IMPACT OF THE DYNAMIC CULTURE SYSTEM FOR 3D HIGH CELL DENSITY NEURAL DIFFERENTIATION OF hESC IN ELECTROSPUN PCL SCAFFOLDS

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The ability of pluripotent stem cells to differentiate into any of the three germ layers have sparked an array of investigations in developmental biology, tissue replacement, drug screening and cellular interactions. A demand has risen for 3-dimensional technologies to improve scalability and better mimic the stem cells niche. With the increasingly complex cultivation platforms and larger scales a limit is rapidly reached in terms of nutrient and waste exchange to the cells. These limitations can be overcome with the use of dynamic culture systems such as bioreactors. However, a culture platform that allows for differentiation of pluripotent stem cells to neural lineages to homogenous macro scale tissue cultures is still challenging.

We have previously developed a protocol for the culture of pluripotent cells in 3D electrospun PCL scaffolds in multi-well format [1]. The purpose of the present was to investigate the potential of different dynamic systems to support the neural differentiation of pluripotent stem cells in 3D electrospun polycaprolactone (PCL) scaffold disks of 35 mm diameter. The dynamic systems were a bioreactor with culture medium flow either longitudinal or orthogonal to the scaffold, alternatively a system with orbital shaking, and absence of agitation. Human embryonic stem cells, hESC, (line HS980 received from Prof. O. Hovatta (Karolinska University Hospital, Huddinge, Sweden), transferred to KTH and used in agreement with ethics approval issued to us by the Regional Ethics Board, Stockholm, Sweden (Ethical Permission Dnr 2013/493-31, amended 2015/824-39)) were submitted to a 3-week neural differentiation protocol. The cells were cultured in 3D Mimetix Air electrospun polycaprolactone (PCL) scaffold disks of 35 mm diameter in small stainless steel bioreactors using a modified version of the dual-SMAD differentiation protocol with small molecular inhibitors. The levels of Sox2, Nestin, ki67, Map2, Doublecortin and beta III tubulin were measured.

Applying dynamic culture conditions for the differentiation of hESC towards neural lineages in 3D electrospun PCL scaffolds greatly increased the cell yield and had a large beneficial impact on the marker expression compared to a static system. Static cultivation resulted in a heterogeneous cell population with cells expressing both early and late differentiation markers. A culture with a flow orthogonal to the scaffold did not sustain a consistent cell culture. By using a flow longitudinal to the scaffold, the cells grew homogeneously across the scaffold and expressed early differentiation markers at a higher level compared to orbital shaking. This latter system increased the cell yield to the highest level, i.e. 120×10^6 cells/scaffold, and led to homogeneously differentiated cells with an increased level of mature neuronal markers.
To our knowledge this was the first time that pluripotent stem cells have been differentiated to neural fate in a 3D scaffold of connected homogeneous macroscopic scale (> cm).

Reference: Leino M, Åstrand C, Hughes-Brittain N, Robb B, McKean R, Chotteau V. J Biomed Mater Res B Appl Biomater. 2018. 106:1226-1236. doi: 10.1002/jbm.b.33928.