APPLICATION NOTE No. 472

Increasing iPSC Numbers through Systematic Culture Process Optimization

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Abstract

Human induced pluripotent stem cells (hiPSCs) are a powerful tool for innovative approaches, such as drug discovery, *in vitro* disease modelling, or regenerative therapies. However, such procedures require high cell numbers to be sufficiently applicable, a criterion that is hard to satisfy with traditional 2D culture methods. Stirred-tank bioreactors on the other hand offer a 3D culture environment suitable to provide, control, and maintain optimal growth conditions for the cell of choice. In this study, DASbox® Mini Bioreactors and the DASbox Mini Bioreactor System were utilized to systematically optimize process parameters of a hiPSC culture in a step-by-step process. This approach led to a more than 10× increase in cell density (almost 35×10^6 cells/mL) compared to uncontrolled conditions while stem cell features and viability were retained.

Introduction

Together with embryonic stem cells (ESC), induced pluripotent stem cells (iPSC) constitute the group of pluripotent stem cells (PSC). Both cell types have the ability to grow indefinitely and to develop into any cell lineage of the body [1, 2]. However, while ESCs are naturally occurring, iPSCs originate from reprogramming somatic cells *in vitro* by delivering specific factors [3]. Therefore, they can be created as needed. This dynamic availability paired with their stem cell properties make iPSCs a powerful tool for innovative approaches, such as drug discovery and *in vitro* disease modelling [4].

Furthermore, clinical trials are under way, opening the doors towards regenerative therapies, such as tissue repair for neurodegenerative diseases or heart failure [5]. In that context, one of the most important aspects of stem cell therapy is achieving high, clinically relevant cell numbers. Estimates for PSC therapies suggest cell doses of 10^9 cells for treatment of a single patient and production of 10^{11} to 10^{14} cells per year for a single product [6].

Such numbers cannot be sufficiently produced by conventional 2D monolayer culture methods in shake flasks or dishes [7]. The limited growth surface combined with the possibility to grow in only two directions can lead to undesired cell proliferation behavior, poor cell differentiation, improper gene and protein expression patterns, and an overall inaccurate representation of the

Disclaimer

All methods and results in this application note are published work by the group of Dr. Robert Zweigerdt, Leibniz Research Laboratories for Biotechnology and Artificial Organs, Hannover Medical School, Germany (doi: 10.1002/sctm.20-0453) [9],

We thank Dr. Zweigerdt for the permission to share these data.

3D environment the cells originate from [8]. On top of that, individual growth parameters, such as pH, dissolved oxygen (DO), culture feeding, or medium perfusion cannot be controlled in 2D cultures.

Bioreactors on the other hand offer an efficient way to mimic the natural habitat of stem cells more closely by providing a 3D setting and precise growth parameter control through bioprocess control units. Furthermore, scalability, the process of increasing the bioprocess dimensions, enables increased yields by transitioning to pilot and production scale once the optimal growth conditions are determined in small scale experiments.

The study described in this application note details such an optimization process for establishing human iPSC (hiPSC) culture at small scale level. Using DASbox Mini Bioreactors and the DASbox Mini Bioreactor System, multiple important growth parameters were systematically adapted in a step-by-step process in order to increase cell numbers during a 7-day-incubation period , as recently published [9,10]. The precise control of pH, nutrient feeding, DO, and agitation by the DASbox Mini Bioreactor System in combination with the perfusion operation mode and pre-culture optimization led to an increase from initially ~3 × 10^6 cells/mL after 7 days of incubation to ~18 × 10^6 cells/mL. Additional implementation of *in silico*-supported culture modelling increased the cell numbers even further to an unmatched cell density of almost 35 × 10^6 cells/mL. During this optimization process, the 60° pitched 8-blade impeller of the DASbox Mini Bioreactors, which is specifically designed for the culture of shear-sensitive cells in suspension or as aggregates, ensured excellent viability of the stem cells throughout the runs [11]. hiPSC cultured this way retained their stem cell properties, as well as the ability to develop into tissues of the endo-, meso-, and ectoderm.

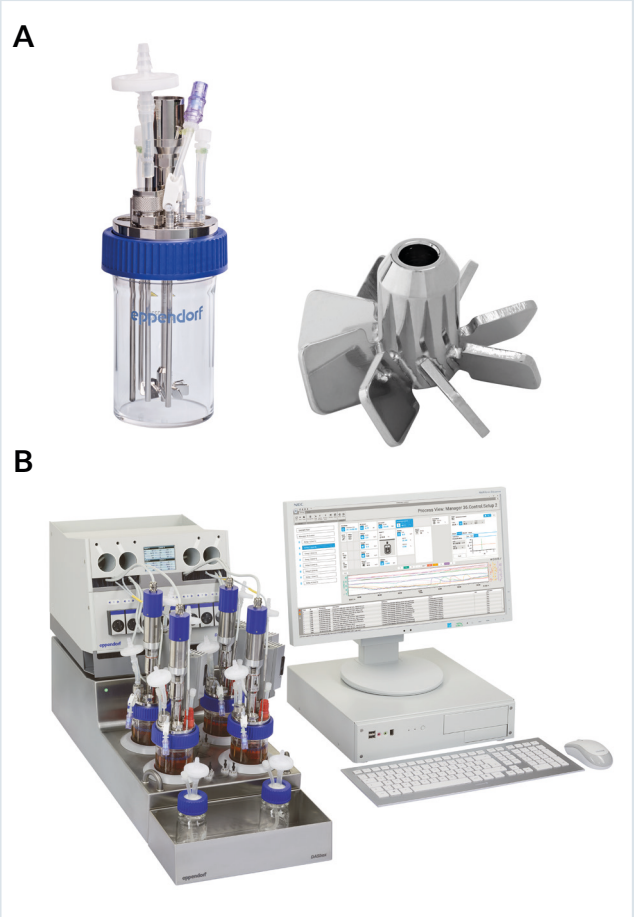

Figure 1: (A) The DASbox Mini Bioreactor is equipped with an 8-blade impeller (60° pitch) specifically designed for the culture of shear-sensitive cells in suspension or as aggregates. **(B)** The DASbox Mini Bioreactor System is designed as a 4-fold system able to handle up to 24 parallel operating bioreactors. It was developed for both cell culture and microbial fermentation applications and can be used with standard glass or single-use bioreactors.

Learn more about of the DASbox Mini Bioreactor System please visit: www.eppendorf.com/dasbox

Material and Methods

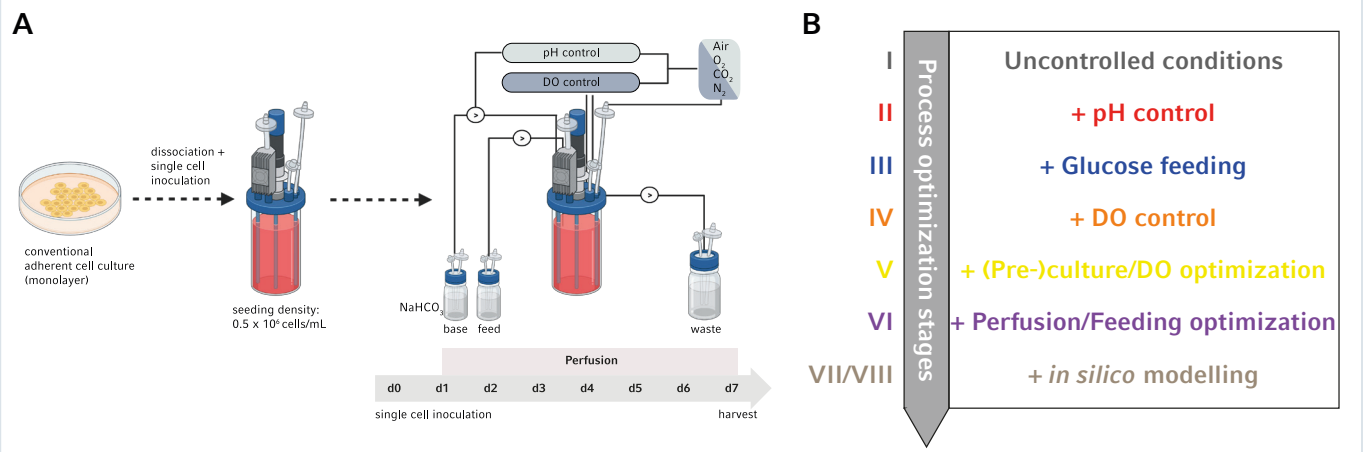

Figure 2: **(A)** Experimental procedure for iPSC culture parameter optimization in DASbox Mini Bioreactors under control of the DASbox Mini Bioreactor System (Created with BioRender.com). **(B)** Overview of the process optimization steps and nomenclature.

The DASbox Mini Bioreactors and DASbox Mini Bioreactor System

Stem cell culturing and culture optimization was performed in DASbox Mini Bioreactors equipped with an 8-blade impeller (60° pitch) optimized for stem cell expansion [11], an overhead drive for agitation, pH and DO sensors, as well as temperature control in order to ensure precise regulation of critical process parameters (Figure 1A).

Perfusion operation mode was enabled by an outflow filter device, allowing medium to flow in and out of the bioreactor while retaining the cells inside. By aligning the influx rate of fresh medium (feed) through a head plate port and the efflux rate of depleted medium (waste) through the outflow filter, the bioreactor volume was kept constant throughout the runs [12]. Medium flow was regulated via the pumps of the DASbox Mini Bioreactor System (Figure 1B).

The system was also used to monitor and adjust process parameters, such as pH, feeding rate, or DO. Pump and sensor calibration was performed according to the protocols by Kempf *et al*. [13] and Ackermann *et al.* [14].

Human induced pluripotent stem cell (hiPSC) lines

The cell culture experiments were conducted using three different hiPSC lines: hHSC_1285i_iPS2 (MHHi006-A) cells derived from hematopoietic stem cells [15], as well as Phoenix (HSC_ADCF_SeV-iPS2; MHHi001-A) and GMPDU_8 (CD34+hPBHSC_GMPDU_SeV-iPS8; MHHi008-A) cells, both derived from $CD34^+$ human cord blood hematopoietic stem cells [16], [17]. GMPDU_8 cells were derived under GMP-compatible conditions.

hiPSC pre-culture

Prior to the bioreactor experiments, the cells were expanded as a feeder-free monolayer culture in flasks at 37°C with 5% CO_2, using Essential 8 (E8) medium (Thermo Fisher Scientific®) supplemented with ROCK inhibitor (RI) Y-27623 (10 µM) (Figure 2A). The medium was changed after 48 hours for a passaging interval of 3 days and after 48 and 72 hours for a passaging interval of 4 days. See Manstein *et al.*, 2021 for the exact passaging protocol [9].

Culturing hiPSCs in DASbox Mini Bioreactors under control of the DASbox Mini Bioreactor System

All main experiments were performed as stem cell aggregate culture without the use of scaffolds for cell attachment. In order to inoculate the DASbox Mini Bioreactors, single-cell suspension was achieved by detaching the cell monolayer *via* StemPro™ Accutase™ (Thermo Fisher Scientific) for

the hHSC_1285i_iPS2 and Phoenix cell lines, or by EDTA-containing versene solution in case of the GMPDU_8 cells. Inoculation was performed with a viable cell density of 5×10^5 cells/mL E8 medium + RI to a final volume of 150 mL (75×10^6 cells/bioreactor) (Figure 2A).

Bioreactor cultivation conditions were 37°C with agitation speeds of either 60, 80, 100, or 120 revolutions per minute (rpm), and headspace gassing with 3 standard liters (sL) per hour with 21% O_2 and 5% CO_2.

If applied, pH control was initiated once the pH within the bioreactor reached ≤7.0. Hereafter, it was maintained at pH 7.0 first by reducing CO_2 in the gas stream and afterwards by addition of 1 M $NaHCO_3$.

Medium was not changed during the first 24 hours after inoculation. If not stated otherwise, the medium was then constantly replaced with E8 without RI at a flow rate of 150 mL/day (one culture volume/day or 6.25 mL/hour) while the cells were retained *via* the cell retention device.

During the optimization process, different compounds, such as glucose (either 3.15 - 6.15 g/L from day 3 or 6.15-7.65 g/L from day 4 onwards) or the shear force-reducing surfactant Gibco™ Pluronic™ F-68 (Thermo Fisher Scientific) were added to the medium.

The exact parameters of pH control, perfusion rate, or compound addition will be detailed within the respective results section. All the described processes were conducted for 7 days in the bioreactor. An overview of the optimization steps is given in Figure 2B and Table 1.

Table 1: Parameters controlled by the DASbox Mini Bioreactor System and the equivalent abbreviations used in this application note.

Process optimization steps	Abbreviations used in text
None	**I**
pH 7	**II**
pH 7, glucose feeding	**III**
pH 7, glucose feeding, DO control	**IV**
pH 7, glucose feeding, optimized pre-culture, optimized DO control, 80 rpm	**V**
pH 7, optimized pre-culture, optimized DO control, 80 rpm, optimized perfusion, optimized glucose feeding	**VI**
pH 7, optimized pre-culture, optimized DO control, 80 rpm, optimized perfusion, optimized glucose feeding, *in silico* modelling 1	**VII**
pH 7, optimized pre-culture, optimized DO control, 80 rpm, optimized perfusion, optimized glucose feeding, *in silico* modelling 2	**VIII**

Cell sampling, aggregate analysis, and cell counting

Cell samples were received from the bioreactor without interrupting the stirring. Aggregate formation was monitored by taking up to five independent light microscopic images per sample. Aggregate size was determined by the ImageJ software (Wayne Rasband, National Institute of Health). Mean diameters represent arithmetic averages of 45 to 1096 single aggregates.

Cell counting was performed after dissociating the aggregates into single cells via accutase-treatment [13]. Cell supernatants were stored at -20 °C for subsequent metabolite analyses (glucose and lactate).

Definitive endoderm and intestinal differentiation

Definitive Endoderm and Intestinal Differentiation was performed as described previously [18].

In brief, after bioreactor expansion cell density was determined and seeded at 10×10^5 cells/mL for differentiation in RPMI 1640 supplemented with 100 ng/mL of the transcription factor activin A and 3 µM of the GSK3beta inhibitor CHIR99021 in Erlenmeyer flasks (20 mL working volume).

Precisely 24 hours later the medium was replaced by 100 ng/mL activin A and 0.8% KnockOut Serum Replacement (Thermo Fisher Scientific) in RPMI 1640 (Thermo Fisher Scientific).

Again 24 hours later the medium was replaced by 100 ng/mL activin A and 8% KnockOut Serum Replacement in RPMI 1640. On day 3 of differentiation, aggregates were dissociated with Accutase for 3 minutes in a water bath at 37 °C, analyzed for definitve endoderm (DE) markers, and further differentiated into intestinal cells by plating down cells at 2×10^5 cells/cm^2 in intestinal medium consisting of DMEM/F12 (Thermo Fisher Scientific), 2% fetal bovine serum, 500 ng/mL Fibroblast Growth Factor (FGF) 4, 3 µM CHIR99021 and 1% penicillin/streptomycin. Medium was changed every other day until day 7, when cells were analyzed.

Statistical analyses

Statistical analysis for three to four independent bioreactor runs per culture condition were performed by the GraphPad Prism 6 software (GraphPad Software, Inc). One-way ANOVA or two-way ANOVA followed by Bonferroni's post-test were used to determine statistical significance. P-values of <0.05, <0.01, <0.001 were considered significant. Results are reported as mean and standard error of mean (SEM).

Results and Discussion

Influence of pH, glucose feeding, and DO control on hiPSC bioreactor culture

In order to increase cellular yields, the DASbox Mini Bioreactor System was employed to introduce precise monitoring and control over pH, glucose feeding, and DO to an otherwise uncontrolled setting (I, see Table 1). With the integrated DASware software, the bioprocess controller was able to automatically adjust and maintain a given growth condition. First, pH control with a setpoint of pH 7.0 (II, see Table 1) was initiated once a certain pH threshold was undercut, resulting in a steady culture pH value of 7.0 throughout the run while higher fluctuations and a generally lower pH were present in the uncontrolled setting (Figure 3A). The pH-controlled culture displayed a much higher glucose consumption over time compared to the uncontrolled setting where glucose levels stagnated 1 day post-inoculation (Figure 3B).

To compensate for the decrease of this important carbohydrate source, glucose feeding (3.15 - 6.15 g/L) was initiated from day 3 onwards in addition to the pH control (III, see Table 1) which resulted in higher culture glucose concentrations (Figure 3B) but also increased levels of lactate, a growth-inhibiting by-product of anaerobic glycolysis (Figure 3D). To provide a steadier oxygen supply, DO-control was introduced with a setpoint of 40% starting from the first day of culture (IV, see Table 1). This resulted in more stable culture oxygen levels, especially during later timepoints. However, initial DO levels (day 0-2) were lower compared to the other conditions (Figure 3C).

The resulting cell densities obtained from the different growth parameter settings imply an influence of the initial lowered oxygen levels under condition IV (see Table 1). Each other optimization step led to a significant increase in cell density compared to the 3.04×10^6 cells/mL from the uncontrolled situation at 7 days post-inoculation. Introduction of pH control achieved about 4.03×10^6 cells/mL and additional glucose supplementation even 6.13×10^6 cells/mL (Figure 3E). Additional maintenance

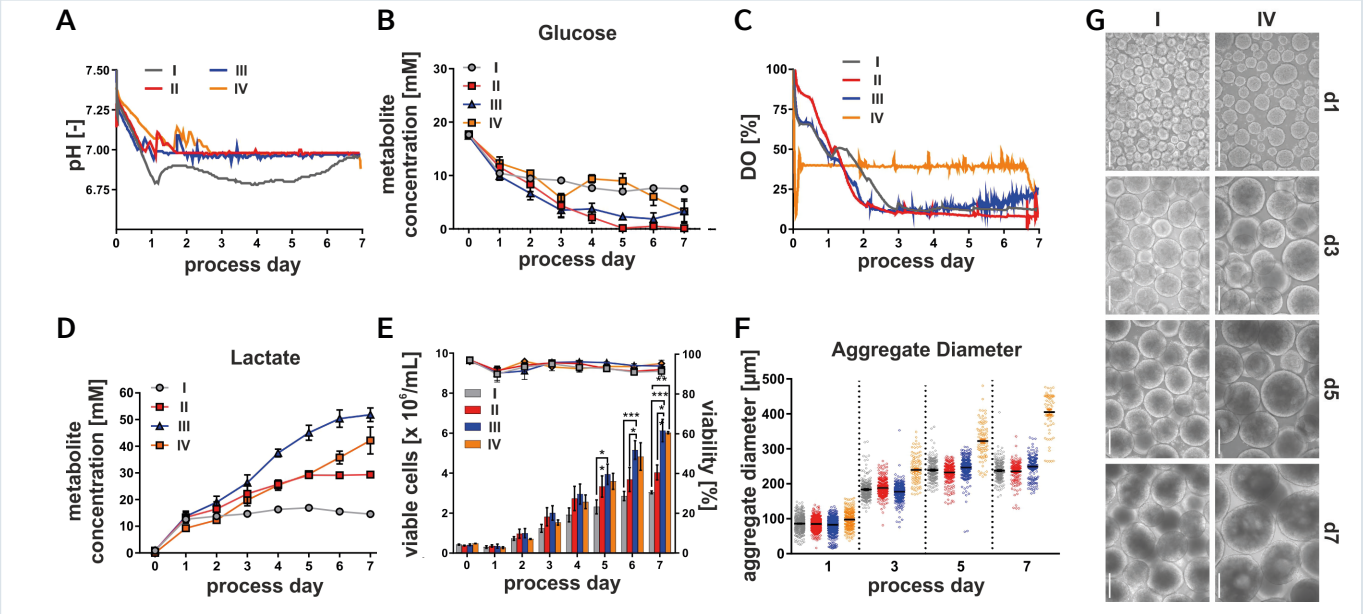

Figure 3: Effects of **(A)** pH (II, see Table 1), as well as additional **(B)** glucose feeding (III, see Table 1) and **(C)** DO control (IV, see Table 1) on **(D)** lactate levels, **(E)** cell density, and **(F/G)** cell aggregate size of hiPSCs in stirred-tank bioreactor culture in comparison to uncontrolled culture conditions (I, see Table 1).

of 40 % DO from the first day of culture however yielded only about the same levels as the glucose-supplemented culture alone (6 × 10^6 cells/mL) (Figure 3E). This might be explained by lower cell counts during the first days of culture, coinciding with the reduced DO levels compared to the other culture modes (Figure 3C/E).

Also, the employed DO-strategy resulted in larger cell aggregates by the end of the run (Figure 3F/G), possibly promoted by cell debris derived from a larger number of dead cells [19]. As oversized aggregates (>300 µm) can lead to detrimental oxygen and nutrient gradients, the size needs to be decreased.

Nevertheless, these experiments show already the positive impact of controlled pH values and glucose feeding with up to double the cell density compared to an uncontrolled culture system.

Furthermore, the observation that approach IV with additional DO control (see Table 1) yielded final cell densities comparable to the ones obtained from setting III with glucose feeding alone (see Table 1) suggested a possible compensatory effect of DO supplementation at later timepoints able to make up for the initial cell loss. Still, further DO strategy adaptation was necessary to reduce early cell loss and control aggregate size.

Controlling cell aggregate size and viability by pre-culture optimization, DO adaptation, and agitation adjustment

In order to overcome the initial cell loss and increased aggregate size in setting IV (see Table 1), a four-stage parameter adaption approach was conducted (Figure 4A):

(i) Pre-culture optimization was carried out by shortening the 2D culture period from 96 hours to 72 hours, resulting in a cell confluence of 65-75% instead of 90-95%.

(ii) A DO-level cascade was introduced, with an initial DO level of 100 % that was automatically stabilized at 40 % once this value was undercut within the medium.

(iii) The shear protectant Gibco Pluronic F-68 (Thermo Fisher Scientific) was added during inoculation in order to (iv) provide a better cell environment for aggregate size control achieved by increasing the agitation speed from the initial 60 to either 80, 100, or 120 rpm.

While all three agitation speed adjustments successfully reduced the initial cell loss (now resulting in a typical lag phase during the first 24 hours of incubation) (Figure 4B), 80 rpm were already sufficient to keep the aggregate size below the critical limit of 300 µm for the entire run (Figure 4C). These measures combined with the gentle stirring properties of the pitched 8-blade impeller led to cell densities comparable to the 9.5 × 10^6 cells/mL achieved by 60 rpm at 7 days of incubation in this setting (Figure 4B). It is also worth noting, that despite the higher agitation speed cell viability remained comparable between all conditions (Figure 4B). Thus, in order to strike a balance between aggregate size control and minimal shear forces on the cells, 80 rpm were used from here on out for the following experiments (V, see Table 1).

Optimized feeding and perfusion rate

As stated earlier, addition of glucose is beneficial to the cell culture growth but also increases the levels of the growth-inhibiting metabolite lactate. Thus, in order to enable increasing glucose levels and to prevent the rise of lactate to cell growth-inhibiting levels, the medium perfusion rate was stepwise increased from 1 to 2 culture-volumes/day between culturing day 2 and 5. At the same time, glucose concentration was increased from 3.15 to 6.15 g/L between

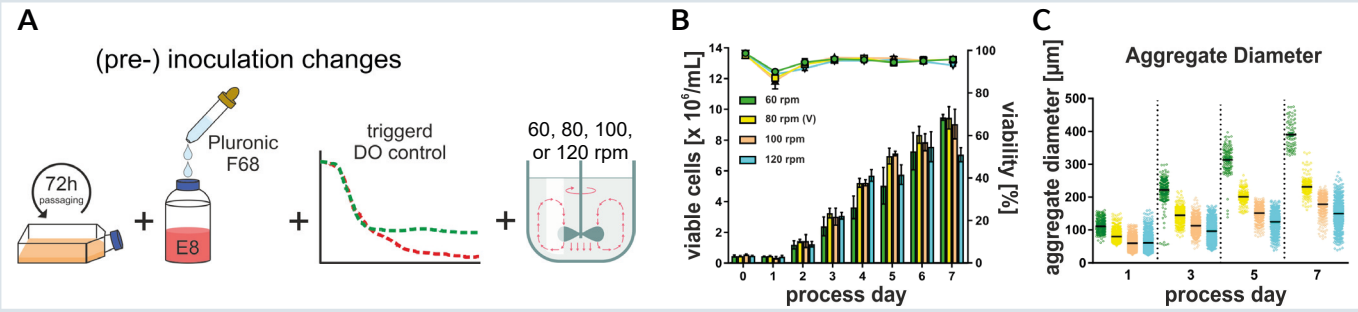

Figure 4: Preventing initial stem cell loss and controlling cell aggregate size by **(A)** pre-culture optimization, as well as DO and agitation control. Effect of the optimization steps described in **(A)** on **(B)** viable cell density and **(C)** aggregate size.

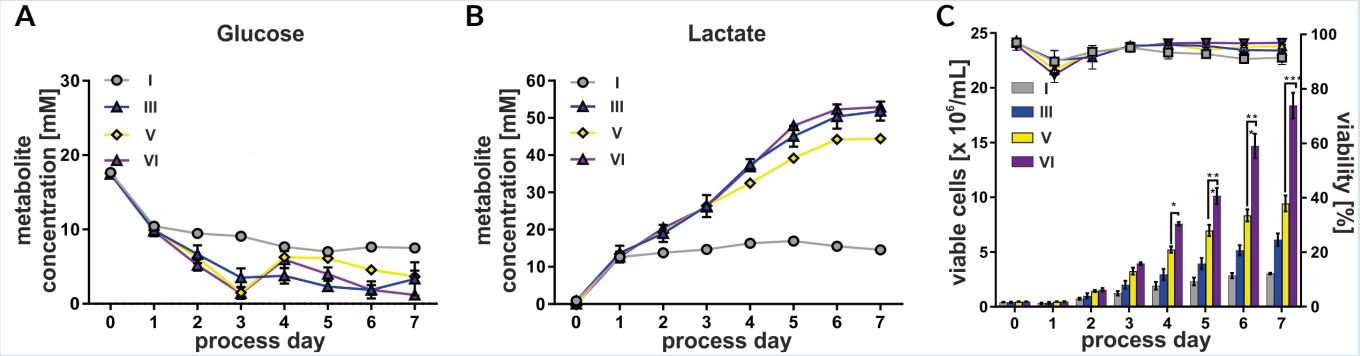

Figure 5: Effects of optimized medium perfusion (VI, see Table 1) and glucose feeding on **(A)** glucose and **(B)** lactate levels, as well as **(C)** viable stem cell density.

day 1 and 3 to 6.15 to 7.65 g/L from day 4 onwards (VI, see Table 1) (Figure 5A). With this optimized glucose feeding strategy, also stronger lactate production was to be expected that should be counteracted by the elevated medium exchange facilitated by the higher medium flow rate.

Indeed, lactate levels in this setting remained comparable to the non-feeding-optimized approaches (Figure 5B). Yet, cell density increased once more to 18 × 10^6 cells/mL (Figure 5C), almost double the amount achieved during the last optimization step.

Thus, compared to uncontrolled conditions (I, see Table 1) with 3.04 × 10^6 cells/mL, the combination of pH, glucose feeding, DO, agitation, and perfusion rate control along with an optimized pre-culture resulted in a sixfold increase in cell density at day 7 post inoculation.

Further culture optimization by *in silico* modelling

At this point, the precise parameter control provided by the DASbox Mini Bioreactor System and the gentle cell mixing with the 8-blade impeller of the DASbox Mini Bioreactor

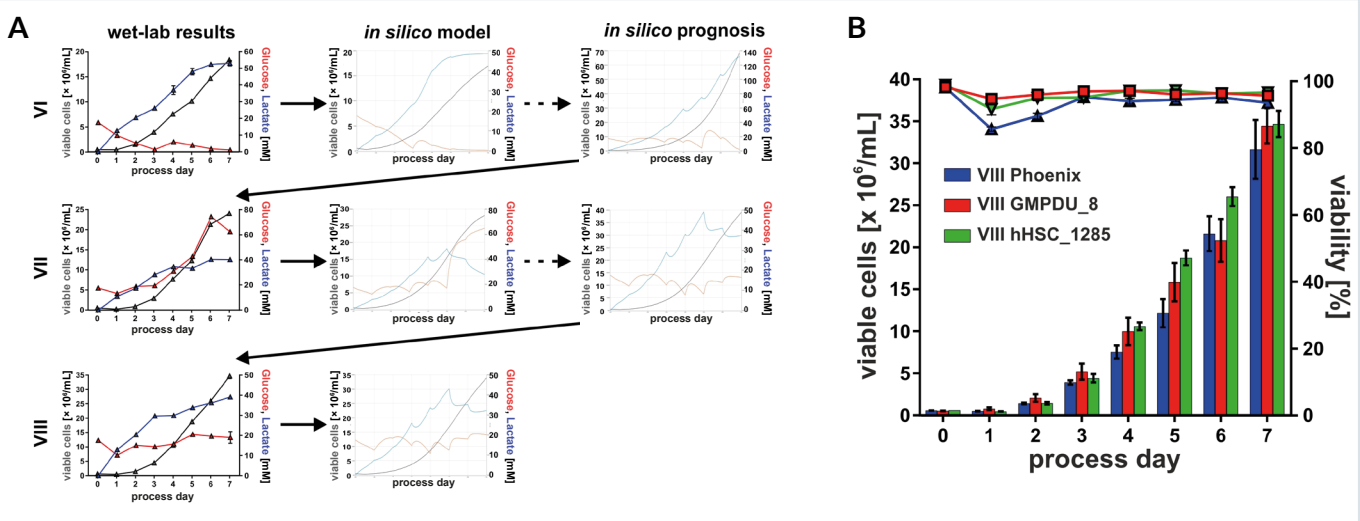

Figure 6: **(A)** *In silico* modelling-supported cell density increase shown **(B)** for 3 different stem cell lines.

resulted already in a remarkable stem cell number increase. To further push the achievable cell density and to reduce the workload of testing each parameter adaptation in a wet-lab setting, culture optimization was next supported by *in silico* modelling.

Using the Berkely-Madonna software (https://berkeley-madonna.myshopify.com/), the latest wet-lab results achieved by setting VI (see Table 1) were fed into an algorithm to predict further parameter optimization approaches (Figure 6A, first row).

This resulted in model VII (see Table 1) with a predicted cell density of 70×10^6 cell/mL on day 7 of culture. However, wet-lab results of the suggested model reached cell densities of 23×10^6 cells/mL (Figure 6A, second row). Even though these numbers surpassed all the previous approaches, they were lower than expected, probably due to an unexpected glucose peak by day 6 of culture, indicating the need for further modelling adaptation.

To further focus the precision of the modelling process, model VII (see Table 1) wet-lab results were fed into the software once more, resulting in the model VIII approach (see Table 1). Now, the wet-lab results resembled the predicted 40×10^6 cells/mL much closer, reaching 33×10^6 cells/mL, almost 10× the density of uncontrolled conditions (Figure 6A, third row).

These unmatched numbers of almost 5×10^9 cells in 150 mL culture volume were confirmed by using three different stem cell line culture runs under the model VIII (see Table 1) conditions with cell viabilities comparable to previous runs (Figure 6B).

Stem cell properties of hiPSCs cultured in a stirred-tank bioreactor

The previous results demonstrate that process optimization through precise parameter control in a stirred-tank bioreactor enables both higher cell numbers and robust cell viability.

Now, the next step was to test if the resulting stem cells were still retaining their expected stem cell properties after such a strong growth period. For that, cells were analyzed for the typical pluripotency markers TRA-1-60, SSEA-4, OCT-3/4, NANOG, SOX2 and KI-67 after 7 days of culture. As shown in Figure 7A and B, cells postive for each marker could be identified by flow cytometry and immunofluorescence microscopy.

Another important property of iPSC is the ability to differentiate into various cell types of the endo-, meso- and ectoderm germ layer. Therefore, the presence of cell differentiation capabilities was tested by conventional undirected differentiation, which revealed the expression of germ layer-specific markers Desmin (DES), SOX17 and TUBB3 (Figure 7C). Furthermore, application of specific differentiation protocols to hiPSCs cultured for 4 and 7 days to generate definitive endoderm, intestinal progenitors and cardiomyocytes resulted in the reproducible induction of 85 – 95 % of these progenies (Figure 7D). Additionally, it is worth noting that no chromosomal abnormalities were observed for the process-derived cells after 7 days of culture, as exemplarily shown for hHSC_1285 in Figure 7E. Together, these data suggest that the pluripotent stem cell population maintained all expected key properties after cultivation to high cell densities achieved by process optimization in a stirred-tank bioreactor.

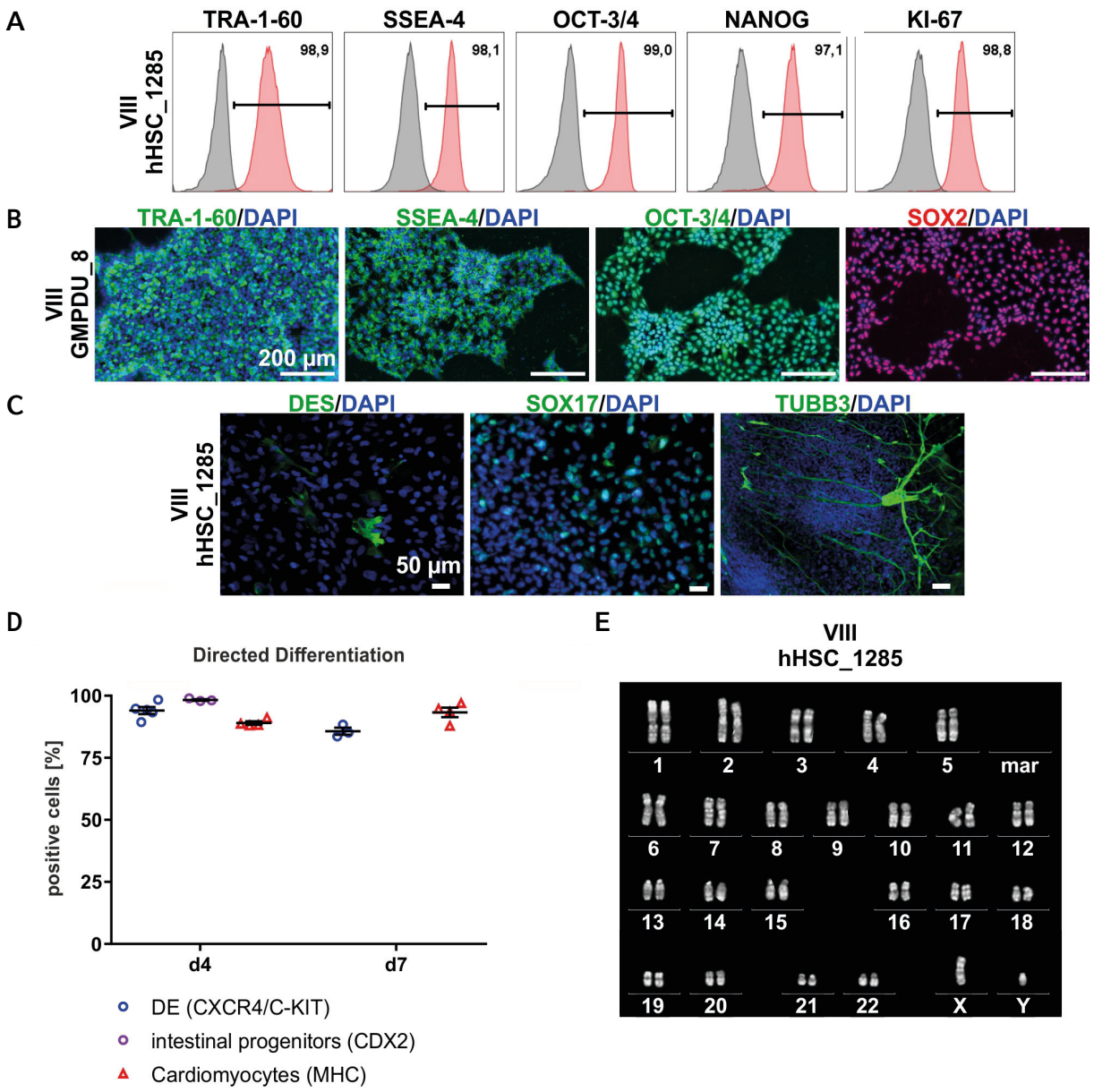

Figure 7: Stem cell properties of hiPSCs cultured in a stirred-tank bioreactor. Analysis of pluripotency-associated marker expression in undifferentiated hiPSCs by **(A)** flow cytometry and **(B)** immunofluorescence microscopy, as well as **(C)** in hiPSCs differentiated towards the three germ layers. **(D)** Differentiation of process-derived (d4 and d7) GMPDU_8 aggregates into definitive endoderm (DE), intestinal progenitors and cardiomyocytes. **(E)** Karyotype of cells cultured for 7 days under condition VIII (see Table 1).

Conclusion

The data described in this study demonstrate the power of a controllable and adjustable growth environment. Using the precise parameter control capabilities of the DASbox Mini Bioreactor System in combination with systematic adaptation and *in silico* process modelling enabled the increase of stem cell densities from about 3 × 10^6 cells/mL in an uncontrolled setting to almost 35 × 10^6 cells/mL (Figure 8). This translated to a total cell number of almost 5 × 10^9 cells within the whole culture volume. Furthermore, the specifically designed 60° pitched 8-blade impeller of the DASbox Mini Bioreactor enabled efficient mixing, aggregate size control, and high cell viability, while keeping shear force stress low at the same time.

We believe that this step-by-step parameter adaptation represents one of the most efficient ways to approach process optimization and development for stem cell culture in bioreactors. The procedure described here can act as a roadmap to identify and overcome cultivation bottlenecks, increase stem cell numbers, and advance the field of stem cell applications.

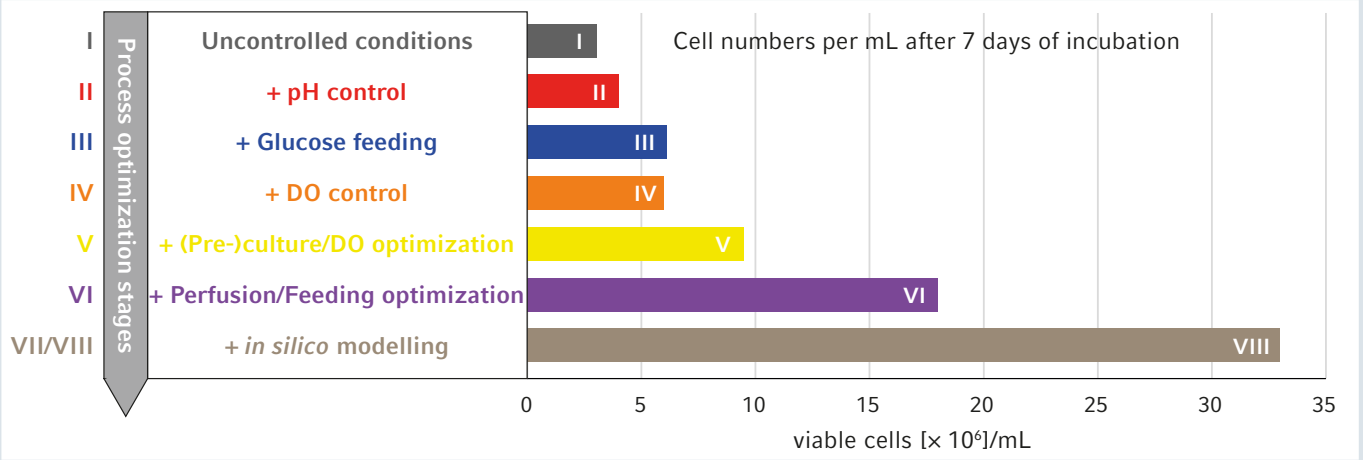

Figure 8: Summary of the process optimization steps along with the achieved cell densities.

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Supplementary Information

Supplementary Table 1: : Culture condition abbreviations used in this application note and the equivalent from the original publication [9].

Abbreviations used in text	Nomenclature in the original publication
I	pUC
II	p7
III	p7G
IV	p7GO
V	p7GOS80
VI	p7GOS80oF
VII	Stg1M
VIII	Stg2M

Ordering information

Description	Order no.
DASbox® Mini Bioreactor System, for cell culture, 4-fold system	76DX 04C C
DASbox® Mini Bioreactor, cell culture, glass, stainless steel head plate, temperature, pH, DO, and level sensors	76DS 025 0OD SS
Pitched-Blade Impeller, 8-blade, 60° pitch, stainless steel, O.D. 34 mm, I.D. 5 mm	7810 060 4
DASbox® overhead drive,	76DX OHD
DASbox® exhaust system,	76DX OFF
DASbox® exhaust condenser, Peltier	76DX CON D
Compression Fitting, complete, with Pg 13.5 male thread, I.D. 12 mm	7853 228 4
Triple Port, Pg 13.5 thread, 3 tubes with O.D. 4 mm × L 85 mm, all parts included	7870 641 4
Pipe, stainless steel, with barb, O.D. 4 mm/I.D. 2 mm, L 225 mm	7810 702 3
Compression Fitting, complete, with Pg 13.5 male thread, I.D. 6 mm	7853 228 3
L-Sparger, stainless steel, complete, O.D. 6 mm, L 300 mm, W 63 mm	7710 202 2
Pump Head Tubing, for DASGIP MP8 pump, Bioprene, I.D. 0.5/W 1.05 mm, female/female	7851 011 8
Pump Head Tubing, for DASGIP MP8 pump, Bioprene, I.D. 1.0/W 1.05 mm, male/female	7851 010 9
Feed Line, with 2× Luer lock fittings, male/male, C-Flex, I.D. 0.8 mm, L 1 m	7851 030 9
Feed Line, with 2× Luer lock fittings, male/male, C-Flex, I.D. 0.8 mm, L 2 m	7851 031 0
Sampling Accessory, with swabable valve	7851 014 5
DASware® control software, including PC, OS, and licenses, for 4-fold DASbox® Mini Bioreactor System	7860 016 7
Eppendorf Safe-Lock Tubes, 1.5 and 2.0 mL	0030 120 086

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AA472-020-01-092023-STS