Peristaltic pump-based circulation can promote insulin precipitation in hPSC culture media

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***Abstract*—In the perspective of automated processes for human pluripotent stem cell (hPSC) production, we investigated the sensitivity of specific hPSC culture media to peristaltic pump-based circulation. Low protein media circulated overnight were affected by insulin precipitation. hPSC aggregates, cultured as relevant assay, revealed unreported sensitivity of hPSCs to reduced insulin concentrations. Notably, the addition/presence of human serum albumin (HSA) in the media stabilized insulin and supported hPSC culture.**

***Keywords*—Pluripotent stem cells bioprocessing, Peristaltic pumping, Insulin, Albumin.**

I. Introduction

HPSCs represent a unique cell source for the unlimited production of functional human cell types in vitro [1]. For their envisioned clinical use, standardised mass production is indispensable but still a challenge [2]. Recently, it was demonstrated that "matrix-free cell only aggregate" suspension culture in stirred-tank bioreactors represents a promising strategy [3], however the high sensitivity of hPSCs to physicochemical culture parameters requires additional efforts for the development of stable chemically-defined culture media and advanced culture technologies essential for standardized GMP/GCP-compliant bioprocesses.

Aiming at automated production in suspension culture, we investigated the sensitivity of specific hPSC culture media to peristaltic pump-based circulation, a technology extensively used in bioprocessing and tissue engineering.

II. Materials and Methods

Culture media E8, TeSR-E8, mTeSR1, StemMACS iPS-Brew XF, DMEM/F12, E8 lacking one protein at a time (i.e. E8 minus bFGF, -TGFβ1, -transferrin, -insulin), and E8 + 0.01-0.1% HSA were conditioned overnight in the incubator under (Fig.1a): 1) static condition in 50 mL tube (SC); 2) continuous circulation (5-100 ml/min) within a peristaltic pump-based circuit (PC). Single hHSC_1285_T-iPS2 and HES3 NKX2-5eGFP/w cells were cultured 24h on orbital shaker to form aggregates ($1x10^6$ cells, 3mL E8 + RI) [3], which were successively cultured for 3 days in conditioned media (Fig.1b). Light microscopy, flow cytometry (propidium iodide (PI) marker for hPSC viability, NANOG and OCT4 markers for hPSC pluripotency), UPLC-MS, and SEM analyses were performed.

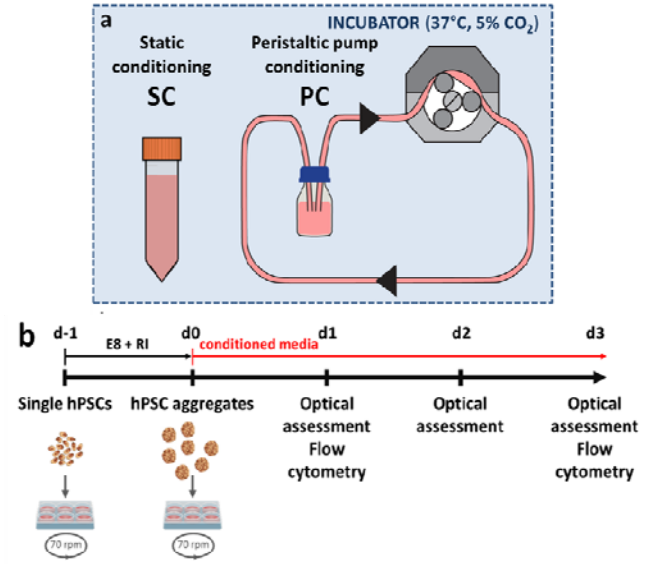

Figure 1. Schematics of (a) hPSC culture media conditioning and (b) hPSC aggregate suspension culture with conditioned media.

III. Results

hPSC aggregates cultured in SC E8 maintained their spherical morphology and increased in diameter over the 3 days (Figs 2a-c), in accordance with [3]. Unexpectedly, hPSC aggregates cultured in PC E8 were characterized at day 1 by irregular morphology and reduced size compared to the SC E8 controls, followed by increase in cell debris and disaggregation on days 2-3 (Figs 2d-f).

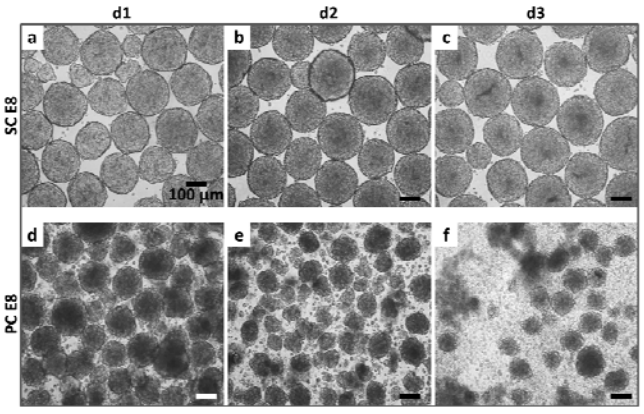

Figure 2. Light microscopy images of (a-c) hPSC aggregates cultured in SC E8 and (d-f) hPSC aggregates cultured in PC E8 over 3 days.

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Light microscopy of SC and PC E8 and DMEM/F12 (Fig.3) revealed the presence of precipitated particles of up to ~50 µm in size in PC E8, only (Fig.3c).

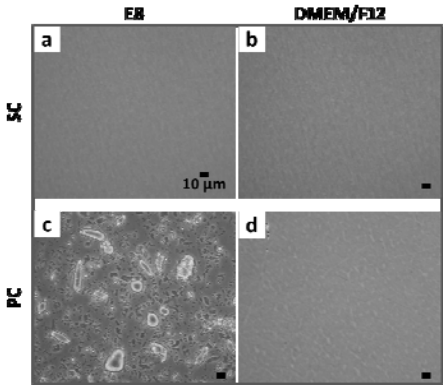

Figure 3. Light microscopy images of (a) SC E8, (b) SC DMEM/F12, (c) PC E8, and (d) PC DMEM/F12.

This suggested that particle formation was related to E8 protein components in combination with PC. Therefore, E8 lacking one protein at a time was tested. No particles were detectable in any SC medium. In contrast, applying PC resulted in substantial particle formation in all media, except in the absence of insulin. Semi-quantitative UPLC-MS analysis of SC and PC E8 revealed that less than 10% of dissolved insulin was present in PC E8 compared to SC E8 controls. Together, this strongly suggested that insulin in E8 was underlying the formation of precipitates induced by PC.

Inspired by pharmacological strategies [4], HSA was added to E8 before conditioning. Surprisingly, although particle formation was detectable in the PC E8 + 0.1% HSA (Fig.4b), hPSC aggregates cultured with PC E8 + 0.1% HSA maintained their morphology (Fig.4d).

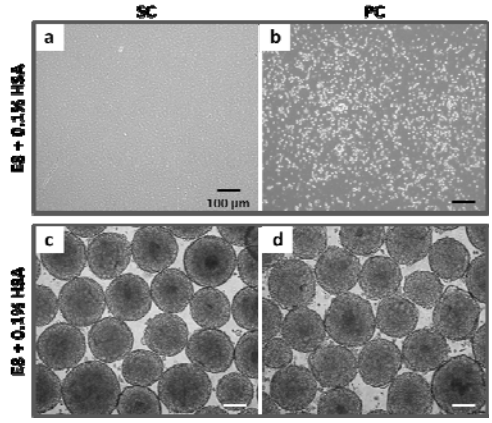

Figure 4. Light microscopy images of (a) SC E8 + 0.1% HSA, (b) PC E8 + 0.1% HSA, (c) hPSC aggregates cultured with SC E8 + 0.1% HSA, and (d) hPSC aggregates cultured with PC E8 + 0.1% HSA.

UPLC-MS analysis revealed maintenance of high dissolved insulin content in PC E8 + 0.1% HSA (~80%) in contrast to the PC E8 (~8%), while dissolved insulin was slightly preserved in PC E8 + 0.01% HSA (Fig.5). This supported the view that (1) insulin availability is the key factor for hPSC survival in the adopted assay, and (2) HSA is beneficial for insulin preservation and stabilization. Similar results were obtained with commercial media containing human or bovine serum albumin (i.e. mTeSR1 and StemMACS iPS-Brew XF).

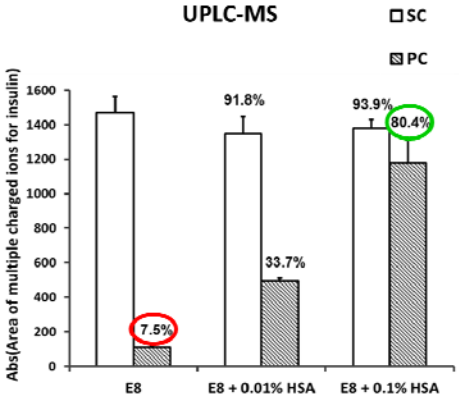

Figure 5. UPLC-MS analysis of SC and PC E8, E8 + 0.01% HSA and E8 + 0.1% HSA.

Lastly, quantitative PI-based flow cytometry revealed, on day 3, ~57% dead cells for hPSC aggregates cultured with PC E8, representing a drastic loss of viability compared to 4-6% for hPSCs cultured in SC E8. In presence of HSA, cell death at day 3 was limited to ~8. Pluripotency assessment at day 3 revealed that more than 90% of cells expressed NANOG and OCT4 with negligible differences among SC E8 with/without HSA and PC E8 + HSA, confirming that HSA did not affect pluripotency.

IV. DISCUSSION

The study revealed that, when the low-protein media E8 and TeSR-E8 were circulated in a peristaltic pump-based circuit, precipitation of insoluble particles and a marked insulin depletion were observed. The drastic reduction of soluble insulin induced severe viability loss in hPSC aggregates cultured in suspension. The addition of HSA did not prevent the precipitation of particles upon PC, but rescued soluble insulin, ultimately supporting hPSC culture. This study highlights the potential and the requirement of media optimization for automated hPSC processing and has broad implications on media development and bioreactor-based technologies, within and beyond hPSC manufacturing.

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