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APPLICATION NOTE No. 499

A Beginner's Guide to Mesenchymal Stem Cell Culture with the SciVario® twin Bioprocess Control Station

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Abstract

This publication is intended to provide a straightforward workflow to help users to culture human mesenchymal stem cells (MSCs) using Eppendorf stirred-tank bioreactors. We describe simplified workflow steps using the Eppendorf SciVario twin control station, including the preparation of MSCs for inoculation, expansion of MSCs in T-flasks and shake flasks, and MSC culture in bioreactors for cell production. To demonstrate the ease of use to beginners, we chose a bench-scale BioBLU® 1c Single-Use Bioreactor as the main vessel for this guide. The principles discussed in this document provide a foundational approach for scaling up MSC cultures, addressing the increasing demands for MSC-based cell therapy.

Content

Introduction

Stem cell-based therapy is at the forefront of cell and gene therapy (CGT), offering transformative potential in treating human diseases through personalized and regenerative medicine [1]. Mesenchymal stem cells (MSCs) are particularly prominent due to their accessibility from various tissue sources, such as bone marrow, adipose tissue, and umbilical cords, as well as their ability to differentiate into a wide range of tissue types [2]. MSCs play crucial therapeutic roles, including reducing inflammation through cytokine release, aiding tissue repair via growth factors, modulating immune responses, and functioning as structural cells in tissues like bone [2]. Their safety and efficacy have led to over 1,100 clinical trials, with Prochymal® being the first market-approved MSC product for allogeneic stem cell therapy [3].

While in vitro expansion of MSCs is well-established, transitioning these cells to stirred-tank bioreactor cultures requires substrates for attachment due to their adherent nature [4]. Microcarriers, typically 100-300 µm in size, are used to facilitate the transition from 2D to 3D suspension culture [5]. This adaptation from conventional suspension cultures, such as those used for CHO cells, necessitates specific medium exchange processes to separate the cell-microcarrier complex from the liquid before removal. This guide demonstrates an accessible MSC-microcarrier culture process in a stirred-tank bioreactor, enabled by the user-friendly SciVario twin controller.

The SciVario twin is a versatile bench-scale bioprocess controller capable of independently operating two bioreactors, making it ideal for cell culture and fermentation applications in R&D. Supporting both glass and BioBLU Single-Use Bioreactors, it offers a modular, customizable design that can be easily upgraded to meet evolving needs. Advanced process monitoring and control are achieved through the DASware® control 6 software, with the BioNsight® cloud solution allowing remote access and connectivity with other labs for data analytics. Its intuitive user interface simplifies process setup, guided by intelligent workflows, thus requiring no complex training.

Material

Cell lines, medium and microcarriers

MSCs for bioprocessing can be primary cells isolated from human body such as muscle, umbilical cord, bone marrow, or differentiated from induced pluripotent stem cells (iPSCs). Many commercial sources can provide cell source and medium for MSCs. Commercial sources, such as the American Type Culture Collection (ATCC), are available to obtain cryopreserved MSC vials, typically as 1-2 million cells per vial.

MSC culture medium originally started with DMEM supplemented with fetal bovine serum (FBS) in the early stages of development. In the CGT context, animal sourced reagents like FBS are discouraged for human use; in addition, chemically defined medium is preferred for better control of batch-to-batch consistency [6]. Accordingly, specialized MSC culture medium has been developed, using clinical grade growth factors to replace serum. MSC medium can be acquired from commercialized sources like the ATCC. Because MSCs are adherent cells, MSC cultures require a surface to attach to. Microcarriers have been favored for adherent cell cultures in bioreactors. Microcarriers are 100-300 µm beads made of biocompatible materials, including glass, polystyrene, dextran, and biological polymers like collagen [5]. Their structure and surfaces can be engineered for enhanced coverage per unit volume for maximized cell adhesion. Cell-laden microcarriers are mixed in stirred tank bioreactors as suspension culture. We found that the collagen-coated microcarriers performed better for supporting MSC cultures than non-coated polystyrene microcarriers (see Eppendorf Application Note 334, source 13).

As our application was primarily a proof-of-concept study, we had used DMEM plus 10% fetal bovine serum (FBS) in the beginning and transitioned to MSC specialized medium such as ATCC's MSC Basal Medium supplemented with IGF, EGF, 7% FBS and glutamine. We used collagen-coated polystyrene microcarriers.

In the following sections, we describe the basic steps of MSC bioprocessing from flask cultures to bioreactor runs.

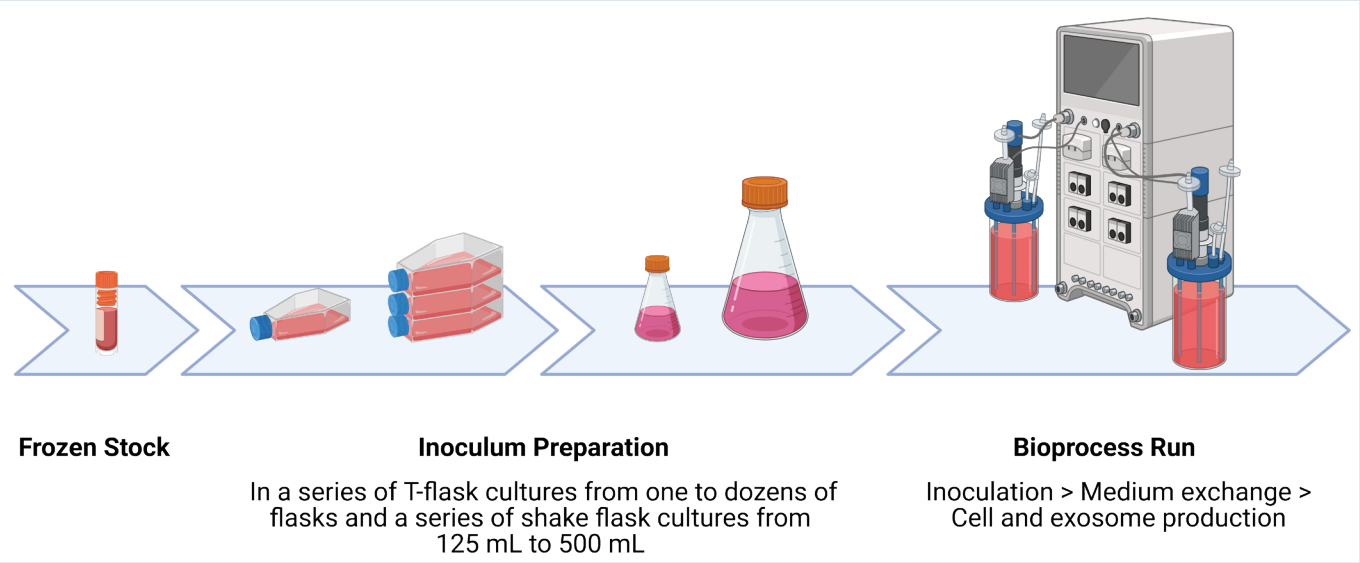

Fig. 1: MSC initial culture and bioreactor inoculum preparation workflow. Image created with BioRender.com

1. MSC initial culture and bioreactor inoculum preparation

From our experience, for the inoculation of a one liter bioreactor culture an inoculum of at least 200 million cells is needed based on an inoculation density of 0.2 million cells per milliliter. Compared to a 1 million cell cryovial stock, the 200-fold expansion needs to be realized in pre-culture before the bioreactor run. As illustrated in Figure 1, the MSC pre-culture expansion involves 2D T-flask culture and 3D shake flask culture.

1.1. 2D T-flask culture

MSCs are expanded from cryo vials in T-flasks, progressing to larger culture volumes. The initial culture is illustrated in Figure 1. T-flask culture is typical of all adherent cells with a seeding density of 1×10^4 and 5×10^4 cells/mL for routine subculture [7]. We passaged MSC T-flask cultures at split ratios of 1:3 to 1:5 upon confluency. T-flask cultures allow for visual examination of cell confluency, therefore, are useful tools for estimating cell saturation density, e.g., the maximum number of cells attainable per unit surface area (cm^2) or per unit volume (mL) of culture [7].

1.2. 3D Shake flask culture with microcarriers

Because of the limited surface area of T-flasks, ultimate yields are limited. Therefore, 3D microcarrier cultures in shake flasks are used as the next step for further expansion of MSCs. Microcarrier cultures benefit from a significantly larger surface area to volume ratio compared to monolayer culture. For example, a series of microcarrier cultures can proceed from 125 mL to 500 mL shake flasks and generate hundreds of millions of cells in one flask, compared to handling dozens of T-flasks for the same total yield.

The maximum MSC yield can be calculated in theory based on the total growth area of the substrate, and MSC surface density at confluency. For example, based on our T-175 cultures of MSCs, we determined a saturation density of 50,000 cells/cm^2 for the MSC line we used. Based on that, we can calculate the theoretical maximum cell densities for different microcarrier types given the same total surface area (Table 1).

Microcarriers are typically provided as dry powder. Preparation of microcarriers for cell culture involves washing with PBS, sterilization by autoclaving, and conditioning with cell culture medium. The most critical step of microcarrier cell culture is the initial cell adhesion, controlled by the cell and microcarrier mixing ratio and the mixing process.

Table 1: Example for MSC and microcarrier mixing calculation.

	SoloHill microcarriers	Cytodex 3 microcarriers	References & Notes
Material	Polystyrene (collagen-coated)	Dextran (collagen-coated)	
Area (cm^2) per g	360	2700	Product brochure
Size (µm)	125-212	120-180	Product brochure
Microcarrier loading density (g/L)	15	15	Examples based on published literature [8]
Total surface area (for 1 L of culture)	5400	40,500	
Cell seeding density by area (per cm^2)	3000	3000	Examples based on published literature [8]
Cell seeding density by volume ($\times 10^6$ per mL)	0.016	0.122	
MSCs maximum density by area (per cm^2)	50,000	50,000	Internal 2D T-175 studies; will depend on MSC source and culture medium
MSCs maximum density by volume ($\times 10^6$ per mL)	0.27	2.0	

1.2.1. MSC and microcarrier mixing ratio

In our workflows, microcarriers are loaded at a density between 10-20 g/L in bioreactors. With the manufacturer specs of the microcarrier, one can calculate the total surface area; based on the expected total cell numbers at full confluence. For each cell and microcarrier combination, initial experiments are needed to determine the optimal mixing ratio of cells and microcarriers upon cell seeding. Too few cells per microcarrier would result in too long lag times for cell expansion, or failure of cell growth especially for sensitive stem cells. Too many cells per microcarrier may not allow for much further cell growth, thus defeating the purpose of cell expansion.

1.2.2 MSC and microcarrier adhesion

Agitation is used to facilitate cell and microcarrier mixing; however, the speed of agitation needs to be carefully controlled to allow for efficient cell adhesion onto the microcarrier. If the agitation is too fast it may delay cell attachment to the microcarrier, resulting in fewer viable cells upon seeding. If it is too slow, the agitation may result in insufficient mixing and incomplete coverage. Shear stress from agitation also impacts MSC expansion and quality [9]. It has been found that intermittent fast agitation with steady agitation at a lower speed has the best outcome compared to a continuous agitation [10]. The initial cell adhesion step for each cell and microcarrier combination needs optimization to determine the appropriate cell-to-microcarrier ratio and agitation schedule.

For a beginner, success in shake flasks is fundamental before going into bioreactor. An example of MSC microcarrier culture in shake flasks is shown in the Eppendorf Application Note 259 "A Novel Method for the Expansion of Mesenchymal Stem Cells using a New Brunswick S41i CO_2 Incubator Shaker" (Figure 2) [11].

1.3. Inoculum preparation

The transition from flask cultures involves dissociating MSCs from seed train substrate (T-flasks or microcarrier in shake flasks), re-mixing and seeding MSCs onto new microcarriers, and introducing them to the bioreactor. The latter two processes can also be carried out directly in a bioreactor with optimized initial adhesion parameters.

We have tried several ways and found bead-to-bead transfer as an effective method for propagating MSC microcarrier cultures in bioreactors. Here we describe this method in comparison to the conventional method of mixing dissociated cells with microcarriers for the inoculum preparation.

1.3.1. Simplified cell inoculation with bead-to-bead transfer

The bead-to-bead transfer method uses a mixture of cell-populated microcarriers and the addition of fresh microcarriers to promote spontaneous cell migration to the fresh microcarriers, effectively subculturing MSCs without enzymatic dissociation [12]. This can avoid the laborious task of enzymatically detaching and re-seeding the cells.

In Application Note 334, we described the bead-to-bead transfer method for MSC and microcarrier cultures in BioBLU 5c Single-Use Bioreactors [13]. Essentially, an inoculum was generated in shake flask microcarrier cultures (e.g., 15-day culture). For bioreactor inoculation, the cell-laden microcarriers were mixed with fresh microcarriers

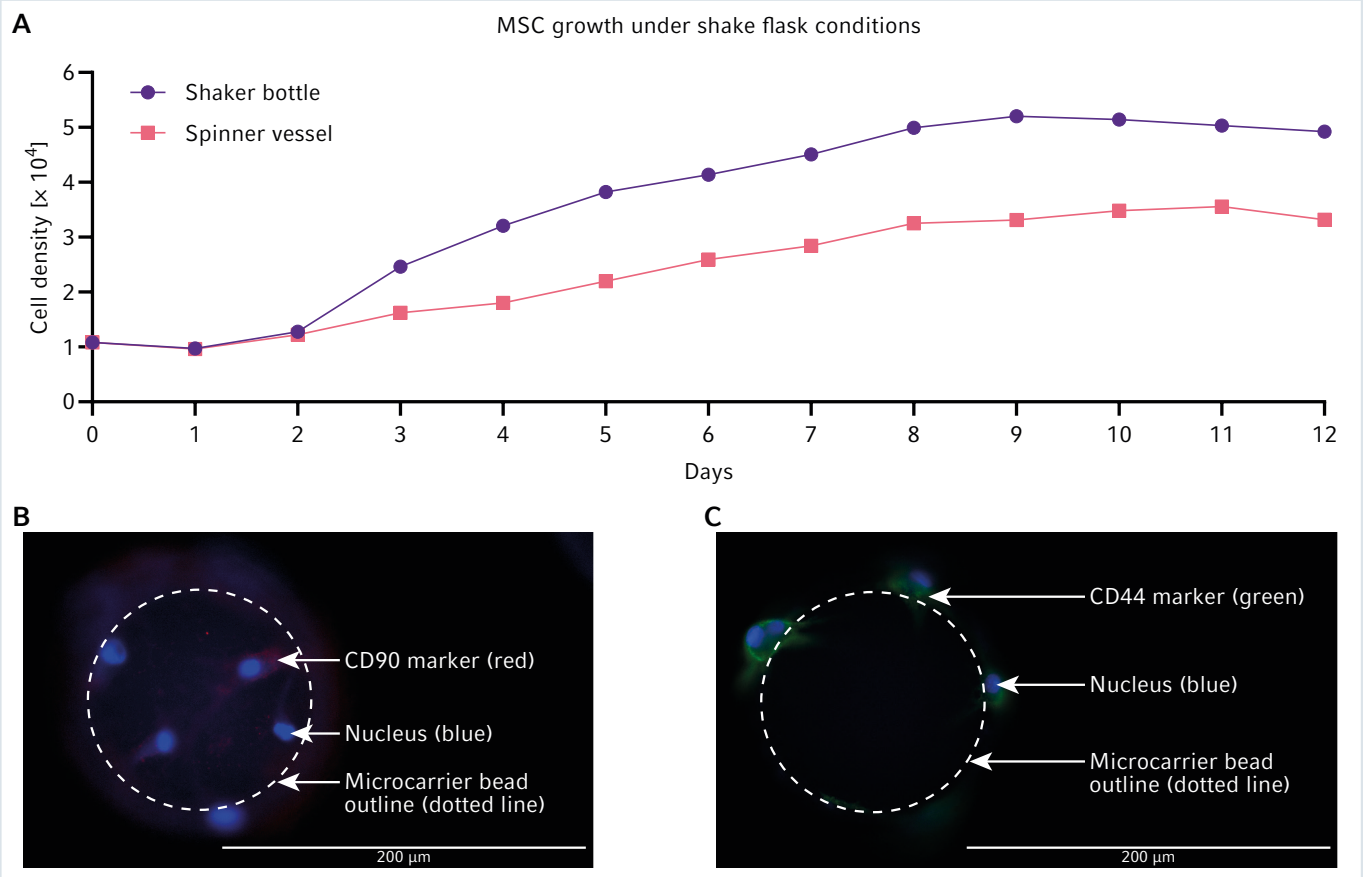

Fig. 2: Adipose-derived mesenchymal stem cells (AdMSC) cultured on microcarriers either in shake flasks or spinner flasks. Mixing of shake flask cultures was achieved by incubation in an incubator with shaking capabilities (Eppendorf New Brunswick S41i CO_2 Incubator Shaker). Mixing of the spinner flask culture was achieved by placing it on a magnetic stirrer in a static incubator. For more information see [11]. **(A)** Growth curves of the shake flask culture (purple) compared to the spinner flask (red) culture. **(B)** AdMSCs positive for CD90 stem cell marker. **(C)** AdMSCs positive for CD44 stem cell marker.

to reach a target microcarrier loading density (e.g., 17 g/L of microcarrier loading density and 0.02×10^6 cells/mL cell seeding density) and introduced into the bioreactor. Intermittent agitation can be introduced to promote cell migration between the beads. This method enabled serial subculture of MSCs without using proteolytic enzymes.

1.3.2. Traditional cell inoculation – Enzymatically released MSCs to mix with fresh microcarriers

Alternatively, one can continue to use the traditional method of inoculation by mixing dissociated cells with fresh microcarriers. Alternative enzymes to trypsin, such as dispase or accutase, should be tested for more gentle enzymatic dissociation of cells from the substrate to minimize cell loss [14]. Upon cell dissociation, the enzymes must be thoroughly deactivated and washed off the cell suspension to avoid residual activity. The dissociated cells are then mixed with fresh microcarriers at an optimized ratio, as discussed in the earlier section, to prepare the inoculum. We have found that co-incubation of cells and microcarriers prior to bioreactor inoculation for at least 2 hours is helpful for the cell adhesion.

2. Bioreactor and Control Station Setup

2.1. Bioreactor setup

BioBLU Single-Use Bioreactors with working volumes ranging from 0.1 to 40 liters are supported by the SciVario

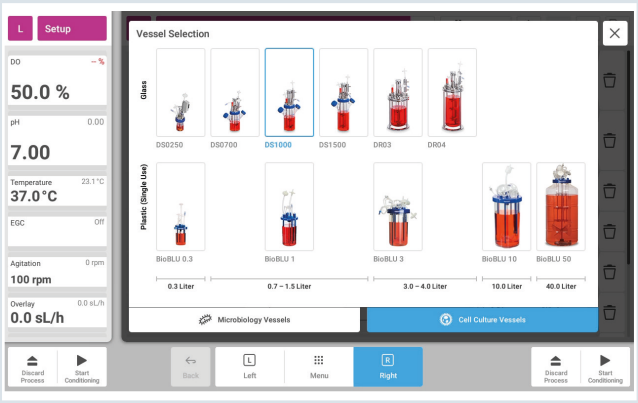

Fig. 3: Bioreactor setup screen of the SciVario twin bioprocess controller.

twin control station (Figure 3). The wide range supports easy scale-up of upstream bioprocess with only one controller, providing flexibility for R&D and process development.

Polarographic sensors for dissolved oxygen (DO) and electrochemical ones for pH are used. Prior to the vessel preparation, the ISM gel-filled pH sensors (Mettler Toledo) need to be calibrated, by connecting them to the SciVario twin controller. The pH sensors are automatically detected by the software of the controller. The calibration can be performed by following the dialogue window on the controller, using buffer solutions of pH 7 and pH 4 as "zero" and "span", respectively. Afterwards, the sensors are disconnected and sterilized in autoclavable pouches. The autoclaved pH and DO sensors are inserted into the vessels in a biosafety cabinet via the spare Pg13.5 ports.

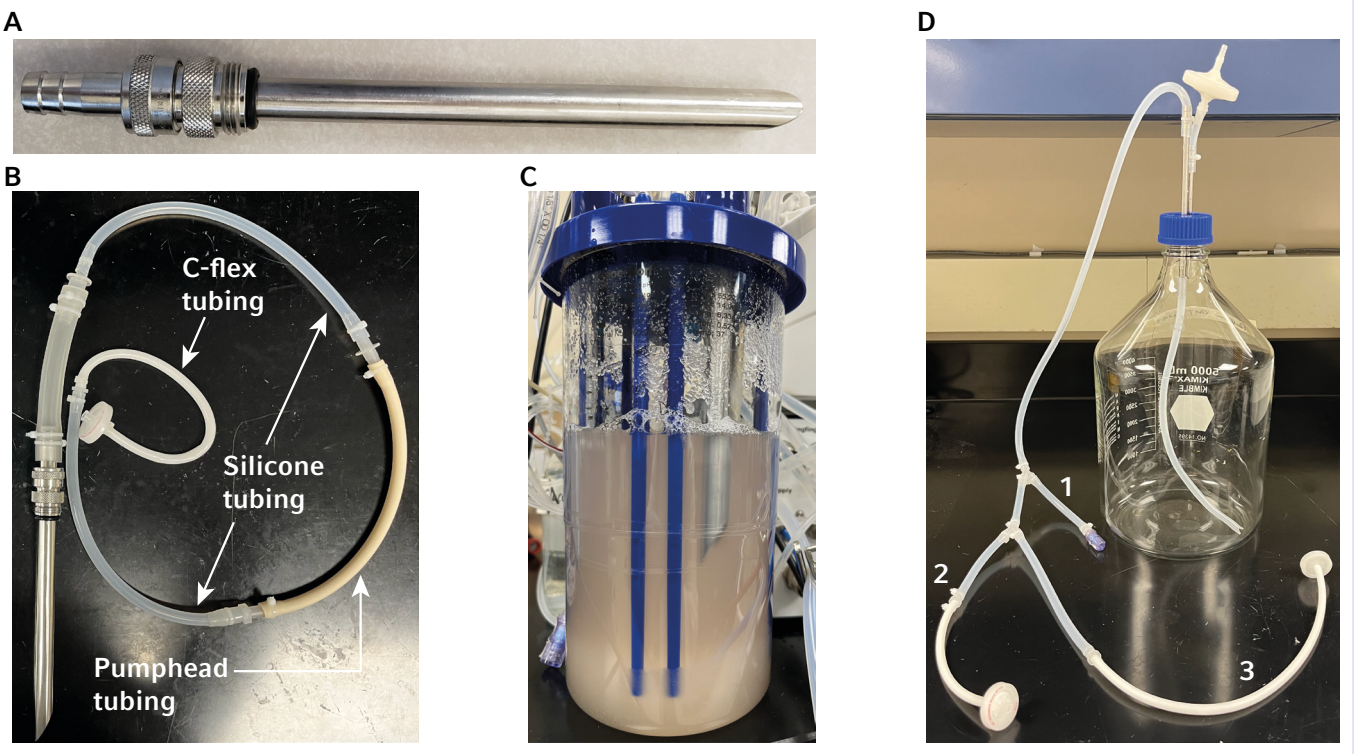

Fig. 4: Medium removal dip tube and perfusion out bottle assembly. **(A)** The medium removal dip tube is equipped with **(B)** tube extensions which include C-flex tubing for welding, silicone tubing, and pumphead tubing for the perfusion out pump on the controller. The tubing assembly is autoclaved before being inserted into the **(C)** bioreactor through an available Pg13.5 port. Prior to perfusion, the opposite end of the medium removal dip tube is welded to a **(D)** collection bottle. The tubing extension features a series of Y-junctions for (1) sampling the perfusate via a needleless port (2) C-flex tubing for welded connection to the bioreactor, and (3) C-flex tubing for an optional welded connection for additional medium removal.

Tube extensions, consisting of a pump head tubing (Feed Line Set for SciVario twin double pump drawer, C-Flex®, I. D. 0.5 mm, Eppendorf, Order No.: 7600 252 012) with the Luer connector at one end capped, are prepared, autoclaved and connected to the BioBLU 1c Single-Use Bioreactor via the Luer lock in a biosafety cabinet. Two overlay lines are used for base addition (0.45 M sodium bicarbonate) and anti-foam addition (0.1%, Antifoam-C Emulsion, Merck®, Cat. No. A8011-600ML), respectively. Two submerged lines are used for inoculum, fresh medium addition and/or glucose bolus feed (200 g/L). The third submerged line is the designated "Harvest" line.

The Medium removal dip tube (Figure 4) is prepared for the manual medium exchange step. In order not to disturb the microcarrier settlement during the process of medium removal, the dip tube opening should be positioned in the middle of the BioBLU Single-Use Bioreactor. The medium removal dip tube can be an open pipe (Length 8.25 inch/209.55 mm, Eppendorf, Order No.: M1287-9085 or Length 9.25 inch/234.95 mm, Order No.: M1287-9083) that fits in one of the standard Pg13.5 open ports with a compression fitting (Eppendorf, Order No.: M1287-5030).

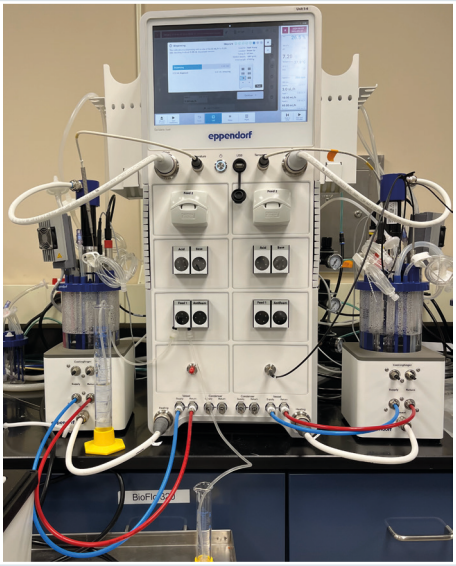

Fig. 5: SciVario twin bioprocess controller (middle) controlling two BioBLU Single-Use Bioreactors placed in the temperature control blocks

The bioreactor assembly can be installed in a biosafety cabinet, filled with PBS or culture medium at the minimum working volume (e.g., ~320 mL for BioBLU 1c), brought out of the biosafety cabinet and connected to the SciVario twin control station. BioBLU 1c Single-Use Bioreactors can sit in the temperature control block that enables electric heating and water-based cooling (Figure 5). Additional connections include the gas lines (submerse and overlay), sensors (temperature/RTD, pH and DO) and pump head tubing. For bottle connections (base, anti-foam, glucose, fresh medium addition, spent medium removal), the pump head tubing is mounted to the corresponding pump. Care is needed to prevent the Luer lock from accidently disconnecting, and to ensure that the flow direction is correct (clockwise "CW" as the default).

The liquid addition bottles of base, anti-foam and glucose are welded to the vessel assembly with a tube welder (Terumo). The fresh medium addition and spent medium bottles can be welded at a later point right before medium exchange. It is recommended to have multiple Y-junctions attached to the bottles (Figure 4D) to allow for aseptic sampling as well as liquid transfer to other bottles.

2.2. SciVario twin bioprocess controller setup

2.2.1. Process states and template

The SciVario twin bioprocess controller has an intuitive user-interface that guides the user step-by-step throughout the equipment use; once the user is familiar with the basics, no additional training is necessary. A key concept is the process state, as shown in Figure 6, of "Setup", "Conditioning" or "Running". These Process States follow the typical workflow of a bioreactor run, permitting certain control parameters to be changed while locking the process parameters during an actual run, thus ensuring process consistency and traceability.

In the "Setup" process, the first step is to choose a template, such as the default "Cell Culture fed-batch (concentration based)". The default template has been tested during its development, therefore, is a reliable process for standard cell cultures. Since the default template contains all the process parameters for a successful run, a user can easily build custom templates with modified parameters. User-built templates can be saved and re-used for the next time, ensuring process consistency.

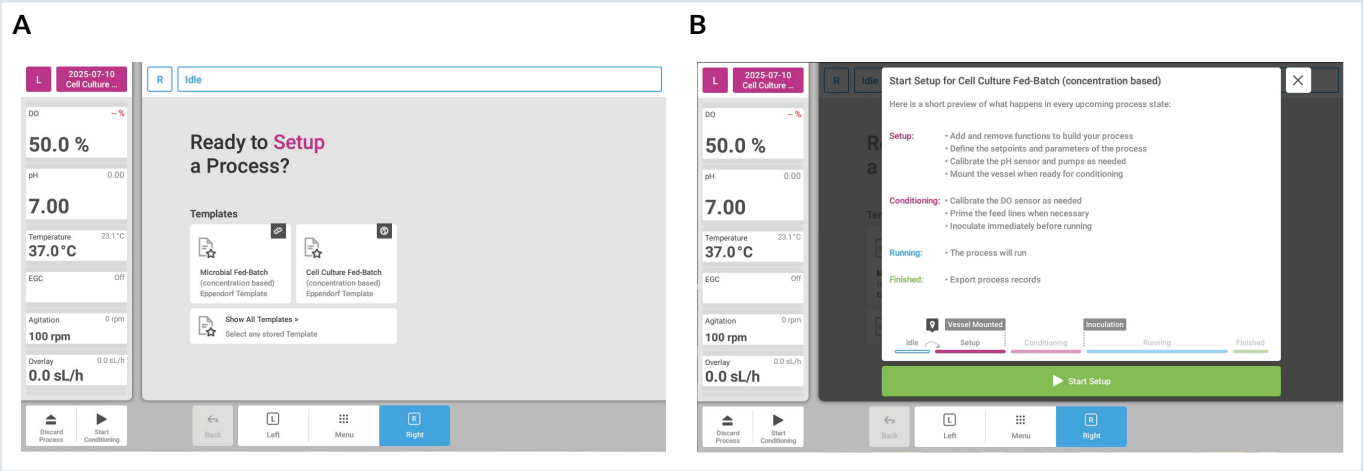

Fig. 6: SciVario twin bioprocess controller setup screen.

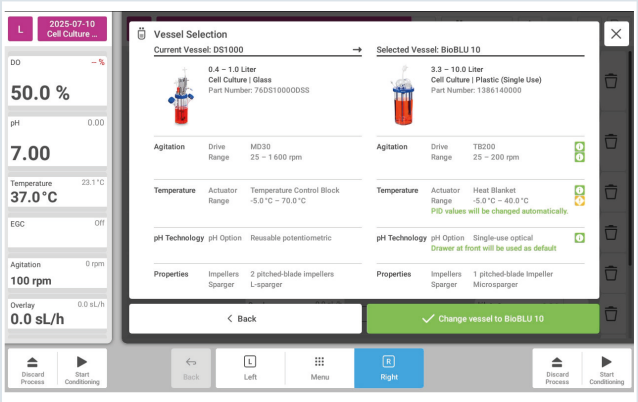

Fig. 7: SciVario twin bioreactor selection screen

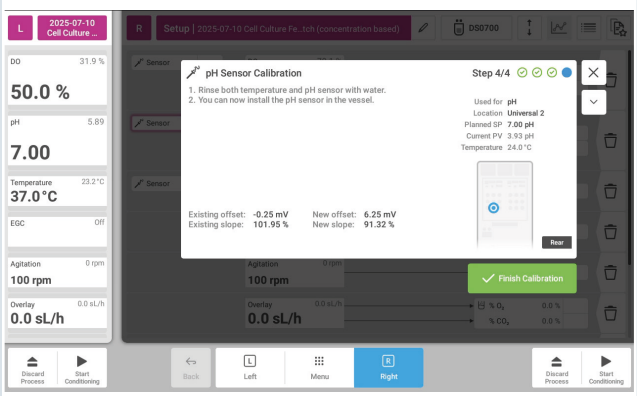

Fig. 8: SciVario twin bioprocess controller pH sensor calibration screen.

2.2.2. Vessel selection

The SciVario twin bioprocess controller user interface (UI) provides a well-arranged layout of all the vessels that are supported (Figure 7). In addition to the vessel type, the UI also provides key parameters of each vessel, including agitation, heating, and pH options. These features allow for easy comparison of vessel types and scale-up options.

2.2.3. pH and pump calibration

Before calibration, the correct sensor type needs to be selected from the dropdown menu. The selection is made easy by the straightforward visual guide of the sensor port layout. Similarly, the pumps may need to be assigned, in addition to the default assignment such as the base. Figure 8 shows the pH sensor calibration screen.

Calibrations of the pH sensor and the pumps need to be carried out during setup. The SciVario twin bioprocess controller provides dialog popups to guide the user through the process step-by-step, without needing specialized training. For the optical pH sensor, the values accompanying the single-use bioreactor can be inputted into the controller.

Fig. 9: SciVario twin bioprocess controller overview screen.

2.2.4. Agitation, temperature sensor, exhaust condenser

Additional functions other than sensors and pumps to be equipped include an agitation motor, temperature sensor, Peltier exhaust condenser, and other user-defined functions such as an anti-foam sensor. The SciVario twin bioprocess controller provides a process overview at the main page that lists all the key functions at one glance. This view shows each key function as a row, and each associated feature of the function as a column (e.g., sensor, setpoints, control parameters, cascades, and actuators) (Figure 9). Therefore, a user can go through each row systemically to check on each function, ensuring setup completeness and accuracy.

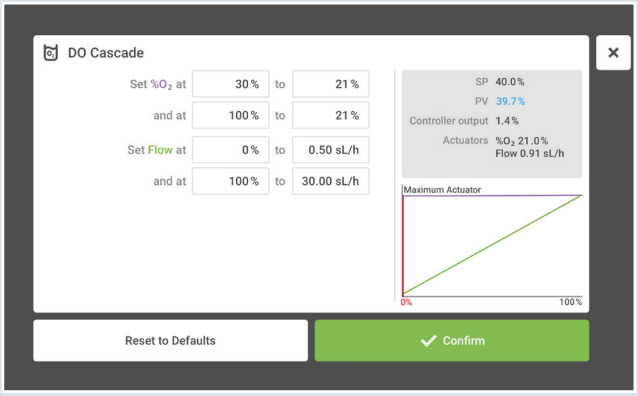

Fig. 10: Exemplary DO cascade for MSC culture controlled by the SciVario twin bioprocess controller as described in the Eppendorf Application Note 435.

2.2.5. Gas sparge and overlay control

The SciVario twin bioprocess controller provides an advanced and powerful mass flow controller module for bench-scale bioreactors, with a wide range of gas flow rates. Currently, the SciVario twin can support 4 gases (air, O_2, CO_2, N_2) in "Submerged" at 0.1 to 1200 sL/h for air, O_2, as well as 0.1 to 12 sL/h for CO_2, N_2, and in "Overlay" at 0.1 to 12 sL/h for all 4 gases. This means that with SciVario twin, a user can run a bioprocess from small scale (0.1 L) to 40 L on a single controller as opposed to two different ones in the past.

2.2.6. Template setup for MSC

MSCs are particularly sensitive to oxygen levels and shear stress [9, 14]. Therefore, the default template of "Cell Culture Fed-Batch (concentration-based)" needs to be modified for MSC bioreactor culture. The following changes from the default values are needed:

> DO.SP as "40". Under "DO Controller", "Proportional P" set as "0.1", "Integral I" set as "3.6/h"
> pH.SET as "7.6". Under "pH Controller", activate "deadband" of 0.1 and "automatically reset the integral memory if the process crosses the deadband or the set point"
> Once the Peltier condenser is attached to the vessel, activate "EGC" function
> All default "Feed" flow rates changed to 0

The modified template can then be saved by "Create Template Based on Current Settings" with a new "Template Name" and used in future.

3. Bioreactor conditioning with the SciVario twin bioprocess controller

3.1. Medium conditioning

The bioreactor is filled with MSC complete medium, maintaining a working volume, pH set point and agitation speed, for example, as shown in Figure 12. The medium in the bioreactor is conditioned for 24 hours prior to inoculation.

3.2. DO calibration and cascade.

DO sensor calibration can only be performed in the "Conditioning" state. For polarographic DO sensors, it is important to polarize the sensor for at least 6 hours by

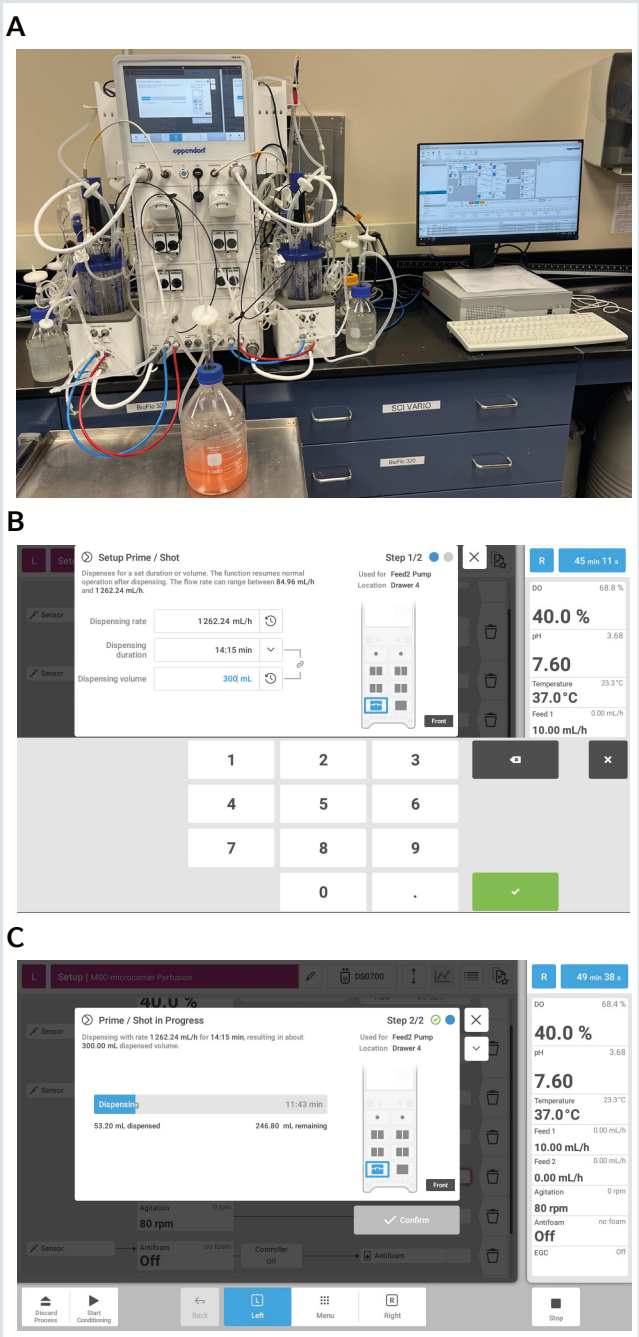

Fig. 11: Inoculation with SciVario twin bioprocess controller. **(A)** Inoculum bottle connection to both bioreactors and inoculum transfer in parallel. **(B/C)** The use of the "Prime/Shot" function to transfer the inoculum **(B)** to the bioreactor **(C)** with controlled timing.

keeping it connected to the controller prior to calibration. The DO Cascade default setting needs to be changed. For example, in our Application note 435, the DO cascade for a MSC culture with the SciVario twin bioprocess controller was set up in the following way [16]: Set O_2% to 21 when DO.OUT = 30%, and O_2% to 21% when DO.OUT = 100% (Figure 10).

3.3. Bioreactor Inoculation

After conditioning, once the bioreactor reaches the setpoints of the process parameters, it is ready for inoculation.

Figure 11 shows an example of an inoculation setup. The two bioreactors shared one inoculum. The inoculum bottle was welded to the two vessels via a Y-junction. It was found that efficient and smooth transfer into the bioreactor is key to post-inoculation cell viability. A maximum flow speed of the big pump can be used for simultaneous inoculum transfer into two bioreactors in less than 15 minutes. The use of the "Prime/Shot" function with the pump is an efficient method to transfer the inoculum to the bioreactor with controlled timing.

3.4. DASware® control software connection and BioNsight® cloud

It is at the SciVario twin control station's "Conditioning" stage that the DASware control software can be connected. Integration with DASware control allows for SCADA (Supervisory Control and Data Acquisition) control for remote process monitoring. A detailed procedure can be found in DASware control 6 Operation Manual. Cloud-based monitoring functions by the BioNsight cloud solution are also integrated in the DASware control software, further enhancing the possibility of process monitoring and analytics.

4. Bioreactor run

In this step, the SciVario twin bioprocess controller is in the process state of "Running". In-process adjustments can be made as needed. For example, in-process pH adjustment allows for off-line pH measurement validation.

4.1. Process parameters for MSC culture in bioreactors

As a sensitive cell line, MSC culture needs control of key parameters, such as pH, DO and gassing. The SciVario twin bioprocess controller provides the precision of bioprocess control in an easy-to-use manner. Figure 12 provides an examplary table of process parameters for MSC culture in BioBLU 1c, as reported in our Application Note 435 (Figure 12).

4.2. Manual medium exchange

Medium exchange is needed after five days of batch culture of MSCs in the bioreactor. As described in Application Note 435, the medium removal dip tube along with a compression fitting adapter is inserted in a spare Pg13.5 port allowing for medium exchange without disturbing the cell culture. Medium exchange starts with 10%-25% total volume every two days and then daily as required [16].

To do manual medium exchange, the agitation and gas flow are stopped for 5-10 minutes. Once the microcarriers settle at the bottom of the BioBLU 1c Single-Use Bioreactor, the old medium is removed using the medium removal dip tube. Subsequently, agitation and gas flow resume, and fresh medium is added using one feeding port of the vessel. The "Prime/Shot" function with the big pump on the SciVario twin bioprocess controller is an efficient method for liquid transfer from and to the bioreactor at a maximum speed of 6,000 mL/h (~100 mL/min).

Table 1: Process parameters and setpoints of the first and second experiments.

	First Experiment	Second Experiment
Parameters	**Setpoints**	
Starting volume	700 mL	
Ending volume	1 L	
Initial agitation	80 rpm (0.2 tip speed)	
Temperature	37 °C	
Inoculation density	3 x 10^4 cell/mL	10.4 x 10^4 cells/ mL
Cell culture medium	DMEM/F12 medium	ATCC complete medium
DO Setpoint	40% (P=0.1; I=3.6/h)	
pH Setpoint	7.2 (deadband = 0.1), cascade to CO_2 (acid) cascade to 0.45 M sodium bicarbonate (base)	7.6 (deadband = 0.1), cascade to CO_2 (acid) cascade to 0.45 M sodium bicarbonate (base)
Overlay N_2 gas flow	0.20 SLPM	0.25 SLPM
Gassing range	0.1 SLPH-30 SLPH	
Gassing cascade	Set O_2 % at 30 % controller output to 21 % and at 100 % controller output to 21 %. Set flow at 0 % controller output to 0.1 SLPH, and at 100 % controller output to 30 SLPH.	

Fig. 12: Exemplary process parameters for MSC culture controlled by the SciVario twin bioprocess controller as described in the Eppendorf Application Note 435 [16].

4.3. Glucose bolus and metabolite monitoring

As regular bioprocess monitoring, daily samples are taken for metabolite measurement, i.e. glucose, lactate and ammonium. As cells expand in number, it is expected to have glucose concentration decrease, and lactate and ammonium concentrations build up. To prevent toxic levels of lactate and ammonium, medium exchange may need to be adjusted for ratio and frequency; for example, to limit ammonium to less than 4 mM [16].

Additionally, it is important to monitor glucose consumption to supplement glucose as needed. Glucose bolus addition can be easily achieved with the "Prime/Shot" function associated with the assigned glucose addition pump on the SciVario twin bioprocess controller.

Results

From our experiences, MSC cultures with non-porous microcarriers often peak at around 5 × 10^5 cells/mL. MSC marker expression is used for cell phenotype validation. Finally, functional validation of MSCs involves trilineage differentiation (adipogenic, chondrogenic, osteogenic) of the cell product. The following figures provide some examples of MSC results from bioreactor cultures using Eppordorf equipment.

MSC Growth Curve

An example of a MSC culture growth curve under control of the SciVario twin is shown below (Figure 13) [16].

3D MSC-microcarrier morphology

An example of MSC morphology during microcarrier culture controlled by the SciVario twin bioprocess controller is shown below (Figure 14) [16].

MSC marker expression

MSCs during or after bioreactor cultures can be characterized for their marker expression for quality control. Below is an example showing the cells were positive for CD90 and CD29 and negative for CD11b and CD34, maintaining their cell phenotype (Figure 15) [16].

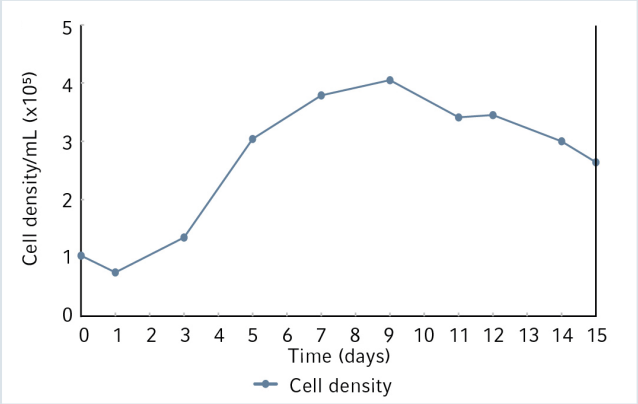

Fig. 13: Example of a MSC growth curve over a culture period of 15 days controlled by the SciVario twin bioprocess controller as described in the Eppendorf Application Note 435.

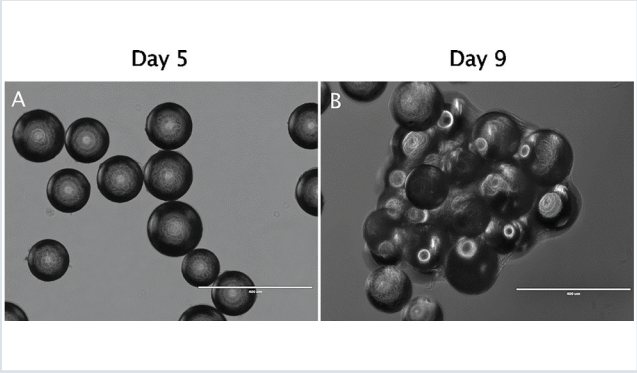

Fig. 14: Bright-field images (10x magnification) of human iPSC-derived MSC (small light circles) on collagen-coated microcarriers after (large dark spheres) **(A)** 5 days and **(B)** 9 days of culture as described in Application Note 435.

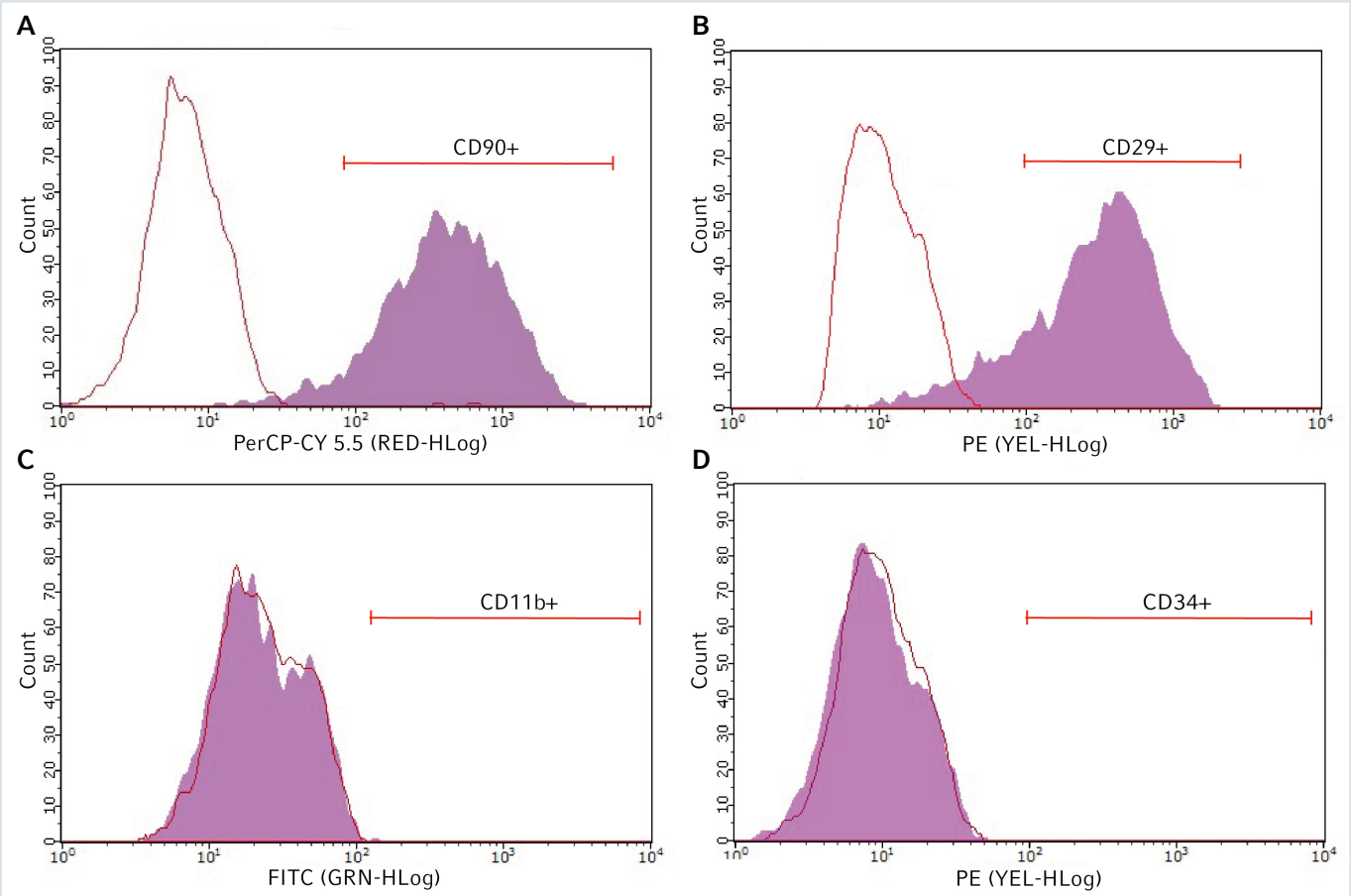

Fig. 15: Immunophenotyping of human iPSC-derived MSC as described in the Eppendorf Application Note 435. Filled areas depict the cells that were positive for the MSC markers CD90, CD29, and negative for the hematopoietic markers CD34 and CD11b.

MSC differentiation assays

After the bioreactor culture, MSCs can be characterized for their differentiation potential for further quality control. For example, MSCs can be dissociated from the microcarriers and seeded into 24 well plates that contain either adipocyte or osteocyte differentiation medium. The plates can be stained with Oil Red O or Alizarin Red S staining solutions, respectively. Below is an example of MSCs differentiated into either adipocytes or osteocytes successfully [11].

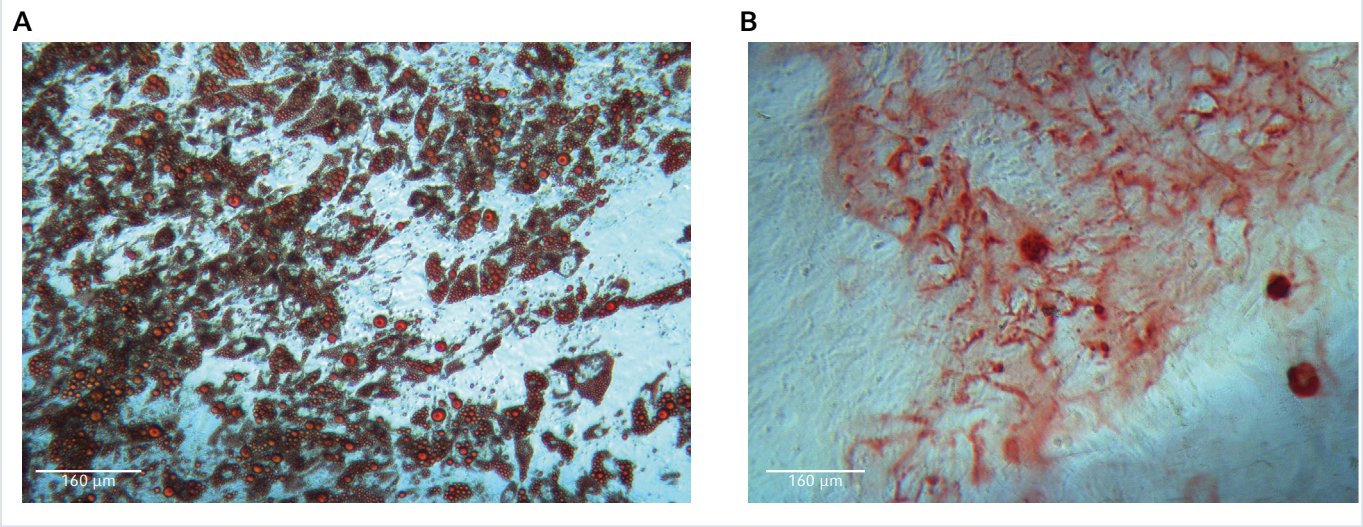

Fig. 16: Differentiation assays for adipose-derived MSCs expanded on microcarriers as describes in Eppendorf Application Note 259. After dissociation from the microcarriers, the cells were seeded into 24 well plates that contain either adipocyte or osteocyte differentiation medium. **(A)** Adipogenic differentiation formed lipid droplets as indicated by Oil red O positive staining. **(B)** Osteogenic differentiation caused calcium mineralization of extracellular matrix as indicated by Alizarin Red S positive staining.

Conclusion

This beginner's guide walks through the basic steps of MSC culture, starting from an initial cryopreserved cell stock, progressing to expansion in conventional flask cultures, and scaling up production in stirred-tank bioreactors. It details the critical transition from 2D to 3D microcarrier cultures, supported by the Eppendorf S41i shaker incubator, offering successful examples and strategies like bead-to-bead transfer to facilitate laborious working steps. 3D MSC production is made accessible with the Eppendorf SciVario twin control station, which simplifies medium exchange and controls critical process parameters such as dissolved oxygen and gassing. The examples shown here demonstrate high yields and consistent stem cell marker expression in cultures carried out in a BioBLU Single-Use Bioreactor, showcasing the power of the SciVario twin and BioBLU Single-Use Bioreactors for R&D and technology transfer approaches. A beginner in MSC bioprocessing can confidently use this guide to tailor their approach based on specific cell sources and microcarrier types to their bioprocessing needs.

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Ordering information

Description	Order no.
SciVario® twin bioprocess controller	7600 100 001
BioBLU® 1c Single-Use Bioreactor, cell culture, open pipe, 2 pitched-blade impellers, no pH, X-ray, 4 pieces	1386 111 100
New Brunswick S41i, 170 L, CO_2 incubator shaker with inner shelf and touch screen control, stackable	Inquire*
*Inquire the part number for your country	

Your local distributor: www.eppendorf.com/contact
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