BIOMANUFACTURING CARDIAC CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Sean Palecek, Professor of Chemical & Biological Engineering, University of Wisconsin, USA
palecek@engr.wisc.edu

ACOUSTIC CELL PROCESSING (ACP) AN INNOVATION IN CELL THERAPY MANUFACTURING

Nina Bauer, FloDesign Sonics
n.bauer@fdsonics.com

Key Words: Acoustic Cell Processing Concentrate Affinity Selection

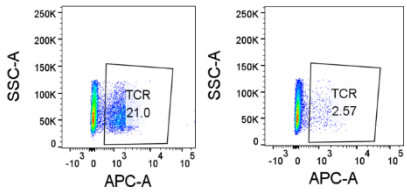
With a significant portion of cell therapy manufacturing costs being attributed to consumables and inefficient processes, there is an enormous industry-wide focus on the industrialization and overall improvement of cost of goods. To date, the focus has been to develop closed, integrated systems with automation. Therapeutic innovators are sometimes creating these systems on their own or through collaboration with equipment vendors. Although incremental improvements have been made through this approach, there is concern that these ad-hoc solutions will not meet the demands of production for large patient populations. This is due to the inherent technologies for each unit operation and their technical limitations.

Acoustic waves have long been known to have the capability to manipulate particles of all sizes in suspension in a closed, continuous manner. Now this technology, now called Acoustic Cell Processing (ACP), is being applied to key steps in the cell therapy manufacturing process as discrete unit operations such as cell selection, transduction, and concentrate/wash.

This presentation will highlight data generated by FloDesign Sonics in collaboration with several clients showing the capability of ACP. It will specifically focus on marked improvements in yield, shear force reduction and reduction in processing time. These improvements all lead to a reduction in the cost of goods, which will ultimately lead to increased access to life-saving therapies. Case study data for both Acoustic Concentrate / Wash (ACW) and Acoustic Affinity Cell Selection (AACS) with be provided (sample data below). Will be supplemented this process data with cost of goods analysis to highlight the real world impact of ACP in the production of therapeutics.

Representative AACS Data

Cell Surface Marker	Cell Conc. (x10^6/mL)	Feed Vol. (mL)	Initial % TCR-cells	Final % TCR-cells	Recovery % TCR-cells
TCR	10	100	79	96	89
TCR	30	100	79	94	95



Representative ACW Data

Process Inputs	Low Cell Density						High Cell Density
Kit Used	FDS-1LE	FDS-1LH	FDS-1LH	FDS-1LH	FDS-1LH	FDS-1LH	FDS-1LH
Volume (mL)	1105	750	1000	1000	1000	1250	950
Viable Cell Density (million/mL)	1.86	40.0	5	7	9	20	35.3
Process Outputs							
Volume (mL)	6.9	100	12	30	100	100	48.9
Cell viability Δ (%)	-1.2	-3.2	-0.2	+0.2	+0.4	-0.2	-0.8
Process Performance							
Viable Cell Recovery (%)	84	83	80	89	93	84	86
Volume Reduction Factor	160-fold	7.5-fold	83-fold	33-fold	10-fold	160-fold	19-fold
Process Time (minutes)	51	60	90	90	90	90	33

PROCESS DEVELOPMENT AND MANUFACTURE OF PRIMARY HUMAN T-CELLS IN SCALABLE, AUTOMATED STIRRED-TANK BIOREACTORS

Elena Costariol, Biochemical Engineering Department, University College London, United Kingdom
elena.costariol.16@ucl.ac.uk
Marco Rotondi, Biochemical Engineering Department, University College London, United Kingdom
Martina Micheletti, Biochemical Engineering Department, University College London, United Kingdom
Qasim Rafiq, Biochemical Engineering Department, University College London, United Kingdom

Key Words: T-cell manufacturing, stirred-tank bioreactor, T-cell phenotype, metabolite flux.

Engineered Chimeric Antigen Receptor (CAR) T-cell products have recently gained FDA and EMA approval and have demonstrated significant clinical efficacy against non-Hodgkin lymphoma and pediatric B-cell acute lymphoblastic leukemia. Despite the significant clinical and commercial progress these products represent, the high costs associated with patient-specific cell therapy manufacture needs to be addressed.

The work presented here focuses on the growth of human primary T-cells and CAR-T cells across a range of commercially available expansion platforms, including stirred tank bioreactors, which although routinely employed for the production of biologics, are not commonly used for the manufacture of T-cells. Initial experimental studies were carried out in an automated ambr® 250 single use bioreactor system which has demonstrated significant success for suspension and adherent mammalian cell culture applications. Building on previous work undertaken in the group which developed a new bioreactor vessel for microcarrier culture, both the new and existing bioreactor vessels were characterized with respect to cell yield, fold expansion, viability, metabolite profile, T-cell subpopulations and kLa. The comparison between the two vessels was performed based on power per unit volume, kLa and stirring speed, ranging from 100 to 200 rpm, using at least 3 different donors per condition.

T-Flask expansion of human primary T-cells was carried out as a static control and results were compared with the dynamic culture conditions (Figure 1). Results revealed a significantly higher fold expansion ($p<0.05$) in the ambr® 250 bioreactor at 200rpm (24.53 ± 1.50) compared to the static platform (16.38 ± 2.91). Moreover, the final product composition in terms of cell phenotype was not affected by the stirring regime. The dO_2 concentration, pH, and metabolite flux was measured throughout enabling for a better understanding of culture performance.

Further studies have compared the growth and quality of human primary T and CAR-T cells across a range of different expansion platforms including a rocking-motion (WAVE®) bioreactor, G-Rex® cell culture device, gas-permeable cell culture bags and the ambr15® microbioreactor. For each platform, the same culture conditions were used including cell source, medium formulation, and seeding density. The findings from this extensive comparability study will be presented, including an overview of the cell phenotype and quality. The findings will be used to identify the capability of each expansion platform for CAR-T process development and manufacture with the aim of developing a cost effective process for both autologous and allogeneic CAR-T cell therapies.

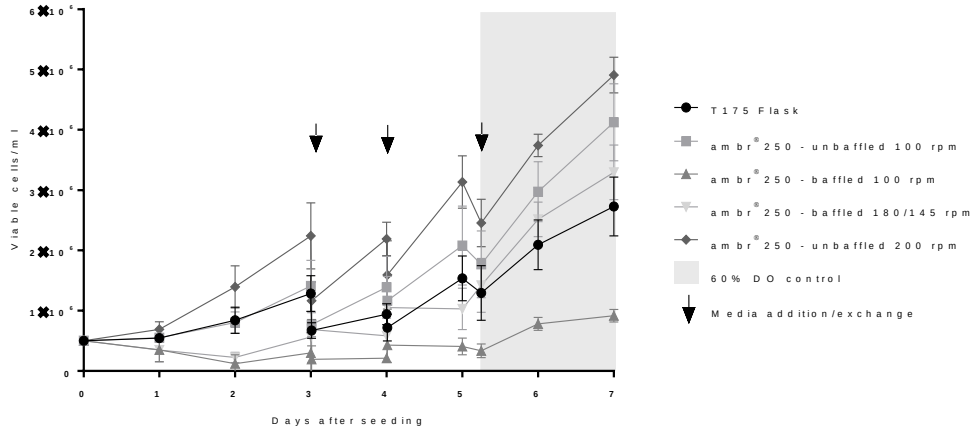


Figure 1 – Growth curves showing the viable cells density in two different vessels. The stirring speed range was varied between 100 and 200 rpm. Media addition was performed on day 3, 4 and 5, while a 60% DO control was introduced from day 5 until day 7. The cells were then harvested and stained for flow cytometry analysis.

ANIMAL COMPONENT FREE CELL CULTURE MEDIA DEVELOPMENT: THE APPROACH FROM THE NORTH

Panos Chrysanthopoulos, Centre for Commercialization of Regenerative Medicine (CCRM), Canada
panos.chrysanthopoulos@ccrm.ca
Sagar Kokal, GE Healthcare, Toronto, Canada
Anthony Eiliazadeh, Centre for Commercialization of Regenerative Medicine (CCRM), Canada

Key Words: Animal component free media, mass spectrometry, DOE, high-throughput screening.

Cell therapies have been recognized as a promising treatment for various diseases including a wide range of malignancies. Hundreds of studies investigating the efficacy of cell infusions in terms of response level, duration and survival are currently in Phase1-2 clinical trials. Several research groups have developed successful protocols for the ex vivo expansion of therapeutic progenitor cells. However, upon the establishment of good manufacturing practice (GMP) standards, most established protocols are problematic mainly due to the presence of xenogeneic or human-derived components which introduce another level of product variability and regulatory complexity. At CCRM, we established a top down process for the development of cGMP-compliant, animal component free (ACF) cell culture media using a combination of mass spectrometry based multi-omics media interrogation and a DOE based, fully automated, high-throughput cell culture screening. Briefly, our media development pipeline can be described in three phases. Firstly, we define the solution space by interrogating a complete, fully supportive, serum-containing cell medium. Mass spectrometry based, open profile proteomics and metabolomics analyses is what allows us to identify the components that are available to the cells. We then reduce the solution space by utilizing the same multi-omics approach on depleting media during a time series interrogation of batch cell cultures. Based on the concentration fluctuations of the different media components, we identify the ones that are possibly correlated with key outcomes, such as cell growth. Finally, once we identify the correlated components, we design a multifactorial DOE based high-throughput screening (HTS) experiment that allows us to pick the critical media components and optimise their concentrations using a cutting edge, entirely enclosed, liquid handling robot. A few HTS iterations are usually necessary for the identification of the optimal medium formulation. For the validation of our approach we developed an in-house ACF formulation able to support the ex vivo expansion of a primary cell type at a similar level as the serum containing gold standard in the field. During the first phase of the media development we identified 1000s of unique media components that we utilised to construct an internal library for the interrogation of the time series multi-omics data. In the second phase and during the multivariate statistical analyses, multiple Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) models indicated that a total of 48 identified components were correlated with cell growth. Finally, after an exhaustive literature mining, 28 components were promoted as potential key factors that could support cell growth in the absence of serum. Based on a custom 2 level, 28 factor, fractional factorial DOE model, we designed and ran a fully automated, high-throughput cell culture screening assay which allowed us to monitor the growth of over 2000 cell cultures in parallel. Using the total cell number as the model's response (Figure 1), we promoted 15 factors for a second HTS iteration upon which, 6 factors had a significantly positive effect and were promoted for a final HTS iteration. Based on a mixed, 2-3 level, 6 factor, fractional factorial design we were able to optimize the concentrations of the final 6 media components. Three variations of the top performing formulation were chosen to be validated across 5 donors and up to a 1L scale bioreactor. Our fully chemically defined, ACF media formulation was consistently able to support cell growth at a comparable level to serum supplemented media while maintaining the same cell phenotype. To our knowledge, this is the first time that a top-down, multi-omics media interrogation is combined with high level automation and cell culture HTS towards the media development for a cell therapy.

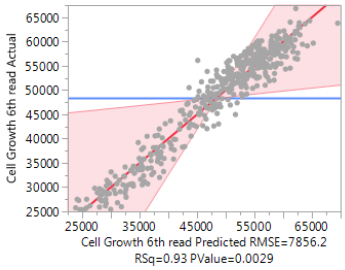


Figure 2 – Multiple Regression Model Fit on Growth Data of 1910 Cell Cultures

TRANSITIONING FROM MANUAL TO STIRRED-TANK BIOREACTOR MANUFACTURING OF IDCT, AN ALLOGENEIC CELL THERAPY TO TREAT LUMBAR DEGENERATIVE DISC DISEASE

Lara I. Silverman, DiscGenics, USA
lara@discgenics.com
Gary M. Pigeau, GE Healthcare, USA
gary.pigeau@ge.com
Terry Tandeski, DiscGenics, USA
Gala Dulatova, DiscGenics, USA
Daniel Rodriquez-Granrose, DiscGenics, USA
Will Heaton, DiscGenics, USA
Isaac Erickson, DiscGenics, USA
Azher Razvi, GE Healthcare, USA
Zoe Anderson-Jenkins, CCRM, Canada
Meghan Rooney, CCRM, Canada

Key Words: Degenerative disc disease, allogeneic cell therapy, process development, scale-up, commercialization

DiscGenics is a clinical stage regenerative medicine company focused on developing cell therapies that alleviate pain and restore function in patients with degenerative disc disease (DDD), a major cause of low back pain which is a driver of disability worldwide. The Company's lead product candidate, IDCT, is a homologous, allogeneic, off-the-shelf, injectable cell therapy under investigational use in the US (ClinicalTrials.gov Identifier: NCT03347708). The manufacturing process for IDCT involves isolating cells from donated intervertebral disc tissue and expanding them into proprietary progenitor cells known as discogenic cells. For preclinical and early clinical testing, cell production was a manual process which relied on pooling individual flasks to achieve the desired lot size. For successful scale-up and commercial production, DiscGenics seeks to modify the IDCT manufacturing process to utilize one large, single vessel per lot, while also applying bioprocess controls and more robust analytical methods to ensure consistent and optimal production of drug product. Once these changes are implemented, the product critical quality attributes (CQAs) must be maintained. DiscGenics has engaged GE Healthcare (GEHC) and the Centre for Commercialization of Regenerative Medicine (CCRM) for assay, media, and process development at the Centre for Advanced Therapeutic Cell Technologies (CATCT) in Toronto, ON., Canada. In partnership with the Federal Economic Development Agency for Southern Ontario (FedDev Ontario), CATCT accelerates the development, industrialization, and adoption of cell manufacturing technologies to improve patient access to cell and gene therapies. In this collaborative project, discogenic cells were generated in traditional static culture using CellStacks (Corning), in PBS-MINI bioreactor systems (PBS Biotech), and in stirred-tank reactors (STRs) (Eppendorf), which was led by the GEHC/CCRM team. Parameters such as cell viability, fold growth, and identity via flow cytometry were compared across modalities. For the STRs, multiple control parameters were evaluated to improve cell growth and assess successful maintenance of a consistent environment for cell quality. In this study, we found that we are able to maintain CQAs between the production modalities, with cell growth being significantly improved in the STR platform. In the STRs, in-process measurements of metabolites aligned with cell growth found using a custom sampling method. Increased cell expansion was facilitated by modified agitation, inoculation, and perfusion feeding strategies. Additionally, the process-controlled STRs provide non-invasive, continuous process data monitoring which allow for development of specified control ranges of manufacturing parameters. The quality by design (QbD) approach taken for the STR process development and improvement has allowed an increase in the lot size, process knowledge, and data-driven process definition. This presentation describes the approach and benefits of transitioning from a manual process to a suspension-based, process-controlled, stirred-tank reactor to produce allogeneic cell therapies.

SCALE UP OF IPSC-DERIVED PRODUCT MANUFACTURING

Peter Fuhrken, Director of Process Engineering, Fujifilm Cellular Dynamics, USA
peter.fuhrken@fujifilm.com

ENABLING LARGE-SCALE EX VIVO PRODUCTION OF MEGAKARYOCYTES AND PLATELETS FROM CD34+ CELLS USING GAS-PERMEABLE SURFACES AND MICROFLUIDIC BIOREACTORS

Andres Martinez, Chemical and Biological Engineering, Northwestern University,
a.martinez@u.northwestern.edu
William M. Miller, Chemical and Biological Engineering, Northwestern University

Key Words: platelets, megakaryocytes, cell therapies, gas-permeable, microfluidic bioreactor

Patients suffering from acute or sustained thrombocytopenia require platelet transfusions, which are entirely donor-based and limited by challenges related to storage and fluctuating supply. Developing cell-culture technologies enables ex vivo and donor-independent platelet production. However, the ability to generate large numbers of platelets is limited by the challenges of (1) producing many megakaryocytes (Mks) from each input CD34+ cell and (2) efficiently generating platelet-like-particles (PLPs) from Mks. To address the first challenge, we evaluated Mk production from mobilized peripheral blood CD34+ cells cultured on a commercially available gas-permeable silicone membrane, which provides efficient gas exchange, and investigated the use of fed-batch media dilution schemes. Starting with a cell surface density of 4×10^4 CD34+ cells/cm^2 (G4), culturing cells on the membrane for the first 5 days and employing media dilutions yielded 38 ± 25 Mks per input CD34+ cell by day 11 – a 2.2-fold increase compared to using standard tissue culture surfaces with full media exchanges. By day 7, G4 conditions generated 1.5-fold more CD34+ cells due to greater CD34 retention. Media dilution schemes for G4 and standard tissue culture surfaces improved culture viability, leading to a 3.6-fold increase in Mks produced per mL of media for G4 and 2.8-fold for tissue culture compared to controls. G4-Mks exhibited lower mean ploidy yet the number of high-ploidy Mks was equal to or greater than controls. Finally, G4-Mks produced proplatelets and PLPs that activated and aggregated upon stimulation. Further optimization is required to take full advantage of the gas-permeable system for Mk production. Additional studies would include refining surface densities, adjusting cytokine concentrations and initial media volumes and evaluating cord blood CD34$^+$ cells.

To address the second challenge, we applied computational fluid dynamics (CFD) modeling to assess published microfluidic platelet bioreactors and used those results to develop an improved 7-µm slit bioreactor with well-defined flow patterns and uniform shear profiles (USRB-7µm). The slits mimic fenestrations in endothelial cells lining sinuses in the bone marrow through which Mks extend cytoplasmic projections, called proplatelets (proPLTs), that are sheared off (50-200 s^{-1}) into platelets. In the USRB-7µm, a center channel flow pushes Mks into 7-µm slits, with shear rates of 5000 s^{-1}. Two outside flows converge at the slits exerting near uniform shear rates (250-350 s^{-1}) to fragment extending proPLTs, similar to the in vivo process. The USRB-7µm permits real-time visualization of proPLT formation and the rapid-release of individual platelet-like-particles (PLPs), which has been observed in vivo, but not previously reported for bioreactors. Collected PLPs exhibited characteristics similar to fresh blood platelets. Surprisingly, using only the center flow without the outside flows led to a 6-fold increase in PLP production. Based on this, we scaled-up the USRB-7µm using only a single flow to carry Mks into high-shear slit regions, mimicking in vivo observations of Mks being processed directly into platelets within the lung capillary bed (>2600 s^{-1}). The new lung-USRB retained uniform shear rates with a 93-fold capture area increase to allow more Mks to be processed into PLPs. The USRB-7µm and lung-USRB will be useful tools for the analysis of proPLT/PLP formation to further understanding of how to increase ex vivo platelet production.

These results highlight distinct improvements in Mk cell-culture and demonstrate how new technologies and techniques are needed to enable clinically-relevant production of Mks for platelet generation and cell-based therapies.

FUNCTIONALIZED MICROCARRIERS FOR ENHANCED CAR T CELL MANUFACTURING

Nate Dwarshuis, Department of Biomedical Engineering, Georgia Institute of Technology and Emory University
ndwarshuis3@gatech.edu
Hannah Wilson Song, Department of Biomedical Engineering, Georgia Institute of Technology and Emory University
Anokhi Patel, Department of Biomedical Engineering, Georgia Institute of Technology and Emory University
Krishnendu Roy, Department of Biomedical Engineering, Georgia Institute of Technology and Emory University

Key Words: microcarrier chimeric antigen receptor bioreactor

Adoptive cell therapy using chimeric antigen receptor (CAR) T cells has shown immense promise in treating cancer. In 2017, Novartis and Kite Pharma both acquired FDA approval for their CAR T cell therapies targeted toward B cell malignancies. Despite these successes, manufacturing high-quality T cells at scale has proven challenging. Notably, current processes rely on bead-based expansion and suspension-cell bioreactors, which do not recapitulate the high cell density and robust signaling found in the lymph nodes.

We hypothesized that recreating the human lymph node using functionalized biomaterials could lead to increased T cell expansion. This strategy involved functionalizing macroporous, gelatin microcarriers (commonly used throughout the bioprocess industry) with CD3/CD28 antibodies to provide activation signals. Cells can adhere to these antibodies and grow along the surface and throughout the interior, enabling efficient auto/paracrine signaling. Gelatin, as a collagen derivative, also provides matrix signals.

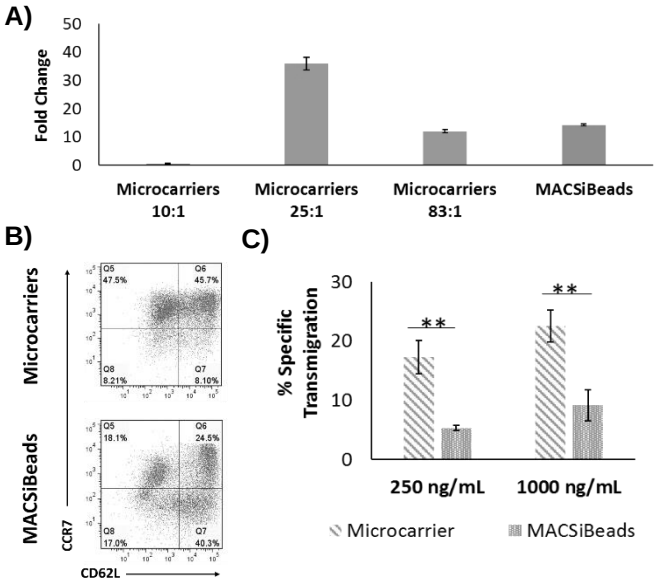


Figure 3 - a) fold change after 14 days b) surface expression of CCR7/CD62L c) chemotaxis transwell assay

Primary human T cells were incubated with carriers or conventional magnetic activation beads. Carriers cultures showed a 3-fold increase at the 25:1 ratio as compared to bead cultures (fig 1a), indicating that microcarriers can provide superior expansion if optimized for culture conditions. Furthermore, the phenotype of microcarrier-expanded T cells had a greater frequency of CD62L+CCR7+ T cells than beads, indicating higher frequency of memory T cells (fig 1b). This trend held true across multiple experiments and donors, with carriers producing 11% more memory cells than average. The functionality of memory T cells was confirmed using a CCL21 transwell assay (fig 1c). We also transfected T cells with an anti-CD19 CAR lentivirus and confirmed anti-tumor functionality after 14 days of expansion using a CD107a degranulation assay.

We further hypothesized that the microcarrier culture could be optimized using design of experiments (DOE) methodology to interrogate meaningful process parameters. The effect of IL2 concentration, initial cell density, media type, activation signal strength, and microcarrier concentration on the resulting fold change and memory phenotype were investigated. Linear regression showed that all factors except initial cell density had a significant effect on fold change, indicating that our process is not dependent on obtaining a fixed number of cells from patients. Furthermore, we found that only IL2 had an effect on memory phenotype, where an optimum was found at an intermediate setting. In summary, this information is useful in industrial settings where the concern is producing high quality cells with minimal resources.

We have demonstrated that microcarriers can provide robust expansion of high-quality memory T cells compared to state-of-the-art methods while maintaining anti-tumor functionality. Furthermore, we demonstrated that DOE methodology can be meaningfully applied to determine optimal process parameters. Further studies will be conducted in scalable bioreactors to develop translatable processes for this technology.

SCALE UP OF ALLOGENEIC CELL THERAPY MANUFACTURING IN SINGLE-USE BIOREACTORS: CHALLENGES, INSIGHTS AND SOLUTIONS

Brian Lee, President, PBS Biotech, CA, USA
blee@pbsbiotech.com
Yas Hashimura, PBS Biotech, CA, USA
Sunghoon Jung, PBS Biotech, CA, USA
Maximilian Lee, PBS Biotech, CA, USA

Key Words: single-use bioreactors; cell therapy; scale up; mesenchymal stem cells; human induced pluripotent stem cells; Vertical-Wheel bioreactors; microcarriers; aggregates.

Allogeneic cell therapy products have enormous potential to treat a wide range of unmet medical needs, with various drug candidates getting closer to commercialization. However, the limited manufacturing capacity of 2D planar technology to meet commercial demands could be a potential bottleneck for the future success of the emerging cell therapy industry. With the benefits of high volumetric productivity, operational controllability, and scalability of cell culture processes for therapeutic protein manufacturing, single-use bioreactors are recognized as promising solutions for large-scale manufacturing of cell therapy products. However, cell therapy products have unique bioprocessing requirements that are different from protein manufacturing. Not only is the final product the cell itself, but many cell therapy products are anchorage-dependent and grow on microcarriers or as cell aggregates. These microcarriers and cell aggregates are much larger than free-floating single cells and require comparatively greater power input to be suspended in a bioreactor, which can lead to hydrodynamic sheer stress and damage to cells. While various cell types require different bioreactor processes, a single-use bioreactor with the following capabilities would be greatly beneficial for reliable scale up of cell therapy manufacturing. One capability is to homogeneously and efficiently suspend large particles in a low shear environment. Another is to provide evenly distributed dissipation energy inside the vessel, leading to uniform aggregate size formation. Finally, both of these capabilities should be reproducible at larger volumes so that micro-environments inside the bioreactors are consistent across various working volumes.

A novel, single-use Vertical-Wheel bioreactor system was designed and introduced in an attempt to provide these capabilities. Experiments with various cell types such as human mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), pluripotent stem cells (PSCs), and chondrocytes have been performed in different sizes of Vertical-Wheel bioreactors with the following results. Comparable cell growth of MSCs (550,000 to 600,000 cells/mL on day 5) in a xeno-free microcarrier culture was achieved in three different scales of bioreactors (0.1L, 3L, and 15L). After the cell expansion phase, in-vessel cell dissociation from microcarriers using Trypsin was performed in the 15L Vertical-Wheel bioreactors with a greater than 90% harvest yield. Aggregates of ESCs grown in 0.5L Vertical-Wheel bioreactors were shown to be more uniform in size compared to aggregates grown in stirred-type spinner flasks. Furthermore, the narrow range of aggregate sizes (150-250 microns) after three days of cultivation in 0.5L Vertical-Wheel bioreactors was reproduced at larger 3L scale. The size of PSC aggregates could also be controlled by adjusting agitation rate, with higher speeds resulting in smaller aggregates and lower speeds in larger aggregates. In addition, pluripotency of PSC aggregates was maintained after cell expansion, as indicated by specific surface marker identifiers. Directed differentiation of PSCs in a single-use bioreactor is another important challenge of manufacturing scale up. Vertical-Wheel bioreactors have been used to successfully differentiate PSCs into different types of target cells, such as insulin-producing SC-islets or cerebellar organoids.

In order for single-use bioreactors to become the standard manufacturing platform for cell therapy products, an ideal bioreactor system should be able to deliver highly productive, reliable, and reproducible cell culture performance at commercial scale. Further details regarding manufacturing challenges and experimental data will be discussed in this presentation.

COMPARATIVE ANALYSIS OF FBS CONTAINING MEDIA AND SERUM FREE CHEMICALLY DEFINED MEDIA, CELLCOR FOR ADIPOSE DERIVED STEM CELLS PRODUCTION

Joo Youn Lee, Xcell Therapeutics, Hanhwa Biz Metro Building, Korea
jylee@xcell.co.kr
Min Hee Kang, Xcell Therapeutics, Hanhwa Biz Metro Building, Korea
Ji Young Choi, Xcell Therapeutics, Hanhwa Biz Metro Building, Korea
Yuri Chun, Xcell Therapeutics, Hanhwa Biz Metro Building, Korea
Hong-Seok Kang, Xcell Therapeutics, Hanhwa Biz Metro Building, Korea
YoungJung Kim, Xcell Therapeutics, Hanhwa Biz Metro Building, Korea
So Min Lee, Xcell Therapeutics, Hanhwa Biz Metro Building, Korea
Sang Gyu Park, Department of Pharmacy, College of Pharmacy, Ajou University, Korea

Background:
As a result of the aging society, the average OECD life expectancy has grown to about 80 years, yet the average health life still remains at only 65 years, leaving more than 15 years of life in an uncertain health state. Regenerative medicine is a new concept of medicine that combines cells and biomaterials to restore the functions of aged or damaged tissues or organs. It is also a good treatment for chronic diseases and incurable diseases, receiving attention as a new paradigm for treating diseases.

Problems:
As the market for regenerative medicine grows, mass production of consistent quality cells is required. Media is the most important thing in mass production of consistent quality cells. However, the fetal bovine serum (FBS) containing media that is currently wide used has many problems, such as unidentified viral infection, immunogenicity, lot variations, unstable supply, and ethical issues. To solve these problems and make rapid progress in regenerative medicine, a high-performance serum free chemically defined media (CDM) is needed.

Solution:
CellCor is a serum free CDM that provides excellent performance, safety, economy and consistency in stem cell production. CellCor allows higher-speed cell production rate than current FBS containing culture media (Figure 1). Compared to the FBS containing media, CellCor is able to maintain stem cell markers, higher population homogeneity, genetic stability, and excellent differentiation potency even at later passage.

Conclusion:
To sum up, by cutting the incubation time of cells with CellCor, the raw material costs of culture can also be reduced accordingly. In addition, far more stable and safer cells for therapy can be obtained compared to growth medium containing FBS and animal originated compounds. CellCor provides better position for commercialization owing to higher quality of cells.

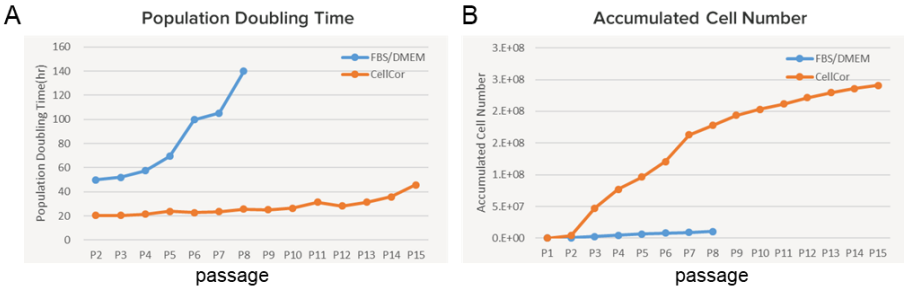


Figure1. Comparison of population doubling time and accumulated cell number of adipose derived stem cells cultured in DMEM containing FBS and CellCor

Adipose derived stem cells (ADSCs) were cultured in DMEM containing 10% FBS and CellCor, respectively. Population doubling time (A) and accumulated cell number (B) were compared. After passage 9, it was no longer possible to culture ADSCs using DMEM containing FBS.

DEVELOPING A TOOLKIT TO ENGINEER VIRAL VECTOR MANUFACTURING AND NEXT GENERATION GENE THERAPIES

Fernanda Masri, University Collge London
Michael Greene, iVexSol, USA

TAKING AN ENGINEERING APPROACH TO EMPOWER BREAKTHROUGH THERAPIES

Anil Narasimha, Chief Scientific Officer, Mekonos Biotech, USA
anil@mekonos.com

A SINGLE-USE CHROMATOGRAPHIC PURIFICATION PLATFORM FOR VIRAL GENE TRANSFER VECTORS & VIRAL VACCINES

Pavel Marichal-Gallardo, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
marichal-gallardo@mpi-magdeburg.mpg.de
Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Kathleen Börner, Center for Infectious Diseases, Virology, Heidelberg University Hospital & BioQuant, Heidelberg University & German Center for Infection Research (DZIF), Partner Site Heidelberg, Germany
Dirk Grimm, Center for Infectious Diseases, Virology, Heidelberg University Hospital & BioQuant, Heidelberg University & German Center for Infection Research (DZIF), Partner Site Heidelberg & Cluster of Excellence CellNetworks, Heidelberg University, Germany
Michael Wolff, Max Planck Institute for Dynamics of Complex Technical Systems, Germany & Institute of Bioprocess Engineering and Pharmaceutical Technology, Technische Hochschule Mittelhessen, Germany
Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems, Germany & Chair of Bioprocess Engineering, Otto-von-Guericke University Magdeburg, Germany

Key Words: Membrane chromatography; single-use; virus purification; downstream processing; platform technology

In steric exclusion chromatography (SXC), a crude sample is mixed with polyethylene glycol (PEG) and fed onto a single-use cellulose column. In this operation, selectivity is influenced strongly by the target species' size, so SXC is well suited for purification of virus particles. The purified product is recovered at physiological pH and conductivity. We have observed recoveries above 95% for several cell culture-based virus particles used as viral gene transfer vectors or as viral vaccines, including: adeno-associated virus (AAV), Modified Vaccinia Ankara (MVA) virus, influenza virus, and yellow fever virus. Preliminary data for purification of lentiviruses suggests recoveries exceeding 60%. Host cell DNA and protein depletion are typically above 90% and infectivity is not compromised thanks to the inert character of PEG towards biomolecules and the mild elution conditions.

Several AAV serotypes and display mutants were produced using HEK cells and purified with up to 95% recovery. Elution fractions had $\leq 2\times10^{14}$ viral genomes$\cdot L^{-1}$ and, depending on the specific AVV particle, the purified viruses successfully transduced or induced gene knockdown *in vitro*. Elution pools from MVA virus produced in continuous bioreactors with an avian cell line contained about 3.7×10^{9} infectious virions measured by $TCID_{50}$. For influenza virus, four strains were produced in MDCK cells. Full recovery of all strains was observed using identical SXC conditions for both infectious and chemically inactivated viruses. The column capacity in terms of the viral hemagglutinin antigen was > 50 mg$\cdot m^{-2}$. In the case of yellow fever virus, two attenuated strains were produced in Vero cells. Here, full recovery of infective titers was also achieved: the elution fraction was concentrated more than 100-fold to a titer of $>6\times10^{9}$ plaque forming units (≈100 000 doses).

In summary, SXC capture with PEG and unmodified cellulose membranes seems to perform very well for a broad range of viruses from different production processes. Thanks to the high degree of success in a relatively narrow operational range, SXC can drastically reduce process development. The high recoveries obtained so far, enable subsequent polishing operations with minimum risk to low overall process yields.

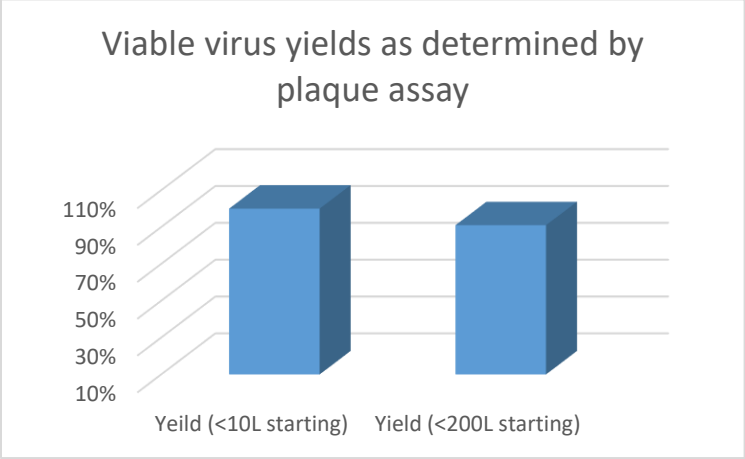
TANGENTIAL FLOW FILTRATION AND SCALABILITY FOR GENE THERAPY VIRUS PURIFICATION

Eni Sterjanaj, Pall Biotech
Enas_sterjanaj@pall.com
Heather Mallory, Pall Biotech
Jacky Dang, Pall Biotech
Rachel Legmann, Pall Biotech

Key Words: TFF, concentration, diafiltration, gene therapy, virus purification

Pall Biotech has linearly scalable tangential flow filtration technology that can cover processing volume ranges from less than a liter to over 2000 liters. Manual assemblies can be used with Pall TFF filters in scales under 20L and programmable skids are available for larger scales. In this document TFF linear scalability of up to 200 liters is covered starting from a 7L initial volume and maintaining similar pressures, processing times and yields at all scales. Using this scalable technology Pall biotech can help biologics companies go to clinical trials and to commercial scale manufacturing successfully.

Column1	Small scale (~7L Clarified bulk)	Large Scale (~200L) Projected values
Processing time (concentration)	1hr 32 min	~ 1hr 30 min
Processing time (buffer exchange)	1hr 10 min	~ 1hr 20 min
Feed flow rate (concentration)	150 ml/min	190L/H (recommended)
Feed flow rate (buffer exchange)	150 ml/min	190L/H (recommended)
Membrane surface area	0.1 m2	2.5m2
Volume processed	7 L	~200
Titer (yield by qPCR)	1.51E+10 gc/cm2 or 100%	< similar
Flow decay	-47%	< similar
Cross flow flux (concentration)	99 LMH	< similar



SCALING UP LENTIVIRAL VECTOR PRODUCTION FROM STABLE PRODUCER CELLS

Vanja Misic, CCRM
Vanja.misic@ccrm.ca
Nadejda Andreev, CCRM
Howard Kim, CCRM
Aaron Dulgar-Tulloch, GE Healthcare

Key Words: Lentiviral vector, scaleup, stable producer, perfusion

Lentiviral vectors (LVs) are commonly used for gene and cell therapies where long-term, sustained expression of therapeutic genes is needed. Legacy methods for LV production include the use of adherent cell lines, transiently transfected with viral packaging genes and the gene of interest (GOI), and cultured in media supplemented with animal sera. However, large scale production is severely limited in adherent cell culture and commercial manufacturing of LV is moving to scalable, serum-free suspension systems. In addition, stable-inducible producer cell lines may eventually replace transient systems for LV production, as this approach circumvents the costs of continually obtaining high quality or cGMP-grade plasmid DNA, the cost of the transfection reagents and the inherent variability of transfection efficiencies. Accordingly, we focused our efforts on workflows that are likely to define the LV manufacturing space in the future and to develop strategies for process development that could enable their uptake and application sooner. For our model, we used an HEK293 derived stable-inducible LV producer cell line (developed by the viral vector production team at the National Research Council Canada in Montreal and described in Manceur et al., 2017) that has been engineered to produce a third generation LV harboring the GFP transgene. A double-inducible system tightly controls the transcription of the envelope glycoprotein *VSV-G* and the viral *Rev* genes and allows for normal maintenance and expansion of cultures during seed train development, without loss of viability. This cell line is stable over many passages in culture and no antibiotics were used to maintain a selection pressure. Our goal was to bring the baseline production protocol closer to an industrial workflow that could be closed, scaled and make use of fully chemically defined media and supplements. Optimization of media formulations and feeding regimes at small scales (in shake flasks) led to the development of a multiple harvest, perfusion enabled process in a 1 L stirred tank reactor (STR) as well as a 5-25 L batch process in single use STRs. We demonstrated that to achieve high yields in multiple harvests, cell density needed to be intensified prior to induction and the medium regularly replaced with fresh medium during the production window. To avoid perfusion in the lead up to induction, while reaching a relatively high density of cells (5E6 cells/mL) in the exponential growth phase, we added GE HyClone Cell Boost 5 Supplement (3.5 g/L) to the basal media. After induction, medium was exchanged by continuous perfusion, using an acoustic filter for cell retention, at a rate of one reactor volume per day. High titer (≥1E7 TU/mL) harvests were observed at three, four and five days post induction, including the reactor contents on the fifth day, resulting in four reactor volumes of high titer product at the end of an 11-day process (including the pre-induction culturing time). For higher production scales (5 L and greater) in single use bioreactors, we failed to identify suitable single-use filtering technology that allowed LV to pass freely into the harvest, while retaining the cells in the culture vessel. Therefore, we developed a simple batch process for large scale production that consisted of inoculation, induction and a single harvest at the end of a 6-7-day process (including the pre-induction culturing time). To reach an acceptable volumetric titer (≥1E7 TU/mL) in a batch process, we supplemented the basal medium with GE HyClone LS250 lipid supplement, which resulted in a greater than 3-fold improvement in LV yield over basal media alone. A multiple harvest production mode is higher yielding than a single harvest mode for equivalent culture vessel volumes, however, the single harvest is technically simpler, uses five times less media and supplements, requires less specialized equipment, and has advantages for downstream processing. Therefore, each harvesting mode offers unique advantages and can be used to address specific production needs. In conclusion, we successfully demonstrated a process development path for an industrial workflow for LV manufacturing based on a stable-inducible producer line in fully chemically defined, serum-free media with high volumetric titers.

Reference:

Manceur, A.P., Kim, H., Misic, V., Andreev, N., Dorion-Thibaudeau, J., Lanthier, S., Bernier, A., Tremblay, S., Gélinas, A.M., Broussau, S., Gilbert, R., Ansorge, S. (2017) Scalable Lentiviral Vector Production Using Stable HEK293SF Producer Cell Lines. Hum Gene Ther Methods. 28 (6):330-339.

KEY ENGINEERING CHALLENGES IN THE BIOMANUFACTURING OF LENTIVIRAL VIRAL VECTORS

Peter Jones, Head of Technical Operations, Oxford BioMedica, UK
p.jones@oxb.com

TECHNOLOGY TRANSFER OF CELL AND GENE THERAPY PRODUCTS INTO A NEW GMP FACILITY: SUCCESS FACTORS AND KEY CHALLENGES

LiYing Yang, Head of MSAT, Lonza
LiYing.yang@lonza.com

IDENTIFICATION OF CAR T CELL CRITICAL QUALITY ATTRIBUTES AND CRITICAL PROCESS PARAMETERS

Ryan Larson, Director, Cell Therapy Product Sciences, Juno Therapeutics, USA
Ryan.Larson@junotherapeutics.com

THE DEVELOPMENT OF A 14-DAY NON-VIRAL ENGINEERED CAR T-CELL PROCESS

Emi Tokuda, Seattle Children's Research Institute
emi.tokuda@seattlechildrens.org
Joshua Gustafson, Seattle Children's Research Institute
Michael Jensen, Seattle Children's Research Institute

Key Words: Electroporation, CAR T cell, immunotherapy

Immunotherapy utilizing chimeric antigen receptor (CAR) T cells is a promising strategy for the treatment of several types of cancer. Many preclinical and clinical studies engineer CAR T cells through a viral vector, presenting the potential for genotoxicity or insertional mutagenesis. We propose a 14-day non-viral process where we introduce the gene of interest via electroporation; integration can be achieved with the Sleeping Beauty transposon system. Minicircle (MC) DNA constructs containing the CAR, a surface marker (EGFRt), and a double mutant of dihydrofolate reductase (DHFRdm) are electroporated into previously frozen, unstimulated CD4/CD8 T cells with an RNA construct coding for the Sleeping Beauty transposase. After electroporation, cells are bead-stimulated with CD3/CD28 without the use of feeder cells throughout the process. CAR+ cells expressing DHFRdm are rendered insensitive to an FDA-approved small molecule drug, methotrexate (MTX), which allows for chemical selection of the cells of interest while avoiding a magnetic bead sort. The entire process is completed in 2 weeks with a media formulation that contains a serum-free replacement.

First, we standardized electroporation conditions and observed that lower DNA concentrations resulted in lower basal levels of integration within T cells based on flow cytometry. Conversely, increased cell concentrations did not necessarily result in higher proportions of transgene positive cells. We also found that altering the concentration of MTX for chemical selection can select for cells that have a higher level of expression of the gene of interest based on MFI. Using this 14-day process and chemical selection, we were able to reach purities of >90% CD19CAR+ T cells where the majority of cells at the end of culture were of a minimally differentiated phenotype, expressing both CD45RA and CD62L. Preliminary characterization of metabolic phenotype showed that at the end of the 14-day process, cells were able to maintain a spare respiratory capacity with or without MTX selection. Initial studies showed that CD19CAR+ cells were able to produce cytokines in response to antigen-expressing target cells; preliminary analysis showed CAR+ cells respond markedly by the production of IFNγ alone or in combination with TNFα. CAR+ cells expressed the degranulation marker CD107a specifically in response to target antigen or TCR stimulation. We propose this process as a means to shorten the timeline and cost for production by using a nonviral method to engineer CAR T cells, avoid the use of feeder cells, and chemically select for cells of interest. Ultimately, this workflow is also applicable to CARs of any specificity and allows for multiplexing.

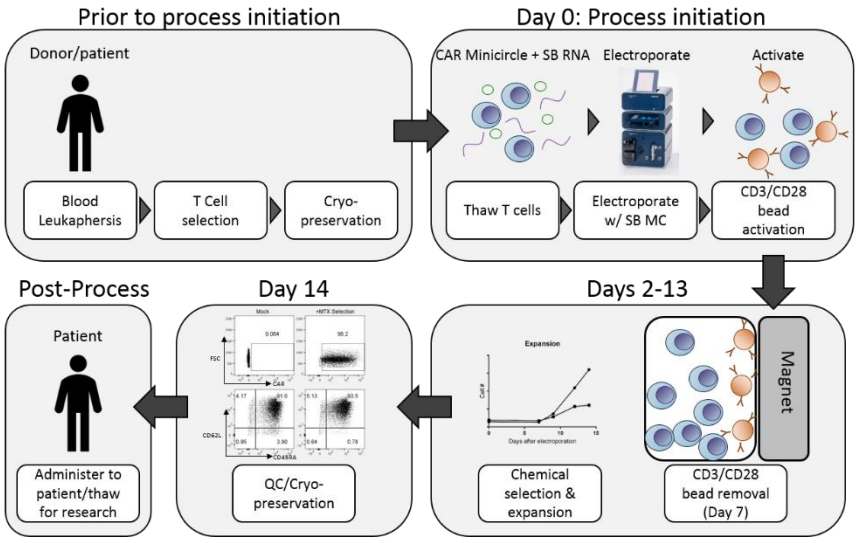


Figure 4. Schematic of 14-day nonviral process of CAR T cell production. Prior to starting a manufacturing process, T cells are isolated from a leukapheresis product and cryopreserved. Thaw, electroporation, and stimulation of the primary T cells occurs on day 0, where cells are electroporated with CAR-containing MC constructs that will be integrated into the genome by the SB transposase RNA. Chemical selection begins on day 2 of the process and occurs through the remaining 12 days, with a bead removal step at day 7. On day 14, cells are characterized and then cryopreserved for later studies.

EFFECTING CLINICAL STARTING MATERIAL QUALITY AND THE IMPACT TO DOWNSTREAM PROCESSING

Dominic Clark, Global Head of Cell Therapy, HemaCare Corporation, USA
DClarke@hemacare.com

RAMAN SPECTROSCOPY OF CELLS AS A PROCESS ANALYTICAL TECHNOLOGY

Jamie Piret, Professor of Chemical & Biological Engineering, University of British Columbia, Canada
james.piret@ubc.ca

THE DYNAMIC MASS SPECTROMETRY PROBE (DMSP) – ADVANCED PROCESS ANALYTICS FOR THERAPEUTIC CELL MANUFACTURING, HEALTH MONITORING AND BIOMARKER DISCOVERY

Andrei G. Fedorov, Georgia Institute of Technology
AGF@gatech.edu
Mason A. Chilmonczyk, Georgia Institute of Technology
Austin L. Culberson, Georgia Institute of Technology
Peter A. Kottke, Georgia Institute of Technology
Gian C. Rivera Crespo, University of Puerto Rico Mayagüez
Robert E. Guldberg, University of Oregon

Key Words: Microfabricated Sampling Platform, Biomarker Monitoring and Discovery, Online ESI-MS

Spatially and temporally resolved *in situ* monitoring of biochemical cell culture environments, e.g., in application to therapeutic cell bioreactors, is of critical importance for facilitating the development of new and reliable quality control methodologies for cell therapies. Identifying and monitoring secreted biomolecular critical quality attributes (CQAs) to enable online feedback control will enable large scale, cost-effective manufacturing of therapeutic cells. These CQA biomarkers have varying concentrations within a bioreactor, both in time and space. Current methods for monitoring these diverse biomolecules are generally ex-situ, time consuming, destructive, provide bulk measurements, or lack the ability to reveal the complete secretome/metabolome composition. The Dynamic Mass Spectrometry Probe (DMSP) synergistically incorporates a sampling interface for localized intake of a small fluid volume of the cellular content, a micro-fabricated mass exchanger for sample conditioning and inline separation, and an integrated electrospray ionization (ESI) emitter for softly ionizing (i.e. preserved biochemical structure) extracted biomolecules for mass spectrometry (MS). ESI-MS via DMSP treatment enables both biomarker discovery and transient (~1 min) analysis of biochemical information indicative of cell health and potency. DMSP is manufactured using advanced batch microfabrication techniques, which minimize dead volume (~20 nL) and ensure repeatable operation and precise geometry of each device. DMSP treatment removes 99% of compounds that interfere with mass spectrometry analysis, such as inorganic salts, while retaining biomolecules of interest within the sample for ESI-MS analysis. DMSP has demonstrated the ability to substantially increase signal to noise ratio in MS detection of biomolecules, and to further enhance sensitivity for probing lower biomarker concentrations via introduction of ESI-MS enhancing molecules (i.e. proton donating chemicals, protein denaturing solvents, and supercharging agents) into the sample within the integrated mass exchanger. To exemplify the DMSP's unique capabilities, Fig. 1 demonstrates detection of multiple low-concentration protein biomarkers sampled from a biochemically-complex cell media solution serving as a proxy to samples taken directly from cell growth bioreactors [1].

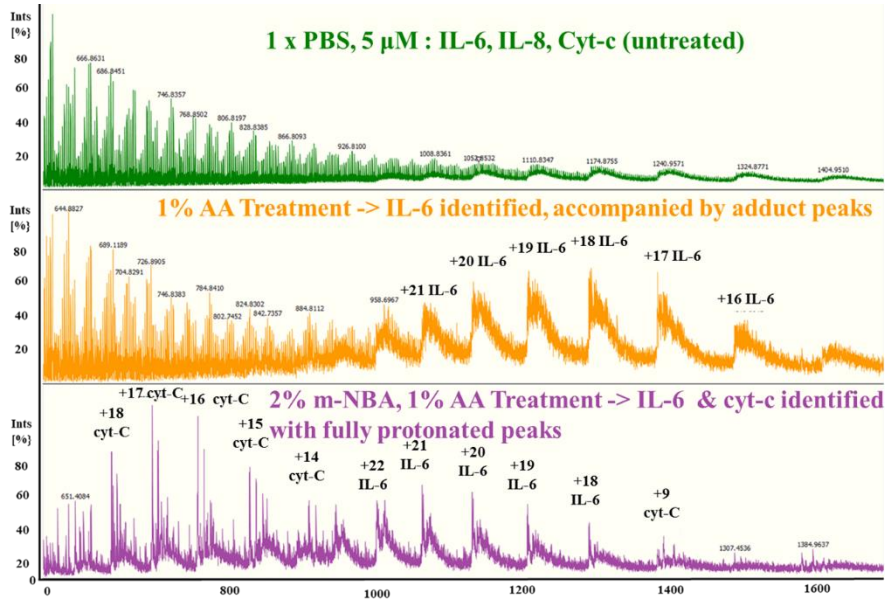


Figure 1 - Mass spectra produced via direct infusion ESI-MS through DMSP A. Untreated 1xPBS with 5 uM cytochrome-c (12 kDa), 5 µM IL-6 (21 kDa), and 5 µM IL-8 (8.4 kDa) shows no identifiable peaks associated with protonation of biomolecules. B. 1% AA treatment reveals multiple charge states associated with IL-6 only. C. 1% AA 2% m-NBA treatment reveals fully protonated charge states of cytochrome-c and IL-6.

[1] Chilmonczyk, M. A., Kottke, P. A., Stevens, H. Y., Guldberg, R. E., Fedorov A. G Dynamic mass spectrometry probe (DMSP) for ESI-MS monitoring of bioreactors for therapeutic cell manufacturing, *Biotech & Bioeng*. (subm).

IMPROVING FUNCTIONAL MATURATION OF HUMAN PLURIPOTENT STEM CELLS DERIVED CARDIOMYOCYTES THROUGH METABOLIC UNDERSTANDING

Margarida Serra, iBET/ITQB-UNL, Portugal
mserra@ibet.pt
Cláudia Correia, iBET/ITQB-UNL, Oeiras, Portugal
Alexey Koshkin, iBET/ITQB-UNL, Oeiras, Portugal
Patricia Duarte, iBET/ITQB-UNL, Oeiras, Portugal
Maria João Sebastião, iBET/ITQB-UNL, Oeiras, Portugal
Patrícia Gomes-Alves, iBET/ITQB-UNL, Oeiras, Portugal
Ana Teixeira, iBET/ITQB-UNL, Oeiras, Portugal
Paula M Alves, iBET/ITQB-UNL, Oeiras, Portugal
David Elliott, Children's Research Institute, The Royal Children's Hospital, Australia
Dongjian Hu, Harvard Stem Cell Institute, USA
Ibrahim Domian, Harvard Stem Cell Institute, USA

Key Words: cell therapy, hPSC-derived cardiomyocytes, three-dimensional culture, bioenergetics, omics technologies

In vitro differentiation of human pluripotent stem cells into cardiomyocytes (hPSC-CMs) is a crucial process to enable their application in cell therapy and drug discovery. Nevertheless, despite the remarkable efforts over the last decade towards the optimization of cardiac differentiation protocols, generated hPSC-CMs are still immature, closely reminiscent of fetal cardiomyocytes in what regards structure, metabolism and function. In this study, we aim to overcome this hurdle by devising a novel metabolic-based strategy to improve hPSC-CMs generation and functionality. Noteworthy, we integrated structural and functional analyses of hPSC-CM with powerful "omics" technologies (proteomics, transcriptomics, metabolomics and fluxomics) as complementary analytical tools to support process optimization and product characterization.

We relied on the aggregation of hPSC-derived cardiac progenitors to establish a scalable differentiation protocol capable of generating highly pure CM aggregate cultures. Whole-transcriptome analysis and ^{13}C-metabolic flux analysis demonstrated that a three-dimensional (3D) and agitated-based culture environment enhances metabolic maturation of hPSC-CMs. When compared to static monolayer, 3D cultures of hPSC-CMs displayed down-regulation of genes involved in glycolysis and lipid biosynthesis and increased expression of genes involved in OXPHOS. Accordingly, 3D hPSC-CMs had lower fluxes through glycolysis and fatty acid synthesis and increased TCA-cycle activity.

We then assessed if alteration of culture medium composition to mimic *in vivo* substrate usage during cardiac development improved further hPSC-CM maturation *in vitro*. Our results showed that shifting hPSC-CMs from glucose-containing to galactose- and fatty acid-containing medium promotes their fast maturation into adult-like CMs with higher oxidative metabolism, transcriptional signatures closer to those of adult ventricular tissue, higher myofibril density and alignment, improved calcium handling, enhanced contractility, and more physiological action potential kinetics. "-Omics" analyses showed that addition of galactose to culture medium improves total oxidative capacity of the cells and ameliorates fatty acid oxidation avoiding the lipotoxicity that results from cell exposure to high fatty acid levels.

This study demonstrated that metabolic shifts during differentiation/maturation of hPSC-CM are a cause, rather than a consequence, of the phenotypic and functional alterations observed. The metabolic-based strategy established herein holds technical and economic advantages over the existing protocols due to its scalability, simplicity and ease of application. Improved maturation and functionality of *in vitro* generated hPSC-CM will excel their application in cell therapy, drug discovery and cardiac disease modeling.

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SINGLE CELL ANALYSIS OF VIRAL TRANSDUCTION AS A NOVEL TOOLBOX FOR AN IMPROVED CHARACTERISATION OF CELL THERAPY PRODUCTS

Nicole Nicholas, Cell and Gene Therapy Catapult
nicole.nicholas@ct.catapult.org.uk
Vincenzo Di Cerbo, Cell and Gene Therapy Catapult
Ilaria Santeramo, Cell and Gene Therapy Catapult
Enas Hassan, Cell and Gene Therapy Catapult
Beata Surmacz-Cordle, Cell and Gene Therapy Catapult
Damian Marshall, Cell and Gene Therapy Catapult

Key Words: Single cell analysis, viral copy number, droplet digital PCR

Ex-vivo gene modified cell therapies are increasingly being developed for the treatment of monogenic diseases and various forms of cancer. In the last few years three gene modified cell therapy products have been granted regulatory approval and there are currently over 600 clinical trials being conducted using this type of cell therapy. However, the risks associated with therapies based on genetically modified cells are not fully understood and safety concerns could hamper their ongoing development.
Retroviral or lentiviral vectors are often used in the generation of genetically modified cells, and these vectors integrate into the host genome for a long lasting and efficacious therapeutic effect. Due to their random integration, viruses may disrupt key genes in the host cells leading to therapy-induced secondary diseases. Hence, ensuring consistency during the transduction process through controlled delivery and integration of the viral vectors is critical to controlling the product quality.
Current methods are limited to the analysis of viral copy number on populations of cells. Whilst this gives an estimate of the viral cell copy number across the population, it does not account for cell-to-cell variations in the number of viruses integrating into the genome and the risk that rapidly proliferating clonal populations could compromise the efficacy and the safety of the product. Though this method is a regulatory requirement, the limitation of population-based assessment has prevented its use as an effective in-process control and development tool. New techniques to accurately determine the copy number per cell will be key to improving product characterisation and the regulatory criteria for product safety.
To address this need we have designed a single cell capture, processing and analysis approach to measure the viral copy number heterogeneity of a human T-cell immunotherapy model. Our unique and novel approach uses cutting-edge droplet digital PCR to evaluate the cell-to-cell variability within the population by accurately measuring the number of viral integrations per single cell. This method provides a robust and reliable toolbox for the analysis of integration events in single cells and can be fully customisable for the analysis of any viral or non-viral transgene.
The incorporation of this method within a process development pathway means that key factors that impact transduction and promote homogeneity in viral integration, including viral input, can be effectively optimised, improving a critical step in the immunotherapy manufacture workflow.
In addition, the use of this method as a replacement of the current population-based safety test, ensures that cell therapy products meet a high safety standard with a known number of cells containing a desired number of viral copies.
This assay is currently being incorporated into the manufacturing process of a cell therapy company and has the potential to play a key role in supporting the development of safe and efficacious cell therapy products for the treatment of a range of diseases.

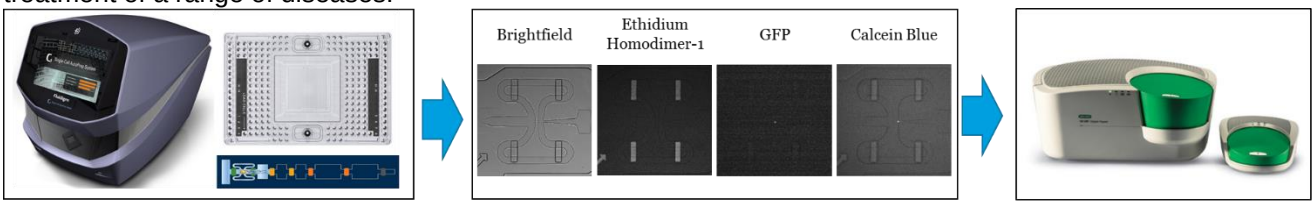

Figure 1: Single cell viral copy number workflow.

EVALUATING THE IMPACT OF CULTURE CONDITIONS ON HUMAN MESENCHYMAL STEM/STROMAL CELL-DERIVED EXOSOMES THROUGH FTIR SPECTROSCOPY

Ana Fernandes-Platzgummer, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal; The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Portugal
ana.fernandes@tecnico.ulisboa.pt
Maria João Canha Pereira, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
Luís Ramalhete, ISEL-Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Portugal
Sandra Aleixo, ISEL-Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Portugal; Centro de Estatística e Aplicações, Faculdade de Ciências, Universidade de Lisboa, Portugal
Cláudia Lobato da Silva, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal and The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Portugal
Joaquim M. S. Cabral, Department of Bioengineering and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal and The Discoveries Center for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Portugal
Cecília R.C. Calado, ISEL-Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Portugal

Key Words: Mesenchymal Stem Cells, Exosomes, Purification, FTIR Spectroscopy, Molecular Fingerprint.

In the last decade, the therapeutic effects of mesenchymal stem/stromal cells (MSCs) have been attributed to a paracrine activity exerted by extracellular vesicles secreted by MSCs, as exosomes. Their properties as intercellular communication vehicles have led to an increase interest in their use for cell-free therapeutic applications. The present work aimed to evaluate how different culture conditions, as culture medium (xenogeneic -free (XF) vs serum-containing medium), conditioning time (1, 2 and 3 days) and different MSC donors (n=6), affect the chemical characteristics of exosomes. For that, purified MSC-derived exosomes were characterized by Fourier-Transform InfraRed (FTIR) spectroscopy, a highly sensitive, fast and high throughput technique. The principal component analysis (PCA) of pre-processed FTIR spectra of purified exosomes was conducted, enabling the evaluation of the replica variance of the exosomes chemical fingerprint in a reduced dimensionality space. For that, different pre-processing methods were studied as baseline correction, standard normal variation and first and second derivative. It was observed that the chemical fingerprint of exosomes is more dependent of the medium used for MSCs cultivation than the MSC donor and conditioning days. Exosomes secreted by MSCs cultured with serum-containing medium presented a more homogenous chemical fingerprint than exosomes obtained with XF medium. Moreover, for a given medium (XF or serum-containing medium), the exosomes chemical fingerprint depends more of the MSC donor than of the conditioning days. The regression vector of the PCA enabled to identified relevant spectral bands that enabled the separation of samples in the score-plot of the previous analysis. Ratios between these spectral bands were determined, since these attenuate artifacts due to cell quantity and baseline distortions underneath each band. Statistically inference analysis of the ratios of spectral bands were conducted, by comparing the equality of the means of the populations using appropriate hypothesis tests and considering the significance level of 5%. It was possible to define ratios of spectral bands, that can be used as biomarkers, enabling the discrimination of exosomes chemical fingerprint in function of the medium used for MSC grown and the MSC donor. This work is therefore a step forward into understanding how different culture conditions and MSC donors affect MSC exosomes characteristics.

THE EMERGING ROLE FOR AI IN CELL AND GENE THERAPY MANUFACTURE

Damian Marshall, Director, New & Enabling Technologies, Cell & Gene Therapy Catapult, UK
damian.marshall@ct.catapult.org.uk

EFFICIENT MODEL DRIVEN DESIGN OF CELL BASED PRODUCT MANUFACTURING

Robert Thomas, Loughborough University, Advanced Bioprocess Design Ltd
R.J.Thomas@Lboro.ac.uk
Katie Glen, Loughborough University, Advanced Bioprocess Design Ltd
Elizabeth Cheeseman, Loughborough University, Advanced Bioprocess Design Ltd
Maryam Shariatzadeh, Loughborough University
Ben Diffey, Loughborough University
Adrian Stacey, Loughborough University

Key Words: Bioprocess, Model, Optimize, Risk, Manufacture

Advanced Therapy Medicinal Products (ATMPs) pose a continuing challenge to manufacturing process development. Despite the adoption of a structured approach to development through systematic frameworks such as Quality by Design, a costly rate of process failure or underperformance is encountered at key transitions such as transfer to contract manufacture organizations or changes of scale or equipment. The complexity of the products, particularly the cell culture step, is frequently a contributor to such issues; this complexity challenges many process development tools, in particular the number of potential process variables and consequences results in a lower quality of evidence base informing early risk assessments, creates difficulties in experimental prioritization and efficiency, and can result in poor experimental coherence over the course of product development (i.e. a failure to efficiently harness/record all data and apply to manufacturing goals). We have proposed that rooting development in a suitable process model would lessen these issues. We have developed a framework that takes advantage of the commonality across ATMP manufacturing processes[(1)]. For example, features such as cell growth, paracrine inhibition or lineage selection, and cell death are representable by a limited set of mathematical building blocks. These behaviors interact with process operation to determine critical manufacturing outcomes such as product cost and identity. From an operational perspective there are a limited number of common process operations such as dilutions, purification or factor additions. This enables a modelling framework that can be constrained whilst still representing a wide range of process dynamic hypotheses and associated manufacturing scenarios. Case studies will be presented across a variety of platforms. These include intensification of hematopoietic lineage cell processing in suspension bioreactors (ambr15) including erythroblast and T-cell processing. In each of these cases a model of cell population growth was developed to optimize short term cell volume productivity. This was applied over a longer timeframe to quantify risks (on yield and phenotypic selection) of longer term operational strategies and control such as feed rates or variability in timings and volumes. This provides a basis to specify manufacture based on cost targets, operational constraints (e.g. feed frequency, reactor size) and risk tolerance. We will further present application of the same approach to gain insight into optimization of specific culture phenomena, such as lag phase and growth factor delivery, that have a potentially high impact on manufacturing outcomes.

(1) AJ Stacey, EA Cheeseman, KE Glen, RLL Moore, RJ Thomas. Experimentally integrated dynamic modelling for intuitive optimization of cell-based processes and manufacture. Biochem Eng J. 2018. 132: 130-138

IMPLEMENTATION OF AUTOMATED PROCESS MONITORING AND DATA CAPTURE

Dina Ibrahim, Kite Pharma
dibrahim@kitepharma.com
Kevin Boomhouwer, Kite Pharma

Key Words: Process Monitoring, Data, Visualization.

Kite Pharma is implementing an automated data capture and visualization tool for automated continued process verification (CPV) to align with FDA best practices on process validation. The increased volume and variety of data associated with autologous therapies add significant scalability challenges for traditional process monitoring workflows. The tool's intent is to reduce the time spent sourcing, aggregating, and transforming the data into a form ready for consumption by operations management, and provides unified views of joint patient/process data for improved knowledge management. The system utilizes an enterprise data lake cloud solution in combination with a variety of big data technologies to ingest and integrate data from disparate source systems, transform the data per defined business rules, and finally load the data into a publishing layer for consumption by various visualization and analytical tools. The platform will be fully GMP compliant and meet 21 CFR Part 11 guidelines with full audit capability. The visualization tools will dashboard key analytics against control limits, with the capability of providing timely alerts to enable earlier identification of process deviations leading to faster response. The tool is built upon advanced analytics platform, which will be capable of machine learning, predictive analytics, and pattern recognition. A series of case studies will support the key advances achieved from this approach.

USING GAUSSIAN MIXTURE MODELS AND MACHINE LEARNING TO PREDICT DONOR-DEPENDENT MEGAKARYOCYTIC CELL GROWTH AND DIFFERENTIATION POTENTIAL EX VIVO

William M. Miller, Chemical and Biological Engineering, Northwestern University, Evanston, IL
wmmiller@northwestern.edu
Jia J. Wu, Darryl A. Abbott, Neda Bagheri; Chemical and Biological Engineering, Northwestern University
Meryem K. Terzioglu, Dolores Mahmud, Nadim Mahmud; Hematology/Oncology, University of IL at Chicago

Key Words: culture-derived platelets, flow cytometry, cell therapies, time-course data, cell-surface markers

The ability to analyze single cells via flow cytometry has resulted in a wide range of biological and medical applications. Currently, there is no established framework to compare and interpret time-series flow cytometry data for cell engineering applications. Manual analysis of temporal trends is time-consuming and subjective for large-scale datasets. We resolved this bottleneck by developing TEmporal Gaussian Mixture models (TEGM), an unbiased computational strategy to quantify and predict temporal trends of developing cell subpopulations indicative of cellular phenotype.

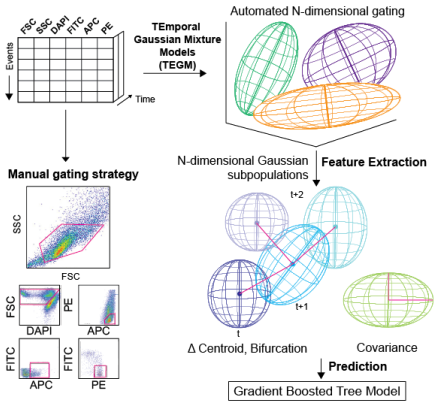

Figure 1 – Gaussian mixture models characterize each population subset in MK culture time-course data.

TEGM applies Gaussian mixture models and gradient boosted trees for cell engineering applications. TEGM enables the extraction of subtle features, such as the dispersion and rate of change of surface marker expression for each subpopulation over time. These critical, yet hard-to-discern, features are fed into machine-learning algorithms that predict underlying cell classes. Our framework can be flexibly applied to conventional flow cytometry sampling schemes, and allows for faster and more consistent processing of time-series flow cytometry data.
As a proof-of-concept, we applied our method to the analysis of *ex vivo* megakaryocytic (Mk) differentiation and maturation of hematopoietic cells from donors with greatly varying potential to generate $CD41^+CD42^+$ mature Mk cells. We illustrate the major steps of the computational approach by predicting peak $\%CD41^+CD42^+$ MK maturation of $CD34^+$-selected umbilical cord blood (CB) cells from 16 independent donors (Figure 1). Cells were cultured over a 19-day multi-phase differentiation culture, consisting of a pre-expansion phase and a differentiation phase. The novel dataset comprised 720 measurements from 80 perturbations of 16 individual donors, with 9 time-point measurements sampled every 2-5 days for each donor. We constructed an automated gating strategy to extract surface marker expression of various clusters of $DAPI^{low}CD41^+$ Mk cells. Notably, we demonstrate that estimation of the $\%CD34^+$ and $\%CD42^+$ cells was within 1% of manual gating estimates, thus illustrating the consistency and accuracy of the technique. A gradient boosted tree model was trained using an explanatory matrix describing early characteristics and tested to predict peak $CD41^+/CD42^+$ marker expression. We then performed feature extraction for each flow cytometry time-course dataset on several descriptors, such as growth rate, viability, production, percentage positivity of each surface marker, covariance of mean fluorescence intensity, rate of change, and bifurcation of each subpopulation. A gradient boosted tree model was trained using an explanatory matrix describing early characteristics (Day 0 to Day 9) and tested to predict peak $CD41^+CD42^+$ marker expression, which typically occurs on Day 14 to Day 17.
Overall, we identify several influential early culture factors that are predictive of peak $\%CD42^+$ expression. We show that $\%CD41^+$ on Day 5 and Day 7 is highly predictive, while cell viability and $\%CD34^+$ are comparatively less predictive of peak $\%CD42^+$. We are able to identify the best performing cultures with high sensitivity and specificity (AUROC = 0.92, where 1 denotes perfect accuracy). Predicted and actual $CD41^+CD42^+$ responses are highly correlated using three independently selected partitions of test/training sets of our data (Figure 2; p = 7.4e-09, R = 0.87). Identifying CB units with high and low MK potential early in the 19-day culture process can save expensive resources and time, and provides the potential to intervene during the culture process.

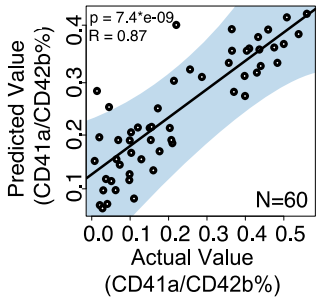

Figure 2 – TEGM with gradient boosted tree model predicts peak $\%CD41a^+/CD42b^+$ of Mk cultures.

ESTABLISHING SUCCESSFUL COMMERCIAL CAR T MANUFACTURING ON A SHORT TIMELINE: A PROCESS DEVELOPMENT PERSPECTIVE

Thomas Brieva, Celgene
tbrieva@celgene.com

Key Words: Cell therapy, commercial manufacturing, GMP

The transformational impact of CAR T cell therapies on serious diseases enables a rapid path to licensure. Although many challenges of reliably delivering autologous CAR T therapies have been managed at clinical scale and quality, the transition to commercial scale and widespread availability to desperate patients presents many additional challenges. The process development contribution of pragmatic and engineering-based operations science with respect to facility fit, scale-out, and patient variability is essential to the success of this transition.

Whether adapting an existing facility or constructing a new one, GMP workspace design requires detailed process evaluation. In addition to maximizing efficient use of space, equipment, and labor, addressing facility impact on process is critical. For example, the scale or layout of a large commercial facility can increase the time necessary to perform a process step. As a result, the process runs within a different range of the parameter space within the facility. We describe time-related process characterization data and closed-system solutions to facility fit challenges along with the process expertise which contributes to successful technology transfer to new facilities. The task of facility fitting becomes increasingly complex as multiple facilities are implemented, as it is desirable to minimize facility-to-facility process variation.

A dramatic increase in scale (number of patient-batches) is associated with establishment of commercial manufacturing. Due to an individual batch being performed for each patient, an increase in the necessary facilities, equipment, and staff accompanies the scale increase. As new manufacturing, QC, and QA talent ramps up the workforce, a robust training program that imparts both process instruction and understanding is critical. Further, process design and batch record clarity must be improved to minimize process variability. We describe process and batch record improvements that address rapid workforce expansion and training. Autologous cell therapies present patient variability, which results in variable process performance and product characteristics. As the number of patient-batches increases, the number of occurrences uncommon process phenotypes also increases. This requires extensive instructions on how to consistently adjust the process for patient variability as well as process design that minimizes the effect of patient variability on performance while ensuring a consistent, high quality product. In addition, process performance data must be monitored to assess process performance and variability. We discuss examples of patient variability, troubleshooting, and batch history data.

NOVEL SUPPLY CHAIN AND PROCESS MODELING FOR CELL THERAPY MANUFACTURING AND DISTRIBUTION

Chip White, Georgia Institute of Technology
chip.white@isye.gatech.edu
Ben Wang, Georgia Institute of Technology
Kan Wang, Georgia Institute of Technology
Kris Saha, University of Wisconsin-Madison
Reid Bishop, Georgia Institute of Technology
Tava Das, University of Wisconsin-Madison
Aaron Levine, Georgia Institute of Technology
Brian Liu, Georgia Institute of Technology
Bhavya Divvela, Georgia Institute of Technology
Aubrey Incorvaia, Georgia Institute of Technology

Key Words: supply chain; simulation; risk mitigation; logistic cost reduction; scale-up manufacturing and distribution

We present a two-level hierarchical supply chain model of (autologous) CAR-T cell therapy that serves as the basis for the development of strategies to: 1) deliver cell therapy products that are safe and have a high level of efficacy, 2) minimize fulfillment time and variability, and 3) reduce total manufacturing and logistics costs while reducing the risk of patient morbidity and mortality. The model consists of two integral components: (1) an agent-based program for a “single manufacturing facility” that simulates the manufacturing and quality control process of cell therapy; and (2) a supply chain network program that evaluates different supply chain configurations and sourcing strategies. The two-level hierarchical supply chain model can be used as a decision support system to explore manufacturing, quality assurance, and supply chain and logistics ‘what if’ questions. Using the model, we explored the impact of reagent supply chain disruptions to manufacturing and evaluated the effectiveness of different tools that can mitigate unexpected supply disruptions. We intend to use this model to support the design and operation of supply chains for end-to-end manufacturing and logistics of large-scale, low-cost, reproducible and high-quality cell therapy products.

A ROADMAP TO SUCCESSFUL COMMERCIALISATION OF AUTOLOGOUS CAR T-cell PRODUCTS WITH CENTRALISED AND BEDSIDE MANUFACTURE

Tania Pereira Chilima, Department of Biochemical Engineering, University College London, UK
tania.chilima.10@ucl.ac.uk
Suzanne S. Farid, Department of Biochemical Engineering, University College London, UK

Key Words: Cost of goods (COG), net present value, process economics, supply chain, reimbursement, centralised, decentralised, bedside, GMP-in-a-box, market access

The availability of two CAR T-cell therapies on the market has cemented the therapeutic potential of these products to treat oncology patients. However, in order for CAR T-cell therapies to be available to a wide number of patients, cell therapy developers must carefully design their manufacturing and commercialisation strategy. This analysis must take into account multiple factors related to the target market characteristics (EU v USA), the product features (e.g. dose size), manufacturing process (e.g. automated v manual platforms) as well as facility network (e.g. centralised v bedside manufacture) and supply chain requirements (e.g. fresh v frozen products). This presentation aims at assessing the implications of the choices made for each of these critical factors to provide a clear framework for decision-making during early stages of the development process of autologous CAR T-cell products. The resulting roadmap enables the successful commercialisation of these powerful therapeutics. This analysis was carried out using an advanced decisional tool developed at University College London. The case study assesses the economic and operational effects of the decisions made at the different levels of manufacturing and commercialisation strategy by computing metrics such as cost of goods, fixed capital investment, net present value, personnel requirement and facility footprint, while considering potential constraints relating to technology capacity, viral vector stock availability, product shelf life, market access and reimbursement strategies.

AUTOLOGOUS CELL THERAPY CAPACITY PLANNING AND DEBOTTLENECKING USING SIMULATION AND OPTIMIZATION

David Zhang, Bioproduction Group, Inc.
david.zhang@bio-g.com

Key Words: autologous cell therapy, CAR-T, modeling, simulation, optimization, manufacturing, commercialization

Autologous cell therapy is a groundbreaking treatment for patients that also has distinct scale-up and commercialization challenges. In this presentation, we discuss the differences between traditional life science versus autologous manufacturing, the underlying mathematical theory, and the use of optimization and simulation to accurately identify and resolve the capacity issues unique to this market. We also look ahead to the future of autologous manufacturing and the issues that must be addressed as more drugs become available commercially worldwide.

AUTOMATED DATA MANAGEMENT STRATEGIES DRIVE CELL THERAPY SUCCESS

Christophe Suchet, Vineti, USA
christophe.suchet@vineti.com

When the patient *is* the product, personalized therapies require advanced IT strategies. Safe, efficient, cost-effective cell therapies demand constant, finely tuned collaboration across dozens of stakeholders and multiple geographies. Automated data management systems, including cloud-based technologies and real-time feedback loops, can solve key challenges, and make the difference between a therapy that reaches a few patients and one that reaches many.

THE TECH ENABLED PHARMA COMPANY

Neil Tiwari, Chief Digital Officer, Novartis, USA
neil.tiwari@novartis.com

LEVERAGING DIGITAL SOLUTIONS TO PREDICT DISEASE AND PATIENT ACCESS TO TRANSFORMATIVE THERAPIES

Ben Newton, Chief Digital Officer, GE Healthcare, USA
ben.newton@ge.com