POSTER OF DISTINCTION

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HIGHLY EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO LONG-TERM EXPANDABLE “MINI-GUT” ORGANOIDS

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Background: The differentiation of human pluripotent stem cells (hPSC) into intestinal organoids represent an attractive mechanism for generating cells for disease modelling, drug screening and cell-replacement therapy. Although it is possible to generate intestinal organoids from adult stem cells, the derivation of organoids from hPSCs has been shown to result in a broader variety of cells as the starting material.

Aims: Introduce novel PSC to intestinal differentiation kit and protocols.

Methods: We developed the STEMdiff™ Intestinal Organoid Kit, a specialized serum-free and fully-defined medium formulation that efficiently and reproducibly promotes differentiation of human embryonic stem (ES) and induced pluripotent stem (iPS) cells through developmental stages of 1) definitive endoderm, 2) mid-/hindgut, and 3) small intestine.

Results: Here we demonstrate that monolayers generated with multiple human ES (WA01, WA07, WA09) and iPS (WLS-1C, STiPS-FO16, STiPS-MOO1) lines maintained in mTeSR™ with Corning Matrigel™, differentiate into FOXA2+/SOX17+ endoderm cultures with an efficiency of 81.6% ± 8.6% (n=21). Further differentiation into posterior endoderm to promote the formation of mid-/hindgut resulted in 71.4 ± 8.5% (n=13) of cells expressing the hindgut marker CDX2, but none of the cells expressing the anterior gut tube marker SOX2. Twenty-four hours after the emergence of CDX2+ cells, clusters in the flat cell sheet monolayers changed their morphology to tightly packed epithelial tubes that generated budding spheroids which detached from the monolayer. These detached hindgut spheroids are composed of CDX2+/E-cadherin+ epithelia and adjacent CDX2+/VIM+ mesenchyme. When these spheroids were collected, embedded in Corning™ Matrigel™ and cultured in fully defined IntestiCult™-hPSC Organoid Growth Medium (OGM), they generated intestinal organoids composed of a polarized intestinal epithelium patterned into villus-like structures, and a surrounding niche factor-producing mesenchyme. Organoids cultured for > 25 days in vitro and analyzed by immunohistochemistry and/or qRT-PCR demonstrate the presence of enterocytes (villin), goblet cells (MUC2), paneth cells (lysozyme), and intestinal stem cells (LGR5). These organoids can be further dissociated and passaged every 7 to 10 days for multiple passages in IntestiCult™-hPSC OGM. Our results demonstrated that a starting population of approximately 200,000 hPSCs seeded in a single well of a 24-well plate gave rise to 216 ± 19.7% (n=10) intestinal organoids, which could be passaged and expanded long-term (> 8 months, n=3) using IntestiCult™-hPSC OGM.

Conclusions: In summary, STEMdiff™ Intestinal Organoid Kit is an easy to use kit for the derivation of large quantities of human intestinal organoids from hPSC in a highly efficient and reproducible manner.

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