IMPACT OF THE HYDRODYNAMIC ENVIRONMENT ON CARDIOMYOCYTE DIFFERENTIATION OF IPSC

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Key Words: iPSC, cardiomyocyte, hydrodynamic, stirred bioreactors, differentiation

Ventricular-like cardiomyocytes have the potential to be used for cardiac repair in cases of cardiovascular diseases, most commonly myocardial infarction, which can ultimately lead to heart failure. The regenerative capacity of the adult myocardium is approximately 1% per annum, therefore, novel solutions are being explored where cardiomyocytes are derived from pluripotent stem cells (PSCs). From the initial promise of PSCs (including embryonic and induced pluripotent stem cells-ESC and iPSC, respectively) for clinical application, their use in therapies has already covered heart, eye diseases and diabetes (www.clinicaltrials.gov/) Furthermore, iPSC have been revolutionizing pre-clinical research as a tool for disease modelling and drug discovery. Biochemical and physical cues have been demonstrated to have a significant impact on the PSC differentiation yield, i.e. the number and quality of the resulting cardiomyocytes. In particular electrical and mechanical stimulation emulates the *in vivo* heart environment and has proven to have significant effects on morphology, cell density, cardiomyogenesis, maturity and functionality of differentiated cardiomyocytes. This is dependent on the type of mechanical cue applied, commonly cyclic strain/stretch or fluid shear stress (hydrodynamic forces), in addition to magnitude, duration and time of application (Acimovic et al., 2014; Geuss and Suggs, 2013; Savla et al., 2014). Correia et al. (2014) developed an iPSC-based differentiation protocol in a stirred tank bioreactor and mechanically varied the environment by using different agitation modes. It was found that combining hypoxic culture conditions with intermittent agitation resulted in 1000-fold increase in the cardiomyocytes yield compared to normoxic and continuous agitation mode. This work combines rigorous fluid dynamics investigation and flow frequency analysis with iPSC differentiation experiments to identify and quantify the flow characteristics leading to a significant increase of the differentiation yield in the aforementioned study, towards a better understanding of the physical relationship between frequency modulation and embryoid bodies suspension, and the development of dimensionless correlations applicable at larger scales.

Laser Doppler Anemometry (LDA) and Fast Fourier Transform analysis were used to identify characteristic flow frequencies under different agitation modes. The use of intermittent agitation resulted in a pattern of low intensity frequencies at reactor scale. It was found that the observed frequencies and their intensity can be controlled by varying rotation time, i.e. the impeller stirrer speed (rpm), the dwell time, during which the motor is stopped and the interval time between in-motion and stationary phases. It can be inferred that the presence of such low frequencies is responsible for the observed improvement in differentiation yield and that this can be further optimised by tuning three significant parameters identified in this study. The results of the engineering studies have informed the biological verification experiments using iPSC. This work introduces the concept of fine-tuning the physical hydrodynamic cues within a three-dimensional flow system to improve cardiomyocyte differentiation of PSC.

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