A novel bioreactor for the perfusion of electrospun fibre scaffold supporting 3D engineered tissue production – Application of human skeletal satellite muscle cells and human embryonic stem cells differentiated towards neural cells

H Hussein[1], M Leino[1], B Robb[2], J Thilo[3], M Huang[5], M Stich[4], E Lindmark[5], C Åstrand[1], Y Zhang[1], R McKean[2], P Stobbe[3], C Krause[4], A Shokri[1], V Chotteau[1]

[1]*Division of Industrial Biotechnology, Cell Technology Group, KTH, Stockholm, Sweden;* [2]*The Electrospinning Company Ltd, Oxford, United Kingdom;* [3]*Stobbe Tech A/S, Holte, Copenhagen, Denmark;* [4]*PreSens Precision Sensing GmbH, Regensburg, Germany;* [5]*3HBiomedical, Uppsala, Sweden.*

INTRODUCTION: Electrospinning fibre creates fibrous scaffolds of the size of the extra cellular matrix highly suitable for the 3D culture. To fully benefit of this advantage, the scaffold needs to be perfused by an orthogonal continuous medium flow in a bioreactor environment.

METHODS: 4 or 8 bioreactors equipped with polycaprolactone (PCL) fibre scaffolds (up to 3 mm thick) were cultured in parallel. The cells were inoculated in the scaffold, cultured overnight in incubator and then transferred in the bioreactor where the medium recirculation was operated. The glucose consumption and lactate accumulation were monitored during the perfusion culture as an indicator of cell growth. At the end of the culture, the following analyses were performed: DNA quantification after papaine digestion, scaffold staining by calceine to analyze the distribution of alive cells, immunocytochemistry and qPCR.

RESULTS: A new system including four small bioreactors was created to support the growth and differentiation of human stem cells in perfused 3D fibre scaffold, see Fig 1. This system was used in two different applications: the expansion of human skeletal muscle satellite cells (hSkMSC) and the expansion of human embryonic stem cells (hESC) as well as their neural differentiation. It enabled the orthogonal perfusion of scaffold of volumes in a range between 15 uL and 3 mL. pH and pO_2 were monitored by miniaturized single-use sensors.

Process optimization was carried out for the expansion of hSkMSC cultured in electrospun PCL fibre scaffolds in two bioreactor systems, i.e. eight bioreactors. Several factors were investigated: the scaffold surface treatment by plasma and/or coating, the scaffold porosity, the initial cell seeding density, and the recirculation rate, see Fig 1. It was observed that the higher porosity significantly increased the cell in-depth penetration into the scaffolds. A considerable influence of the seeding density on the cell proliferation was observed. After a 7-day culture period, the highest cell fold increase, i.e. 25 fold, was obtained in the scaffolds seeded with the lowest density. It was showed that human skeletal muscle satellite cells cultured in this system were not carcinogenic in a mouse model. This system was then used for the culture of hESC. The effect of the recirculation rate, the porosity and the cell density were re-visited for these cells. It was showed that the cell pluripotency was completely maintained. A successful neural differentiation of 2 weeks nicely showed the early expression of Pax6 at day 5 followed by beta-III tubulin expression at day 10.

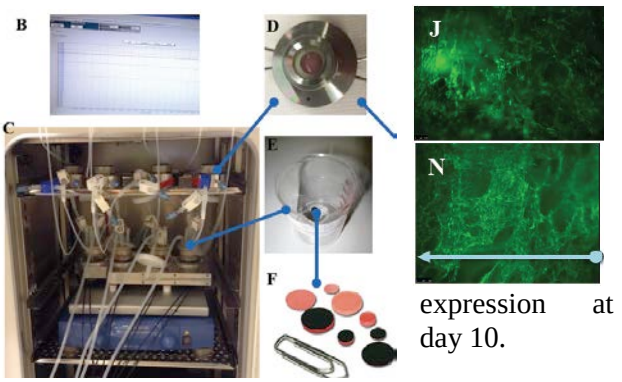

Fig 1: Bioreactor: (D) top; (C) overview; (B) sensors of pH and pO_2 ((F) detail); (E) reservoir; (J) top of the scaffold with SkMSC in 3 mm thick electrospun fiber PCL scaffold (calcein); (N) orthogonal view of the scaffold showing a uniform cell distribution – arrow head and tip pointing at the top and the bottom edge of the scaffold

DISCUSSION & CONCLUSIONS: To our knowledge, this is the first presentation of a bioreactor enabling the orthogonal perfusion of thick electrospun fibre scaffold. These bioreactors can be used as stand alone or as parallel scale-down of a larger bioreactor. This system is easy to operate and generates reliable data in parallel cultures allowing the direct comparison of the different parameter values. The cells cultures in this system have proven to be healthy and to maintain their markers as well as to be safe.

ACKNOWLEDGEMENTS: This work was supported by FP7-Health, HESUB, #601700.