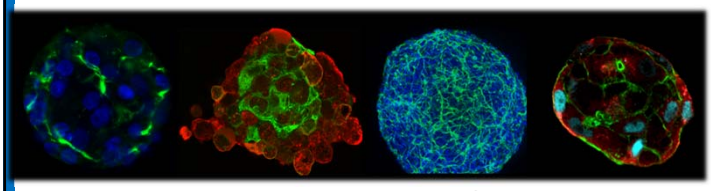
Células Estaminais em investigação biomédica: modelos celulares derivados de células estaminais humanas

Catarina Brito
anabrito@itqb.unl.pt

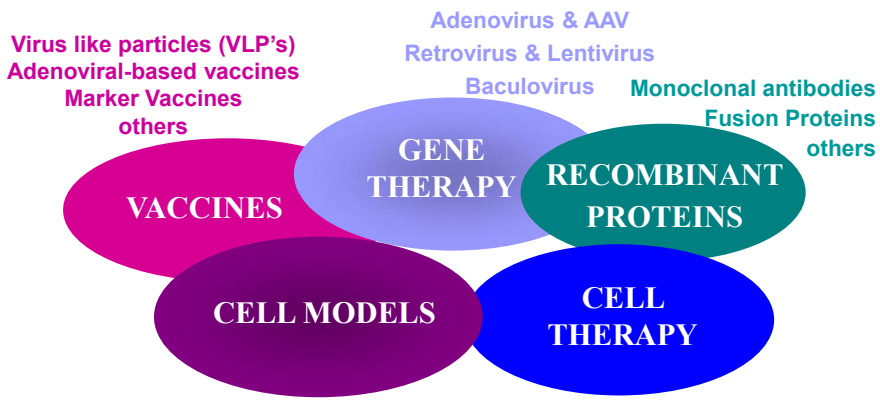
Advanced Cell Models Lab
Animal Cell Technology Unit
iBET & ITQB-NOVA



www.ibet.pt

1 www.ibet.pt

Focus of the Animal Cell Technology UNIT



PRIMARY CULTURES:
- Human Hepatocytes
- Human Cancer cells

Human STEM CELLS:
- Human ESC, human iPSC
- Adult Stem cells – neural, mesenchymal and cardiac

neuronal, cardiac & hepatic differentiation

ANIMAL CELL CULTURES

R&D

Production

Biopharmaceuticals
- recombinant proteins
- growth factors, blood factors
- vaccines

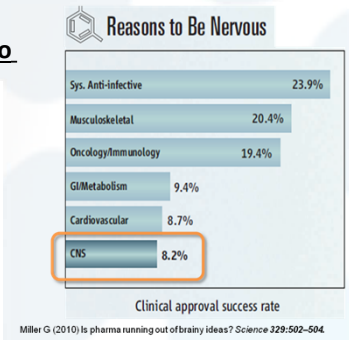
Gene therapy
- adenovirus, AAV
- retrovirus, lentivirus

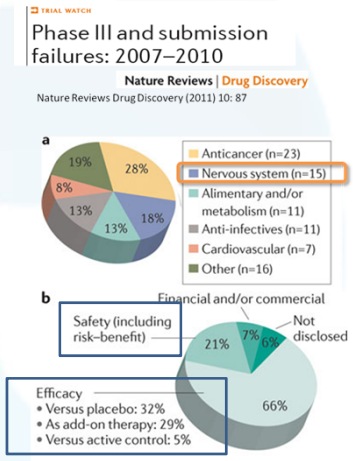
Cell Therapy
- stem cells

Model systems
- Disease models (cell physiology & metabolism)
- Drug Testing
- *in vitro* toxicology

I&D industria farmacêutica

taxas de atrito elevadas





~ 90% dos novos medicamentos para doenças do SNS falham em ensaios clínicos fase II and III

Causas dos insucesso em ensaios clínicos fase III

Eficácia (66%) **Segurança (21%)**

Falta de poder predictivo dos modelos utilizados em fases de desenvolvimento pré-clínico

5

Modelos em I&D

Identificação de alvos | **Validação de alvos** | ***Drug Screening*** | **Toxicologia**

Modelos in vivo

Animal models

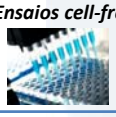
- **Complexidade elevada**
- **Disponibilidade limitada** (Guidelines para redução da experimentação animal)
- **Divergência do humano**– em termos de desenvolvimento, anatomicamente e fisiologicamente; diferenças nos mecanismos de doença

Modelos celulares in vitro

2D Cell Cultures

- **Não recapitulam as características dos tecidos-alvo**
  interações célula-célula e célula-matriz, diferentes tipos de células, etc

Ensaios cell-free



-**Abordagem direccionada para proteínas e vias bem caracterizadas**
-Sem capacidade de prever contribuições/consequencias de outros potenciais intervinientes

Mudança de paradigma: relevância humana

6

THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – WHY?

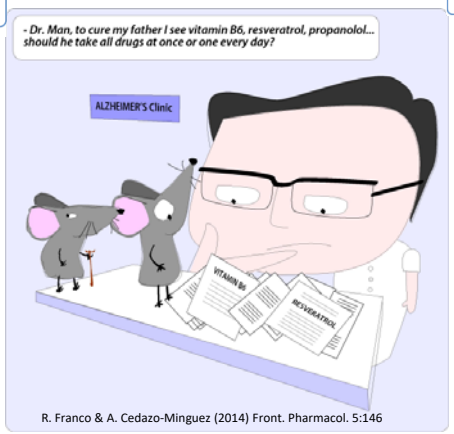
Prevalent models in R&D

in vivo models

Animal models

in vitro models

2D Cell Cultures



R. Franco & A. Cedazo-Minguez (2014) Front. Pharmacol. 5:146

http://xkcd.com/1217/

Paradigm shift towards human relevant translation
- **Increased human relevance of disease models**
- **Increased biological complexity & microenvironment recapitulation**

7 www.ibet.pt

Modelos em I&D

Identificação de alvos | **Validação de alvos** | ***Drug Screening*** | **Toxicologia**

O novo paradigma: ***translação com relevância humana***
- *Aumentar a relevância humana dos models de doença*
- *Aumentar a complexidade biológica dos ensaios de screening*

novos modelos *in vitro*

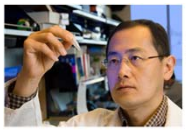
✓ *Que consigam recapitular o tecido/doença alvo*
✓ *Com maior relevância fisiológica e molecular*
✓ *Disponibilidade & robustez (de forma a serem implementados em laboratórios de investigação e indústria)*

As novas ferramentas:
Células estaminais humanas & Sistemas de cultura 3D

8

THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

Human Induced Pluripotent Stem Cells (iPSC)



Nobel Prize **in Physiology/ Medicine 2012**

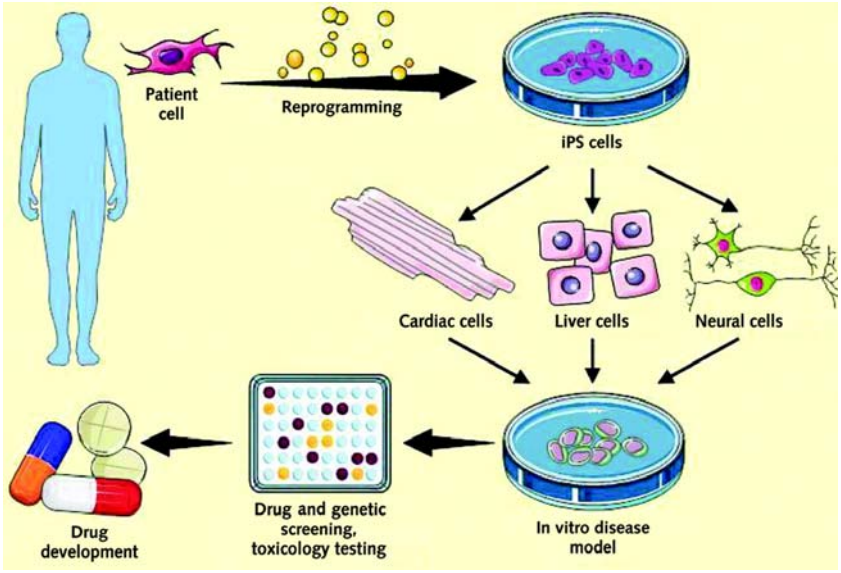
Yamanaka's Lab (Japan)
Takahashi, K. et al. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872

Thomson's Lab (EUA)
Yu, J. et al. (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917–1920.

9 www.ibet.pt

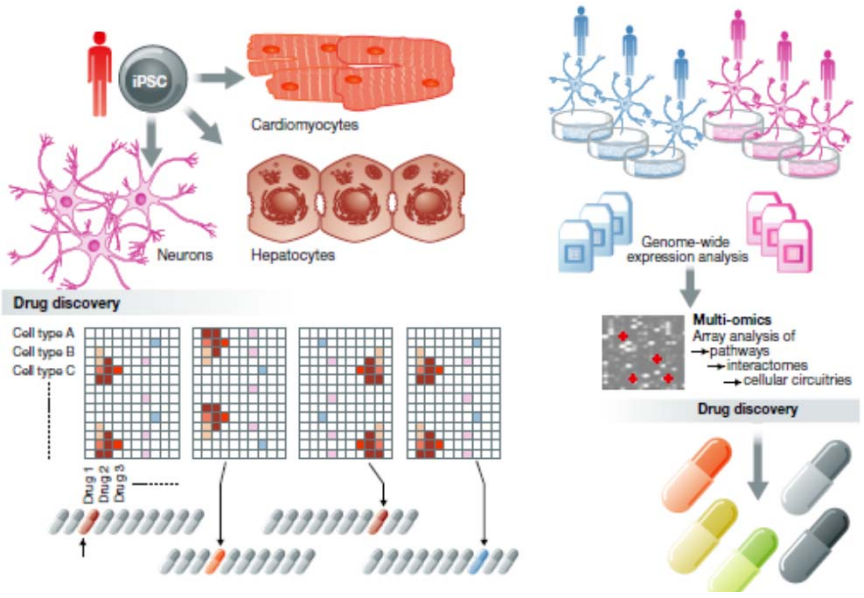
THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

Human Induced Pluripotent Stem Cells (iPSC)



10 www.ibet.pt

THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?



Inoue et al 2014 EMBO J (2014) 33, 409-417

11

THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

Gene Editing tools for specific genome disruption & replacement

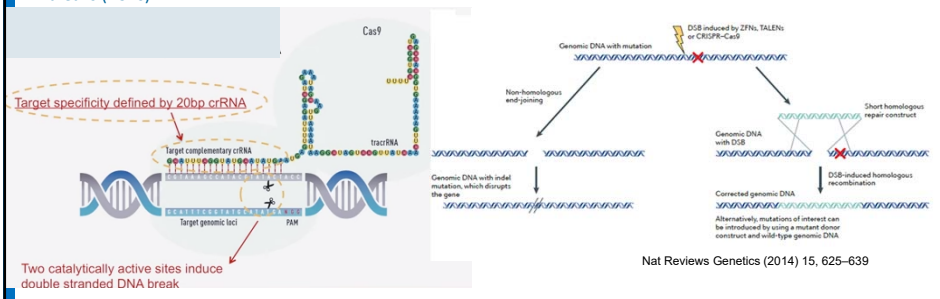
CRISPR/Cas9 - components of the bacterial immune response to bacteriophages

Clustered Regularly Interspaced Short Palindromic Repeat

- **Cas9 DNA nuclease**
- **Guide RNA (gRNA)= short CrisprRNA (crRNA) + transactivating CRISPR RNA (tracrRNA)**

crRNA - recognizes a complementary stretch of nucleotides (the protospacer) within foreign DNA, thus conferring sequence specificity

tracrRNA - required to form ribonucleoprotein complexes with the Cas9 nuclease, generating site-specific double-strand breaks (DSBs)

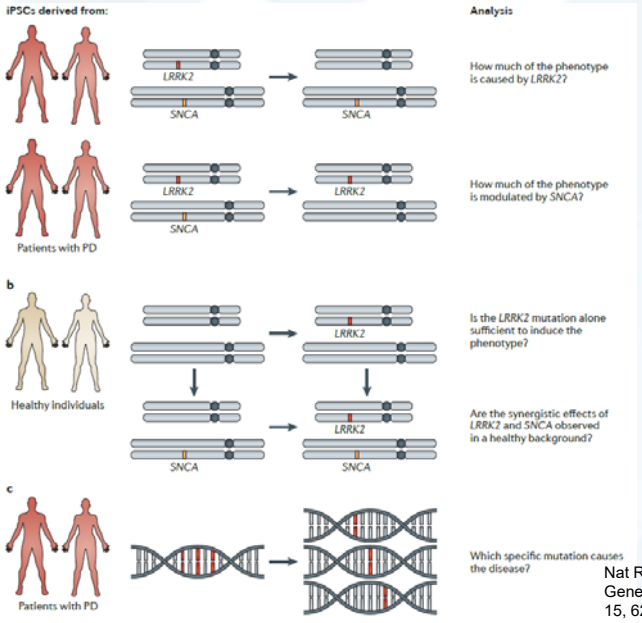


Nat Reviews Genetics (2014) 15, 625–639

12

www.ibet.pt

Using stem cell disease models to evaluate the contribution of specific loci to complex polygenic disorders.



Nat Reviews Genetics (2014) 15, 625–639

Table 1. Patient-Specific iPS Lines that Have Been Differentiated into a Disease-Relevant Cell Type

Cellular Disease Phenotype Identified

Type	Disorder	Publication
Genetic	α1-antitrypsin deficiency	(Rashid et al., 2010)
Genetic	Adrenoleukodystrophy, X-linked	(Jang et al., 2011)
Genetic	Alzheimer's disease, familial	(Yagi et al., 2011)
Genetic	Amyotrophic lateral sclerosis, familial	(Mitne-Neto et al., 2011)
Genetic	Atypical Werner syndrome	(Cy Ho et al., 2011)
Genetic	Chronic granulomatous disease, X-linked	(Zou et al., 2011b)
Genetic	Down syndrome	(Baek et al., 2009)
Genetic	Duchenne muscular dystrophy	(Kazuki et al., 2010)
Genetic	Dyskeratosis congenita	(Agarwal et al., 2010)
Genetic	Epidermolysis bullosa	(Tolar et al., 2011b)
Genetic	Familial dysautonomia	(Lee et al., 2009)
Genetic	Fragile X syndrome	(Urbach et al., 2010)
Genetic	Gaucher's disease	(Mazzulli et al., 2011)
Genetic	Glycogen storage disease type 1A	(Rashid et al., 2010)
Genetic	Huntington's disease	(Zhang et al., 2010)
Genetic	Hurler syndrome	(Tolar et al., 2011a)
Genetic	Hutchinson-Gilford progeria	(Liu et al., 2011)
Genetic	Hypercholesterolemia, familial	(Rashid et al., 2010)
Genetic	Inherited dilated cardiomyopathy	(Cy Ho et al., 2011)
Genetic	LEOPARD syndrome	(Carvajal-Vergara et al., 2010)
Genetic	Long QT syndrome	(Moretti et al., 2010; Itzhaki et al., 2011; Lahti et al., 2011)
Genetic	MPS type IIIB	(Lemonnier et al., 2011)
Genetic	Myeloproliferative disorder	(Ye et al., 2009)
Genetic	Parkinson's disease, familial	(Seibler et al., 2011; Nguyen et al., 2011; Devine et al., 2011)
Genetic	Retinitis pigmentosa	(Jin et al., 2011)
Genetic	Rett Syndrome	(Marchetto et al., 2010)
Genetic	Sickle cell disease	(Zou et al., 2011a)
Genetic	Spinal muscular atrophy	(Ebert et al., 2009)
Genetic	Timothy syndrome	(Ebert et al., 2009)
Genetic	Wilson's disease	(Zhang et al., 2011b)
Multifactorial	Schizophrenia	(Brennand et al., 2011)

No Cellular Disease Phenotype Determined

Type	Disorder	Publication
Genetic	ADA-severe combined immunodeficiency	(Park et al., 2008a)
Genetic	Becker muscular dystrophy	(Park et al., 2008a)
Genetic	Crigler-Najjar syndrome	(Ghodsizadeh et al., 2010)
Genetic	Cystic fibrosis	(Somers et al., 2010)
Genetic	Gyrate atrophy	(Howden et al., 2011)
Genetic	Osteogenesis imperfecta	(Khan et al., 2010)
Genetic	Progressive cholestasis, familial	(Ghodsizadeh et al., 2010)
Genetic	Shwachman-Bodian-Diamond syndrome	(Park et al., 2008a)
Genetic	Tyrosinemia type 1	(Ghodsizadeh et al., 2010)
Multifactorial	Diabetes type 1	(Maehr et al., 2009)
Multifactorial	Parkinson's disease, sporadic	(Soldner et al., 2009; Hargus et al., 2010; Swistowski et al., 2010)
Multifactorial	Scleroderma	(Somers et al., 2010)

Most lines to date that have been differentiated into disease-relevant cell types have been from patients with genetic disease. Identifying a disease-

Objective: Reproduce disease molecular hallmarks in cell types relevant in human diseases

Cherry & Daley 2012, Cell 148, 1110-1122

14

www.ibet.pt

Table 1. Patient-Specific iPS Lines that Have Been Differentiated into a Disease-Relevant Cell Type

Cellular Disease Phenotype Identified

Type	Disorder	Publication
Genetic	α1-antitrypsin deficiency	(Rashid et al., 2010)
Genetic	Adrenoleukodystrophy, X-linked	(Jiang et al., 2011)
Genetic	Familial Alzheimer's Disease	
Genetic	Chronic granulomatous disease, X-linked	(Zou et al., 2011b)
Genetic	Familial Amytrophic lateral sclerosis	
Genetic	Epidermolysis bullosa	(Tolar et al., 2011b)
Genetic	Familial Parkinson's Disease	
Genetic	Glycogen storage disease type 1A	(Rashid et al., 2010)
Genetic	Huntington's Disease	al., 2010)
Genetic		l., 2011a)
Genetic		al., 2011)
Genetic	Down Syndrome	(Rashid et al., 2010)
Genetic		(Cy Ho et al., 2011)
Genetic		(Carvajal-Vergara et al., 2010)
Genetic	Long QT syndrome	(Moretti et al., 2010; Itzhaki et al., 2011; Lahti et al., 2011)
Genetic	MPS type IIIB	(Lemonnier et al., 2011)
Genetic	Myeloproliferative disorder	(Ye et al., 2009)
Genetic	Parkinson's disease, familial	(Seibler et al., 2011; Nguyen et al., 2011; Devine et al., 2011)
Genetic	Retinitis pigmentosa	(Jin et al., 2011)
Genetic	Rett Syndrome	(Marchetto et al., 2010)
Genetic	Sickle cell disease	(Zou et al., 2011a)
Genetic	Spinal muscular atrophy	(Ebert et al., 2009)
Genetic		(Ebert et al., 2009)
Genetic	Schizophrenia	(Zhang et al., 2011b)
Multifactorial		(Brennand et al., 2011)

No Cellular Disease Phenotype Determined

Type	Disorder	Publication
Genetic	ADA-severe combined immunodeficiency	(Park et al., 2008a)
Genetic	Becker muscular dystrophy	(Park et al., 2008a)
Genetic	Crigler-Najjar syndrome	(Ghodsizadeh et al., 2010)
Genetic	Cystic fibrosis	(Somers et al., 2010)
Genetic	Gyrate atrophy	(Howden et al., 2011)
Genetic	Osteogenesis imperfecta	(Khan et al., 2010)
Genetic	Progressive cholestasis, familial	(Ghodsizadeh et al., 2010)
Genetic	Shwachman-Bodian-Diamond syndrome	(Park et al., 2008a)
Genetic	Tyrosinemia type 1	(Ghodsizadeh et al., 2010)
Multifactorial	Diabetes type 1	(Maehr et al., 2009)
Multifactorial	Parkinson's disease, sporadic	(Soldner et al., 2009; Hargus et al., 2010; Swistowski et al., 2010)
Multifactorial	Scleroderma	(Somers et al., 2010)

Most lines to date that have been differentiated into disease-relevant cell types have been from patients with genetic disease. Identifying a disease-

Genetic Diseases

Multifactorial Diseases

Genetic Diseases

Multifactorial Diseases

Objective: Reproduce disease molecular hallmarks in cell types relevant in human diseases

Success in genetic diseases mainly

Cherry & Daley 2012, Cell 148, 1110-1122

15

www.ibet.pt

THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

Table 1 (cont.) | **Summary of a subset of published human iPSC models, including gene-corrected controls**

Disease	Mutant locus	Description
Parkinson's disease	Sporadic	Impaired autophagy; aberrant neuronal morphology; increased production of cleaved CASP3 with prolonged culture[37]
	LRRK2	• Increased levels of α-synuclein[13,14,16], MAPT[16] and phosphorylated MAPK[16]; neurodegeneration under oxidative stress[16,27,28,121]; impaired neurite outgrowth and autophagy[25,27,29,122]; aberrant nuclear morphology[30], aberrant mitochondrial activity; increased mitochondrial DNA damage[121,123] • Gene correction reversed the phenotypes[16,30,121]
	PARKIN	Aberrant dopamine use[124]; mitochondrial dysfunction[125]; α-synuclein accumulation[125]
	PINK1	Aberrant recruitment of the PARKIN protein to mitochondria[126]
	SNCA	• Increased α-synuclein[43,127,128]; increased neurodegeneration with stress[43]; aberrant mitochondrial activity[43,127]; increased nitrosylation of MEF2C[43]; decreased expression of PGC-1α[43] • Gene correction reversed the phenotypes[43]
Pompe disease	*GAA*	Differentiated cardiomyocytes had high glycogen levels and aberrant ultrastructure[128]
Pulmonary alveolar proteinosis	*CSF2R*	Impaired GM-CSF signalling[130,131], surfactant clearance, phagocytosis[130,131] and inflammatory cytokine production[130] in macrophages
Rett syndrome	*MECP2*	• Aberrant neuronal morphogy[132,133]; defect in neuronal maturation[134] • Gene correction reversed the phenotypes[132]
Schizophrenia	Familial cases, but unknown gene (or genes)	Decreased neuronal connectivity and neurite number[135]
Spinal muscular atrophy	*SMN1*	• Reduced SMN protein levels[11]; decreased survival of MNs[11,41,136,137]; fewer synapses after long-term culture[11]; decreased levels SMN-positive nuclear aggregates[11,41,137] • Gene correction reversed the phenotypes[26]
Timothy syndrome	*CACNA1B*	Defects in Ca^{2+} signalling[138,139]; abnormal neuronal differentiation[138]; dendrite retraction[140]; cardiomyocte arrhythmia[125]
Wilson's disease	*ATP7B*	Abnormal localization of mutant ATP7B protein; defective Cu^{2+} transport in differentiated hepatocytes[19]
Alzheimer's disease	Sporadic	Oligomerization of $A\beta^{28}$; increased secretion of Aβ40 (REF. 102); phosphorylation of MAPT[102]; activation of $GSK3\beta^{102}$; stress response[28]; large and very early endosomes[102]
	APP	• Dominant mutation: increased secretion of Aβ40 (REF. 102), increased Aβ42/Aβ40 ratio[28,103]; phosphorylation of MAPT[102]; activation of $GSK3\beta^{102}$; large and very early endosomes[102] • Recessive mutation: decreased secretion of Aβ40 (REF. 28); increased oligomerization of Aβ; increased stress response[28]
	PS1 or *PS2*	Increased Aβ42/Aβ40 ratio[103,104]
	Trisomy 21	Increased secretion of Aβ40 (REF. 105); formation of amyloid aggregates[105]; phosphorylation of MAPT[105]

Nat Reviews Genetics (2014) 15, 625–639

16

www.ibet.pt

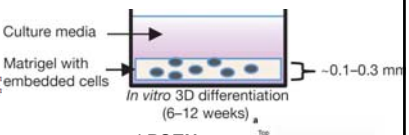
THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

LETTER

doi:10.1038/nature13800

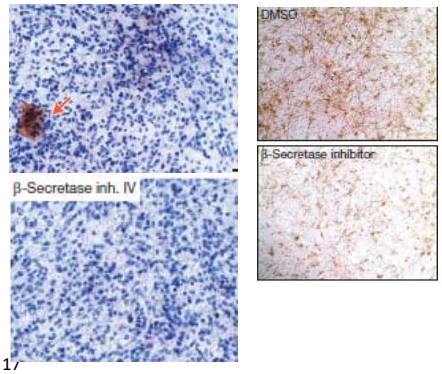
A three-dimensional human neural cell culture model of Alzheimer's disease

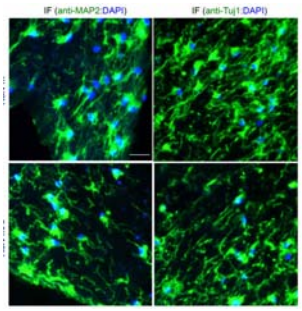
Se Hoon Choi[1]*, Young Hye Kim[1,2]*, Matthias Hebisch[1,3], Christopher Sliwinski[1], Seungkyu Lee[4], Carla D'Avanzo[1], Hechao Chen[1], Basavaraj Hooli[1], Caroline Asselin[1], Julien Muffat[5], Justin B. Klee[1], Can Zhang[1], Brian J. Wainger[4], Michael Peitz[3], Dora M. Kovacs[1], Clifford J. Woolf[4], Steven L. Wagner[6], Rudolph E. Tanzi[1] & Doo Yeon Kim[1]

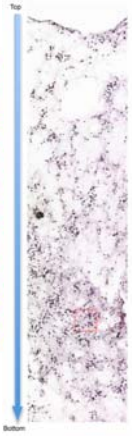


hNPC transduced with Familial AD mutated forms of **APP** (K670N/M671 & V717I) and **PSEN** (ΔE9)

Extracellular amyloid-b deposits **aggregated p-tau in cells**



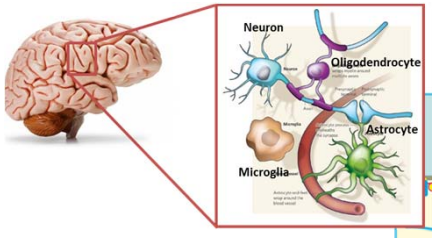


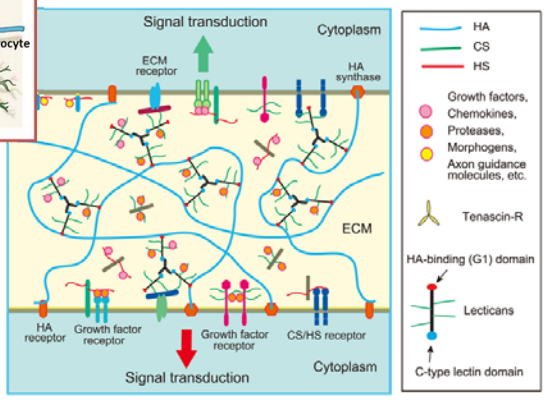


17

www.ibet.pt

MODELLING CNS NEURAL MICROENVIRONMENT





Highly complex system:

- Architecture
- Cellular composition
- Secreted Factors

Network of Cell-Cell/ECM Interactions

Tissue Function

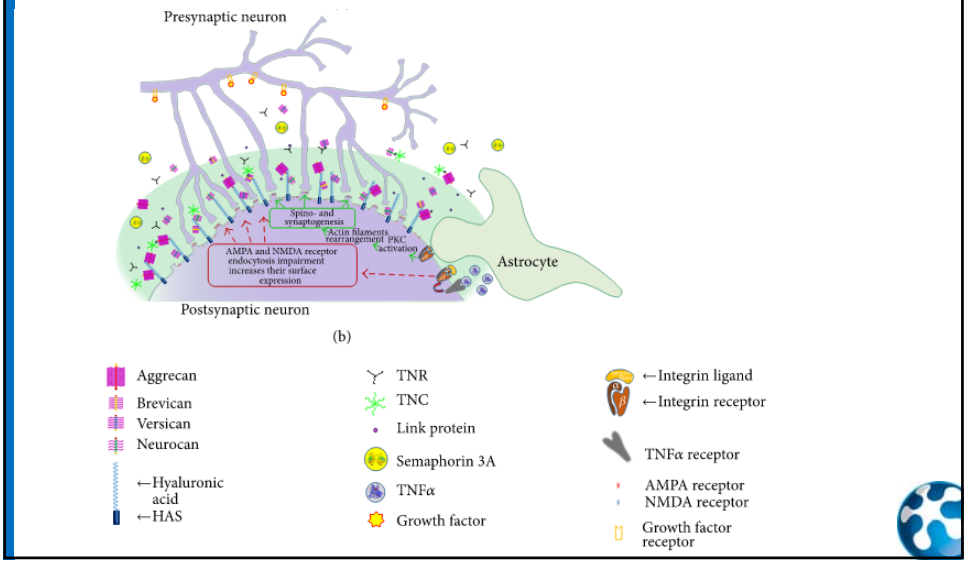
Common endeavor of biomimetic models = place the target cell in its environment

18

www.ibet.pt

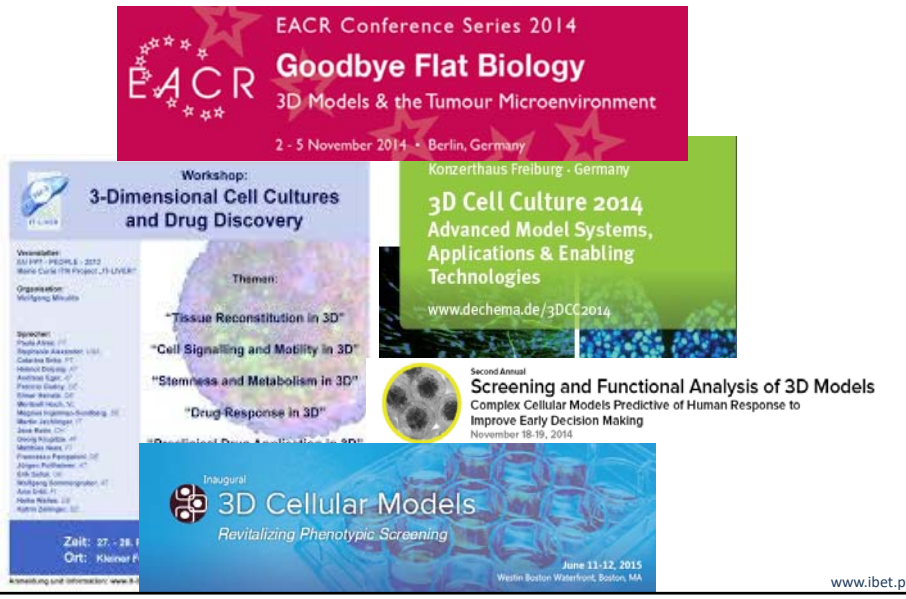
MODELLING CNS NEURAL MICROENVIRONMENT

Astrocytes and olygodendrocytes required for neural maturation – physical, protein-mediated and metabolic interactions



THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

The rise of 3D Cell Models

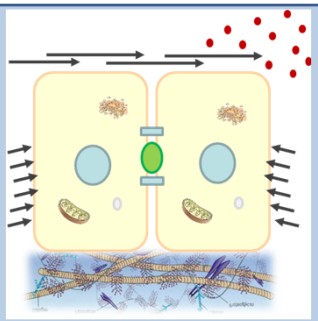


www.ibet.pt

THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

Minimal components for recreation of tissue microenvironment

Soluble factors
- Growth factors, hormones, cytokines



Cell-cell interactions
- Monotypic or heterotypic
- Maximization of interactions
- Polarization of epithelial tissues

Extracellular matrix
- Anchorage, support, mechanical stimuli
- Modulate cellular pathways and differentiation
- Migration

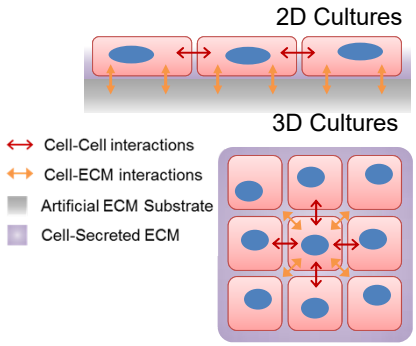
Physical Forces & Physicochemical parameters
- Hydrodynamic shear; Compressive strains: Hydrostatic pressure; diffusion rates
- Temperature, pH, oxygen

21 www.ibet.pt

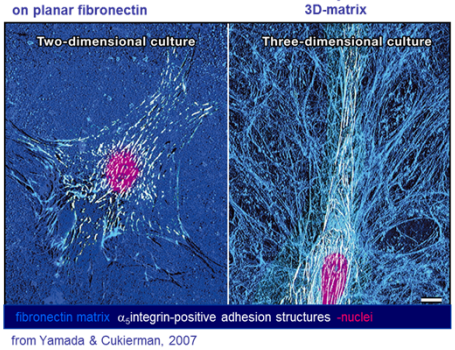
THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

to mimic the architecture & cell interactions occurring within the tissue/organ:

- ✓Morphology & polarity (e.g. axonal branching)
- ✓Cell-adhesion events
- ✓Cytoskeletal organization
- ✓Intracellular signaling



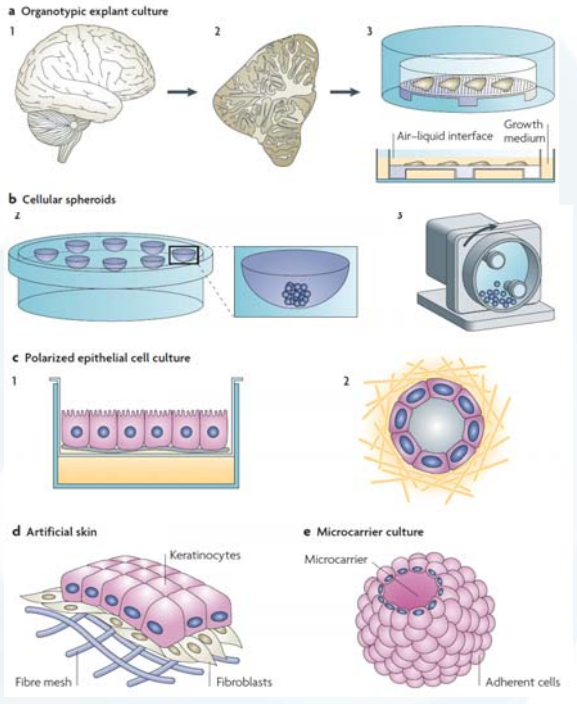
Fibroblasts:



from Yamada & Cukierman, 2007

22 www.ibet.pt

3D *in vitro* models:

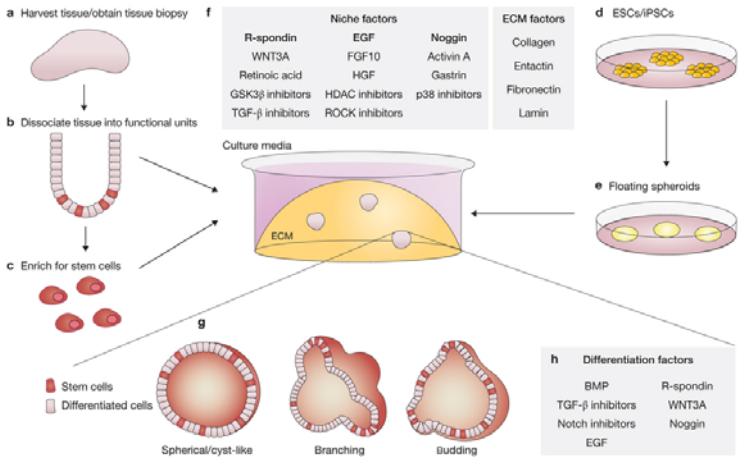


Nature Reviews Molecular Cell Biology (2007) 8, 839-845

23

iBET THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

ORGANOIDS 'R-spondin method'

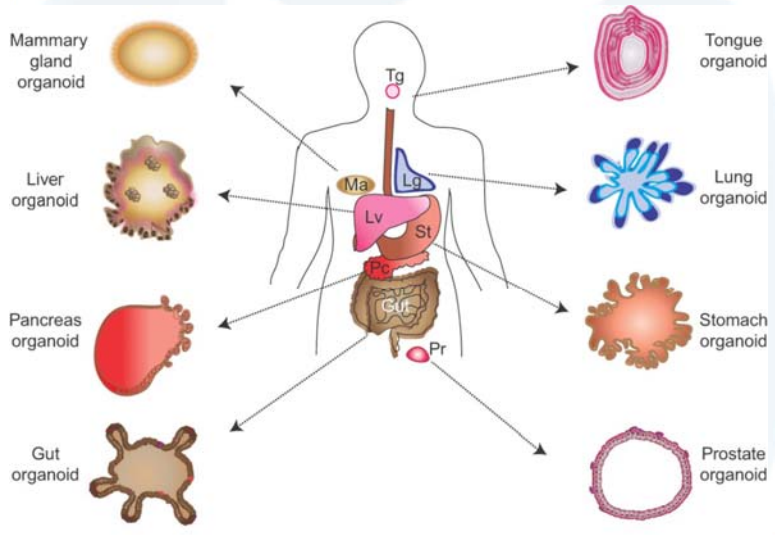


Nature Cell Biology18, 246–254 (2016) doi:10.1038/ncb3312

24

www.ibet.pt

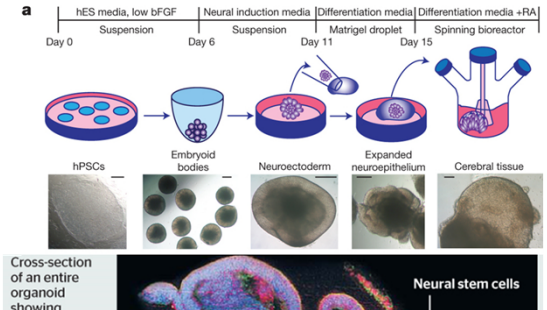
Adult stem cell (AdSC)-derived organoids.

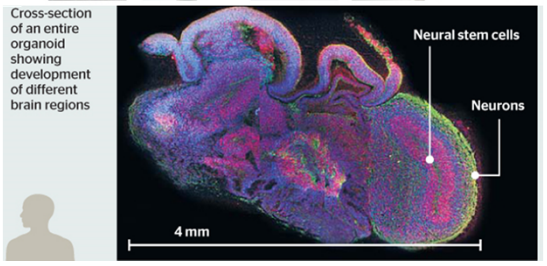


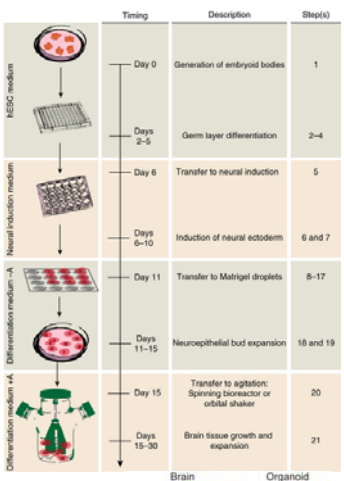
Meritxell Huch, and Bon-Kyoung Koo Development 2015;142:3113-3125

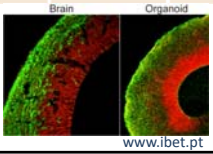
THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

The brain "organoid" model





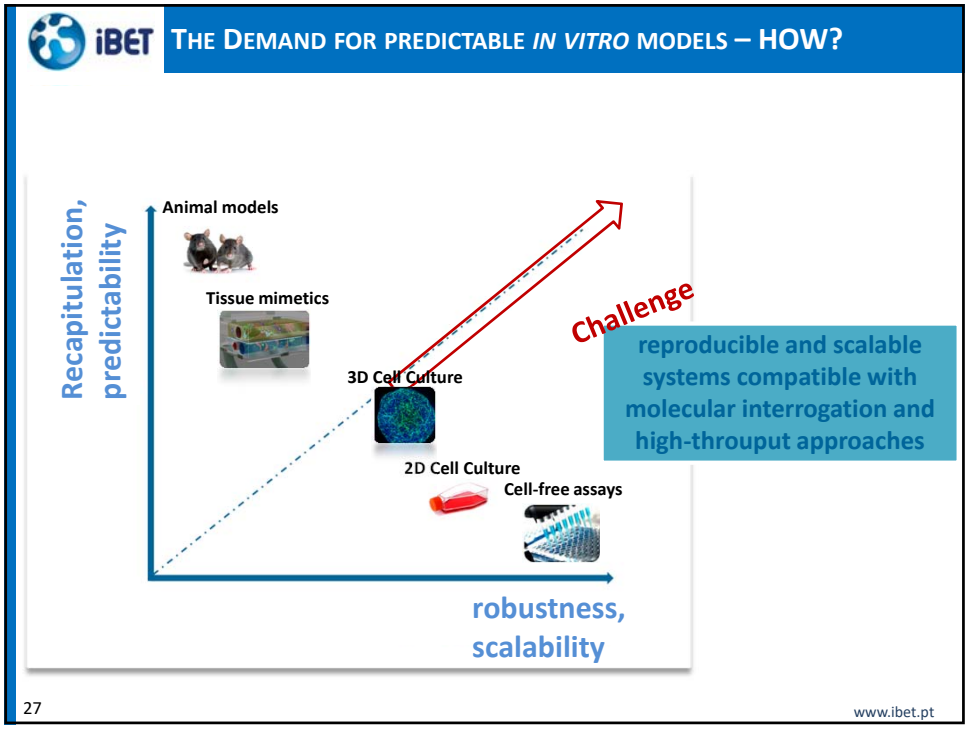


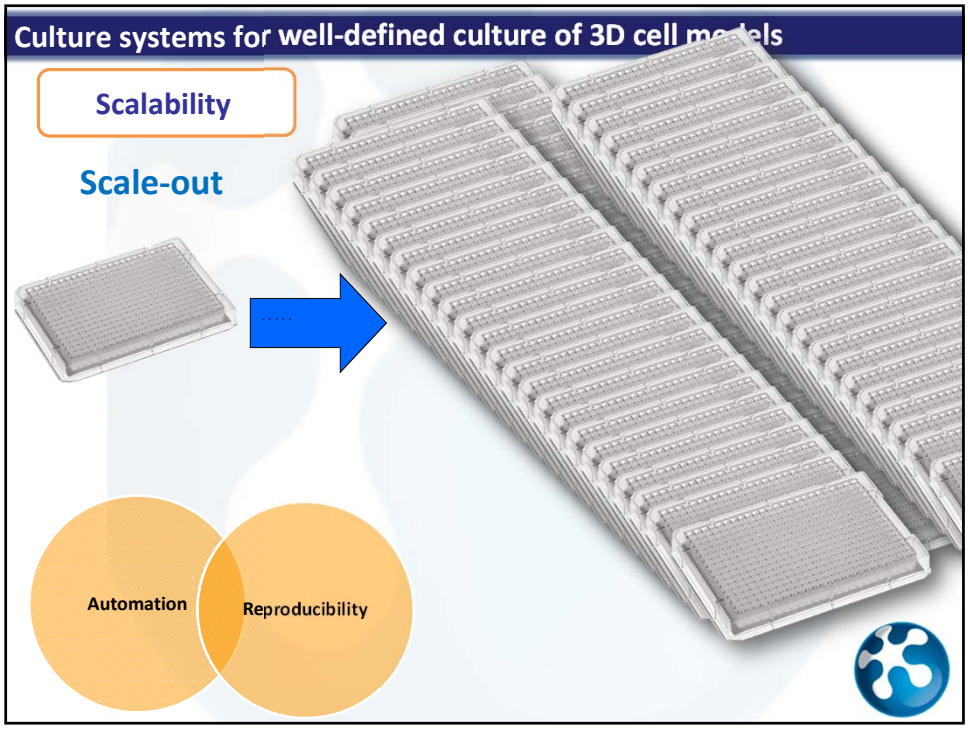


Lancaster et all: Nature 501, 373–379 (2013); Nature Protocols 9, 2329–2340 (2014)

26

www.ibet.pt


iBET
THE DEMAND FOR PREDICTABLE IN VITRO MODELS – HOW?
Recapitulation, predictability
Animal models
Tissue mimetics
3D Cell Culture
2D Cell Culture
Cell-free assays
Challenge
reproducible and scalable systems compatible with molecular interrogation and high-throuput approaches
robustness, scalability
27
www.ibet.pt

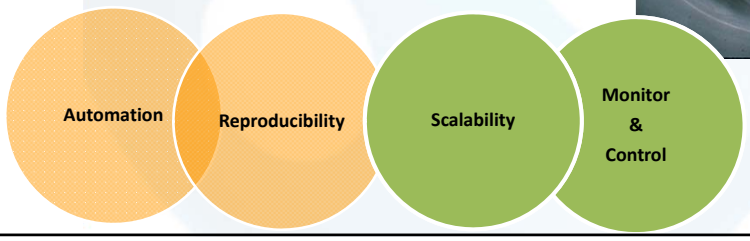

Culture systems for well-defined culture of 3D cell models
Scalability
Scale-out
Automation
Reproducibility

Culture systems for well-defined culture of 3D cell models

Scalability

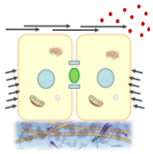
Scale-up

Photo: courtesy A.Amanullah



iBET THE DEMAND FOR PREDICTABLE *IN VITRO* MODELS – HOW?

3D cell models: Physicochemical parameters



- **Bioreactors**

Stirred-tank bioreactors

- **efficient** & **well-characterized** mass transfer;
- on-line **monitoring** and automated **control** of physicochemical variables (temperature, pH, dissolved oxygen).
- **perfusion operation modes**: simulation *in vitro* of consequences of circulatory system connections
  - shear stress and pressure
  - supply of O_2
  - supply of cytokines/ growth factors
  - metabolite clearance
  - accessibility of drugs/compounds

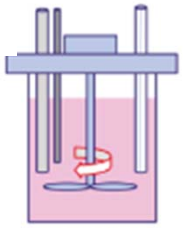
microfluidic bioreactors

30

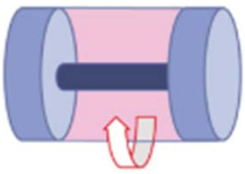
www.ibet.pt

BIOREACTOR TECHNOLOGY: Configurations

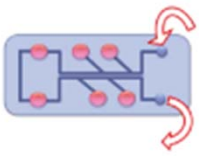
Controlled Culture Systems for recapitulation of tumour microenvironment



Stirred Culture Vessels



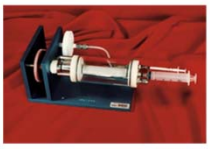
Rotatory Cell Culture Systems



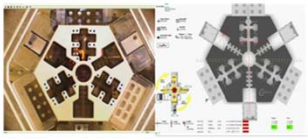
Microfluidic Devices

John A. Hickman et al. Biotechnol. J. 2014, 9, 1115–1128.

DASGIP EPPENDORF



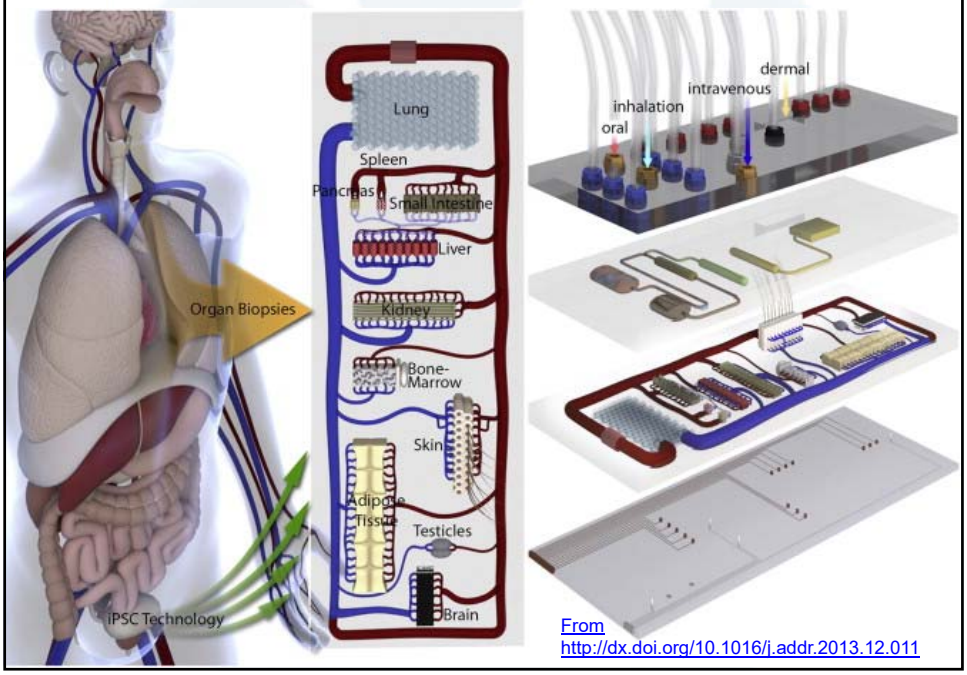
NASA



Images courtesy of FhG-IBMT

31 www.ibet.pt

human on a chip

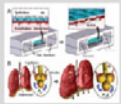


From http://dx.doi.org/10.1016/j.addr.2013.12.011

Living systems on a chip – state of the art

Nature March 31st 2011: Technology feature: A living system on a chip. Vol 471 pp. 661-665

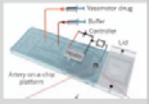
miniaturized single-organ-chips



Hepregen

CellASIC

Hµrel



Huh D. et al Science 328, (2010)

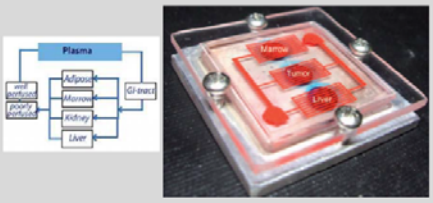
Guenther A. et al Lab Chip 10, (2010)

systemic multi-organ-chip concepts

Michael Shuler, Cornell University, U.S.A.
Sung J.H. et al Lab Chip 11, 389-392 (2011)
Sung J.H., Kam C.; Shuler, M.L. Lab Chip 10 446-455 (2010)
Mahler G..J. et al. Biotechnol Bioeng. 104, 193-205 (2009)

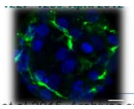
Kiichi Sato, University of Tokyo, Japan
Imura Y. et al Anal. Chem. 82, 9983-9988 (2010)

Shuichi Takayama, University of Michigan, U.S.A.



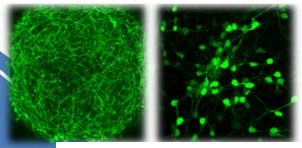
Uwe Marx, Technische Universität Berlin, Germany
Sonntag F. et al , J. Biotechnol 148, 70-75 (2010)

iBET HUMAN 3D MODELS @ iBET



- Tostões et al Hepatology 2012
- Leite SB at al. Toxicol Sci. 2012
- Rebelo SP et al Arch Toxicol 2014
- Rebelo SP et al 2016

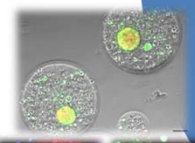
Long-term cultures of functional human Hepatic cells

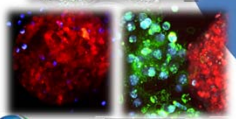


3D neural models derived from human stem cell lines

- Brito C et al Methods, 2012
- Simão D et al Tissue Eng A, 2014
- Gualda EJ, Fronters in Neuro 2014
- Terrasso AP et al J Biotecnol 2015
- Simão et al Gene Therapy 2015

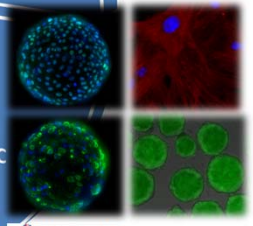
IGMM - CNRS ,France; Cancer Research UK; UCL, UK: ; Università La Sapienza, Italy





Breast & Lung cancer 3D models: microenvironment recapitulation

3D cultures of cardiomyocytes derived from iPS cells and hCSC



predect imi

- Estrada et al Biomaterials 2016
- Santo VE et al J Biotech 2016

cellectis cellartis

- Gomes-Alves et al 2014, 2016
- Correia C et al 2014, 2016

34

www.ibet.pt

WORKING HYPOTHESIS & STRATEGY

Microenvironmental recapitulation can potentially be attained via accumulation of endogenous biomolecules *(i.e., without addition of exogenous bioactive components – ECM and soluble factors)*

Requirements:

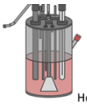
- Relevant cell types of the specific cell microenvironment
- Physiologically relevant cell interactions

3D co-culture

- Cell-cell interactions
- tumour & stromal cell components

- Physico-chemical: oscillations in pH, pO_2
- 4th dimension: time

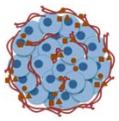
perfusion bioreactors



- Scalable
- Characterized mass transfer
- Perfusion
- Continuous non-invasive monitoring
- Non-destructive sampling

Homogeneous long-term cultures

Scalable and reproducible process



Built-up & remodeling of endogenous ECM & soluble factors along culture time

reflect the native tissue context and mimic disease progression in vitro

35 www.ibet.pt

HUMAN 3D MODELS @ iBET – STRATEGY

Strategy

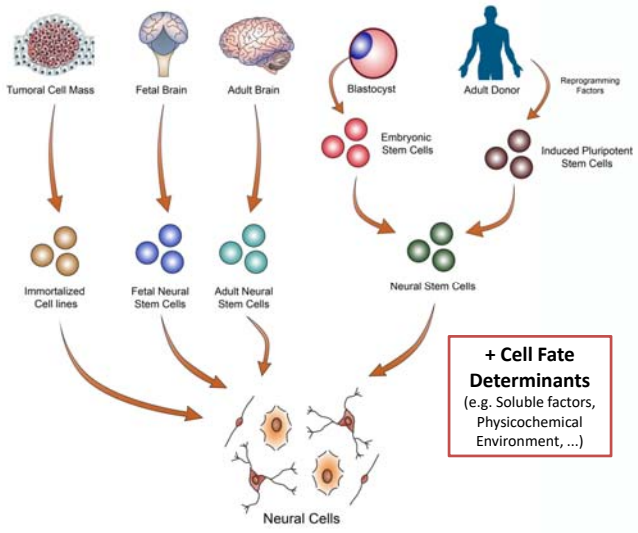
Neural Stem Cell Sources

- Human Embryonic Stem Cells (hESC)
- **Induced Pluripotent Stem Cells (iPSC)**
- Embryonal Carcinoma Stem Cells (hECS)
- Human fetal Neural Stem Cells (hNSC)

NEURAL STEM CELLS:

✓ Multipotent and proliferative

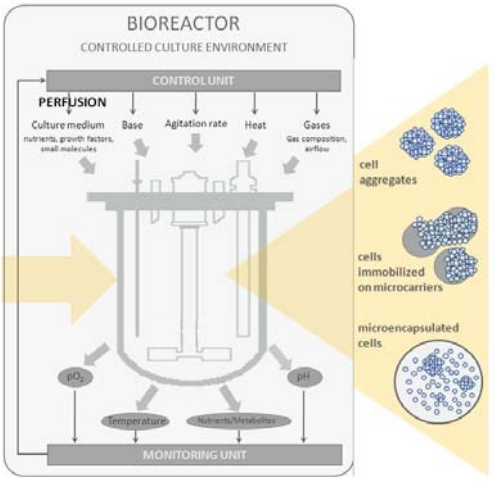
✓ Differentiation into neurons, astrocytes and oligodendrocytes



+ Cell Fate Determinants
(e.g. Soluble factors, Physicochemical Environment, ...)

36 Adapted from Jakel RJ et al (2004) *Nat Rev Genet* www.ibet.pt

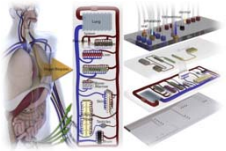
BIOREACTOR TECHNOLOGY – *STIRRED TANK BIOREACTORS*



Feeding high-throughput systems:

- High content screening
- Functional assays
- Toxicological assays
- Drug screening & drug-drug interactions

Feeding multi-organ chips:



- **Dynamic, homogeneous environment**
- **Tight control of culture parameters**

37 www.ibet.pt

HUMAN 3D MODELS @ iBET – STRATEGY

Strategy

Neural Stem Cell Sources

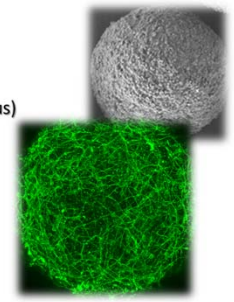
- Human Embryonic Stem Cells (hESC)
- **Induced Pluripotent Stem Cells (iPSC)**
- Embryonal Carcinoma Stem Cells (hECS)
- Human fetal Neural Stem Cells (hNSC)

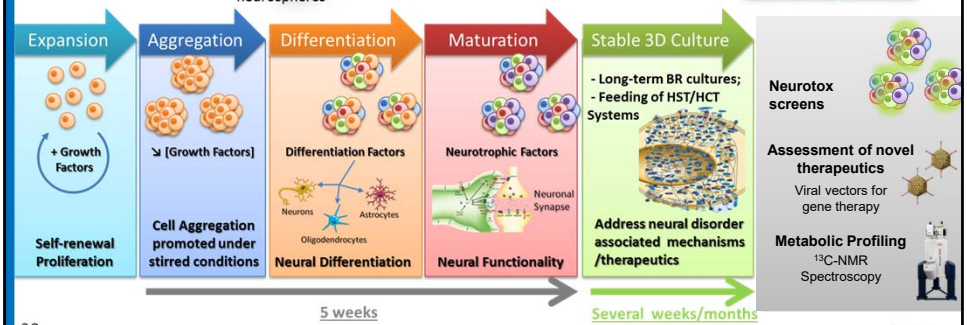
Controlled Stirred Systems:

Systems: DasGip, BIOSTAT Qplus (Sartorius)

- **Working volumes:** 100-500 mL
- **Low Oxygen Tensions:** pO_2=15-25% (Air Saturation)

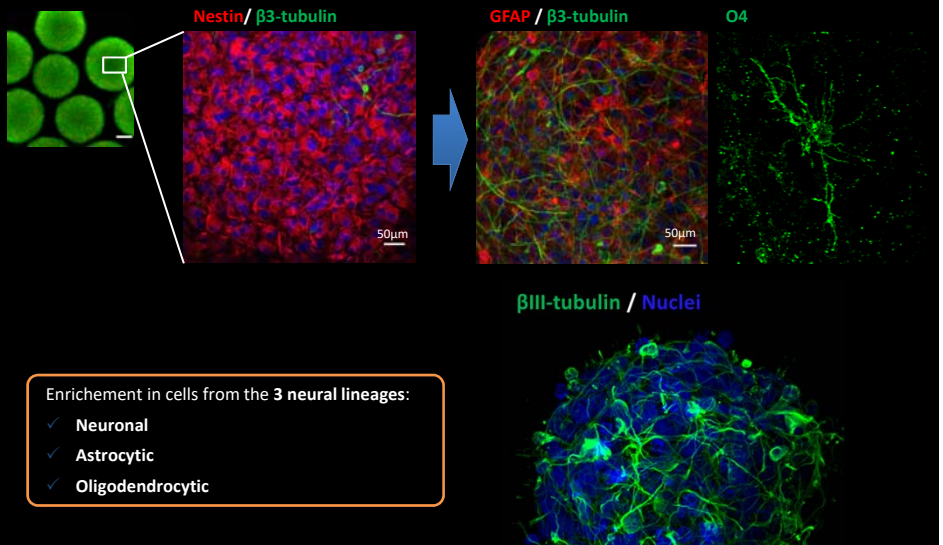
neurospheres





38 www.ibet.pt

NEURONAL AND GLIAL DIFFERENTIATION IN 3D



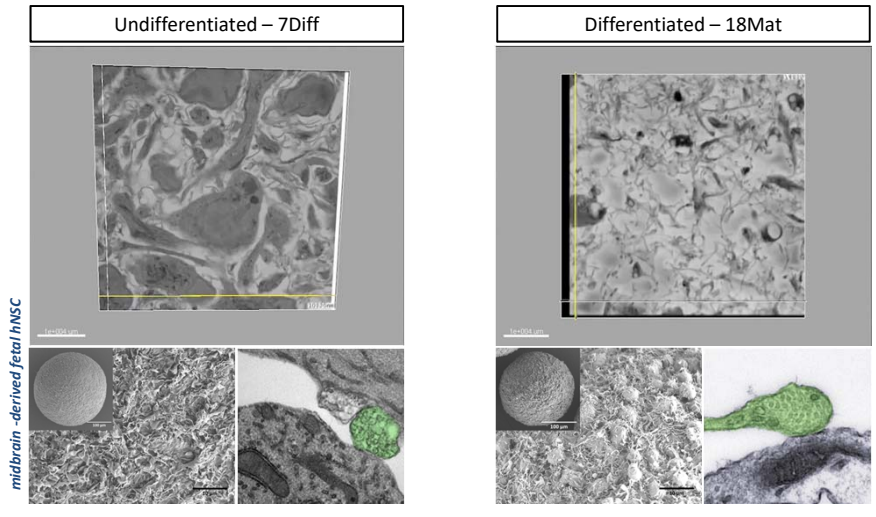
Enrichement in cells from the **3 neural lineages**:

- ✓ **Neuronal**
- ✓ **Astrocytic**
- ✓ **Oligodendrocytic**

Brito *et al. (2012) Methods*
Simão & Pinto *et al. (2015), Tissue Eng. Part A*
Terrasso *et al.* (2015) *J. Biotech*
Simão *et al.* (2016) *Bioreactors in Stem Cell Biology*

39 www.ibet.pt

NEURAL DIFFERENTIATION IN 3D



- ✓ **Absence of necrotic centers**
- ✓ **Complex neurite network** in differentiated neurospheres
- ✓ Presence of mature synaptic sites

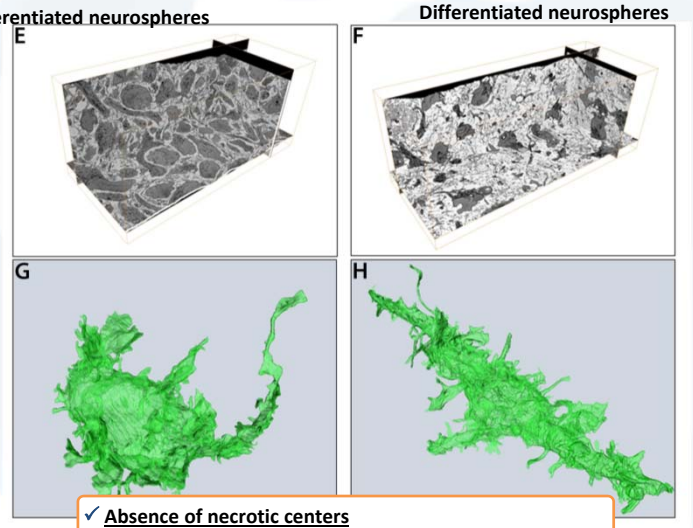
40 Simão & Pinto *et al. (2015), Tissue Eng. Part A* www.ibet.pt

Ultrastructural characterization

Serial block-face SEM

Undifferentiated neurospheres

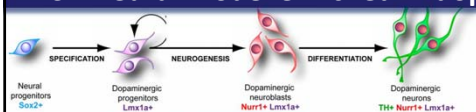
Differentiated neurospheres



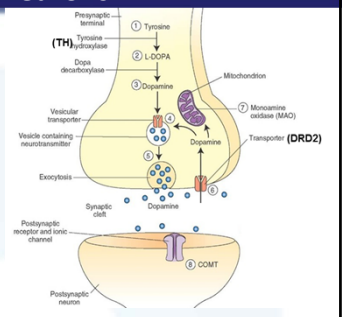
✓ **Absence of necrotic centers**

✓ **Complex neurite network** in differentiated neurospheres

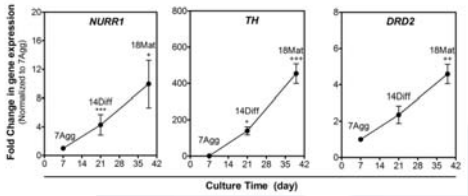
3D neural model enriched in dopaminergic neurons



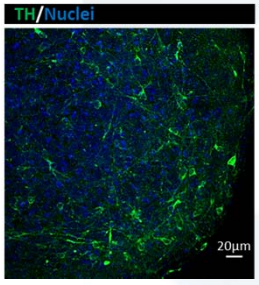
Emma R. Andersson et al. PNAS 2013;110:2450-2451



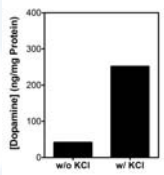
mRNA



Protein



Neurotransmitter Synthesis/Release

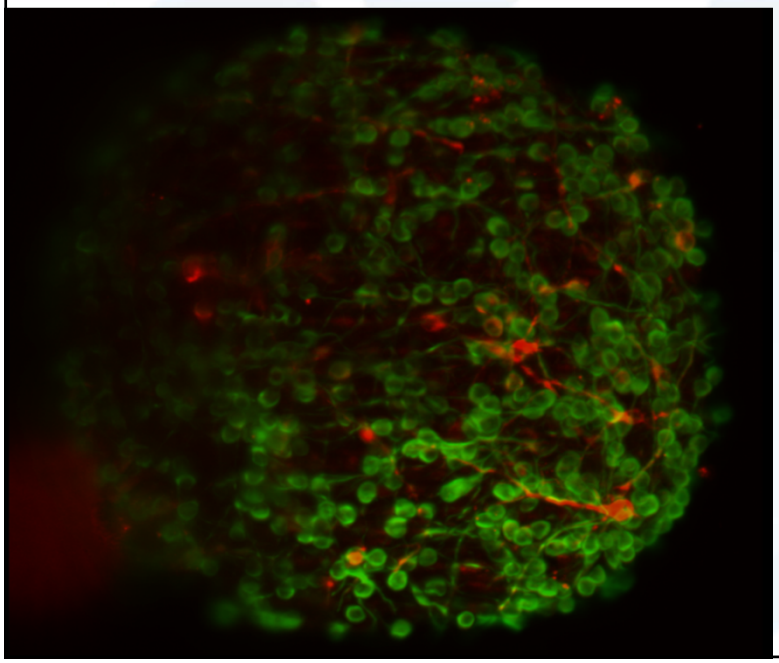


✓ Differentiated neurospheres enriched in **DOPAminergic neuronal markers**

Simão & Pinto *et al.*, (2015) *Tissue Eng– Part A*, 21(3-4):654-68

NURR1: Nuclear receptor related 1; **TH:** Tyrosine hydroxylase; **DRD2:** Dopamine receptor D2

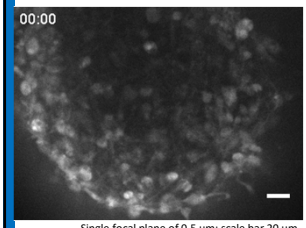
Neuronal dopaminergic differentiation in 3D



43

NEURAL DIFFERENTIATION IN 3D

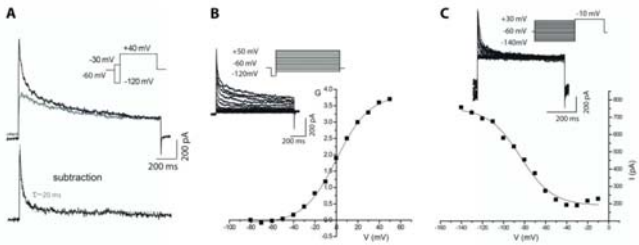
Ca^{2+} Signaling



Single focal plane of 0.5 µm; scale bar 20 µm

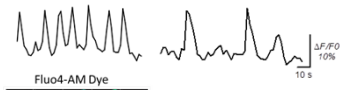
↑ [Ca^{2+}] → ↑ Fluorescence

Electrophysiological Properties

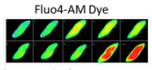


Neuronal-like firing

Glia-like firing



Fluo4-AM Dye



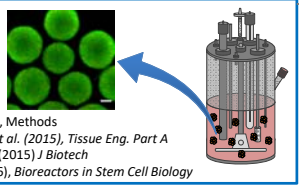
↑ [Ca^{2+}] → ↑ Fluorescence

- ✓ Detection of spontaneous Ca^{2+} firing patterns typical of neurons and astrocytes
- ✓ Presence of voltage-activated ionic currents

Simão & Pinto *et al. (2015), Tissue Eng. Part A*
Gualda E *et al. (2014) Front Cell Neurosci*

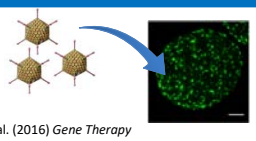
44 www.ibet.pt

Human 3D neural models



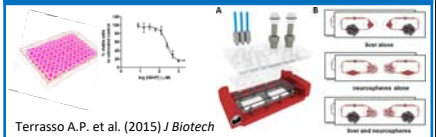
Brito C *et al.* (2012), Methods
Simão D &Pinto C *et al. (2015), Tissue Eng. Part A*
Terrasso A.P. *et al.* (2015) *J Biotech*
Simão D *et al.* (2016), *Bioreactors in Stem Cell Biology*

Preclinical evaluation of gene therapy vectors



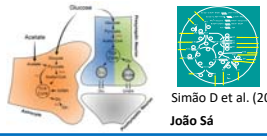
Simão D et al. (2016) *Gene Therapy*

Neurotoxicity/neuroprotection assessment



Terrasso A.P. et al. (2015) *J Biotech*
Terrasso A.P. et al. (2016) J Pharmacol Toxicol Methods
Materne et al. (2015) J Biotech

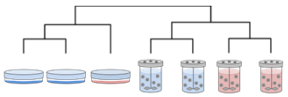
Neuron-astrocyte metabolic interactions



Simão D et al. (2016) *Scientific Reports*

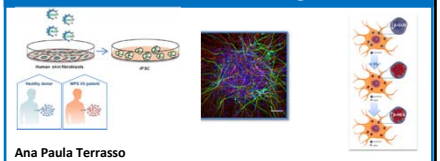
João Sá

Neural microenvironment remodeling



Daniel Simão

Disease Modeling



Ana Paula Terrasso

45 www.ibet.pt

PRECLINICAL EVALUATION OF VIRAL VECTOR FOR GENE THERAPY

Canine Adenovirus type-2 (hdCAV-2)

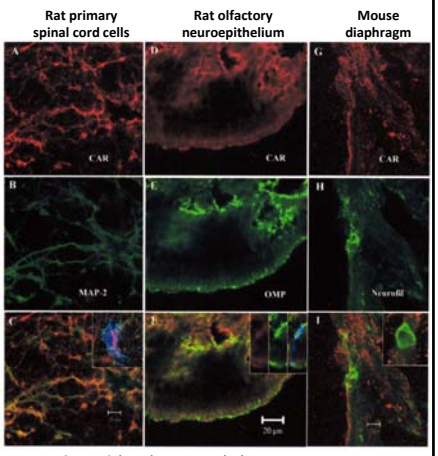
- ✓ **Low immunogenicity**

(negligible levels of neutralizing anti-CAV-2 antibodies in healthy humans)

In animal models:

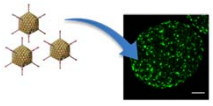
- ✓ **Long-term transgene expression**
- ✓ **Neuronal tropism**
- ✓ **Long-distance targeting via axonal transport**

The molecular basis for CAV-2 neuronal tropism likely relies on the exclusive binding to CAR receptor for internalization



Soudais et al. (2001) FASEB J. 15(12), 2283-5

Human midbrain 3D neural model

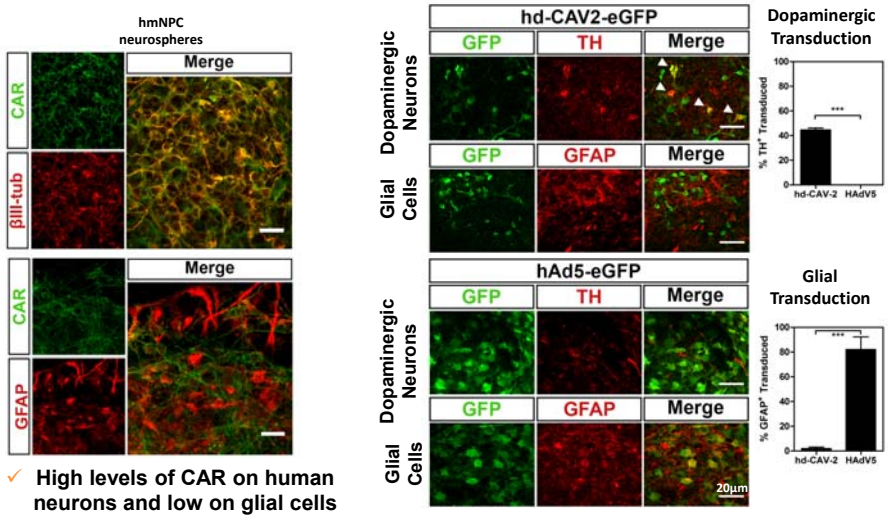


- **Vector Toxicity**
- **Vector Tropism**
- **Long-term transgene expression**

46 www.ibet.pt

PRECLINICAL EVALUATION OF VIRAL VECTOR FOR GENE THERAPY

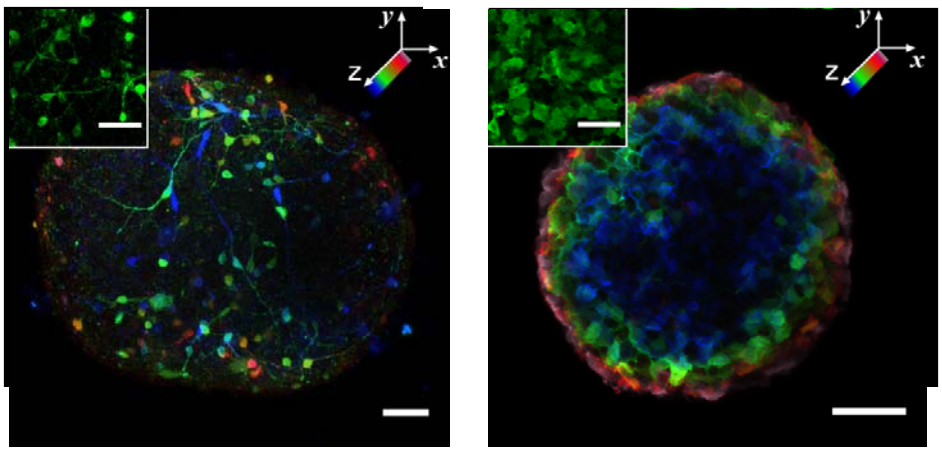
Tropism of Canine Adenovirus type-2 (hdCAV-2)



- ✓ High levels of CAR on human neurons and low on glial cells
- ✓ High transduction of dopaminergic neurons (TH^+)
- ✓ Low tropism towards glia and progenitors

 Simão et al. (2016) Gene Therapy www.ibet.pt

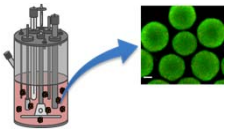
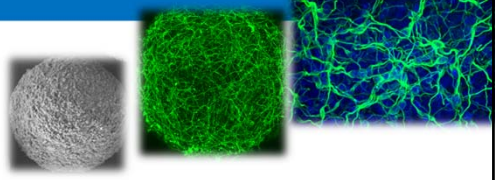
PRECLINICAL EVALUATION OF VIRAL VECTOR FOR GENE THERAPY



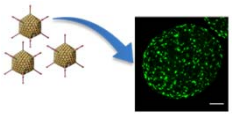
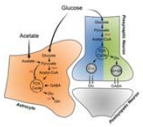
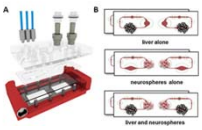
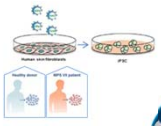
- ✓ hd-CAV transduced cells present neuronal-like morphology while hAd5 present glia-like morphology
- ✓ Presence of hd-CAV transduced cells in inner layers of neurospheres

 Simão et al. (2016) Gene Therapy www.ibet.pt

CONCLUDING REMARKS

- Homogeneous & reproducible differentiation of hNPC as 3D neurospheres in perfusion stirred-tank bioreactors
- Differentiation into neurons, astrocytes, oligodendrocytes
- Neuronal and glial maturation and synaptic functionality
- Remodeling of ECM and cell surface signatures towards higher recapitulation of neural microenvironment
- Applications in preclinical drug discovery, toxicology and disease modeling

49 www.ibet.pt

iBET ITQB

ANIMAL CELL TECHNOLOGY UNIT
Advanced Cell Models Lab

Daniel Simão
Ana Paula Terrasso
Catarina Pinto
Francisca Arez
Teresa Mendes
Marta Estrada
Tatiana Martins
Sofia Abreu
Sofia Rebelo
Vítor Espírito Santo

Cellular Bioprocesses Lab

Paula M. Alves
Patrícia Alves
Ana Teixeira
Margarida Serra
Marcos Sousa
João Sá

Goettingen Medical University, Germany
Nuno Raimundo

IGMM, France
Eric J. Kremer
Sara Salinas
Neus Bayó-Puxan

Cancer Research UK, UK
Lucy Collinson
Anne Weston

Università di Roma, Italy
Isabella Saggio
Stefania Piersanti

UCL, UK
Giampietro Schiavo

NOVA Medical School, Portugal
Pedro A. Lima
André Bastos

IGC, Portugal
Emílio Gualda
Nuno Moreno

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR
PTDC/EBB-BIO/112786/2009
PTDC/EBB-BIO/119243/2010

European Commission
BrainCAV (FP7-222992)
BrainVectors (FP7-286071)

iNOVA4Health


iBET
ITQB