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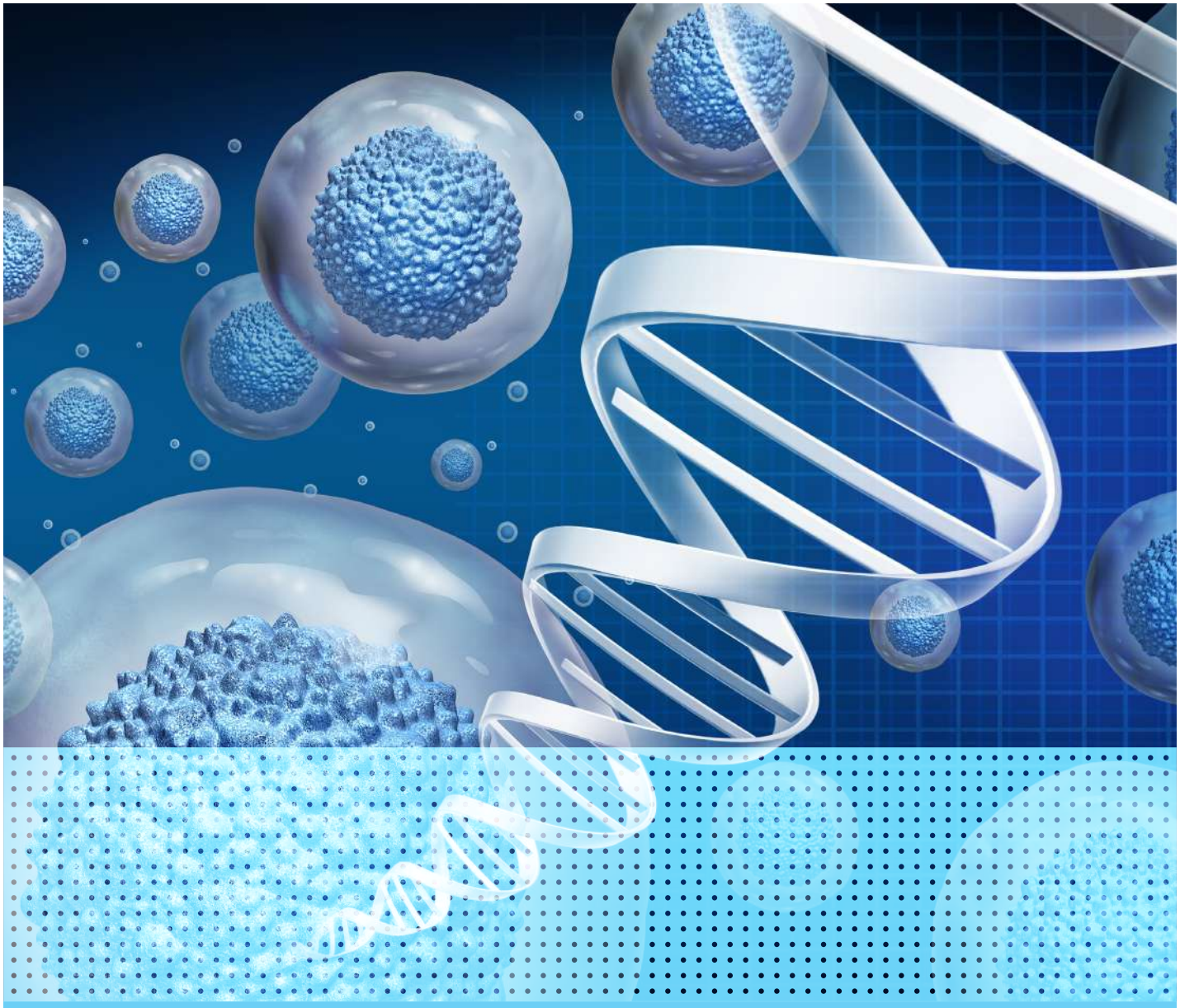

Optimizing Cell and Gene Therapy Workflows

Bioprocessing Resource Guide

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Cell and Gene Therapy—A Perspective

Traditional medicine and surgery have improved the lives of people around the world but are limited in their treatment of monogenic diseases. In many cases, the traditional approach can moderate the course of a disease in an individual but falls short of providing a cure.

Cell and gene therapies (CGT) are emerging fields of transformative medicine where diseases are treated by restoring or reconditioning cells or genes. Cells and genetically engineered cells demonstrate vastly different properties than traditional medicines and surgery. Akin to "living drugs", they can heal and replace diseased organs and thus have the potential to provide curative therapies for various diseases.

Although applications of CGT in medicine remain in their infancy, research, and development of novel CGT are rapidly expanding. With an aim to develop treatments that may prevent, treat, and cure genetic and acquired diseases, CGT comprise overlapping fields of biomedical applications where cell and tissue cultivation are fundamental techniques for the generation of CGT technologies.

Cellular Therapy (CT) and Regenerative Medicine—Stem Cells

CT techniques treat diseases by transplanting human cells to replace or repair damaged tissue and/or cells. Cells are cultured and modified before being infused into the patient and can originate from the patient (autologous cells) or a healthy donor (allogeneic cells). Various types of cells have been utilized as part of therapy or treatment for a variety of diseases and conditions. Among them, stem cells are one of the most popular options thanks to their value for regenerative medicine, characterized by their ability to differentiate into almost any type of cell in the body.

Various stem cell types can be developed into treatments as novel cell therapies and for potential applications. Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) and their derivatives, such as hematopoietic stem cells (HSCs), skeletal muscle stem cells, mesenchymal stem cells, etc., are considered promising cell sources for novel regenerative therapies. HSCs or blood-forming stem cells have been utilized in clinical therapies for over 40 years, forming the base of adult stem cell therapies, and are used to treat a variety of blood cancers and hematologic conditions. CT involving stem cells caters to an array of therapeutic applications involving the treatment of autoimmune and infectious diseases, urinary problems, skeletal and spinal cord injuries, weakened immune systems, and neurological disorders, that cannot be healed by established, conventional treatments. Furthermore, specific human cell types derived from hPSCs can assist in the development of novel, *in vitro* disease models and drug discovery strategies, and further the development of predictive drug safety assays.

Research and development of these therapies require consistent and constant production of large numbers—ranging in the billions—of high-quality lineage-specific cells. Though popular, two-dimensional cultivation systems, such as bags or T-flasks, are limited in terms of control and scalability, and often suffer from lower yields, lack of real-time bioprocess information and parameter control, and poor adaptability.

As such, the development of cell culture systems that can generate large culture populations and facilitate a straightforward approach in scaling up towards larger process dimensions is integral.

Stem Cell Bioprocessing— Stirred-Tank Bioreactors

Depending on the scale of cell production, researchers can choose a range of equipment. Bioreactors are particularly invaluable in this regard and were first created in 1944, for compressed yeast production, by George De Becze and A.J. Libemann. Since then, several design features were added that are now available to meet the diverse cell production needs of researchers. Nowadays, different types of bioreactors exist. Stirred-tank bioreactors are most suitable for suspension cells and microcarrier cultures and are also scalable to suit the needs of R&D as well as production. Packed-bed bioreactors are optimal for culturing adherent cells. Single-use plastic bioreactors become increasingly popular for the cultivation of chimeric antigen receptor T-cells and other high-quality cells for use in therapy development because they eliminate the need for time-consuming cleaning and sterilization processes. This saves time and enables faster turnaround times. BioBLU® c Single-Use Bioreactors from Eppendorf cover working volumes from 100 mL to 40 L. Like this, processes can be developed in small volumes and then be transferred from larger volumes.

Eppendorf single-use as well as reusable bioreactors are compatible with the various bioreactor controllers of the company and allow efficient cell expansion where conventional 2D-cell culture systems reach their limits. One prominent example is the DASbox® Mini Bioreactor System for parallel bioprocessing at small-scale. Widely used in the biopharmaceutical industry, bioreactor systems come with a host of advantages for process development. They allow for online monitoring and control of key process parameters including oxygenation, pH, and temperature.

Optimizing upstream bioprocesses is an essential aspect of CT development. The Eppendorf Bioprocess Unit, built on decades of experience in upstream bioprocessing, has contributed to the advancement of stem cell cultivation in stirred-tank bioreactors for many years. With extensive options for real-time monitoring and control of critical process parameters and scalable bioreactor design, Eppendorf provides a platform to transfer small-scale bioprocess results to larger working volumes.

Beyond enabling efficient cell expansion, stirred-tank bioreactors and bioprocess control software help reproduce initial culture success, accelerating development timelines and time to market. They also automate parameter control and routine tasks like culture feeding to free up time for valuable research.

BioBLU® Single-Use Bioreactors

Combine all the advantages of single-use technology with the trusted performance and scalability of a stirred-tank design. BioBLU disposable bioreactors eliminate autoclaving, improve turn-around time, and reduce overall costs.

RIGID-WALL DESIGN

Simplify installation. No risk for damages due to the folding of bioreactor bags, protecting your bioprocess run. Even better, the consistency of a rigid wall stirred–tank design mimics traditional bioreactors and makes scale-up easy. Sizes range from 100 mL to 40 L working volume.

REDUCE RISK FROM LEACHABLES AND EXTRACTABLES

Made from monolayer virgin plastics, the Eppendorf BioBLU Single Use Bioreactors address the widely discussed problems associated with leachables and extractables, helping to make laboratories more efficient and safe.

ELIMINATE CONTAMINATION AND LOSS

Unique non-invasive pH and DO sensor technology drastically reduces contamination risks (industry standard autoclavable pH sensors available for pH measurement). Lost runs due to sensor failure are also reduced with non-invasive sensor options. Bioreactors come preassembled with sparger, overlay, and gas filters for inlet and exhaust as well as penetrations for pH, DO, temperature, liquid additions, sampling, and harvest. A sealed magnetic drive with fully enclosed bearings maintains vessel sterility.

BIOBLUc BIOREACTORS

These rigid-wall stirred-tank single-use bioreactors have been specifically designed and optimized for the cultivation of mammalian cells.

BIOBLU 5P BIOREACTOR

A packed-bed bioreactor perfectly suited for adherent cells and perfusion culture. Pre-loaded with Fibra-Cel® disks, it provides a solid support growth matrix, e.g. for the production of secreted proteins.

To learn more, visit **www.eppendorf.com/biobluc**

Eppendorf Bioreactors in Action

Eppendorf DASbox Mini Bioreactor System with BioBLU Single-Use Bioreactors brings together the benefits of single-use bioreactor technology and the reliability of stirred-tank design into one system. BioBLU Single-Use Bioreactors have demonstrated their applicability in the expansion of different stem cell types, including human pluripotent stem cells and mesenchymal stem cells.

To learn more on the DASbox Mini Bioreactor System, visit:

Scalable Expansion of Human Pluripotent Stem Cells

Advanced bioreactor systems help facilitate the scaling up of cell cultures to larger dimensions. The cultivation and differentiation of hPSCs in stirred-tank bioreactors particularly require the adaptation of 2D surface-adherent cell cultivation to 3D suspension cultures.

Equipment used:

> Parallel DASbox® Mini Bioreactor System
> BioBLU® 0.3 Single-Use Bioreactors
> Cord blood derived hiPSC line hCBiPSC2
> mTeSRTM1 (STEMCELL Technologies®, Vancouver, Canada) supplemented with the ROCK inhibitor Y-27623 (10 µM)

Over a 7-day lasting expansion process, a 4-fold increase in viable cell count can be achieved of hPSCs in a fed-batch process—where nutrients are continuously or intermittently fed to supplement and maximize the reactor's contents, in this case, the cells and their growth—with a total cell yield of 2.3 x 10^8 cells/100 mL as shown in a case study. Flow cytometry confirmed that a majority of the yielded cell population retained expression of established, pluripotency-associated cell surface markers in the cultivation process. These results proved that the combination of BioBLU 0.3 Single-Use Bioreactors with the DASbox system provides an excellent platform for process optimization and adaptation to lineage-specific hPSC differentiation processes. Further building on these results, Dr. Robert Zweigerdt and his team from Hannover Medical School, Germany have greatly increased the hiPSC yield in bioreactors with a total culture yield of 35 million hiPSCs per mL.

To read the full study and review the protocol, visit:

Stem Cell Cultivation in Bioreactors: Increasing hiPSC Yield

Robert Zweigerdt, Lab Academy

Zweigerdt from Hannover Medical School explains how his team greatly increased the hiPSC yield in bioreactors.
Human induced pluripotent stem cells (hiPSCs) and their differentiated progeny provide the toolset for innovative approaches in regenerative medicine. Stirred-tank bioreactors are promising cultivation systems, as they have the capacity to produce high cell numbers, allowing scale-up. They provide cutting-edge opportunities for improving the control of growth parameters, bringing numerous benefits, the foremost of which is improved cultivation reproducibility. Increasing cell density is a vital component of research in stem cell bioprocessing. In this interview, Dr. Robert Zweigerdt from Hannover Medical School, Germany explains how his team reached a culture yield of 35 million hiPSCs per mL.

Eppendorf:
Your group has established expertise in cultivating hiPSCs as cell aggregates in stirred-tank bioreactors. In your latest publication, you reported attaining a cell density of 35 million cells per mL. That is a big leap! Which major hurdles did you have to overcome to reach this milestone?

RZ:
The first hurdle, which we attacked a decade ago, was to support the survival and proliferation of hiPSC seeded in a three-dimensional (3D) matrix-free suspension culture, in contrast to the established cultivation protocols employing 2D matrix-dependent monolayer culture on conventional culture dishes and platforms [1, 2].

The second big step, accomplished in collaboration with your company, was the design of a modified stirring impeller design supporting a more homogeneous hiPSC aggregation [3] and, subsequently, a "retention-filter" system. Such retention systems enable keeping the hPSC cells, which form multicellular aggregates in stirred suspension culture, in the bioreactor upon automated perfusion feeding, defined as the constant replacement of used by fresh media [4]. Subsequently, perfusion feeding was the prerequisite for our latest step:

that is, the identification of growth-limiting parameters such as pH dependence, glucose consumption, and lactate accumulation. Having identified these growth-limiting bottlenecks, feedback-based monitoring was performed which requires the control of the overall medium throughput via perfusion feeding. Dr. Felix Manstein, of our department, who was driving these investigations in recent years, has also implemented in silico process modeling and optimization strategies, which facilitate rational process development of high-density bioprocessing of hiPSCs [5].

Eppendorf:
For prospective use in advanced therapies, hiPSCs need to be differentiated into the desired cell type. How straightforward was it to translate differentiation protocols which have been designed for monolayer cultures to cell aggregates in bioreactors?

RZ:
Since we initiated the development of lineage-specific differentiation strategies in suspension several years ago, shortly after the first successful hiPSC culture in 3D [6], we have established a substantial degree of competency in that area as well. The most significant challenges regarding directed differentiation in suspension culture include the impact of cell aggregates size, its heterogeneity, overall cell density, and defining mechanical and hydrodynamic parameters [7].

However, we also noted that the standard culture media components and differentiation-directing molecules that we are applying, for example, the WNT pathway modulators, used for mesendoderm-induction and cardiac differentiation, have equivalent effects in 2D and in 3D [8]. Therefore, the process transition from 2D, which is often applied for cell differentiation basic research, to 3D suspension culture is typically straightforward. However, we are convinced that in the future many differentiation strategies will benefit from advanced process control abilities enabled by bioreactor technologies, still in the early stages of development [9].

Notably, we demonstrated that stirred-tank bioreactor-based hiPSC differentiation is efficiently applicable not only for cardiac diffraction (as highlighted by references above) but also for the differentiation and production of numerous other functional hiPSC progenies, including endothelial cells [10] macrophages [11] and endodermal derivatives [12].

Eppendorf:
In upstream bioprocessing, the feeding strategy strongly impacts cell growth and viability. Repeated batch and perfusion are two options for removing byproducts and replenishing nutrients. What do you consider the pros and cons of these two strategies?

RZ:
As mentioned above, our experience suggests that perfusion feeding, despite its complexity, is the optimal tactic for advanced hPSC cultivation [13]. This is due to the highly glycolytic metabolism of the rapidly growing hPSC, which, on the one hand, requires an enormous supply of extra glucose to avoid growth-limiting starvation. Moreover, on the other hand, high glucose supplementation results in a massive accumulation of secreted lactate, which may become toxic and which induce proliferation-inhibiting acidification of the culture. These issues increase exponentially in parallel to the exponential increase in cell density [5]. For these reasons, we feel that perfusion feeding is the most successful strategy to control growth-limiting parameters, if the goal of the protocol is to optimize high-density cultivation of hiPSC. Notably, in parallel to the 10-fold increase in cell density, the amount of medium required to generate a given number of cells was reduced by 70 % as a consequence of the process optimization steps.

Eppendorf:
Within a few years, you were able to increase hiPSC culture density more than tenfold. How did you optimize your process to obtain this value?

RZ:
A couple of years back, we gained 2.85 million hiPSCs per mL following inoculation with 0.5 million cells per mL. Recently, we obtained a more than 10-fold higher cell density following a comparable inoculation density. We follow a step-by-step strategy, systematically analyzing the challenges and then applying the bioreactor-enabled combined control of the parameters, thereby overcoming the growth-limiting hurdles. Specific bottlenecks include promoting efficient survival and aggregation of hiPSC after single cell-based process inoculation, an appropriate adaptation of the stirring speed to ensure no hiPSC-clumping, and reducing aggregate diameter below ~300 µm. Next, avoiding pH drop below circa 6.7, ensuring constant glucose supply to avoid cell starvation, and adapting the perfusion speed (and promoting thus the optimal medium throughput) to avoid peak accumulation of lactate and toxic osmolality levels as well as several additional parameters [5]. However, once these limitations are identified, they can be systematically controlled via the bioprocess control software, and optimized in combination with in silico process modeling; details in our most recent protocol [14].

Eppendorf:
You were able to generate 5.25 billion hiPSCs in a 150 mL volume. How does that number compare to the number of cells required for cell therapy applications, for example for the heart? Do you see the need for future scale-up?

RZ:
Despite the substantial progress of the bioprocessing of undifferentiated, pluripotent hiPSCs, we are still working on further increasing the cell density and thus the yield of differentiated cells including hiPSC-derived cardiomyocytes. While we have achieved very high lineage purity e.g. of >95% iPSC-cardiomyocytes, the cell density obtained from the differentiation protocol is still relatively low; currently ca. 1-2x10^6 cells per mL [8]. Since estimations suggest that for the replacement of disease-depleted heart myocytes about 1-2 x

10^9 iPSC-cardiomyocytes will be required for each patient, we would currently require about a 1-liter culture to provide the appropriate cell dose for an individual patient. Regenerative medicine researchers are discussing the possibility of generating very large cell batches for an allogeneic – non-patient specific – transplantation approach, so we believe it would be appropriate to pursue a program of substantial upscaling in the future. This goal would target volumes of five, ten, twenty, one hundred-fold, and eventually even greater levels.

Such upscaling strategy is also highly attractive from a commercial perspective, which includes the transition to fully controlled GMP conditions required for regulatory compliance and clinical translation.

Another promising approach is "blood cell farming" for example the differentiation of functional macrophages from hiPSC. As we recently demonstrated in collaboration with the group of Nico Lachmann at the Hannover Medical School campus, this approach, in contrast to the batch-production of hiPSC-cardiomyocytes, is compatible with the continuing production of macrophages in stirred tank bioreactors over several weeks or even months [11].

Eppendorf:
Considering the cell density, do you still see room for improvement? Which are the limiting factors?

RZ:
As indicated above, we see room for improvement in the bioprocessing of differentiated hiPSC progenies. The (limiting) factors for differentiation are even more complex compared to the expansion of hiPSC at the pluripotent state. The reasons for this include the higher complexity of differentiation processes since the cells are constantly changing their progenitor status and phenotype and thus their physiology and proliferation properties. We are working intensively to develop lineage-specific process conditions expressing numerous different lineages. It is a challenging task but therefore inspiring and exciting!

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Scalable Expansion of Human Bone Marrow-Derived Mesenchymal Stem Cells

Originating from a large variety of tissues including bone marrow, adipose tissue, placenta, muscle, or umbilical cord, mesenchymal stem cells (MSCs) are adult stem cells that are capable of differentiating into more than one, but not all, cell types. Compared to embryonic stem cells, MSCs can be isolated from the various aforementioned sources and reduce the risk of rejection reactions deeming them particularly attractive for therapeutic applications in the field of regenerative medicine. For clinical trials, it is estimated that between one and 200 million cells per patient are the required dose of human MSCs (hMSCs) depending on the relevant disease to be treated. Unfortunately, no matter the tissue source, the number of hMSCs that can be extracted is generally very low and insufficient for clinical use. Thus, one of the major challenges in the application of hMSCs for regenerative medicine involves the production of these cells in large quantities.

Equipment Used:

> DASbox® Mini Bioreactor System
> BioBLU® 0.3 Single-Use Bioreactor
> Cell Culture Flasks T-175
> Cytodex® type 1 (GE Healthcare® Bio-Sciences, Sweden)
> Cytodex® type 3 microcarriers (Sigma-Aldrich®, USA)
> MSCGM Mesenchymal Stem Cell Growth BulletKit Medium (Lonza®, Switzerland)
> 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) solution
> 0.25 % Trypsin-EDTA
> CASY® Cell Counter and Analyzer, model TT (Omni Life Science®, USA)
> Glucose RTU Kit (Biomerieux®, France)
> L-Lactate Assay Kit (Abcam®, United Kingdom), BD BioCoat Fibronectin Cellware, 24-well plates (Corning®, USA) hMSC Osteogenic Differentiation Bulletkit Medium (Lonza®, Switzerland)

> OsteoImage Mineralization Assay (Lonza®, Switzerland)
> Anthraquinone dye (Alizarin Red S Staining Kit, ScienCell, USA)
> hMSC Chondrogenic Differentiation BulletKit Medium (Lonza®, Switzerland)
> Alcian Blue staining (Sigma-Aldrich®, USA)

Rigid-wall, stirred-tank bioreactors provide precise control of critical process parameters like pH and dissolved oxygen, temperature, gas sparging, and agitation, and enable a homogeneous distribution of nutrients and gases alongside high process control for maintenance of cells and molecules in suspension. As such, these bioreactors offer a suitable environment for the cultivation and expansion of stem cells and T cells. The Eppendorf DASbox Mini Bioreactor System equipped with BioBLU 0.3 Single-Use Bioreactors demonstrated successful expansion of hMSCs. Cytodex type 1 and Cytodex type 3 microcarriers were evaluated as growth surfaces for adherent stem cells. A 17.5-fold expansion corresponding to a maximum cell density of 1 x 10^8 cells/batch and an 11.5-fold expansion with maximum cell numbers of 7 x 10^7 cells/bioreactor were observed for Cytodex type 1 and type 3 carriers, respectively. The resultant hMSCs successfully retained their multipotency and maintained their ability to differentiate into osteocytes and chondrocytes.

To read the full study and review the protocol, visit:

Optimization of CD4+ T Cells for Long Term Expansion

The combination of DASbox Mini Bioreactor System equipped with BioBLU 0.3 Single-Use Bioreactors has also been used successfully for optimizing the long-term expansion of CD4+ T cells.

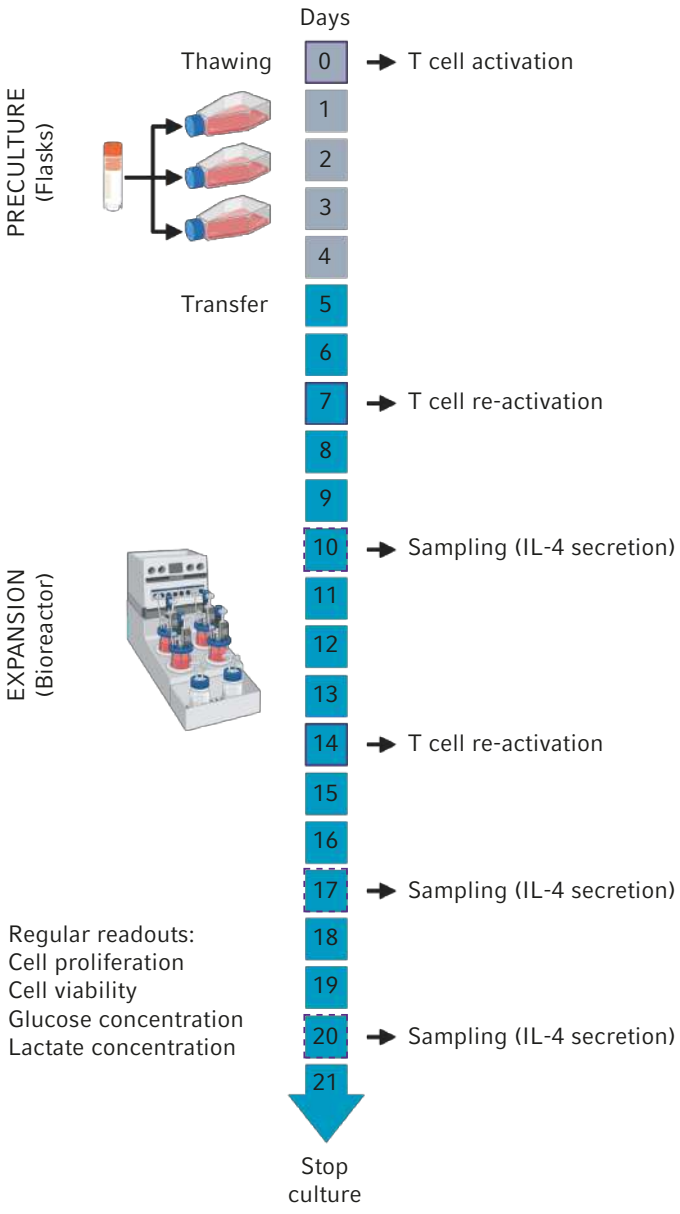
Adoptive cell therapy (ACT) tackles chronic viral infections and malignant diseases such as cancer. Here, treatment revolves around the autologous or allogenic transplantation of immune cells, or more specifically, T cells, into the patient's body. T cells are sourced from patients, modified genetically, when necessary, expanded *ex vivo*, and reinfused into the patients to target viral or tumor antigens. As T cells are highly sensitive to their culture environment and can react easily by modifying their receptor/ligand repertoire, changes surrounding their metabolic properties, cell proliferation, redox status, oxygen tension, and apoptosis activation can have immediate consequences for the product quality. With around five adoptive cell therapies approved by the US Food and Drug Administration (FDA), all involving the use of chimeric antigen receptors (CAR) T cells (that have been greatly effective in the eradication of various tumors), the optimization of protocols and cell culture conditions for the scalable manufacture of high-quality T cells has been an important challenge.

Equipment Used:

- DASbox® Mini Bioreactor System
- BioBLU® 0.3 Single-Use Bioreactors
- CellXpert® C170i Incubator (Eppendorf, 6731)
- Lonza® human peripheral blood CD4+ T cells (Lonza, Switzerland, 2W-200)
- T75 CellBIND® flasks (Corning®, USA, 3290)
- ImmunoCult® Human CD3/CD28/CD 2 T Cell Activator (StemCell Technologies®, Canada, 10990)
- ImmunoCult XF Cell expansion medium (StemCell Technologies®, Canada, 10981)
- Recombinant human interleukin 2 (rhIL-2) (StemCell Technologies®, Canada, 78036)
- Vi-CELL® automated cell counting device (Beckman Coulter®, USA, 731050)
- YSI® 2900 Biochemistry analyzer (YSI®, USA)
- Ionomycin (2.5 µM) (StemCell Technologies®, Canada, 73722)
- Phorbol 12-myristate 13-acetate (PMA) (10 ng/ml) (StemCell Technologies®, Canada, 74042)
- Invitrogen® IL-4 Human ELISA kit (ThermoFisher Scientific®, USA, BMS-225-2)

The DASbox Mini Bioreactor System's ability to provide fully controllable culture platforms also aided in the efficient proliferation of high-quality and functional CD4+ T cells following a culture timespan of 16 days. Considering the impact of different oxygen tensions on cell proliferation, sample incubations were performed at 70% and 20% dissolved oxygen levels. The corresponding results suggested the positive impact of lower oxygen tension at 20% for CD4+ T cell proliferation rates (an average fold-expansion of 2.35 x 10^6 versus that of 9.63 x 10^5 for 70% DO) without any negative impact on cell functionality. Quantitative evaluation of the latter was achieved by measuring the production of interleukin 4 (IL-4) secretion, a signature cytokine involved in many immunological processes. By testing T cells from different donors, the reproducibility of these results and the overall optimization of scalable bioprocessing of T cells were further confirmed.

The approaches presented thus far illustrate the advantages of controllable bioreactor-based culturing systems for the development of scalable stem cell and T cell production processes over static cultures in bags or flasks. These results solidify the substantial potential of the Eppendorf DASbox Mini Bioreactor System used in combination with BioBLU 0.3c Single-Use Bioreactors to optimize T cell culture conditions.



Schematic of CD4+ T cell expansion in BioBLU 0.3c Single-Use Bioreactors using a DASbox Mini Bioreactor System for 21 days with a preculture of 5 days in flasks.

To read the full study and review the protocol, visit:

Exosomes Produced in Bioreactors

BIOREACTORS PROVIDE THE IDEAL ENVIRONMENT FOR THE EXPANSION AND LARGE-SCALE PRODUCTION OF EXOSOMES

*◂**Dr. Jorge Escobar** is a senior research scientist in the applications lab of Eppendorf, Inc., in Enfield, Connecticut, USA. Having joined the company in 2019, he has worked on applied research focused on efficient expansion of adherent and non-adherent cells, such as stem cells and adult primary cells, in cell culture bioreactors.*

Q: Please tell us about your journey in the field so far, what drives your research motivation, and your current focus.

A: My main area of research after college was the design and development of medical devices for multiple biomedical applications. In fact, it was a collaborative effort that involved scientists from different areas and healthcare professionals with the aim of finding innovative solutions that could have a positive impact on the lives of patients. Subsequently, I had the opportunity to work on a multi-institutional research project during my PhD, where we focused on the development of three-dimensional (3D) organotypic models for cartilage and bone tissue regeneration, using biocompatible polymeric scaffolds and mesenchymal stem cells with the goal of replacing damaged tissue and mimicking the architecture of native tissues. As an Assistant Research Professor in the Department of Chemical & Biomolecular Engineering, School of Engineering at UConn Storrs, and a faculty member in the Institute for Regenerative Engineering my primary goal was to study the therapeutic potential of adipose-derived stem cells in cartilage regeneration through preclinical animal studies. The cells delivered exosomes that acted as delivery vehicles for anti-inflammatory factors and helped modulate the inflammatory environment in the knee joint caused by cartilage degradation itself. But the avascular nature of cartilage in the knee joint is known to cause oxygen and nutrient deprivation creating a hostile environment for transplanted stem cells. Finally, Eppendorf offered me the opportunity to develop an off-the-shelf example of exosome production workflow to potentially help our customers implement solutions that can improve the living conditions of patients suffering from multiple ailments. My current focus is to research and understand bioprocess customer requirements and user needs to help develop solutions, especially with a focus on cell and gene therapy applications.

Q: What do you believe are the major concerns surrounding the field of cell therapy and regenerative medicine?

A: While regenerative medicine and cell therapy offer enormous potential to improve human living conditions, there are several considerations that need to be addressed. First, it is very important to ensure that cells and cell-derived byproducts to be administered to patients are safe and effective to maximize their therapeutic benefits. Studying immune rejection and immunogenicity is also crucial in the field of cell therapy to better understand the host's immune response to transplanted cells and to develop solutions to overcome these challenges. Another concern is the source of the cells (embryonic stem cells or embryo-derived induced pluripotent stem cells (iPSCs)) used for these applications, which always raises debates about ethical issues. To address these ethical concerns, adult stem cells or iPSCs reprogrammed from adult somatic cells, such as skin or blood cells, are different alternative cell sources used to avoid stringent regulations. Finally, achieving scalable production of high-quality cells is a great concern since the manufacturing process for cell therapies is complex and challenging. The development of robust and standardized manufacturing protocols, as well as quality control measures, are critical to ensure in-process reproducibility, which is critical for the widespread adoption of cell therapies.

Q: What are exosomes and how do they factor in this picture? What role do they play as potential substitutes for cell therapy?

A: Exosomes, often referred to as a type of cell-free therapy, are small extracellular vesicles (EVs) ranging in size between 30 and 150 nanometers that are released by cells and contain a variety of bioactive molecules (such as proteins, lipids, nucleic acids, and metabolites). The composition of exosomes can vary depending on various factors, including cell type, physiological state of the cell, and environmental influences (cell stress and activation, pathological conditions, therapeutic interventions, etc.). Although exosomes were initially considered cell waste products, subsequent research revealed that exosomes play several important roles in intercellular communication and several physiological and pathological processes such as tissue regeneration, immune regulation, and intercellular communication. In addition, they can be used as therapeutic agents by engineering or loading them with specific cargo (proteins, drugs, nucleic acids), making them ideal for regenerative medicine and drug delivery applications. It is important to say that although exosomes have the potential to overcome the limitations associated with cell-based therapies, ongoing research should help to understand their full therapeutic potential.

Q: How do bioreactors facilitate the large-scale production of exosomes?

A: Bioreactors play a key role by offering a controlled environment under specific conditions (including pH, temperature, nutrient availability, and oxygen supply) where cells (mesenchymal stem cells among other cell types) expand under optimal conditions and produce a high yield of desired products such as exosomes. Media optimization is another area where the bioreactor can help to customize cell culture composition by precisely monitoring and adjusting components such as glucose, amino acids, essential factors, etc., required for cells to efficiently produce exosomes. Also, to produce a large number of exosomes required for clinical trials and therapeutics purposes, bioreactors allow for scalability by providing a larger yield per volume compared to traditional cultivation methods such as flasks or rocking motion bioreactors. In terms of process monitoring and control, real-time monitoring is essential to optimize cell culture conditions and consistent exosome production. This is possible because bioreactors are equipped with sensors to track the cell fitness during the production process and automated controlled systems can adjust various parameters (pH, temperature, or nutrient supply) based on monitored data. As you can see, by providing control over the scale-up process, it is possible to significantly improve large-scale exosome production.

Q: What does Eppendorf bring to the table for researchers and laboratory personnel for their cell culture needs conncering exosomes?

A: The success of our customers is very important to Eppendorf. In this sense, our company provides solutions in upstream and downstream processes, supporting our customers in various aspects of exosome production and helping them to study and use these fascinating extracellular vesicles for various applications in research and medical fields. Eppendorf offers in-house developed application notes that add significant value to our solutions, serving as key resources for our company's customers, providing detailed protocols, best practices, and offering insights into experimental setups, troubleshooting tips, and data analysis methods, empowering our customers to optimize their workflows, save time and achieve more reliable results.
Our core strength lies in bioreactor solutions for small and bench-scale processing. This is especially important in the development of cell therapies that takes place in small volumes where precision is key. For well over a decade, we have also provided BioBLU single-use rigid-wall bioreactors, which are a game changer to the market enabling our customers to grow high-quality cells in single-use solutions with the proven design of traditional stirred bioreactors. Our solutions cover the perfect volume range, when we think of current CGT applications.

Q: What is next for Eppendorf and what does the future hold for applied research concerning exosomes?

A: We know that there is an increasing demand for expert partners in the field of extracellular vesicle research in the cell therapy market, and Eppendorf will continue to add value and solutions for our customers. We are attentive to our customers' needs, to their challenges and pain points, and we aim to develop solutions to overcome these challenges. Our mission continues to be to "Help bring life-saving treatments to the world." The Eppendorf Bioprocess team is striving to be at the forefront of the cell and gene therapy industry and will supply innovative solutions to our customers to help overcome development challenges.

Closing Testimonial

User at a Glance

Eppendorf knows the users of its products and their specific requirements for various applications very well. Here, we would like to introduce some of our valued customers—or rather, let them introduce themselves by answering five questions about themselves, their employer, and current challenges they face in their market.

For this issue we have interviewed Kfir Molakandov about his work and private life. He is Head of Diabetes Cell Therapy Department at Kadimastem (Israel).

What three words would your colleagues use to describe you?
I believe my colleagues would describe me as creative, thoughtful, and cooperative.

Where and how did you spend your last vacation?
Though Israel is a relatively small country, the landscape is very versatile, from the desert down south to the snowy mountains up north. On our last family vacation, we went to the northern part where the temperatures are cooler and the rivers are flowing. We enjoyed hiking and experienced a thrilling adventure rafting down the beautiful Jordan River. We also visited Nimrod's Fortress, a 13th century medieval crusader's fort.

What do you especially like about your job?
I am grateful, that I am part of the leading team of a clinical stage biotech company. A company that is engaged in the development and production of innovative regenerative cell therapies intended to treat diseases currently considered incurable. The dynamic work environment in our company is full of enthusiasm that provides ongoing learning opportunities. Heading the Diabetes Cell Therapy Department, together with my team, we feel honored for having the opportunity to further develop our cell therapy solutions intended to provide treatment to millions of diabetes patients.

How did you get in touch with Eppendorf bioprocess equipment?
Our company, Kadimastem, is part of the interdisciplinary TECHNOBEAT consortium consisting of renowned experts working in different fields from cell therapy to tissue engineering. The TECHNOBEAT project aims to provide new treatment options for patients suffering from heart failure due to the loss of heart muscle tissue following myocardial infarction (heart attack). Within this project, we have developed unique

cGMP protocols for large scale expansion of pluripotent stem cells (PSCs) in bioreactors. For these expansion processes, we have used the DASGIP and the DASbox systems that enable online monitoring, sampling, and a wide range of culture volumes. Since then, we are utilizing these technologies for the generation of large quantities of high-quality PSC-derived pancreatic islets for the treatment of insulin-dependent diabetes.

In your opinion, what is the most exciting challenge in your area of science at the moment?
The cell therapy industry presents a range of scientific and clinical challenges. The major challenges in PSC-derived pancreatic islets therapy have to do with appropriate delivery systems, cell protection from host immune response, as well as the need for cell retrieval. The ideal product is expected to enable long term satisfactory function, maintaining the capacity to restore normal blood glucose levels. Research and development activities of both industry and academia are focused on finding the appropriate mechanisms and solutions. We are happy to lead such efforts through different projects and collaborations, promoting cell therapy as a viable solution for millions of patients suffering from diabetes.

Kfir Molakandov was interviewed by Eppendorf in February, 2020

Conclusion

Cell and gene therapies are at the forefront of efforts toward transformative medicine where diseases can be treated by the restoration or reconditioning of cells or genes. Crucial to their development is the large-scale cultivation and production of stem cells thanks to their ability to differentiate into many cell types in the body. Eppendorf's extensive portfolio of stirred-tank bioreactor systems allows efficient cell expansion where conventional 2D-cell culture systems reach their limits. Culture vessels such as BioBLU Single-Use Bioreactors and DASbox Mini Bioreactor Systems combined with supplementary bioprocess control software demonstrate their applicability for the expansion of different stem cell types, including $CD4^+$ T cells, human pluripotent stem cells, and mesenchymal stem cells. Moving forward, Eppendorf provides a reliable platform for accelerating cell culture development while optimizing the time spent on research.

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