Induced Pluripotent Cell Culture Using Single-Use Vertical-Wheel Bioreactors Under Xeno-Free Conditions

Carlos A.V. Rodrigues[1], Teresa P. Silva[1], Tiago G. Fernandes[1], Yas Hashimura[2], Robin Wesselschmidt[2], Maria Margarida Diogo[1], Brian Lee[2] and Joaquim M.S. Cabral[1]

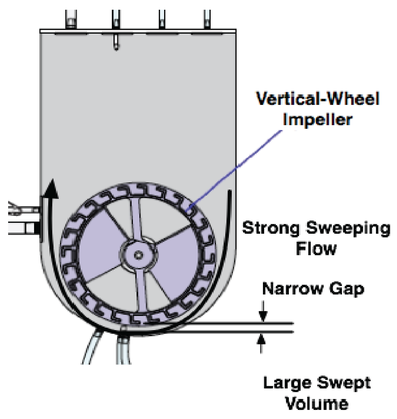
[1] Department of Bioengineering and Institute for Bioengineering and Biosciences; Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

[2] PBS Biotech, CA, USA

E-mail: carlos.rodrigues@tecnico.ulisboa.pt

Introduction

- Induced Pluripotent Stem Cells (iPSC) are able to self-renew indefinitely and have the remarkable ability to differentiate into virtually all cell types of the human body
- iPSC technology holds great potential for the development of regenerative medicine therapies and also for drug discovery.
- A significant constraint for the success of these approaches is the difficulty to generate large quantities of cells, maintaining biological functionality and safety
- The novel single-use Vertical-Wheel bioreactors (PBS Biotech) are designed with vertical mixing orientation, allowing homogenous and gentle particle suspension and higher mass transfer rate, under lower shear forces.
- The work here presented consists in the culture of human iPSC under xenogeneic product-free conditions using Vertical-Wheel bioreactors

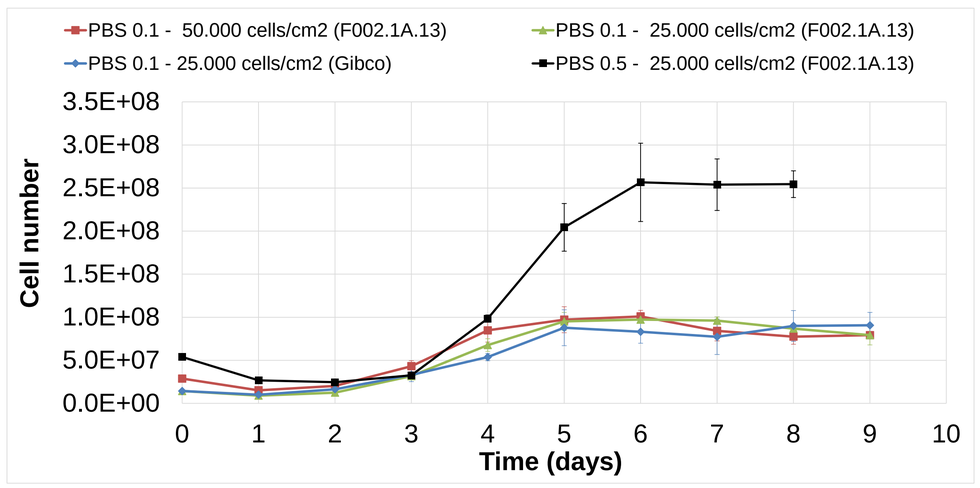


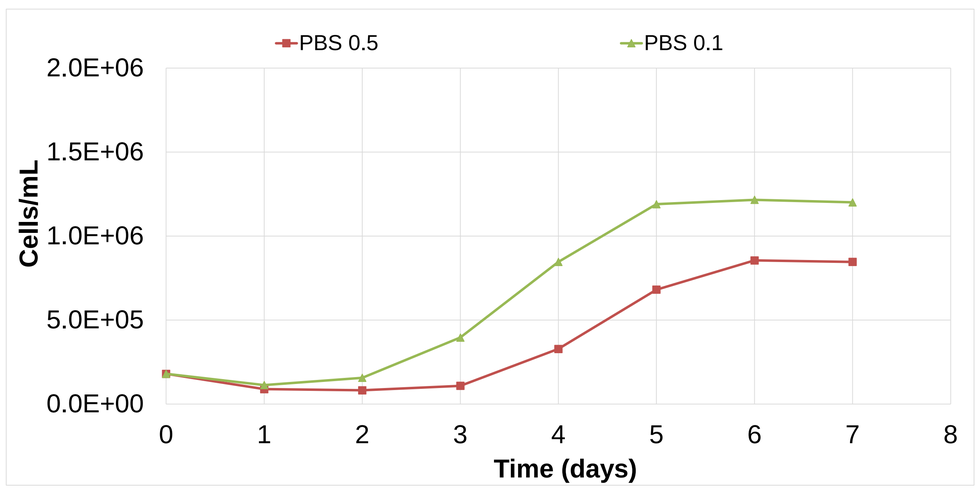
Materials and Methods

- The human iPSC line F002.1A.13 (TCLab, Portugal) was used as cell model
- Cells were cultured using the Vertical-Wheel bioreactors PBS 0.1 (80mL working volume) and PBS 0.5 (300mL working volume), using a maximum 26 RPM agitation speed
- Xeno-free culture conditions were used, including culture medium (Essential 8, Thermo Scientific) and microcarriers (Plastic, Pall Corporation), coated with a recombinant extracellular matrix protein (VTN-N, Thermo Scientific)
- The samples were analyzed in an automatic analyzer (YSI7100MBS, Yellow Springs Instrument) to determine the concentration of nutrients (glucose, glutamine) and metabolites (lactate and ammonia)
- To characterize the cells cultured in the Vertical-Wheel Bioreactor, cells were analyzed by immunocytochemistry (on the microcarriers and after replating in culture plates) and by flow cytometry with pluripotency markers (including Oct4, Sox2, Nanog, SSEA4)

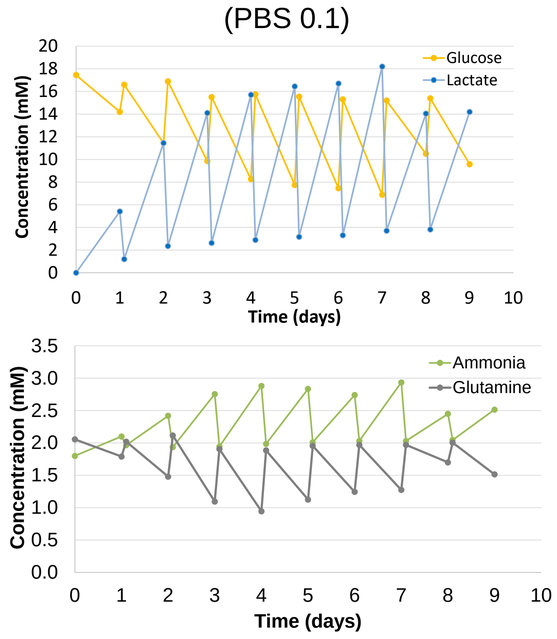
Results and Discussion

Growth Kinetics of hiPSC cultured on microcarriers using Vertical-Wheel Bioreactors

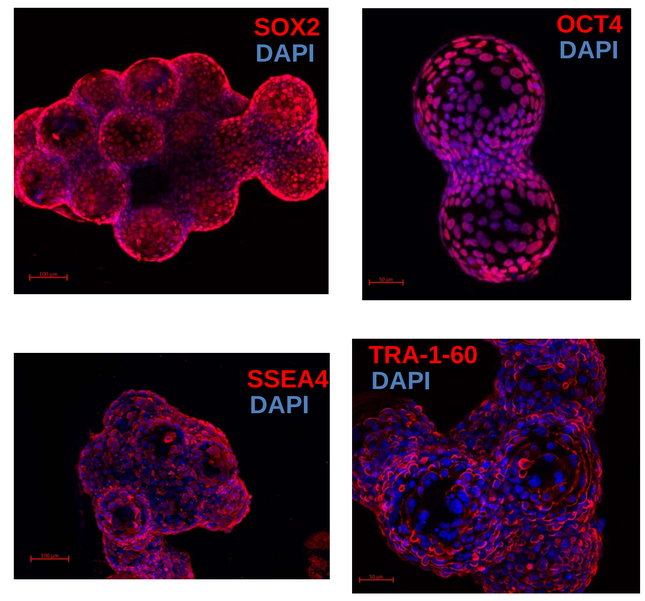




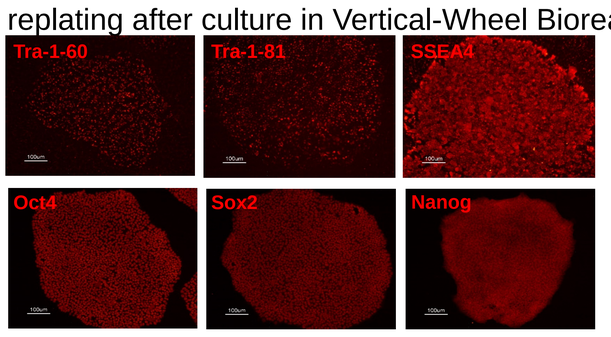
Nutrient consumption/metabolite production of hiPSC cultured in Vertical-Wheel bioreactors (PBS 0.1)



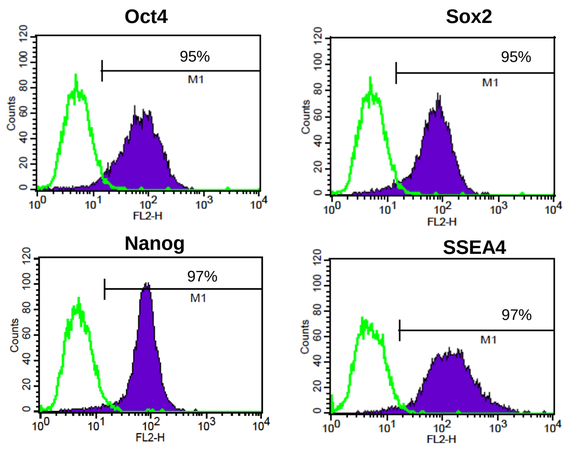
Pluripotency marker expression on cells attached to microcarriers



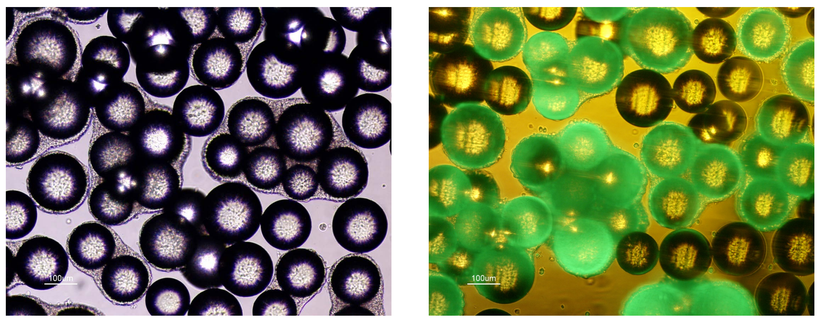
iPSC replating after culture in Vertical-Wheel Bioreactor



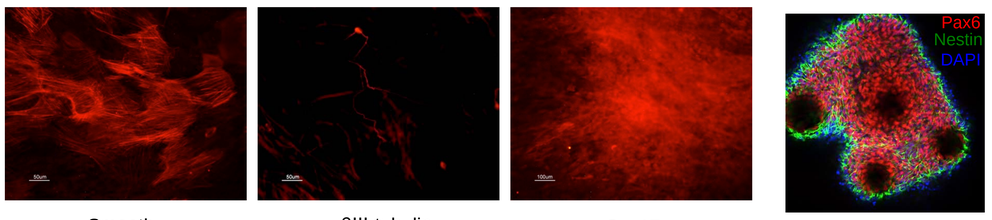
Flow Cytometry (day 9)



Day 6 (left: bright field; right: Calcein AM)



Differentiation potential after culture on Vertical-Wheel Bioreactor



Smooth Muscle Actin (mesoderm) | βIII-tubulin (ectoderm) | Sox17 (endoderm) | Neural Differentiation (on microcarriers)

Conclusions

- iPSC culture in Vertical-Wheel bioreactors led to a maximum of 7-fold increase in cell number after 6 days (PBS 0.1) and to the production of ≈2.6x10^8 cells (PBS 0.5)
- After culture in the PBS Mini bioreactors the iPSC maintained expression of pluripotency markers and differentiation into cells of the three germ layers
- Glucose or glutamine depletion was not observed but metabolite accumulation was observed to some extent (lactate: 18mM, ammonia 3mM) which may be detrimental for iPSC expansion
- The methodologies here developed, combining single-use bioreactors and chemically defined xeno-free reagents for iPSC production may constitute a basis for future regenerative medicine applications of hiPSC-derived cells

Acknowledgments

Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, INOVAÇÃO E DO ENSINO SUPERIOR
SFRH/BPD/82056/2011

Funding received by iBB-Institute for Bioengineering and Biosciences from FCT - Portuguese Foundation for Science and Technology (FCT) under the project UID/BIO/04565/2013 and from Programa Operacional Regional de Lisboa 2020 (Project N. 007317) is acknowledged