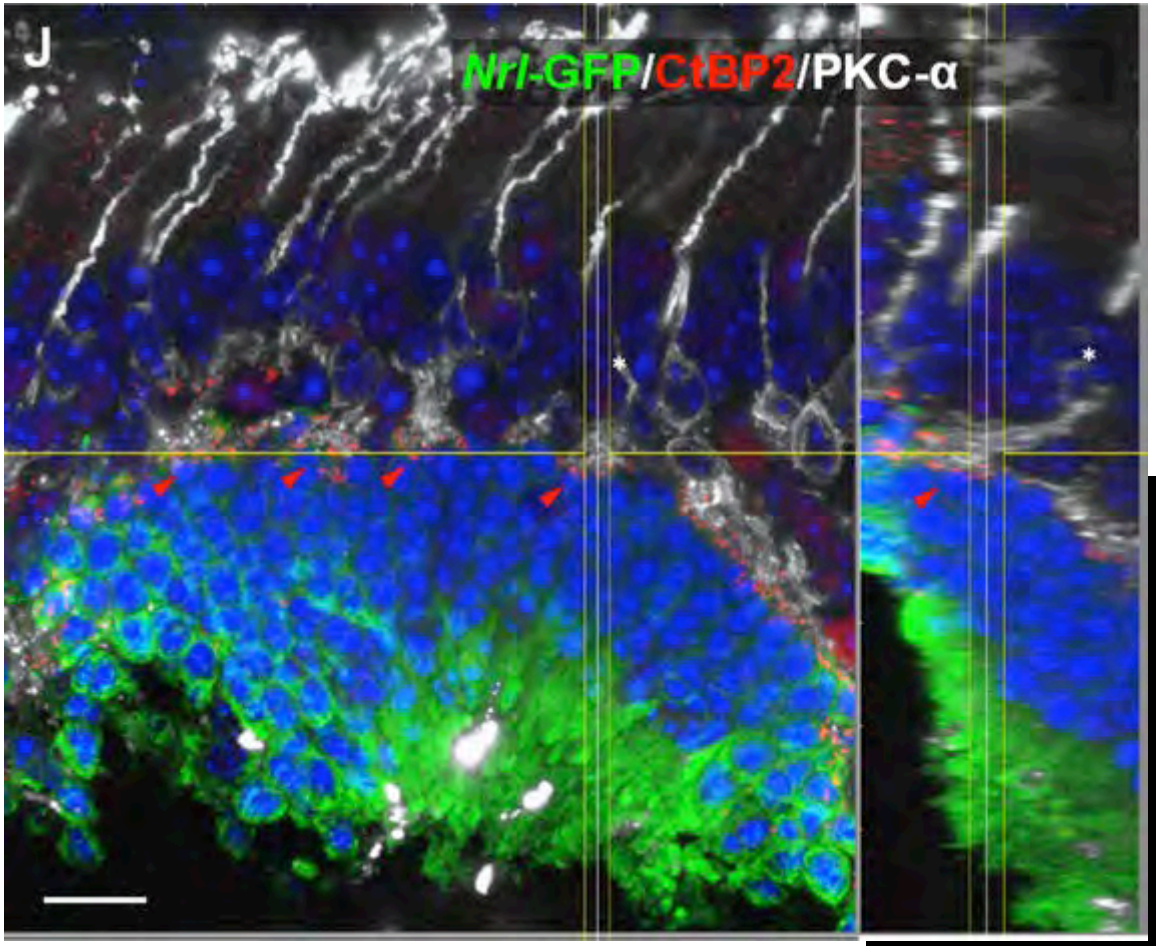


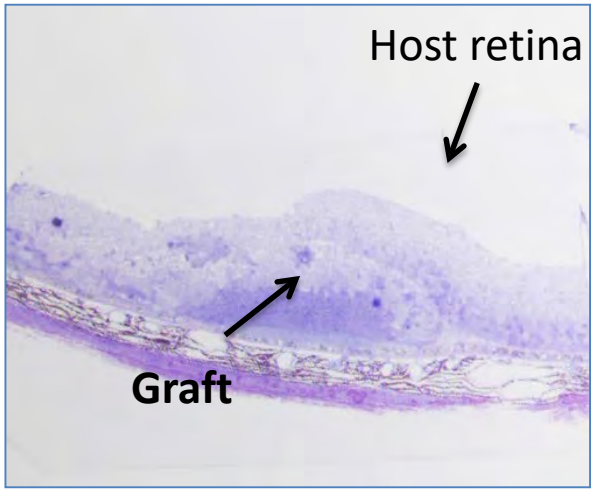


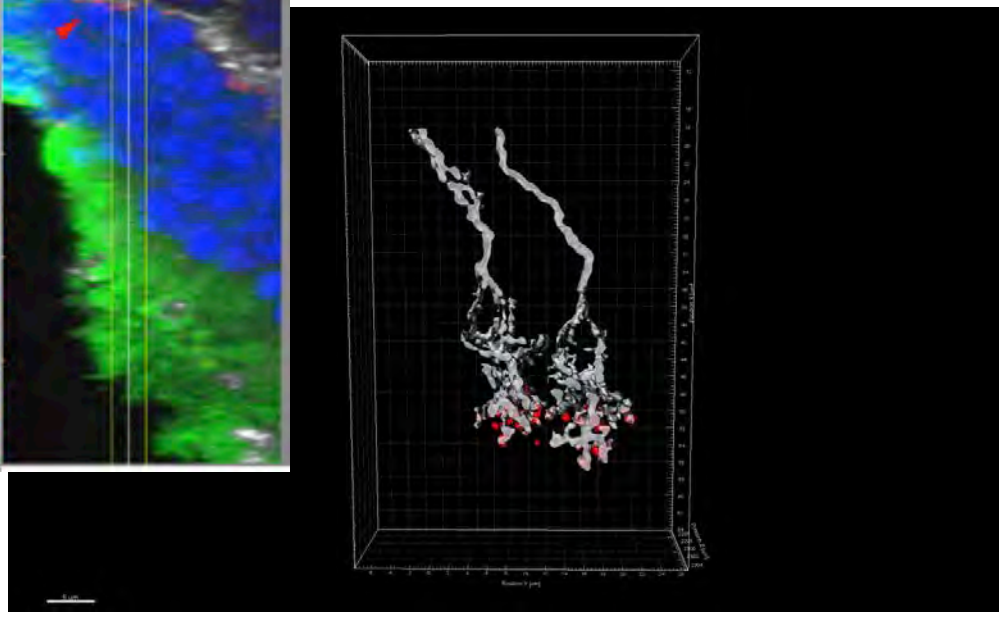
Stem Cell Reports 2014

Mouse retinal sheet transplantation

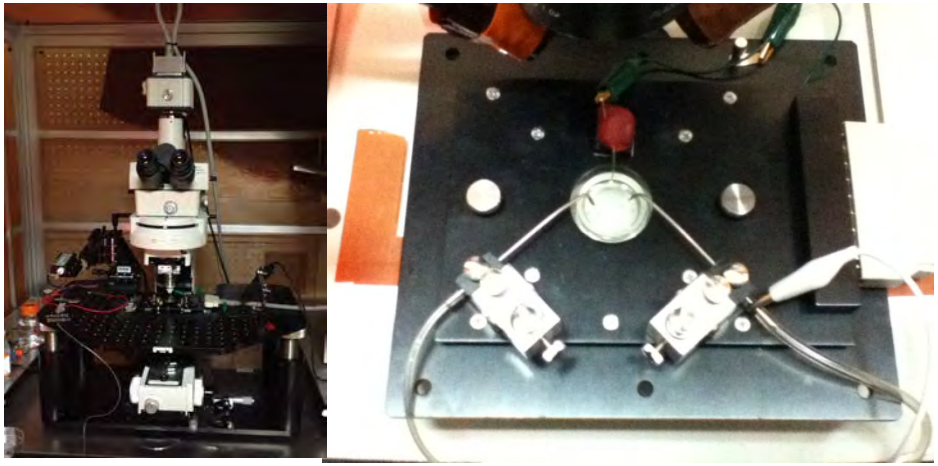
Host-graft synaptic contact in direct contact pattern



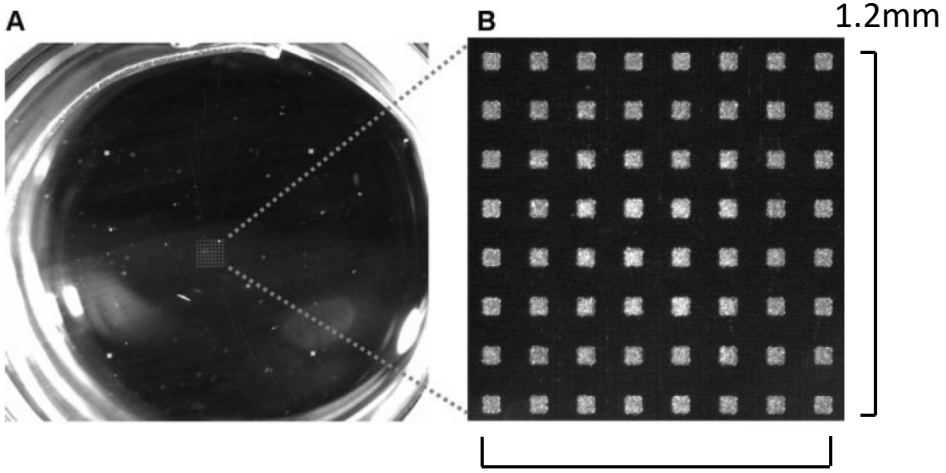




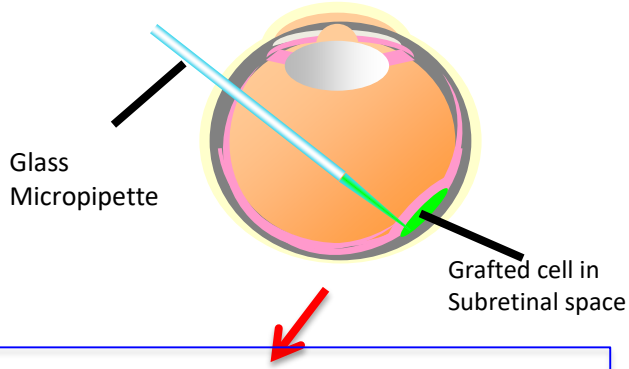
Evalutation of Function after transplantation

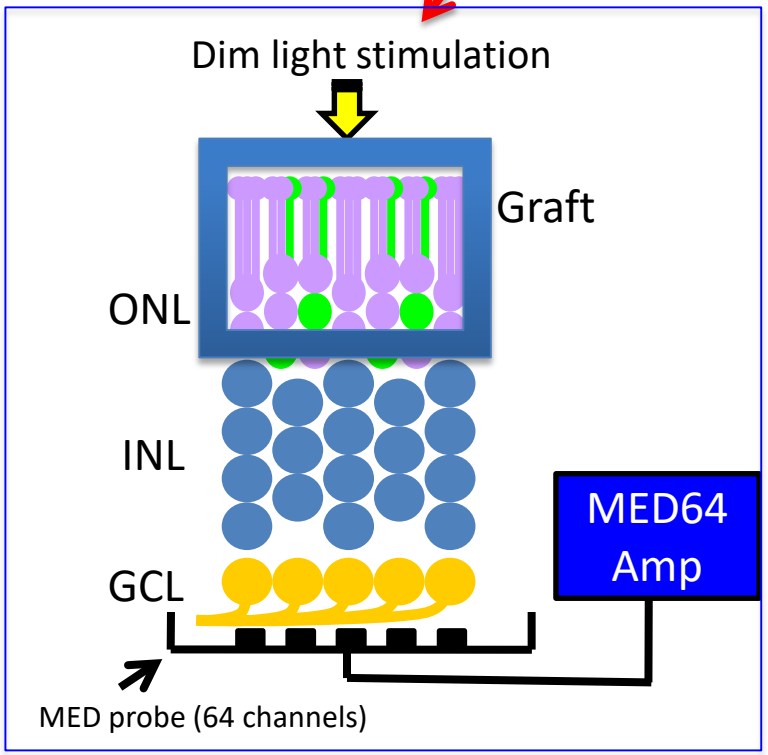


MED probe (MED-P5155)

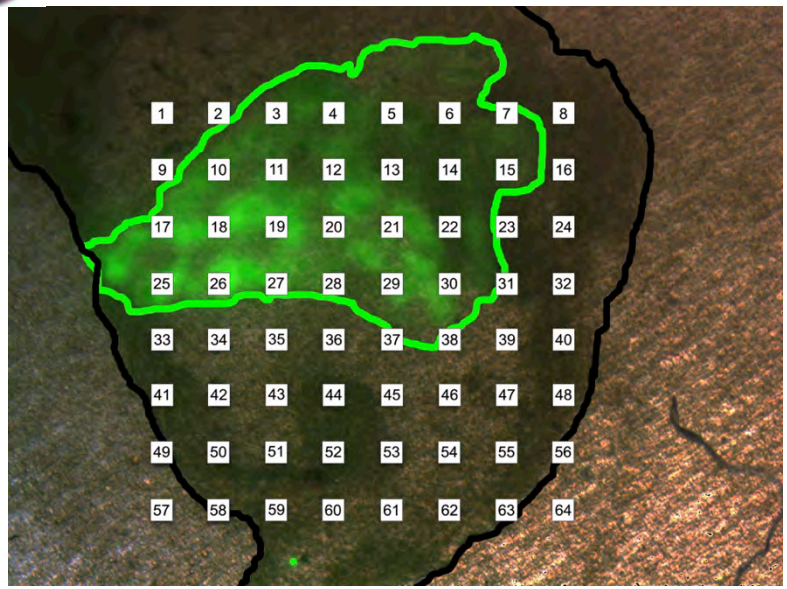


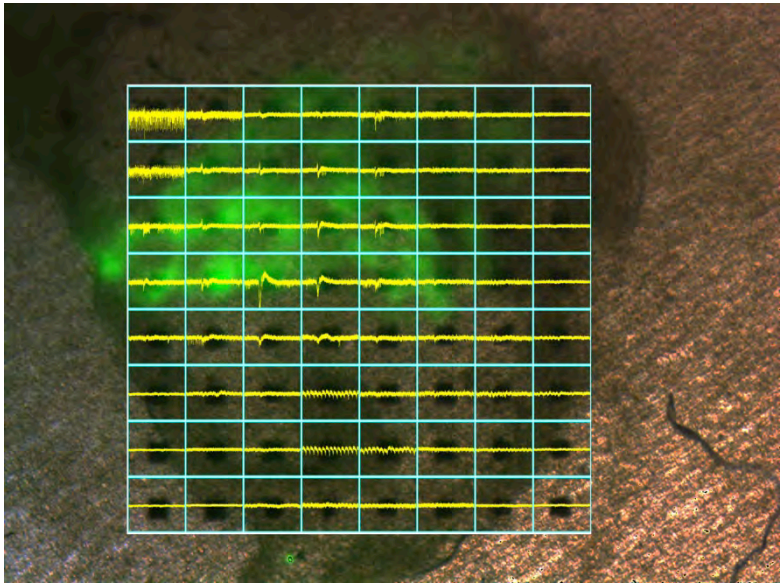
Electrode size: 50x50μm



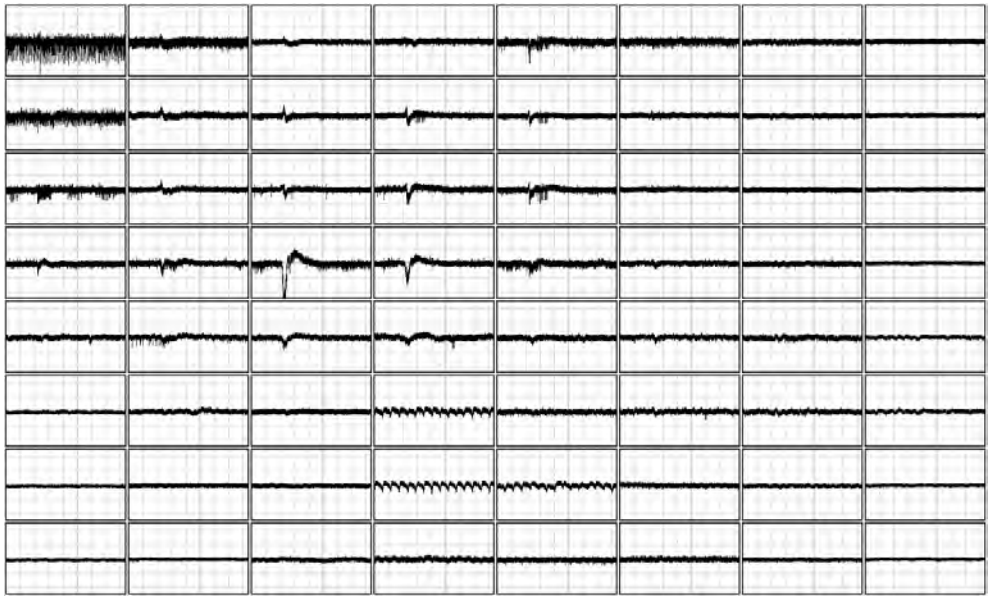


Response to the light stimuli after transplantation





microERG

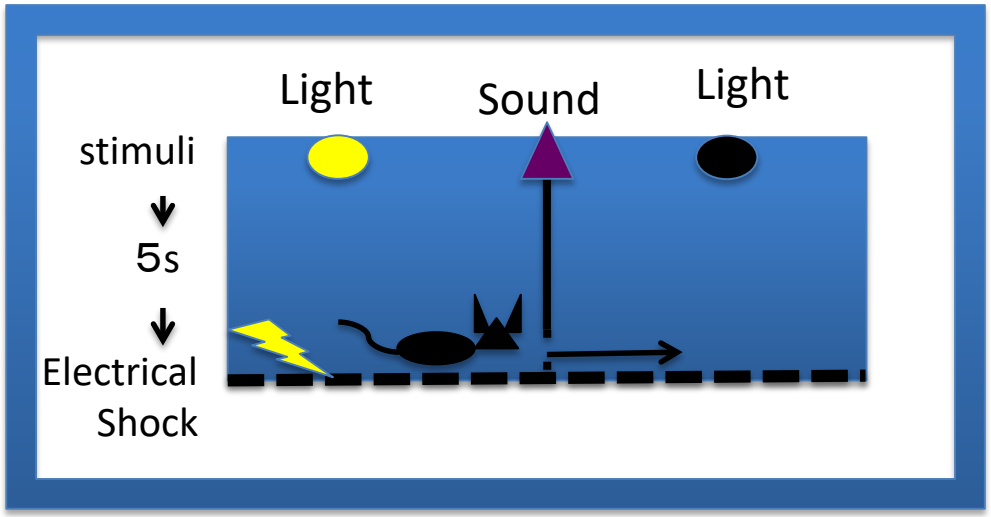


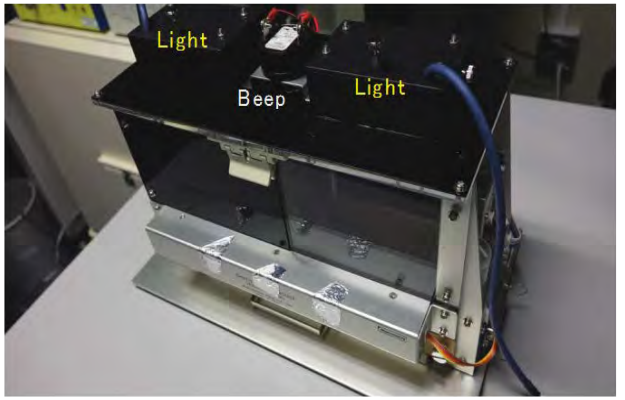
光刺激のタイミング

Light intensity:750cds/m2
Stimulus duration:10ms
1Hz low cut filter

X-axis:Time (500ms/DIV)
Y-axis:Amplitude(0.05mV/DIV)

Behavior test : shuttle avoidance test





Training with light & sound

↓

Within 2w
(avoidance ratio:70%<)

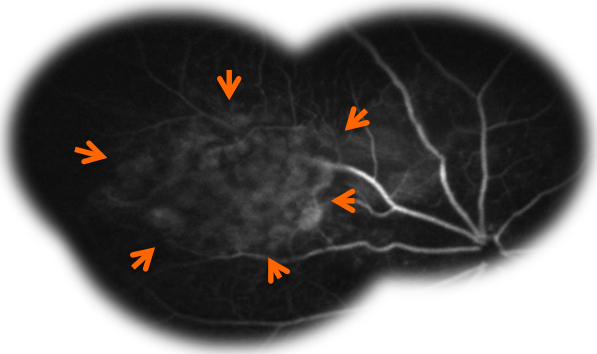
OK → Test with light only

stop

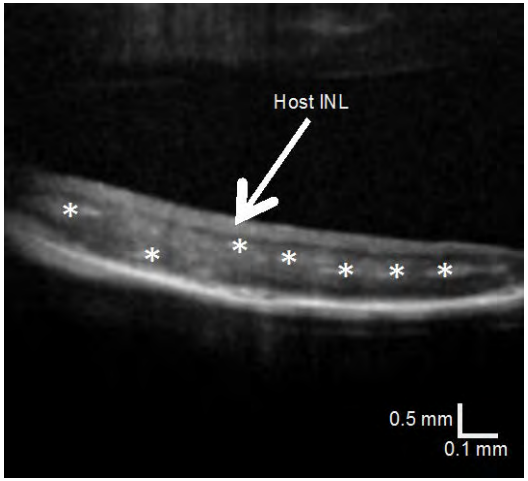
↓

Analysis

Select the nicely grafted mice with OCT images

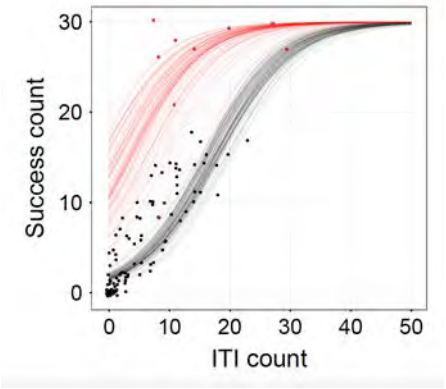


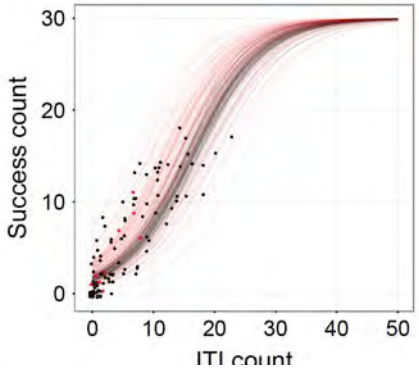
Grafted part is too small for ERG

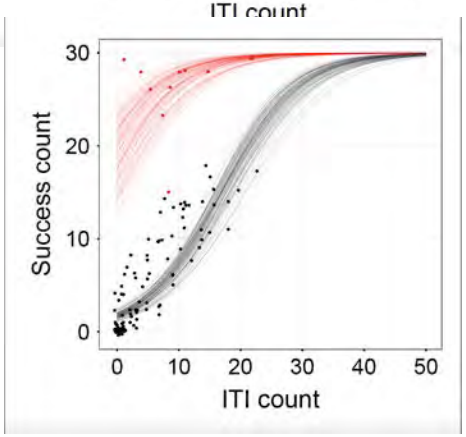


Host with good transplants（post OCT selection）

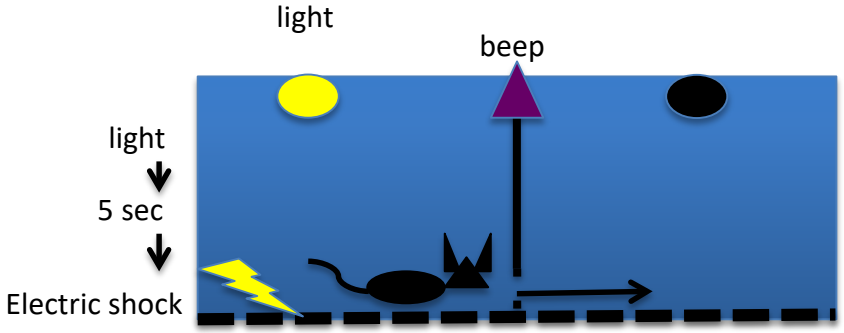
Light only wild type rd1

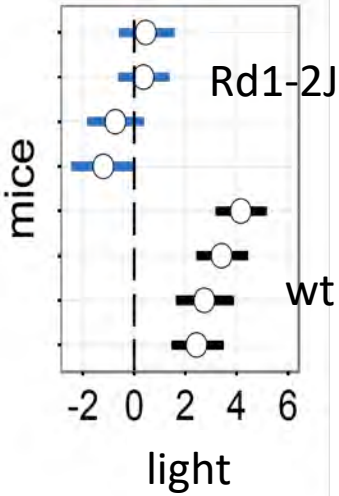


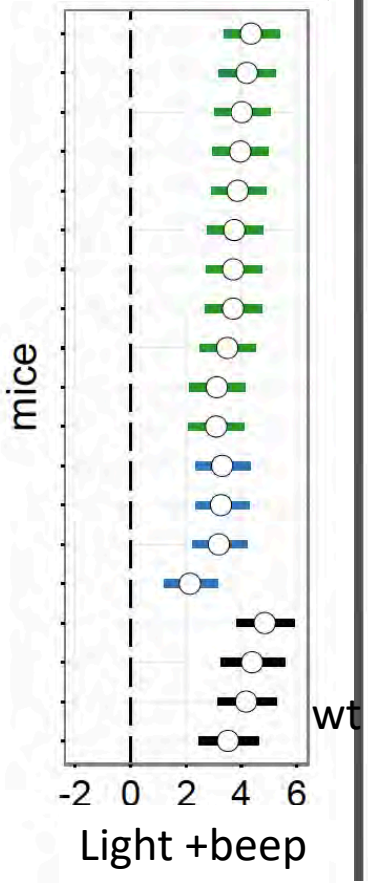


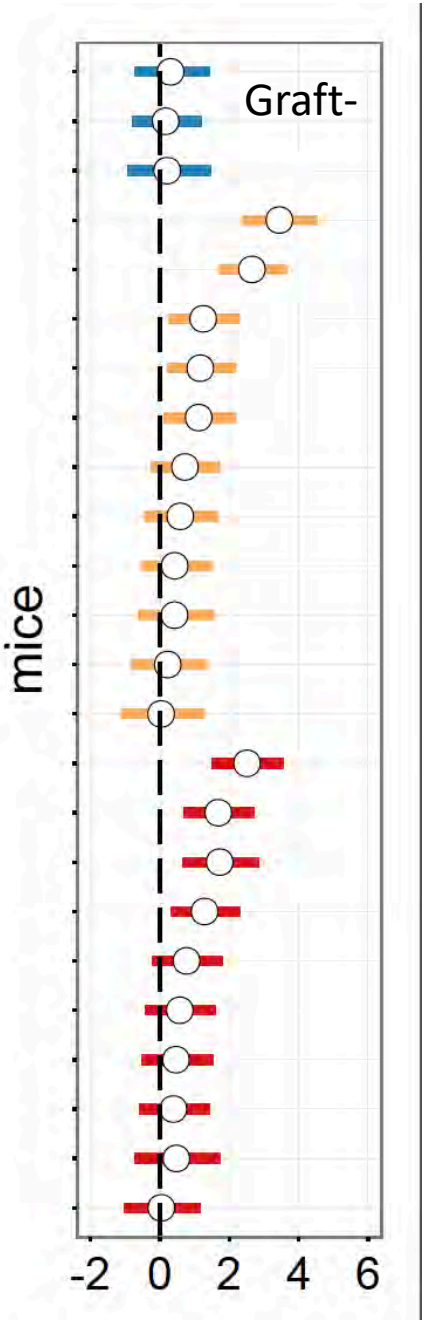


After transplantation….

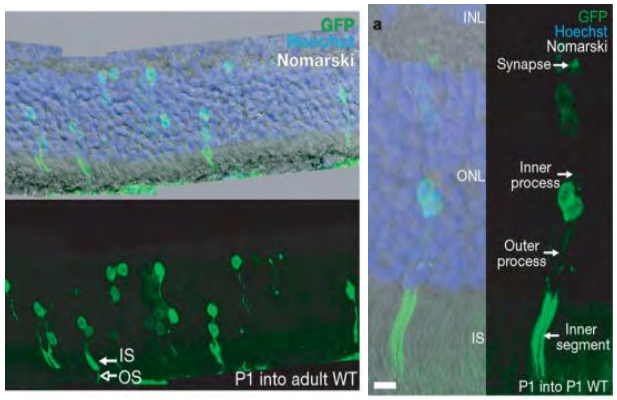








nature COMMUNICATIONS



It was cytoplasmic exchange !!

Published 4 Oct 2016

Retinal transplantation of photoreceptors results in donor–host cytoplasmic exchange

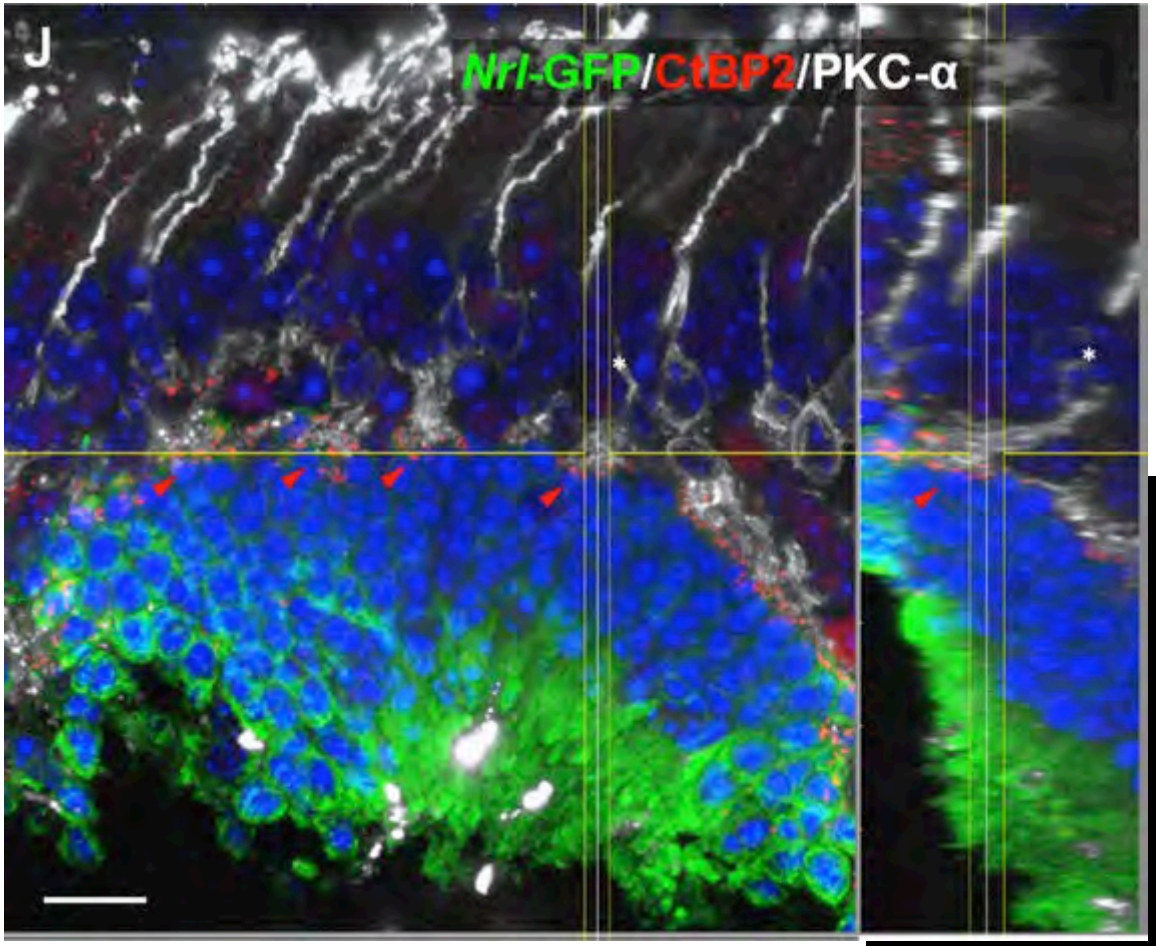
Tiago Santos-Ferreira1,*, S´ılvia Llonch1,*, Oliver Borsch1,*, Kai Postel1, Jochen Haas1 & Marius Ader1

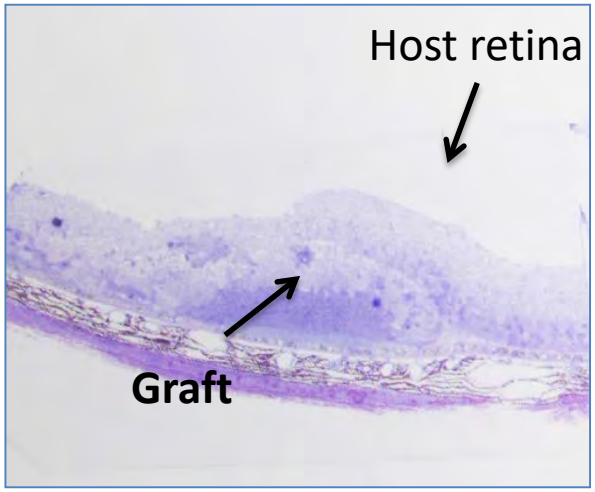
Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors

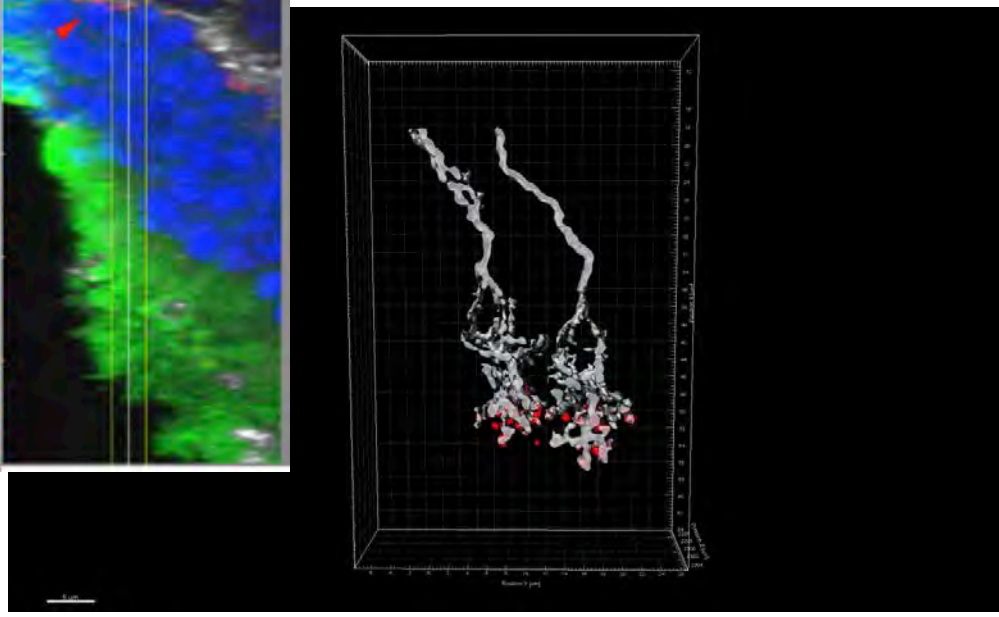
R.A. Pearson1,*, A. Gonzalez-Cordero1, E.L. West1,**, J.R. Ribeiro1,**, N. Aghaizu1,**, D. Goh1, R.D. Sampson1, A. Georgiadis1, P.V. Waldron1, Y. Duran1, A. Naeem1, M. Kloc1, E. Cristante1, K. Kruczek1, K. Warre-Cornish1,w, J.C. Sowden2, A.J. Smith1 & R.R. Ali1,3,*

Mouse retinal sheet transplantation

Host-graft synaptic contact in direct contact pattern







Effect of Regenerative medicine

1. Donor cells

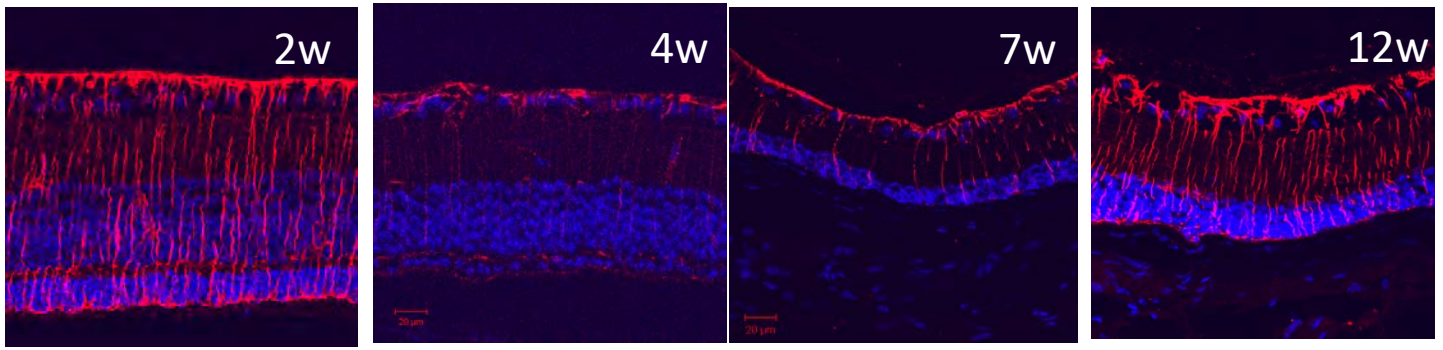
2. Host environment

Host environment and transplantation

（rd mice：rapid photoreceptor degeneration model mice）

Glial scar

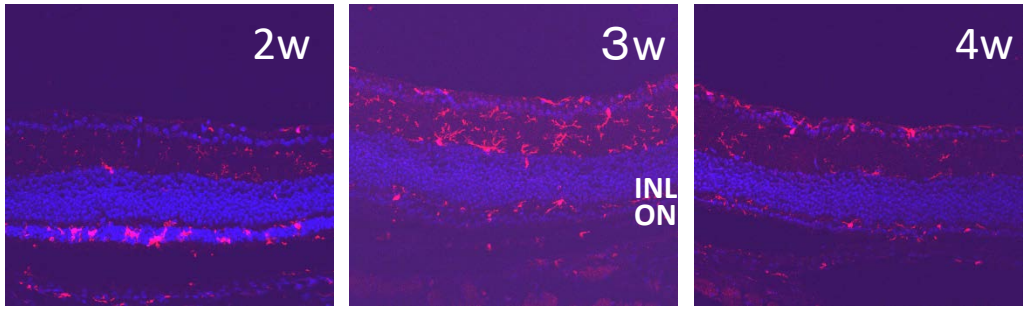
Red: GFAP
Muller glia



Time window

Immflamaion

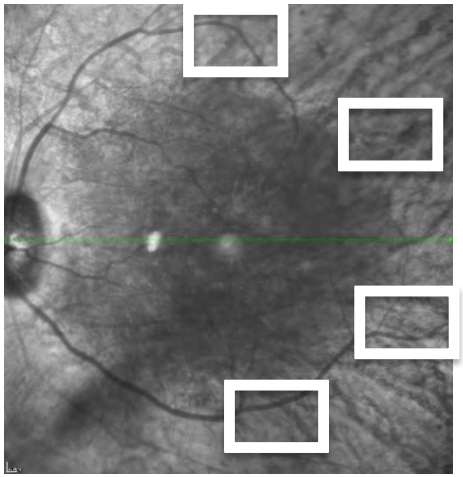
Red: Iba1
Microglia

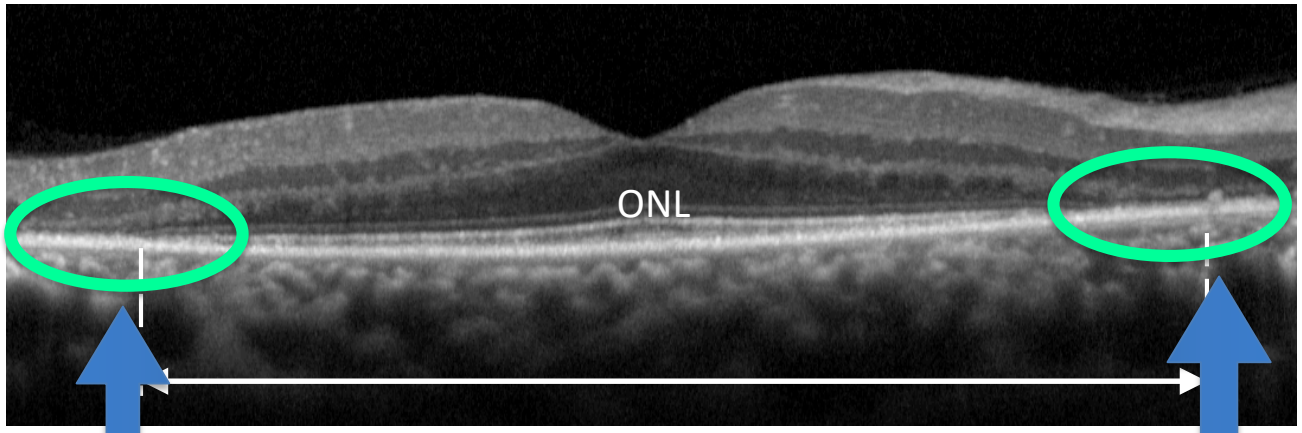


Rod degeneration → Cone degeneration → Bipolar degenration

Retinitis Pigmentosa

In human patients, various stages exist in one eye

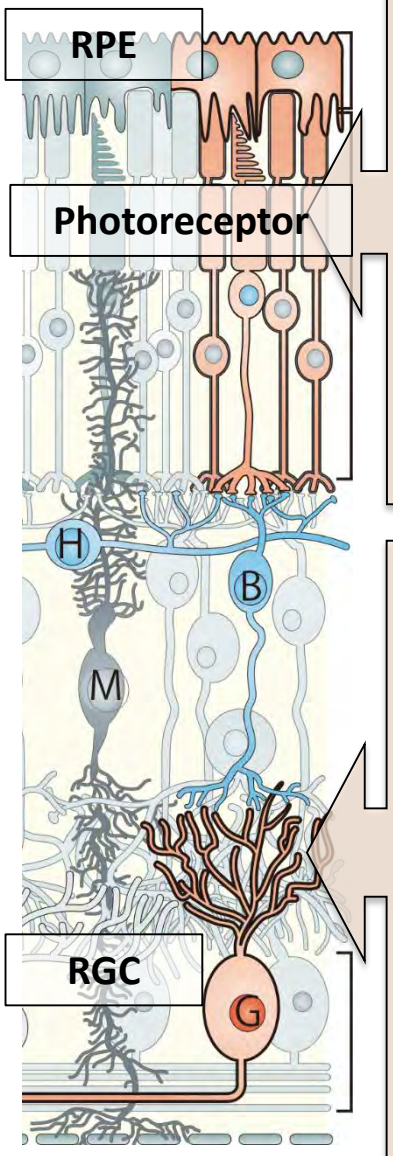




ONL (Photoreceptor cell layer) +

Transplantation

Patient-derived iPSCs studies



(Mofiried from Nat Rev Neurosci.)

Photoreceptor dysfunction/degeneration

Retinitis Pigmentosa

- 1 in 4000～8000 individuals
- ~50 causal genes identified
- ~60% are sporadic/unknown

①	RHO	outer segment
②	RP1	clila
③	PRPH2	outer segment
④	RP9	splicing factor
⑤	PRPH2	outer segment
⑥	RP2	cilia
⑦	EYS	outer segment/cilia
⑧	PRPF31	splicing factor/cilia

RGC dysfunction/degeneration

Leber hereditary optic neuropathy

- 1 in ~50000 individuals
- 80% of Japanese LHON patients

⑨ MT-ND4 ComplexI

Normal tension glaucoma

- 70~90% in Japanese patients
- Causal genes characterized (MYOC WDR36 OPTN)

⑩ NTG (uncharacterized)

Regulation for cell therapy in Japan

Enforcement 2015.11.25~

Preclinical research

Clinical research
(Act on the safety of regenerative medicine) forced on Nov 25, 2014

Clinical trial
(revised Pharmaceutical affairs act)
forced on Nov 25, 2014

【Regenerative medicine 2014.11.25~】

※Earlier Patient Access !

Informed Consent and Post Market Safety Measures

* Probable benefit: Confirmation of efficacy with small patient population.
** Safety: Earlier detection and evaluation of adverse events.

Project Leader

Masayo Takahashi

Deputy Project Leader

Michiko Mandai

Sunao Sugita

Research Scientist

Akishi Onishi
Akiko Maeda
Genshiro Sunagawa
Ojos Matsuyama
Yuki Arai
Akiko Yoshida

Student Trainee

Shiniciro Ito
Satoshi Iraha
Kaori Ueda
So Goto
Kenichi Makabe
Wataru Kobayashi
Cody Kime
Anouk Georges
Ryutaro Akiba

CPC team

Hiroyuki Kitajima
Noriko Sakai
Yumiko Shibata
Motoki Teragda
Mitsuhiro Nishida

Thank You !