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

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

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THE DEMAND FOR PREDICTABLE MODELS

- Highly characterized, reproducible and scalable systems
- Compatible with molecular interrogation and drug testing

Animal models
Tissue mimetics
3D Cell Culture
Organoids
Spheroids
2D Cell Culture
Cell-free assays

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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/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.

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THE ANIMAL CELL TECHNOLOGY UNIT

Ultrasound

Cell characterization
- Phenotypic & functional
- Transcriptomics
- Metabolomics

BIOPROCESSING OF HUMAN PLURIPOTENT STEM CELLS PRODUCTS @ iBET

EXPANSION
hESCs & hiPSC

DIFFERENTIATION & MATURATION
Neural
Cardiac
Hepatic

POSTER #140

Queiroz-Aires et al 2016, Protocols;
Queiroz-Aires et al 2015, Tissue Eng Mech;
Correia et al 2015, Stem Cell Transl Med;

Queiroz-Aires et al 2016, Protocols;
Queiroz-Aires et al 2015, Tissue Eng Mech;
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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

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**CNS MICROENVIRONMENT**

Highly complex system:
- Architecture
- Cellular composition
- Extracellular matrix
- Secreted factors

Network of Cell-Cell/ECM Interactions

Tissue Function

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Maeda N (2015) Front Neurosci
INTEGRATED BIOPROCESS DEVELOPMENT

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

I. Aggregation
Cell Source: iPSC-derived human Neural Stem Cells

II. Neural differentiation
Differentiation Factors

III. Neural maturation
Maturation Factors
NEUROSPHEROIDS

In process analytics for characterization of cell quality attributes

NEURONAL AND GLIAL DIFFERENTIATION IN 3D NEUROSPHEROIDS

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

Cell Source: iPSC-derived human Neural Stem Cells

Simão et al. (2016) Bioreactors in Stem Cell Biology
Simão & Pinto et al. (2015), Tissue Eng. Part A
Preclinical evaluation of gene therapy vectors

Neurotoxicity/neuroprotection assessment

Disease Modeling

**NEURAL MICROENVIRONMENT REMODELING**

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

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?
Transcriptome-Proteome Correlation

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

Enriched in 2D:
- Collagen organization

Enriched in 3D:
- Synaptic machinery & neural development
- Cell morphogenesis/migration

Impact of the 3D Culture System on the Microenvironment

- Clear separation of non-differentiated and differentiated samples
- Neurospheres closer to fetal cortex
**CASE STUDY: MUCOPOLYSACCHARIDOSIS TYPE VII - MPS VII**

(i) 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
- Deficient β-glucuronidase (β-GUS) activity

### Clinical hallmarks
- Damage in several organs
- Neurological defects
- Impaired cognition

**Vesicular accumulation of GAGs in lysosomes:**
Chondroitin, heparan and dermatan sulphate

**Secondary alterations:**
Elevation of β-hexosaminidase (β-HEX) activity
Accumulation of GM2 and GM3 gangliosides

Link between lysosomal defects and progressive neurologic dysfunction not clear

Identification of novel therapeutical targets/approaches

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**MODELING MPSVII – NEUROSPHEROID STRATEGY**

Healthy donor (wt)

*MPS VII patient cl.8*

*MPS VII patient cl.13*

Perfusion Stirred-tank Bioreactors

Data from Eric J Kremer laboratory

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

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

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

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http://www.itqb.unl.pt/research/technology/advanced-cell-models
http://tca.itqb.unl.pt
**MPSVII NEUROSPHEROIDS RECAPITULATE DISEASE HALLMARKS**

**Stable β-GUS Expression**
- Fold change in gene expression over time (days)
- Control vs. MPS VII

**Intracellular GAGs accumulation**
- % of GAGs/ug protein
- Time (day) 7 vs. 28

**Deficient β-GUS Activity**
- Enzyme activity (%)
- Time (day) 7 vs. 28

**Secondary elevation of βHEX**
- Enzyme activity
- Time (day) 7 vs. 28

**MPS VII NEUROSPHEROIDS HAVE DECREASED NEURONAL ACTIVITY**

**Spontaneous calcium imaging**
- Single focal plane of 0.5 µm; scale bar 20 µm

**Lower number of active MPSVII cells**
**Active MPSVII cells have similar numbers of calcium events per cell but lower peak amplitudes**

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

[Links]
- http://www.itqb.unl.pt/research/technology/advanced-cell-models
- http://tca.itqb.unl.pt
MPS VII NEURONAL NETWORKS HAVE CONNECTIVITY DEFECTS

Spontaneous calcium imaging

- Lower connectivity and synchronization of MPS VII neurons

Synchronization

Connectivity

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
Thank you

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http://www.itqb.unl.pt/research/technology/advanced-cell-models
http://tca.itqb.unl.pt