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ENABLING BIOLOGY IN VITRO

Application note No. 620

Efficient microcarrier-assisted 3D culture with beadMATRIX⁺

Summary

Microcarrier-assisted 3D culture of iPSCs enable the manufacturing of clinical relevant number sof iPSCs and their descendants for therapeutic applications. We previously developed a chemically defined coating for iPSC culture in 2D. In the further course we used our knowledge of biomaterial design to create beadMATRIX+. This product was qualified for the dynamic culture in 3D for iPSCs over three passages and the resulting iPSCs were characterized for pluripotency. We found that iPSCs from beadMATRIX+ proliferate fast and reliable over 3 passages (21 days) and remain pluripotent and genetically stable. The results show that beadMATRIX+ supports dynamic culture of iPSCs and can be implemented for efficient iPSC manufacturing for clinical applications.

Introduction

Induced pluripotent stem cells (iPSC) and their descendants carry the therapeutical potential for the amelioration of several diseases. To serve the vast amount of cells required for iPSC-derived therapies large-scale manufacturing is key. However, iPSCs are very sensitive to in vitro culture conditions. They require an optimal, fine tuned microenvironment to proliferate efficiently while maintaining their vulnerable stem cell state and genetic stability. As anchorage-dependent cells, the survival and pluripotency of iPSCs is highly dependent on the underlying substrate. For improved 2D culture of iPSCs denovoMATRIX has developed a chemically defined biomatrix called myMATRIX iPSCs. Nevertheless, large-scale culture in 2D remains a cost-, labor-, and space-intensive approach and leads to unfavorable media and waste gradients. Dynamic suspension culture in which iPSCs can be cultures as aggregates, microencapsulated, or on microcarriers, may overcome the aforementioned limitations. Those modalities protect to different extents the sensitive iPSCs from shear stress and vary in price, dissociation strategies, efficiency, and maintenance of the pluripotent state. High-density microcarrier-assisted dynamic culture of iPSCs has been shown as highly effective, especially for downstream differentiation (Eicke et al., 2018); (Sivalingam et al., 2021); (Sivalingam et al., 2018). Similarly, we have recently demonstrated that using microcarriers coated with our mesenchymal stromal cell-specific biomatrix in an upscale manufacturing procedure would result in the production of clinically required amounts of cells (White paper I PBS-0.1 MINI S.U.B, PBS Biotech; White paper 3D Manufacturing of Therapeutic hMSCs with RoosterNourish™-XF & beadMATRIX, RoosterBio, Inc.).

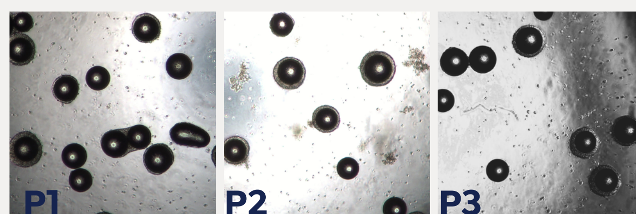

Figure 1. Appearance of iPSCs on beadMATRIX+ throughout the individual passages P1-P3.

Hence, we used our knowledge and expertise in biomatrix development to transfer myMATRIX iPSC from 2D to 3D by creating a biomatrix-coated microcarrier that supports iPSCs expansion in a bioreactor setup. Here, we show that the culture of iPSCs on beadMATRIX+ in the CERO bioreactor for 3 passages leads to an efficient cell expansion while maintaining their pluripotent state.

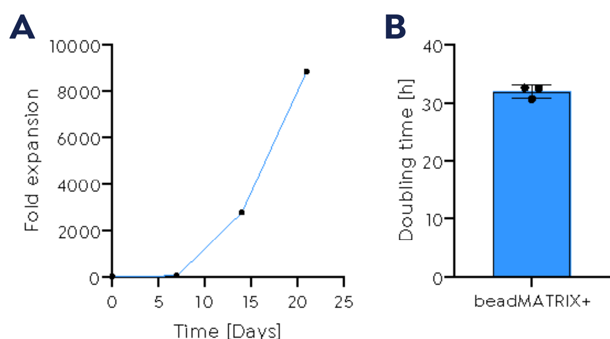

Figure 2. Growth kinetics of iPSCs on beadMATRIX+ in dynamic culture over 3 passages. A) Calculated fold increase over 3 passages corresponding to 21 days in culture, B) Doubling time of iPSCs on beadmMATRIX+ (Mean ± SD).

Results

In order to assess the performance of beadMATRIX+ for 3D iPSC culture we set up a 3-passage experiment (21 days) in the CERO bioreactor (OLS – OMNI Life Science). Cells have been thawed and passaged 2 times before seeding into the 3D setup. After each passage, cells were counted and characterized for 2D and 3D morphology, expression of pluripotency-related genes, and genetic abnormalities. Throughout the 3 passages, the iPSCs attached to the beads and built a thick cell layer around them (Fig. 1). We often observed agglomerates of cells with beads that increased in number during cultivation time.

During the 3D culture period, iPSCs showed highly comparable doubling times between individual passages 31.9±1.1h (Fig.2B). This would lead to a 9000-fold increase of the number of iPSCs within 21 days of culture in this small-scale setup, illustrating the potential for iPSC manufacturing (Fig.2A).

The successful and efficient expansion of iPSCs

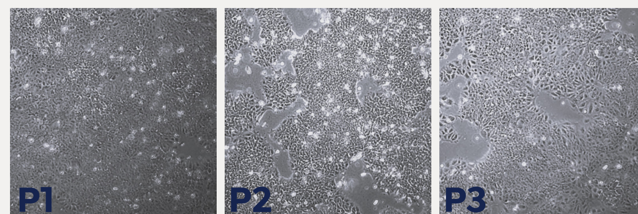

Figure 3. Morpholgical appearance of iPSCs in 2D after individual passages in dynamic culture on beadMATRIX+. After A) P1 B) P2 C) P3.

maintained their proliferation characteristics and the cells remained pluripotent after the dynamic culture. When cells were transferred onto the myMATRIX iPSC 2D surface after dissociation from the beads, they exhibited typical colony morphology with a high nuclear-to-cytoplasmic ratio (Fig. 3). In addition, we looked at the expression of OCT4, SOX2, SSEA-4, and TRA-1-60 after each passage of 3D culture. Over 90% of the iPSCs expressed these genes throughout the 21 days of culture (Fig. 4). We then assessed the genetic integrity of the iPSCs by karyotypic analysis and PCR of known copy number variations (Stemgenomics ddPCR, data not shown). After the 3 passages of microcarrier-assisted 3D culture, we found no numerical or structural abnormalities in the iPSCs (Fig. 5 A). In order to show the cells' capacity to differentiate into the 3 germ layers, we differentiated them in 2D. The qualitative assessment showed a high differentiation potential of iPSCs at P3 after dynamic culture (Fig. 5 B-D).

Methods

2D culture

Maintenance culture of iPSCs, fluorescence-activated cell sorting (FACS), karyotyping, and differentiation were performed as previously described (White paper No. 472).

3D culture

For 3D culture, cells were harvested from 2D using Accutase and subsequently inoculated in one CERO tube containing mTeSR1 supplemented with ROCK inhibitor Y-27632. The inoculation phase was set to: rotation period 3 sec, agitation pause

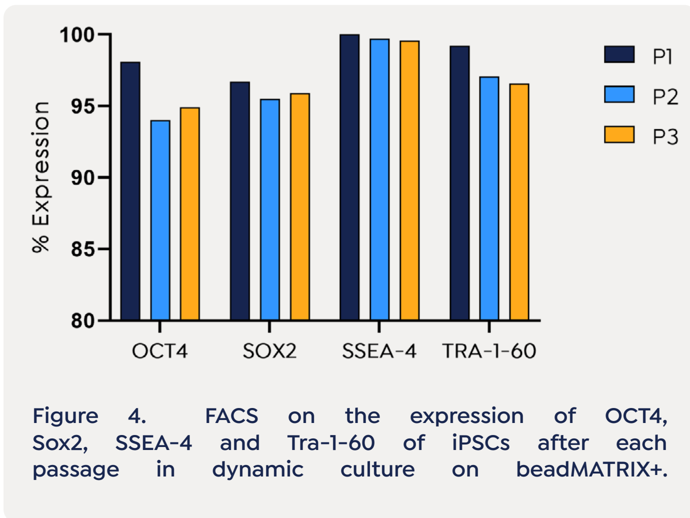

Figure 4. FACS on the expression of OCT4, Sox2, SSEA-4 and Tra-1-60 of iPSCs after each passage in dynamic culture on beadMATRIX+.

1 min, rotation speed 100 rpm, rotation pause 3 sec, agitation period 0 min, protocol duration 12 hours. The culture period was conducted for 7 days at the rotation period 1 sec, agitation pause 0 min, rotation speed 70 rpm, rotation pause 0 sec, agitation period 0 min. Cells were harvested from 3D using rotation period 1 sec, rotation speed 80 rpm, rotation pause 2 sec for 15 mins.

References

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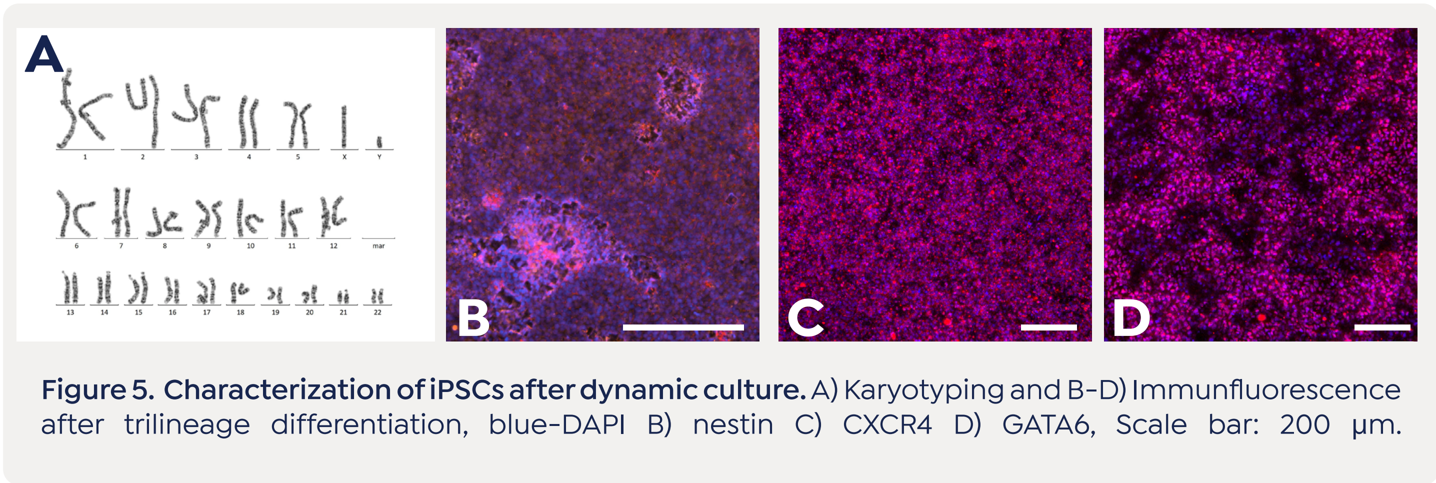

Figure 5. Characterization of iPSCs after dynamic culture. A) Karyotyping and B-D) Immunfluorescence after trilineage differentiation, blue-DAPI B) nestin C) CXCR4 D) GATA6, Scale bar: 200 µm.

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