HIGH DENSITY EX VIVO EXPANSION OF STEM CELL AGGREGATES IN STIRRED PERFUSION BIOREACTORS

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Stem cell based products are expected to emerge as a major paradigm in the development of novel therapies for the regeneration of functional tissues and organs [1]. Although somatic stem cells display both multipotency and self-renewal features, their natural *ex vivo* proliferative capability is rather limited. An alternative approach with great potential involves the use of pluripotent stem cells (PSCs), which can self-renew *in vitro* for prolonged periods while maintaining their intrinsic differentiation potential for all three embryonic germ lineages. In order to overcome the limited scalability of standard monolayer cultivations and to establish more defined cell culture conditions, bioreactor expansions in suspension using microcarriers, aggregated cell clumps and microencapsulation have been proposed [2]. Typically applied fed-batch and repeated batch cultures are limited in terms of maximum cell densities given the inability of hPSCs to perform oxidative phosphorylation [3], leading to excessive lactic acid accumulation and reduced proliferation after approximately one week of cultivation.
In this study we adapted the technology of continuous stirred tank suspension culture for the production of large numbers of high quality human PSCs. Stem cells were cultivated as multicellular spheroids to guarantee survival in suspension, maintain their native microenvironment and prevent harmful harvesting procedures, as required for microcarrier cultures. The scale up was achieved in a 1L scale bioreactor equipped with an alternating tangential flow (ATF) system for cell retention to allow the renewal of spent medium and maintenance of key operating parameters (pH, oxygen pressure and temperature). The continuous exchange of medium prevented lactic acid accumulation as well as glucose limitation. The adaption of the perfusion rate to account for the increasing cell number led to very high cell densities, above $1 \cdot 10^7$ cells/mL. By controlling the agitation rate, we were able to maintain a constant size of the aggregates, eliminating the need for passaging procedures and the use of Rho kinase inhibitors, thus bringing the system closer to a fully automated operation. The maintenance of hPSCs' differentiation potential was confirmed by expression for pluripotency markers and differentiation into endo-, meso- and ectodermal lineages. Our work reports the achievement of high density human PSCs *ex vivo* expansion as suspended cell aggregates by controlled culture in perfusion mode. The fabrication of 10 billion pluripotent stem cells in a 1L fully automated bioreactor would be sufficient for the complete reconstruction of major organs (e.g. heart and liver [4]), and for large scale toxicology screenings.

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