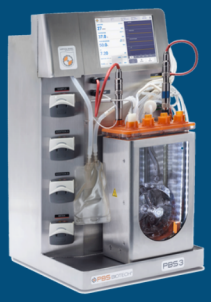
WHITE PAPER | PBS-0.1 MINI & PBS-3 S.U.B



Superior microcarrier-based iPSC expansion in PBS-3 Vertical-Wheel® Bioreactor

INTRODUCTION

Induced pluripotent stem cells (iPSCs) are used for a variety of cellular applications including toxicity studies, drug screening, disease modeling, and regenerative medicine. Although the number of cells required for each application might differ, an efficient expansion phase that reliably generates a large number of high-quality cells in a defined time period is a prerequisite for all of them. The most common method for the expansion of iPSCs is adherent culture on plastic-ware coated with commercially available matrices such as Matrigel™, Geltrex™, vitronectin, and laminins, or synthetic matrices such as Synthemax™. However, the cultivation of iPSCs in 2D is cost- and labor-intensive, restricted in scale-up potential, and requires highly trained personnel. Alternatively, 3D suspension cultures in bioreactors allow the production of clinically relevant cell quantities in a safe, scalable, reproducible, and cost-effective way. A dynamic suspension bioreactor provides a highly controlled environment in which important physiochemical parameters, such as dissolved oxygen level and pH, can be closely monitored and controlled. The dynamic system of bioreactors also improves the mixing of dissolved gasses and nutrients, avoiding unwanted gradients, and enables the use of alternative medium feeding methods such as perfusion. Despite many benefits, expansion in a bioreactor can introduce complex hydrodynamic forces that could adversely affect cell viability and pluripotency. Vertical-wheel bioreactors have been demonstrated to offer improved mixing characteristics such as a more uniform energy dissipation rate throughout the culture volume. They also reduce shear stress due to their unique vessel and impeller geometry thereby providing an optimal culture environment for shear-sensitive stem cells such as iPSCs[1] [2].

Different culture modalities have been employed for the expansion of iPSCs in bioreactors, e.g. aggregates, microcarriers, and microencapsulation. Aggregates form spontaneously when iPSCs are grown appropriately in suspension culture. However, this process requires a highly controlled setup to drive the formation of homogenously sized aggregates and to prevent the development of large entities (⩾500 µm) with high nutrient and oxygen diffusion gradients. Oversized aggregates can lead to decreased cell viability, induce spontaneous differentiation, reduce proliferation, and negatively affect the pluripotent state[3] [4]. Nevertheless, aggregate suspension cultures of 1 L-scale were successfully used to produce large quantities of iPSCs (up to 2×10^9 cells) with preserved pluripotent state[5]. While aggregates have been intensively studied as a 3D culture modality for iPSCs in suspension culture over the past decades, microcarrier and encapsulation culture of iPSCs must still be explored in depth.

Recently, the encapsulation of iPSCs as single cells or preformed cell aggregates in natural or synthetic hydrogels gained attention[6]. The formation of spherical capsules protects the cells against agglomeration and shear forces in suspension culture systems, improving viability and expansion rates while maintaining pluripotency. While natural hydrogels often suffer from batch-to-batch variety, a narrow property range, and a short degradation time, the mechanical properties and degradability of synthetic hydrogels are highly modular and tunable. However, the mechanical properties and other characteristics of synthetic hydrogels are used to regulate the stem cell fate of iPSCs, and thus for a reproducible outcome these characteristics must be tightly controlled. In addition, the complexity of regulating the size of cell aggregates used for encapsulation can also compromise the reproducibility of this technology.

Microcarriers can also facilitate the growth of iPSCs in 3D culture but have been utilized to a lesser extent, in part due to the lack of ready-to-use microcarriers for iPSCs and the laborious coating protocols for existing polystyrene microcarriers.In addition, microcarriers must be filtered from the cells upon harvest, which may result in shear stress, clogging of the filtration device, and kinetic damage to the cells. To circumvent these drawbacks a variety of biodegradable materials were used to produce digestible microcarriers, including dextran, collagen, and gelatin. The microcarrier system also bears multiple advantages such as providing a solid surface to support the growth and attachment of iPSCs while maintaining the benefits of a dynamic suspension culture. Their cell-free cores also reduce the formation of unwanted gradients of nutrients or gasses throughout the cell layers. Microcarriers could also improve differentiation efficiencies of iPSCs compared to aggregate culture, most likely caused by the more uniform exposure of the cells to cytokines and growth factors. For example, Eicke et al found a significantly increased yield of megakaryocytes when using laminin-coated microcarriers[7].

Due to their high sensitivity to culture conditions, iPSCs require an optimal, fine-tuned environment to proliferate efficiently while maintaining their vulnerable stem cell state and genetic stability. denovoMATRIX optimized the 2D microenvironment for iPSCs in vitro by developing a chemically defined biomatrix, called myMATRIX iPSC. To create a surface that supports iPSC cultivation in a bioreactor, this biomatrix was transferred to a polystyrene-based microcarrier. The beadMATRIX+ microcarriers showed efficient iPSC expansion when used in a small-scale bioreactor (up to 50 mL) for 3 passages while cells maintained their pluripotent state (denovoMATRIX application note 620).

In this study, we demonstrate beadMATRIX+ microcarrier culture of iPSCs in 0.1 L and 3 L Vertical-Wheel bioreactors (VWBR, PBS Biotech). We also compared their performance to aggregate culture (68-fold expansion) and found that beadMATRIX+-assisted iPSC expansion is more efficient (100-fold expansion).

RESULTS

Comparable cell yields of aggregate and microcarrier culture in a PBS-0.1 Mini

For autologous or allogeneic stem cell-based therapies 10^8–10^{10} cells per patient are theoretically required, demanding reliable culturing modalities, reproducible protocols, and cell therapy compliant materials. Here, we compared the proliferation of iPSCs on the newly developed beadMATRIX+ microcarriers versus aggregates in the PBS-0.1 Mini Vertical-Wheel® Bioreactor (PBS-0.1 Mini). First, PLX 1.0 hiPSCs (Pluristyx) were expanded in mTesR1™ (STEMCELL Technologies) on myMATRIX iPSC T-flasks for 2 passages before seeding into the PBS-0.1 Mini. The beadMATRIX+ culture was inoculated as single cells with 10 g/L microcarriers and 20,000 viable cells/mL with an agitation rate of 24 rpm. After 24 hours, the agitation rate was increased to 54 rpm. Aggregate culture was inoculated at the same cell concentration at an agitation rate of 60 rpm during the entire culture duration, 8 days. The expansion of iPSCs on beadMATRIX+ microcarriers showed a comparable growth profile to aggregate culture with a total cell concentration of 1.2 ± 0.06 x10^6 cells/mL and 1.4 ± 0.16 x10^6 cells/mL, resembling a 58- and 68-fold expansion, respectively (Figure 1A and C). Similarly, Vallabhaneni et al. observed comparable growth performance between vitronectin-coated microcarriers and aggregates although with a lower fold expansion rate[8].

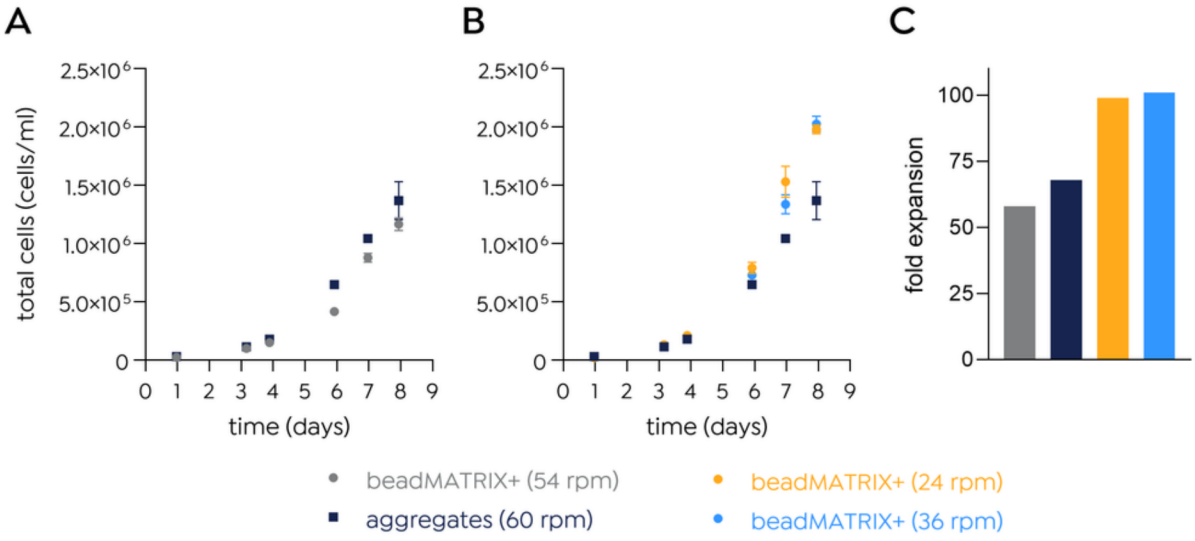


Figure 1. Establishment of beadMATRIX+-assisted iPSC culture protocol in PBS-0.1 Mini. iPSCs were grown either as aggregates or on beadMATRIX+ microcarriers in PBS-0.1 Mini Vertical-Wheel bioreactors. To optimize cell growth on beadMATRIX+, different agitation rates were tested: (A) 54 rpm and (B) 36 rpm versus 24 rpm. (C) Calculation of fold increase of cells/ml after 8 days in suspension culture. n=1

Optimization of agitation rate improves microcarrier culture cell yield

The optimization of suspension expansion protocols is crucial to achieve maximum proliferation of high-quality cells. One important parameter is agitation rate, since resulting shear stresses could exert a strong impact on cell quality, pluripotency, and growth. Although shear stress effects are reduced in VWBR, optimization of the agitation rate could improve culture outcomes dramatically. To identify the optimal agitation rate for beadMATRIX+ culture, we tested the expansion of iPSCs in the PBS-0.1 Mini at 36 rpm and 24 rpm and compared it to the standard protocol for aggregate culture (60 rpm). Both microcarrier conditions resulted in a higher growth rate with a final cell concentration of 2.0 ± 0.05x 10^6 cells/mL compared to aggregate culture with 1.4 ± 0.16 x10^6 cells/mL (Figure 1B). Figure 1C illustrates the improvement in cell expansion rates (x-fold) with decreasing agitation rates. Moreover, the comparable growth performance of iPSCs on beadMATRIX+ at 36 rpm and 24 rpm illustrates the compatibility of this microcarrier culture with various downstream processes, e.g., differentiation or extracellular vesicle production, by providing a large operating window for agitation rate adjustments.

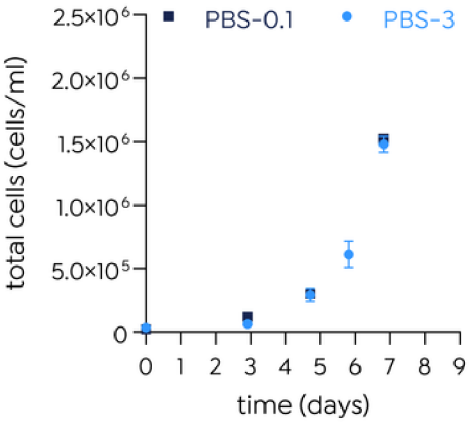


Figure 3. The beadMATRIX+ microcarrier-assisted iPSC expansion protocol demonstrates scalability. iPSCs were expanded on beadMATRIX+ microcarriers in VWBRs at the 0.1 L and 3 L scale, i.e., in PBS-0.1 Mini and PBS-3 bioreactor.

Seed train conditions do not impact subsequent suspensionculture

The production of iPSCs at scale requires a 2D seed train to generate the quantities of cells needed to inoculate a bioreactor. Therefore, we investigated the impact of the seed train conditions on subsequent cell expansion as aggregates or on beadMATRIX+ microcarriers. iPSCs were expanded for 2 passages either on Matrigel-coated T-flasks or on myMATRIX iPSC T-flasks and then seeded in PBS-0.1 Minis for either aggregate or beadMATRIX+ culture. Irrespective of the seed train conditions, the cells showed similar proliferation behavior on beadMATRIX+ illustrating the robustness of the process (Fig. 2A). The expansion of iPSCs in aggregate culture resulted in slightly reduced cell growth but again no impact of the seed train conditions was observed (Fig. 2B).

beadMATRIX+ culture in VWBRs provides consistency across scales

Next, we tested the scalability of our 3D culture beadMATRIX+ microcarriers and VWBR protocol. Cells were expanded on myMATRIX iPSC T-flasks for 2 passages and subsequently seeded on beadMATRIX+ microcarriers in a PBS-0.1 Mini and a PBS-3 VWBR. The agitation rates for the initial 24 hr post-inoculation were 24 and 15 rpm for the PBS-0.1 Mini and PBS-3 bioreactor,

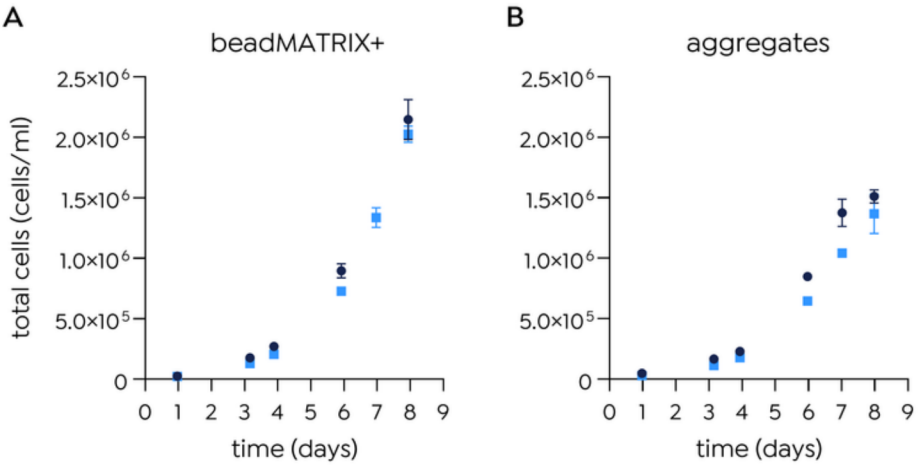


Figure 2. Impact of seed train conditions on suspension culture. iPSCs were cultured on myMATRIX iPSC or Matrigel-coated T-flasks for 2 passages before they were expanded (A) on beadMATRIX+ or (B) as aggregates in a PBS-0.1 Mini.

respectively. After 24 hr, the agitation rates were increased to 36 and 19 rpm, respectively. As shown in Figure 3, iPSCs expanded at the 0.1 L and 3 L scale showed comparable growth performance. This straight-forward scale-up was facilitated by the consistency of mixing patterns and hydrodynamic forces over scale in the VWBRs. As shown by Dang et al, this holds true up to the 15 L VWBR[9], and suggests the process developed in this study has the potential to be scaled up further, thereby simplifying the production of clinically relevant numbers of iPSCs.

Cells maintain iPSC-specific characteristics after beadMATRIX+ expansion

To evaluate the cell quality after their expansion on beadMATRIX+ in the PBS-3 VWBR, we analyzed their pluripotency phenotype, screened for genomic defects, and tested their differentiation potential. Flow cytometry analysis showed that iPSCs strongly express the pluripotency markers OCT4, SOX2, SSEA-4 and TRA-1-60 (>99%) after expansion in beadMATRIX+ microcarrier-mediated suspension culture (Fig. 4A). In addition, we confirmed the genetic integrity of the iPSCs by karyotypic analysis and PCR of known copy number variations (Stemgenomics ddPCR). After expansion on beadMATRIX+ microcarriers in PBS-3, we found no numerical or structural abnormalities (Fig. 4B) and no abnormal copy numbers in the iPSCs (data not shown). In order to show that beadMATRIX+ microcarriers-expanded iPSCs maintained their pluripotency, the cells were harvested and cryopreserved and then freshly thawed cells were differentiated into mesoderm, endoderm and ectoderm in 2D planar culture. The qualitative assessment showed a high differentiation potential of iPSCs after beadMATRIX+ culture at 3L scale. In summary, iPSCs expanded on beadMATRIX+ in the 3L VWBR produced cells in high quantity and high quality.

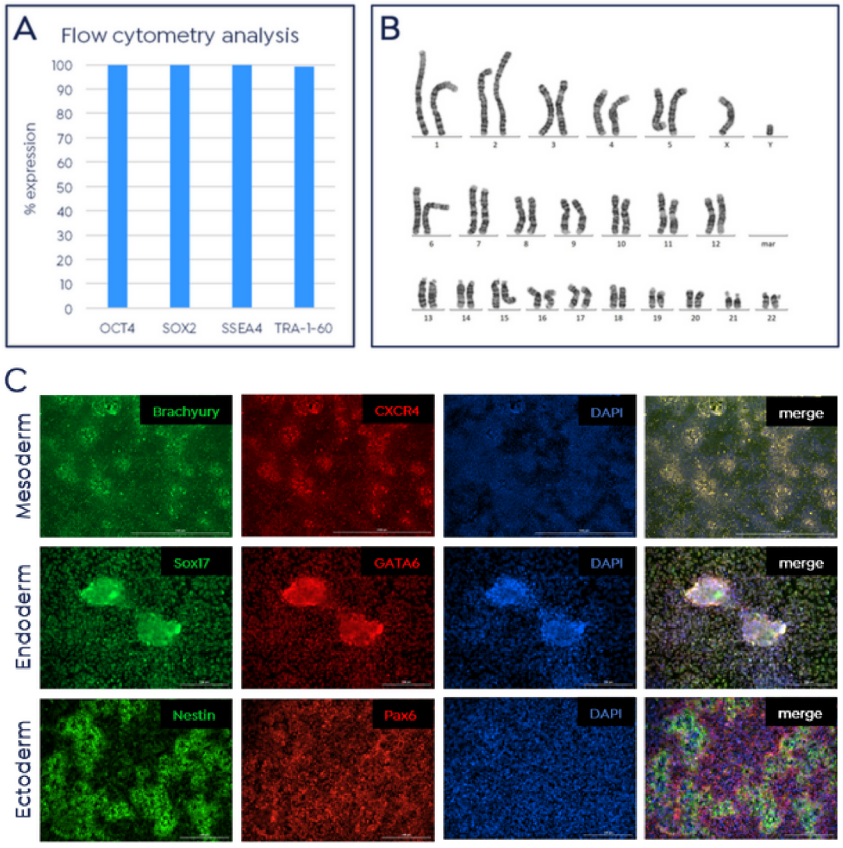


Figure 4. Characterization of iPSCs after suspension culture. (A) Flow cytometry on the expression of OCT4, Sox2, SSEA-4 and Tra-1-60, (B) karyotyping and (C) immunofluorescence after trilinieage differentiation. Scale bar, 1000 µm (mesoderm); 200 µm (endoderm/ectoderm).

CONCLUSION

In this study, we showed that beadMATRIX+ microcarrier-assisted iPSC expansion resulted in the production of a larger number of high-quality cells compared to aggregate culture in PBS-0.1 Mini. The efficient cultivation of iPSCs on microcarrier is dependent on the optimization of the agitation rate to establish an optimal culture environment that is beneficial for the proliferation of shear-stress sensitive iPSCs. Here, we identified a large operating window that enables the use of beadMATRIX+ at different agitation rates, allowing for process adaptation to various downstream processes, e.g., differentiation or extracellular vesicle production. The robustness of the beadMATRIX+ culture is further illustrated by our seed train culture experiment, which showed stable subsequent microcarrier culture and offers flexibility in the pretreatment of cells. Most importantly, beadMATRIX+ microcarrier-assisted iPSC expansion is consistent across scales since the expansion in the 3L VWBR resulted in comparable cell yields as in the PBS-0.1 Mini. Lastly, the culture did not influence the viability, pluripotency, genetic integrity, and differentiation potential of the expanded iPSCs.

In summary, we successfully developed a bioprocess to scale-up the beadMATRIX+ microcarrier-assisted expansion of high-quality iPSCs from 0.1 L to 3 L VWBR supporting the realization of iPSC-derived cell therapy applications.

REFERENCES

Borys BS, Dang T, So T, et al. Overcoming bioprocess bottlenecks in the large-scale expansion of high-quality hiPSC aggregates in vertical-wheel stirred suspension bioreactors. 2021. doi:10.1186/s13287-020-02109-4

2. Lee B, Jung S, Hashimura Y, et al. Cell Culture Process Scale-Up Challenges for Commercial-Scale Manufacturing of Allogeneic Pluripotent Stem Cell Products. 2022. doi:10.3390/bioengineering9030092

3. Lee B, Borys BS, Kallos MS, Rodrigues CA V, Silva TP, Cabral JMS. Challenges and Solutions for Commercial Scale Manufacturing of Allogeneic Pluripotent Stem Cell Products. doi:10.3390/bioengineering7020031

4. Bogacheva MS, Harjumäki R, Flander E, et al. Differentiation of Human Pluripotent Stem Cells Into Definitive Endoderm Cells in Various Flexible Three-Dimensional Cell Culture Systems: Possibilities and Limitations. Front Cell Dev Biol. 2021;9:726499. doi:10.3389/FCELL.2021.726499/BIBTEX

5. Keong Kwok C, Ueda Y, Kadari A, et al. Scalable stirred suspension culture for the generation of billions of human induced pluripotent stem cells using single-use bioreactors. 2017. doi:10.1002/term.2435

6. Poorna MR, Jayakumar R, Chen JP, Mony U. Hydrogels: A potential platform for induced pluripotent stem cell culture and differentiation. Colloids Surfaces B Biointerfaces. 2021;207:111991. doi:10.1016/J.COLSURFB.2021.111991

7. Eicke D, Baigger A, Schulze K, et al. Large-scale production of megakaryocytes in microcarrier-supported stirred suspension bioreactors OPEN. Sci REpoRtS |. 2018;8:10146. doi:10.1038/s41598-018-28459-x

8. Vallabhaneni H, Shah T, Shah P, Hursh DA. Suspension culture on microcarriers and as aggregates enables expansion and differentiation of pluripotent stem cells (PSCs). Cytotherapy. 2023;25(9):993-1005. doi:10.1016/J.JCYT.2023.05.002

9. Dang T, Borys BS, Kanwar S, et al. Computational fluid dynamic characterization of vertical-wheel bioreactors used for effective scale-up of human induced pluripotent stem cell aggregate culture. Can J Chem Eng. 2021;99(11):2536-2553. doi:10.1002/CJCE.24253

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