Investigating 2D Cell Culture Optimization for Large-Scale Production of IPSCs

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Introduction

Large-scale culturing of induced pluripotent stem cells (iPSCs) is a prerequisite to fulfil the great potential these cells hold in regenerative medicine, drug discovery and disease modelling. For adherent culture of iPSCs, the interaction with substrate material is critical for the proper growth and maintenance of pluripotency. However, it is unclear how various parameters affect cellular growth and phenotype. In this study, we employed design-of-experiments (DoE) methodology to investigate the influence of coating, seeding density and exposure to ROCK inhibitor (ROCKi) on iPSC growth and pluripotency.

Significant interactions between these critical parameters were found, revealing that specific combinations affect the survival and proliferation of iPSCs. Characterization revealed that even under these conditions, expression of pluripotency markers was retained.

Objectives

To fully realize the therapeutic potential of iPSCs, billions of high-quality iPSCs must be generated per patient with consistent, reproducible and automated methods. With technological advances like the SCINUS Cell Expansion system, together with microcarrier culture, cell numbers can be upscaled in a controlled manner to generate clinically relevant numbers. However, optimization of iPSC culture on microcarriers to ensure high-quality expansion remains a significant barrier.

This study aimed to elucidate effects of critical culture parameters on iPSCs in Laminin-511 (LN511), Laminin-521 (LN521) and Synthemax II-SC coating environments to optimize iPSC culture for further applications with microcarrier culture. This information can direct further optimization efforts of iPSCs culture, such as those using microcarriers where bead-to-bead transfer is a requirement for optimal growth.

Methods

iPSCs were adapted to LN511, LN521 and Synthemax II-SC™(SM2) coated surfaces, respectively. Using Design-of-Experiments software, central composite (face-centered), randomized designs were created using three factors: seeding density, coating concentration and ROCKi exposure. For all points of the design, iPSCs were seeded in the corresponding density (range: 500 – 2000 cells/cm^2) and exposed to ROCKi for 1 – 3 days in 6-well plates. Coating concentrations depended on the coating material used (Table 1). Population doubling time (PDT) after 7 days was used as the primary response of the model to generate surface response models (RSM).

Table 1. Range of coating concentrations respective to substrate type.

Coating Material	Highest Coating Limit	Mid-Range Coating Limit	Lowest Coating Limit
LN511	0.25 ug/cm^2	0.0833 ug/cm^2	0.05 ug/cm^2
LN521	2.00 ug/cm^2	1.20 ug/cm^2	0.40 ug/cm^2
Synthemax II-SC	5.21 µg/cm^2	2.73 µg/cm2	0.52 µg/cm2

Resultant significant models were used to determine optimal combinations of all factors. Using point prediction, two conditions were investigated based on reducing PDT of cultures (Cond. 1) and reducing PDT along with ROCKi exposure and coating material (Cond. 2). These conditions were used to validate each model and resultant cell populations were characterized using flow cytometry (Tra-1-60, SSEA-4 and CD13) and immunocytochemistry (Oct3/4 and Sox2).

Results

DoE Analysis and Model generation

Significant response surface models (RSMs) were generated for all coatings. For biological coatings (LN511 and -521), significant interaction was observed between coating concentration and ROCKi exposure. While for the synthetic coating (SM2), two significant interactions were observed between seeding density and coating concentration, and between coating concentration and ROCKi exposure. These results depict the effect of each factor interaction on the proliferative ability of iPSCs under various conditions.

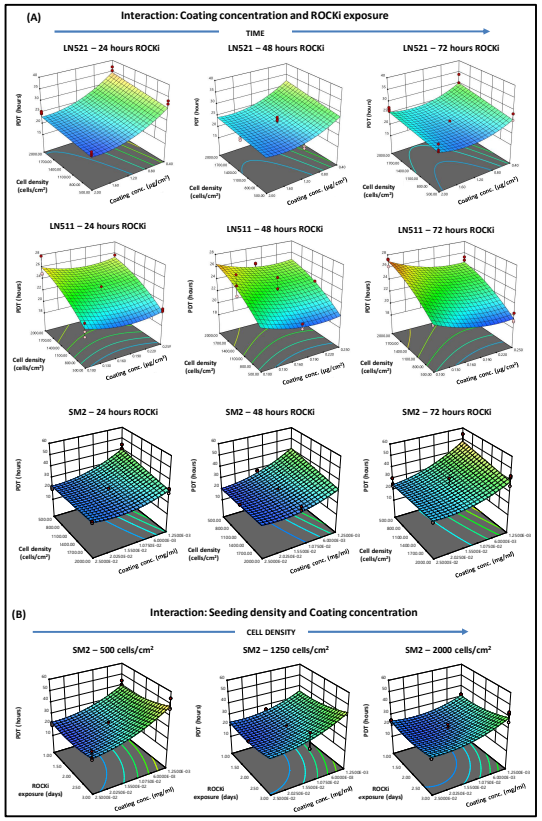

Figure 1. (A) RSMs depicting the significant interaction between ROCKi exposure and coating concentration on PDT values for LN511, LN521 and Synthemax II-SC. (B) RSMs depicting the interaction for iPSC PDTs cultured on Synthemax II-SC surfaces, between seeding density and coating concentration.

Results

Model Validation

For every coating, via point prediction analysis the RSM was navigated to predict the combination of factors that resulted in:

Condition 1: Lowest PDT

Condition 2: Lowest PDT and minimized use of ROCKi and coating material

These combinations were then used to culture iPSCs (n=3) and evaluate whether the resultant PDTs were in the range predicted by the model. For almost all combinations, the model correctly predicted PDT values (Figure 2). The PDT for LN521 Condition 2 was slightly overestimated by the model.

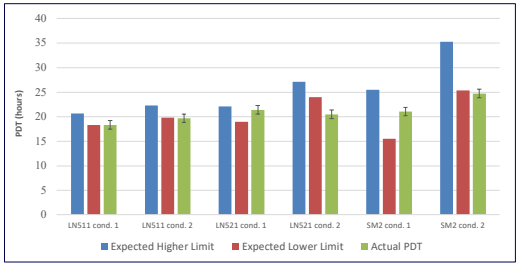

Figure 2. Model validation depicting the predicted lower and higher limits of PDT responses versus actual PDTs obtained using the results of the models generated with LN511, LN521 and SM2 substrates (n=3) (confidence = 95%).

Pluripotency Marker Retention – iPSC characterization

iPSCs were cultured in parallel with validation cultures for each condition. Cultures were then processed for analysis of marker expression when they were approximately 70-80% confluent.

With flow cytometry, hiPSC-specific markers SSEA-4 and TRA-1-60, and differentiation-specific marker CD13 were investigated. All samples showed high expression of pluripotency-specific markers (>99%) and little expression of differentiation-specific CD13 (<2%, see Figure 3).

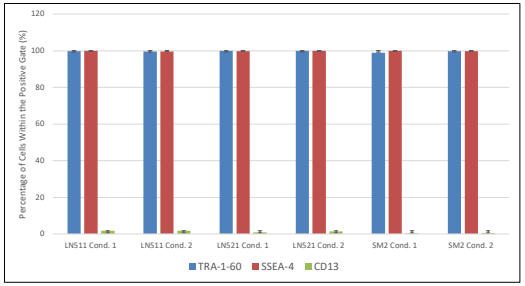

Figure 3. Pluripotency makers TRA-1-60 and SSEA-4, and the differentiation marker CD13 were investigated for iPSCs on LN511, LN521, SM2 using flow cytometry. Cultured cell populations retained their pluripotency characteristics .

Results

With immunofluorescence, expression of nuclear, hiPSC- specific markers OCT3/4 and SOX2 were investigated. All samples depicted high expression of the pluripotency markers, except for LN521 samples (both conditions), where OCT3/4 expression was lower compared to cell bank control samples.

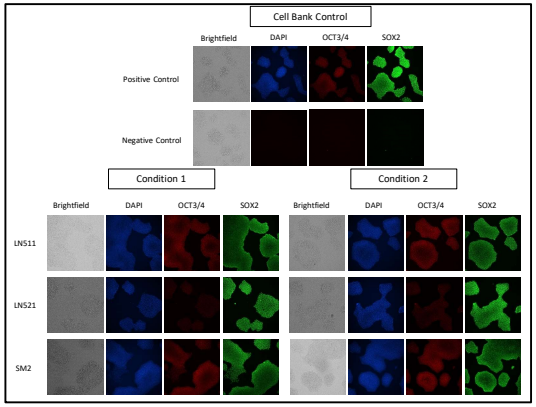

Figure 4. Cultured cell populations retained their pluripotency characteristics according to immunofluorescence staining.

Conclusion

This study optimized the culture parameters for iPSC cultivation using DoE methodology. The method yielded significant models with which combinations of critical culture parameters (coating concentration, seeding density and ROCKi exposure) could be optimized, with retention of pluripotency. These results can be used to direct large-scale iPSC cultivation, such as microcarrier-based expansion.

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