Hepatic Differentiation of Human Induced Pluripotent Stem Cells in a Perfused 3D Porous Polymer Scaffold for Liver Tissue Engineering

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Publication date:
2014

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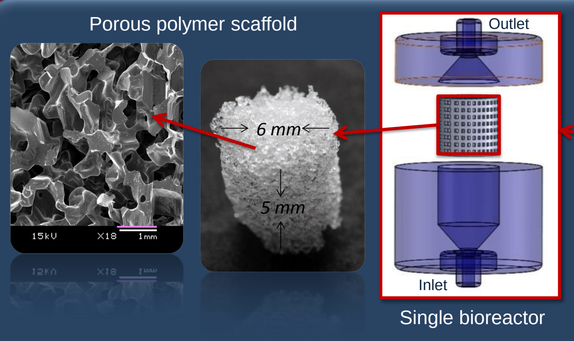
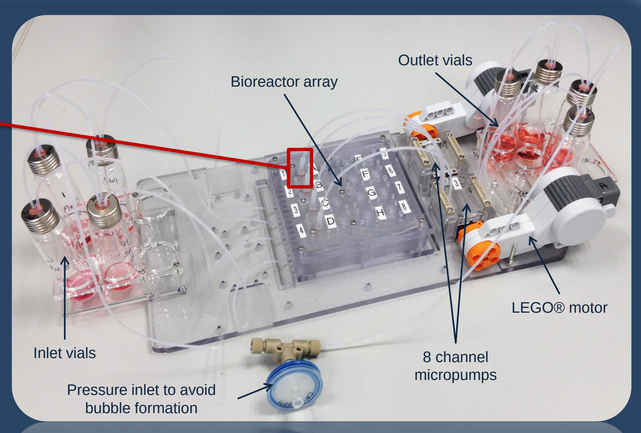
Introduction and Aim

A huge shortage of liver organs for transplantation has motivated the research field of tissue engineering to develop bioartificial liver tissue and even a whole liver. The goal of NanoBio4Trans is to create a vascularized bioartificial liver tissue, initially as a liver-support system. Due to limitations of primary hepatocytes regarding availability and maintenance of functionality, stem cells and especially human induced pluripotent stem cells (hIPS cells) are an attractive cell source for liver tissue engineering. The aim of this part of NanoBio4Trans is to optimize culture and hepatic differentiation of hIPS-derived definitive endoderm (DE) cells in a 3D porous polymer scaffold built-in a perfusable bioreactor. The use of a microfluidic bioreactor array enables the culture of 16 independent tissues in one experimental run and thereby an optimization study to be performed.

Bioreactor Array System for Tissue Culture



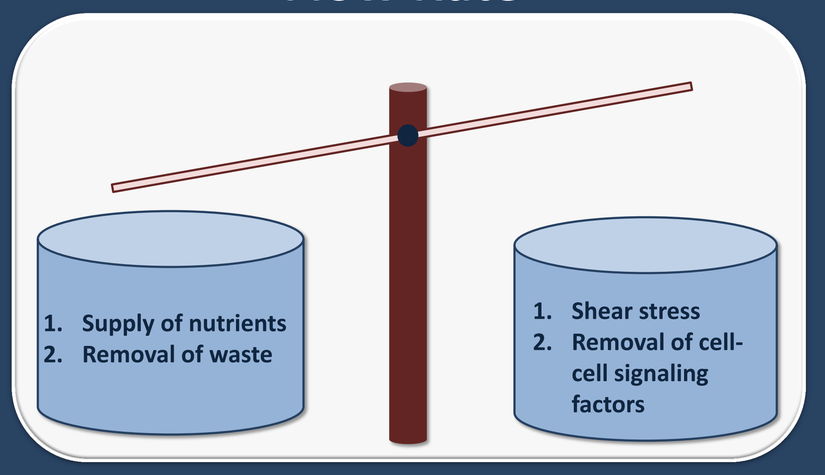
Our approach for engineering liver tissue is to culture and differentiate hIPS-derived DE cells in a 3D porous polymer scaffold housed in a perfusable bioreactor to ensure supply of oxygen and nutrients and removal of waste.



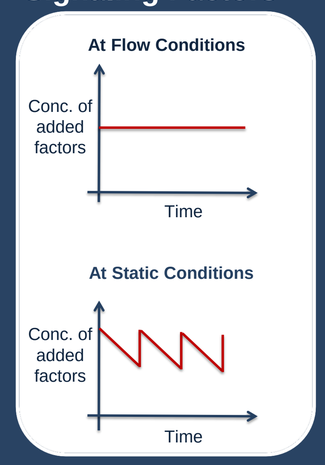
The bioreactor array enables culture/differentiation of 16 independent tissues in one experimental run.

Issues at Flow Conditions

Flow Rate



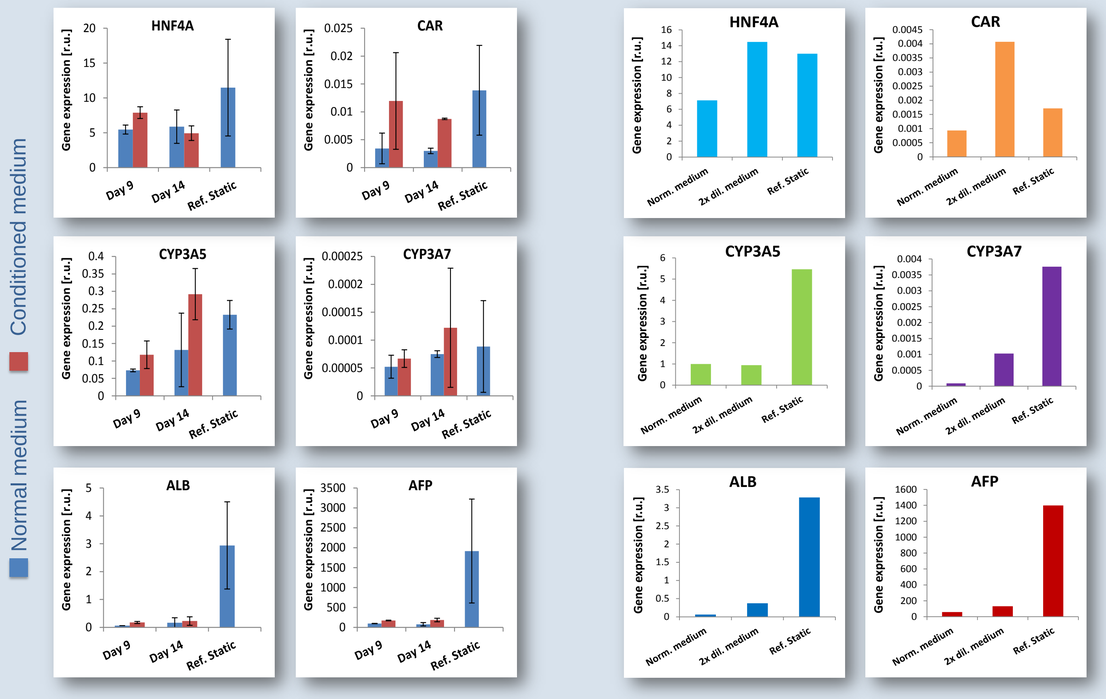
Concentration of Added Signaling Factors



One important parameter when optimizing conditions for flow cultures is the flow rate. The flow rate is a balance between ensuring sufficient supply of nutrients and oxygen, but at the same time avoiding to much shear stress onto the cells and removal of cell-to-cell signaling factors. Besides testing different flow rates, we apply different scaffold designs with a different flow profile. Another issue to consider is the concentration of added signaling factors. At flow the concentration of added factors is constant, while at static conditions in a batch culture, the concentration decreases between each exchange of medium. Thus, a lower concentration than the one optimized for batch cultures might be better at flow conditions.

Gene Expression of Liver Markers

Similar gene expression level of the hepatocyte nuclear transcription factor 4α was observed at perfusion cultures compared to conventional static cultures, whereas a decreased expression was seen for the transcription factor CAR and the CYP enzymes CYP3A5 and CYP3A7. Furthermore, expression of albumin and α-fetoprotein was almost knocked down. However, expression of most of the markers was increased by the use of **A)** conditioned medium or **B)** medium with a two times lower concentration of the added signalling factors than those optimized for conventional batch cultures.

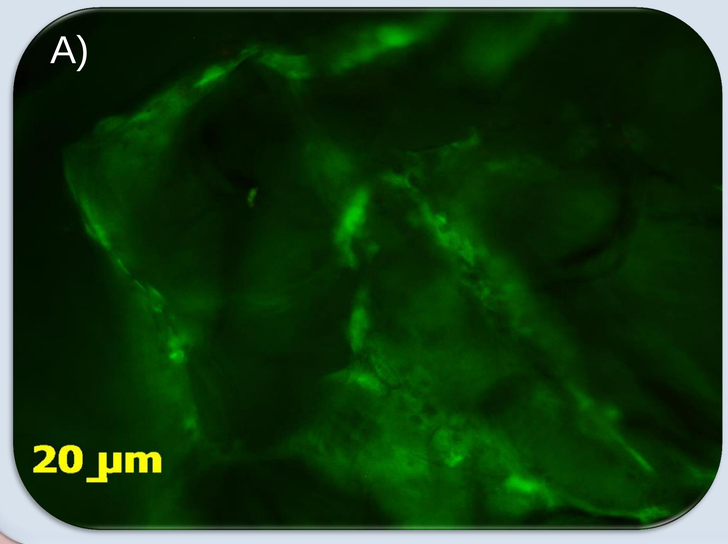


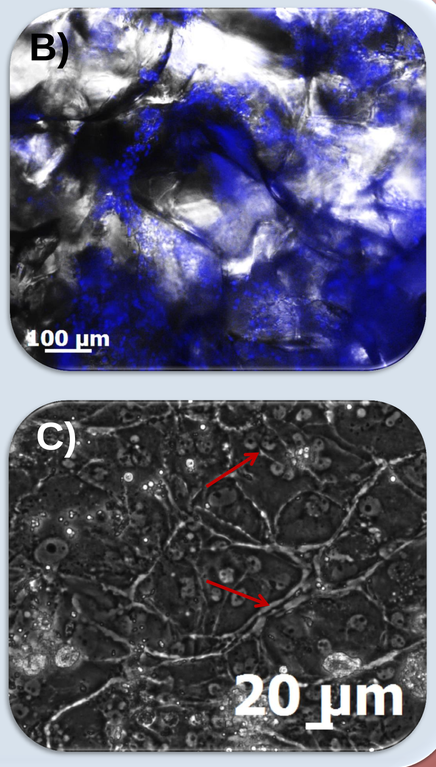
A) Conditioned medium collected from a parallel static batch culture increased the gene expression of liver markers. Analysed at day 9 and day 14 after the DE stage.

B) Differentiation medium with a two times lower concentration of added signalling factors than those optimized for conventional batch cultures (2x dil. medium) resulted in increased expression of most liver markers at day 14 after the DE stage.

Cell morphology

A hepatocyte-like cell morphology was obtained with polynucleated cells and ”bile-canaliculi”-like structures (shown by the red arrows). Imaging at day 14 of differentiation after DE stage. Live stained cells **A)** and Höchst stained cells **B)** in the 3D scaffold. **C)** Phase contrast image of differentiated cells in a 2D perfusion chamber.





Conclusion

The results suggest that the flow conditions affect cell-to-cell signalling necessary for liver differentiation and/or functionality, as well as the concentration of added signalling factors in the differentiation medium has to be adapted to the different environment at flow culture with constant renewal of the culture medium.

Funding provided by EU Program HEALTH.2012.1.4-2 [Medical technology for transplantation and bioartificial organs], Contract:304842