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iPSC-derived neurospheroids recapitulate development and pathological signatures of human brain microenvironment

Catarina Brito

iBET - Instituto de Biologia Experimental e Tecnológica, Portugal, anabrito@ibet.pt

Ana Paula Terrasso

iBET - Instituto de Biologia Experimental e Tecnológica, Portugal

Daniel Simão

iBET - Instituto de Biologia Experimental e Tecnológica, Portugal

Francisca Arez

iBET - Instituto de Biologia Experimental e Tecnológica, Portugal

Marta M. Silva

iBET - Instituto de Biologia Experimental e Tecnológica, Portugal

See next page for additional authors

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<http://dc.engconfintl.org/ccexvi/228>

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Authors

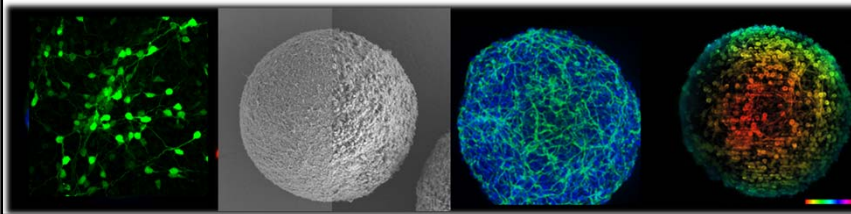
Catarina Brito, Ana Paula Terrasso, Daniel Simão, Francisca Arez, Marta M. Silva, Patrícia Gomes-Alves, Paula M. Alves, Marcos F. Sousa, Nuno Raimundo, Neus Bayó-Puxan, Sophie Creysells, and Eric J. Kremer

Human iPSC-derived neurospheroids: recapitulating developmental and pathological signatures of human brain microenvironment

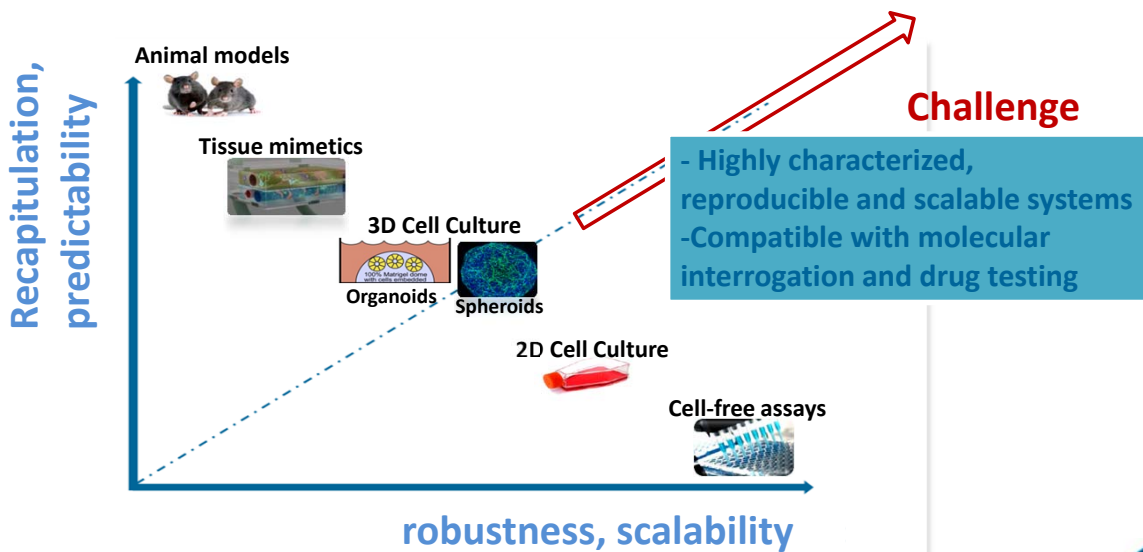
Catarina Brito

anabrito@ibet.pt

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THE DEMAND FOR PREDICTABLE MODELS



2

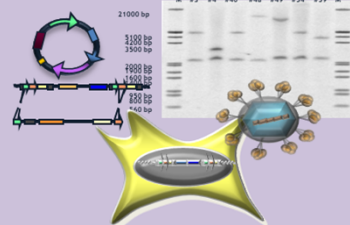
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THE ANIMAL CELL TECHNOLOGY UNIT

Biopharmaceuticals &

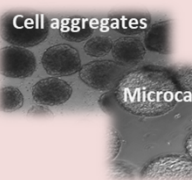
Human Cells & Models

MOLECULAR BIOLOGY

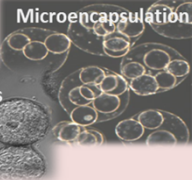


- Expression vector design
- Transient and stable expression
- Viral vector design/production
- Cell line development

CULTURE APPROACHES



Microcarriers




Microencapsulation

Cell aggregates

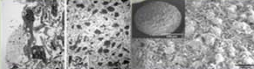
- Microcarriers/scaffolds;
- Cell aggregates;
- Microencapsulation (inert biocompatible matrices or defined ECM components);
- Monocultures and co-cultivation strategies

CULTURE SYSTEMS ENVIRONMENTALLY CONTROLLED BIOREACTORS

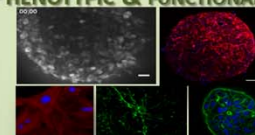


- for cell expansion and differentiation;
- for improved functionality, productivity and quality of the complex biopharmaceuticals.


ULTRASTRUCTURE



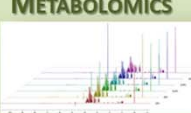
CELLULAR CHARACTERIZATION PHENOTYPIC & FUNCTIONAL




TRANSCRIPTOMICS



METABOLOMICS

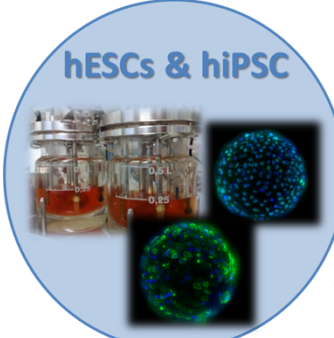


PROTEOMICS



BIOPROCESSING OF HUMAN PLURIPOTENT STEM CELLS PRODUCTS @ IBET

EXPANSION

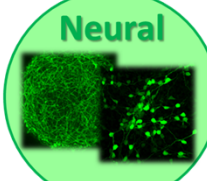


hESCs & hiPSC

Serra et al 2010, J Biotech;
 Serra/Correia et al 2011, PLoS ONE;
 Silva et al 2015, Stem Cells Transl Med;
 Abecasis et al 2017, J Biotech
 Cunha et al 2017, J Membrane Science

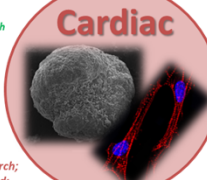
DIFFERENTIATION & MATURATION

Neural



Simão D et al 2015, Tissue Eng Part A
 Terrasso AP et al 2015, J Biotechnol
 Simão D et al 2016, Gene Therapy
 Sá JV et al 2017, Neurochem Res.
 Simão D et al 2016, Sci Rep
 Terrasso AP et al 2017, J Pharm Toxicol Meth

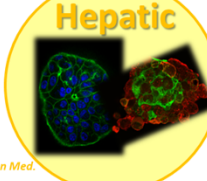
Cardiac



POSTER #140

Serra et al 2012, Trends in Biotech;
 Correia et al 2014, Stem Cell Rev Rep;
 Gomes-Alves et al 2014, Proteomics;
 Gomes-Alves et al, 2015, Transl Research;
 Correia et al 2016, Stem Cell Transl Med;
 Correia et al 2017, Sci Rep.

Hepatic



Tostões RM et al 2011, Biotech Bioeng
 Leite SB et al 2011, Toxicol In Vitro
 Tostões RM 2012, Hepatology
 Leite SB et al 2012, Toxicol Sci.
 Rebelo SP et al 2015, Arch Toxicol.
 Rebelo SP et al 2017, J Tissue Eng Regen Med.

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WORKING HYPOTHESIS

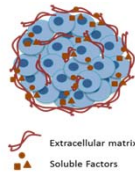
1. Can microenvironmental recapitulation be attained via accumulation of endogenous biomolecules...?

(i.e., without addition of exogenous bioactive components)

2. ...to (i) reflect the native tissue context & (ii) mimic disease in vitro?

Requirements:

- Relevant **cell types** of the specific cell microenvironment
- Physiologically relevant **cell interactions**
- **Physico-chemical**: oscillations in pH, pO₂
- 4th dimension: **time**

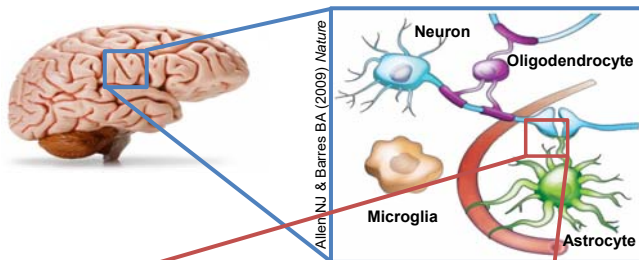


Built-up & remodeling of endogenous ECM & soluble factors along culture time

5

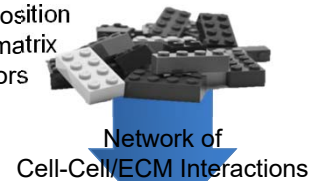
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CNS MICROENVIRONMENT

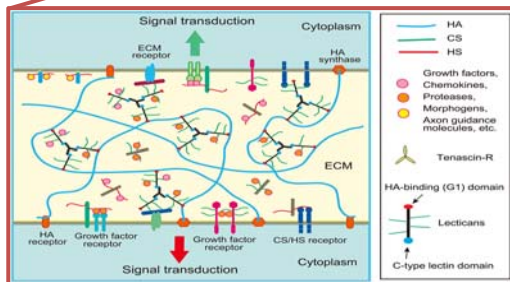


Highly complex system:

- Architecture
- Cellular composition
- Extracellular matrix
- Secreted factors



Tissue Function



Maeda N (2015) Front Neurosci

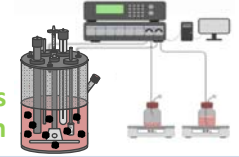
6

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INTEGRATED BIOPROCESS DEVELOPMENT

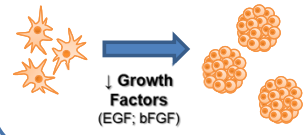
Human Neural Stem Cells:
AGGREGATION, NEURAL DIFFERENTIATION & MATURATION, as 3D neurospheroids
Robust Scalable Integrated Efficient Reproducible

Stirred-tank Bioreactors
in perfusion



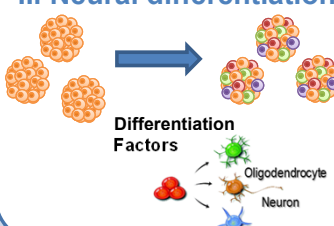
I. Aggregation

Cell Source: iPSC-derived human Neural Stem Cells



↓ Growth Factors
(EGF; bFGF)

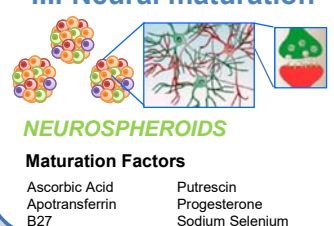
II. Neural differentiation



Differentiation Factors

Oligodendrocyte
Neuron
Astrocyte

III. Neural maturation



NEUROSPHEROIDS

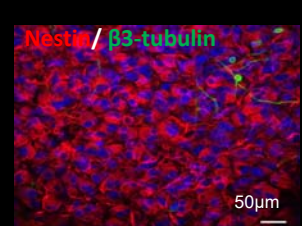
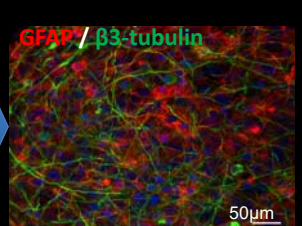
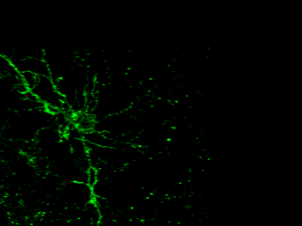
Maturation Factors

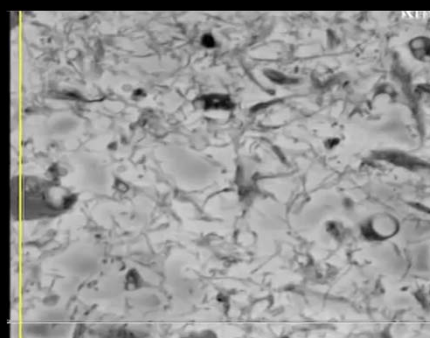
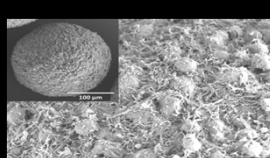
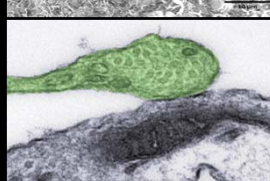
Ascorbic Acid Apotransferrin B27	Putrescin Progesterone Sodium Selenium
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In process analytics for characterization of cell quality attributes

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www.ibet.pt
Simão et al. (2016) Bioreactors in Stem Cell Biology

NEURONAL AND GLIAL DIFFERENTIATION IN 3D NEUROSPHEROIDS

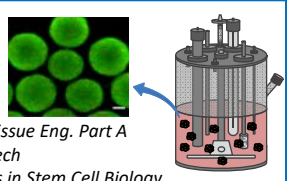




- ✓ Neurons, astrocytes & oligodendrocytes
- ✓ Complex neurite network
- ✓ Mature synaptic sites
- ✓ Absence of necrotic centers

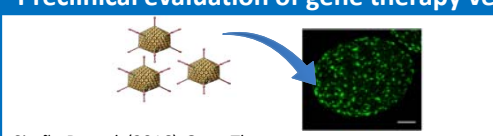
8
www.ibet.pt
Simão & Pinto et al. (2015), Tissue Eng. Part A

human Neurospheroids



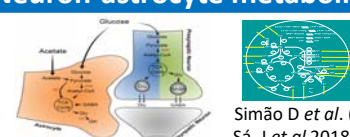
Brito C *et al.* (2012) *Methods*
 Simão D & Pinto C *et al.* (2015) *Tissue Eng. Part A*
 Terrasso A.P. *et al.* (2015) *J Biotech*
 Simão D *et al.* (2016) *Bioreactors in Stem Cell Biology*

Preclinical evaluation of gene therapy vectors



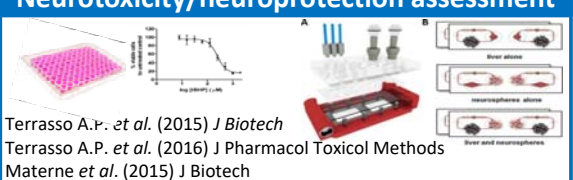
Simão D *et al.* (2016) *Gene Therapy*

Neuron-astrocyte metabolic interactions



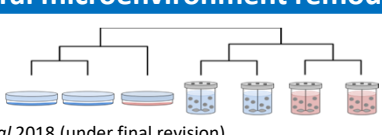
Simão D *et al.* (2016) *Scientific Reports*
 Sá J *et al.* 2018 (in preparation)

Neurotoxicity/neuroprotection assessment



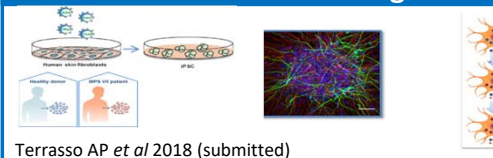
Terrasso A.P. *et al.* (2015) *J Biotech*
 Terrasso A.P. *et al.* (2016) *J Pharmacol Toxicol Methods*
 Materne *et al.* (2015) *J Biotech*

Neural microenvironment remodeling



Simão *et al.* 2018 (under final revision)

Disease Modeling

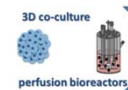


Terrasso AP *et al.* 2018 (submitted)


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NEURAL MICROENVIRONMENT REMODELING

(i) Can microenvironmental recapitulation be attained via accumulation of endogenous biomolecules, reflecting the native tissue context ?



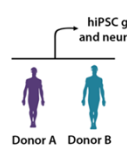
3D co-culture
perfusion bioreactors



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

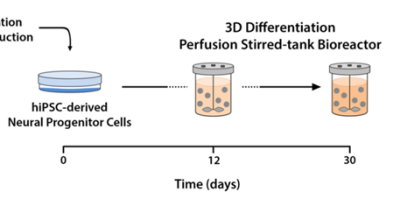
Specific questions:

- Can endogenous ECM be accumulated during neurospheroid differentiation?
- Is neurospheroid ECM composition similar to brain ECM?
- What is the impact of the 3D culture system on ECM secretion/accumulation?



hiPSC generation and neural induction

Donor A Donor B



3D Differentiation
Perfusion Stirred-tank Bioreactor

hiPSC-derived Neural Progenitor Cells

0 12 30
Time (days)

RNA Extraction


↓

NTGCCAATCAAGG
GTTGGCCGACAGC
TTTGGAGAGAGCTT
TSMATGAAAGAGC
ACTATCTGATGTC

NGS Analysis

Protein Extraction/Digestion

↓



SWATH-MS Analysis

DATA ANALYSIS

↓

Available Datasets

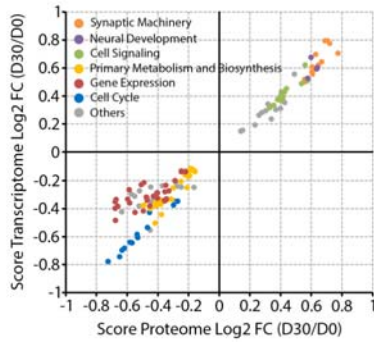
10 www.ibet.pt

Simão et al. (2018), under final revision

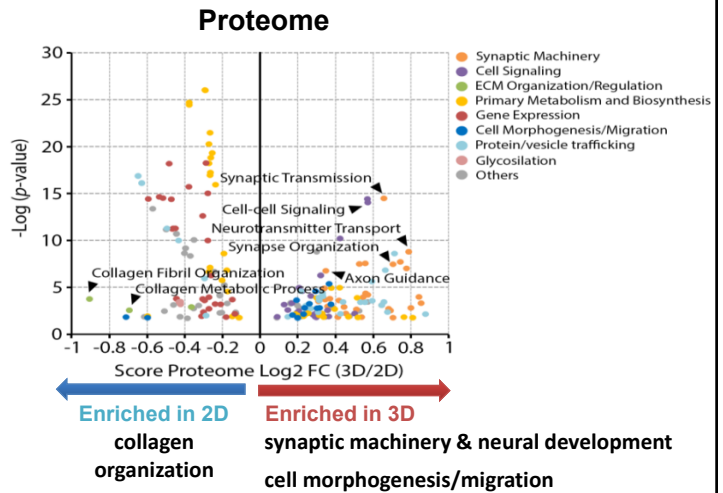
ECM AND PLASMA MEMBRANE REMODELING IN NEUROSPHEROIDS

PATHWAY MODULATION: GENE ONTOLOGY – BIOLOGICAL PROCESSES

Transcriptome-Proteome Correlation



- ✓ High correlation transcriptome/ proteome
- ✓ Enrichment in genes/proteins related with synaptic machinery and neural development



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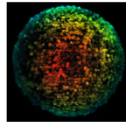
www.ibet.pt

Simão et al. (2018), under final revision

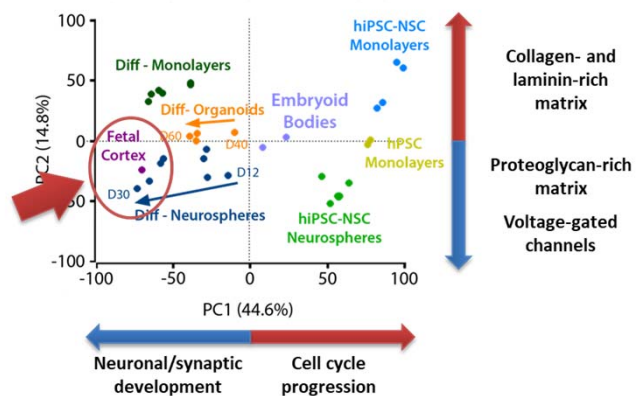


IMPACT OF THE 3D CULTURE SYSTEM ON THE MICROENVIRONMENT

Neurospheroids



Transcriptome Comparative Analysis (Principal Component Analysis)



- ✓ Clear separation of non-differentiated and differentiated samples
- ✓ Neurospheres closer to fetal cortex

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Simão et al. (2018), under final revision



CASE STUDY: MUCOPOLYSACCHARIDOSIS TYPE VII - MPS VII

(ii) Can microenvironmental recapitulation contribute to mimic disease in vitro?

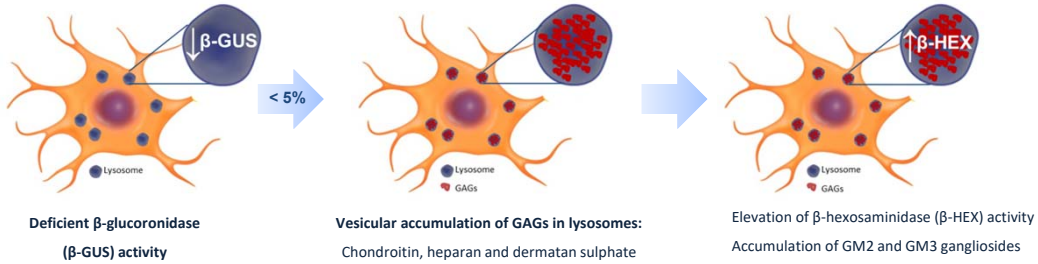
Neuropathological hallmarks

- Lysosomal storage disease
- Rare metabolic disorders (1 in 250 000)
- Recessive mutation on *GUSB* gene

Clinical hallmarks

- ✓ Damage in several organs
- ✓ Neurological defects

➔ Impaired cognition



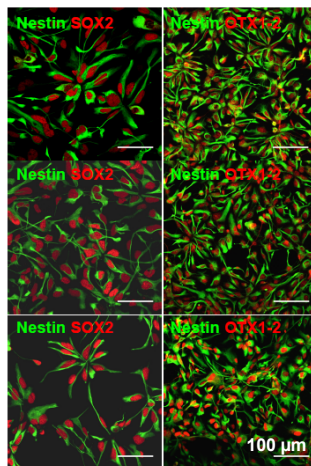
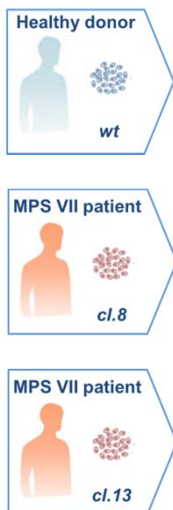
Link between lysosomal defects and progressive neurologic dysfunction not clear

Identification of novel therapeutical targets/approaches

13

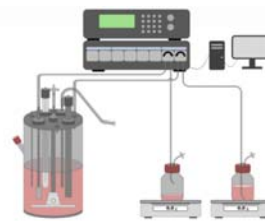
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MODELING MPSVII – NEUROSPHEROID STRATEGY



Data from Eric J Kremer laboratory

Perfusion Stirred-tank Bioreactors



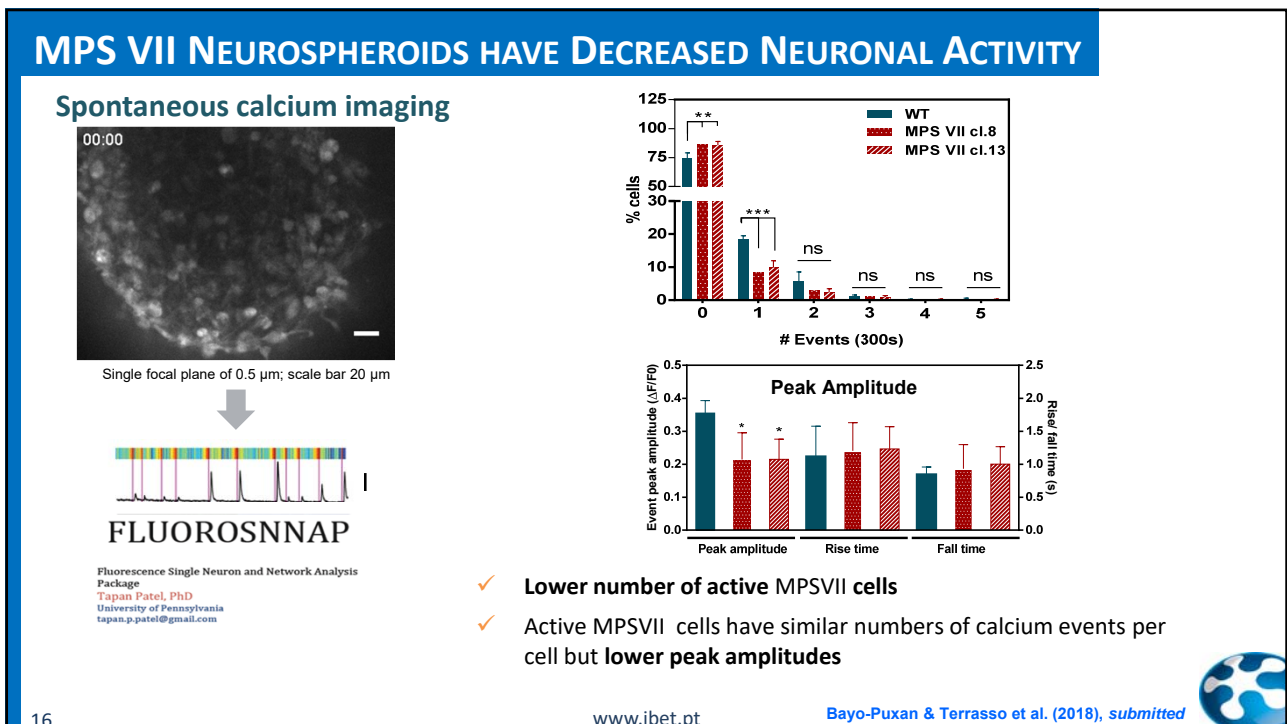
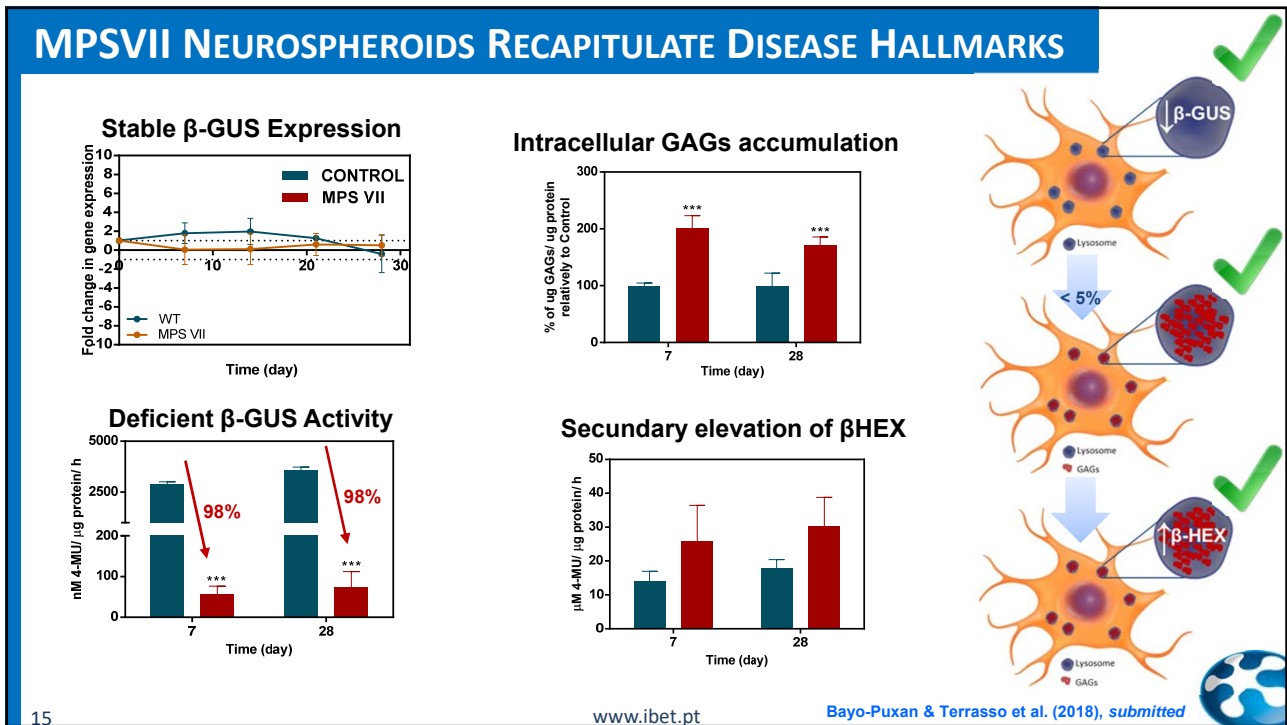
Simão D. (2016) in *Bioreactors in Stem Cell Biology*

MPS VII iPSC-NSC express typical neural progenitors markers (Nestin, SOX2, OTX1-2)

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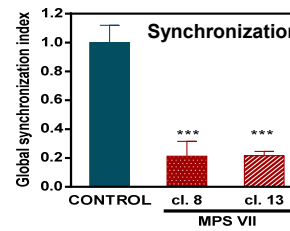
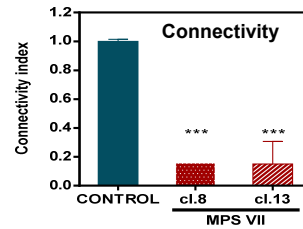
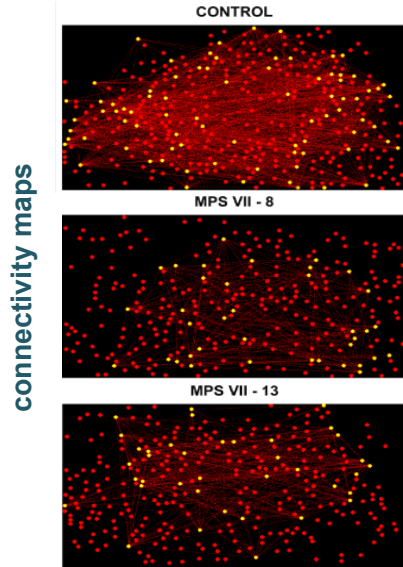
www.ibet.pt

Bayo-Puxan & Terraso et al. (2018), submitted



MPS VII NEURONAL NETWORKS HAVE CONNECTIVITY DEFECTS

Spontaneous calcium imaging



✓ Lower connectivity and synchronization of MPS VII neurons

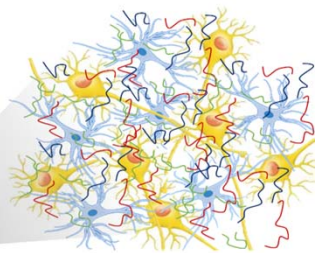
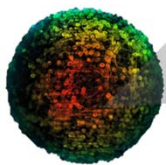
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www.ibet.pt

Bayo-Puxan & Terrasso et al. (2018), *submitted*



CONCLUDING REMARKS



Modeling neural microenvironment features in neurospheroids:

- ✓ neuronal and glial differentiation of hiPSC-NSC in perfusion stirred-tank bioreactors
- ✓ Enrichment in synaptic machinery proteins
- ✓ Higher expression of neural proteoglycans and cell adhesion molecules

Recapitulation of disease pathological hallmarks in neurospheroids:

- Molecular hallmarks of MPS VII
- Neurological defects of MPSVII

Further understand the correlation between disease-induced molecular alterations and neurological defects



Identification of novel therapeutic targets & Preclinical testing of novel therapeutic approaches

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ACKNOWLEDGMENTS

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Manuel Carrondo, PhD
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Dr Nuno Raimundo
University of Cologne

Dr Tomo Saric

Mass Spec facility UniMS

Dr Ricardo Gomes

Funding



iNOVA4Health



UID/Multi/04462/2013

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A large, abstract graphic on the left side of the slide, consisting of several overlapping, rounded shapes in various shades of blue, creating a dynamic, organic form.

Thank you

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Catarina Brito
anabrito@ibet.pt