DIELECTRIC SPECTROSCOPY MONITORING OF A BIOREACTOR PROCESS FOR hiPSC EXPANSION AND DIFFERENTIATION

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Key Words: dielectric spectroscopy; bioprocess monitoring; human pluripotent stem cells; stirred tank bioreactors; cell aggregates

Bioprocessing strategies using 3D cell culturing approaches, such as cell aggregates, are promising solutions to achieve efficient and scalable bioprocesses for stem cell expansion and differentiation. However, tracking viable and total cell numbers in such culture systems is not straightforward. It requires cell detachment, disaggregation or disruption, which results in measurements that are laborious, biased and with high variability.

In this work, we used a commercially available capacitance probe to explore the applicability of dielectric spectroscopy for in situ monitoring of a multistep process for expansion and differentiation of human induced pluripotent stem cells (hiPSC) cultivated as cell aggregates. After 5 days of cell expansion in a bioreactor, the hepatic differentiation step was integrated by addition of different levels of specific soluble factors at various stages of the process to promote growth and generate populations successively enriched for definitive endoderm, hepatoblasts, hepatocyte progenitors and mature hepatocytes. While this differentiation procedure has been previously validated for monolayer cultures, this was the first time it was carried out in a stirred tank bioreactor operated in perfusion mode. Phenotype analysis confirmed a marked increase in key hepatic differentiation markers culminating at day 21 of differentiation.

Our data shows a good correlation between total volume of the cell aggregates and permittivity measured by the probe ($R^2 = 0.84$). However, there was a delay between changes in cell concentration and the permittivity signal. This suggests that cell expansion requires a few days to result in increased volume of the cell aggregates and that each aggregate behaves as one overall inducible dipole. The β-dispersion curve shape also appears to change over culture time and could eventually be used as an indicator for differentiation progression.

Dielectric spectroscopy has been used successfully to monitor viable cell concentration in different single-cell suspension cultures, but there are few published applications to 3D cultures. Our results demonstrate the potential of dielectric spectroscopy to monitor complex bioprocesses for human stem cell aggregates in stirred cultures.

Acknowledgements: Funding provided by ERA-NET/E-Rare3 programme through research project ERAdicatPH (E-Rare3/0002/2015). The authors acknowledge Dr Juan Rodriguez-Madoz (University of Navarra, Spain) and Dr Anders Aspegren (Takara Bio Europe – Cellartis AB, Sweden) for helpful discussions on hepatic differentiation of hiPSC.