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

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HESUB

SEVENTH FRAMEWORK PROGRAMME

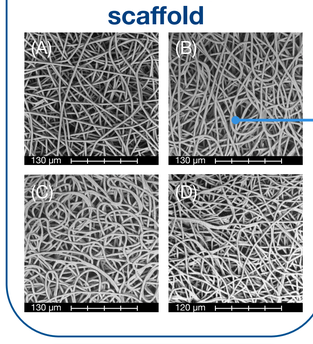
Introduction

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 expansion 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 goals are stem cell amplification while maintaining cell property, as well as directed cell differentiation in the scaffold for transplantation in human. A new perfusion bioreactor supporting the culture of human stem cells adhering on electrospun fiber (EF) scaffold of biocompatible and biodegradable polymer is under development. 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 EF.

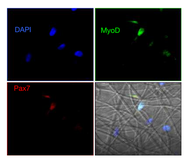
ELECTROSPINNING COMPANY

3-D electrospun scaffold



3H Biomedical
More innovation, Less preparation.

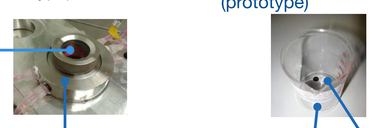
Media, Protocols, Cells
Human satellite for muscle stem cell therapy

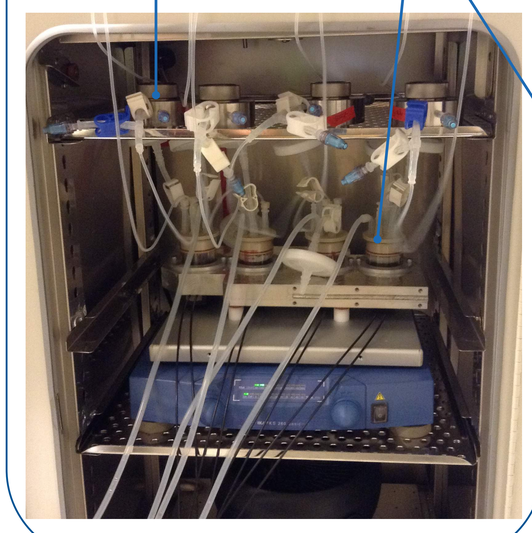


KTH
Design & optimization of bioprocess and bioreactor

Bioreactor where cells sitting in the scaffold (prototype)

Reservoir where medium is perfused and process is monitored (prototype)



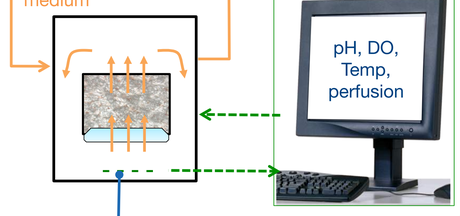


Stobbe Tech **Bioreactor design and production**

Next generation design: bioreactor and reservoir integrated (and larger volume)

Cell-free waste medium

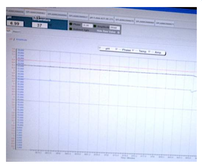
Fresh medium



PreSens
PRECISION SENSING

Single-use Sensors

pH, DO, glucose, lactate, ammonia



on-line pH monitoring

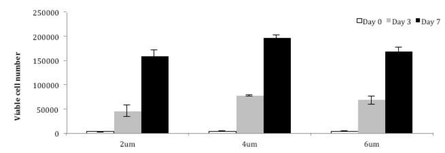
on-line DO monitoring

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, 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, dissolved oxygen (DO), etc. Analyses for cell quantification and staining methods inside the scaffolds during the cultivation and/or at end-point are investigated and set up.

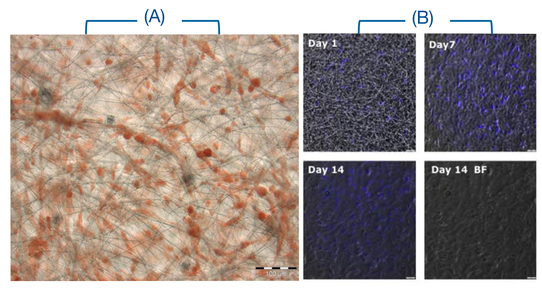
Results

❖ 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.

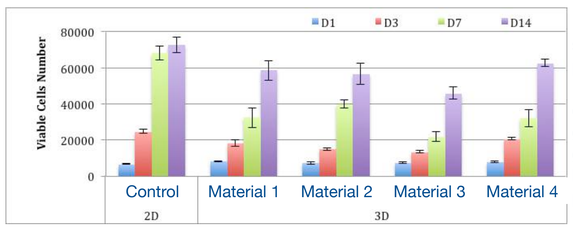
❖ Cell distribution on EF



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

The cells were expanding inside the EF scaffold and an increased coverage of the fibers occurred until a point when the scaffold fibers were almost invisible (Day 14) and the cells became more apparent in BF.

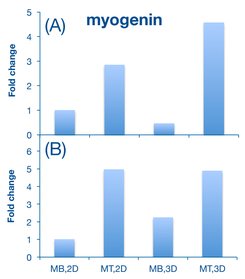
❖ Scaffold material selection

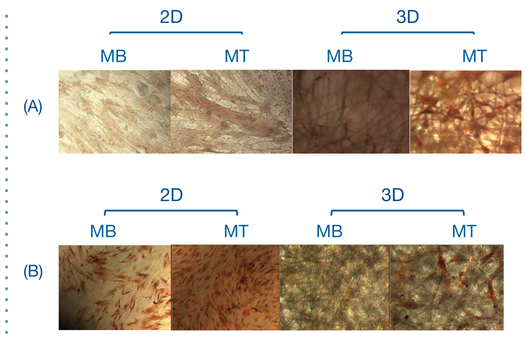


Four biodegradable and biocompatible scaffold materials (4 µm fibre diameter/50 µm scaffold thickness) were evaluated as EF scaffolds for the cell proliferation and differentiation. Materials 1, 2 and 4 generated somewhat comparable cell growth. Material 2 was chosen for bioreactor studies because the cell proliferation was higher at day 7 compared to the other material and due to a higher material flexibility.

❖ Myoblasts and the differentiation into myotubes on EF

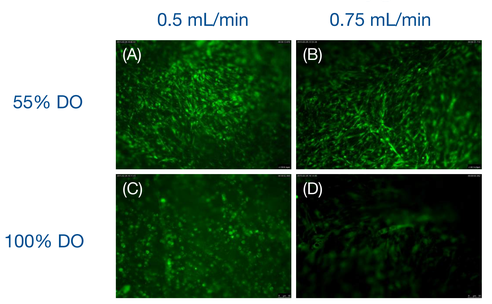
Myogenin is a marker for the entry of myoblast into myogenic differentiation.
Below is the expression of myogenin from 2 DSk cell objects (donor A and donor B). It is clear that the myogenesis in 3D culture environment is equivalently good or even better than 2D control cultures.





Light microscopy images of A) HSk and B) DSk myoblast (MB) showing the expected differentiation into myotubes (MT) as elongated cells on 2D control and 3D scaffolds (neutral red staining).

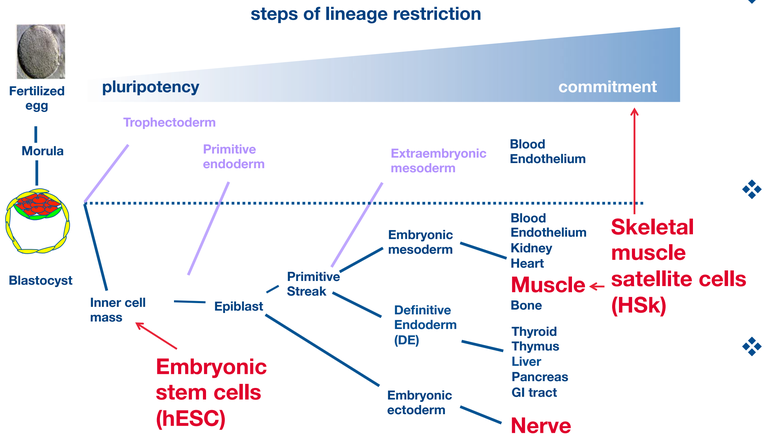
❖ Recirculation rates and oxygen level



The effects of oxygen level and the recirculation rates on the cell growth in 3D EF scaffold were studied in mini-bioreactors: (A) 0.5 mL/min recirculation rate, 55% DO; (B) 0.75 mL/min, 55% DO; (C) 0.5 mL/min recirculation rate, normal incubator atmosphere; (D) 0.75 mL/min recirculation rate, normal incubator atmosphere. The recirculation rate refers to the flow rate of the medium between the bioreactor and the reservoir. (calcein dye, staining the living cells only).
Reduced oxygen level is more favorable than 100% DO that is uncontrolled incubator atmosphere.

Discussion and Perspective

Mammalian development proceeds through a series of sequential steps of lineage restriction



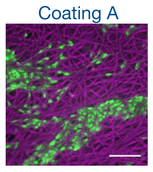
❖ The project focuses on the development of perfusion bioreactor and process for the production of human myogenic precursors, but includes also other type of cell fates.

❖ We work with satellite skeletal muscle cells, human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSCs).

❖ We also work with directed differentiation inside the scaffolds (myogenic and neuronal differentiation)

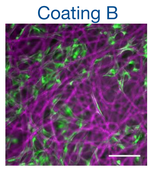
❖ Effect of coating of the scaffold fibers on hESC

Coating A



Coating A generates compact colony growth

Coating B

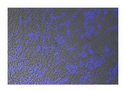


Coating B generates single cell growth, preferable for a homogenous bioreactor cultivation.

❖ Pluripotency of hESC in EF scaffold

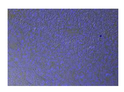
Nuclear staining (DAPI) | Staining for pluripotency markers

16 h



72 h Nanog

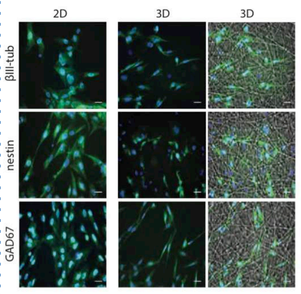
72 h



72 h Oct3/4

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

❖ Neuronal differentiation



Neural progenitor differentiation from human embryonic stem cells in culture dish (2D) or in EF scaffold (3D). Expressed markers: early neuronal marker BIII-tubulin and Nestin, postmitotic neuronal 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

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The hESC were obtained according to Bjuresten K and Hovatta O. Donation of embryos for stem cell research - how many couples consent? Hum Reprod 2003; 18:1353–1355.