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APPLICATION NOTE No. 480

Metabolic Phenotype Preservation and Suitability of Human Mesenchymal Stem Cells Cultivated in Stirred Tank Bioreactors for Cell Therapy Applications

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Abstract

Human mesenchymal stem cell (hMSC) therapy holds promise in various fields of medicine due to its potential regenerative and immunomodulatory properties. The success of hMSC therapy depends on the ability to produce high amounts of metabolically fit cells capable of surviving and functioning *in vivo*. MSCs need to undergo substantial proliferation to produce enough cells for therapeutic applications. Understanding their metabolic pathways is essential for optimizing culture conditions, nutrient supply, and oxygen levels to support rapid and efficient cell expansion. Proper metabolic control can help maintain the quality and viability of MSCs during expansion. Imbalanced metabolism can lead to cellular stress, increased apoptosis, and reduced cell quality, which may impact their therapeutic potential. Growing MSCs on microcarriers in stirred-tank bioreactors provides the high surface–volume ratio and controlled microenvironment providing conditions well suited for successful expansion of metabolically active MSCs.

In this study, we tested the expansion of human adipose-derived stem cells (hADSC) on microcarriers in six independent BioBLU® 0.3c Single-Use Bioreactors controlled by the DASbox® Mini Bioreactor System. hADSC are mesenchymal stem cells with the capacity for self-renewal and multipotent differentiation. After 5 days of culture, the amount of hADSCs produced ranged from 1.4-1.9 × 10^8 cells per bioreactor with a high cell growth reproducibility between bioreactors and an average expansion of 28-fold at the end of the culture. Glucose consumption and lactate secretion were consistent between different bioreactors. The average glucose consumption after the 5 days of culture ranged from 1.8-2.2 g/L with corresponding average lactate secretion from 1.1-1.7 g/L. The consistent conversion of glucose to lactate, averaging 67%, throughout the cell expansion process, confirmed the glycolytic phenotype of hADSCs. Furthermore, the changes in other nutrients within the culture medium, such as glutamine and glutamate, demonstrated reproducibility across all six bioreactors, signifying uniform cell growth.

After 5 days of expansion in the bioreactors, intracellular concentrations of key metabolites, as well as the activity of mitochondria and pentose phosphate pathway dehydrogenases, were found to be slightly higher compared to the cells used for inoculation. This increase in metabolite concentrations and enzyme activity might be attributed to enhanced proliferation and growth rates observed during the expansion process.

In cooperation with Promega

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Introduction

Mesenchymal stem-cell (MSC)-based therapy involving both autologous and allogeneic MSCs has emerged as an effective therapeutic approach in the field of regenerative medicine (1). The importance of MSCs in cell therapy lies in their ability to modulate the immune system, promote tissue repair, reduce inflammation, secrete growth factors, and provide therapeutic options for a wide range of medical conditions (2,3).

More than 1400 hMSC-based clinical trials covering diverse therapeutic areas (more than 14) are registered on ClinicalTrials.gov and have been completed or are ongoing (4). It has been reported that a typical cell therapy clinical trial requires 60 billion hMSCs and the demand will continue to increase (5). Therefore, to achieve its potential, standardized cost-effective manufacturing protocols capable of producing large quantities of high-quality hMSCs must be established.

2D planar MSC cultures and 3D stirred bioreactors represent two different approaches to growing and expanding (6,7). Although, 2D culture is easy to set up, it has limited expansion capabilities and is labor-intensive. 3D stirred-tank bioreactors involve culturing cells in a three-dimensional environment where the culture is continuously stirred to ensure homogenous distribution of cells and nutrients. MSCs cultured in 3D bioreactors often exhibit greater proliferation and expansion potential. Stirred-tank bioreactors have the potential for scale-up, and microcarriers provide a high surface–volume ratio, resulting in greater cell yields (8,9). In addition, these systems can better mimic the *in vivo* microenvironment, which is important for maintaining cell characteristics.

For hMSCs to demonstrate a therapeutic effect, they must be able to self-renew and adjust to the *in vivo* microenvironment, processes that are highly regulated by the metabolic state of the cells (10). Like all cells, MSCs require energy for various cellular processes, including self-renewal, proliferation, differentiation, and their immunomodulatory functions. Energy is primarily generated in the form of adenosine triphosphate (ATP) through glycolysis and OXPHOS. The metabolic state of MSCs, particularly the balance between glycolysis and OXPHOS is important for keeping their stemness and maintaining an undifferentiated state (11).

In the early stage of expansion, MSCs rely on glycolysis for energy production. The shift towards glycolysis helps to keep MSCs in a stem cell-like condition, inhibits spontaneous differentiation, contributes to cellular fitness, and helps to delay senescence. To optimize the expansion of MSCs in bioreactors, it is essential to monitor and control the metabolic environment, ensuring that the culture conditions are conducive to cell growth and maintaining the desired cell characteristics. This involves fine-tuning parameters such as nutrient supply, oxygen levels, pH, and waste product removal to support MSC metabolism and achieve the desired cell yield and quality (12,13).

In the present study we examined the expansion of human Adipose Derived Stem Cells (hADSC) in six independent single-use bioreactors and used viability and metabolic readouts to characterize the expansion and metabolic status of hADSC. The uniform expansion was shown between different bioreactors that was confirmed by consistent nutrient consumption and secretion rates. The cells retained their high glycolic rate during the expansion and no significant shifts of key metabolic pathways were measured.

Material and Methods

Culture of hADSC on microcarriers in BioBLU® 0.3c Single-Use Bioreactors

The human Adipose Derived Stem Cells (hADSC) (Lonza, PT-5006,) were thawed directly in RoosterNourish™-MSC-CC medium (RoosterBio®, SU-022) supplemented with 10 mL Booster (RoosterBio®, SU-016) per 500 mL and cultured for one passage (5 days) in planar format (T75 CellBIND flasks, Corning, 3290). Cells were incubated in the CellXpert® C170i incubator (Eppendorf, 6731) at 37°C in a humidified atmosphere with 5% CO_2. For passaging to the bioreactor, cells were trypsinized (0.025 % Trypsin-EDTA & Trypsin Neutralizing Solution, Lonza, CC-5012 & CC-5002) and counted using the Vi-CELL™ automated cell counting device (Beckman Coulter, Vi-Cell XR 12). A total amount of 6×10^6 hADSC (2.4×10^4 cells/ml) combined with 3.4 g of Synthemax II low density microcarriers (Corning, 3781), representing a cell-to-bead ratio of 3.7 cells/bead, were cultured in suspension in a volume of 250 ml RoosterNourish_MSC-CC media using the DASbox Mini Bioreactor System (Eppendorf, 76DX04CCSU) equipped with BioBLU 0.3c Single-Use Bioreactors (Eppendorf, 1386100000). To promote initial cell adhesion, the suspension of cells and microcarriers was not agitated for the first 4 hours. Later, the agitation speed was set to 80 rpm. The cells were cultivated at 37°C and the dissolved oxygen (DO) level was set to 40 %. The pH of the growth medium was controlled at 7.2 by automatic addition of CO_2 in the vessel headspace and NaOH (1N). At day three, 5 ml of RoosterReplenish™-MSC-XF (RoosterBio, SU-023)

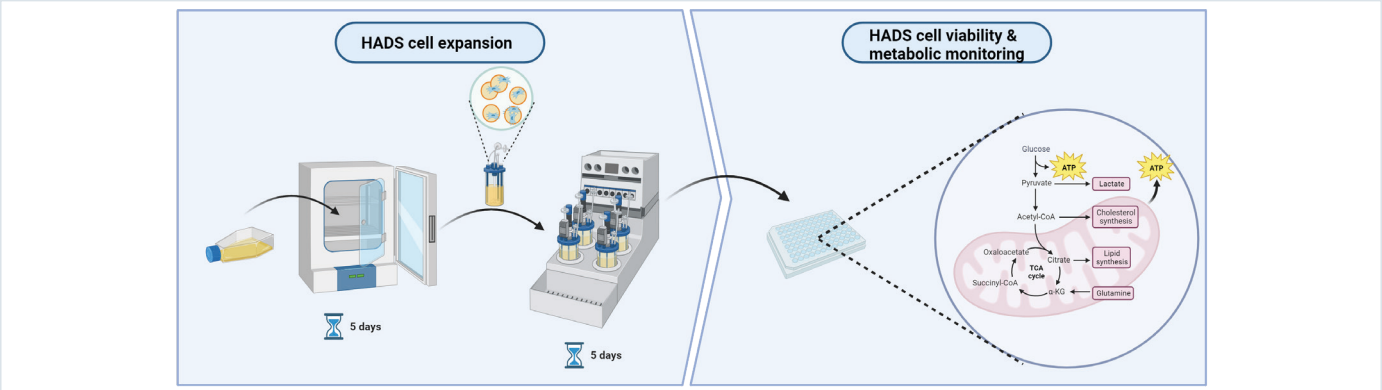

Fig. 1: Schematic representation of (A) cell expansion and (B) viability and metabolic monitoring during cell culture in BioBLU 0.3c Single-Use Bioreactors controlled by a DASbox Mini Bioreactor System.

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were added to each bioreactor to provide additional growth factors to the cells. The culture was extended to a total of 5 days in 6 independent BioBLU 0.3c Single-Use Bioreactors (Figure 1A).

Bioreactor sampling

For analyzing cell growth and metabolic characterization (Figure 1B), samples from each bioreactor were collected every day by connecting a sterile 10 ml syringe to the Luer Lock sample port and removing 6.5 ml of cell suspension aseptically. The collected samples were analyzed as described below in respective sections.

Microcarrier removal

hADSC were separated from the microcarriers by trypsinization as follows. A volume of 5 ml of culture removed from each BioBLU vessel was left undisturbed for 5 minutes to allow the microcarriers to settle. The medium was then removed and the microcarriers were washed with Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS, Gibco, 14190-094). The microcarriers were settled again for 5 minutes before the PBS was removed and 2.5 ml of 0.025 % Trypsin-EDTA was added. Cells were then incubated at 38°C for 8 minutes while mixing at 500 rpm in an Eppendorf Thermomixer (Eppendorf, 5382000.015). On day 4 and 5 of cultivation, cell aggregation increased, therefore a second round of trypsinization was performed to ensure an optimal separation of cells from the microcarriers. After incubating the cells with trypsin, 5 ml of Trypsin Neutralizing Solution was added. The cells were then passed through a cell strainer (Fisher Scientific, 10282631) for separation from the microcarriers. The sample tube was subsequently washed with 2 ml PBS and put through the cell strainer to ensure all cells were harvested. Collected cells were centrifuged at 200 x *g* for 5 min using the Eppendorf centrifuge 5810R (Eppendorf, 5811000010) and resuspended in 5 ml of fresh culture medium.

Cell viability and proliferation

Cell proliferation was evaluated each day using the Vi-CELL™ automated cell counting device after the cells were removed from microcarriers.

For cell viability, the cells were stained with the cell-permeant fluorochrome Calcein AM (Invitrogen™, C3099) and visualized under the Evos™ FL Auto 2 microscope (Invitrogen). Briefly, to a volume of 500 µl of cell culture in a 24-well plate, 1 µl of Calcein AM was added. The plate was then incubated at 37°C and 5% CO_2 (CellXpert C170i) for 30 minutes and the stained cells were visualized by fluorescence microscopy.

For measuring cell viability using luminescence CellTiter-Glo® 3D Cell Viability assay (Promega, G9683), samples were prepared as described for Vi-CELL™ automated cell counting and were analyzed following manufacturer's instructions. Briefly, 50 µl of prepared samples were transferred into 96-well assays plate and 50 µl of CellTiter-Glo 3D Reagent pre-warmed to room temperature was added to the samples and an ATP standard curve. After 5 minutes of mixing and 25 minutes of incubation at room temperature, luminescence was read using the GloMax® Discover Microplate Reader

(Promega, GM300). ATP levels were calculated for each sample from an ATP standard curve, assayed on the sample plate.

Extracellular metabolite levels

Samples were collected at indicated time points as described in bioreactor sampling section. 1 ml of collected samples was centrifuged at 200 x *g* for 5 min using the Eppendorf 5810R centrifuge. After centrifugation, the medium was removed for metabolite detection. A portion of the sample was frozen at -20°C. Part of the sample was used immediately for measuring glucose and lactate. The rest of the sample was frozen and stored and kept at -20°C. Glucose and lactate were immediately measured using YSI 2900 Biochemistry analyzer (YSI,2900D) and bioluminescence Glucose-Glo™ (Promega, J6022) and Lactate-Glo™ (Promega, J5022) assays following the manufacturer's instructions. For bioluminescence glucose and lactate detection assays, the samples were diluted 2,000-fold and 200-fold, respectively.

For glutamine and glutamate detection, the frozen medium samples were thawed and diluted 200-fold in PBS to maintain metabolite concentrations within the linear range of the assays and were measured using the Glutamine/Glutamate-Glo™ assay (Promega, J8022). RoosterNourish™-MSC-CC medium was used to determine starting metabolite concentrations and representative standards were included for metabolite quantification. All assays were performed in 96-well plates following the manufacturer's instructions.

Intracellular metabolite levels

For intracellular metabolite detection, samples were collected on day 0, prior to inoculation into the bioreactors, and day 5 post bioreactor culturing. Cells were separated from microcarriers as described above and resuspended in PBS at 0.9 × 10^6 cells/ml. The cells were lysed by adding one-half volume of 0.3N HCl. The cell lysates were frozen and stored at -20°C.

Cell lysates were assayed for lactate (Promega, J5022), malate (Promega, JE9200), glutamine and glutamate (Promega, J8022). Before assaying, the sample pH was adjusted for each assay based on the manufacturer's instructions. Assays were completed in a 384-well low volume plate with 3,000 cells per well. All assays were performed following the manufacturer's instructions.

Dehydrogenase activity

Samples were collected on day 0 and day 5 and microcarriers were removed as described above. Samples were washed two times with PBS and then resuspended in storage buffer (200mM Tris-HCl pH 7.3, 10% glycerol, 1% BSA) to preserve enzyme activity before frozen them at -20°C until assayed. Samples were assayed using the Dehydrogenase-Glo™ Detection System (Promega, J9020) using 500 cells per well. For dehydrogenase substrates, malic acid (Sigma, M7397), D-Isocitrate (Sigma, 56790), and 6-phosphogluconate (Sigma, P7877) were used for respective dehydrogenases. 200 µM of dehydrogenase substrate and dinucleotide were used to prepare each dehydrogenase detection reagent, as recommended in the manufacturer's instructions. Luminescence was measured every 15 minutes for 90 minutes to ensure the signal is within the linear range. The signal from each dehydrogenase activity assay was linear at 60 minutes and is depicted in Figure 8.

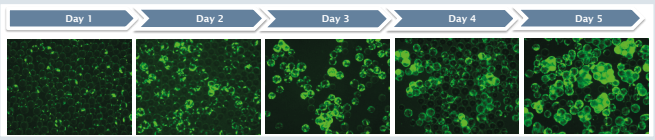

Fig. 2: Cell growth and viability of hADSC on Corning® Synthemax II microcarriers was monitored each day by staining of the cells with a green cell-permeant dye (Calcein-AM). The images show a representative field at each day the cells were cultured in bioreactors.

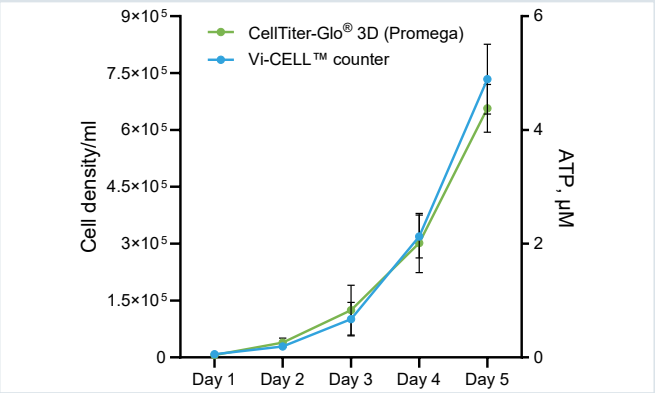

Fig. 3: Cell proliferation curve and ATP content of hADSC at passage 3 (P3) in the BioBLU 0.3c Single-Use Bioreactor controlled by a DASbox Mini Bioreactor system during a 5 day- expansion phase. Data represents the average of six bioreactors (n=6).

Results and Discussion

hADS cell expansion in the BioBLU 0.3c Single-Use Bioreactors

Initially, hADSC were cultivated for a period of 5 days on T75-flasks to provide an appropriate number of cells for the culture in the bioreactors. Subsequently, cells were inoculated at a density of 2.4×10^4 viable cells/ml (6.0×10^6 viable cells/vessel) in 6 stirred-tank single-use bioreactors utilizing Synthemax II low density microcarriers as matrix support. Viability staining during the 5-day expansion of hADSC in the bioreactors showed a successful cell-to-microcarrier attachment and homogeneous distribution (Figure 2).

At the end of expansion phase (at day 5), the cell number increased to an average fold of 28.3x corresponding to a cell density of 7.34×10^5 viable cells/ml (1.7×10^8 viable cells/vessel) with a final average viability > 97 % (Figure 3). The cell growth data demonstrates robust reproducibility between the all six bioreactors, with cell counts ranging from 1.4×10^8 to 1.9×10^8 cells per bioreactor at the end of the 5-day culture. Importantly, the number of cells produced in 5 days for each bioreactor is within the requirement for one single therapeutic dose (0.35 -3.5 $\times 10^8$ hMSC/dose) (14).

Cell viability was also determined using the CellTiter-Glo® 3D Cell Viability Assay. The CellTiter-Glo assay is a homogeneous method of determining the number of viable cells in culture based on quantitation of the ATP present, which signals the presence of metabolically active cells. This assay is designed for use with biological samples where a luminescent signal is generated and is proportional to the amount of ATP present. As observed in Figure 3, the ATP content is directly proportional to the number of cells present in the culture over time.

Nutrient consumption and secretion

Monitoring and maintaining the right nutrient concentrations in medium is crucial for maintaining appropriate energy metabolism within the cells, controlling the supply of nutrients, and adjusting operating conditions to optimize the bioprocess.

Here we used YSI 2900 Biochemistry analyzer and bioluminescent metabolite detection assays to monitor glucose consumption and lactate secretion during the growth of hADSC in six independent bioreactors. Glutamine and glutamate in the medium were measured using bioluminescent Glutamine/Glutamate-Glo™ assay.

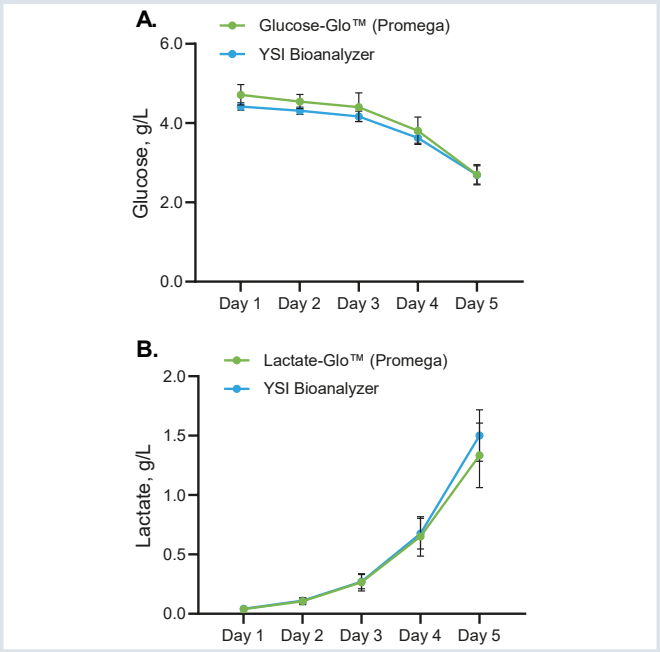

Fig 4: (A) Glucose consumption and (B) lactate secretion profile of hADSC cultured for 5 days in the BioBLU 0.3c Single-Use Bioreactor controlled by a DASbox Mini Bioreactor system. Data represents the average of six bioreactors (n=6).

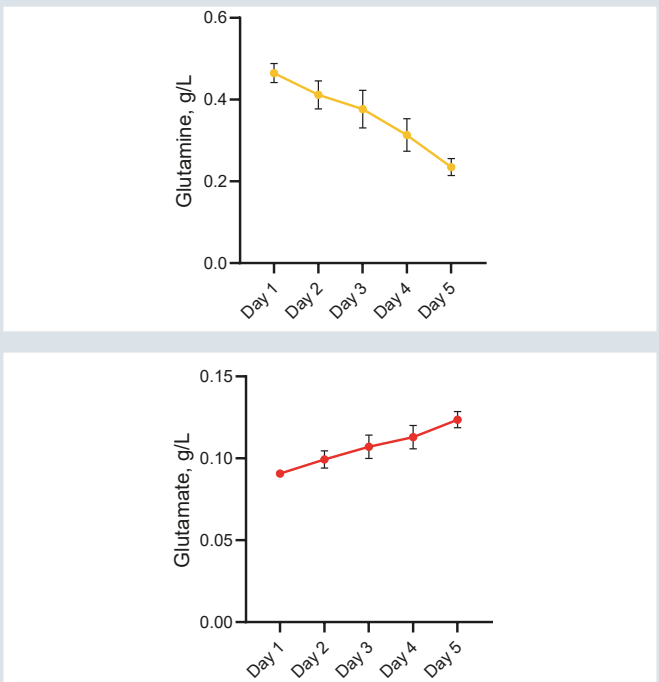

Fig 5: (A) Glutamine consumption and (B) glutamate secretion of hADSC cultured for 5 days in the BioBLU 0.3c Single-Use Bioreactor controlled by a DASbox Mini Bioreactor system. Data represents the average of six bioreactors (n=6).

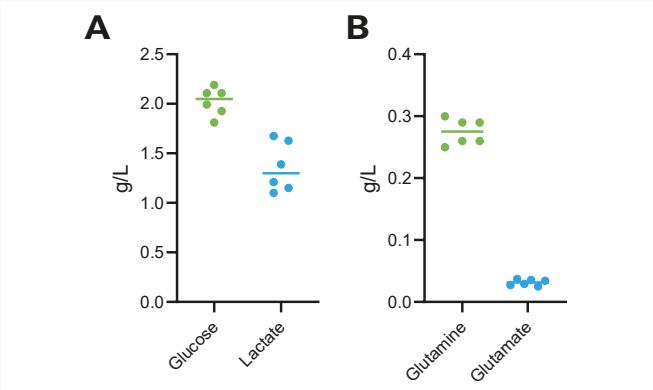

Fig 6: Extracellular metabolite levels throughout growth and proliferation of hADSC. Metabolites consumed from the media (green) or secreted into the media (blue). RoosterNourish™-MSC-CC medium was assayed along with media samples to determine starting metabolite concentrations. Data represents the average of six bioreactors (n=6).

Figure 4 shows the continuous decrease of glucose with concomitant increase of lactate in the medium during five days of hADSC expansion in bioreactors. The changes in glucose and lactate levels measured using bioanalyzers and bioluminescence metabolite assays were in close agreement and both methods indicated consistent nutrient usage between six independent bioreactors.

Glutamine is another important component in cell culture media that can serve as an energy source and a precursor for the synthesis of various important biomolecules required to support their growth and division, including nucleotides, amino acids, and glutathione. Cells often require glutamine to support their growth and division by providing the necessary building blocks for these molecules. As depicted in Figure 5A, constant decrease in glutamine and slower increase in glutamate was measured during continuous growth of hADSC. On day 5, > 50 % of the glutamine present in the media was consumed by growing cells. High glutamine consumption and low glutamate secretion are consistent with high anabolic requirements for rapidly proliferating hADSC cells. As was the case for lactate and glucose, the consumption and secretion rates were reproducible between different bioreactors.

In figure 6, we compared the average amount of glucose, lactate, glutamine, and glutamate consumed or secreted into the medium during five-day cultivation of hADSCs. Glucose consumption ranged from 1.8 to 2.1 g/L with average consumption of 2.02 g/L and lactate secretion ranged from 1.1 g/L to 1.6 g/L with average secretion of 1.35 g/L between 6 independent bioreactors. After 5 days in culture, 67% of the consumed glucose was converted into lactate, indicating the hADSC maintained their aerobic glycolysis profile. Glutamine consumption ranged from 0.21 to 0.26 g/L with average consumption of 0.24 g/L with much slower glutamate secretion ranging from 0.025 g/L to 0.04 g/L with an average of 0.03 g/L. The data clearly show consistent nutrient consumption and secretion rates between different bioreactors that is also reflected in uniform cell growth as shown above.

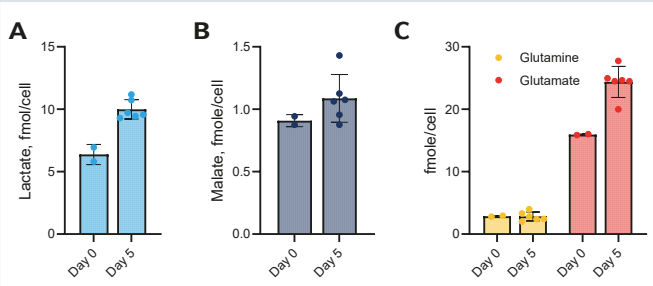

Fig. 7: Intracellular metabolite levels. Cells were removed from microcarriers on day 5 and compared to day 0. A total of 3,000 cells/well were assayed in a 384 well low volume plate. Lactate (A), malate (B), and glutamine/glutamate (C) were assayed using Promega bioluminescent assays. Data represents the average of six bioreactors (n=6).

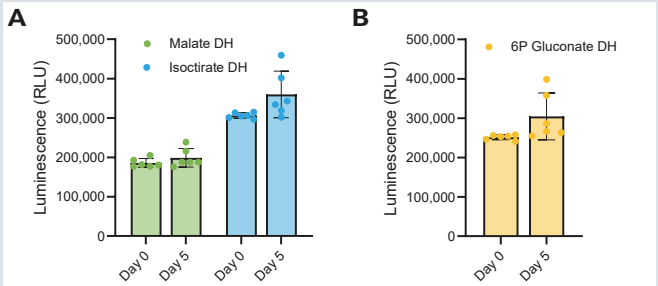

Fig. 8: Endogenous dehydrogenase activity in hADSC. 500 cells/well were assayed for two dehydrogenases in various pathways. Malate DH and Isocitrate DH from the TCA cycle (A) and 6-Phosphogluconate DH from the pentose phosphate pathway (B). RLUs shown are after a 60-minute incubation with respective dehydrogenase detection reagents. Data represents the average of six bioreactors (n=6).

Intracellular metabolite levels

Intracellular metabolite profiling is an informative tool for determining metabolic activity of growing cells, understanding their nutrient utilization and waste production. For example, high levels of glycolytic intermediates suggest increased glycolytic activity, while high levels of citric acid cycle intermediates may indicate active oxidative metabolism. Abnormal metabolite levels can be a sign of cellular stress, damage, or dysfunction. Here we examined the intracellular levels of lactate, glutamine, glutamate, and malate as readout of three key metabolic pathways: glycolysis, glutaminolysis, and the TCA cycle. The levels of intracellular metabolites were measured in cells collected at Day 0, prior to bioreactor culturing and at Day 5, after cell expansion in bioreactors. As expected, glutamate levels were substantially higher (>20 fmol/cell) as compared to glutamine (<3 fmol/cell) consistent with rapid glutamine conversion to glutamate in highly proliferated cells. Intracellular lactate and glutamate concentrations were slightly higher in cells expanded in bioreactor on Day 5 as compared to Day 0, while no significant change was measured for malate. Intracellular levels of all four tested metabolites were close in range between the 6 bioreactors, showing the reproducibility and robustness of cells produced.

Dehydrogenase activity

Dehydrogenases are critical components of multiple metabolic pathways and are essential for maintaining the energy balance, redox state, and metabolic processes within cells. Measuring expression levels or activity of dehydrogenases (DH) is often used to gain more information about metabolic status of the cells. Here we used 6-phosphogluconate DH to evaluate the activity of pentose phosphate pathway or malate and isocitrate dehydrogenases to test the activity of mitochondria TCA cycle. Across the 6 bioreactors cultured throughout 5 days, relative enzyme activity was similar and only had a slight increase in activity for day 5 compared to day 0 for isocitrate DH and 6-phosphogluconate DH. The data are in agreement with cell viability and metabolite analysis confirming uniform cell growth with a slight increase in their metabolism that can be attributed to higher proliferation rates due to the controlled growth environment and increased nutrient availability.

Conclusion

In recent years, there has been an increasing emphasis on the necessity of providing high-quality, viable, and fully functional MSCs for (pre)-clinical investigations (1, 3, 15). Among the key considerations, preserving cellular homeostasis throughout the stages of isolation, culture expansion, and harvesting has been identified as a critical factor in maintaining the cell quality for MSC-based therapies (3, 16). When it comes to *in vitro* expansion within bioreactors, the current challenge lies in optimizing and controlling the primary bioprocess parameters while also finely monitoring the metabolic state of the cells.

In the present study we demonstrate the feasibility of generating high-quality hADSC on microcarriers using the DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Bioreactors. Assessments of cell viability and metabolic indicators provided the means to comprehensively assess cell expansion and metabolic status throughout the entire procedure. The production of cells within each bioreactor, achieved in under a week of culture, provided the quantity necessary for a single therapeutic dose in various clinical applications. Furthermore, in addition to the high reproducibility observed between bioreactors, monitoring of the cell's metabolic status throughout the entire process confirmed the retention of their glycolytic profile as they underwent rapid proliferation, with no significant changes of key metabolic pathways.

Literature

1. Margiana R. et al., "Clinical application of mesenchymal stem cell in regenerative medicine: a narrative review" Stem Cell Research & Therapy, 2022, 13:366.
2. Abbaszadeh H. et al., "Regenerative potential of Warthon's jelly-derived mesenchymal stem cells: A new horizon of stem cell therapy" Journal of Cellular Physiology 2020, 235:12; 9230-9240.
3. Yuan X. et al., "Strategies for improving adipose-derived stem cells for tissue regeneration" Burns & Trauma 2022, 10, tkac028.
4. Rodriguez-Fuentes D.E. et al., "Mesenchymal stem cells current clinical applications: A systematic review" 2021, 52:93-101.
5. Olson TR. et al., "Peak MSC – are we there yet?" Front Med. 5:178. (5)
6. Martin C. et al., "Revisiting MSC expansion from critical quality attributes to critical culture process parameters" Process Biochem. 2017, 59, 231-243. (6)
7. Kusuma GD. et al., Effect of 2D and 3D culture microenvironments on mesenchymal stem cell-derived extracellular vesicles potencies. Front. Cell Dev. Biol. 10:819726. (6)
8. Egger D. et al., "Physiologic isolation and expansion of human mesenchymal stem/stromal cells for manufacturing of cell-based therapy products" Engineering in life Sciences 2021, 27;22(3-4):361-372. (8)
9. Lin Y.M. „Expansion in microcarriers-spinner cultures improves the chondrogenic potential of human early mesenchymal stromal cells" Cytotherapy 2026, 18;740-753. (7)
10. Salazar-Noratto G.E. et al., "Understanding and leveraging cell metabolism to enhance mesenchymal stem cell transplantation survival in tissue engineering and regenerative medicine applications. Stem Cells. 2020:38:22-33.
11. Folmes C.D.L. et al., "Metabolic plasticity in stem cell homeostasis and differentiation." Cell Stem Cell. 2012:11(5):596-606.
12. Zhu H. et al., "Inducible metabolic adaptation promotes mesenchymal stem cell therapy for ischemia." Arterioscler Thromb Vasc Biol. 2014; 34:870-876.
13. Yuan X. et al., "Metabolism in human mesenchymal stromal cells: a missing link between hMSC biomanufacturing and therapy." Front. Immunol. 2019:10:977.
14. Jossen V. et al., "Theoretical and practical issues that are relevant when scaling up hMSC microcarrier production processes" Stem Cell Intl. 2016:4760414
15. Tsai AC.et al., "Influence of microenvironment on mesenchymal stem cell therapeutic potency: from planar culture to microcarriers", Frontiers in Bioengineering and Biotechnology, 2020, 24;8;640.
16. Yin J. et at., "Manufacturing of primed mesenchymal stromal cells for therapy" Nat. Biomed.End. 2019 2:90-104.

Ordering information Eppendorf Products

Description	Order no.
DASbox® Mini Bioreactor System, for cell culture applications, 4-fold system for single-use vessels	76DX04CCSU
BioBLU® 0.3c Single-Use Bioreactor, cell culture, open pipe, 1 pitched-blade impeller, no pH, sterile, 4 pieces	1386 100 000
DASware® control software, including PC, OS, and licenses, for 4-fold DASbox® Mini Bioreactor System	7860 016 7
CellXpert®, inner door with 4 door segments, handle left side	6731010055
Eppendorf ThermoMixer® C, basic device without thermoblock	5382 000 015

Ordering information Promega Products

Description	Kit Size*	Catalog Number
CellTiter-Glo® 3D Cell Viability Assay	10 ml	G9681
Glucose-Glo™ Assay	5ml	J6021
Lactate-Glo™ Assay	5ml	J5021
Malate-Glo™ Assay	5ml	JE9200
Glutamine/Glutamate-Glo™ Assay	5 ml	J8021
Dehydrogenase-Glo™ Detection System	5 ml	J9020
Glomax® Discover Microplate Reader	n/a	GM300

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