XENO-FREE PRODUCTION AND RECOVERY OF HUMAN PLURIPOTENT STEM CELLS USING SYNTHETIC DISSOLVABLE MICROCARRIERS

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The implementation of scalable culture platforms for the large-scale production of human pluripotent stem cells (hPSC) and their derivatives is mandatory to fulfill the requirement of obtaining large numbers of these cells for cell therapies and other *in vitro* biomedical applications, such as drug screening, toxicology assays and disease modeling. Recent progress includes the development of chemically-defined culture conditions for manufacturing of hPSC and their derivatives, namely the development of xeno-free microcarrier platforms to meet good manufacturing practice (GMP) quality requirements [1]. One challenge that remains to be addressed is the establishment of a robust, scalable, and cost-effective downstream processing for cell recovery and removal of the microcarriers. Since hPSC have the tendency to create multilayers of cells on the microcarriers, often forming very large cell-microcarrier aggregates, the process of cell recovery can be technically challenging and time consuming.

In this work, we developed a robust and efficient platform for large-scale production of hPSC using synthetic dissolvable microcarriers, which can be quickly dissolved by a non-proteolytic enzyme. This allows an easy cell recovery without the need of the microcarrier separation step, facilitating the downstream processing. Moreover, these synthetic microcarriers are sterile and ready-to-use, and are functionalized with the Synthemax® surface, based on a peptide-acrylate matrix designed for long-term support of hESC self-renewal [2].

hPSC were able to attach and grow on the dissolvable microcarriers and the expansion process was evaluated in a scalable stirred culture system. The cells growth performance on these microcarriers was comparable with the ones obtained when culturing hPSCs in non-dissolvable microcarriers (backbone of polystyrene coated with different ECM molecules), being possible to obtain 1.3×10^6 cells/mL during 5 days. Importantly, hPSCs cultured on these novel microcarriers were efficiently recovered without the need of the filtration step to separate the microcarriers from the cells and maintained their typical colony morphology and pluripotency-associated marker-expression after re-plating on tissue culture plates. Moreover, their potential for spontaneous differentiation into cells of the three embryonic germ layers was demonstrated through formation of embryoid bodies containing cells expressing typical markers of endoderm, ectoderm and mesoderm.

These novel synthetic dissolvable microcarriers allow an easy and efficient downstream processing for hPSCs recovery after expansion/differentiation, without compromising the quality of the cells (viability, potency and functionality), which are a major process breakthrough for stem cell manufacturing.

[1] Badenes SM, et al., “Defined Essential 8™ Medium and Vitronectin Efficiently Support Scalable Xeno-Free Expansion of Human Induced Pluripotent Stem Cells in Stirred Microcarrier Culture Systems”, PlosOne (2016), 11(3):e0151264.
[2] Melkoumian Z, et al, “Synthetic peptide-acrylate surfaces for long-term self-renewal and cardiomyocyte differentiation of human embryonic stem cells”, Nat Biotechnol (2010), 28(6): 606-10.

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