Introduction

Electrospinning allows for the production of scaffolds mimicking that of in vivo extracellular matrices. With the additional flexibility of altering topography, porosity, protein coating and polymer composition it provides a suitable platform for 3D culture systems. While the scaffold provides excellent support for growing and differentiating cells, the cell density required for production of tissue-like constructs will eventually be limited by insufficient distribution of oxygen and nutrients.

To overcome this problem we have designed and built a bioreactor system that allows the scaffold to be continuously perfused by an orthogonal flow of medium. Furthermore, this system is fully automated with live monitoring of pH, oxygen and temperature allowing for a robust culture with low maintenance.

Our goal in our studies is to evaluate this system with regards to proliferation and differentiation of skeletal muscle cells as well as embryonic stem cells to dense tissue-like constructs.

Cultivation of embryonic stem cells under perfusion

Initially the bioreactor system was tested using embryonic stem cells confirming if it was a suitable platform for culturing and differentiating cells. To create a sufficient microenvironment for the stem cells PCL scaffolds of 200µm was coated in a stepwise manner with the extracellular protein laminin-521 along with the cell-cell adhesion protein E-cadherin-fc. To these scaffolds cells were seeded a density of 1.5x10^6 cells/scaffold. The cells were then cultured for 1 day in static conditions followed by 5 days in bioreactors under a 0.032cm/min perfusion rate. After the cultivation the scaffolds were initially analysed using calcein staining to investigate the viability of the cells. After the 4 day cultivation the cells had grown to a dense layer covering the entire scaffold (Figure 1a). Further investigation of the pluripotency markers Oct3/4 and Nanog using antibody staining revealed a clear expression, indicating that the cells could proliferate without spontaneous differentiation.

Differentiation of embryonic stem cells to neural cells in perfusion bioreactors

To further investigate whether these bioreactors also could support the differentiation of embryonic stem cells, a 15 day protocol based on dual MADH inhibition was tested. The protocol utilizes three inhibitors; IWP-2, LDN193189 and SB431542 to direct differentiation of the stem cells towards a neural lineage. Similar to the stem cell expansion, cells were cultured under a 0.032cm/min flow rate with a daily media exchange rate of 50%. Cells were harvested at day 5, day 10 and day 15. As seen in figure 4a cells at day 15 were healthy and covered the entire scaffold as indicated by the calcein-stain (fig 4a). STaining of markers Tuj1 and Pax6 clarified the differentiation in a neural lineage (fig 4b). Further analysis of gene expression of the forebrain marker FoxG1 showed a clear increase over the 15 days (fig 4c). Pax6 on the other hand had an expression peak at day 5, with a drop towards day 10 and 15 (fig 4d), this hits well with its description as an early neural progenitor marker. Finally the cell number was estimated to be 23x10^6 cells at day 15, which within these scaffolds is a density of 370x10^6 cells/cm^3 (fig 4e).

Conclusions & perspectives

This bioreactor system provided an excellent way to culture both embryonic stem cells and skeletal muscle cells. Furthermore we show that differentiation of the embryonic stem cells was possible and yielded a highly dense culture of neural cells in only 15 days. As we could obtain these densities it is possible to culture the cells longer to obtain tissue like structures in the 3D scaffold environment, these could be useful for a range of studies like developmental biology, drug testing, regenerative medicine etc.

As this system is fully automated and easy to use, long-term cultures and differentiations can be executed with ease.

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