ESACT 2015 Barcelona

Perfusion process of human myogenic stem cells in electrospun nanofiber scaffold-based mini-bioreactor

Zhang Y, Al-Khalili L, Hussein H, Åstrand C, Leino M, Stobbe P, Krause C, Bates N, McKean R, Chotteau V

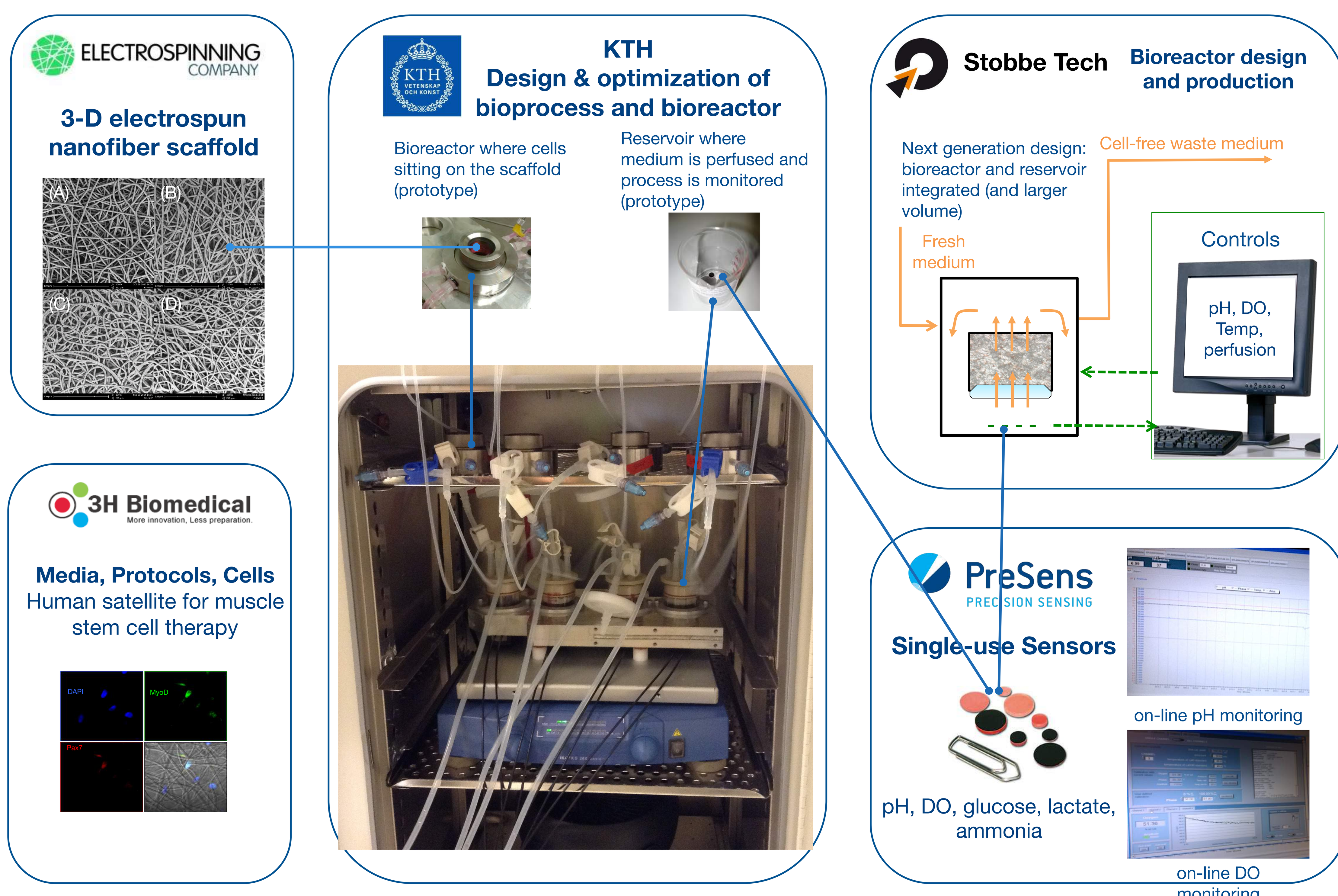
School of Biotechnology, Cell Technology Group (CETEG), KTH - Royal Institute of Technology, Stockholm, Sweden

yezhang@kth.se

Introduction

Stem cells bear an enormous promise for future therapy and have already shown their efficacy in numerous clinical trials. The state-of-the-art methods for stem cells expanding and differentiation rely on 2D static culture protocols, which are highly labour consuming, inefficient and lacking reproducibility. To meet the demand of health care addressing life-threatening diseases by cell therapy, new methods and equipment to enlarge the manufacturing capability of these cells under controlled conditions are urgently needed.

Our ultimate goal is to create a new perfusion bioreactor supporting the culture of human stem cells adhering on electrospun nanofiber scaffold (ENF) of biocompatible and biodegradable polymer. In the present study, we aim at developing scale-down mini-bioreactors, and use them to develop and optimize a perfusion process of human stem cells with myogenic progenitor potential grown in ENF.

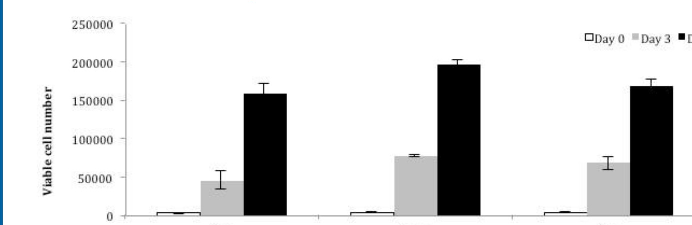


Experimental Approach

Human skeletal muscle satellite stem cells isolated from *pectoral girdle* (HSk cells from ScienCell, USA) and primary human skeletal muscle satellite stem cells isolated from *vastus lateralis* (DSk cells obtained from healthy donors transferred from Karolinska Institute BioBank) are used in the current study. The existing protocols and media applied for myogenic stem cells seeding, proliferation and differentiation are translated into perfusion process. Eight mini-bioreactors are created and used in parallel for the development and optimization of a perfusion process sustaining human myogenic stem cell expansion. The process is optimized for 3D cell seeding, proliferation and differentiation such as the medium recirculation rate, the recirculation direction, DO, etc. Analyses for cell quantification in the scaffolds are investigated and set up. Various staining methods are studied for the cell visualization in the scaffolds during the cultivation and/or at end-point.

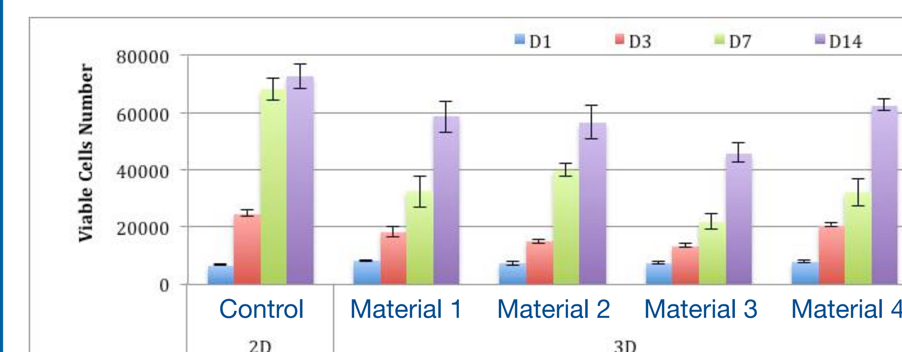
Results

❖ Electrospun fibre diameter selection



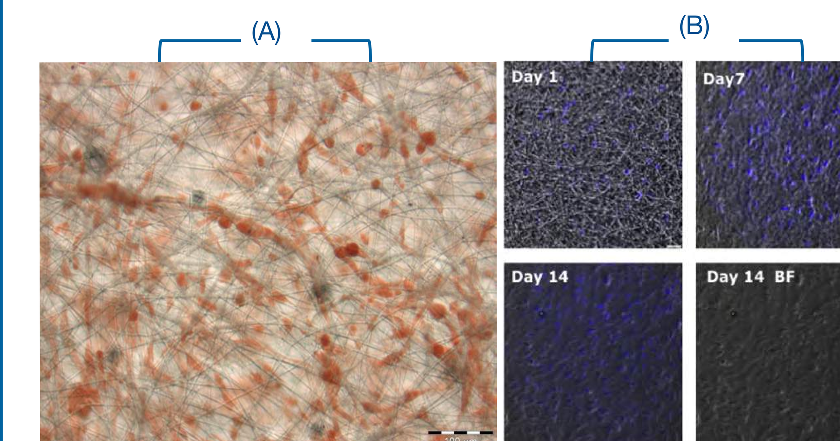
Effect of fibre diameter on HSk cells growth at days 0, 3 and 7. A fibre diameter of 4 µm was selected to potentially better harbour cells of smaller size than HSk cells such as hESCs. The scaffold thickness showed no significant influence on the cell growth rate and cell attachment efficiency (data not shown).

❖ Scaffold material selection



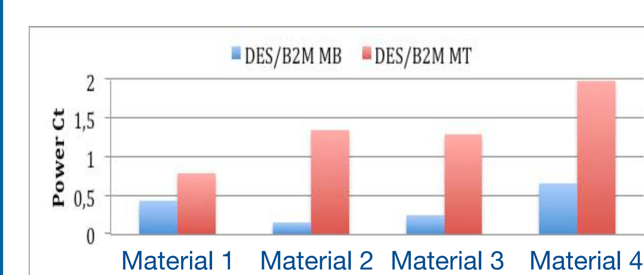
Five biodegradable and biocompatible scaffold materials (4 µm fibre diameter/50 µm scaffold thickness) were evaluated as ENF scaffolds for the cell proliferation and differentiation. Material 5 (data not showed) was too brittle and degraded very rapidly so no conclusive data could be obtained. Material 2 was chosen for bioreactor studies because the cell proliferation was higher at day 7 compared to the other material.

❖ Cell distribution on ENF

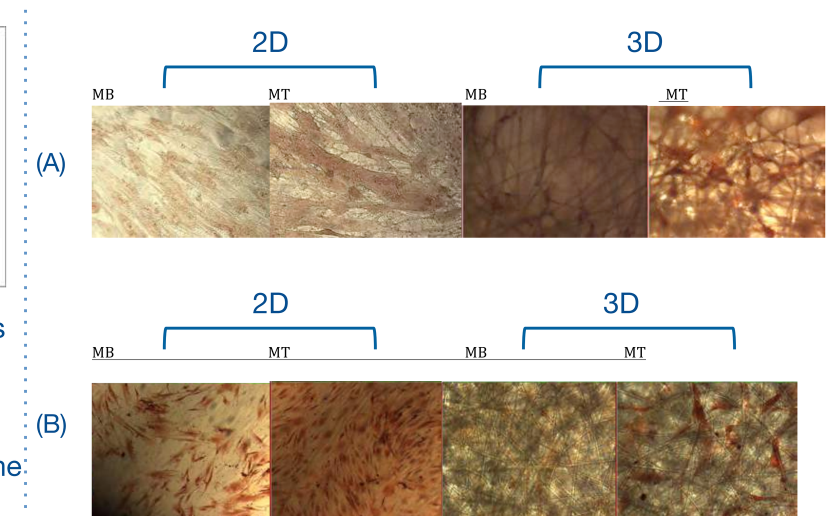


(A) HSk cells growth and distribution on material 1 scaffold shown with invert light microscope (neutral red staining, bar = 100 µm);
(B) Confocal microscopy of HSk cells grown on material 1 scaffold at different days after seeding. DAPI staining of nuclei, BF= Bright Field

❖ Myoblasts and its differentiation into myotubes on ENF



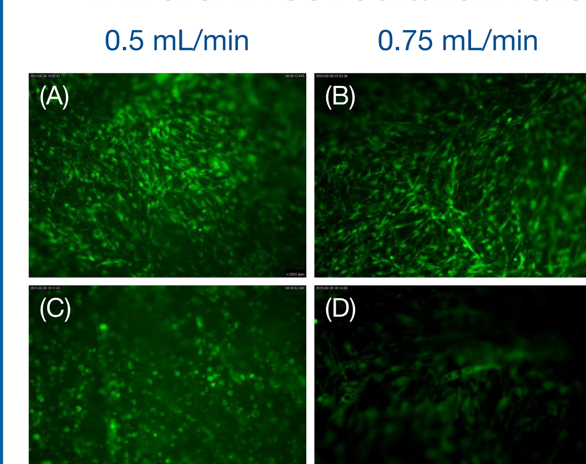
HSk myoblast (MB) differentiation into myotubes (MT) on different materials of 3D scaffolds, determined by mRNA expression of the differentiation myogenic marker Desmin (DES) expression, adjusted over the housekeeping gene Beta 2 microglubulin (B2M)



Light microscopy images of A) HSk and B) DSk myoblast (MB) differentiation into myotubes (MT) on 2D control and 3D scaffolds. Cells were stained with 1:20 neutral red for 15 minutes after 4 % formaldehyde fixation.

❖ Different recirculation rates

0.5 mL/min   0.75 mL/min

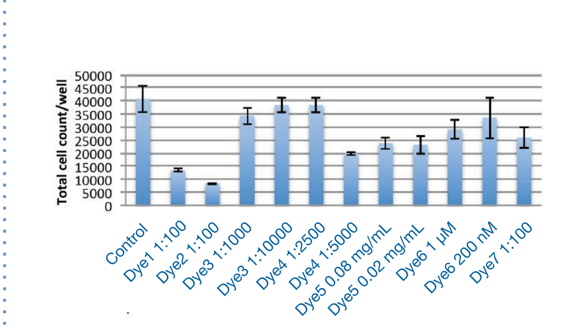


❖ Different DO

55% DO

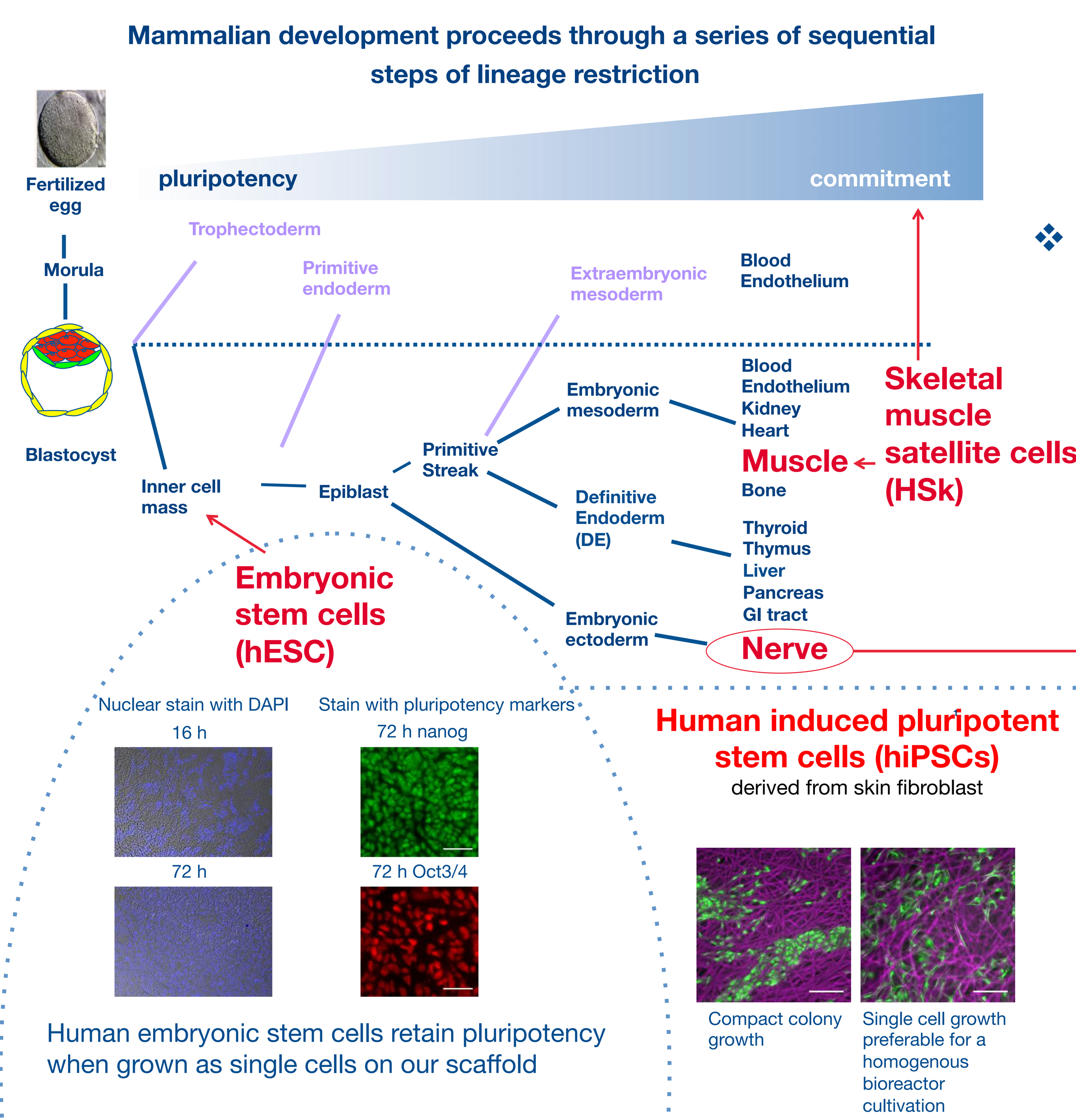
100% DO

❖ Staining methods study



Various staining methods, both cell non-toxic and toxic, with different working concentrations were investigated (part of the data shown).

Discussion and Perspective



Human embryonic stem cells retain pluripotency when grown as single cells on our scaffold

- ❖ In this project, myogenic stem cells (satellite cells) are our main focus in the development of perfusion equipment and process. The perfusion system is also applied to other stem cell systems.
- ❖ We work with different human cell systems: skeletal muscle cells (myogenic), pluripotent stem cell self-renewal, directed neural differentiation (CNS).

2D 3D 3D

BIII-tub
nestin
GAD67

Neural progenitor differentiation from human embryonic stem cells in culture dish (2D) or in our scaffolds (3D). Expressed markers: early neuronal marker BIII-tubulin and Nestin, postmitotic neural marker GAD67 (green), nuclei with DAPI (blue), overlay including bright field (right hand panel, 3D) to visualize the cell nuclei on the scaffold. Scale bar represents 30 µm.

Acknowledgement

This project is supported by FP7-Health-2013-601700 program, acronym HESUB

References

- Al-Khalili et al. "Evaluation of human skeletal muscle cells attachment and growth at surfaces of various types of biocompatible polymeric electrospun fibrous scaffolds", manuscript in preparation;
- Åstrand C, Leino M, et al. manuscript in preparation