Theoretical Grounds of Clinical Medicine

Significance and Perspectives of Stem Cells in Clinical Medicine II

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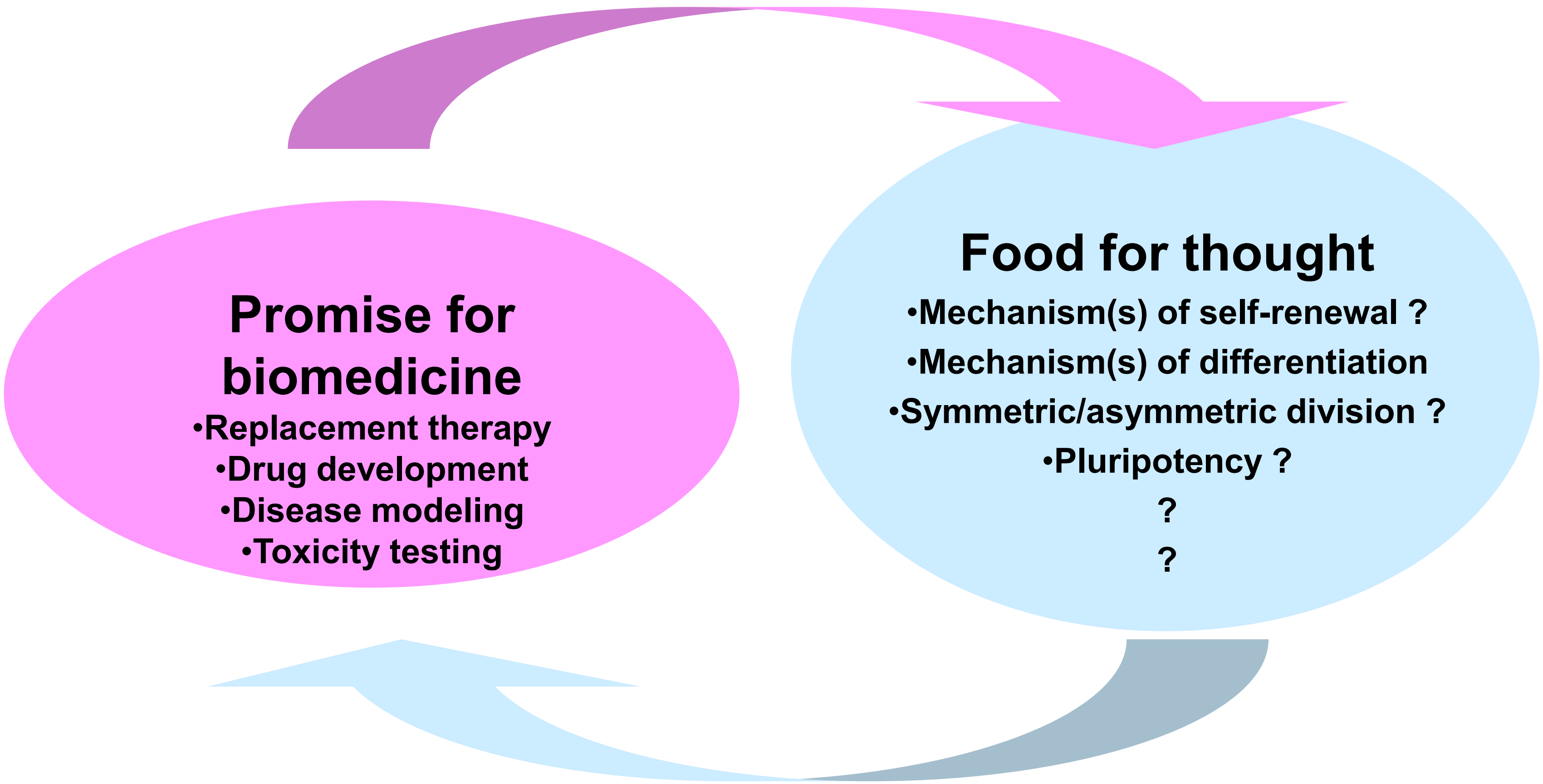
& Dáša Bohačiaková & Tomáš Bárta & Josef Jaroš

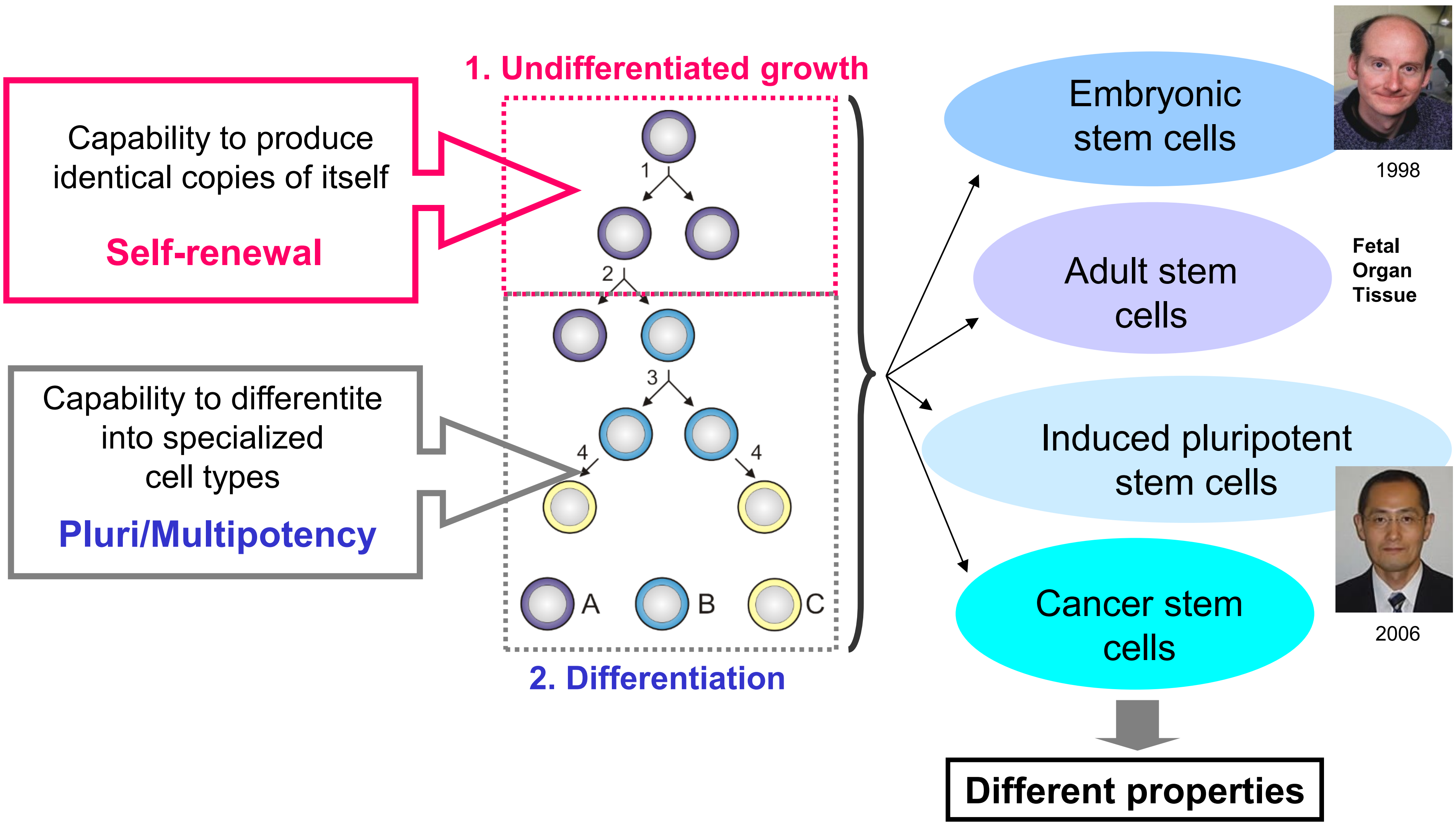
March 2018

Why people fell in love with stem cells?
(Embryonic, Adult, Induced)

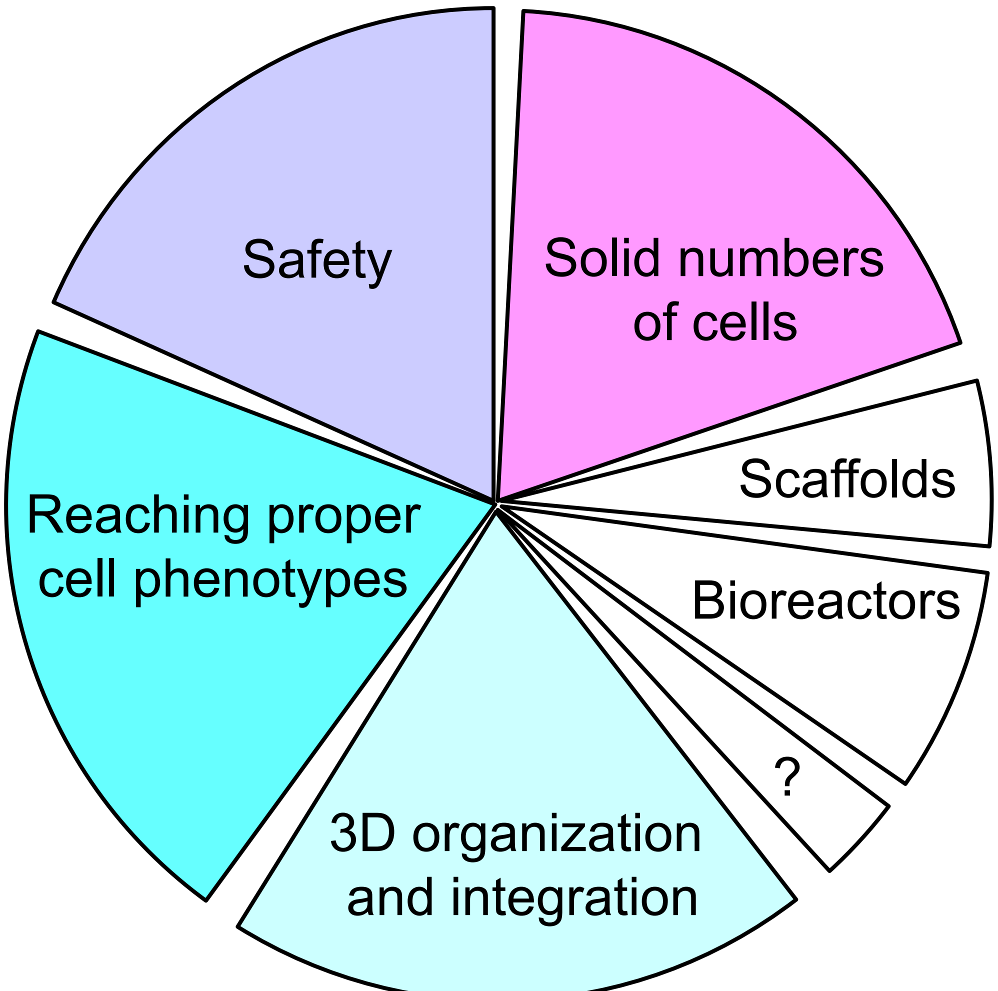


Mother nature and scientists supply us with many

Stem cells generate and regenerate our body



Fulfilling dreams needs solid grounds



What will we discuss today ?

One have to be cautious - representative example of risk associated with propagation of stem cells outside the body

Stem cells in real clinic - example of what stem cells and how they are successfully used in tissue reconstruction

Lungs made from stem cells - two ways to go

Strong nerves made from stem cells - where we stand - example of the story, to which we also contributed

Twisting biology for good – new scenarios for driving stem cells to where we need them

Also stem cells need support and help - how to provide stem cells with the right and caring environment

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Genetic changes develop in self-renewing hESC

2010

OPEN ACCESS Freely available online

PLoS one

A Teratocarcinoma-Like Human Embryonic Stem Cell (hESC) Line and Four hESC Lines Reveal Potentially Oncogenic Genomic Changes

Outi Hovatta[1*], Marisa Jaconi[2], Virpi Töhönen[3], Frédérique Béna[4], Stefania Gimelli[4], Alexis
Frida Holm[1], Stefan Wyder[5], Evgeny M. Zdobnov[5], Olivier Irion[6], Peter W. Andrews[7], Stylia
Antonarakis[4], Marco Zucchelli[3], Juha Kere[3,9], Anis Feki[6,9]

Aging Cell October 2011

Increased dosage of tumor suppressors limits the tumorigenicity of iPS cells without affecting their pluripotency

Sergio Menendez[1], Suzanne Camus[1,§], Aida Herreria[1,§], Ida Paramonov[1], Laura Batlle Morera[1], Manuel Collado[2], Vlad Pekarik[1], Iago Maceda[1], Michael Edel[1], Antonella Consiglio[1,‡], Adriana Sanchez[1,‡], Han Li[2], Manuel Serrano[2], Juan Carlos Izpisua Belmonte[1,3]

DOI: 10.1111/j.1474-9726.2011.00754.x

Issue

Aging Cell

Accepted Article (Accepted, unedited articles published online for future issues)

nature biotechnology

PERSPE

2007

Adaptation to culture of human embryonic stem cells and oncogenesis *in vivo*

Duncan E C Baker[1,4], Neil J Harrison[2,4], Edna Maltby[1], Kath Smith[1], Harry D Moore[2], Pamela J Shaw[3],
Paul R Heath[3], Hazel Holden[3] & Peter W Andrews[2]

…. and also in adult stem cells

ELSEVIER
Cytotherapy
Volume 15, Issue 11, November 2013, Pages 1352–1361

Original paper

Culture expansion induces non-tumorigenic aneuploidy in adipose tissue-derived mesenchymal stromal cells

Marieke Roemeling-van Rhijn[1], Annelies de Klein[2], Hannie Douben[2], Qiuwei Pan[3], Luc J.W. van der Laan[4], Jan N.M. Ijzermans[4], Michiel G.H. Betjes[1], Carla C. Baan[1], Willem Weimar[1], Martin J. Hoogduijn[1]

You have requested the following article:

Expert Opinion on Biological Therapy, Ahead of Print : Pages 1-18

Placental mesenchymal stem cells of fetal origin deposit epigenetic alterations during long-term culture under serum-free condition

Yongzhao Zhu, Xumei Song, Jian Wang, Yukui Li, Yinxue Yang, Tingting Yang, Haibin Ma, Libin Wang, Guangyi Zhang, William C Cho, Xiaoming Liu, Jun Wei

(doi: 10.1517/14712598.2015.960837)

STEM CELLS AND DEVELOPMENT
Volume 00, Number 00, 2014

DOI: 10.1089/scd.2014.0137

ORIGINAL RESEARCH REPORT

Asymmetric Aneuploidy in Mesenchymal Stromal Cells Detected by In Situ Karyotyping and Fluorescence In Situ Hybridization: Suggestions for Reference Values for Stem Cells

Seon Young Kim,[1] Kyongok Im,[2] Si Nae Park,[2] Jiseok Kwon,[2] Jung-Ah Kim,[1] Qute Choi,[1] Sang Mee Hwang,[1,3] Sung-Hee Han,[4] Sunghoon Kwon,[5] Il-Hoan Oh,[6] and Dong Soon Lee[1,2]

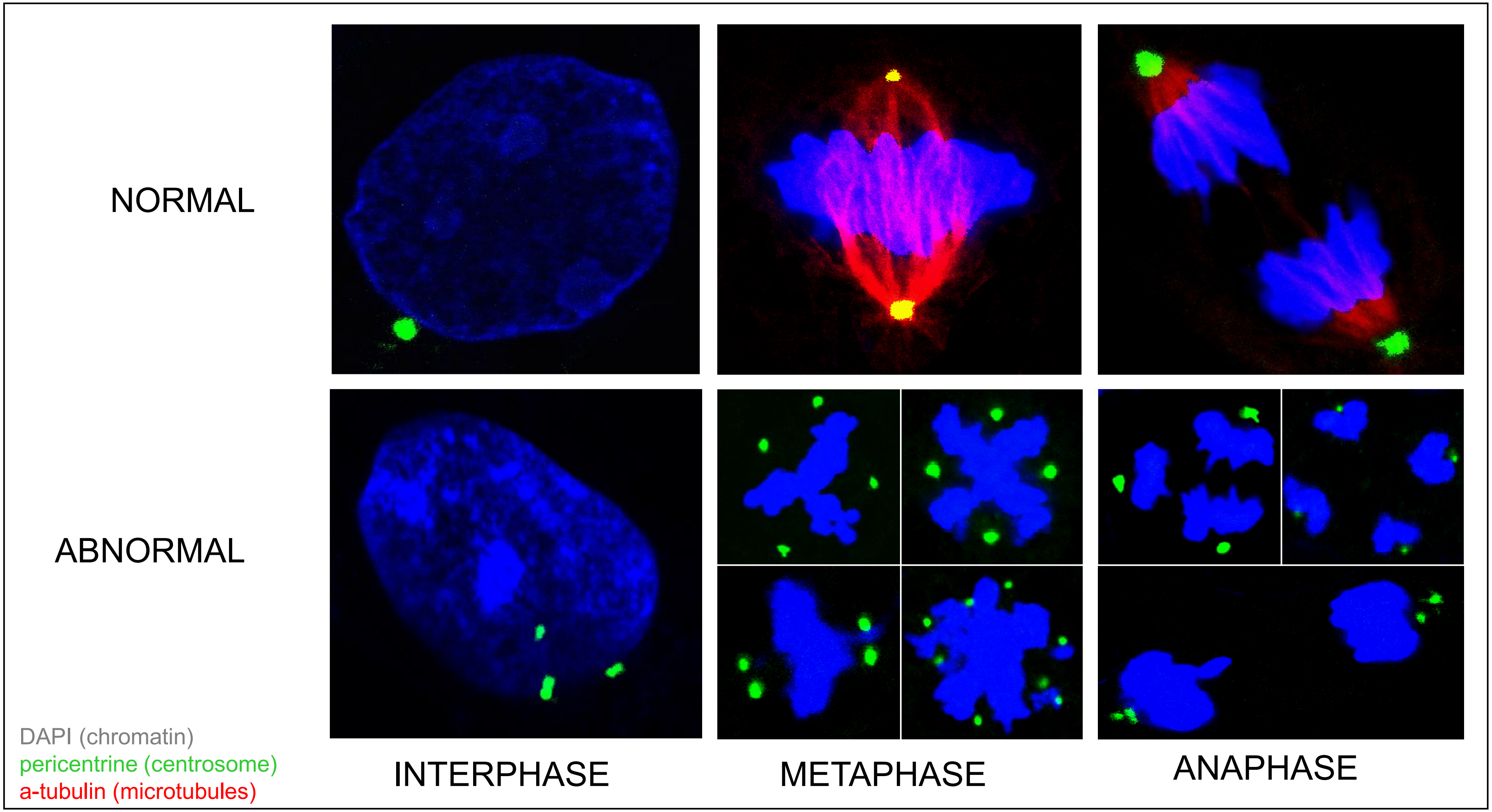
ELSEVIER
Cytotherapy
Volume 15, Issue 11, November 2013, Pages 1362–1373

Original paper

Genomic alterations in human umbilical cord–derived mesenchymal stromal cells call for stringent quality control before any possible therapeutic approach

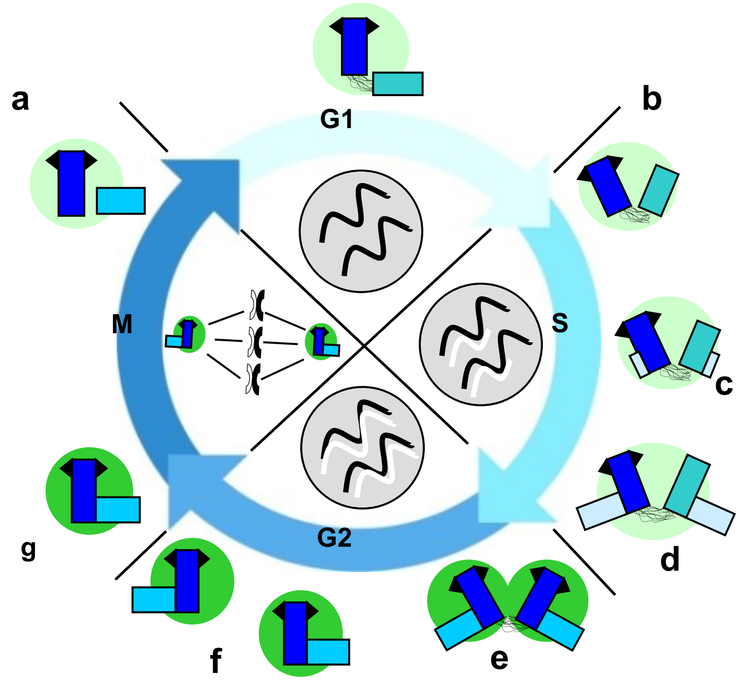
Alessandro Borghesi[1,2,*], Maria Antonietta Avanzini[3,*], Francesca Novara[4,*], Melissa Mantelli[3], Elisa Lenta[3,5], Valentina Achille[3], Rosa Maria Cerbo[1], Chryssoula Tzialla[1], Stefania Longo[1], Annalisa De Silvestri[6], Luc J.I. Zimmermann[7], Paolo Manzoni[8], Marco Zecca[9], Arsenio Spinillo[10], Rita Maccario[3,*], Orsetta Zuffardi[4,11,*], Mauro Stronati[1,2,*]

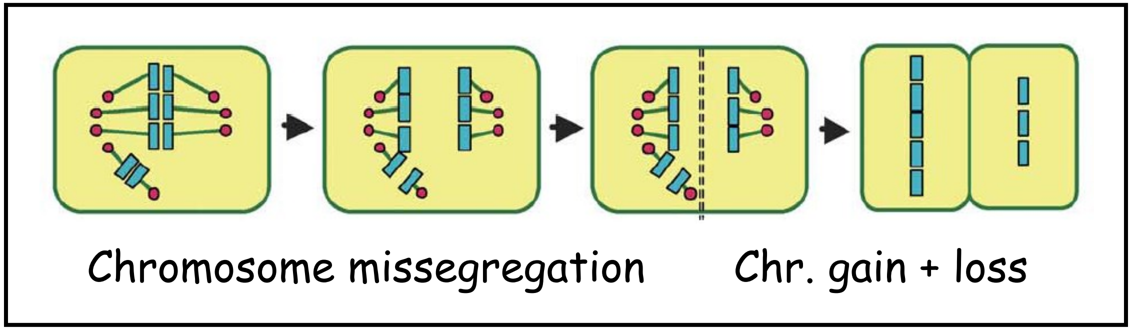
Cultured hESC display centrosomal overamplification that produces abberant mitoses



Holubcova et al., Stem Cells, 2010

Why this may represent a serious problem ?





.... with very high frequency !

Undifferentiated hESCs

	cell line	passage number	mitoses multicentrosomal / total	multicentrosomal mitoses percentage
Brno	CCTL6	P26	18 / 88	20,45 %
	CCTL8	P24	31 / 201	15,40 %
	CCTL10	P14	61 / 260	23,46 %
	CCTL12	P18	17 / 158	10,82 %
	CCTL13	P18	10 / 68	14,70 %
	CCTL14	P19	38 / 237	16,03 %
Stockholm	HS181	P25	18 / 108	16,60 %
	HS420	P31	21 / 172	12,02 %
	HS207	P27	10 / 61	14,75 %
	HS306	P39	21 / 131	16,03 %
	HS401	P23	15 / 146	10,27 %
Boston	HUES9	P27	57 / 544	10,47 %

Holubcova et al., Stem Cells, 2010

In mESC the frequency of multicentrosomal mitoses is low

cell line	passage number	mitoses multicentrosomal / total	multicentrosomal mitoses percentage
B10/CBA_11.1	P8	5 / 120	4,17 %
B10/CBA_11.2	P5	3 / 122	2,45 %
B10/CBA_11.3	P8	3 / 96	3,13 %
B10/CBA_11.4	P5	0 / 104	0,00 %
B10/CBA_11.5	P7	1 / 111	0,90 %
B10/CBA_11.6	P7	0 / 47	0,00 %
B10/CBA_11.7	P3	1 / 125	0,80 %
B10/CBA_11.8	P4	3 / 109	2,75 %

unpublished

In hiPSC the frequency of multicentrosomal mitoses is variable depending on cell line

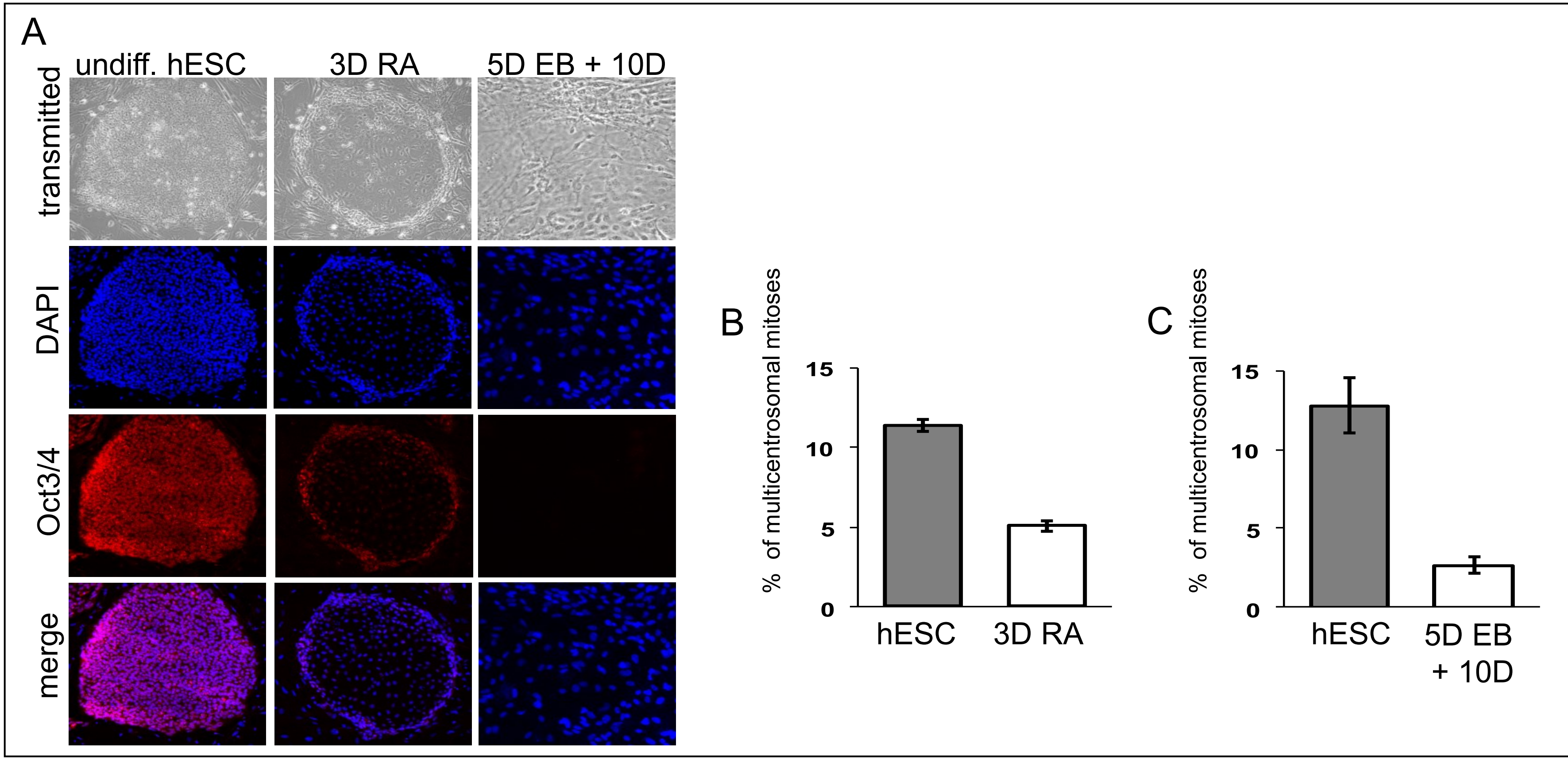
	Somatic cells		hiPSC		
fibroblast source	multicentrosomal / total mitoses	multicentrosomal mitoses percentage	clone ID (passage number)	multicentrosomal / total mitoses	multicentrosomal mitoses percentage
Human foreskin fibroblasts	0/96	0,0%	HFF_L1 (P20)	10 / 110	**9,09%**
			HFF_L2 (P20)	5 / 125	**4,0%**
Normal human dermal fibroblasts (Lonza)	6/60	10,0%	NHDF (P26+7)	14 / 202	**6,9%**
Adult dermal human fibroblasts	2 / 267	0,74%	AHDF_#1 (P36	25/249	**10,07%**
			AHDF_#4 (P35)	29/217	**13,36%**
Ligase IV mutated (patient derived)	0 / 60	0,0%	FO7/614 (P18+10)	5 / 110	**4,5%**
	4 / 111	3,6%	FO7/614_shRNAp53 (P20+11)	29 / 174	**16,6%**
	0 / 52	0,0%	GM16088 (P19+9)	1 / 77	**1,29%**
	0 / 56	0,0%	GM17523 (P18+6)	20 / 160	**12,5%**

unpublished

Supernumerary centrosomes develop only in pristine hESCs

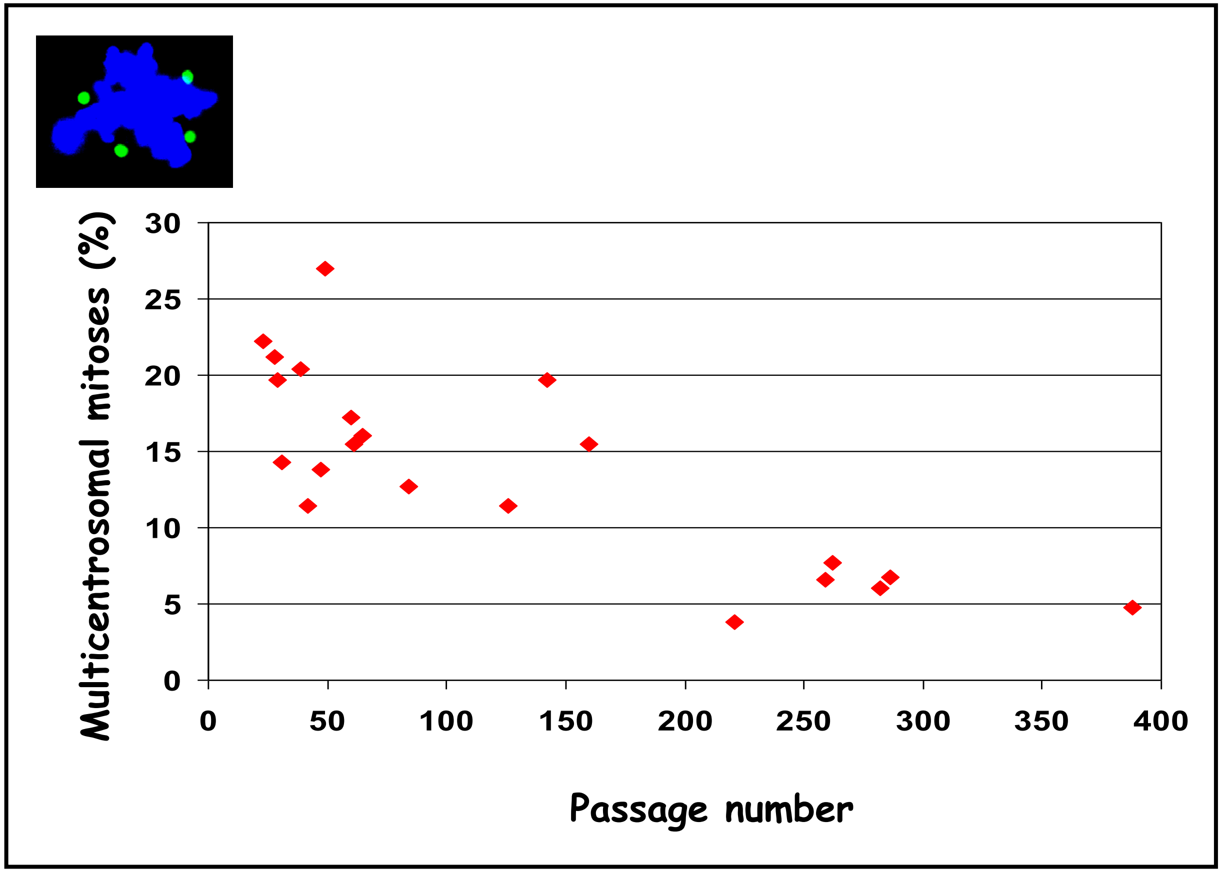
Differentiated cells

cell line	mitoses multicentrosomal / total	multicentrosomal mitoses percentage
human foreskin fibroblasts (hFF) SCRC 1041	5 / 245	2,04 %
hESC derived fibroblast-like cells	1 / 37	2,70 %
β3Tu⁺/Pax6⁺ hESC-derived cells	5 / 106	4,71 %



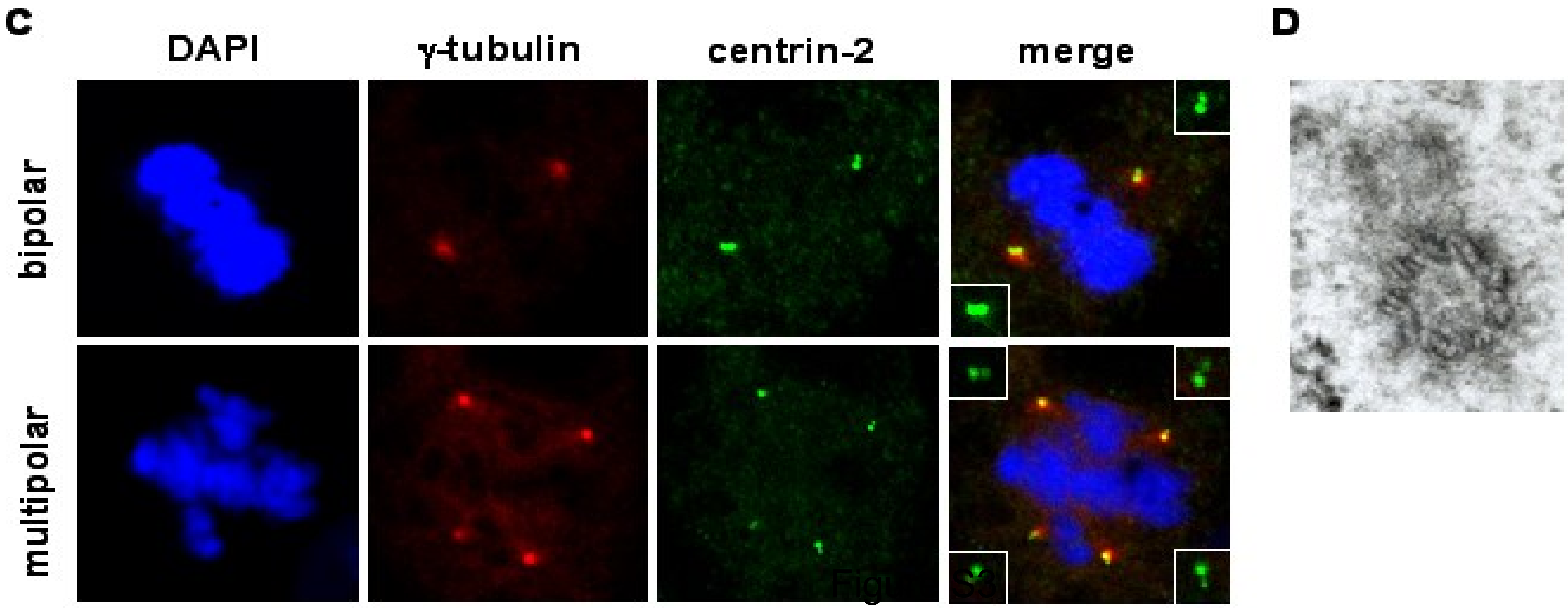
Holubcova et al., Stem Cells, 2010

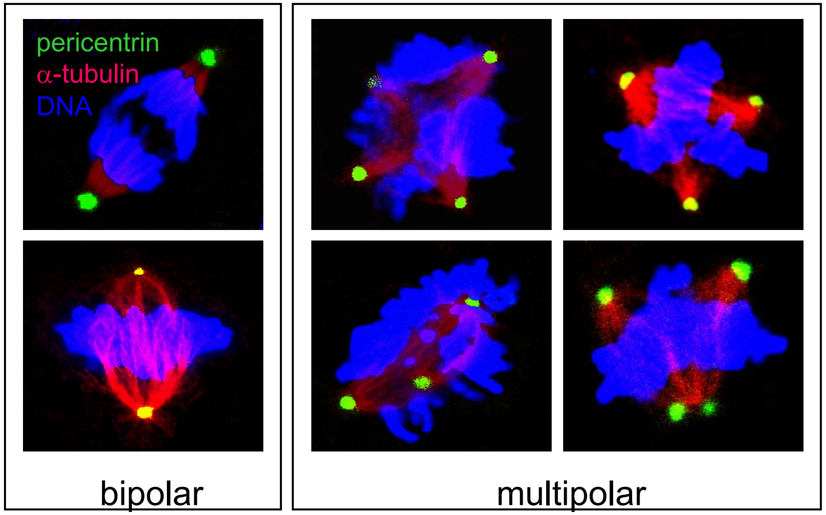
Prolonged culture reduces the frequency of mitoses with supernumerary centrosomes



Holubcova et al., Stem Cells, 2010

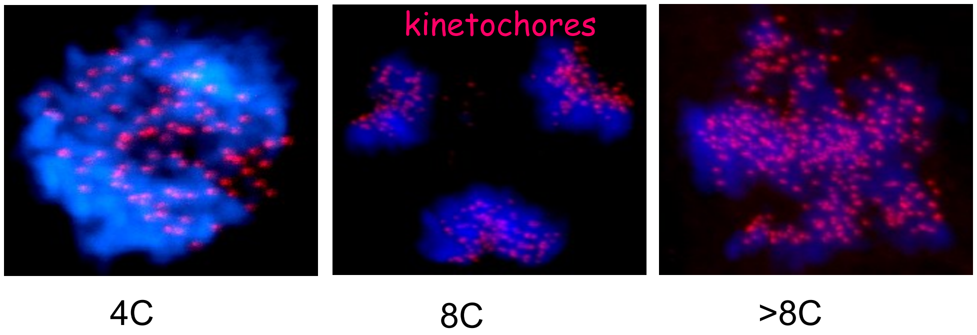
Supernumerary centrosomes have normal structure

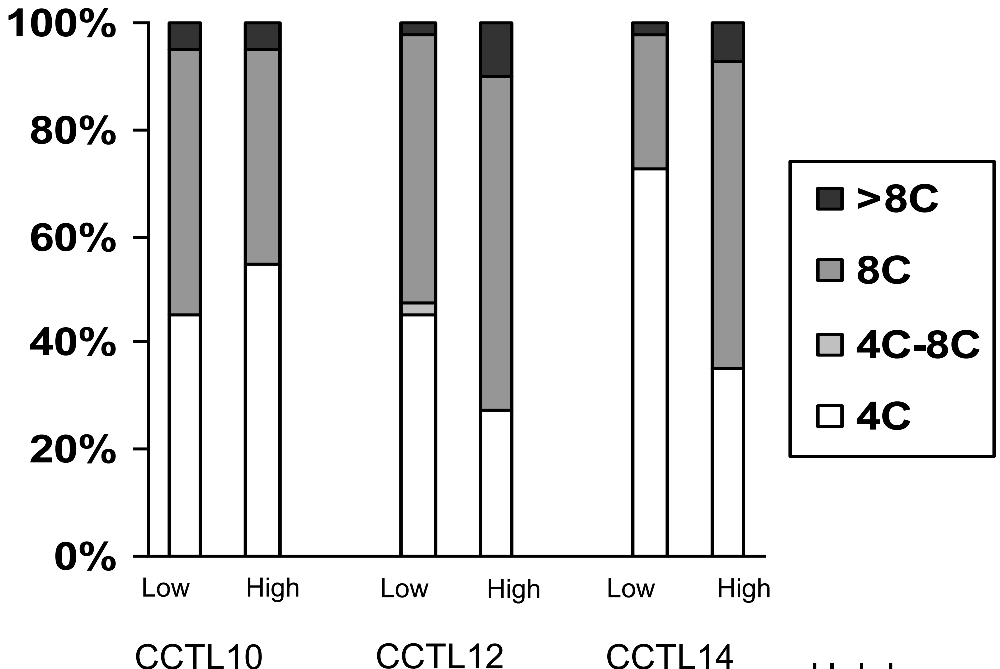




Holubcova et al., Stem Cells, 2010

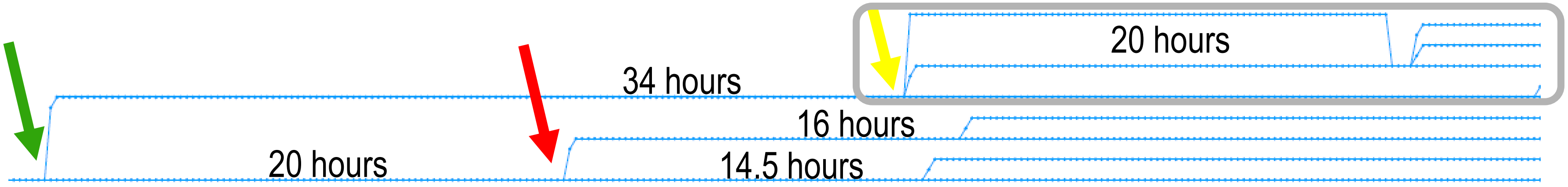
Both endoreduplication and mitotic failure contribute to overamplification of centrosomes in hESC

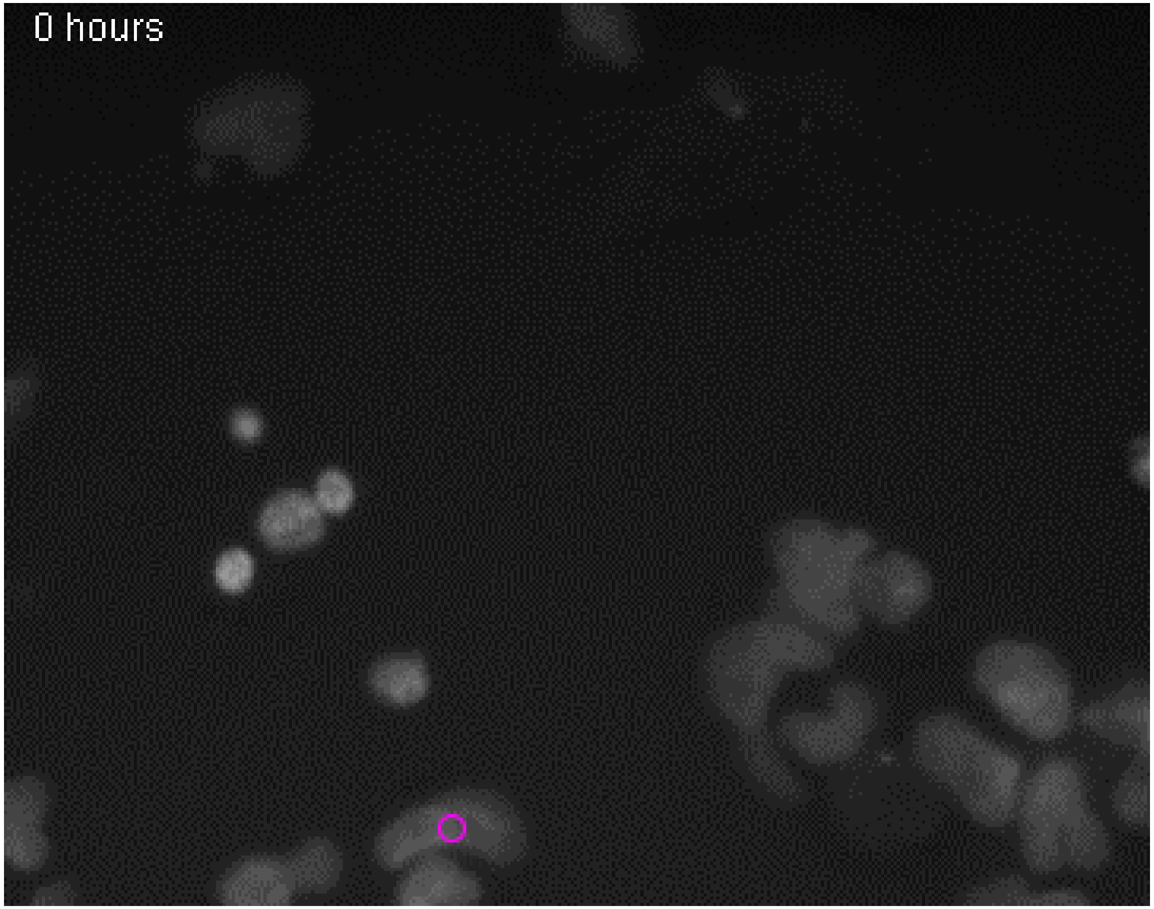




Holubcova et al., Stem Cells, 2010

Cells divide unfaithfuly





unpublished

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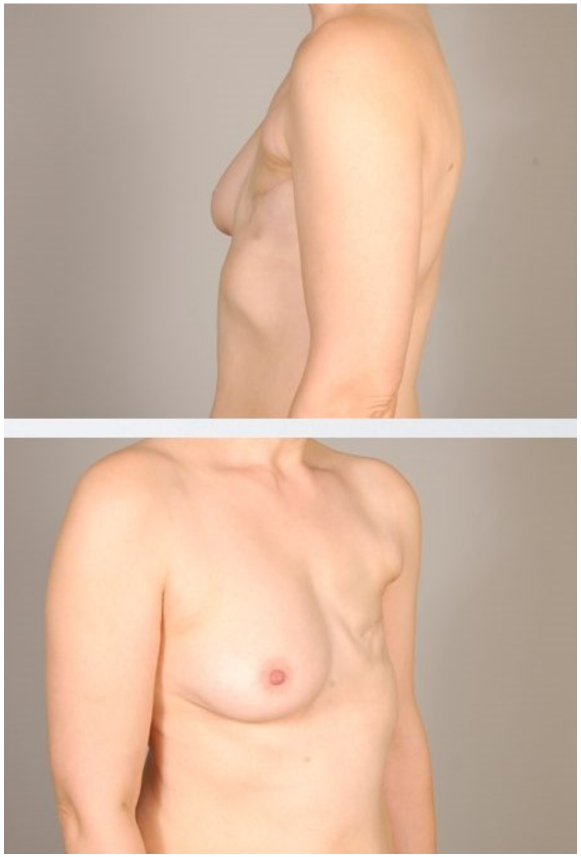
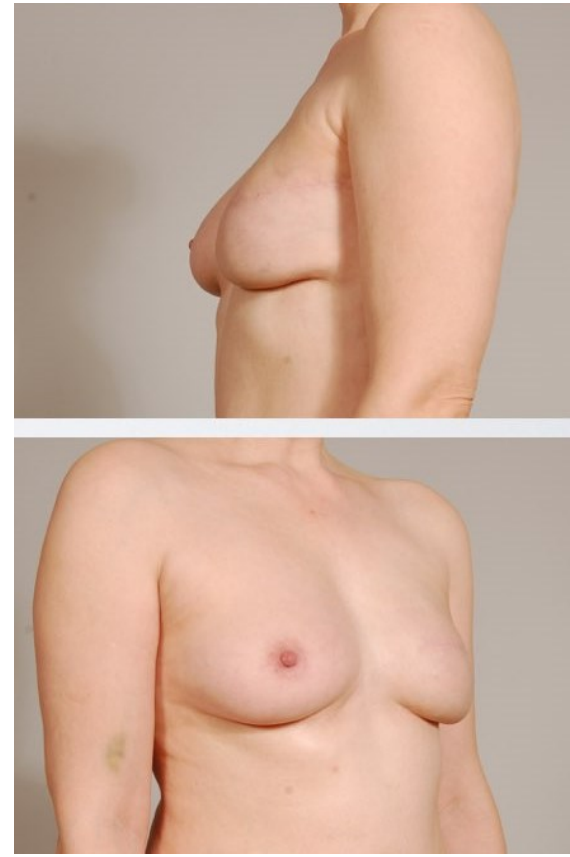
Missing connective tissues can be supplied by grafting adipose tissue



left breast ablated
because of cancer

2x consecutive lipofilling
(lipomodelation)



7 months after lipofilling

Department of Plastic and Cosmetic Surgery
(Dr. Streit)

Missing connective tissues can be supplied by grafting adipose tissue

What is the concept ?

fat grafting = a promising surgical technique that uses patient's own fat for tissue regeneration and augmentation

What is behind the effect ?

adipose-derived stem cells (ASCs) = potent fat tissue cells responsible for regeneration

What is the question ?

various ways of fat processing = do they produce the same cells ?
= do they give the same clinical outcome ?

Teaming up for getting the answer

Department of Plastic and Cosmetic Surgery
&
Department of Histology and Embryology

Patients & Collection of adipose tissue

Liposuction abdominoplasty

Measurement	Gender	Age [years]	Body mass index
I	female	47	23.4
II	female	60	25.7
III	female	60	29.4
IV	female	54	28.4
V	female	30	29.4
VI	female	49	27.1
VII	female	18	25.4
VIII	female	19	24.2
IX	female	49	27.6
X	female	42	30.5
XI	female	34	39.7
XII	female	37	26.6
XIII	female	37	33.9
XIV	male	51	43.`2
Median (minimum, maximum)		**44.5** (18; 60)	**28.0** (23.4; 43.2)

Application

500 ml normal saline
+
epinephrine

Liposuction

150 ml of lipoaspirate

(via 3.5 mm cannula)

Transfer to the lab

average length of ischemia 2.5 hr at 37°C

Processing of lipoaspirate

Sedimentation

- separation by gravity
- still used
- control group
- time consuming
- upper fat layer
- Fat fraction 71%

Centrifugation

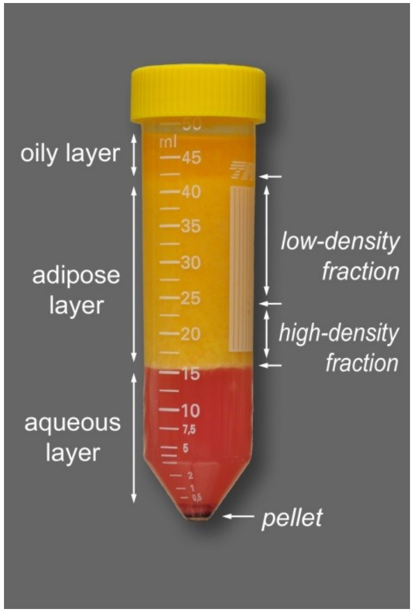
(1200 g, 3 min)

- Upper oily layer
- Fat layer - 56%

Low density (upper 2/3)

High density (bottom 1/3)

- Liquid layer
- Pellet



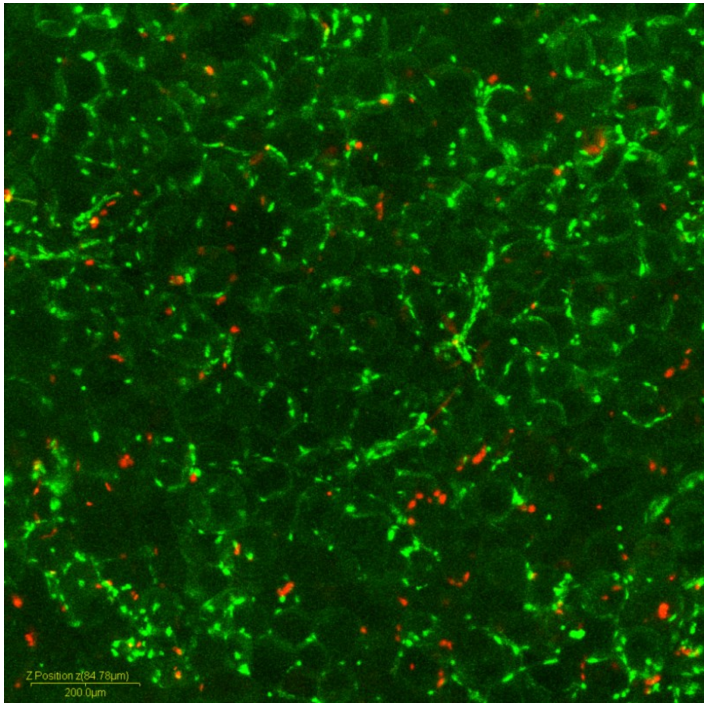
Membrane-based tissue filtration

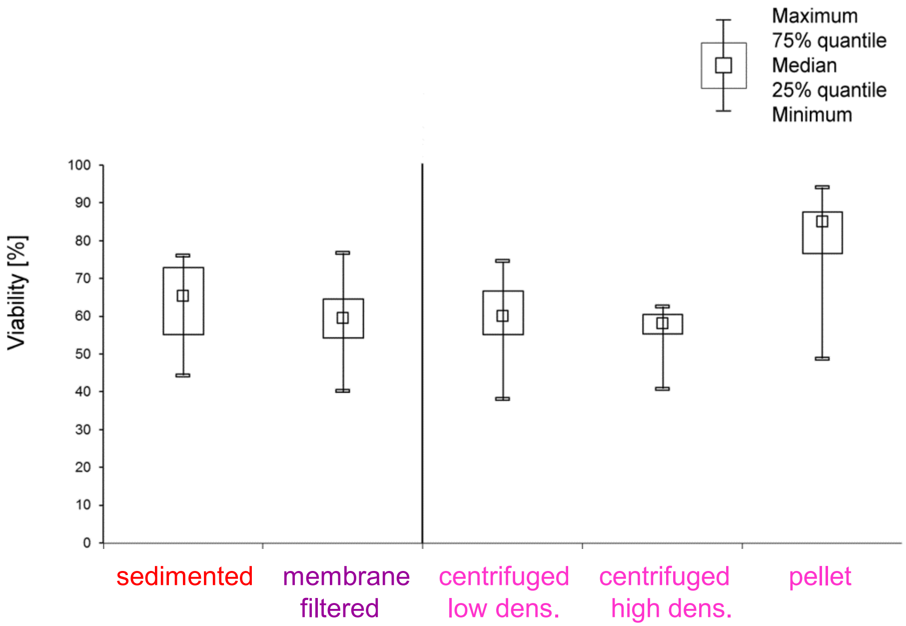
(PureGraft™ membrane)

- 2x washing 2x30 ml PBS
- filtration 2x 5 min
- Fat fraction - 46%

Many different characteristics were determined

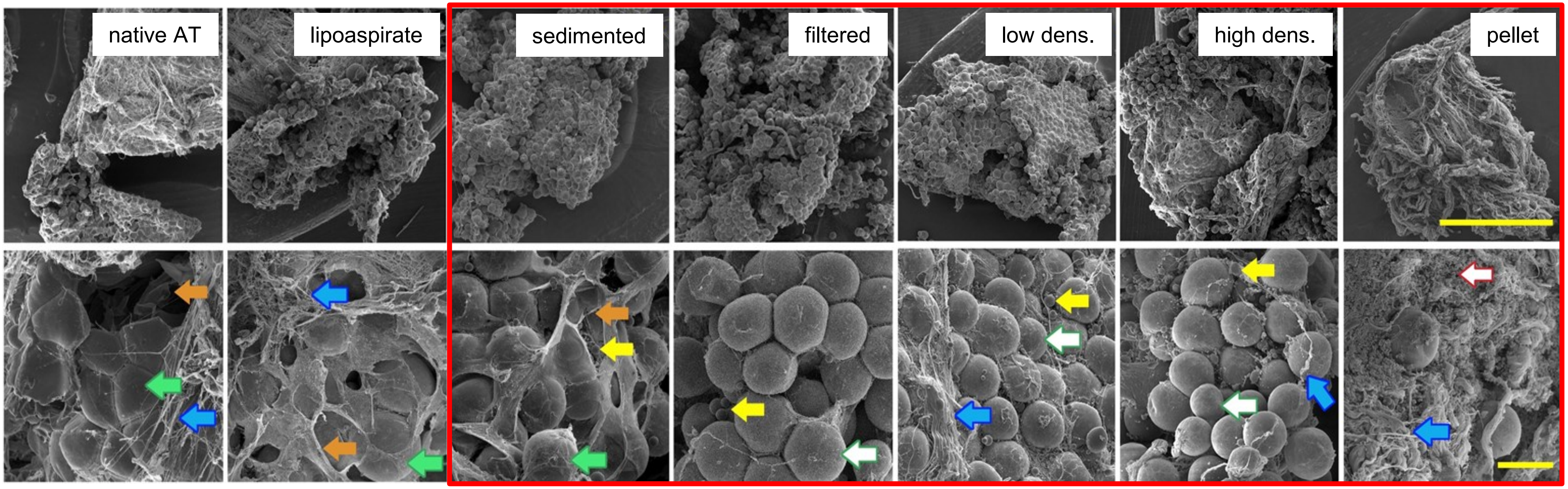
Viability of cells





NO difference

Microstructure of individual preparations



- cell debris (orange arrow)
- lipid droplets (yellow arrow)
- adipocytes + amorphous component of ECM (green arrow)
- adipocytes (open green arrow)
- fibrilar component of ECM (blue arrow)
- erythrocytes (open red arrow)

Significant differences

Some features

Sedimentation:
- abundant debris + oil drops

Centrifugation:
- minimum debris + oil drops
- reduced amount of ECM

Filtration:
- minimum debris + oil drops
- minimum ECM

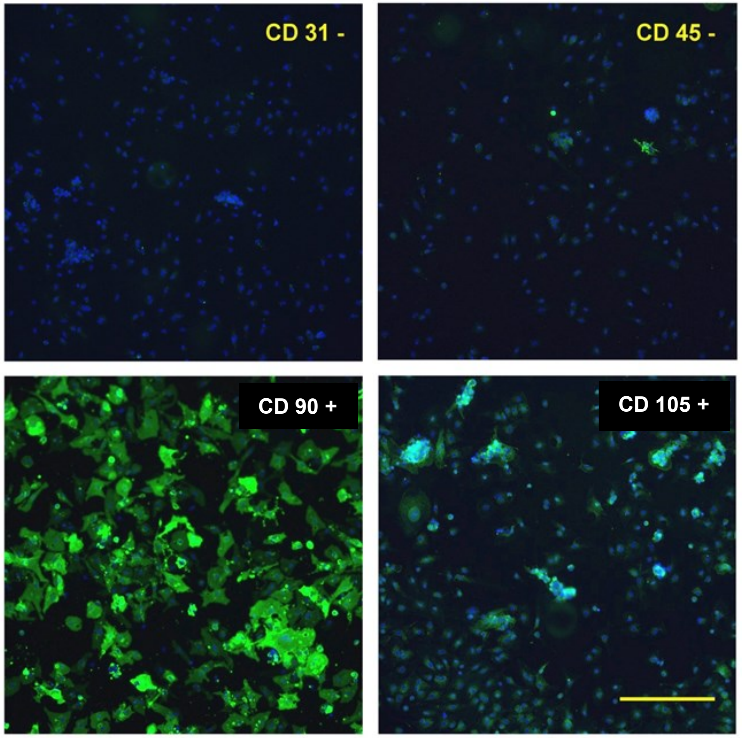
Pellet
- abundant ECM + erythrocytes

Molecular phenotype of adipose-derived stem cells

Author, title, references number	CD marker characteristic of stem/stromal cells
Rigotti, G., et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: A healing process mediated by adipose-derived adult stem cells. (9)	CD 29+, CD44+, CD 73+, ,CD90+, CD105+
Mitchell, J. B., et al. Immunophenotype of human adipose-derived cells: Temporal changes in stromal-associated and stem cell-associated markers. (16)	CD14+, CD34+, CD45+, CD73+, CD90+, CD105+,
Yoshimura, K., et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. (33)	CD 31-, CD34+, CD45-, CD90+, CD105-, CD146-
Yang, X.-F., et al. High efficient isolation and systematic identification of human adipose-derived mesenchymal stem cells. (34)	CD 29+, CD31-, CD34-, CD 44+, CD45-, CD 73+, CD105+, CD166+, HLA-DR-
Yoshimura, K., et al. Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation. (47)	CD 31-, CD34+, CD45-, CD90+, CD105-, CD146-
Dominici, M., et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. (15)	CD14-, CD34-, CD45-, CD73+, CD90+, CD105+
Condé-Green, A., et al. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: A comparative study. (48)	CD 34+, CD45-, CD105+
Condé-Green, A., et al. Effects of Centrifugation on Cell Composition and Viability of Aspirated Adipose Tissue Processed for Transplantation. (50)	CD 34+, CD45-, CD105+

Negative for:
CD31 & CD45

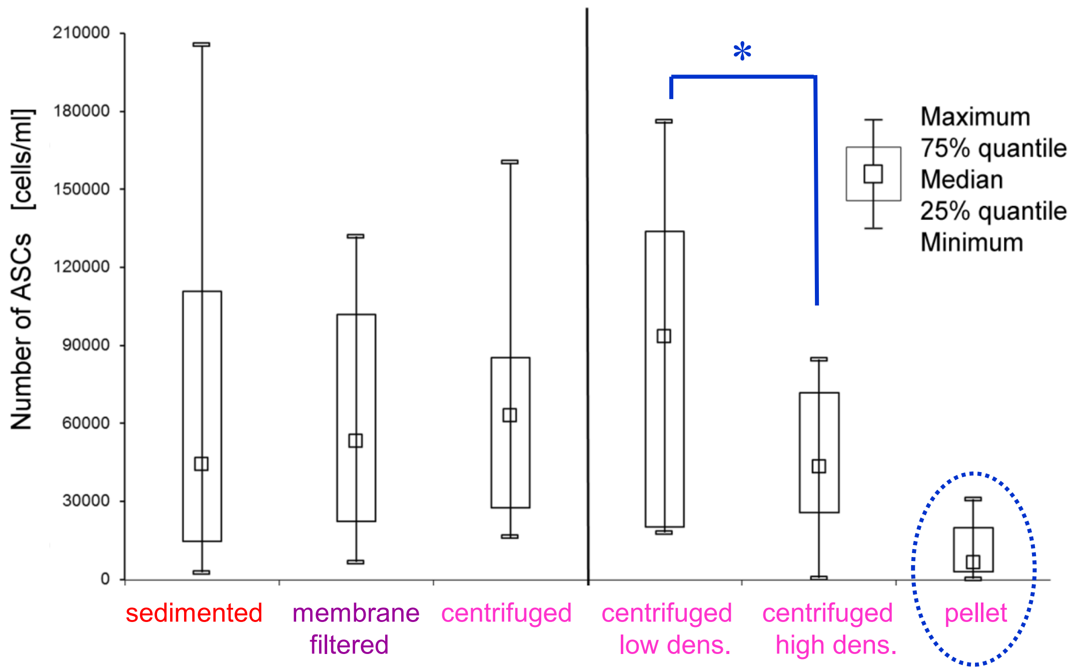
Positive for:
CD90 & CD105



Perfectly normal in all preparations

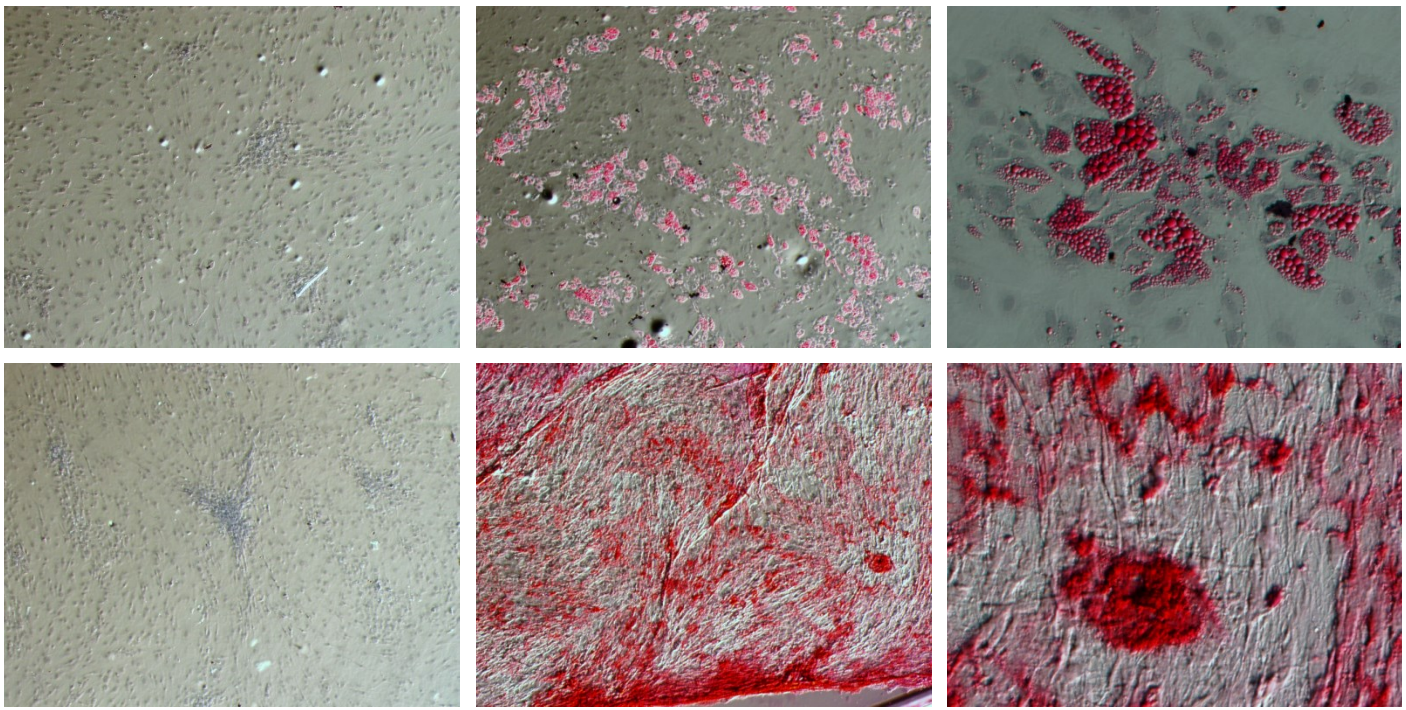
Proportion of adherent adipose-derived stem cells

calculated as number of SC in 1 ml of original processed adipose fraction



SOME differences

Capacity to differentiate towards adipogenic and osteogenic



adipogenic (oil red)

osteogenic (alizarin red)

non-differentiated control   differentiated

Perfectly normal differentiation capacity in all preparations

Key medically relevant findings

Viability of cells

- independent of the procedure
- probably much more affected by the duration of ischemia (manipulation)

Sedimentation

- very gentle – lowest yield of ASC
- time consuming – unpractical for clinical setting

Centrifugation x Membrane-based filtration

- quantitatively and qualitatively very similar outcome

Centrifugation seems to be preferable

uncompromised quality & cost effectiveness

EXPERIMENTAL

A Comprehensive In Vitro Comparison of Preparation Techniques for Fat Grafting

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Josef Jaros, Ph.D.
Veronika Sedlakova, M.D.
Miroslava Sedlackova, M.D., Ph.D.
Lubos Drazan, M.D., Ph.D.
Michal Svoboda, B.Sc.
Jakub Pospisil, M.Sc.
Tomas Vyska, M.D.
Jiri Vesely, M.D., Ph.D.
Ales Hampl, D.V.M., Ph.D.

Brno, Czech Republic

Background: Lipomodeling is a technique that uses the patient's own fat for tissue regeneration and augmentation. The extent of regenerative effect is reported to be determined by the numbers of adipose-derived stem cells and the viability of cells in processed adipose tissue which, together with other factors, influence the degree of graft retention. This study addresses whether differences exist in properties of fat graft obtained by three commonly used techniques.
Methods: Adipose tissue harvested from the hypogastric regions of 14 patients was processed by decantation, centrifugation, and membrane-based tissue filtration. The morphology of each preparation was assessed by electron microscopy and overall cell viability was assessed by live/dead assay. The number of adipose-derived stem cells was determined and their stem cell character was assessed by the presence of cell surface molecules (i.e., CD105, CD90, CD31, and CD45) and by their capacity to differentiate into adipogenic and osteogenic lineages.
Results: First, morphologies of processed fat samples obtained by individual procedures differed, but no preparation caused obvious damage to cellular or acellular components. Second, although the highest numbers of adipose-derived stem cells were contained in the upper fraction of centrifuged lipoaspirates, the difference between preparations was marginal. Third, the maximal concentration of adipose fraction (removal of watery component) of lipoaspirate was achieved by membrane-based tissue filtration. Finally, no significant differences in overall viability were detected.
Conclusions: Properties of processed lipoaspirate were influenced by the preparation procedure. However, the differences were not dramatic; both centrifugation and membrane-based filtration are methods of choice whose selection depends on other criteria (e.g., practicality) for individual surgical settings. (*Plast. Reconstr. Surg.* 139: 670e, 2017.)

It works and a spectrum of applications is wide

persistent postradiation ulceration (sarcoma treament)

2x application of the graft (arround + underneath the ulcer)

7 months after grafting

Unpublished (Dr. Streit)

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Stem cells can repair adult tisues/organs

Reparative behavior

- Constitutive high rate
- Defined hierarchy of stem/progenitor cells

Epidermis

Intestine

Blood

- Low steady-state turnover
- Robust repair after damage

Lung

Liver

Pancreas

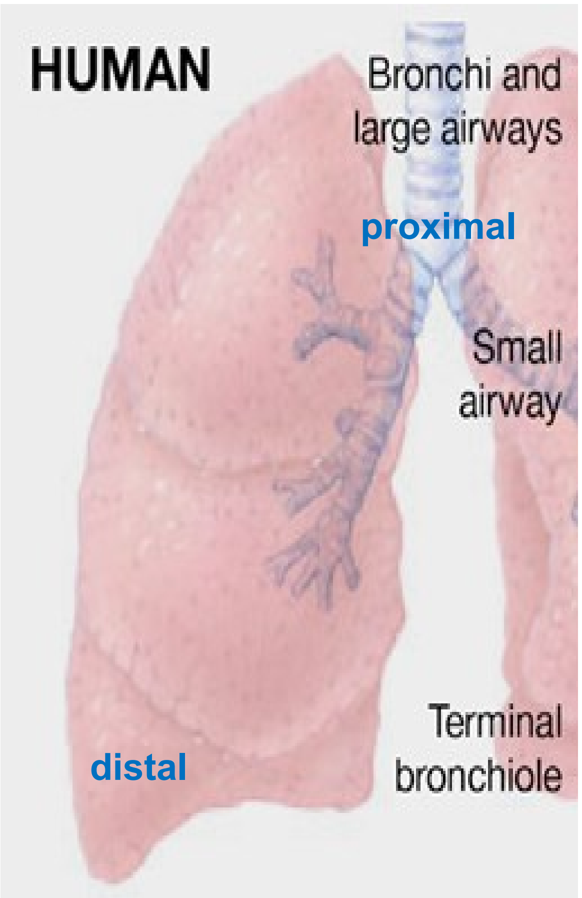
- Inefficient
- Scaring instead of repair

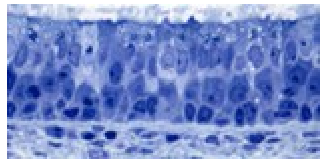
Brain

Heart

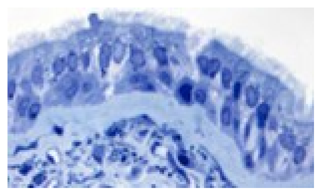
Lungs as one of the targets

Anterior ventral foregut endoderm +

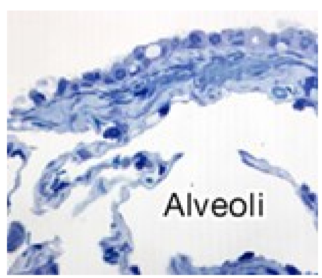




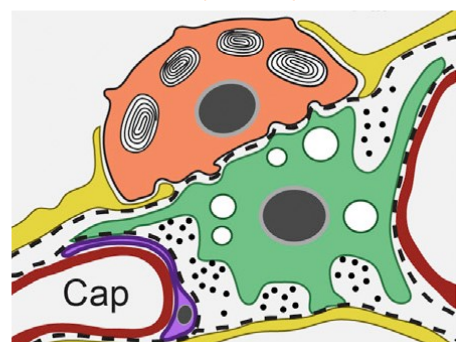
Ciliated cells
Goblet cells
Serous cells
Neuroendocrine cells
Basal cells



Club cells
Ciliated cells
Basal cells



Pneumocytes type I



Pneumocytes type II

Mesoderm

Vascular smoth muscle
Airway smooth muscle
Cartilage
Fibroblasts
Pericytes

More than 40 cell lineages identified in lungs !!!

Lung diseases potentially treatable by cell therapies.

Respiratory diseases are the **third leading cause of death** in the industrialized world.

Lung replacement is often the only solution.

Lung disease	Affected components	Therapeutic target
Respiratory distress syndrome	Alveolar epithelium Capillary endothelium	Epithelia and endothelia regeneration
Asthma	Epithelium Myofibroblast Airway smooth muscle	Inhibition of inflamation Inhibition of airway remodeling, Inhibition of muscle heperplasia
Bronchopulmonary dysplasia	Alveolar epithelium Capillary endothelium Interstitial fibroblasts	Inhbition of inflamation Regeneration of alveolar septa and epithelium
Cystic fibrosis	Airway epithelium	Delivery of CFTR (cystic fibrosis conductance regulator)
Chronic obstructive pulmonary diseases (emphysema)	Alveolar epithelium Capillary endothelium Interstitial fibroblasts	Generate 3D alveolar structure
Bronchiolitis obliterans	Airway epithelium	Regeneration of epithelia
Cancer	All components	Complete replacement of 3D structure

and others

Options for new therapeutic strategies

Acute alveolar damage
- inhalation injury
- blast injury

↓

Activation of healing potential of resident progenitors

Chronic lung damage
- chronic obstructive pulm. disease
- fibrosis
- bronchopulmonary displasia
- and others

↓ Cellular therapy

↓ Lung engineering

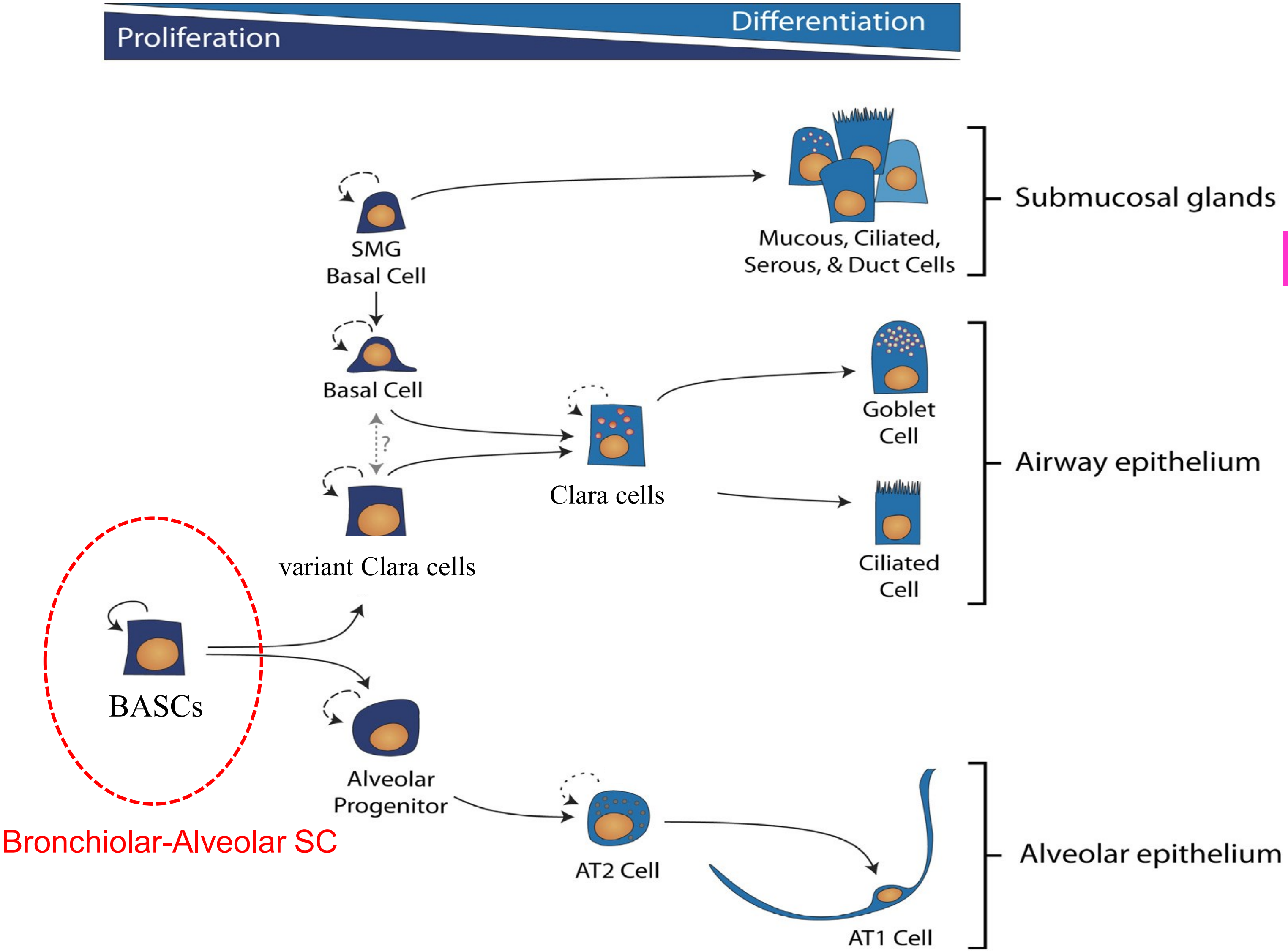
What cell sources we may consider ?

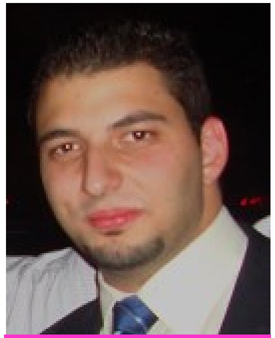
1
Adult stem cells isolated from non-lung compartmets

2
Stem / progenitor cells residing in lung tissues

3
Lung stem / progenitor cells differentiated from pluripotent stem cells

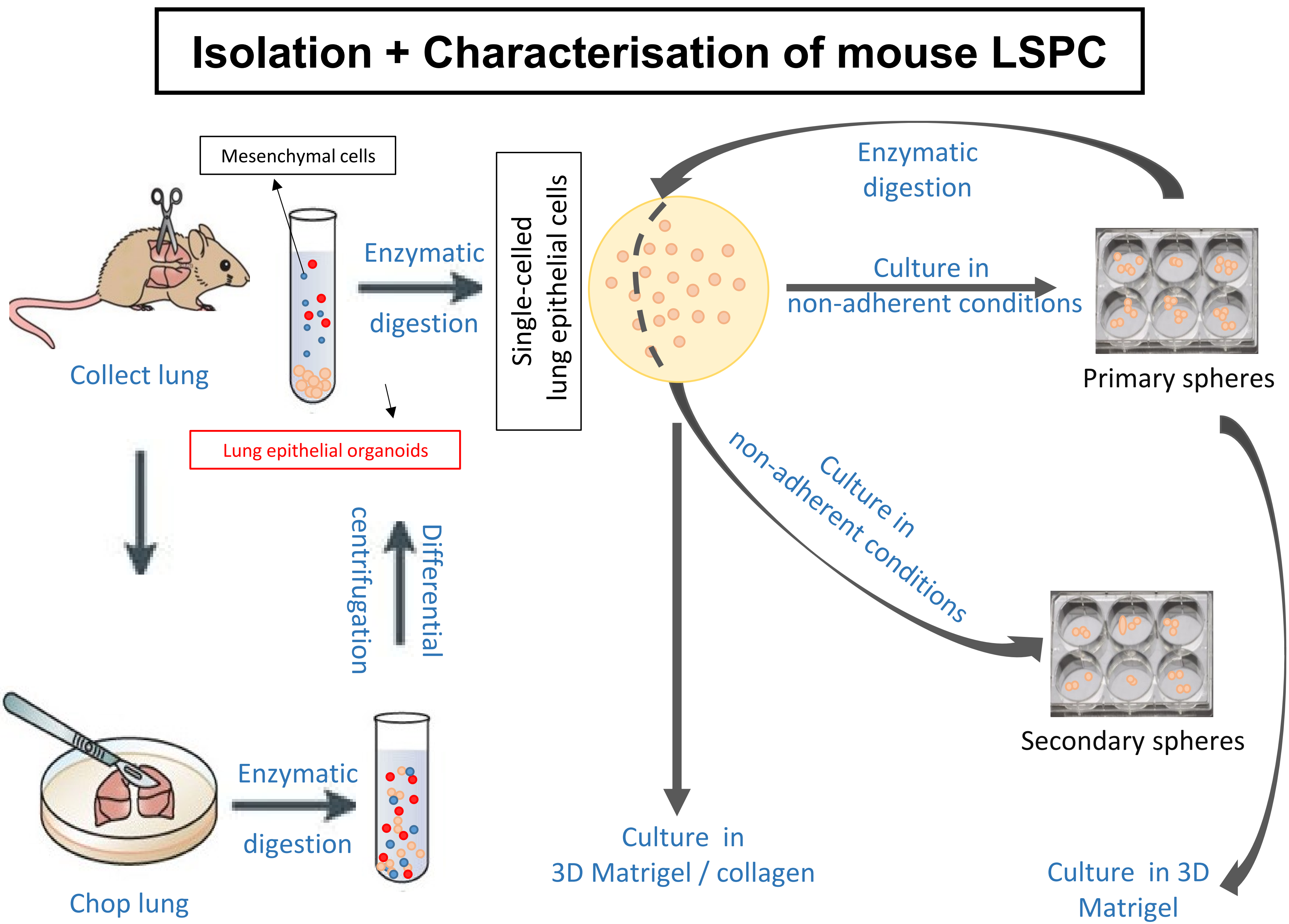
Stem / progenitor cells residing in lung tissues





Anas Rabatta

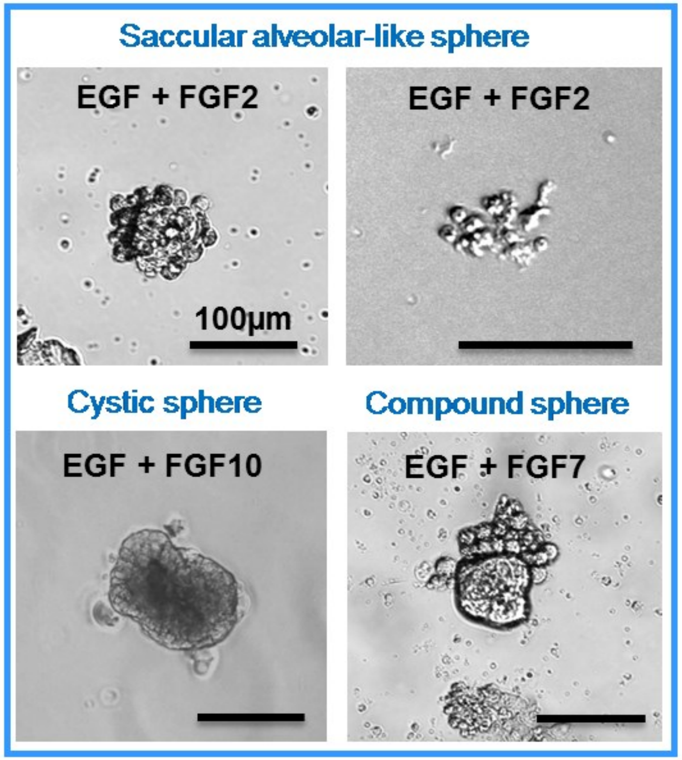
Dr. Zuzana Koledová

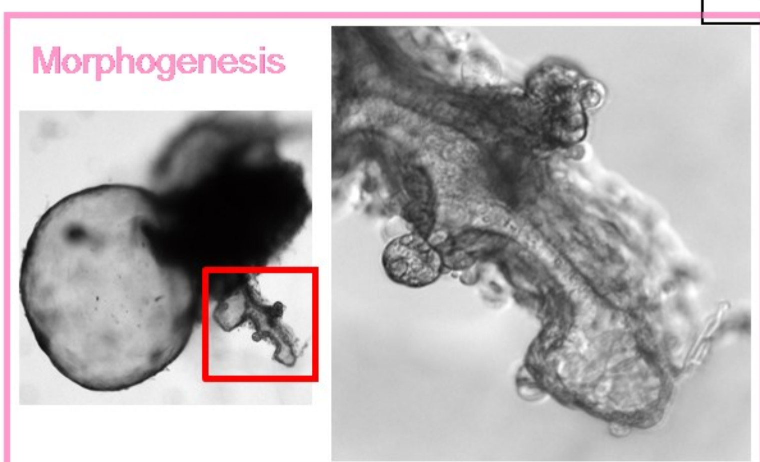


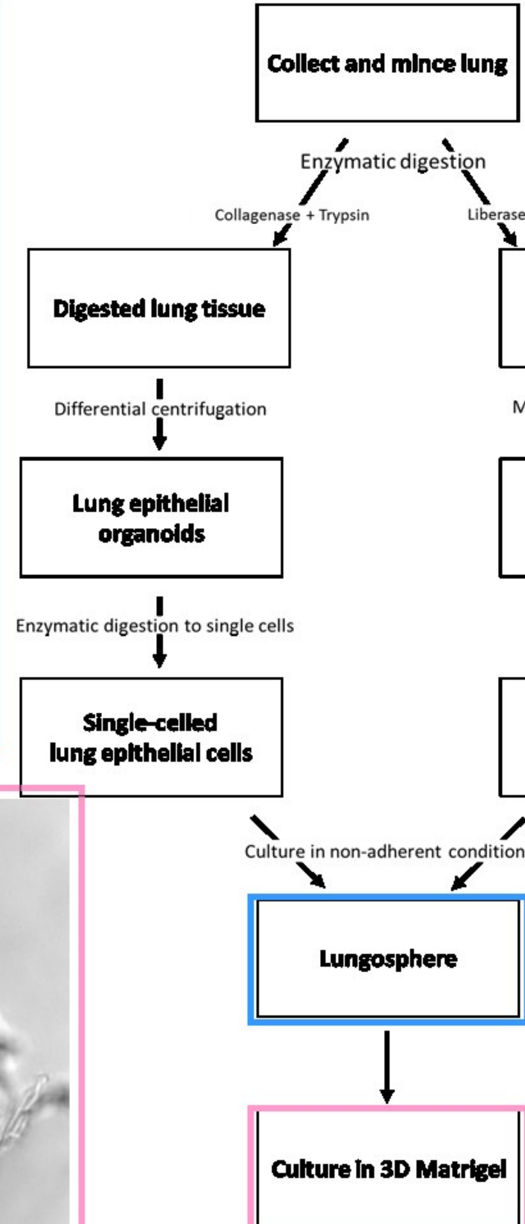
Isolation + Characterisation of mouse LSPC
Mesenchymal cells
Collect lung
Enzymatic
digestion
Single-celled
lung epithelial cells
Lung epithelial organoids
Differential
centrifugation
Chop lung
Enzymatic
digestion
Enzymatic
digestion
Culture in
non-adherent conditions
Primary spheres
Culture in
non-adherent conditions
Secondary spheres
Culture in
3D Matrigel / collagen
Culture in 3D
Matrigel

Global strategies adopted

unpublished







EpCAM^{+}
CD49f^{+}
CD24^{low}
CD104^{+}

PNAS

Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung

Jonathan L. McQualter[a,b,1], Karen Yuen[a], Brenda Williams[a], and Ivan Bertoncello[a,b]

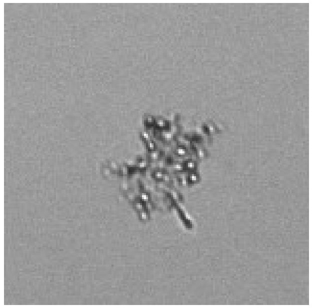
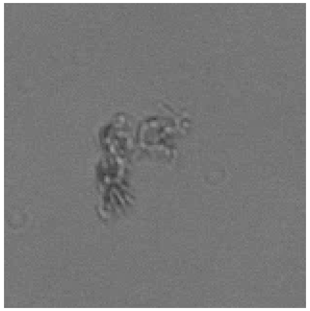
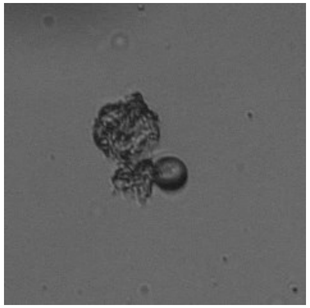
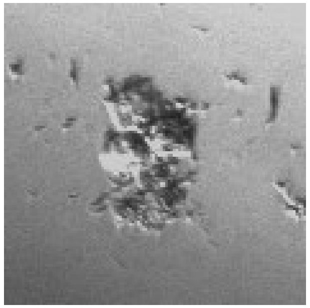
[a]Australian Stem Cell Centre, Clayton, Victoria 3800, Australia; and [b]Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria 3800, Australia

Edited by Darwin J. Prockop, Texas A&M Health Science Center, Temple, TX, and approved December 8, 2009 (received for review August 13, 2009)

The role of lung epithelial stem cells in maintenance and repair of ... in turn, leads to the identification of cellular and molecular ther-

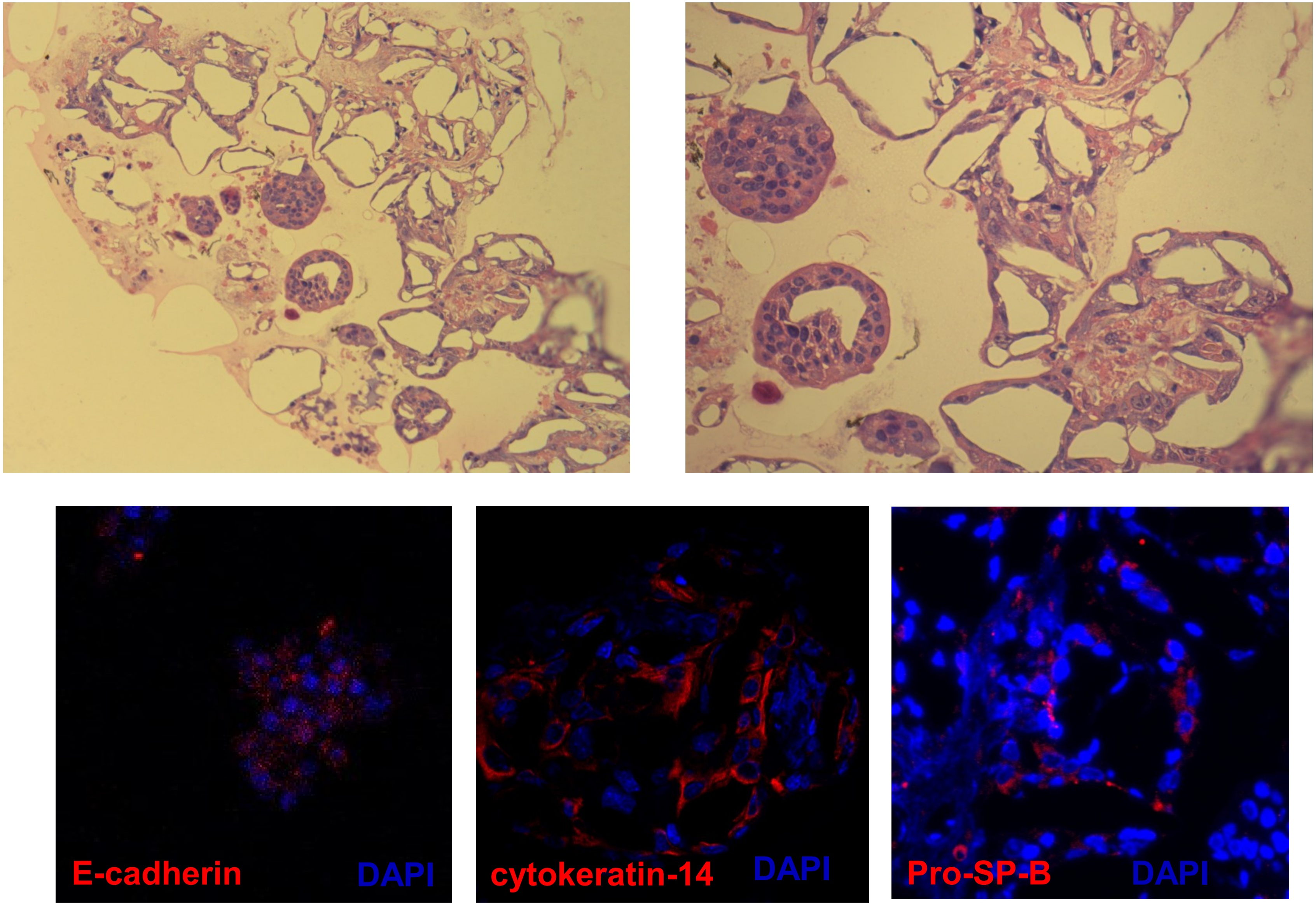
Lungospheres originating from one single cell

	The number of cells before sorting	Viability	The number of ($EpCAM^+$,$CD49f^+$, $CD104^+$,$CD24^{low}$) cells	Efficiency of Primary lungospheres formation
Liberase	$35*10^6$	86.8%	1834	2.1%

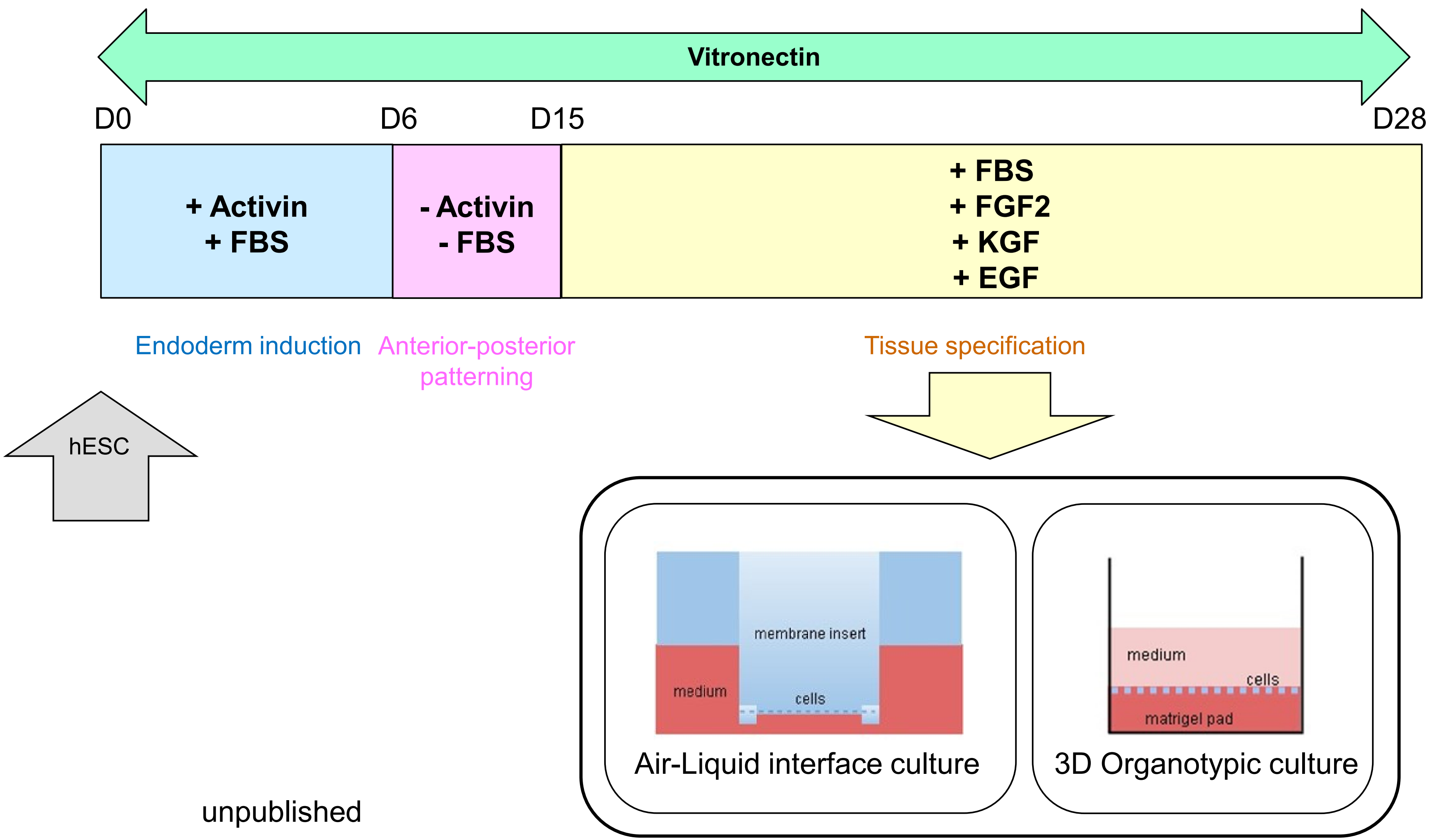
unpublished

Morphogenesis in lungospheres grown for 2 weeks in suspension culture



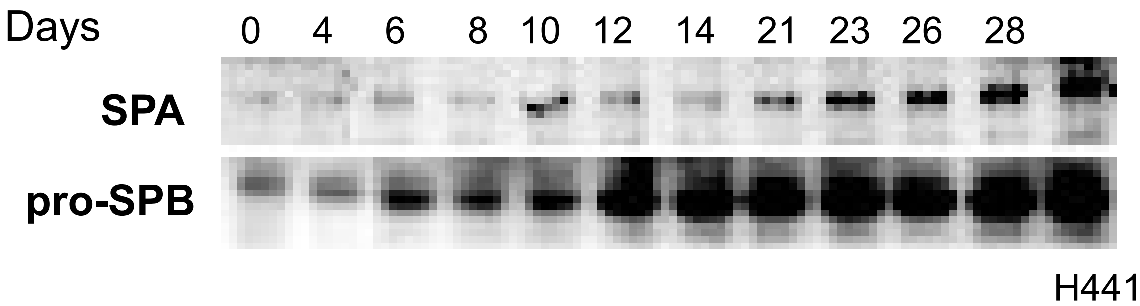
unpublished

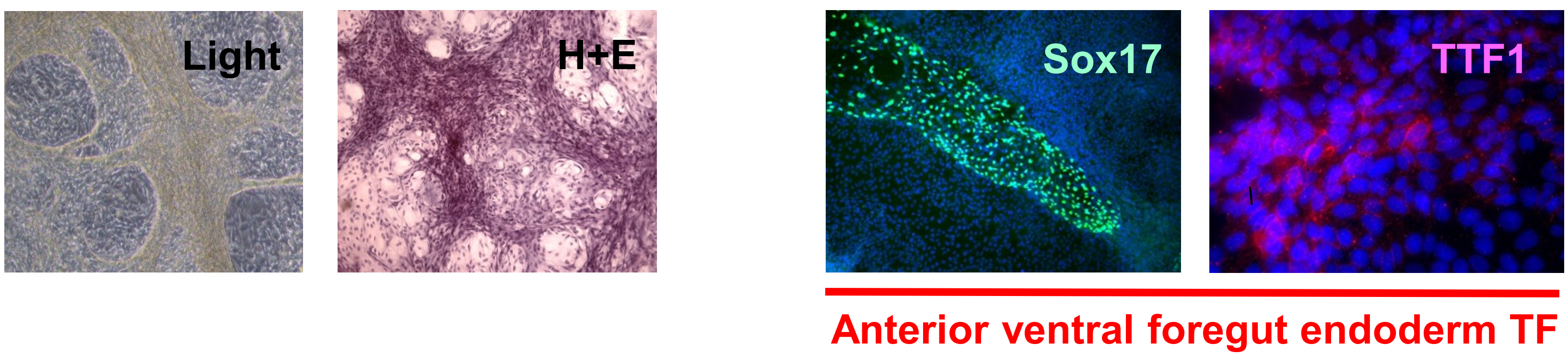
Direct differentiation of pluripotent SC into airway epithelia

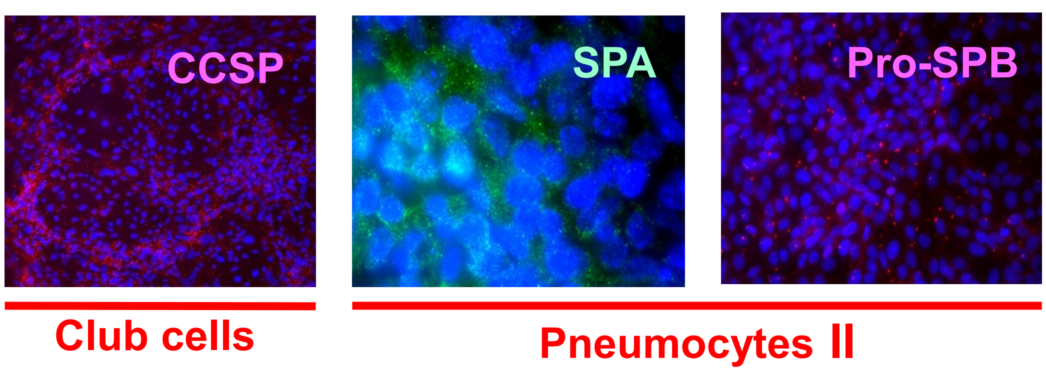


unpublished

Direct differentiation of pluripotent SC into airway epithelia – 15 days







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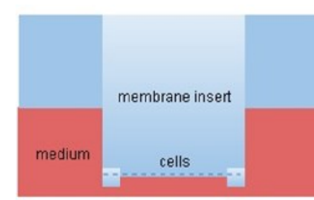
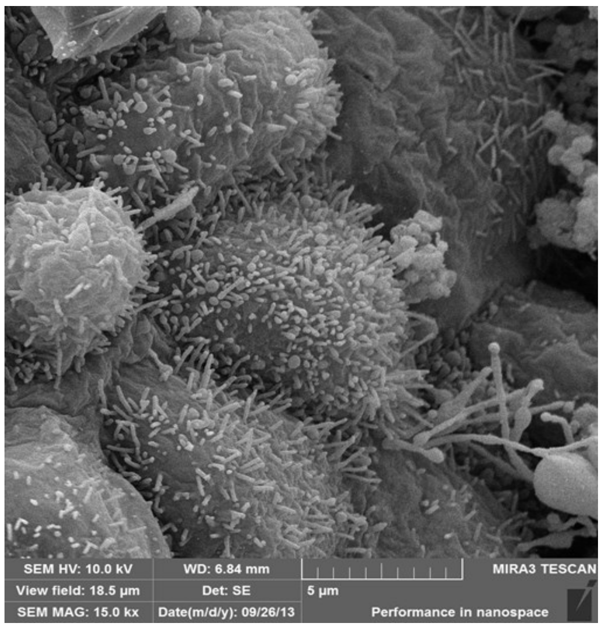
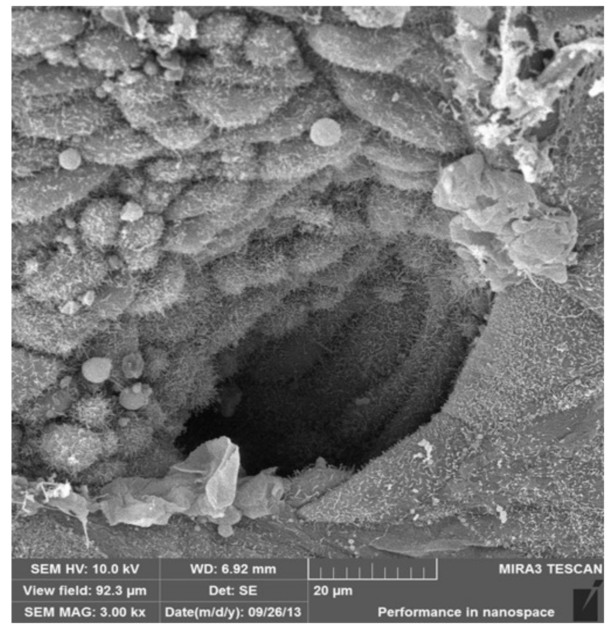


Air-Liquid interface culture

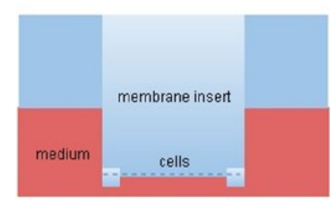
Direct differentiation of pluripotent SC into airway epithelia – 20 days



Microvili



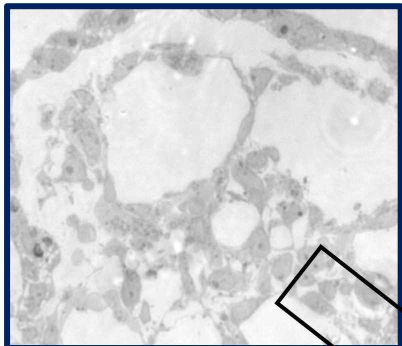
Cavities

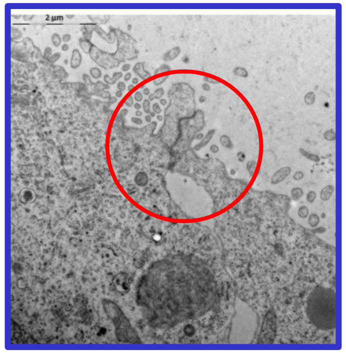


Air-Liquid interface culture

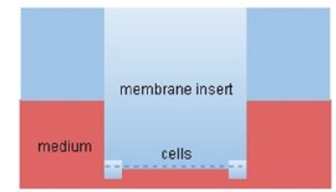
unpublished

Direct differentiation of pluripotent SC into airway epithelia – 20 days

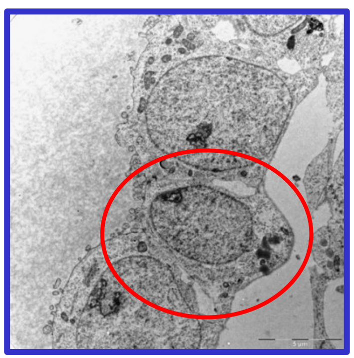




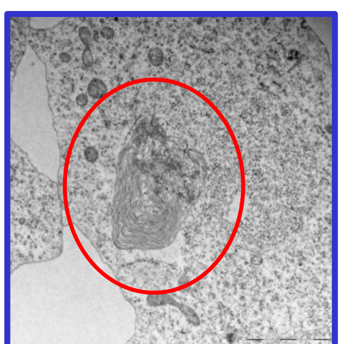
Tight junctions
Microvili



Air-Liquid interface culture

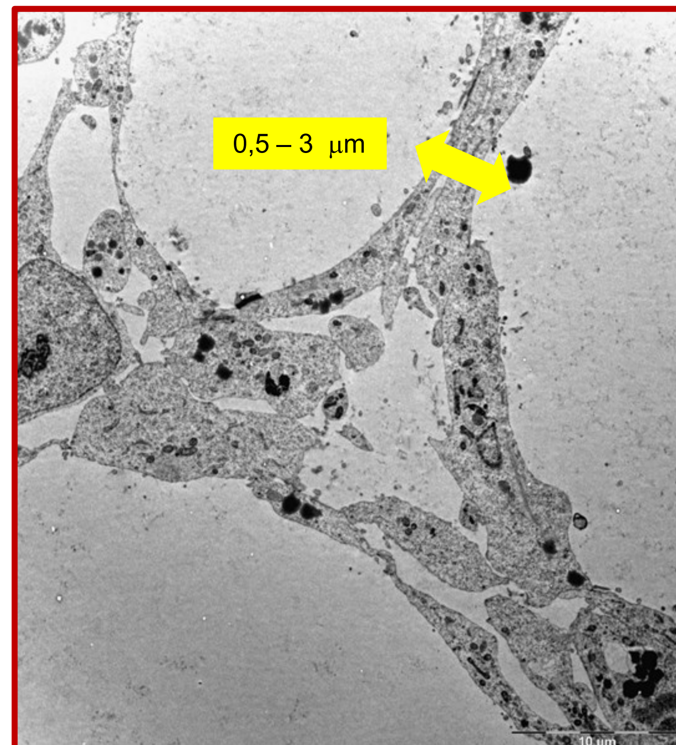


Polarized cells



Lamellar bodies

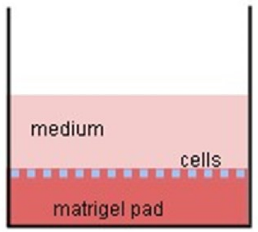
Flattened cells



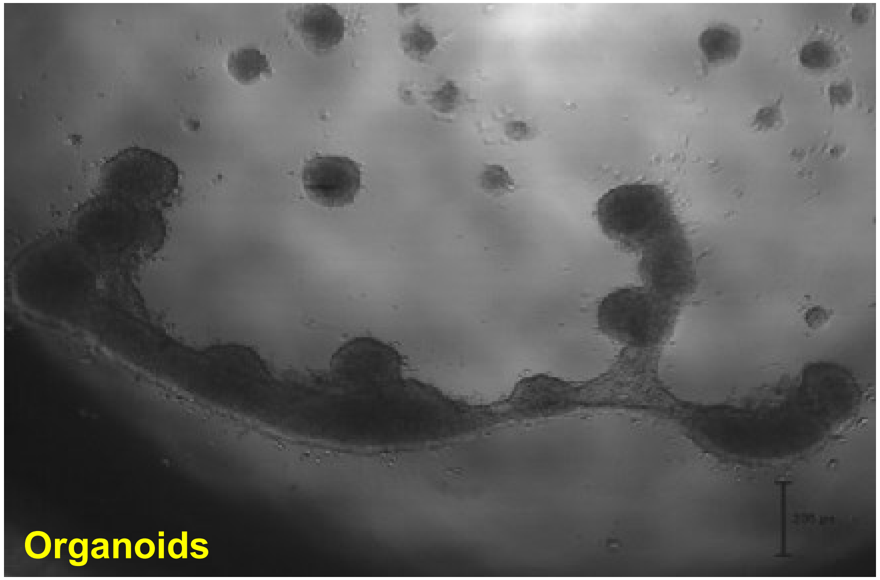
Alveolar-like organization

unpublished

Direct differentiation of pluripotent SC into airway epithelia – 20 days



3D Organotypic culture



Analysis in progress

unpublished

Differentiation of early lung progenitors (ELP) from hESC

FBS	10 %	10 %	0	2 %
Activin A	-	+	-	-
ITS	-	-	+	+
FGF2	-	-	-	+
EGF	-	-	-	+
Heparin	-	-	-	+
Vitronectine	+	+	+	+

Day 0 — 1 — 6 — 15

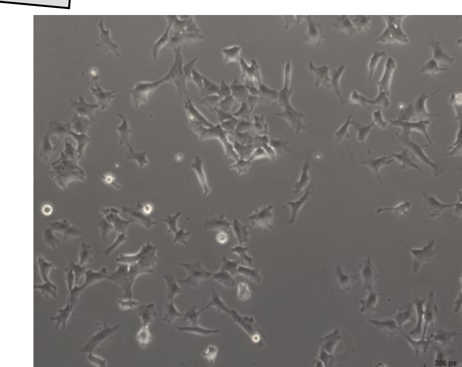
hESC (Day 0)

Definitive endoderm (Day 6)

Anterior ventral foregut endoderm (Day 15)

ELP

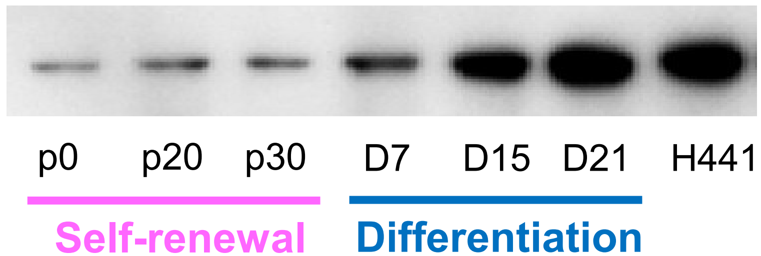
Hana Kotasová

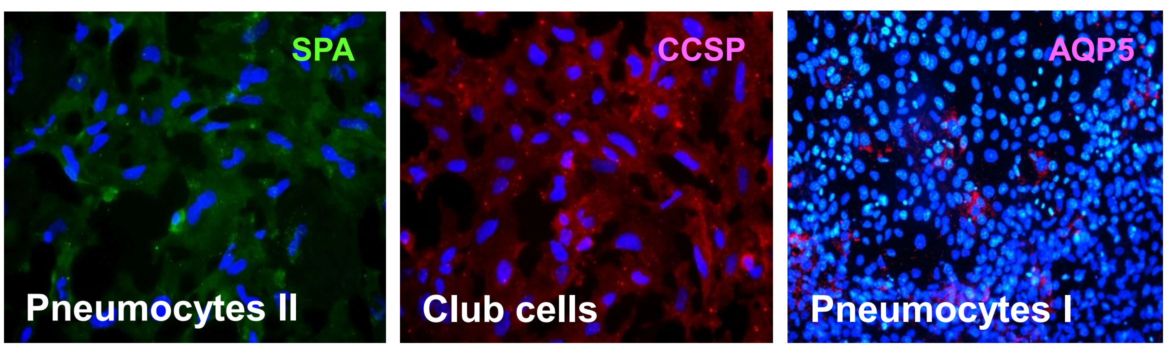


unpublished

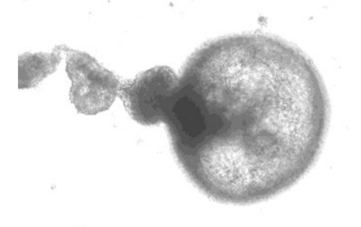
Early lung progenitors X Common endoderm progenitors

Lamellar bodies





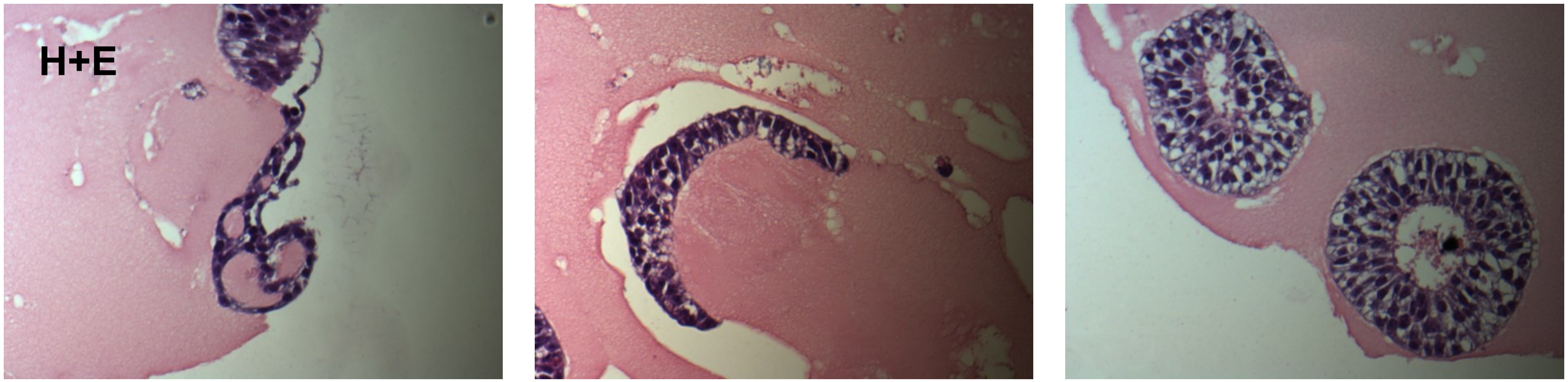
Differentiation for 20 days (ELP in p20)



Organoid (10 days 3D)

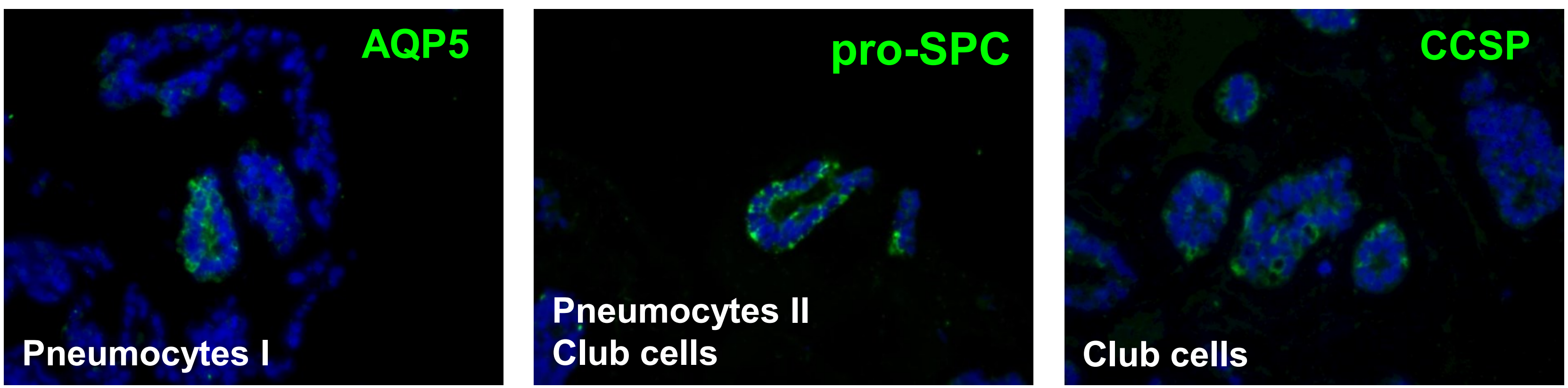
unpublished

Early lung progenitors differentiated for 25 days in matrigel



Simple squamous epithelium (alveolar-like)

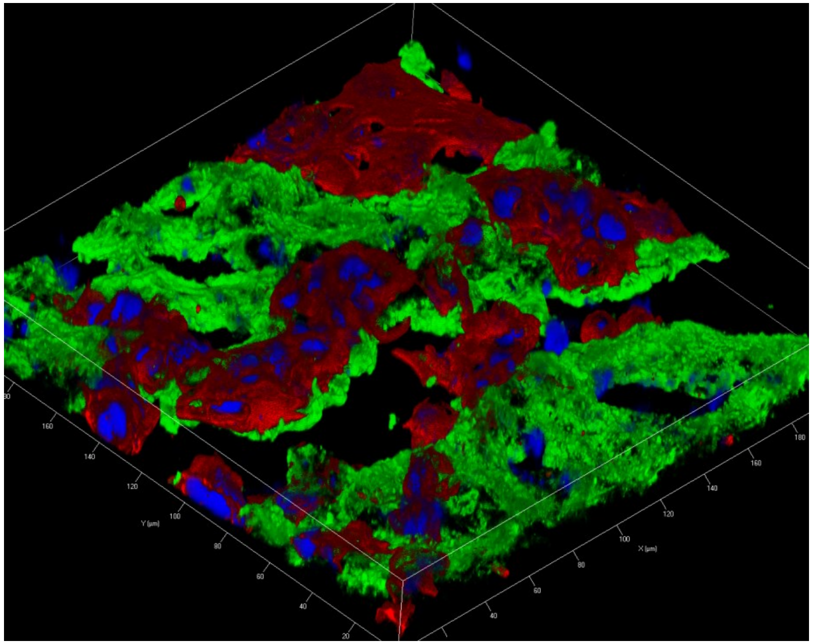
Pseudostratified epithelium in tubular structures (airway-like)

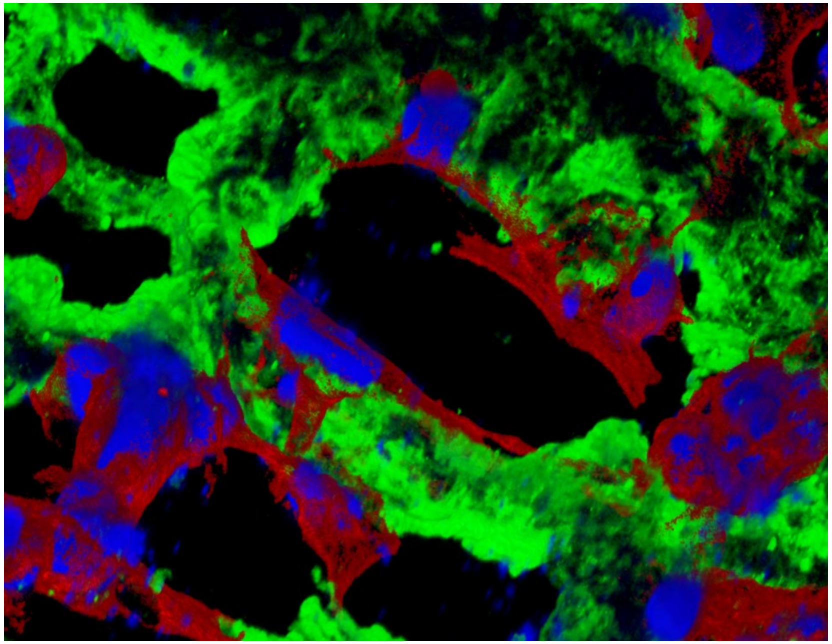


unpublished

Early lung progenitors attach and proliferate on mouse decellularized lung scaffold

+ respond to topography by adjusting their shape

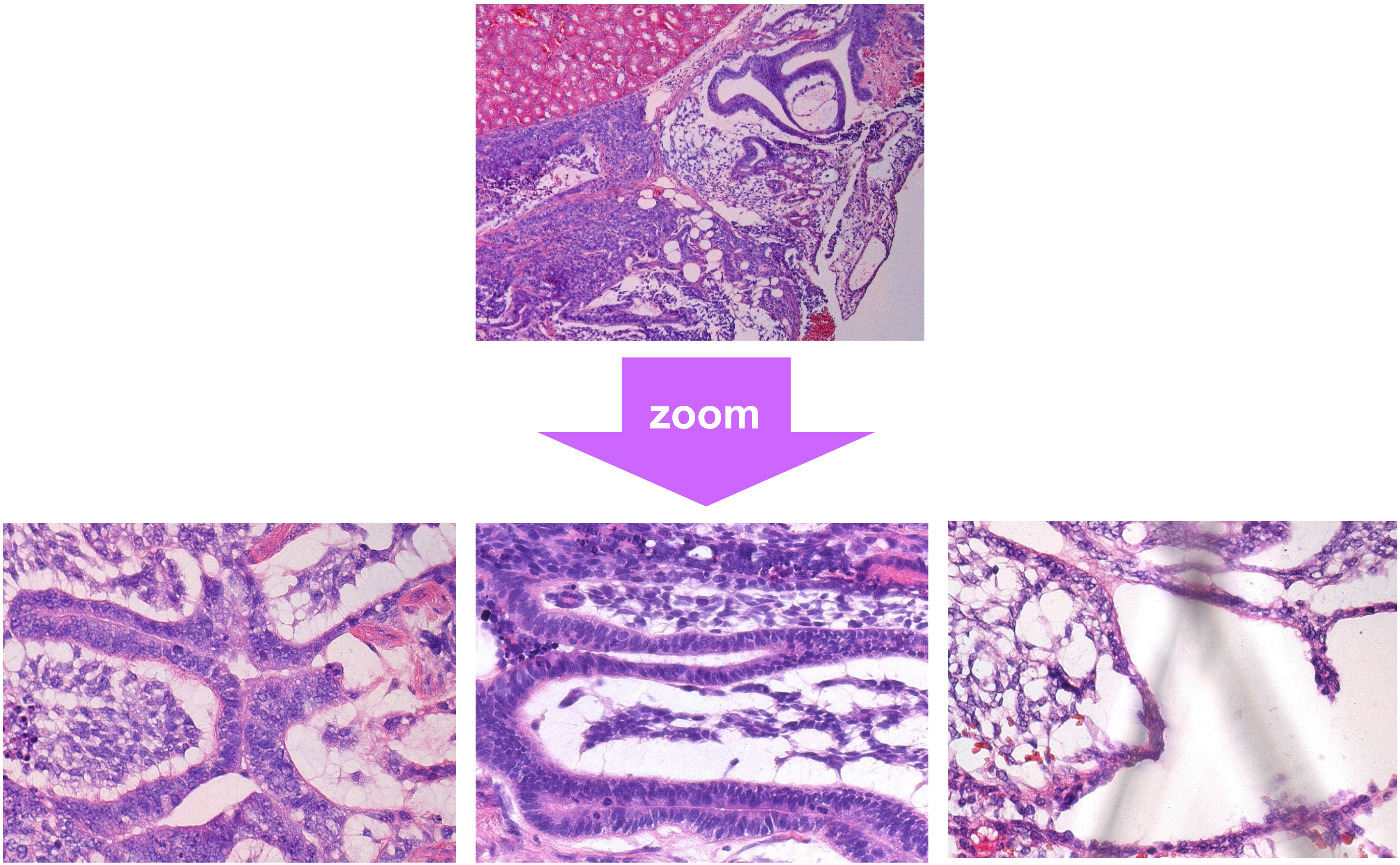




Col IV
Phalloidin
DAPI

unpublished

ELP differentiate when transplanted under kidney capsule



unpublished

ESC can give rise to highly organized organ-like structures

3D culture (EB)
+ integrins
+ laminin
+ entactin
+ Nodal

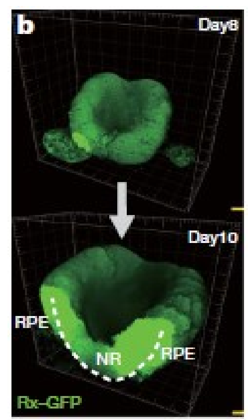
ARTICLE

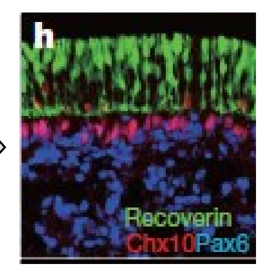
April 2011

doi:10.1038/nature09941

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

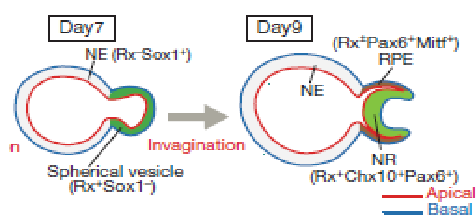
Mototsugu Eiraku[1,2], Nozomu Takata[1], Hiroki Ishibashi[3], Masako Kawada[1], Eriko Sakakura[1,2], Satoru Okuda[3], Kiyotoshi Sekiguchi[4], Taiji Adachi[3,5] & Yoshiki Sasai[1,2]





Internal nuclear layer
External nuclear layer
Ganglion cells

D24



What will we discuss today ?

One have to be cautious - representative example of risk associated with propagation of stem cells outside the body

Stem cells in real clinic - example of what stem cells and how they are successfully used in tissue reconstruction

Lungs made from stem cells - two ways to go

Strong nerves coming from stem cells - where we stand - example of the story, to which we also contributed

Twisting biology for good – new scenarios for driving stem cells to where we need them

Also stem cells need support and help - how to provide stem cells with the right and caring environment

Two sorts of diseases that disable central nervous system

Acute:

- spinal cord injury
- brain trauma
- stroke…

Chronic:

- Parkinson's Disease
- Alzheimer's Disease
- Amyotropic lateral sclerosis

Any posssible help from stem cells ?

Model the disease + discover new drugs

+

Trophic support for the surviving / damaged cells

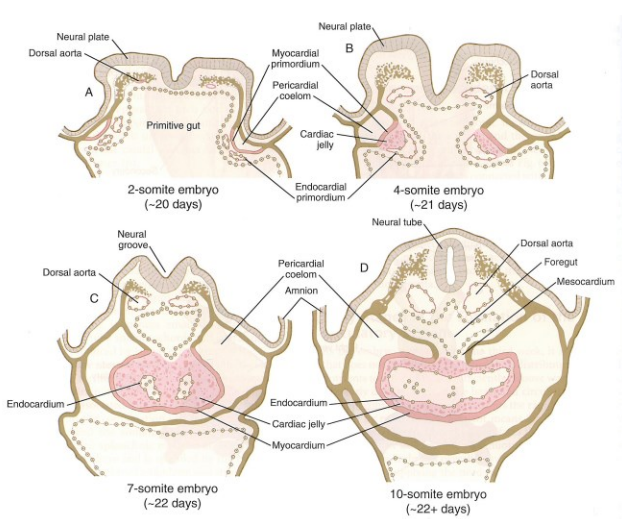
+

Replace lost + non-functional cells

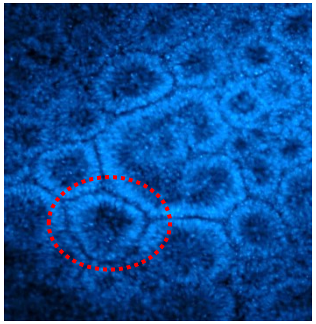
All kinds of cells can be considered:

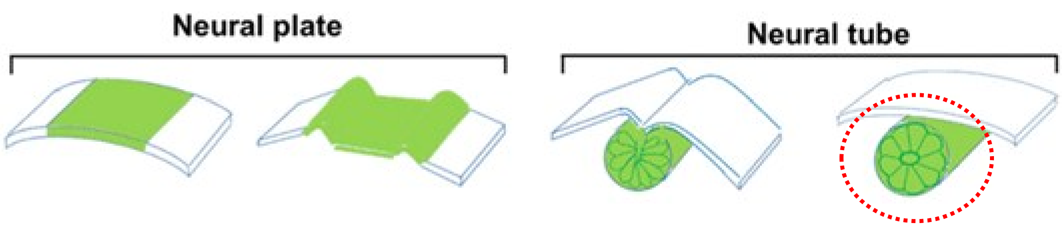
- embryonic + induced pluripotent stem cells
- fetal neural cells - problematic
- adult neural stem cells - problematic

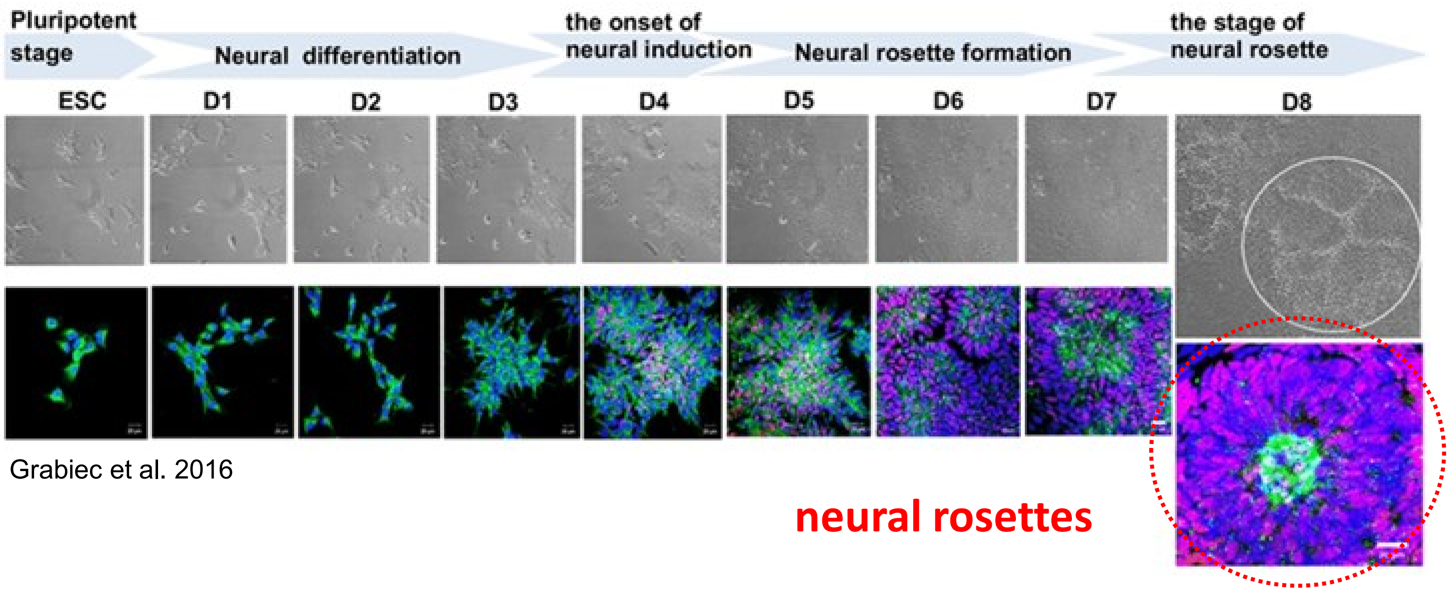
Neural differentiation of pluripotent SC – recapitulating development



Carlson, Human Embryology and Developmental Biology, 2014

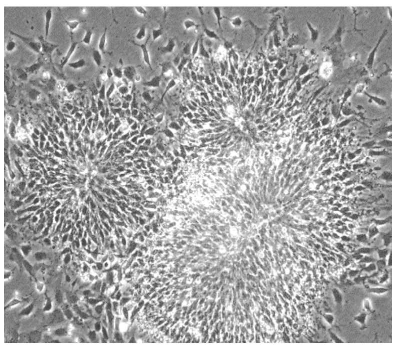






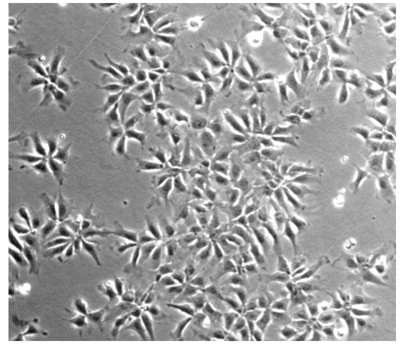
Grabiec et al. 2016

What else you can do?

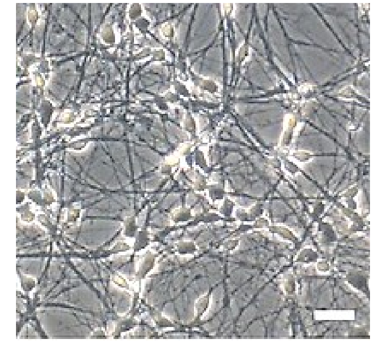


neural rosettes

2D



neural stem cells
(selfrenewing)



mature neurons/glia

3D

Cerebral organoids model human brain development and microcephaly **Nature, 2013**

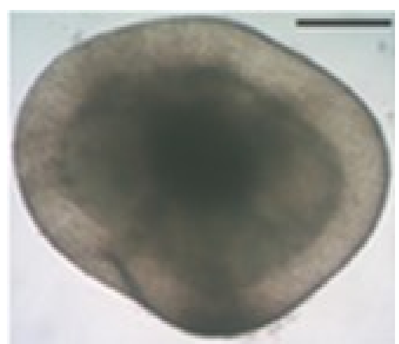
Madeline A. Lancaster[1], Magdalena Renner[1], Carol-Anne Martin[2], Daniel Wenzel[1], Louise S. Bicknell[2], Matthew E. Hurles[3], Tessa Homfray[4], Josef M. Penninger[1], Andrew P. Jackson[2], and Juergen A. Knoblich[1]

[1]IMBA - Institute of Molecular Biotechnology of the Austrian Academy of Science, Vienna 1030, Austria

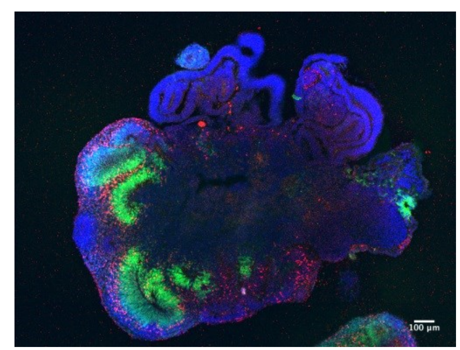
[2]MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK

[3]Wellcome Trust Sanger Institute, Cambridge, UK

[4]Department of Clinical Genetics, St. George's University, London, UK

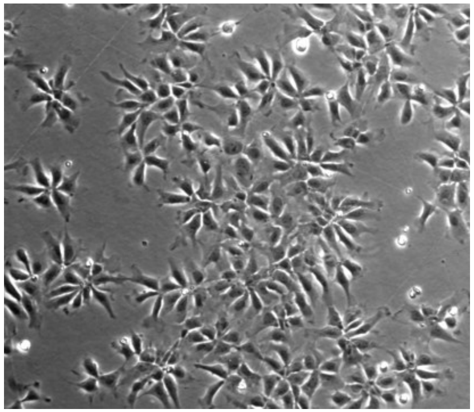


spheroids



cerebral organoids
(whole brains)

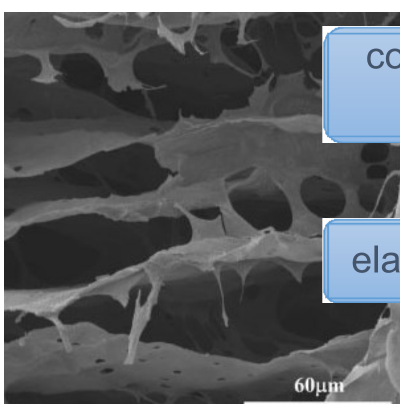
Many diffrent ways of getting there



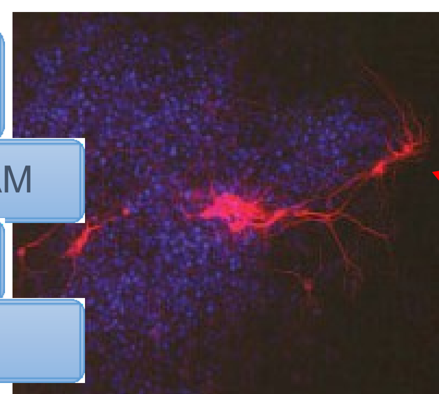
… growth factors:

- Coculture with mouse stromal cell line PA6 → Peripheral neurons
- Neurotrofin-4 → GABAergic neurons
- FGF8/Shh, ascorbic acid + BDNF → Dopaminergic neurons
- FGF2, RA, Shh → Motoric neurons
- CNTF (ciliary neurotrophic factor) → Astrocytes
- CNTF/PDGF → Oligodendrocytes

… culture surface/scaffolds:



- collagen, laminin, fibronectin
- integrins, NCAM
- elasticity / plasticity
- 2D / 3D



Nisbet et al., 2008

… by combination of these

Problem of heterogenous differentiation

Can we convert this knowledge into medical benefit ?

Spinal cord injury

Hans Keirstead
UC Irvine

- spinal cord trauma destroys numerous cell types
- in most cases of injuries, spinal cord is **not** damaged completely and some neuronal connections remain intact
- oligodendrocytes (new myelinisation) may improve the function of motoneurons

1990 – **Hans Keirstead** started his PhD in Neurobiology

2005 – Data published in **Journal of Neuroscience**

2010 – **1st Clinical Trial with hESCs Approved**

Clinical Trial **Cancelled**

2014 – Clinical Trial **Re-Approved**

2015

Sept. 2016 – **1st positive efficacy data form 4 patients**

26 years

11 years

Further study towards cell therapies for acute CNS injuries

The goal:
To obtain clinically applicable Neural Stem Cell line from hESC

Functional Neural Relay Formation by Human Neural Stem Cell Grafting in Spinal Cord Injury

FUNDING TYPE:		Early Translational III
GRANT NUMBER:		TR3-05628
INVESTIGATOR:		
PI	Mark Tuszynski	University of California, San Diego
Co-PI	Lawrence Goldstein	University of California, San Diego
DISEASE FOCUS:		Spinal Cord Injury Neurological Disorders
STEM CELL USE:		Embryonic Stem Cell

Stem Cell-Derived Astrocyte Precursor Transplants in Amyotrophic Lateral Sclerosis

FUNDING TYPE:		Early Translational from Disease Team Conversion
GRANT NUMBER:		TRX-01471
INVESTIGATOR:		
PI	Lawrence Goldstein	University of California, San Diego
Co-PI	Samuel Pfaff	The Salk Institute for Biological Studies
Co-PI	Martin Marsala	University of California, San Diego
DISEASE FOCUS:		Amyotrophic Lateral Sclerosis Neurological Disorders
STEM CELL USE:		Embryonic Stem Cell

Dasa Bohaciakova (Dolezalova), PhD

Martin Marsala, MD

UNIVERSITY OF CALIFORNIA · SAN DIEGO · LET THERE BE LIGHT

Department of Anesthesiology and Stem Cell Program, University of California San Diego, La Jolla, CA

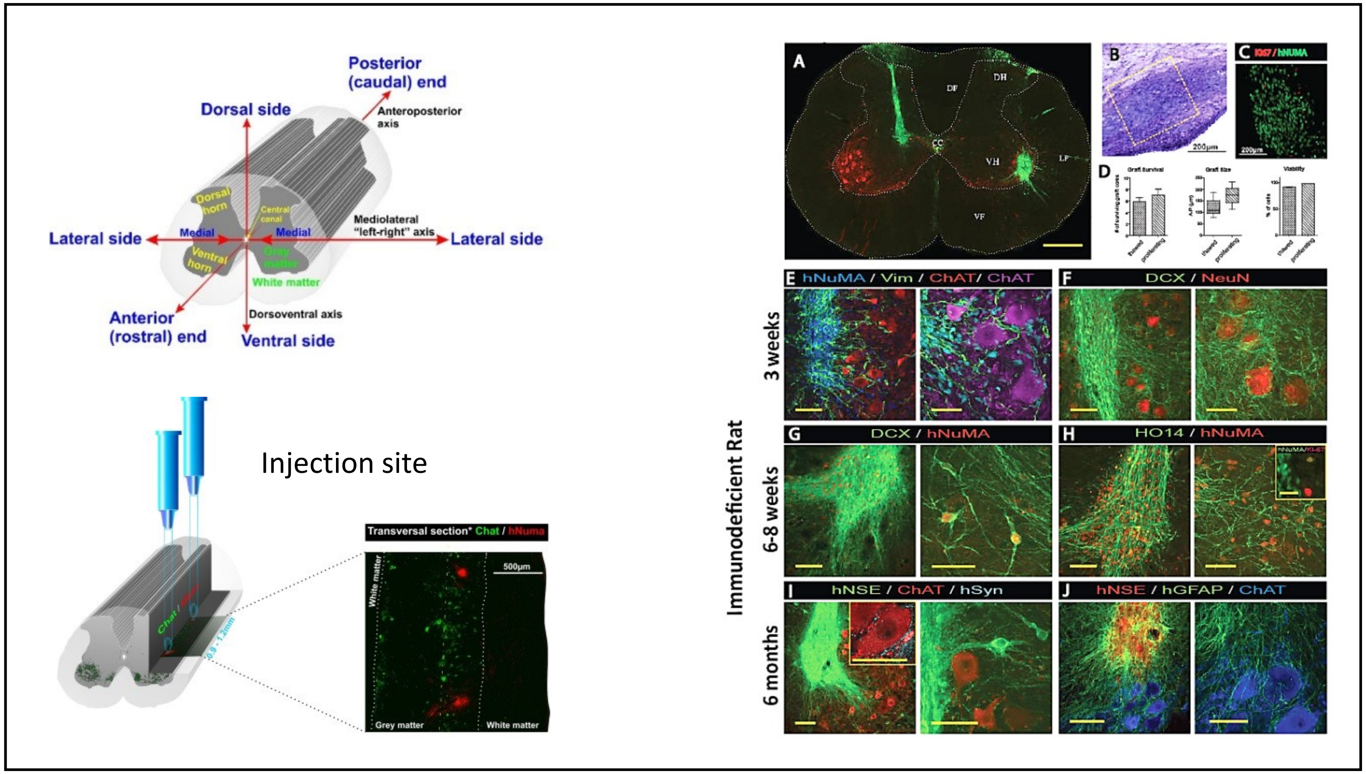
Key requirements on clinically applicable Neural Stem Cells

1) Derivation method is **simple, robust, cost-effective** and can be implemented in **cGMP**
   - no xenobiotic tissue culture components or enrichment methods

2) Cell line of NSCs is expandable (and **karyotypically stable**), well characterized in vitro, **homogenous**, and has **broad differentiation potential**
   - Methods generate heterogeneous population of NSCs enriched with "contaminating" cells with limited differentiation and/or proliferative capacity

3) Extensive ***in vivo* studies**
   - Several reports suggest that tumorigenicity or neural overgrowth in vivo may represent a major obstacle in the future application of hESC-derived NSCs into human therapies

Shortcut to the current stage of the story



- After transplantation *in vivo*, cells survive and integrate into host tissue and differentiate into mature neurons and/or glia and do not form any tumors
- Method has successfully been transferred to cGMP facility at UC Davis
- Several NSC cell lines are currently being tested on Amyotrophic Lateral Sclerosis (ALS) and Spinal cord injury animal models

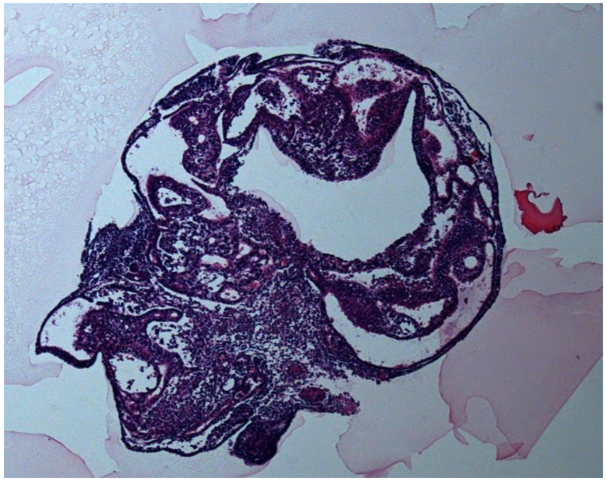
Some more neurobilogy fun here in Brno

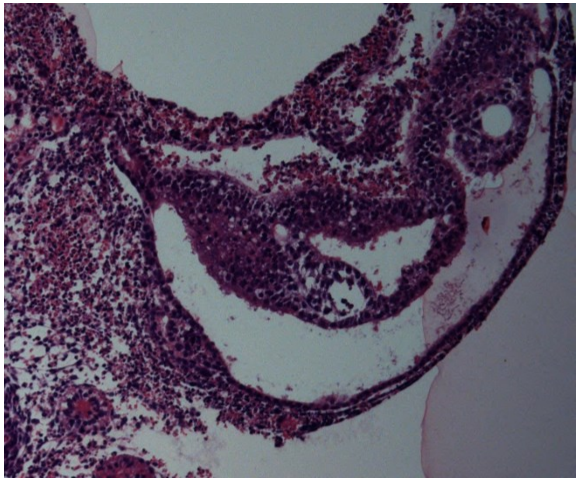
Modeling Alzheimer's disease in the dish

to develop robust protocol for differentiation and culture of 3D cerebral organoids from hiPSC for modeling Alzheimer's disease

Martin Barak
P-POOL

Dasa Bohaciakova
(Dolezalova), PhD





Where we are now ?
Organoids with visible mature structures

What will we discuss today ?

One have to be cautious - representative example of risk associated with propagation of stem cells outside the body

Stem cells in real clinic - example of what stem cells and how they are successfully used in tissue reconstruction

Lungs made from stem cells - two ways to go

Strong nerves coming from stem cells - where we stand - example of the story, to which we also contributed

Twisting biology for good – new scenarios for driving stem cells to where we need them

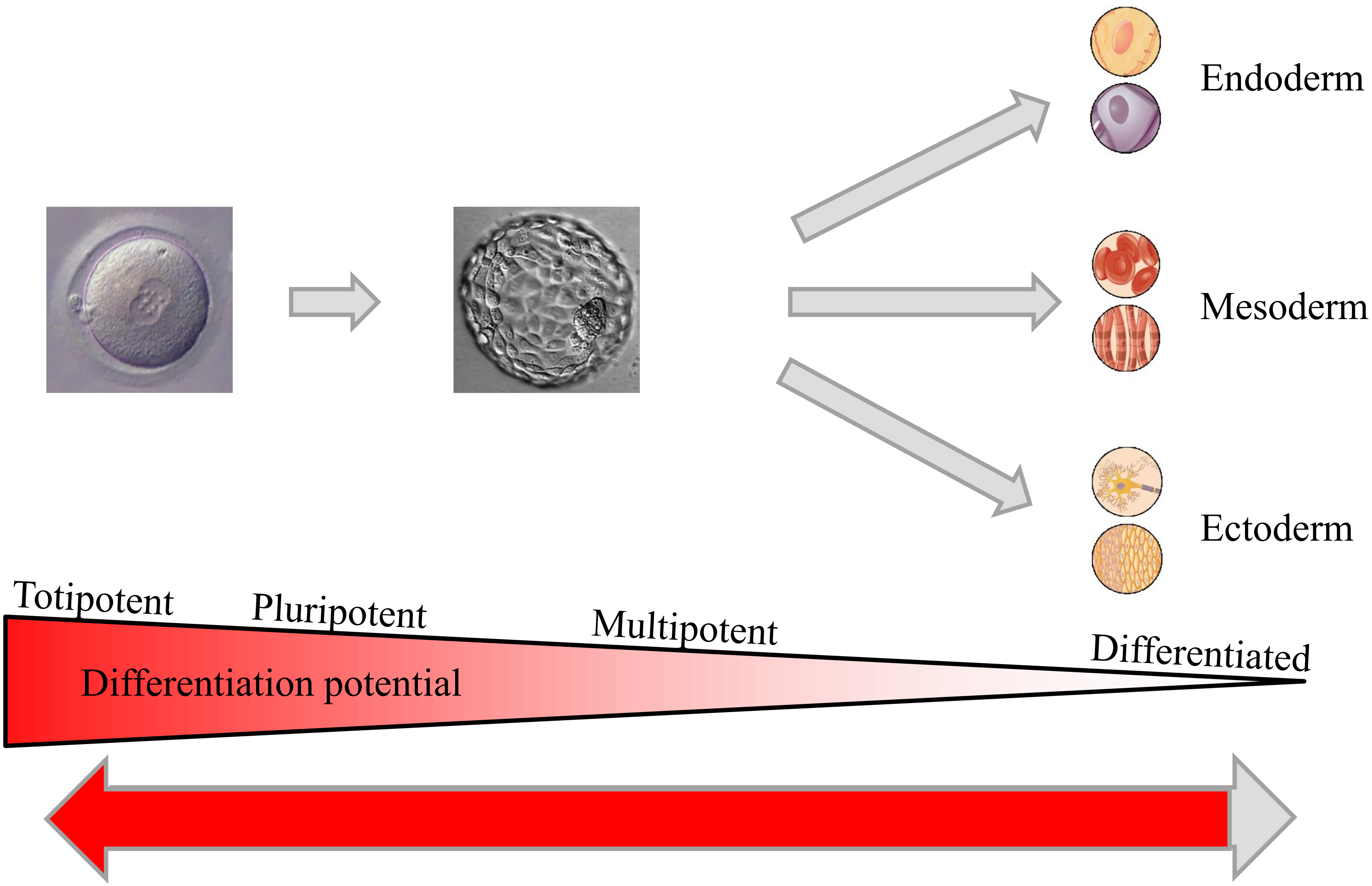
Also stem cells need support and help - how to provide stem cells with the right and caring environment

Twisting biology for good

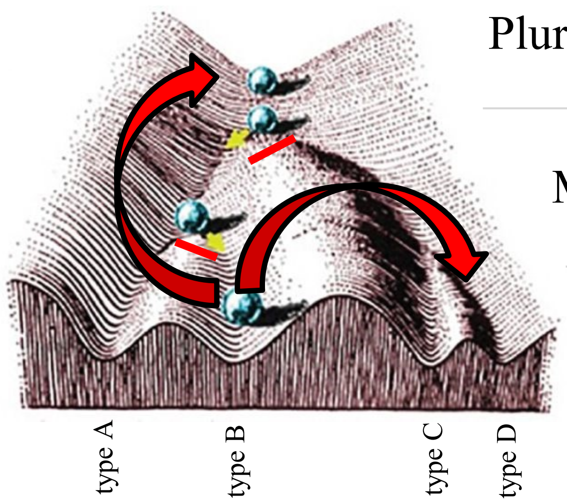
new scenarios for driving stem cells to where we need them

Tomáš Bárta

Differentiation potential



How to change the cell fate?

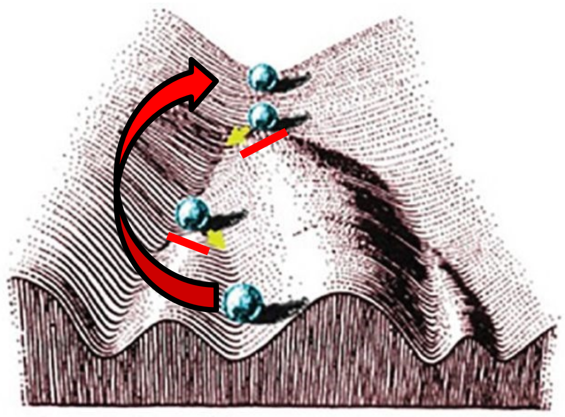


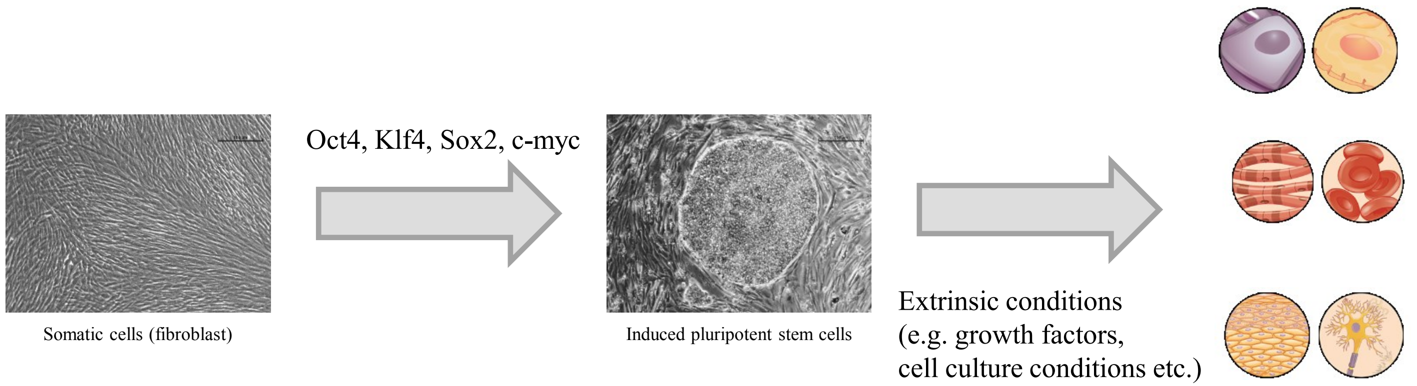
Pluripotent

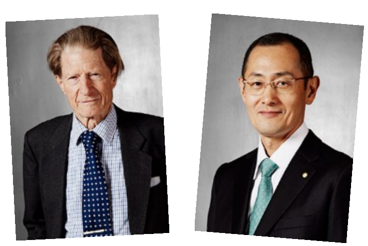
Multipotent

Differentiated

Cell reprogramming into pluripotent state



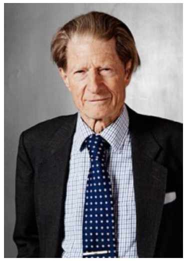




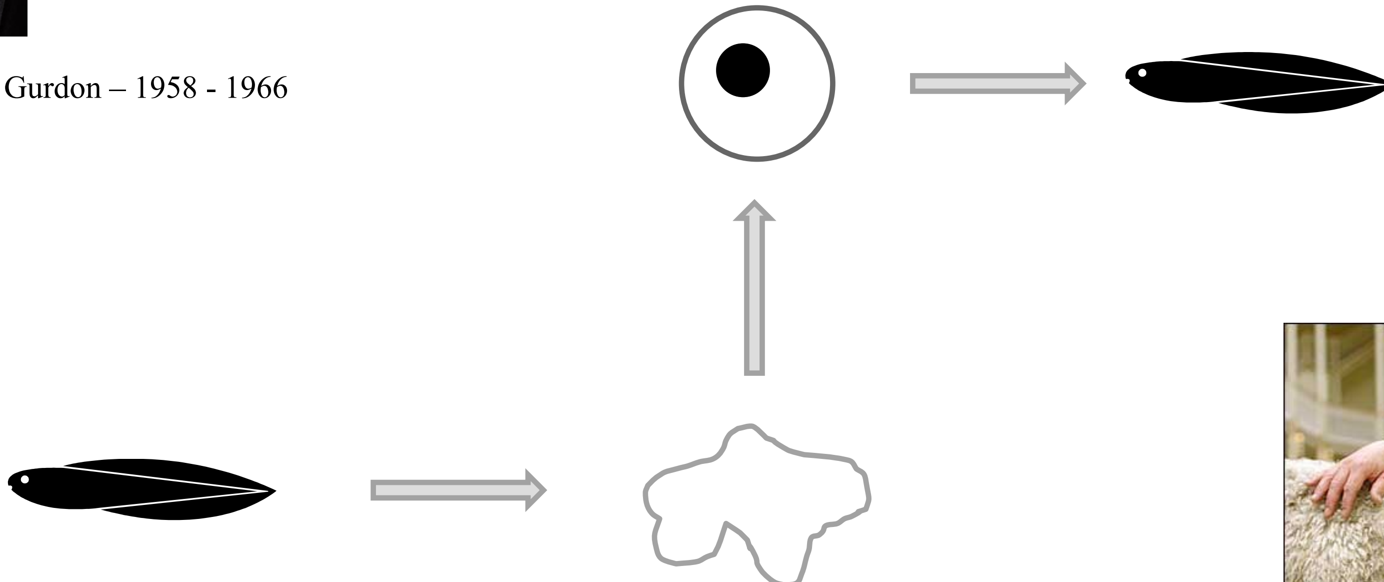
Sir John B. Gurdon Shinya Yamanaka

Advantages: patient-specific cells

Disadvantages: instability of the genome, tumorigenicity, high-costs, safety



Sir John B. Gurdon – 1958 - 1966

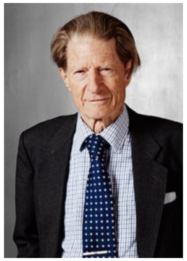


Ian Wilmut - 1997

"This result is of interest since it shows that genetic factors required for the formation of a fertile adult frog are not lost in the course of cell differentiation..."

X

Recipient cell contains factors that are capable to revert somatic cell into embryonic cell.



Sir John B. Gurdon – 1958 - 1966

NATURE July 5, 1958 VOL. 182

ghbouring

(5)

Sexually Mature Individuals of *Xenopus laevis* from the Transplantation of Single Somatic Nuclei

1240 NATURE JUNE 18, 1966 VOL. 210

"FERTILE" INTESTINE NUCLEI

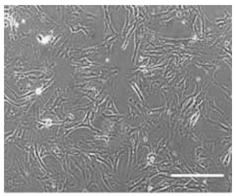
By DR. J. B. GURDON
Department of Zoology, Parks Road, Oxford
AND
V. UEHLINGER
Station de Zoologie Expérimentale, Université de Génève

"We describe here some adult frogs which are derived from transplanted intestine nuclei and some of which are fertile."

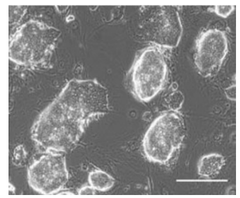
Shinya Yamanaka - 2006

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

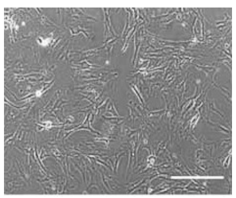
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024



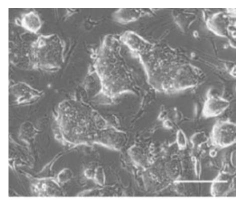
24 genes, specific for pluripotent stem cells



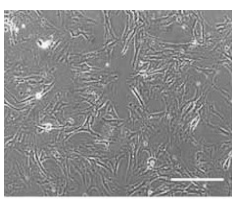
he always took out one gene and followed the efficiency of reprogramming => 10 genes seriously affected the efficiency.



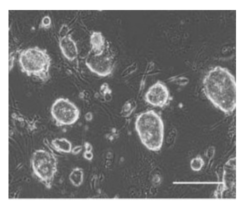
10 genes, specific for pluripotent stem cells

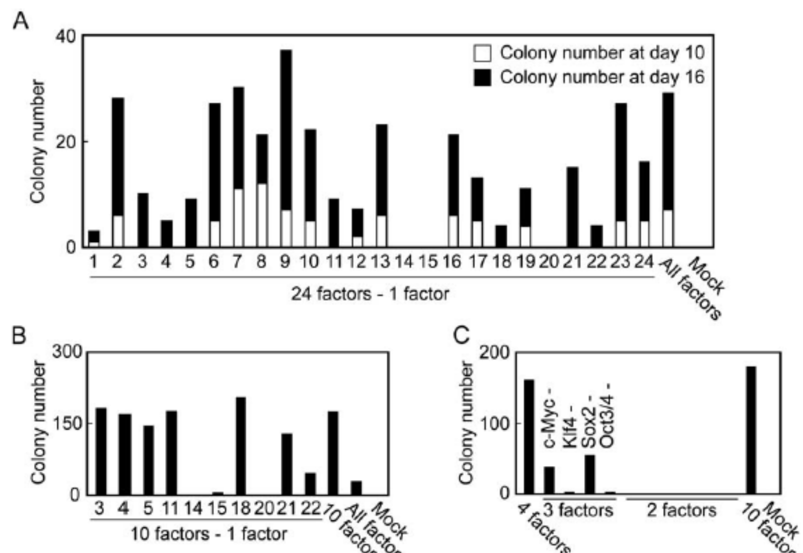


he selected 4 genes

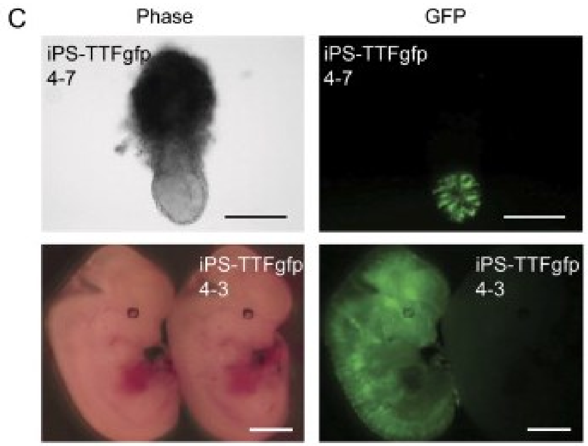


Oct4
Sox2
Klf4
c-Myc





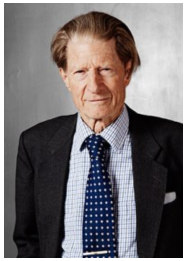
Shinya Yamanaka – 2006 - 2007



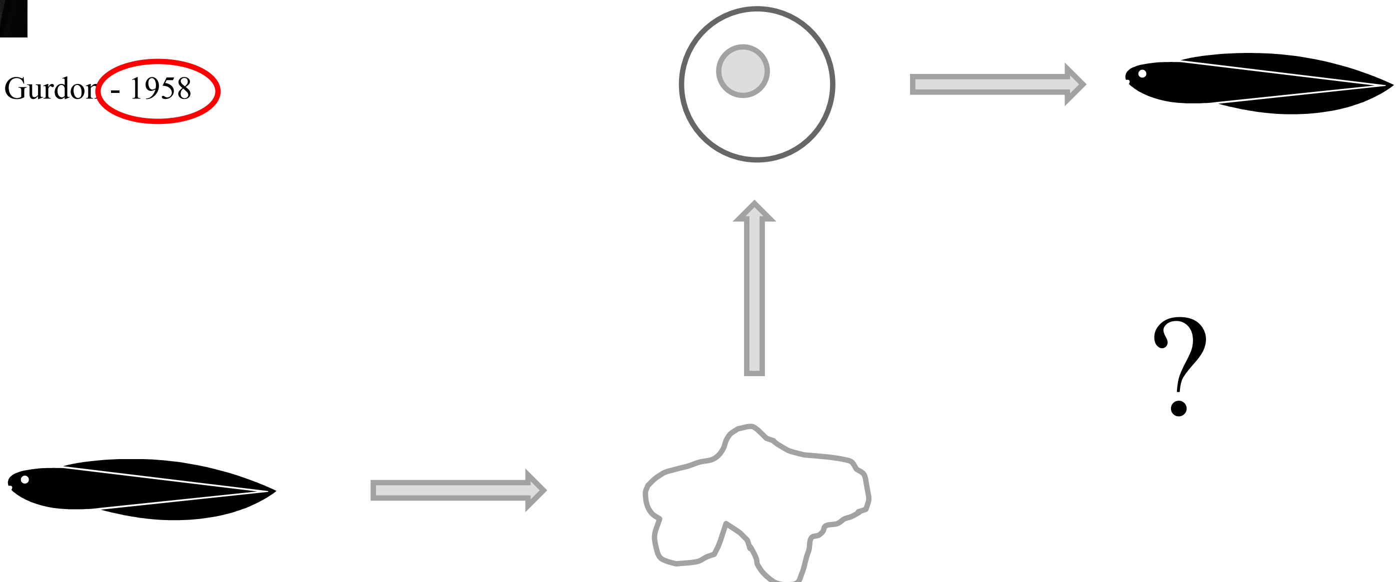
Cell

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

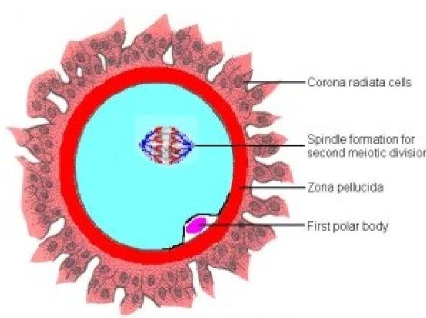
Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
[3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
[4]Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019



Sir John B. Gurdon - 1958

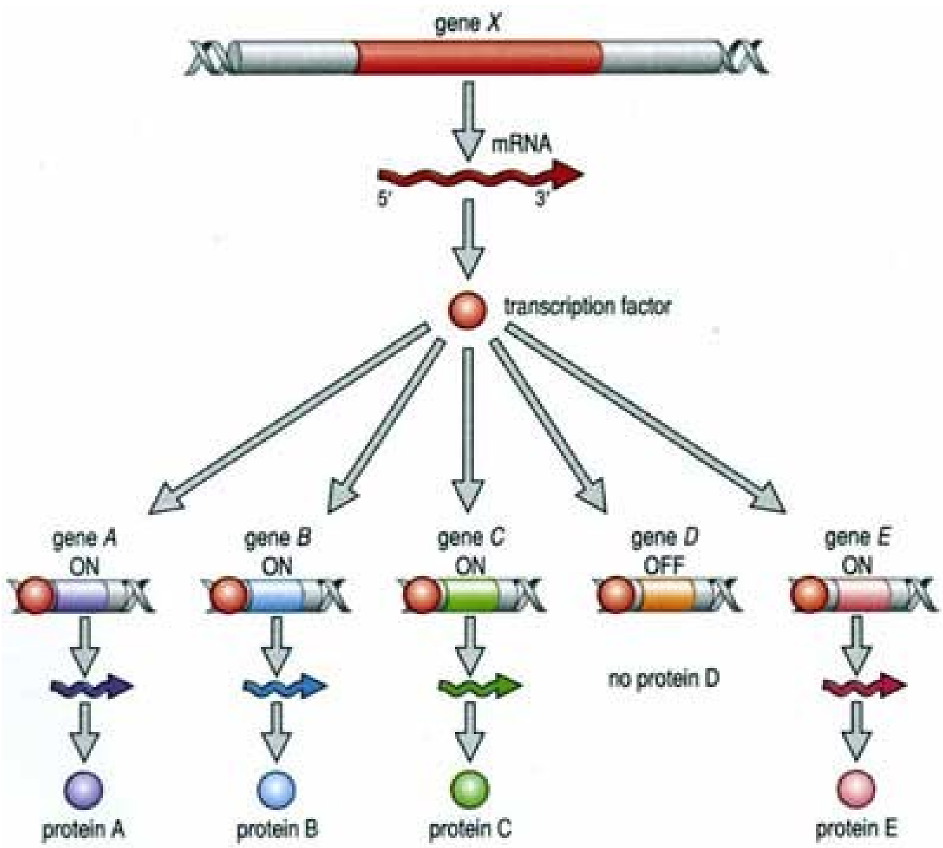


?

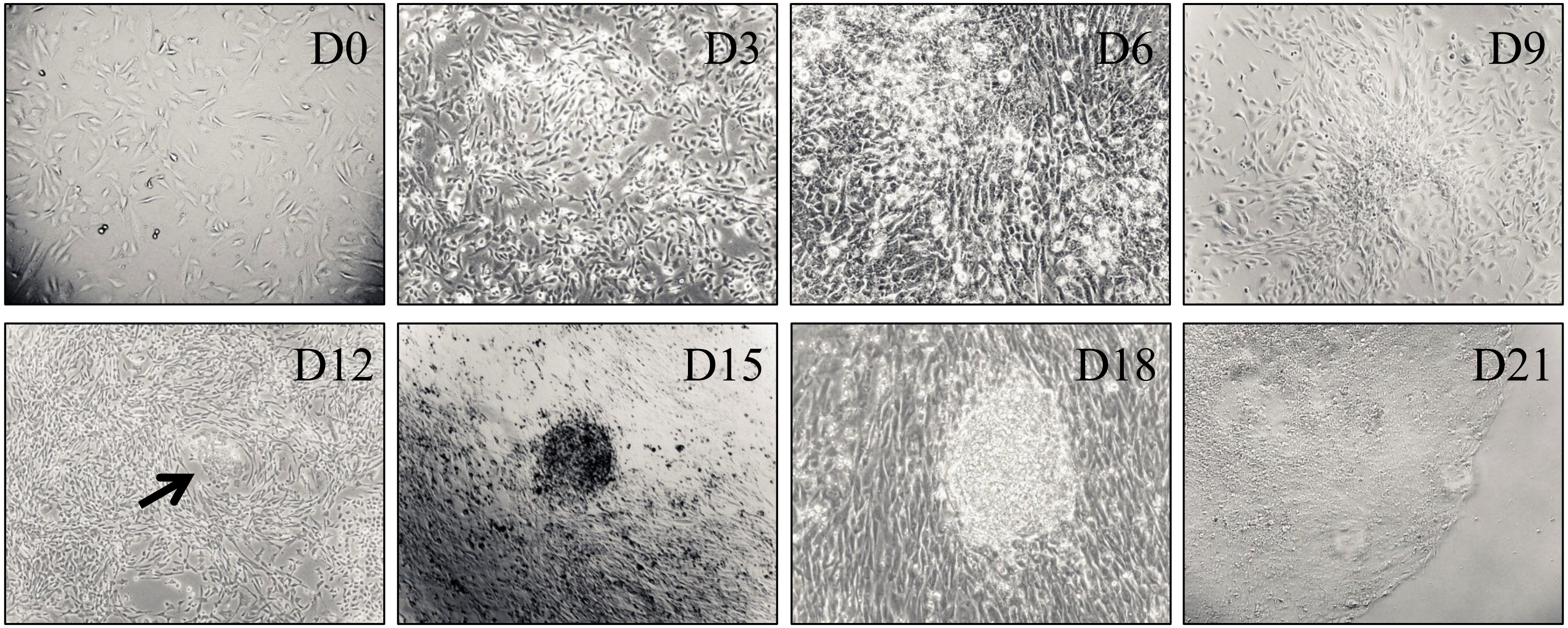


Ian Wilmut - 1997

How only 4 genes can reprogram the fate of a cell?

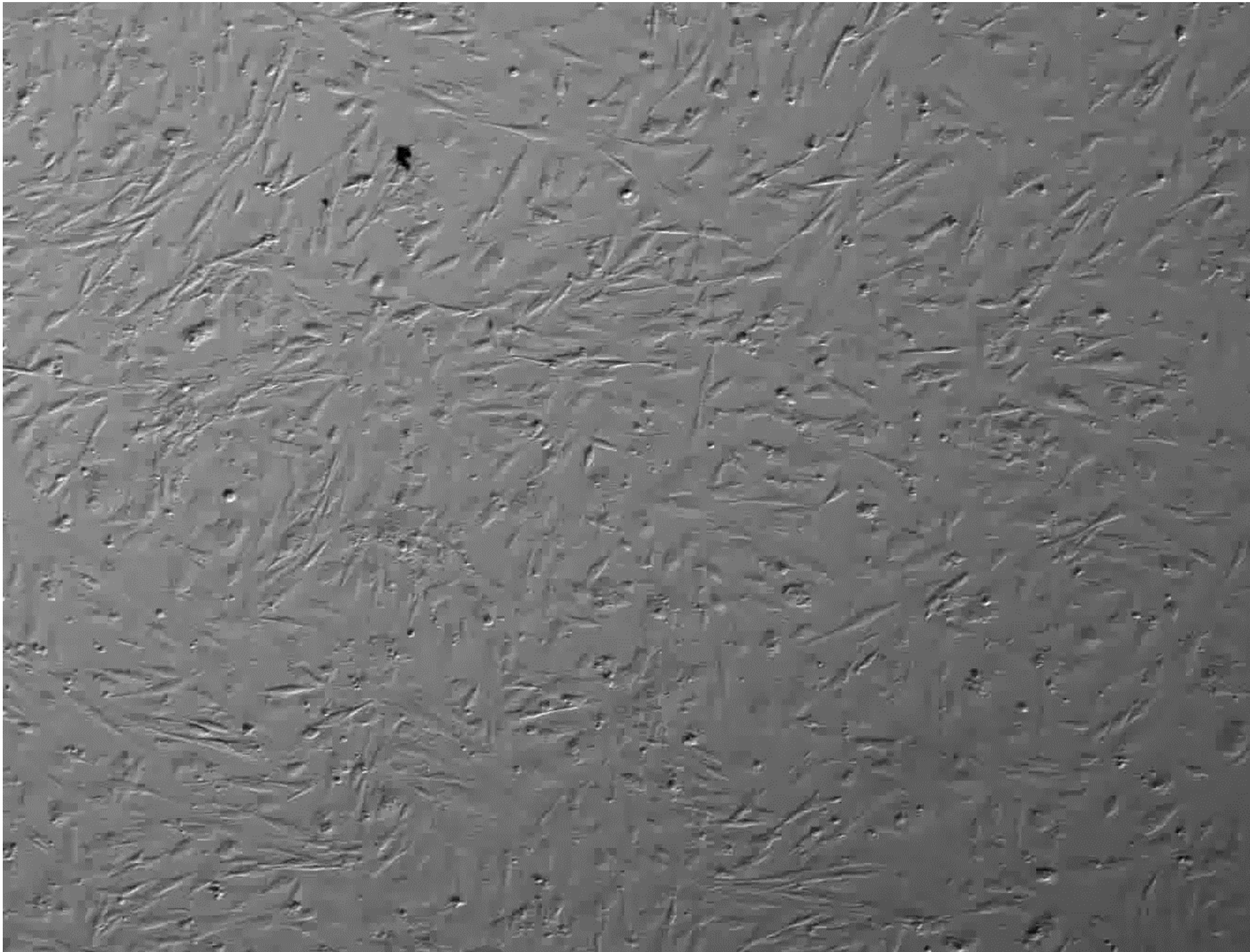


What is happening during the reprogramming?

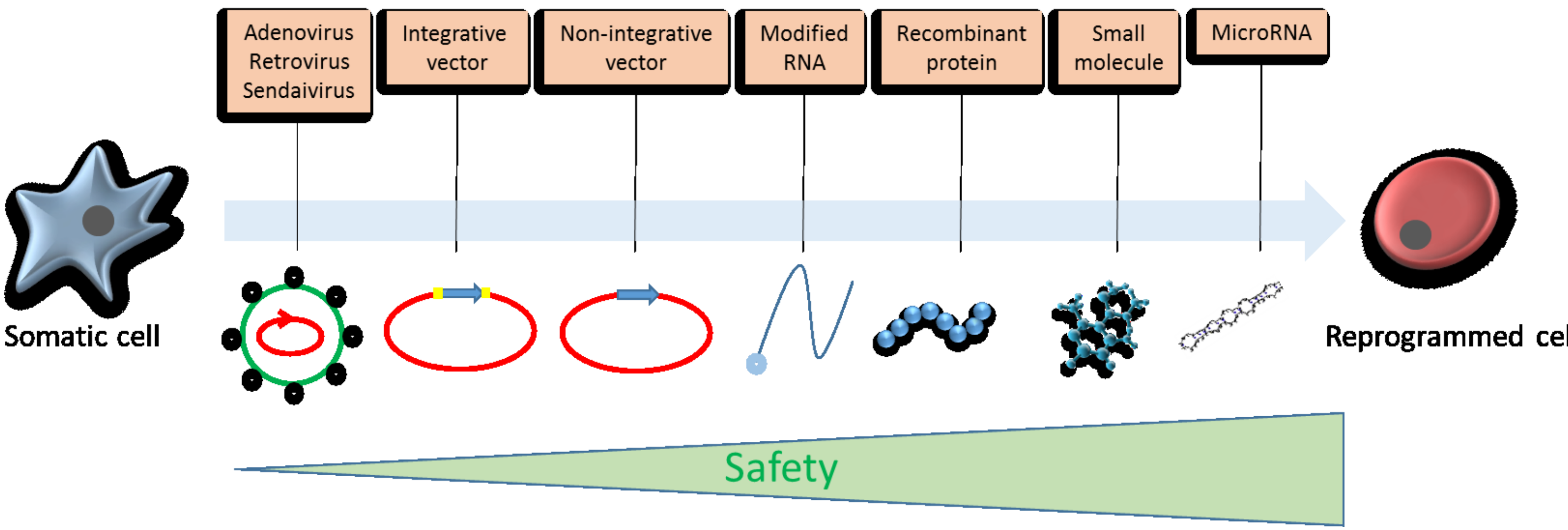


I. Shut down of genes maintaining the "identity" of fibroblasts.
    I. dedifferentiation and upregulation of genes maintaining proliferation

II. MET – transition from mesenchymal to epithelial phenotype.
III. Establishment of pluripotency gene network.



Increasing the safety of the cell reprogramming

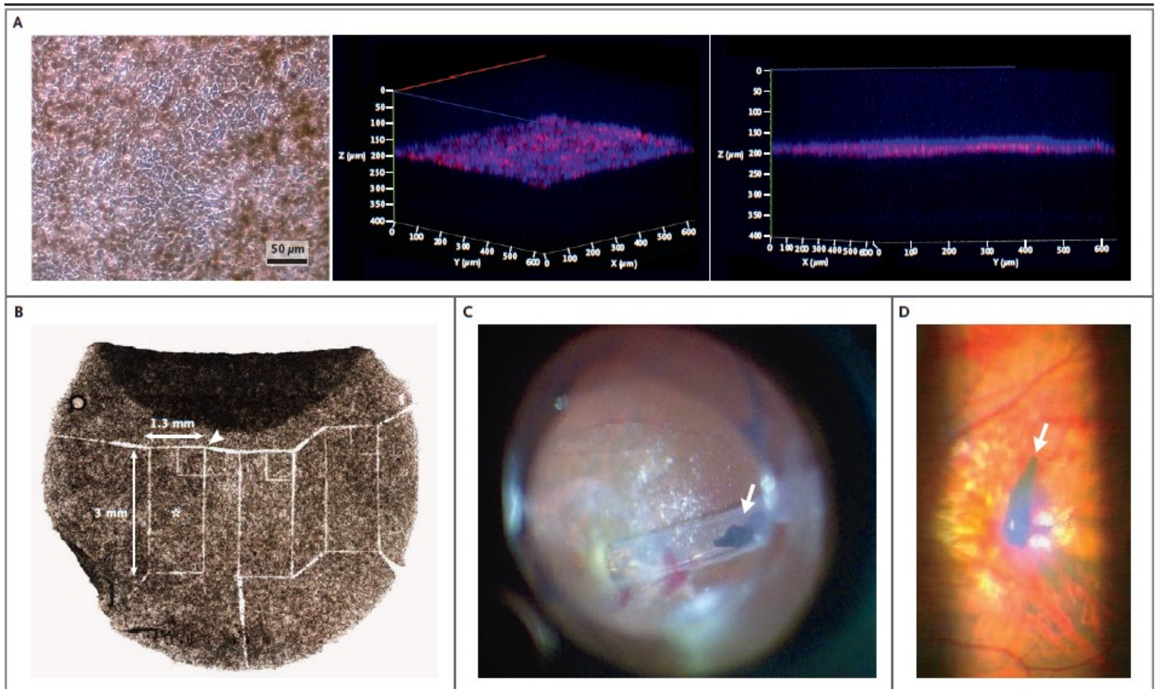


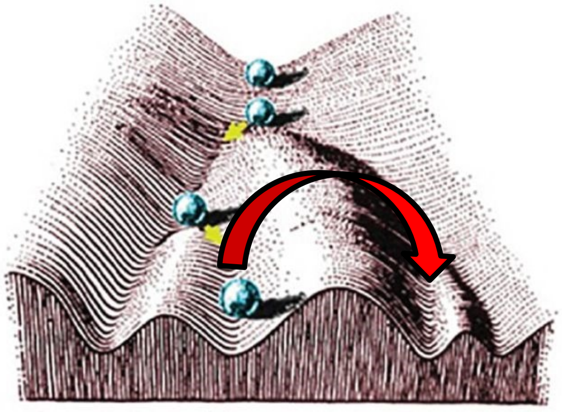
The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

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

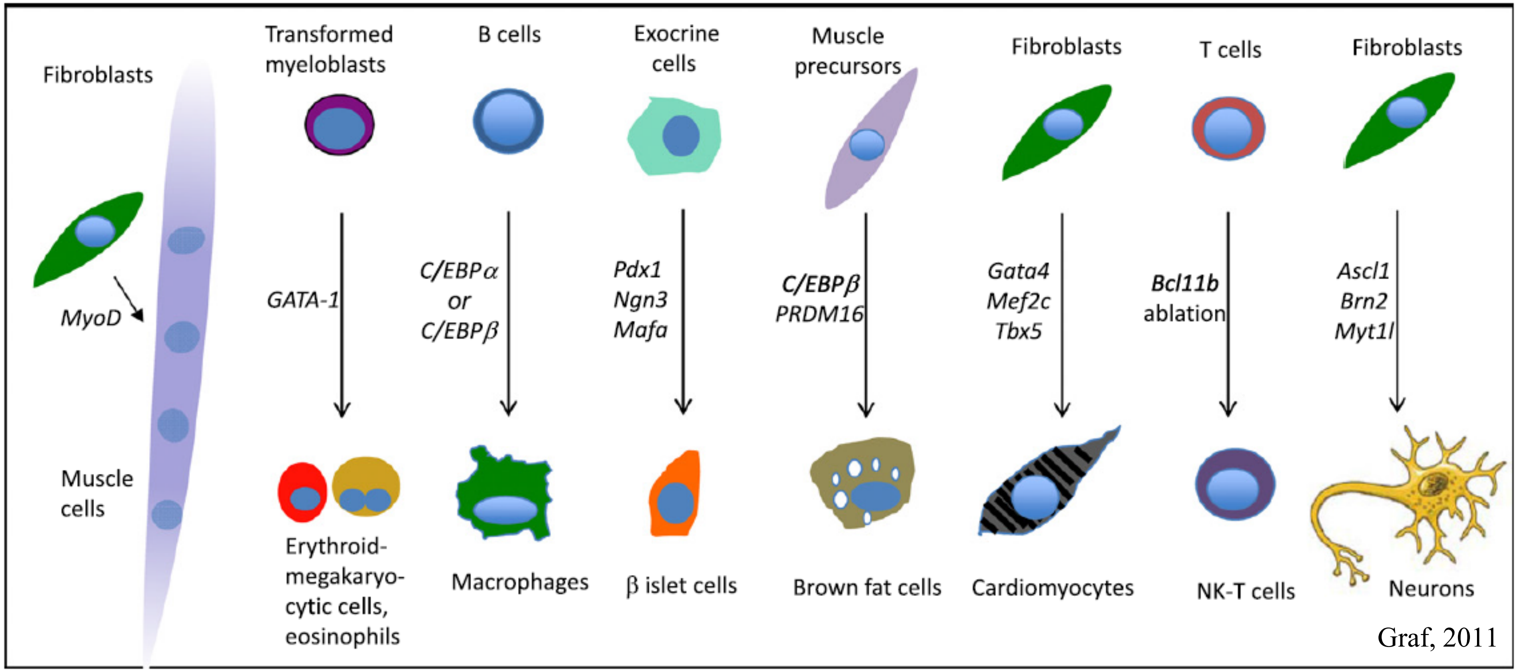
M. Mandai, A. Watanabe, Y. Kurimoto, Y. Hirami, C. Morinaga, T. Daimon, M. Fujihara, H. Akimaru, N. Sakai, Y. Shibata, M. Terada, Y. Nomiya, S. Tanishima, M. Nakamura, H. Kamao, S. Sugita, A. Onishi, T. Ito, K. Fujita, S. Kawamata, M.J. Go, C. Shinohara, K. Hata, M. Sawada, M. Yamamoto, S. Ohta, Y. Ohara, K. Yoshida, J. Kuwahara, Y. Kitano, N. Amano, M. Umekage, F. Kitaoka, A. Tanaka, C. Okada, N. Takasu, S. Ogawa, S. Yamanaka, and M. Takahashi





Transdifferentiation

Conversion of one cell type to another cell type.
Easily accessible and easy-to-cultivate cell types (fibroblasts, blood cells) are often used.



Advantages: patient-specific cells
Disadvantages: low-efficiency, restricted proliferative capacity, limited cell type diversity, senescence, and do not generally produce progenitor cells

Transdifferentiation of hepatocytes into insulin producing cells.

Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia

Sarah Ferber[1], Amir Halkin[2], Hofit Cohen[1], Idit Ber[1,3], Yulia Einav[4], Iris Goldberg[5], Iris Barshack[5], Rhona Seijffers[1,3], Juri Kopolovic[3,5], Nurit Kaiser[6] & Avraham Karasik[1,3]

Cell-replacement therapy for diabetes: Generating functional insulin-producing tissue from adult human liver cells

Tamar Sapir*†, Keren Shternhall*†, Irit Meivar-Levy*, Tamar Blumenfeld*‡, Hamutal Cohen*†, Ehud Skutelsky‡, Smadar Eventov-Friedman§, Iris Barshack¶, Iris Goldberg¶, Sarah Pri-Chen‖, Lya Ben-Dor‡, Sylvie Polak-Charcon‡¶, Avraham Karasik*‡, Ilan Shimon*‡, Eytan Mor‡, and Sarah Ferber*‡††**

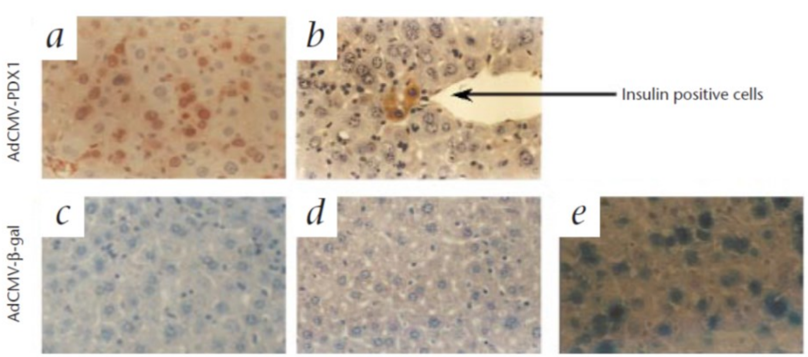
*The Endocrine Institute, ¶The Institute for Pathology, and ‖The Maurice and Gabriela Goldschleger Eye Research Institute, Sheba Medical Center, Tel-Hashomer 52621, Israel; †Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel; ‡Sackler School of Medicine, Tel Aviv University, Ramat-Aviv 69978, Israel; §Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel; **Rabin Medical Center, Beilinson Campus, Petah-Tiqva 49100, Israel

Activation of insulin expression in human hepatocytes infected with virus encoding PDX-1

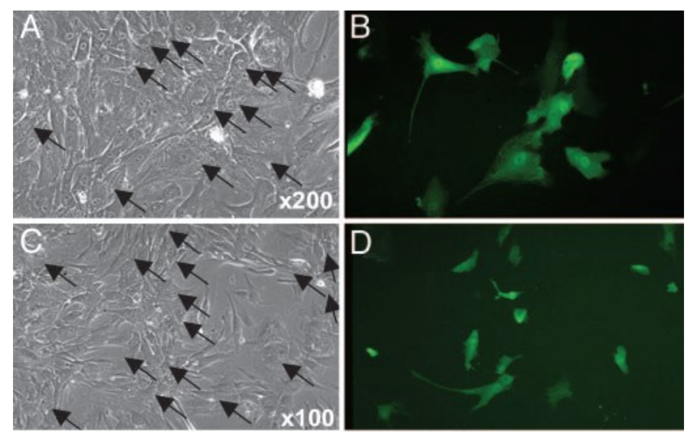
diabetic mice

adenovirus encoding PDX-1 injected to liver

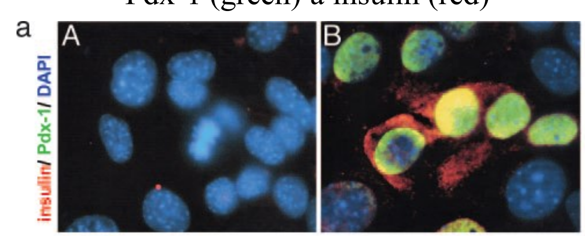
PDX-1 Insulin



Activation of insulin promoter (green)

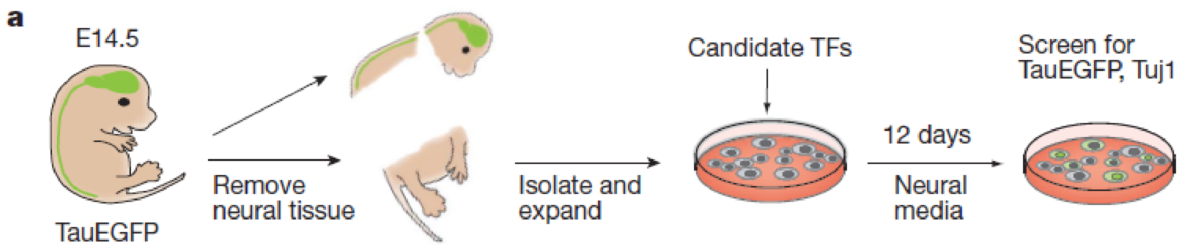


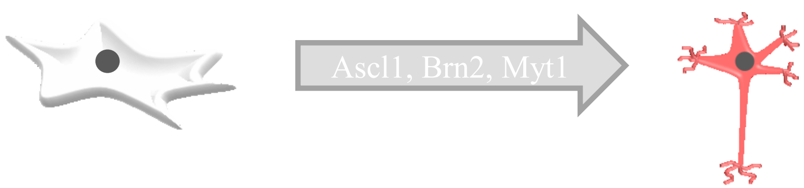
Pdx-1 (green) a insulin (red)

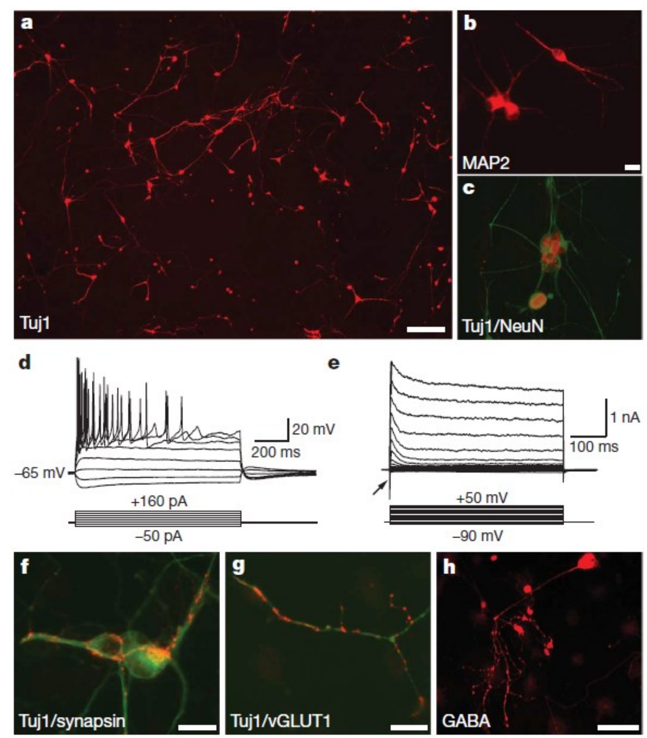


Direct conversion of fibroblasts to functional neurons by defined factors

Thomas Vierbuchen[1,2], Austin Ostermeier[1,2], Zhiping P. Pang[3], Yuko Kokubu[1], Thomas C. Südhof[3,4] & Marius Wernig[1,2]



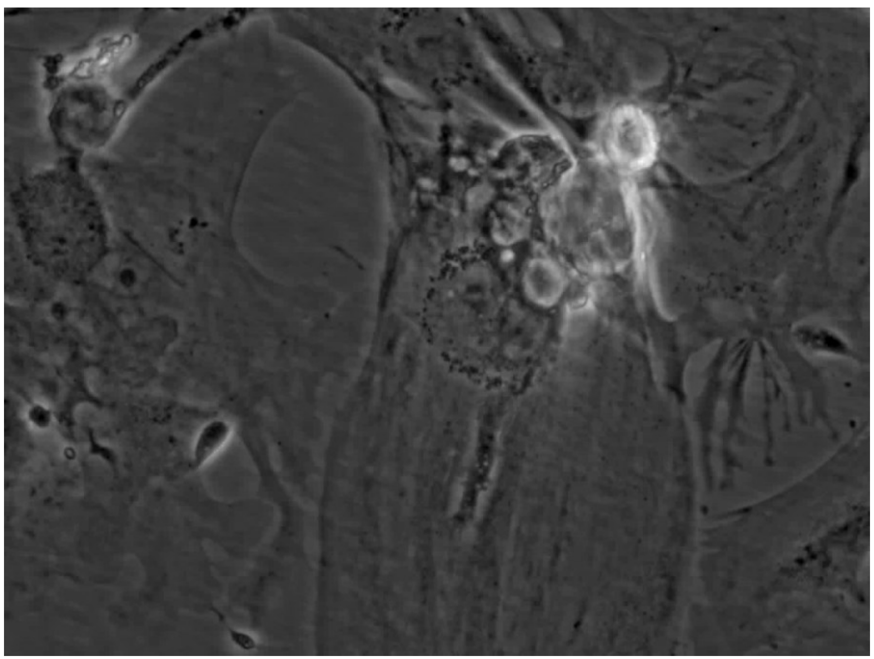


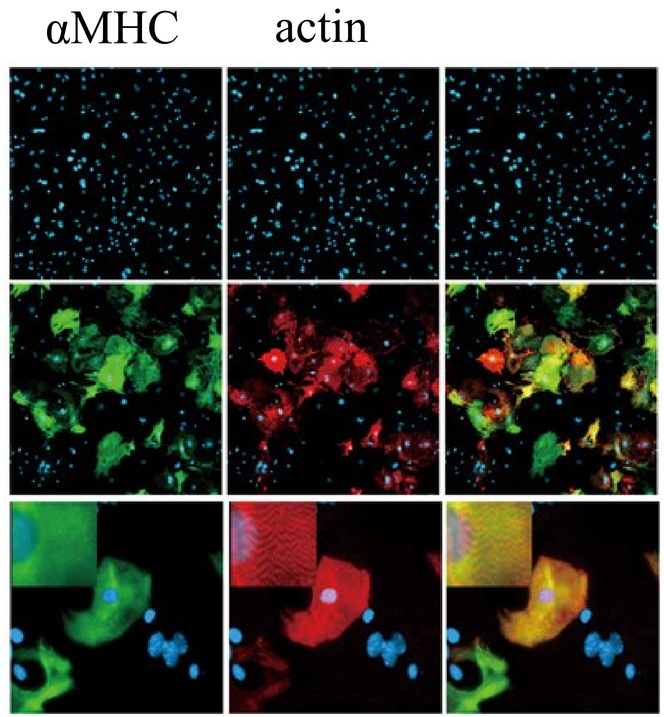


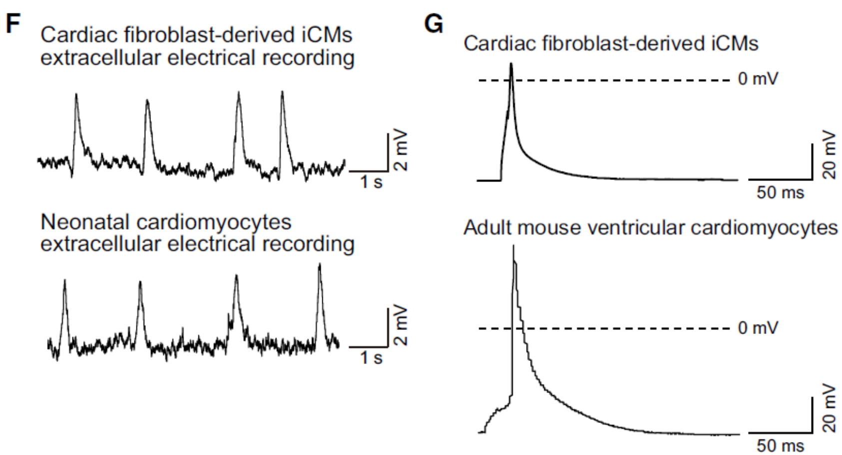
Cell

Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors

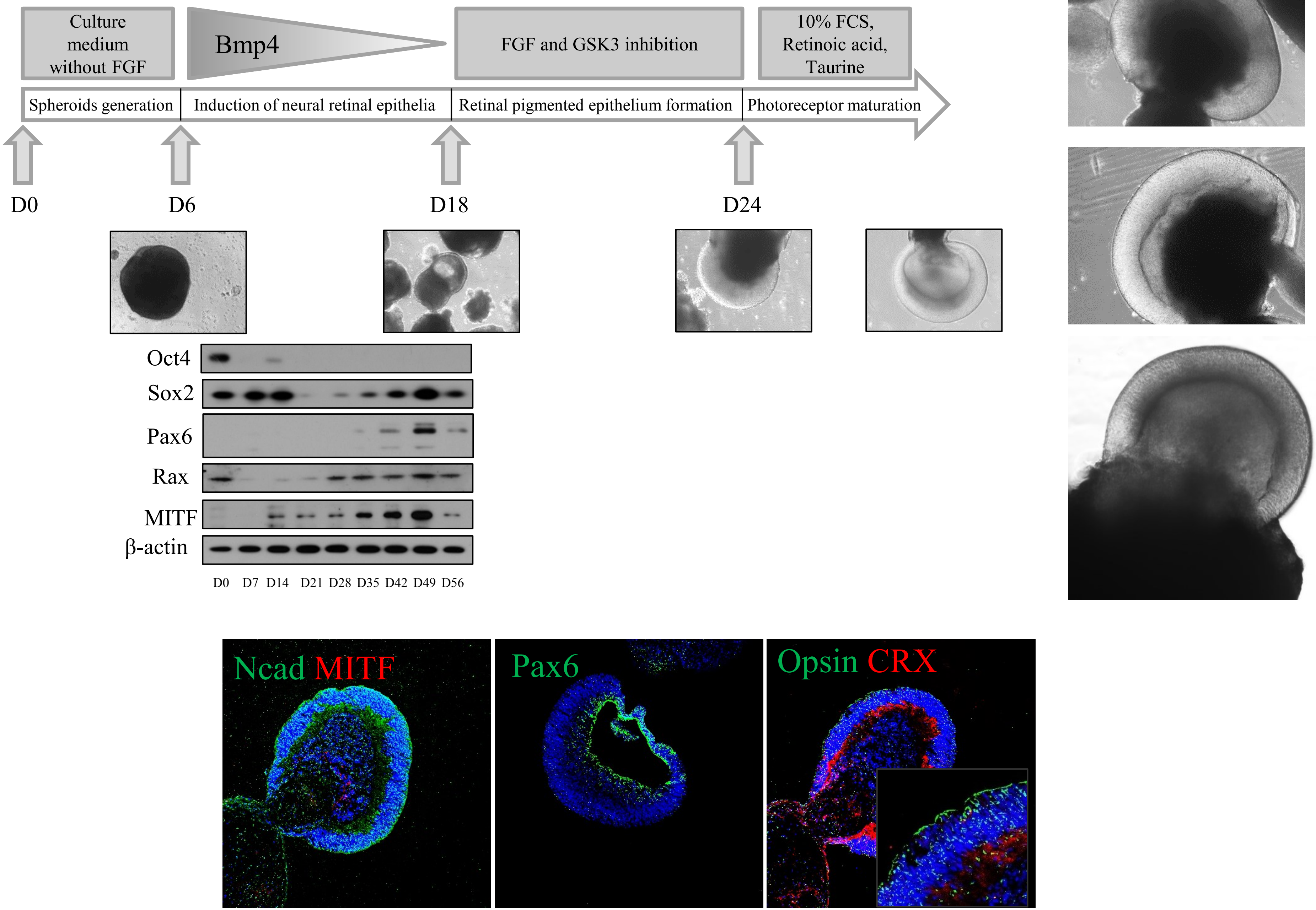
Masaki Ieda,[1,2,3,6,*] Ji-Dong Fu,[1,2,3] Paul Delgado-Olguin,[1,2,4] Vasanth Vedantham,[1,5] Yohei Hayashi,[1]
Benoit G. Bruneau,[1,2,4] and Deepak Srivastava[1,2,3,*]







What we are trying to achieve?



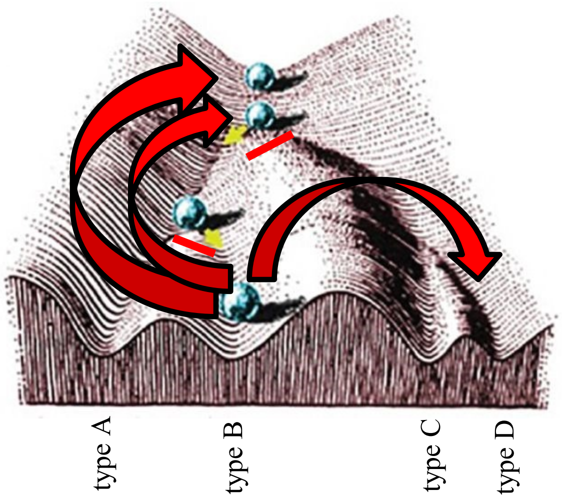
Many issues must be addressed....

Basic research

Clinical applications

Take-home message

There are many ways...



...we are trying to find the safest and cheapest (the best?) way.

What will we discuss today ?

One have to be cautious - representative example of risk associated with propagation of stem cells outside the body

Stem cells in real clinic - example of what stem cells and how they are successfully used in tissue reconstruction

Lungs made from stem cells - two ways to go

Strong nerves coming from stem cells - where we stand - example of the story, to which we also contributed

Twisting biology for good – new scenarios for driving stem cells to where we need them

Also stem cells need support and help - how to provide stem cells with the right and caring environment

Caring MicroEnvironment

how to provide stem cells with the right and caring environment

Josef Jaros

For what it is applicable in medicine?

Vědecké články o cell microenvironment for clinics
... stars of the inflammatory tumour microenvironment - Allavena - Počet citací tohoto článku: 279
... in disease: evolving concepts from the clinic - Martin - Počet citací tohoto článku: 343
Mesenchymal stem cells: heading into the clinic - Koc - Počet citací tohoto článku: 349

The Unique Molecular and Cellular Microenvironment of Ovarian ...
https://www.ncbi.nlm.nih.gov/pubmed/28275576 ▾ Přeložit tuto stránku
autor: T Worzfeld - 2017 - Počet citací tohoto článku: 13 - Související články
22. 2. 2017 - The Unique Molecular and Cellular Microenvironment of Ovarian Cancer. Worzfeld T(1), Pogge von ... (2)Experimental Tumor Research, Clinic for Hematology, Oncology and Immunology, Center for Tumor Biology and Immunology, Philipps University , Marburg , Germany. (3)Institute of Medical ...

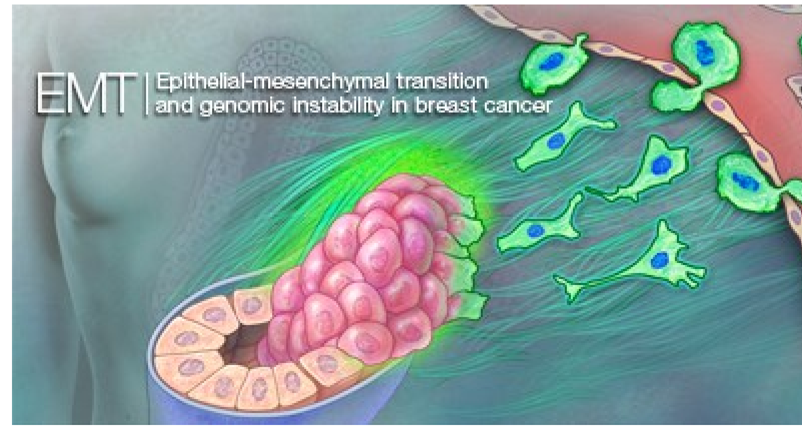
The Unique Molecular and Cellular Microenvironment of Ovarian Cancer
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5319992/ ▾ Přeložit tuto stránku
autor: T Worzfeld - 2017 - Počet citací tohoto článku: 13 - Související články
22. 2. 2017 - Cells of both the innate and adaptive immune system, in particular tumor-associated macrophages (TAMs) and T cells, as well as cancer-associated fibroblasts enter into a malicious liaison with tumor cells to create a tumor-promoting and immunosuppressive tumor microenvironment (TME). Ovarian cancer ...

Cellular composition of the tumor microenvironment. - NCBI
https://www.ncbi.nlm.nih.gov/pubmed/23714465 ▾ Přeložit tuto stránku
autor: SM Ansell - 2013 - Počet citací tohoto článku: 10 - Související články
Am Soc Clin Oncol Educ Book. 2013. doi: 10.1200/EdBook_AM.2013.33.e91. Cellular composition of the tumor microenvironment. Ansell SM(1), Vonderheide RH. Author information: (1)From the Division of Hematology, Mayo Clinic, Rochester, MN; Abrahamson Cancer Center of the University of Pennsylvania, ...

Lymphoma Microenvironment and Immunotherapy - Surgical ...
www.surgpath.theclinics.com/article/S1875...8/abstract - Přeložit tuto stránku
autor: ML Xu - 2016 - Počet citací tohoto článku: 1 - Související články
Understanding of the lymphoma tumor microenvironment is poised to expand in the era of next-generation sequencing studies of the tumor cells themselves. Successful therapies of the future will rely on deeper appreciation of the interactions between elements of the microenvironment. Although the phenotypic, cytogenetic, ...

The tumor suppressor function of mitochondria: Translation into the ...
https://www.sciencedirect.com/science/.../pii/S0925443909000210 - Přeložit tuto stránku
autor: JM Cuezva - 2009 - Počet citací tohoto článku: 89 - Související články
In this review, we summarize most of these findings paying special attention to the opportunity that translation of energetic metabolism into the clinics could afford for the management of cancer patients. ..... other details, see the text. At a certain stage of tumor development cancer cells grow in a hypoxic microenvironment.

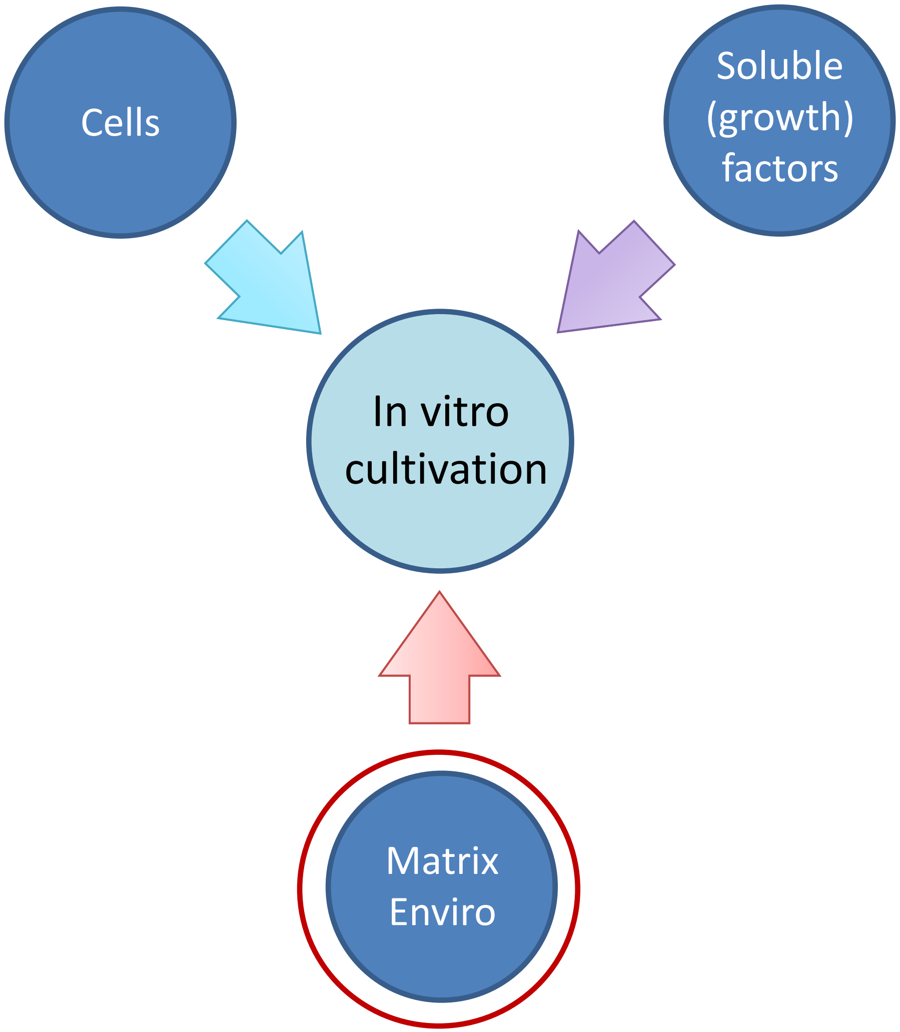
Tumor microenvironment - an overview | ScienceDirect Topics



Cancer biology
Stem cell biology
Disease modelling
Developmental biology

Molecular processes
Drug testing

Factors of environment



Comparison of environments

In vitro

Pros & Cons
+ Manipulation & analysis
- Artificial conditions

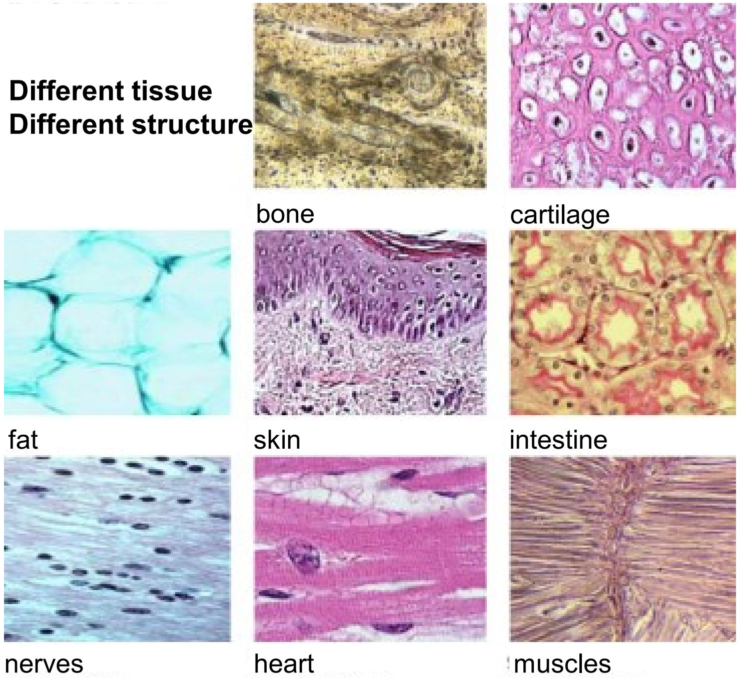
- Cells flat
- Nutrients &soluble factors from 1 side
- Connected to other cells 5-15%
- etc.

⇒ Diverse cells **behavior** and **reactivity** to drugs grown in 2D and 3D environment

Solution
- Self-organization – e.g. organoids, spheroids
- Surface modifications of Petri dish
- Building our own 3D matrix

In vivo

Different tissue
Different structure

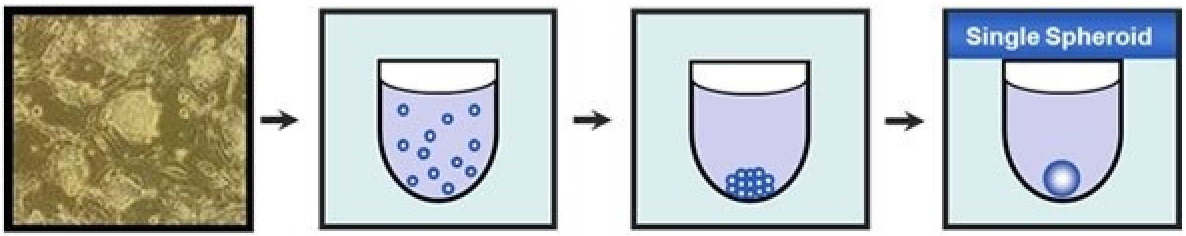


Cells need 3D ECM

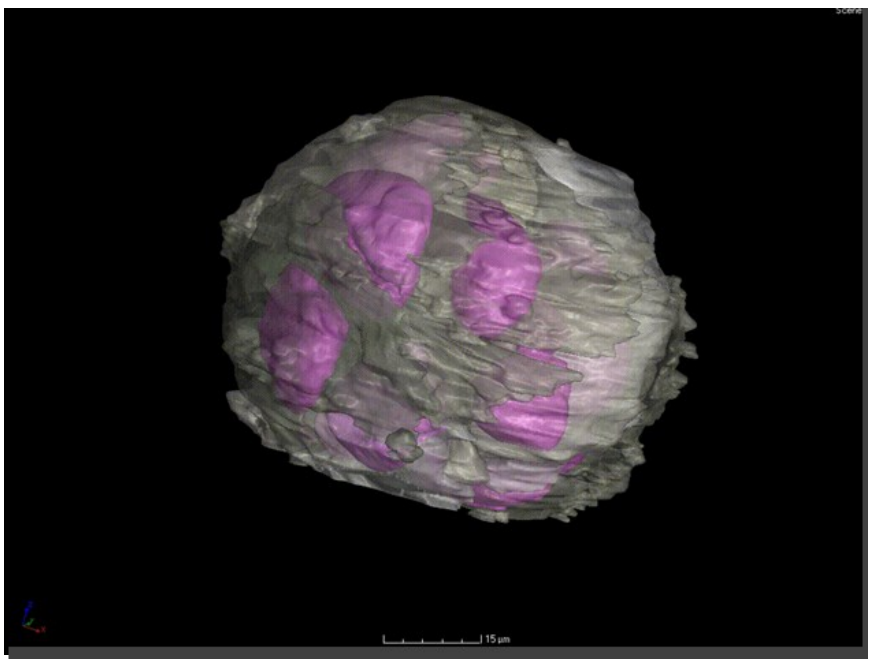
Adhesion – **Proteins, peptides**
Topography – **fiber diameters**
Mechanosensing – **mesh size, stiffness**

Cell Motility – **Porosity**
Molecule Diffusion - **mesh size**
Matrix Degradability - **MMPs**

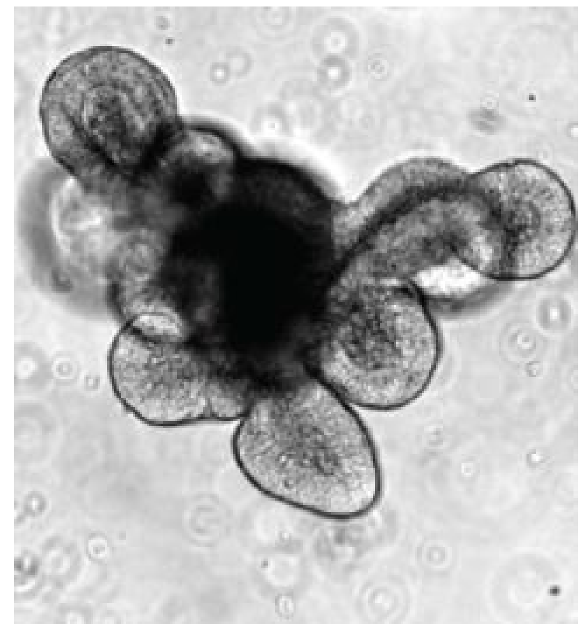
Self-organizing cells
Organoids & Spheroids formation



Non-adherent surface
V, U shaped well



3D Electron microscopy visualization of spheroids (*Jaros et al, 2017*)



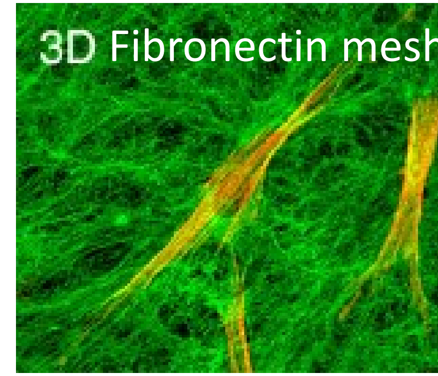
Differentiation in 3D = Organoids in culture
- Intestinal, cerebral, lung…

Clevers, Knoblich, …

Surface modification and 3D matrix

Natural

- Natural recognition by cells
- Expensive isolation
- Availability and purity



– Individual proteins ECM (collagen, fibronectin, etc.)
– Mixture – Matrigel, Geltrex, …
– Decellularized tissue (**Preserved structure**)

Heart

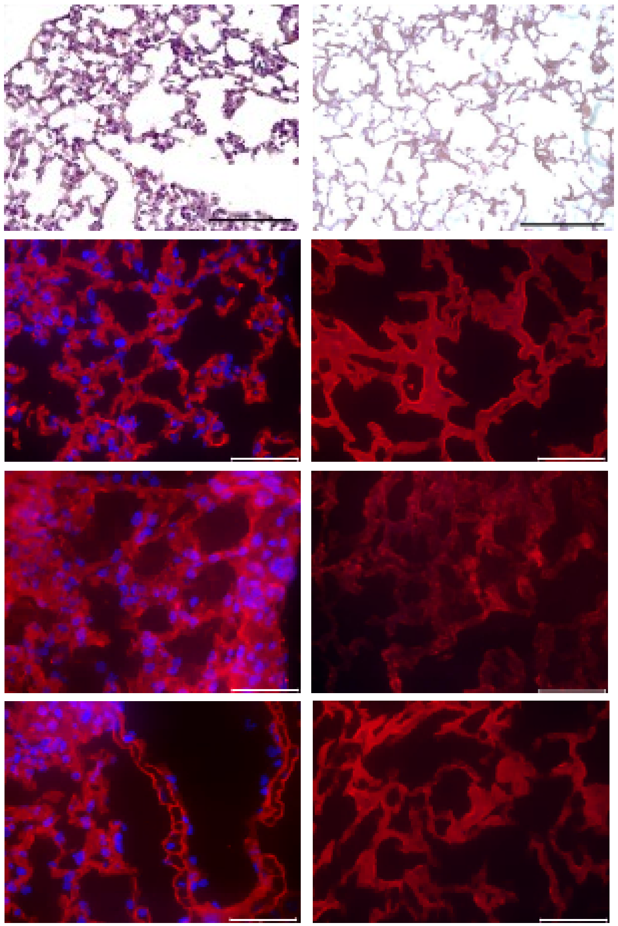
Ott, H.C. et al, Nat Med, 2008

Synthetic

- Precisely defined
- Can be customized
- Large scale production -> low price
- Easy modifications

– Polymers (PLLA, PCL, PU, …)
– Peptides
– Foams, mesh fibers, scaffolds, hydrogels

Decellularized tissue - mouse lungs



Hematoxylin Eosin

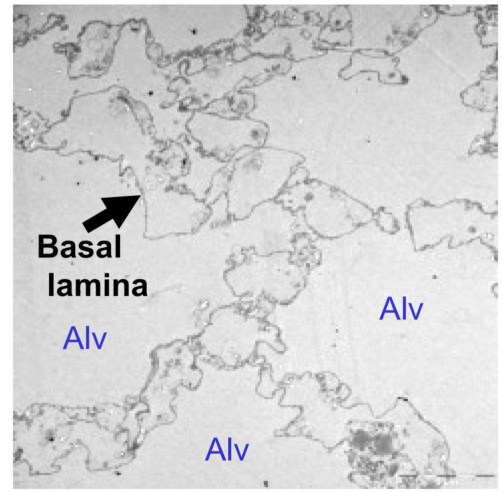
Collagen

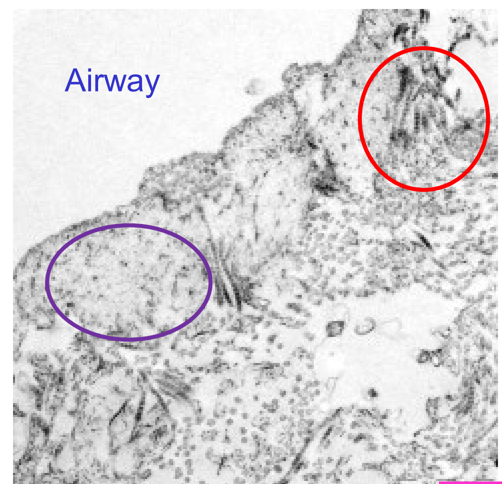
Fibronectin

Laminin

Complete **Decellularized**

Immunostained ECM
Blue – cell nuclei



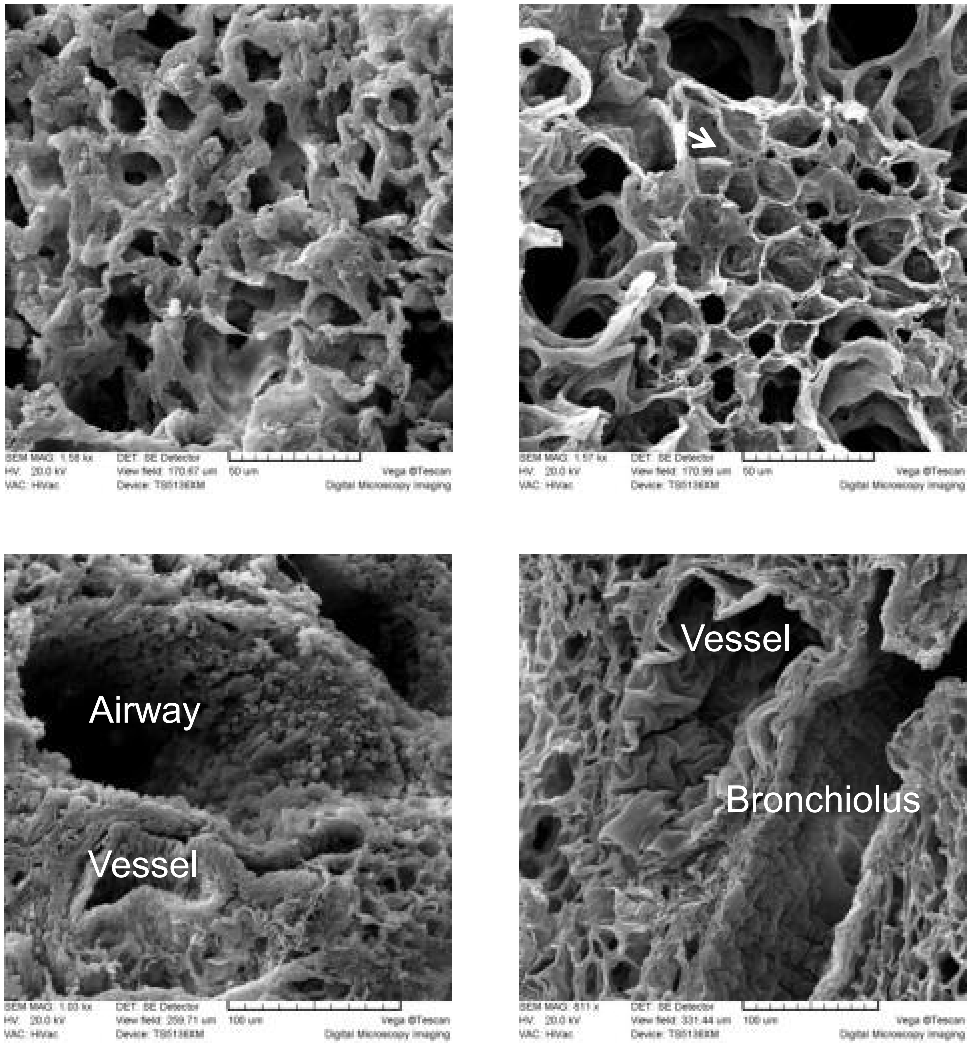


Collagen

Elastin + Fibrilin

**Zuzana Garlíková
Hana Kotasova**

Decellularized lungs preserved anatomical structure



Complete **Decellularized**

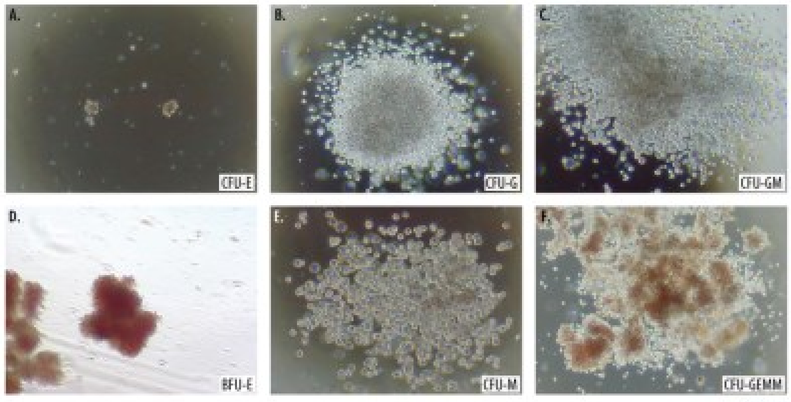
Alveolar region

Garlikova, Hampl et.al. , Tissue Eng., 2018

Man-made structures

Cultivation medium

Viscosity play role for cells



Hematology - MethoCult – growing of colonies

Hydrogels

formed by crosslinking or polymerization

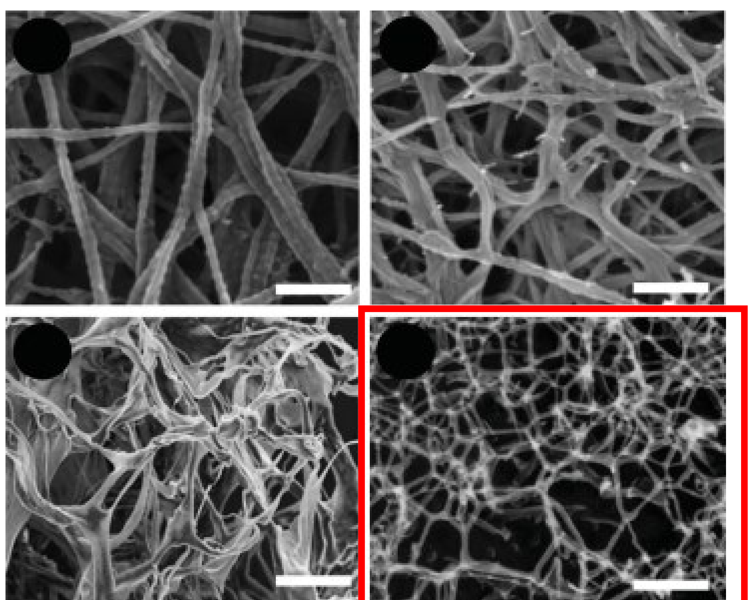
Injectable, >98% of water content

Hyalyronan - Plaster, patch

Effort to produce materials with structure close to natural ECM

Fibrilar structure collagen I

Fibrin matrix



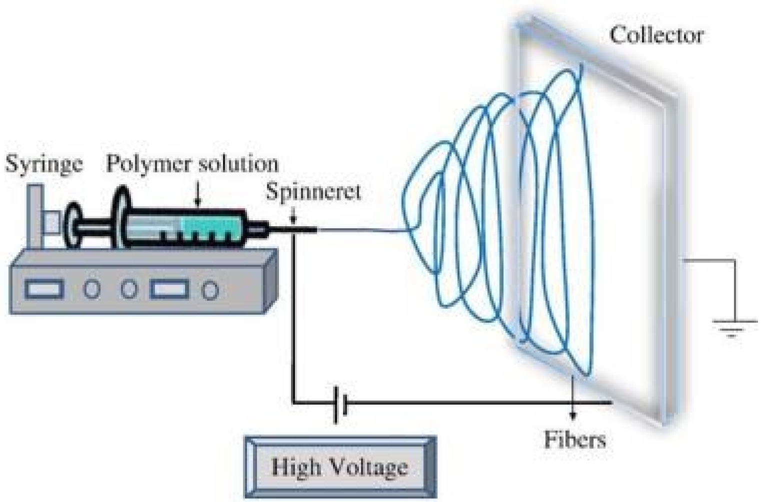
Hydrogel of modified hyaluronic acid

Synthetic peptide Hydrogel RADA

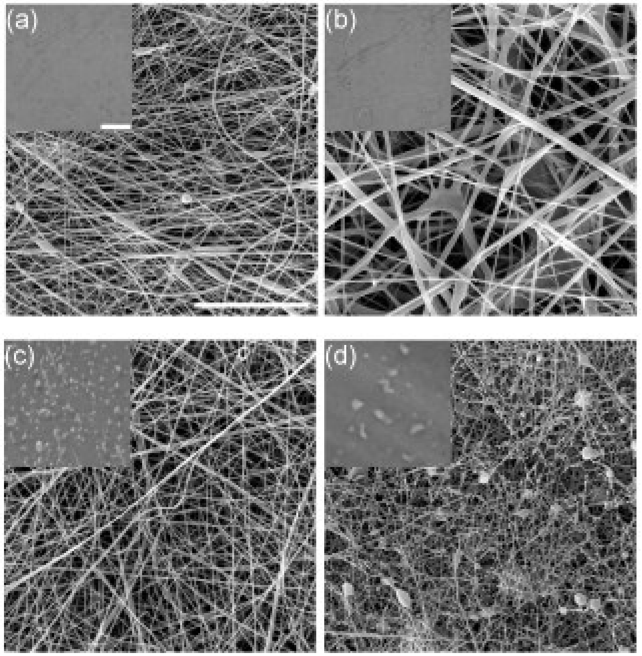
Patterson J. et al, MaterialsToday, 2010

Nanofibers

- Soluble materials, spun fibers under 1 um
- Mostly for covering – skin, endothelium, dura mater…



Modified PCL and PLLA nanofibers

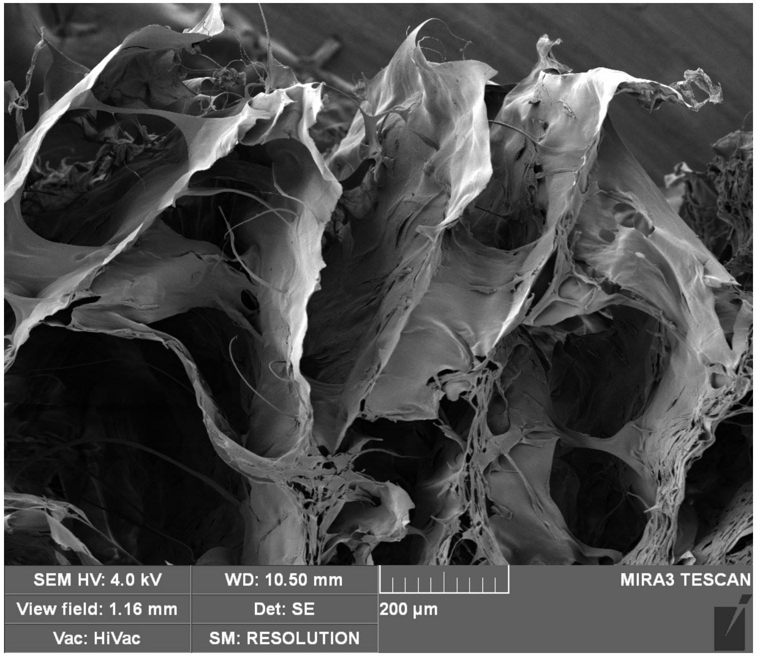


Sedlakova, submitted, 2017

Porous scaffolds

Application in clinics

- Trachea
- Heart valves
- Bones



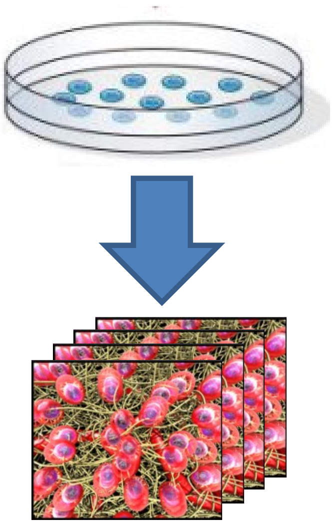
Structure

- *Pores (60-300 um)*
- *Diffusion*
- *Degradability*
- *Dynamics – release of chemicals –drugs, molecules for differentiation (growth factors, morphogens)*

Jaros, unpublished

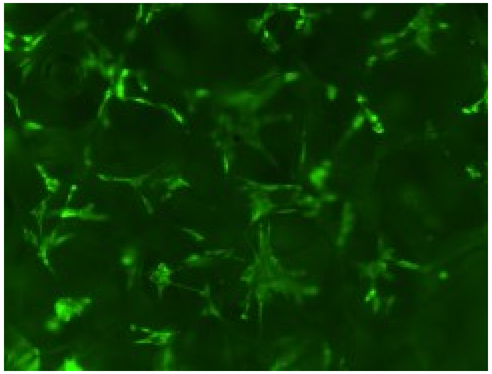
3D cultivation troubles

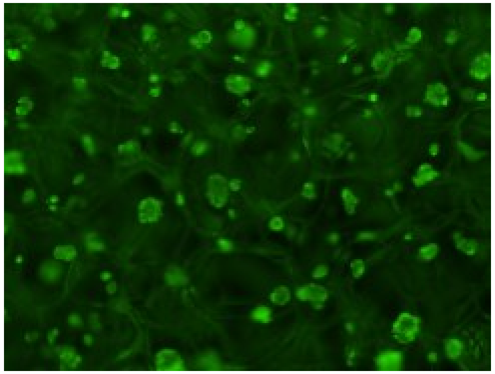
- Manipulation, analysis, sterilization
- How to get cells inside
- How to get cells/proteins out (PCR, WB, etc.)
- How to provide nutrient/waste exchange
- **Organization of cells**



solution ⇨ **3D bioprinting**

3D porous synthetic gel

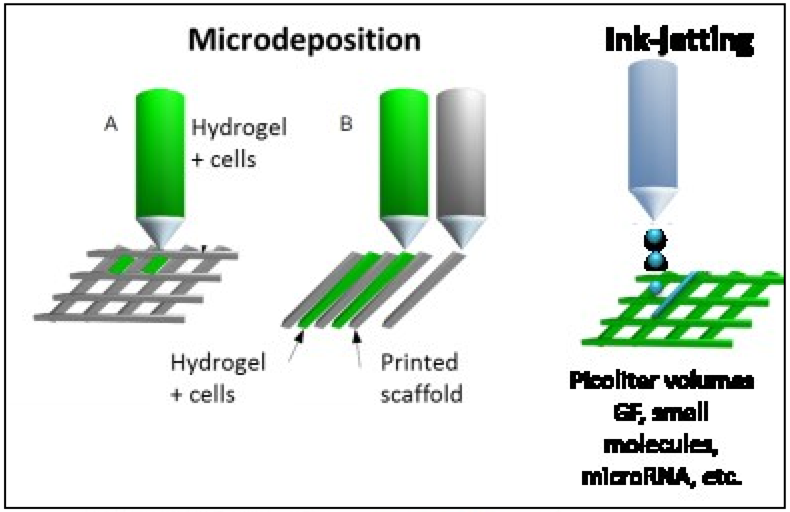




Golunová A., Jaros J., et al., *Biomaterials, 2015*
Proks, V., Jaros, J., et al., *Macromol Biosci 2012*

3D bioprinting allows cell organization and tissue formation

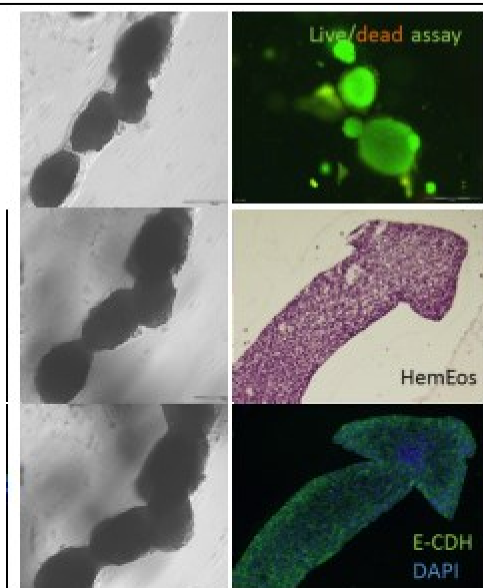
Organization and positioning of cells is important aspect for **controlling cell behavior**



Josef Jaros
Karolina Spustova
Richard Mackovic

- Extruders and ink-jetting
- Hydrogels applied
- Human stem cells and progenitors
- Combination of material and cells

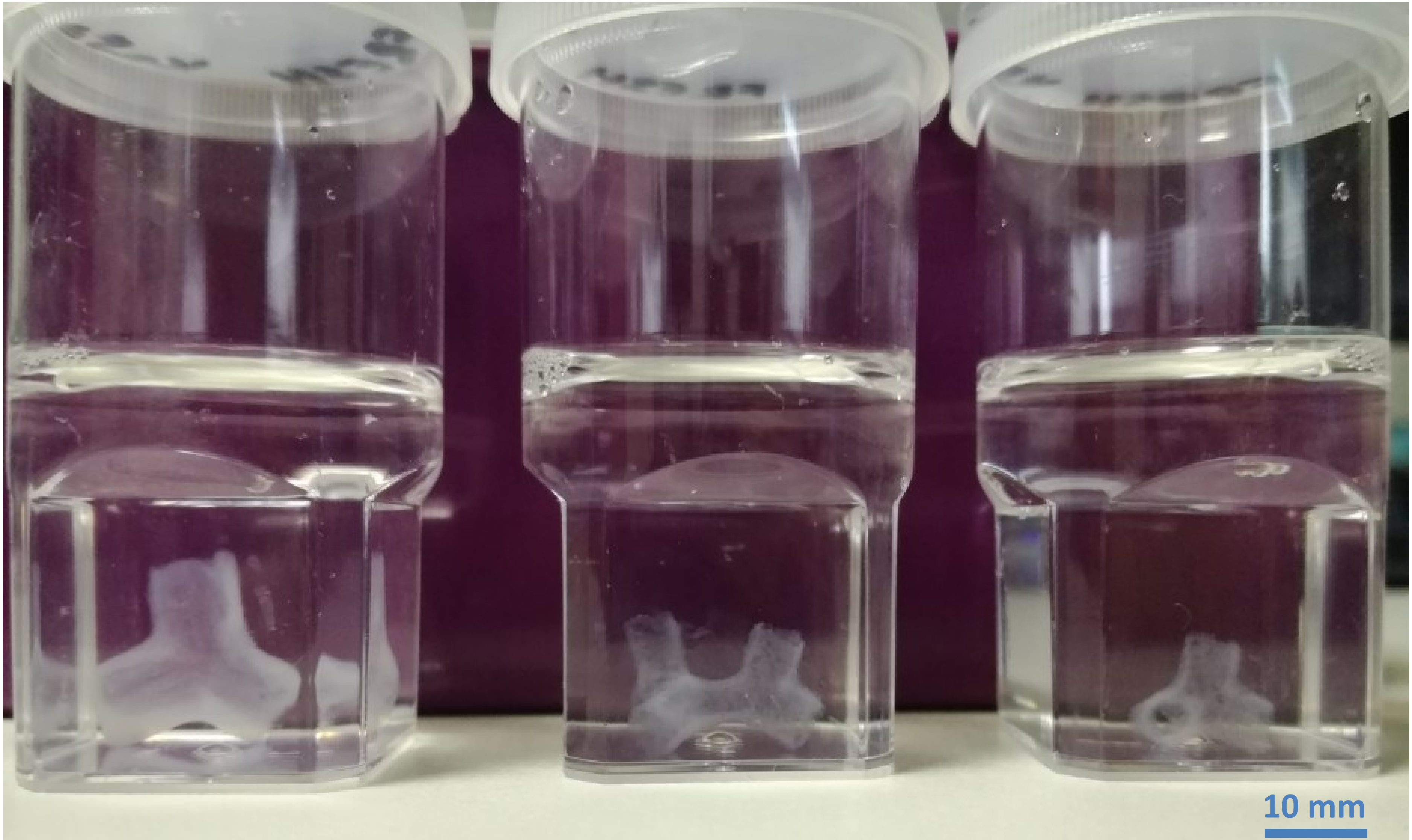
Picoliter drops arrays



Printed spheroids

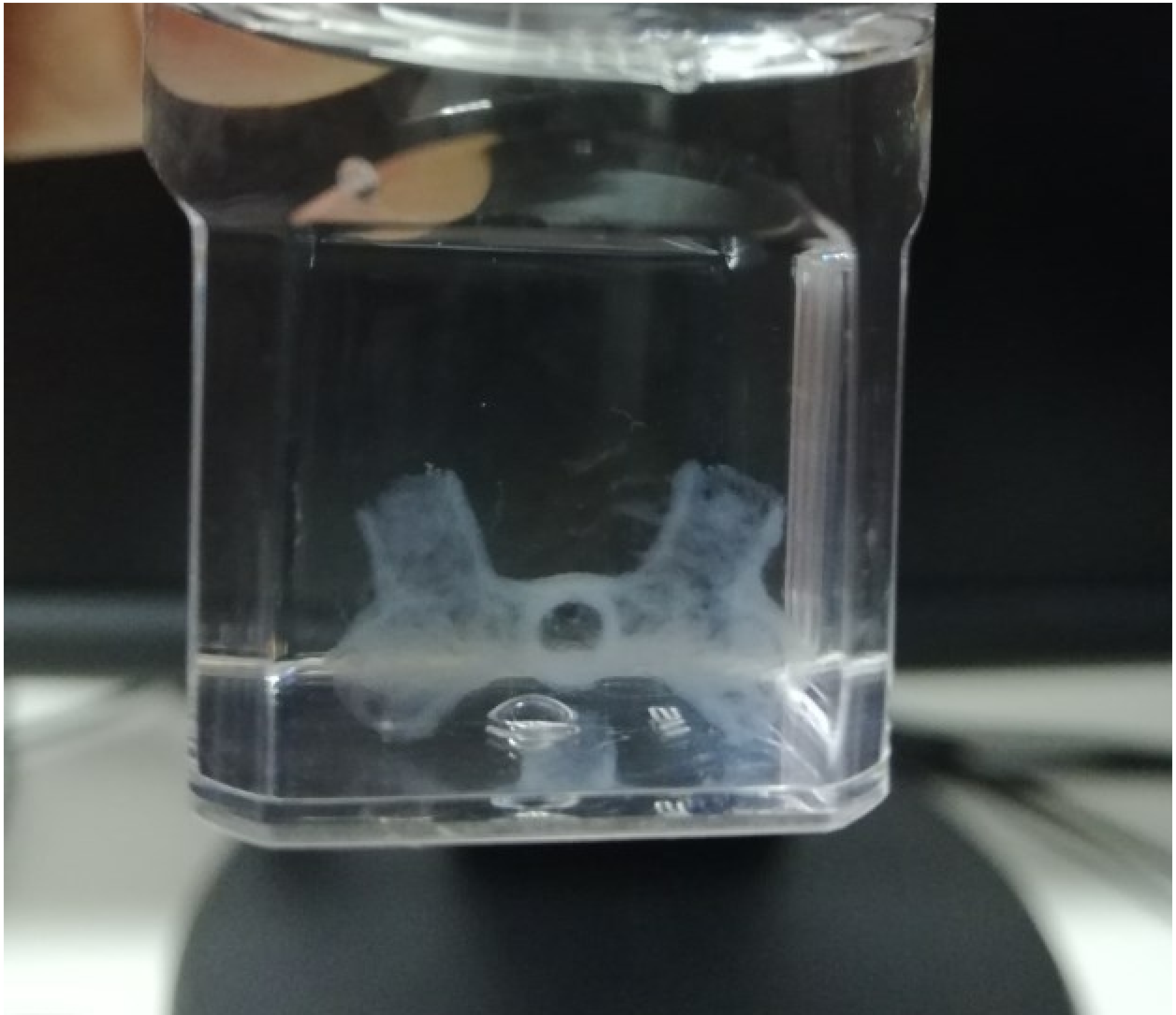
Hydrogel vessels

tubes with walls of 300 um



Jaros, unpublished

Branching structure



Jaros, unpublished

Printing cells
into shapes of branched tubes

unpublished

Take-home message

Caring microenvironment and organization of stem cells help to build tissue structures and to understand biological mechanisms..

Video
Dynamics of growth of hESC spheroids
organized within hydrogels
by 3D bioprinting