BIO REGULATES THE *EX VIVO* EXPANSION AND FUNCTION OF HEMATOPOIETIC STEM CELLS BY INHIBITING GSK-3β

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Key words: $CD34^+$ cells expansion, Wnt pathway, BIO, β-catenin, *in vitro* function

Hematopoietic stem cells (HSCs) have been applied in clinic settings for treating hematologic diseases, including leukemic disorders, immune deficiencies, and hemoglobinopathies. Umbilical cord blood(UCB) is an important source of HSCs. However, the low frequency of HSCs per unit of UCB remains a big hurdle to their wider applications. Wnt/β-catenin pathway plays important roles in the self-renewal of HSCs *in vivo*, but the roles of Wnt/β-catenin signaling on *ex vivo* expansion of HSCs remains controversial. GSK3β is the major regulator of Wnt pathway. Here, we evaluate the effects of 6-bromoindirubin-3'-oxime (BIO), a GSK3β inhibitor, on *ex vivo* expansion characteristics and regenerative potential of (UCB)-derived $CD34^+$ cells.

First, the expansion of $CD34^+$ cells at the presence of different concentrations of BIO was examined. Compared to the control, BIO treatment favored the expansion of $CD34^+$ cells within the 4-day culture, while there was a trend toward repress the expansion of $CD34^+$ cells on day 7. These results indicated that Wnt/β-catenin pathway is potentially related to the expansion of $CD34^+$ cells. Subsequently, western bolt analysis showed that the amounts of β-catenin, phosphorylated GSK3β, and downstream effector Cyclin D1 were unchanged by BIO treatment at days 4 of culture. Notably, the β-catenin accumulated gradually with the prolongation of culture time peaking at day 7 with BIO treated. However, transcription factor TCF showed significant decrease at days 7 compared to days 4 of culture. Remarkable increase in total CFU number was observed in BIO-treated cells relative to control, mainly because the frequency of CFU-GM (granulocyte, macrophage) was significantly higher. The expansion fold of total cells derived from BIO-cultured CD34+ cells favored the secondary expansion of total cells at day 4 and 14.

In conclusion, Wnt/β-catenin signaling agonist BIO regulated the expansion of $CD34^+$ cells and differential expression of key factors in Wnt signaling pathway in a β-catenin-dependent fashion during *ex vivo* culture. Furthermore, BIO-expanded $CD34^+$ cells showed better multilineage commit potential and secondary expansion ability, which provides a valuable guidance for optimizing *ex vivo* culture.

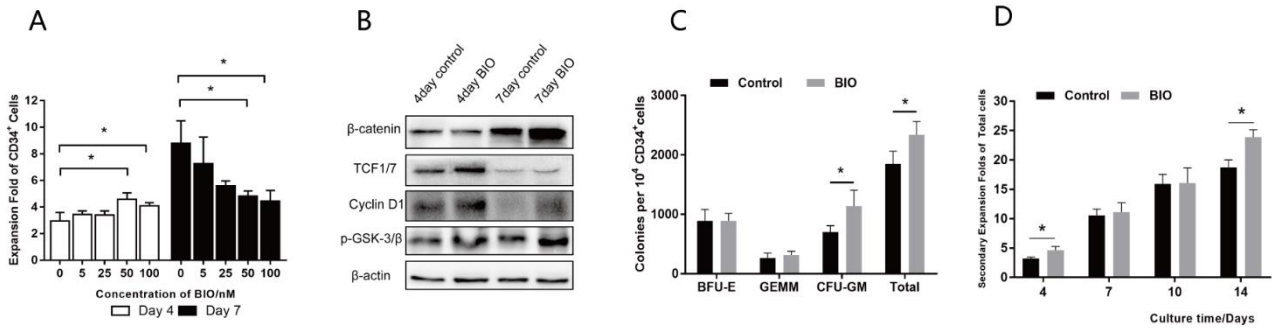

*Figure 1 (A)The effect of BIO on expansion fold of $CD34^+$ cells (*p<0.05 , n=3). (B) Western blot analysis of for β-catenin, TCF, Cyclin D1 and phosphorylated GSK3β in CB $CD34^+$ cells. (C, D) CFU and the secondary expansion ability analysis of expanded-$CD34^+$ cells (*p<0.05 , n = 3 experiments).*

SINGLE USE DISPOSABLE BIOSETTLER REMOVES THE DEAD CELLS AND CELL DEBRIS SELECTIVELY TO INCREASE THE VIABILITY PERCENTAGE OF MAMMALIAN CELLS (E.G. CAR-T) DURING EXPANSION

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Key Words: Increasing viability percentage, Selective removal of dead cells and cell debris, Single use disposable BioSettler, CAR-T cell expansion ex vivo, Perfusion Bioreactor

Current challenge in a FDA approved cell therapy product for adult B-cell leukemia is reported to be the percentage of viable cells after manufacturing is sometimes out of specified range. As the head of a large contract development and manufacturing organization observed, this fall in viability percentage during CAR-T cell manufacturing is a challenge to the whole industry.

We present a simple and powerful off-the-shelf solution to this big challenge in all mammalian cell culture expansion bioreactor system. Our single use disposable BioSettler has been demonstrated to be uniquely capable of removing dead cells and cell debris selectively from the bioreactor and returning or recycling live mammalian cells back to the expansion bioreactor. The mechanism of this very fine separation of dead cells from live cells is the exploitation of their vastly different sedimentation rates during enhanced sedimentation of live cells on inclined surfaces.

This inclined sedimentation technology has been proven extensively with Chinese hamster ovary (CHO) cells used in commercial manufacture of therapeutic antibodies. As the size and sedimentation velocity difference between live and dead cells are similar for CHO cells and CAR-T cells, our off-the-shelf BioSettler will be readily useful for removing the dead cells and cell debris from the rocking or Wave cell expansion bioreactor and increasing the percentage of viable CAR-T cells being expanded for adult cell therapy.

USE OF THE *NANOBRIDGE* SYSTEM FOR THE RAPID PRODUCTION OF PLURIPOTENT STEM CELLS AND NEURAL PROGENITOR CELLS

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Key Words: Scale-up, pluripotent stem cells, neural progenitors, thermoresponsive polymer, extracellular matrix

The novel *Nanobridge* system allows the formation of cellular aggregates of pluripotent stem cells, which can then be grown in suspensions cultures allowing accurate control of the environment in which the cells are growing. The *Nanobridge* system utilizes a thermo-responsive poly N-isopropyl acrylamide (PNIPAM) polymer decorated with extracellular matrix (ECM) protein fragments (fibronectin or vitronectin) to bind to and bridge between adjacent cells and form cell aggregates at 37^0 C. A temperature shift from 37^0 C to 32^0 C causes the PNIPAM to become water soluble weakening the bonding between adjacent PNIPAM chains and allowing the aggregates to be broken down to smaller aggregates by increased shear forces. By returning the temperature to 37^0 C and increasing the culture volume with additional medium, the increased number of smaller aggregates are able to grow to a larger diameter. Repeating this cycle allows for the rapid expansion in cell numbers. In addition, the ability to vary the concentrations and ratios of the two components in the *Nanobridge* system, when coupled with the temperature shift procedure during passaging, allows for tight control over the aggregate diameters at all stages of the expansion process.

In this paper, two examples of using the *Nanobridge* system to culture stem cells will be described: firstly using the system for the rapid expansion of human embryonic stem cells whilst maintaining high viability and pluripotency, and secondly; using the system to develop a process to form neural cell aggregates and maintain and expand cells at a stem/progenitor (NPC) stage, obviating the need for the current cumbersome manual methods to produce larger numbers of NPCs.

In the first example, embryonic stem cells (hESC) WA09 were cultured in spinner flasks with the *Nanobridge* system. At the end of the growth phase, aggregates of 348 micron average diameter were reduced to an average diameter of 139microns after sub-passaging. When this cycle was repeated five times, there was a 500 fold increase in the number of cells produced, with a viability at the end of the process of 90% while maintaining key pluripotent markers NANOG, OCT3/4, SOX2, and DNMT3B. Characterization of the hESC aggregates was performed using the IN Cell Analyser 2200, which demonstrated that there was uniform cell viability and pluripotency marker distribution throughout the aggregates, ie there was no evidence of any diffusional limitations or necrotic regions within the aggregates. At the end of the expansion process it was shown that the cells were able to differentiate into all three germ layers, and that the cells could be converted, to cells types such as cardiomyocytes. The results demonstrate that the *Nanobridge* system is a simple and scalable method of producing large numbers of PSCs without the need for enzymes during passaging.

For the production of the neural progenitors (NPCs), hESC (WA09) cells were formed and cultured as *Nanobridge* aggregates with diameters of 200-300 mm. Differentiation was initiated by culturing the aggregates in mTESR medium with 5uM SB431542 and 100 nM LDN for 5 days. At day 5, the medium was changed to neural basal medium (NBM) supplemented with EGF and FGF2 for the next 5 days of culture. Cultures were maintained in NBM from day 10 onwards. Passaging was performed at day 5 and day 10 and thereafter on a weekly basis for 4 weeks. Temperature shift and mechanical shear were utilized to breakup aggregates and *Nanobridge* components and medium were replaced during passaging. Cells demonstrated upregulation and subsequent maintenance of neural-associated markers (PAX6, SOX1, and NCAM) in aggregate culture. Passaging resulted in an overall seven fold increase in the number of cells expressing the neural-associated markers. Furthermore, neural progenitor cell aggregates exhibited the capacity to differentiate towards a more mature phenotype as demonstrated by the outgrowth of neurites. This demonstrated that the *Nanobridge* system has the potential to facilitate the scale-up of NPC production in bioreactors for applications in regenerative medicine and pharmacological testing.

CHALLENGES AND OPPORTUNITIES FOR CLOSED PROCESSING IN AUTOLOGOUS CAR-T MANUFACTURING

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Key Words: Closed Processing, CAR-T Manufacturing, capacity

CAR-T Cell Therapy manufacturing processes require handling of small volume process and product components without compromising sterility. Historically, in academic settings, aseptic processing is assured via a combination of controls and testing including material, environment and facility components. Such controls can be costly, time-consuming, and present a risk to product sterility. These controls are a challenge for scaling a CAR-T manufacturing process and can significantly limit facility capacity. As an alternative to open processing, all manufacturing steps would be executed using closed system manipulations, eliminating the requirement for costly and time consuming environmental controls to ensure sterility. Here we present a case study for enabling the closed-system processing of a CAR-T manufacturing process. The solutions enabled significant cost-savings, reduced operator safety risk and increased manufacturing capacity by shortening unit-operation duration.

SCALABLE GENERATION OF CEREBELLAR NEURONS FROM PLURIPOTENT STEM CELLS

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Key Words: human induced pluripotent stem cells; Vertical-Wheel Bioreactors; disease modeling; cerebellar differentiation

Human induced pluripotent stem cells (iPSCs) have great potential for disease modeling and provide a valuable source for regenerative approaches. However, generating iPSC-derived models to study brain diseases remains a challenge. In particular, our ability to differentiate cerebellar neurons from pluripotent stem cells is still limited. Recently, we described the long-term culture of cerebellar neuroepithelium formed from human iPSCs, recapitulating the early developmental events of the cerebellum. Additionally, an efficient maturation of replated cerebellar progenitors into distinct types of functional cerebellar neurons was also achieved under defined and feeder-free conditions. However, developing a scalable protocol that allows to produce large numbers of organoids and high yields of mature neurons in a 3D bioreactor culture systems is still a difficult challenge. In this work, we present a new approach for the reproducible and scalable generation of mid-hindbrain organoids under chemically defined conditions by using the novel PBS 0.1 (100 mL) Vertical-Wheel single-use bioreactor. In this system, an efficient cell aggregation with shape and size-controlled aggregates can be obtained, which is important for homogeneous and efficient differentiation. Moreover, a larger amount of iPSC-derived aggregates can be generated without being excessively labour-intensive, achieving 431 ± 53.6 aggregates/mL at 24 hours after seeding. After differentiation, distinct types of cerebellar neurons were generated, including Purkinje cells (Calbindin^{+}), Granule cells (BARHL1^{+} and Pax6^{+}), Golgi cells (Neurogranin^{+} and GAD65^{+}), Deep cerebellar nuclei projection neurons (TBR1^{+}) and Non-Golgi-type interneurons (Parvalbumin^{+} and Calbindin^{-}). These cells show signs of efficient maturation, staining positive for MAP2, and are able to change intracellular Ca^{2+} concentration following KCl stimulation. In this system, human iPSC-derived organoids are able to mature into different mature cerebellar neurons and to survive for up to 3 months, without replating and co-culture with feeder layers.

HUMAN PLURIPOTENT STEM CELL EXPANSION IN VERTICAL-WHEEL BIOREACTORS

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Key Words: human induced pluripotent stem cells; Vertical-Wheel Bioreactors; microcarriers; aggregates.

Human induced pluripotent stem cells (hiPSC) have been regarded as an enormous breakthrough for medicine, since they can be derived from patients and be used to generate virtually all types of cells in the human body. One of the great bottlenecks in the usage of these cells for regenerative medicine or drug discovery applications is their expansion to relevant quantities. The Vertical-Wheel Bioreactors (PBS Biotech) present a novel scalable bioreactor configuration, whose agitation mechanism allows for homogeneous mixing conditions inside the single-use vessel, while conveying less shear stress to the cells when compared to traditional alternatives. These characteristics are advantageous for hiPSC expansion and thus, in this work, hiPSC were expanded in the Vertical-Wheel Bioreactor using different strategies, namely culturing the cells 1) on microcarriers and 2) as floating aggregates.

In the first approach, cells were cultured under xeno-free conditions, using the Essential 8 medium together with microcarriers and coatings devoid of any animal-derived products [1]. The culture conditions were optimized in terms of initial cell/microcarrier ratio, inoculation method and agitation rate, in the PBS 0.1 vessel (working volume: 80 mL). The cells were successfully expanded, maintaining a normal karyotype, up to a 6.7-fold increase in cell number, after 6 days. These optimized culture conditions were successfully repeated in a larger vessel, the PBS 0.5 (300 mL working volume) demonstrating the scalability of the Vertical-Wheel system.
In the second approach, hiPSC were expanded as floating aggregates, a methodology which does not require a separation step at the end of culture, to remove microcarriers, facilitating the downstream processing and Good Manufacturing Practice-compliance of the process. Cells were cultured in the PBS 0.1 (working volume: 60 mL), using mTeSR1, a serum-free medium and were monitored throughout culture regarding growth kinetics, aggregate size distribution and expression of pluripotency markers. The Vertical-Wheel Bioreactors were shown to efficiently keep the cell aggregates in suspension, under lower linear agitation speeds than an equivalent volume spinner flask (7 cm/s vs. 13 cm/s). Following 7 days of culture, cells were expanded up to a 5.2 ± 0.6-fold increase in cell number. The hiPSC aggregates increased in size over time, from an average diameter of 135 ± 61 µm to 397 ± 119 µm after 7 days. Pluripotency was maintained throughout time, as assessed by sustained high (> 80%) expression of pluripotency markers OCT4, SOX2 and TRA-1-60, and low (< 10%) expression of early differentiation marker SSEA-1. The results were validated using a second hiPSC line.
This study revealed that the Vertical-Wheel Bioreactor allows hiPSC growth either on microcarriers and as aggregates and suggested it to have advantages versus other configurations. These results make the Vertical-Wheel Bioreactor a promising platform for hiPSC expansion and, prospectively, differentiation approaches, contributing for the generation of *bona fide* cells for various biomedical applications, namely drug screening, disease modelling, and, ultimately, for Regenerative Medicine.

[1] Rodrigues CAV, Silva TP, Nogueira DES, Fernandes TG, Hashimura Y, Wesselschmidt R, Diogo MM, Lee B, Cabral JMS (2018), “Scalable Culture Of Human Induced Pluripotent Cells On Microcarriers Under Xeno-Free Conditions Using Single-Use Vertical-Wheel™ Bioreactors”, Journal of Chemical Technology and Biotechnology, DOI: 10.1002/jctb.5738

PANCREAS ORGANOIDS FOR TYPE I DIABETES MELLITUS – IS IT FEASIBLE AS A CELL THERAPY?

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Key Words: Type I Diabetes, Organoids, Cost of goods, Scale up, Commercialization

Human pancreas organoids are a promising cell therapy candidate for Type I Diabetes Mellitus (T1DM). T1DM is a disabling chronic disease with a juvenile onset. Auto-immune destruction of pancreatic beta cells results in insufficient insulin production to manage blood glucose. This can lead, on the long-term, to serious complications, such as hypoglycemic episodes, neuropathy, retinopathy, and renal failure. The current standard of care is insulin therapy. However this cannot fully prevent long-term complications from arising, especially in patients suffering from uncontrolled T1DM with severe hypoglycemic episodes. Restoration of the natural blood glucose management can prevent complications, for instance by restoring the beta cell population with a cell therapy. Organoids, proclaimed by the journal Nature as “Method of the year 2017”, are 3D structures that can be generated from progenitor cells of many different organs, such as pancreas, liver, brain, lung, and heart. These miniature organs, when differentiated to produce insulin, can be a 3D cell therapy for T1DM. In case of the pancreas, these can be generated from adult-derived progenitor cells, which have a safer profile than ESC or iPSC based cell therapies for T1DM.

Lonza Netherlands is a partner within the LSFM4LIFE project: a European consortium of universities and industrial partners that aims to produce a GMP batch of human pancreas organoids. To reach this goal, two milestones need to be achieved. First, to transfer the research process of organoid production into GMP. Specific challenges are a scalable GMP compliant platform for 3D culture and replacing research materials (e.g. complex media formulations and culture substrates) with GMP compliant substitutes. Additionally in process controls and QC assays are in its infancy for organoids and need to be developed. The second milestone is to develop a strategy for commercialization of this therapy, for which a number of analyses have been performed. A market and SWOT analysis have been performed (Figure 1). A clinical strategy is proposed to first access the market with an introduction into a small patient cohort and then to stay on the market by reaching all T1DM patients. An assessment of the manufacturing cost of goods is made for the current process, as well as how it could be envisioned in a commercial setting. From these costs, the cost-effectiveness compared to insulin therapy and islet of Langerhans transplantation is evaluated [1]. In conclusion, while the potential for organoids as a cellular therapy is considerable, this paper addresses the progress so far and the major challenges ahead.

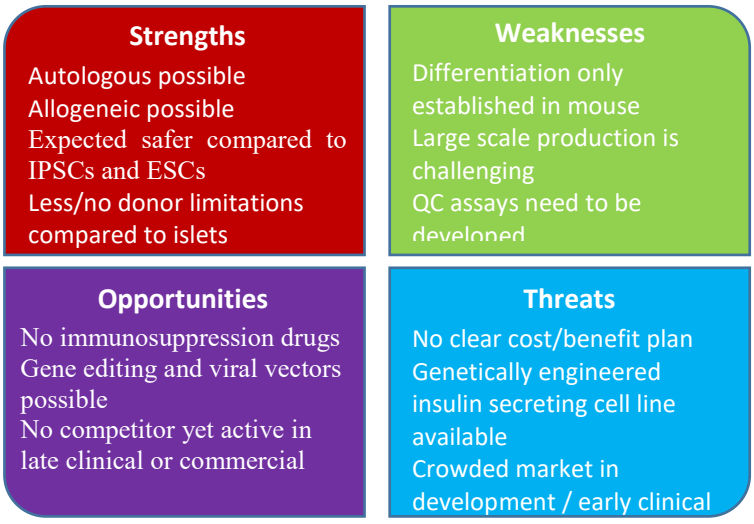

Figure 1 – Strengths Weaknesses Opportunities Threats (SWOT) analysis on pancreas organoid cell therapy.

The unique aspect of this paper is that it describes the commercialization of an early stage 3D cell therapy. Organoids could be envisioned as an autologous ex vivo gene therapy for a subset of T1DM patients, or a donor-matched allogeneic treatment, but could finally be used as an allogeneic treatment. Additionally, the assessments on this therapy are applicable in a broader sense to other therapeutic areas of organoids and to other 3D cell therapies. For some cell therapy applications, 3D structures could be essential to their success (e.g. cardiovascular, organ function replacement) and could be the next generation of cell therapies.

The paper is relevant for the sections “Advances in cell processing: New Techniques for new therapies” and potentially for “Bioprocess modeling for successful commercialization of advanced therapies”

[1] Beckwith, et al (2012). A health economic analysis of clinical islet transplantation. *Clinical transplantation*, *26*(1), 23-33.

ESTABLISHMENT AND EVALUATION OF THE SUSPENSION CULTURE SYSTEM FOR UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELLS

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Key Words: Mesenchymal stromal cells, umbilical cord-derived, bioreactor, single use, micro carrier

Mesenchymal stromal cells (MSCs) derived from various tissues including bone marrow, adipose and umbilical cord tissues have been shown to modulate aberrantly activated immune system. With the features, MSC-based therapies targeting graft-versus-host disease (GvHD) by the administration of bone marrow-derived MSCs (BM-MSCs) have been available in some countries including Japan, and the expectations for the stable and cost effective supply system are getting higher and higher recently. However, the conventional culture systems which usually use plastic flask or multi-chamber equipment require space and manpower, thus the maximal expansion of MSCs at one production is likely to be limited. To compensate the limitation, repetitive productions have been unavoidable, and higher the production cost.

Here, we introduced a new suspension-culture system, using micro-carriers and single-use-bioreactors, for the preparation of MSCs in anticipation of establishment of mass production system. Since the umbilical cord (UC) tissues can be collected through noninvasive procedure, and UC-derived MSCs (UC-MSCs) are shown to present higher proliferation rate and lower immunogenicity in comparison with BM-MSCs, we evaluated the potential and the versatility of UC-MSCs for the treatment of several diseases including GvHD. Results from several in vitro assays demonstrated that our new culture system maintains major key characteristics of MSCs, such as adhesiveness to cell culture surface, the expression of cell surface markers, differentiation capacities toward osteoblasts, chondroblasts, and adipocytes, and immunosuppressive effects on activated T cells. We are currently investigating cellular profiles and characteristics which are specific to the cells prepared in our suspension-culture system through meta-analysis. The established suspension-culture system is presumed to attain the mass production of UC-MSCs, contributing to lower the cost and also providing possible applications for MSCs from other origins.

SCALABLE EXPANSION OF HUMAN UMBILICAL CORD MATRIX- AND ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM/STROMAL CELLS AND DERIVED EXOSOMES IN THE SINGLE-USE, VERTICAL-WHEEL BIOREACTOR SYSTEM USING A HUMAN PLATELET LYSATE CULTURE SUPPLEMENT

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Keywords: mesenchymal stem/stromal cells, expansion, exosomes, vertical-wheel bioreactor, human platelet lysate

Mesenchymal stem/stromal cells (MSC) hold great promise for tissue engineering and regenerative medicine settings due to their multilineage differentiation potential and their intrinsic immunomodulatory and trophic activities. Large cell doses (>1x10^6 cells/kg) are however required for clinical implementation and the success in obtaining those cell numbers is dependent on efficient *ex vivo* expansion protocols able to comply with Good Manufacturing microcarrier-based cultures in scalable bioreactors using serum-/xenogeneic-free (S/XF) culture components. In this context, a S/XF microcarrier-based culture system was successfully established for the expansion of human UCM and AT MSC using an innovative disposable bioreactor system utilizing the Vertical-Wheel™ technology (PBS-0.1 MAG with maximum working volume of 100 mL, PBS Biotech) combined with a commercially available fibrinogen-depleted human platelet lysate-based culture supplement (UltraGRO™-PURE, AventaCell BioMedical). By optimizing the agitation and feeding regimes, UCM and AT MSC were successfully expanded to maximum cell densities of 5.3 ± 0.4 x 10^5 cell/mL (*n*=3) and 3.6 ± 0.7 x 10^5 cell/mL (*n*=3), respectively, after 7 days of culture (cell viability ≥ 94%), while maintaining their identity.

Recently, increasing evidence has proposed extracellular vesicles (EVs), as exosomes, as mediators of many of the MSC-associated therapeutic features. Exosomes are small EVs (30-150nm) of endocytic origin, involved in intercellular communication, through transfer of a cargo of proteins and RNAs. In this context, the platform established for the expansion of MSC is under optimization for exosome production. Dynamic culture systems, as the one presented herein, are expected to allow a higher exosome titer, as well as a better control when fine-tuning the exosomes' properties, by changing culture conditions (*e.g.* shear, oxygen). Preliminary results have shown that human MSC expanded in the Vertical-Wheel™ bioreactor system allowed to obtain a population of EVs with a more homogeneous size distribution profile, when compared to cells cultured in traditional static systems. Overall, we demonstrate that this culture system is able to robustly manufacture human MSC and MSC-based exosomes towards the development of novel therapeutic products.

VIABLE MANUFACTURE OF CELL THERAPIES THROUGHTHE INTEGRATION OF MULTIPLE UNIT PROCESSES ONTO A COUNTER-FLOW CENTRIFUGATION DEVICE

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Key Words: Commercial Manufacture, Scale-Out, Automated, Closed, Integrated

With growing proof of efficacy in varied indications, regenerative medicine has reached a positive inflection point in the investment of time and money by established industry leaders and disruptive startups. With rapid growth comes the need to devote resources to the engineering challenges that currently prevent the quick and cost-effective manufacture of therapies that maintain a consistent, high-level of quality and, in turn, can support commercial manufacturing. This is especially true when looking at patient specific cell therapies that require rapid change over of equipment and benefit little from traditional sterile barriers (i.e. filters and heat inactivation). Counter-flow centrifugation (CFC) presents an intriguing technology that, when implemented using closed and automated system, provides a platform for upstream and downstream processing of cellular therapies by incorporating multiple unit processes and mitigating the risk imposed with manual equipment transfers.

The CFC technology under development by a partnership Hitachi Chemical Advanced Therapeutic Solutions (HCATS) and Invetech compounds the benefits of a fully integrated, closed processing kit with easily customizable procedures to integrate multiple unit processes onto a singular device. Increased integration of unit processes will be integral to addressing the dynamic challenges of commercial cell therapy manufacturing in a scaled-out model. This CFC device has shown the capability to perform platelet wash steps with 99% efficiency and retain 100% of the mononuclear cells. The platform can then harvest a concentrated volume of cells or shift directly into an elutriation protocol to separate hematopoietic cell populations. Further development work is being done to create a CAR-T manufacturing protocol using the device and to establish fill/finish capabilities. By incorporating multiple unit processes onto a device that also meets the need for rapid change-over between lots this device offers a unique solution to the emergent challenges of cell therapy manufacturing.

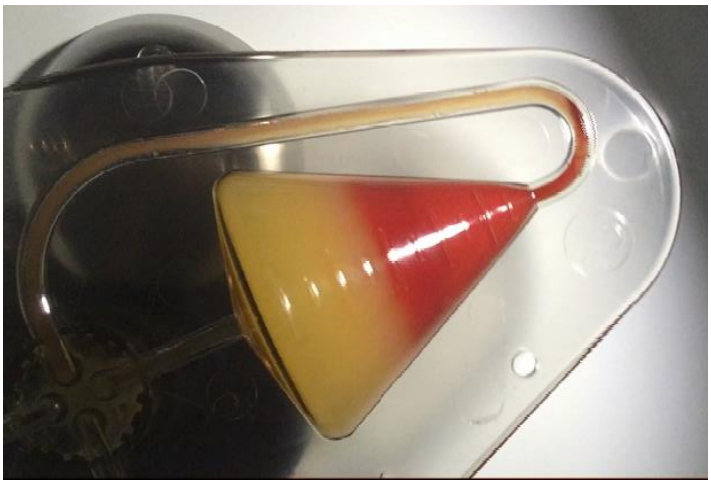

Figure 1. Image of performing a platelet wash on an incoming apheresis collection using counter-flow centrifugation.

WITHDRAWN

DEVELOPING A NOVEL MICROCHANNEL EMULSIFICATION DEVICE FOR DIABETES CELL THERAPY

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Key Words: Encapsulation, Diabetes, Microchannel, Emulsification, Cell Therapy

Type 1 diabetes is a chronic, autoimmune illness, involving immune rejection of the body's insulin-producing pancreatic beta cells. The reduced insulin production results in a dangerous increase in blood glucose levels. Cell therapy is currently being explored as an attractive long-term treatment option for this disease, whereby pancreatic islet cells are isolated from allogeneic donors, and transplanted into affected patients. Islet transplantation is a minimally invasive treatment that has successfully eliminated the need for exogenous insulin in 50-70% of patients for 5 years (Shapiro, 2017). Limitations to this treatment include the requirement of lifelong immunosuppression that may lead to opportunistic infection, and the limited donor islet supply. To overcome these issues, we are investigating islet immunoisolation in alginate microbeads to eliminate the requirement of immunosuppressive drugs and to improve access to islet transplantation as a therapeutic option.

Compared to conventional nozzle encapsulation processes, emulsification and internal gelation in a stirred vessel is a highly effective and scaleable technique for islet immunoisolation (Hoesli, 2010; Hoesli, 2012). However, the alginate beads produced via stirred emulsification are highly polydisperse. The objective of this project was to determine whether insulin-producing beta cells can be encapsulated in monodisperse alginate beads produced by combining internal gelation and microchannel emulsification technologies.

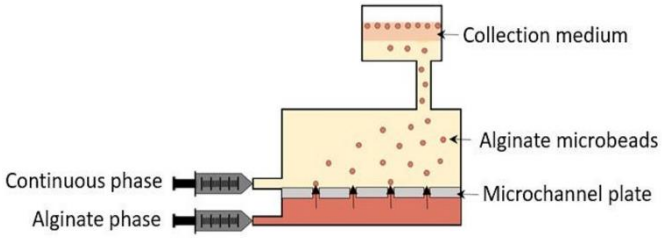

Figure 2 – MCE Encapsulation Device

We developed a novel microchannel emulsification (MCE) device (Figure 1) consisting of an alginate phase flowing below a hydrophobic microchannel plate, with a stagnant continuous oil phase above the plate (Markwick, 2016). Three hydrophobic and non-toxic continuous phase fluids (light mineral oil, glyceryl trioleate, and 3M™ Novec™ 7500 Engineered Fluid) were considered in this study and evaluated based on their density difference with the alginate phase, as well as their surface tensions. Higher density difference between phases and lower continuous phase surface tension both resulted in facilitated droplet formation in the device. Viability assessments of encapsulated mouse insulinoma 6 (MIN6) cells were also conducted via live/dead staining and flow cytometry.

Overall, Novec™ 7500 had the highest density difference with alginate, and the lowest surface tension, compared to the other fluids considered. Using this continuous phase fluid, uniform alginate beads with diameters as low as 1.5 mm were successfully produced with size variations < 10% and production rates of ~100 beads per minute per channel. Preliminary assessments of MIN6 cell survival showed 82 ± 4% viability. Future work will seek to further reduce the minimum bead diameter that can be achieved, to improve cell survival, and to assess encapsulated islet function. The MCE process is a promising low-cost and high-throughput method to encapsulate and transplant a variety of therapeutic cell types.

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A SCALABLE XENO-FREE MICROCARRIER SUSPENSION BIOREACTOR SYSTEM FOR REGENERATIVE MEDICINE BIOMANUFACTURING OF hMSCs

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Key Words: bioreactor, hMSCs, scalable manufacturing

An economical biomanufacturing paradigm for human mesenchymal stem/stromal cells (hMSCs) is in critical need, as indicated by over 800 clinical trials investigating the use of hMSCs for regenerative medicine. To meet the demand for clinical manufacturing, a scalable process and production technology platform that can generate billions to trillions of cells per manufacturing lot is needed. Suspension bioreactors show great promise in reaching commercially-viable working volumes, however, scalability of cell production remains an issue. Overcoming this challenge is necessary to drive widespread adoption of this culture system for hMSCs. We have taken the Quality by Design (QbD) approach to develop a scalable xeno-free (XF) hMSC bioreactor process that maintains the final cell population doubling level (PDL) within the recommended range of 16-20 to ensure product quality. Our strategic XF bioprocess was designed using high volume XF cell banks, an optimized XF fed-batch media system, and XF microcarriers, all combined in a scalable bioreactor system to meet our design criteria and streamlined production at different culture scales.

In this study, we demonstrated the scalability of a XF hBM-MSC microcarrier suspension culture in a low shear, single-use, vertical-wheel suspension bioreactor (PBS Biotech) at small scale (0.1 L), development scale (3 L), and pilot scale (15 L). Cell yields of >0.5M cells/mL were achieved in all bioreactor scales within 5 days of culture with no media exchange. Comparable nutrient and waste metabolite levels, pH, and cell growth curves (Fig. 1) were observed at each scale. In addition, cells harvested from all bioreactor scales maintain the hMSC critical quality attributes of osteogenic, adipogenic, and chondrogenic differentiation potential, as well as functional attributes of angiogenic cytokine (FGF, HGF, IL-8, TIMP-1, TIMP-2, and VEGF) secretion and inducible immunomodulatory potential (as measured by functional IDO activity), which are comparable to 2D control of similar PDL. Our development data supports the expansion of XF hMSCs in a scalable bioreactor culture platform, providing significant time and cost savings as a standardized system for translational researchers and product developers in the regenerative medicine, tissue engineering, and cell therapy fields.

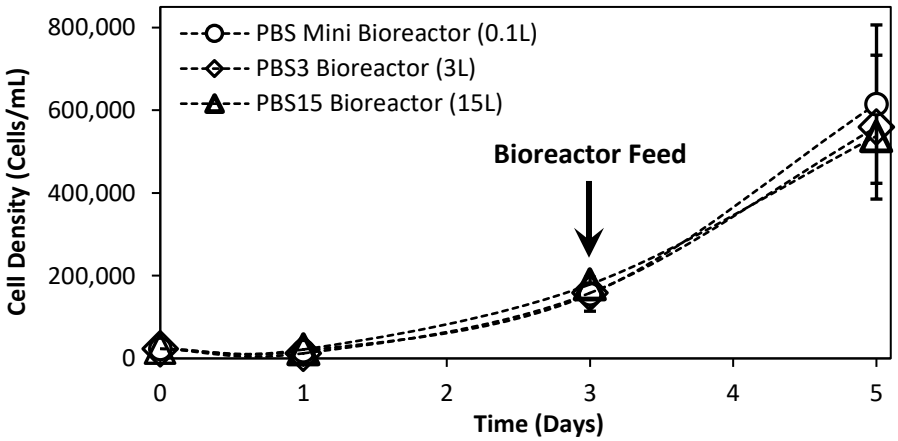

Figure 1 – Growth profiles of XF hBM-MSCs are comparable across various scales of microcarrier suspension culture: 0.1 L, 3L, and 15L. Cell yields of >0.5M cells/mL were achieved within 5 days.

WITHDRAWN

WITHDRAWN

FURTHER EVALUATION OF A NOVEL COP CONTAINER SYSTEM FOR THE CRYOPRESERVATION OF ADHERENT AND SUSPENSION HUMAN CELL TYPES

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Key Words: cryopreservation, MSC, HSC, container closure integrity, cyclic-olefin polymer, packaging

The commercialization of cell and gene-based therapies is driving a critical need for the refinement of handling, storage and transportation procedures to ensure the integrity of the product is maintained throughout its life cycle.

One such area for refinement is in the preservation of cells that is routinely performed at cryogenic temperatures. Cells are typically stored in the vapor phase of Liquid Nitrogen (LN) at approx. -180^{o}C to eliminate the risk of any degradation. For cryogenic storage, there are various container systems that are available for research and commercial use. These include traditional polypropylene (PP) screw-cap cryovials, and container systems with glass or polymer vials that are closed with a rubber stopper and aluminum seal.

Glass/rubber vial systems, although common elsewhere in the pharmaceutical industry for drug containment, are not well suited to the challenges of cryopreservation. Even beyond risk of fracture, the differences in coefficients of thermal expansion of glass and rubber can put these systems at risk of losing container closure integrity (CCI) when stored at cryogenic temperatures. Containment systems that rely on screw-caps are also at risk, as the screw thread can create a pathway that can lead to an ingress of contaminants. In contrast, a polymer/rubber vial system, comprised of materials with more comparable coefficients of thermal expansion, closed with an aluminum seal would be a better suited alternative that could better ensure that CCI is maintained throughout a product life cycle.

This research presented here is a scientific evaluation of a novel cyclic olefin polymer (COP) container system. The performance at cryogenic temperatures has been demonstrated in two separate studies. An investigation conducted without cells but measuring CCI via Oxygen Headspace of vials stored at -180°C over a 90-day period has been combined with a follow up study that compared performance between COP and PP-based container systems when cryopreserving two different cell types: adherent type human mesenchymal stem cells (MSCs) and suspension type umbilical cord hematopoietic (CD34+) stem cells.

The results showed that the polymer/rubber-based container closure system maintains CCI at -180°C, and that the cells were preserved well, as demonstrated by their viability, morphology and biomarker expression post-thaw. These findings, when combined with previously reported advantages of the novel COP container system [1], show that it is a highly suitable alternative to traditional packaging systems for cryopreservation in the field of cell and gene-based therapies.

[1] E.J. Woods, A. Bagchi, R. Nase and V. Vilivalam, Container System for Enabling Commercial Production of Cryopreserved Cell Therapy Products, Regenerative Medicine, 5(4) (2010), 659-667

ENABLING STEM CELL BASED THERAPIES: ADAPTABLE AND SCALABLE MANUFACTURING OF HUMAN PLURIPOTENT STEM CELLS

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Enabling stem cell-base therapies requires innovative solutions to close the gaps which exist between research and commercialization. Allogeneic cell therapy indications that target large patient populations will necessitate the use of flexible cell production platforms to meet required cell quantities. Here we will show how moving away from conventional 2D culture platforms and developing a truly scalable, controlled bioreactor platforms for cell expansion enables meeting cell quantity demand for clinical applications while allowing comparability between the various scales. Likewise, it enhances process automation and allows integration of online monitoring systems. These bioreactor platforms are flexible cell production platforms, applicable to various cell types. Utilizing many common components, such as bioreactor controllers and centralized up-stream and down-stream hardware, while being able to quickly and easily change components such as vessels, media and microcarriers. The capability of effectively culturing adherent stem cells, namely pluripotent stem cells, will be presented. Cells are expanded in suspension, in a controlled bioreactor, obtaining high fold expansion without compromising cell quality, and the capacity to be further differentiated. This achieved through avoiding 2D cell culture steps, reduces footprint, labor and cost, while enhancing process control and cell product quality.

MAINTAINING CD4/CD8 RATIO AND TH1-CTL SUBSETS OF CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELLS IN SERUM-FREE CULTURE CONDITIONS

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Key Words: Chimeric antigen receptor (CAR)-T cells, Serum-free condition, T cell subsets

Chimeric antigen receptor (CAR) T cells therapy is a promising strategy that significantly controlled the progress of cancer diseases. CAR-T cells could kill cancer cells through cellular immune response; therefore, CD8+ cytotoxic T cells are critical for CAR-T cell therapy. However, recent papers reported that CD4+ T helper cells were important for the response and maintenance of CAR-T cells *in vivo*. Here, we developed a serum-free CAR-T cell preparation process that maintained the T cell population and controlled the T cell subsets. The CD4+ and CD8+ T cell population in CAR-T cells were maintained at averagely 59.4 % and 34.6%, and the major T cell subsets were Th1 cells and cytotoxic T lymphocytes (CTLs), implying the potentially high cellular immune response. To verifying whether the prepared CAR-T cells were exhausted, the expression of several immune checkpoint markers was determined. Of interest, only less than 20% of CAR-T cells at endpoint were PD-1+ or CTLA4+, but more than 40% of CAR-T cells at the endpoint were TIM-3+, implying most CAR-T cells were not exhausted. These CAR-T cells produced more than 1 ng/mL of IFN-γ in the response to the antigen. Altogether, CAR-T cells could be prepared in our serum-free process in the controlling of T cell subsets, leading to potential high therapeutic potency.

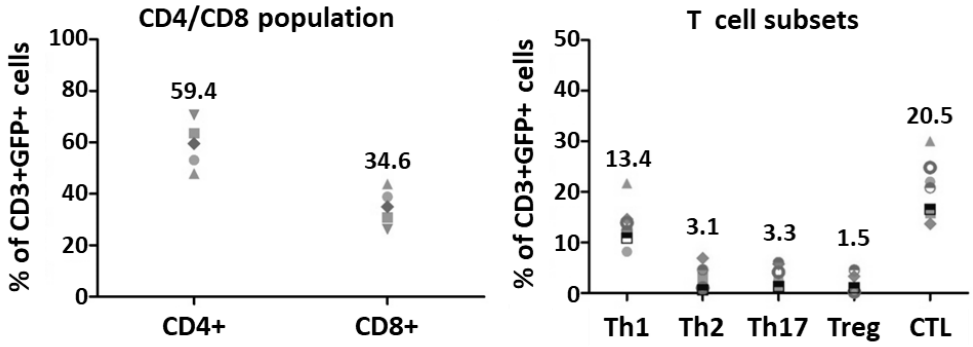

Figure 3 – The major cell subsets of CAR-T cells expanded in the serum-free culture condition for 6 days were Th1 and cytotoxic T lymphocytes (CTLs) that participated in the cellular immune response.

SCALE-UP STUDY FOR *EX-VIVO* EXPANSION OF ALLOGENEIC NATURAL KILLER CELLS IN STIRRED-TANK BIOREACTOR

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Natural killer (NK) cells are a type of lymphocyte in the blood that are responsible for innate and adaptive immune response, and they mature in the liver and bone marrow. Being a key role in host defense system with direct and indirect killing of virus-infected cells or cancer cells, NK cell has been considered an attractive candidate for cancer therapy. Peripheral blood shows the low frequency of NK cells, so *ex vivo* expansion method is important to obtain sufficient NK cells for therapeutic use. Currently, we successfully developed bioreactor process for NK cell expansion on lab-scale. Stirred-tank bioreactor could be considered as optimal alternative system for large-scale NK cell expansion compared with other ones because it is automated, less labor intensive, scalable, well-controlled and cost-effective. In bioreactor process, agitation is one of important parameters for NK cell expansion because it is necessary to provide homogenous culture conditions. So we defined effects of agitation in bioreactor and figured out an optimum condition. After that scale-up studies were carried out with manufacturing-scale bioreactor based on these results. The results in terms of growth rate, viability cytotoxicity and purity, were comparable with lab-scale.

A STEP CLOSER TO INDUSTRIAL SCALE MANUFACTURE OF EXOSOMES – ADAPTATION OF CLINICAL GRADE NEURAL STEM CELLS FROM 2D TO 3D CULTURE

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Key Words: Neural stem cells, exosomes, bioprocessing, scale-up, microcarriers

Exosomes derived from the clinical grade neural stem cell line *CTX* (ReNeuron) are the basis of a new class of therapy for the treatment of degenerative disorders. Thus far we have generated *CTX*-derived exosomes at research scale in 2D planar cultures. Now the cell culture process needs to be scaled up in order to deliver commercially relevant quantities of exosomes that have the correct quality attributes. To meet these demands, *CTX* cells, which are adherent and habitually grown in a 2D static environment, must be adapted for growth in 3D agitated bioreactor systems.

In this research we show that *CTX* cells can be grown on microcarriers in 100mL spinner flasks, a model bioreactor system, with a view to achieving industrial scale cultivation of exosomes. This was informed by preliminary microscale screening of different microcarrier substrates using a low-volume closed system under automated perfusion. Furthermore an innovative semi-automated technique for imaging and analysis is applied so that optimal conditions for bioreactor culture can be predicted in terms of both cell number and phenotype.

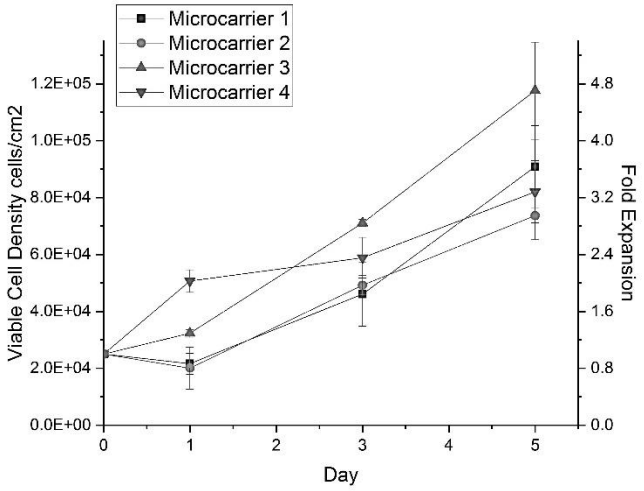

Figure 4 – Validation of CTX cell growth on microcarriers in 100mL spinner flasks in which target cell population of 60,000 cells/cm² within 4 days was achieved in-line with 2D culture system

Using this system we were able to reduce a vast panel of commercially available microcarriers by >80% prior to any scale-up activities, thereby minimising experimental time, cost and risk of failure. We were then able to validate our candidate conditions in 100mL spinner flasks, achieving our target cell population of 60,000 cells/cm² in 4 days, representative of our static 2D system. The resultant exosomes were purified and analysed in terms of particle number, size distribution and CD markers to assist us in assigning critical quality attributes.

Having effectively adapted our cells to our model bioreactor system, we now look to scale-up further and succeed in industrial scale cultivation of exosomes.

IN VITRO HIGH EXPANSION OF CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELLS IN SERUM-FREE PROCESS CONDITIONS

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Key Words: Chimeric antigen receptor (CAR)-T cells, Serum-free condition, process optimization

Manufacturing process is an important and complex factor for preparing chimeric antigen receptor (CAR) T cells for therapy. Although serum was widely applied in the culture or expansion of T cells, the quality of serum could be varied from batch to batch, leading to the variation of T cell expansion and quality. In addition, the safety of pathogens from serum and Chemistry, Manufacturing, and Control (CMC) were required to be considered. To overcome the disadvantages of serum application in T cell culture, serum-free and xeno-free culture conditions were required. We intended to develop a rapid serum-free culture condition for the expansion of immune T cells *ex vivo*. In our optimized serum-free condition, CAR-T cells could be expanded to about 100-200 times to the initial cell number after 6-day culture and the cell viability of all specimens was above 98%. Of interest, the percentage of CAR+ population in all specimens was increases, and the T cell pollutions could be maintained at averagely about 35-40% of CD8+ T cells and averagely about 50-55% of CD4+ T cells after culture. Taken together, our conditions could be applied in the expansion of CAR-T cells for cell therapy to support the minimum requirement of blood or cell samples from patients and to maintain the T cell population.

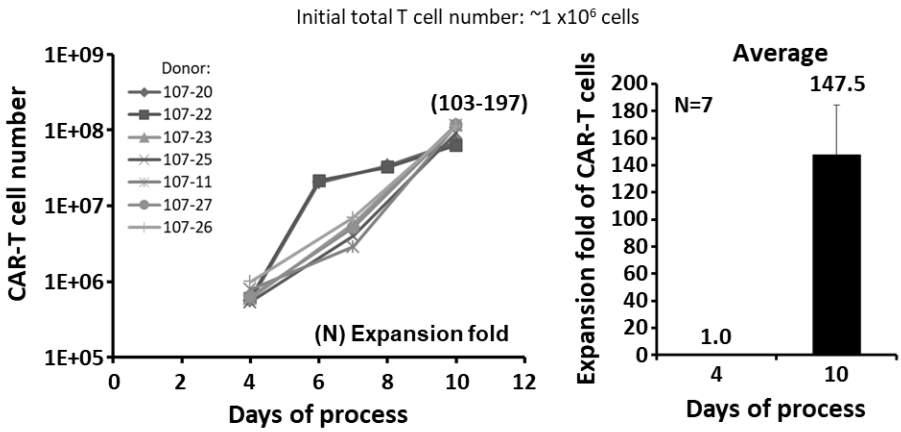

Figure 5 – CAR-T cells prepared from 7 donors' T cells were applied to the serum-free culture condition, and the CAR-T cells grew to averagely 147.5 times to the initial CAR-T cells number.

REDUCING VARIABILITY IN CONDITIONS FOR CELL HANDLING IMPROVES MSC YIELDS

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Efficient cell expansion *in vitro* is essential to commercialization of human MSC as a cellular therapy. The cost of goods sold (COGS) is dramatically affected by how long it takes to expand the cells *in vitro* and the cell yield determines the number of doses generated for profit. Therefore, maximizing MSC growth in culture is critical for the success of MSC-based cellular therapies. Studies by others have shown that temperature differences in cell production can adversely affect cell yields. Here we study the effects of variability in temperature and CO2, like changes seen during routine cell handling in a room air BSC, on human MSC yield. We cultured human bone marrow mesenchymal stromal/stem cells for 8 biweekly subpassages (P4-P12) with conventional room air CO2 incubator conditions (37 degrees C/ 5% CO2). The culture was divided into separate cultures for routine cell handling in two different conditions (1) room air BSC conditions (RT/ 0.1% CO2) (variable) or (2) the same conditions as incubation (constant). At each passage, cells were plated in 96-well plates which were assayed over time for cell growth kinetics. Consistently, MSC incubated and handled in constant conditions recovered more quickly after subpassage and were more likely to continue to divide, improving final cell yields. We conclude that constant conditions for cell handling are critical for maximum MSC cell yield.

IMPACT OF THE DYNAMIC CULTURE SYSTEM FOR 3D HIGH CELL DENSITY NEURAL DIFFERENTIATION OF hESC IN ELECTROSPUN PCL SCAFFOLDS

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Key words: hESC, neural differentiation, bioreactor, PCL, 3D culture

The ability of pluripotent stem cells to differentiate into any of the three germ layers have sparked an array of investigations in developmental biology, tissue replacement, drug screening and cellular interactions. A demand has risen for 3-dimensional technologies to improve scalability and better mimic the stem cells niche. With the increasingly complex cultivation platforms and larger scales a limit is rapidly reached in terms of nutrient and waste exchange to the cells. These limitations can be overcome with the use of dynamic culture systems such as bioreactors. However, a culture platform that allows for differentiation of pluripotent stem cells to neural lineages to homogenous macro scale tissue cultures is still challenging.

We have previously developed a protocol for the culture of pluripotent cells in 3D electrospun PCL scaffolds in multi-well format [1]. The purpose of the present was to investigate the potential of different dynamic systems to support the neural differentiation of pluripotent stem cells in 3D electrospun polycaprolactone (PCL) scaffold disks of 35 mm diameter. The dynamic systems were a bioreactor with culture medium flow either longitudinal or orthogonal to the scaffold, alternatively a system with orbital shaking, and absence of agitation. Human embryonic stem cells, hESC, (line HS980 received from Prof. O. Hovatta (Karolinska University Hospital, Huddinge, Sweden), transferred to KTH and used in agreement with ethics approval issued to us by the Regional Ethics Board, Stockholm, Sweden (Ethical Permission Dnr 2013/493-31, amended 2015/824-39)) were submitted to a 3-week neural differentiation protocol. The cells were cultured in 3D Mimetix Air electrospun polycaprolactone (PCL) scaffold disks of 35 mm diameter in small stainless steel bioreactors using a modified version of the dual-SMAD differentiation protocol with small molecular inhibitors. The levels of Sox2, Nestin, ki67, Map2, Doublecortin and beta III tubulin were measured.

Applying dynamic culture conditions for the differentiation of hESC towards neural lineages in 3D electrospun PCL scaffolds greatly increased the cell yield and had a large beneficial impact on the marker expression compared to a static system. Static cultivation resulted in a heterogeneous cell population with cells expressing both early and late differentiation markers. A culture with a flow orthogonal to the scaffold did not sustain a consistent cell culture. By using a flow longitudinal to the scaffold, the cells grew homogeneously across the scaffold and expressed early differentiation markers at a higher level compared to orbital shaking. This latter system increased the cell yield to the highest level, i.e. 120×10^6 cells/scaffold, and led to homogeneously differentiated cells with an increased level of mature neuronal markers.
To our knowledge this was the first time that pluripotent stem cells have been differentiated to neural fate in a 3D scaffold of connected homogeneous macroscopic scale (> cm).

Reference: Leino M, Åstrand C, Hughes-Brittain N, Robb B, McKean R, Chotteau V. J Biomed Mater Res B Appl Biomater. 2018. 106:1226-1236. doi: 10.1002/jbm.b.33928.

SUPERIOR EXPANSION OF LONG-TERM HEMATOPOIETIC STEM CELLS USING StemPro™ HSC MEDIUM KIT

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Key Words: hematopoietic stem cells, $CD34^+$, expansion, cell therapy, long term HSC.

The use of $CD34^+$ hematopoietic stem cells (HSC) for transplantation has been limited due to the low $CD34^+$ cell numbers in tissue sources such as peripheral blood and cord blood. Two strategies have been employed to increase the $CD34^+$ cell dosage. These include mobilization of HSC into peripheral blood via injection of G-CSF, and *ex vivo* expansion of $CD34^+$ cells. A major limitation of current systems used for the expansion of HSC is that *ex vivo* culture leads to expansion and differentiation of cells, at the expense of the most primitive pluripotent long-term HSC. This has limited the clinical application of *ex vivo* expanded HSC, since short-term progenitor cells only provide transient protection, ultimately reducing the positive health outcomes, increasing the duration of hospitalizations, and health care costs per patient. Development of a culture system that expands, both short term progenitor cells and long-term HSC would enable immune protection during the early phase of recovery, and provide a suitable solution for transfusion-independent hematopoiesis. Therefore, we have developed an HSC culture medium that enables the expansion of both long-term HSC and short-term progenitor cells, while maintaining their functional properties. We conducted several iterative rounds of Design of Experiments (DOE) involving multifactorial analysis, and mathematical modeling methods. The DOEs allowed us to identify optimal combinations and concentrations of essential media components, small molecules, and growth factors. The performance of candidate HSC expansion media were evaluated after 7 days of culture, upon which the $CD34^+$ cells and $CD34^+CD90^+CD45RA^-$ cells (long-term HSC) were quantified. We were successful in developing a media system- StemPro™ HSC Medium Kit-which is xeno-free, serum-free medium that expands both long term $CD34^+CD90^+CD45RA^-$ HSC and short term $CD34^+$. The expression of aldehyde dehydrogenase was conducted to identify primitive stem cells, and colony-forming unit assays were performed to assess the *in vitro* differentiation capacity of expanded cells. We plan to determine whether the expanded cells are engraftable by transplanting the cells into immuno-deficient mice. Taken together, we seek to highlight our design philosophy in HSC culture media development, and we believe our efforts are critical for the successful utilization of hematopoietic stem cell transplants in translational cell therapies.

AN AUTOMATED AND CLOSED SYSTEM FOR PATIENT SPECIFIC CAR-T CELL THERAPIES

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Key Words: automation, manufacturing, autologous, CAR-T, closed

Autologous cell therapies, particularly chimeric antigen receptor T-cell (CAR-T) immunotherapies, are becoming a promising treatment option for difficult diseases. Immunotherapies for blood cancers have dominated the pipeline, while treatments for solid tumors have started to become more successful. However, as the market continues to grow and more clinical trials begin globally, the challenge of manufacturing autologous cell therapies remains significant. A greater number of patients will lead to an increase in cost, labor, and the complexity of logistics for scaling out the commercial production of patient specific therapies. To enable clinical and commercial success, novel manufacturing platforms, such as closed and automated systems, will be required to produce cost effective and robust therapies. This abstract highlights a successful CAR-T process translation from a manual process to an automated patient scale system.

To accomplish a CAR-T process translation, we utilized a platform that automates cell seeding, activation, transduction, real time process monitoring, feeding, washing and concentration, and harvesting. In order to mimic a therapeutic CAR-T cell process, manual research scale processes were optimized, scaled up, and then programmed to run automatically without manual intervention. In these processes, 100 million peripheral blood mononuclear cells (PBMC) were first inoculated with CD3/CD28 activation beads. The following day, cells were transduced with HER-2 lentivirus vector. Cells were then expanded with a defined feeding strategy and IL-2 supplements until harvested when target yields were reached. After harvest, cells were analyzed for cell yield, viability, transduction efficiency, and an array of cell phenotype, potency and functionality via FACS and killing assays. Specifically, CAR-T cells were analyzed for the presence of naïve T cells, T stem cell memory, T central memory, T effector memory, and T effector cells.

We show here how we optimized, scaled up, and automated manual processes to reach clinical requirements. Automated runs using the above process with cells transduced by HER-2 virus yielded an average of 2×10^9 cells post harvest with a viability > 90%. Automated runs and associated controls were able to support the expansion of both CD4+ and CD8+ T cells with 73% CD4+ T cells and 20% CD8+ T cells. Harvested cells yielded approximately 80% NGFR+ cells with a higher detection of NGFR in the CD4+ fraction than in the CD8+ fraction for all samples. Both CD4+ and CD8+ subsets demonstrated T cell phenotype such as naïve T cells, T stem cell memory, T central memory, T effector memory, and T effector cells. Both subsets also only expressed between 15-20% of immunosuppressive regulatory T cells. Cell health was evaluated by the levels of exhaustion marker, PD-1, which was 19% in CD4+ T cells and < 1% in CD8+ T cells. Furthermore, there was a negligible amount of senescent T cells and anergic cells and < 10% expression of the apoptotic marker, Caspase-3. Subsequently, cells from multiple automated runs showed the specific killing of NGFR+ tumor line were correlated with high levels of effector cytokines: TNF-alpha (~34%) and IFN-gamma (20-25%) as compared to a manual control. In summary, automated CAR-T process in the Cocoon system yields a healthy populations of T cell subsets. This system is a viable solution to translate labor-intensive CAR-T process into a fully automated system, thus allowing scalability, high yield, reduction of manufacturing cost, and better process control to yield high quality CAR-T cells.

AUTOMATED MANUFACTURING FOR IPSC-DERIVED RETINAL PIGMENT EPITHELIAL CELLS

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Key Words: cell manufacturability, automation, flexible modular platform, iPSCs, retinal pigment epithelial cells

Cell manufacturing, which is the most critical steps to realize the transplant of cell-based products for cell therapy or regenerative medicine, will be done in terms of safety, stably and cost-saving under the aseptic environment in the cell processing facility (CPF). The cell processing is regarded as the system consisting of target process, input and output, and there are several fluctuations derived from extrinsic noises (environmental errors) against the system, input quality such as starter cells and materials (medium, reagents, substrate, vessel etc.), and intrinsic disorders (in-process errors) from the behavior variance in manual operation (Fig.1). Especially, intrinsic disorders cause the difficulty to make consistency and robust process for stable quality because the cells have uncertainty accompanied by time-dependent and time-delay properties. Therefore, environmental, material, and operational standardizations are required to realize consistency of processes. In addition, long manufacturing period and small lot size for cell production make the low productivity, causing the high cost production.

Cell manufacturability is defined to be "manufacturing design of cell-based products in such a way that they are easy to manufacture through simple, safe and efficient (cost-saving) process with stable product quality and secure to customer by considering transpiration and hospital preparation outside factory". Based on the cell manufacturability, the realization of 3S (safe, stably and cost-saving) is desired for the industrialization, leading to the affordability of the final cell-based products.

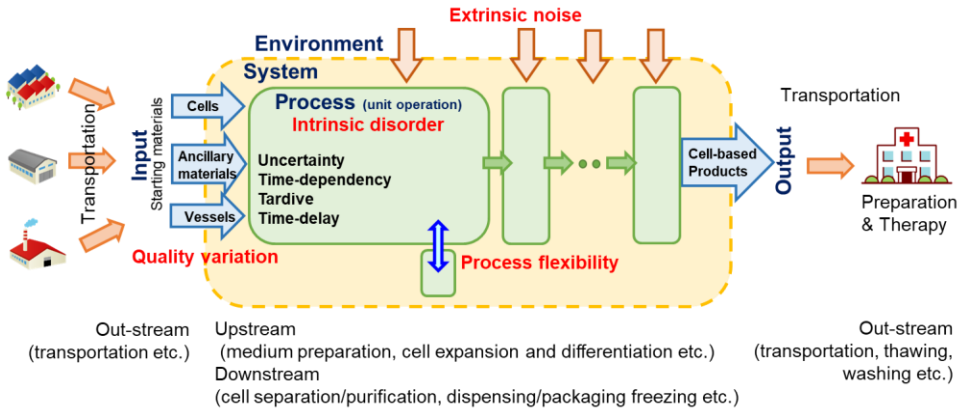

Figure 1 The cell processing system

In the present study, the novel isolator system based on a flexible Modular Platform (fMP) was designed to realize that the individual modules can connect and disconnect flexibly with keeping the aseptic environment in each module(Fig.2). The fMP isolator system may reduce equipment and maintenance/operation costs while providing a reliable aseptic environment for the CPFs handling different cell-based materials. The system performs 4-month culture processing of iPS cells and the derived retinal pigment epithelial cells (RPECs), successfully by considering the cell manufacturability. In addition, the gravity forces in manual handlings were measured by using motion sensor and the set-up of the motion in robotic arm handling. This teaching leads to the stability of cell culture in differentiation of iPSCs to RPECs to realize the automated cell manufacturing, preventing from the intrinsic disorder in cell processes.

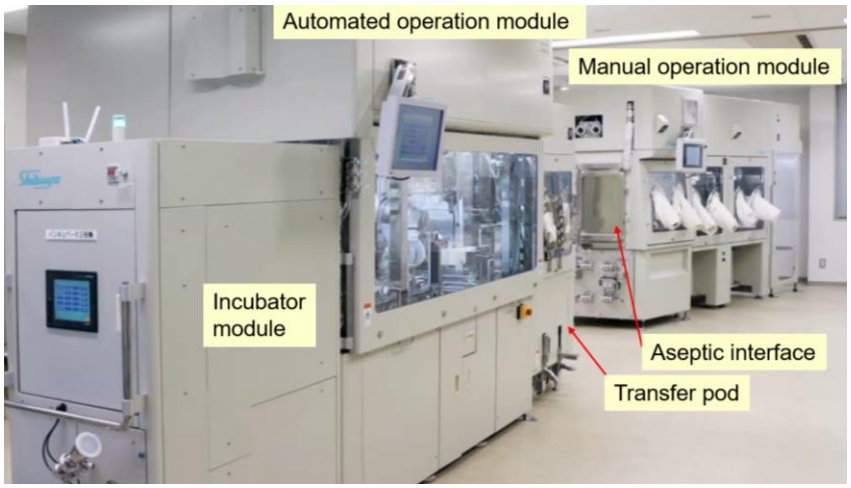

Figure 2 The novel cell processing system based on the fMP

ISOLATION AND EXPANSION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS DIRECTLY ON MICROCARRIERS IN A STIRRED TANK BIOREACTOR

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Key Words: hMSC, bone marrow isolation, expansion, microcarriers, bioreactor

Human mesenchymal stem cells (hMSCs) are emerging as a promising treatment for several diseases including diabetes, heart disease and Parkinson's. However, current manufacturing methods are time consuming, expensive and unable to satisfy the increased patient demand. Upscaling the stem cell isolation and expansion process in a cost-effective manner is therefore the key for future affordable therapies. Typically, the isolation of hMSCs from bone marrow would be realised in monolayer in T-flasks followed by scale-out in devices such as Cell Stacks. More recently, following the monolayer stage, further expansion has been successfully achieved at the litre scale in stirred tank bioreactors used in conjunction with microcarriers. Here, to the best of our knowledge, we report for the first time, a bioprocess in a stirred tank bioreactor with microcarriers for the isolation and the subsequent expansion of hMSCs from fresh bone marrow aspirates. The Mobius® 3L single-use stirred tank bioreactor was operated at 1L scale for the isolation from bone marrow and at 2L for the expansion stage. The entire upstream process was performed on the bench with minimal manual handling, thus mimicking an automation-friendly process by making use of peristaltic pumps, aspiration bottles and sterile welders. Cell counts, imaging and metabolite data at different stages of the bioprocess were obtained in order to assess cell growth. Cell quality post-harvesting at the end of the process was assessed by differentiation and CFU-f assays. The hMSCs were successfully isolated on microcarriers from fresh bone marrow and expanded over a 20 day period in the single use stirred tank bioreactor to achieve a total of $760x10^6$ cells with a viability of 95% (*Figure 1*). Additionally, the cells maintained their differentiation potential and showed a CFU-f efficiency of 35%. Nevertheless, we have demonstrated for the first time, proof of concept of a bioprocess that enables the isolation and subsequent expansion of hMSCs on microcarriers directly from bone marrow aspirates.

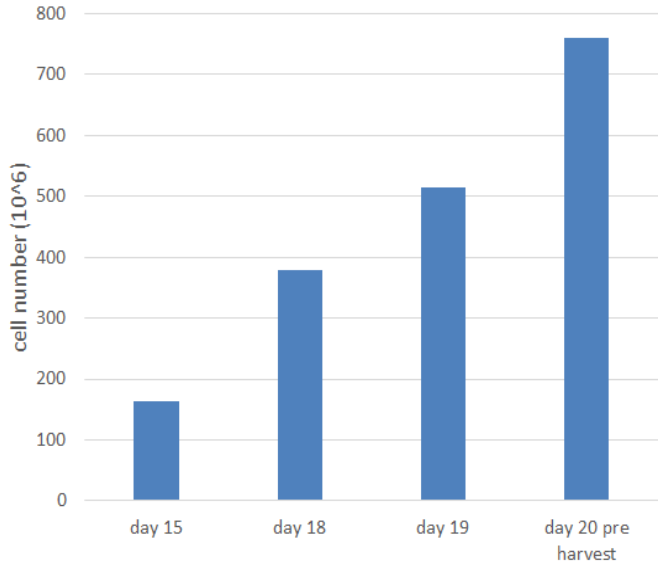

Figure 6 – Cell counts during the 20 day period isolation/expansion culture and post harvesting

MITIGATING THE RISKS OF ADVENTITIOUS AGENTS IN SERUM: ELIMINATION OR VIRAL INACTIVATION

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Key Words: serum, γ-irradiation, serum-free, CAR T, Celgene

Human AB serum (hABs) is used in culture medium for some cell therapies, such as chimeric antigen receptor T cell (CAR T) or engineered T cell receptor therapies, to provide the necessary growth factors and nutrients for cell proliferation. While hABs has been demonstrated to effectively sustain primary cell cultures, hABs has several drawbacks including lot-to-lot variability, supply constraints, and, above all, the risk associated with adventitious agents. hABs is derived from either whole blood or plasma donations that are subject to stringent sourcing controls such as viral screening on individual donations. The addition of a viral clearance step can further mitigate the risk associated with potential adventitious agents. Celgene is exploring two strategies to mitigate the risk associated with adventitious agents: γ–irradiation of hABs to inactivate viruses and serum-free medium (SFM) formulations to eliminate the use of complex animal-derived materials.

γ-irradiation has been proven to be an effective viral reduction method in animal sera, but its application to human serum is currently limited. γ-irradiation is known to impact serum composition, thus risking potential changes to process performance and critical product quality attributes. Additionally, there is a limited number of hABs vendors with γ-irradiation experience, ultimately requiring close collaboration between company and vendor to establish a robust supply chain.

Adoption of a SFM formulation can eliminate some of the challenges associated with serum. SFM formulations are commercially available, well defined, and can serve to replace hABs entirely. Implementation of a new SFM formulation in an existing process risks changes to critical quality attributes of the cell product and may require significant development efforts to achieve comparability. In addition, many commercially available SFM formulations still contain human- or animal-derived materials, such as human serum albumin or transferrin, which also can present viral disease transmission risk and must undergo appropriate viral reduction treatments.

Celgene's strategy is to evaluate both γ-irradiated hABs and SFM for cell therapy applications. Both media containing γ-irradiated hABs and SFM support T cell expansion, though differences have been observed when compared with typical hABs containing media. Moreover, both γ-irradiated hABs and serum-free alternatives may result in differences in CAR T cell quality attributes, such as phenotype and activity. Investment in development and comparability is critical when planning to incorporate γ-irradiated hABs or SFM in cell therapies in order to reduce the risk associated with adventitious agents. Careful selection of media formulation for future programs should also be a consideration given the challenges of changing media formulations for an existing product.

LENTIPRO STABLE PRODUCER CELLS: DELIVERING SCALABLE AND RELIABLE LENTIVIRAL VECTOR MANUFACTURING

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Key Words: Lentiviral Vector, stable cell lines, constitutive production, manufacturing, upstream process

Lentiviral vectors are one of the most currently used viral vectors for gene and cell therapies. Their use in clinical protocols has significantly increased in the past 5 years with the approval of several gene therapeutic products relying on lentiviral vector gene delivery. Capable of transducing non-dividing cells and presenting safer integration profiles as self-inactivating vectors, lentiviral vectors have progressively undertaken gammaretroviral vector use in gene therapies. However the knowledge on lentiviral vector manufacture is far more immature than that of gammaretroviral vectors. While the production of gammaretrovirus rely on stable producer cell lines and perfusion systems, enabling high cell density and longer term productions, most of the bioprocesses for lentiviral bioproducts rely on transient transfections and short term batch productions.

At the upstream process, many of the challenges lentiviral bioproducts present in their manufacturing are related to the apoptosis leading cytotoxicity of some of the vector components. Supported on our long track experience and enabling tools developed for gammaretrovirus manufacturing, we carried out the challenge of establishing a constitutive stable lentiviral producer cell line. To surpass the challenges we proposed to eliminate or reduce the cytotoxicity of the lentiviral vector expression components[1]. Several strategic novelties were introduced in the development of the cell line namely: (i) the use of a modified gag-pro-pol, (ii) introduction of all the third generation lentiviral expression cassettes by chemical transfection instead of viral transduction and (iii) performing only one clone screening step (enabling the use on the 'Single step cloning screening' protocol developed by our group[2]). After establishing a stable producer cell line the culture conditions were developed with the main aim of extending bioreaction culture time and viral vector total yields.

A lentiviral producer cell line constitutively producing infective titers above 10^6 $TU.mL^{-1}.day^{-1}$ was established. Moreover the new protocol to generate the cell line enabled its development in less than six months. The cell line showed to be stable, consistently maintaining vector productivity over one month in the absence of antibiotics. At the bioreaction process it was possible to maintain the cells continuously producing over 10 days[1]. These results validate the transition to continuous or perfusion large-scale production systems qualifying the strengths and advantages of the strategies followed.

This work to be presented will discuss the challenges on the manufacture and scale-up of lentiviral vectors as well the strategies and novel technologies to be adopted to enable effective upstream processes.

[1]Tomás et al. (2018) 'LentiPro26: novel stable cell lines for constitutive lentiviral vector production' Sci Rep. 8(1):5271

[2] Rodrigues et al. (2015) 'Single step cloning-screening method: a new tool for developing and studying high-titer viral vector producer cells' Gene Ther. 22(9):68

THERAPEUTIC GENOME EDITING FOR CHARCOT-MARIE-TOOTH DISEASE TYPE 1A

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Key Words: CRISPR/Cas9, Charcot-Marie-Tooth 1A, Gene editing, Peripheral nervous system

Charcot-Marie-Tooth 1A (CMT1A) is the most common inherited neuropathy without a known therapy, which is caused by a 1.4 Mb duplication on human chromosome 17, which includes the gene encoding the peripheral myelin protein of 22 kDa (PMP22). Overexpressed PMP22 protein from its gene duplication is thought to cause demyelination and subsequently axonal degeneration in the peripheral nervous system (PNS). Here, we targeted regulatory region of human PMP22 to normalize overexpressed PMP22 level in C22 mice, a mouse model of CMT1A harboring multi copies of human PMP22. Direct local intraneural delivery of CRISPR/Cas9 designed to target TATA-box of PMP22 before the onset of disease, downregulates gene expression of PMP22 and preserves both myelin and axons. Notably, the same approach was effective in partial rescue of demyelination even after the onset of disease. Collectively, our data present a potential therapeutic efficacy of CRISPR/Cas9-mediated targeting of regulatory region of PMP22 to treat CMT1A.

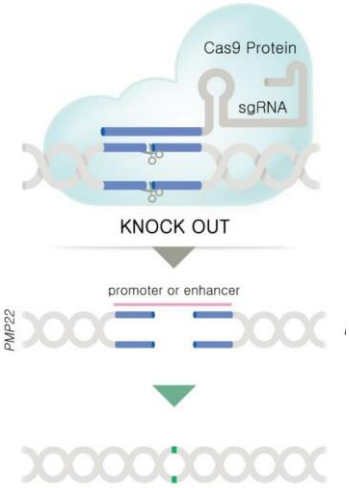

Figure 7 – Schematic diagram describing gene editing strategy for CMT1A

ENGINEERING CHARACTERIZATION OF A VERSATILE VERTICAL-WHEEL BIOREACTOR FOR CELL AND GENE THERAPY

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Key words: bioreactor, power, mixing, solids suspension

An ideal, versatile bioreactor for cell and gene therapy should be capable of growing cells across a range of formats, such as freely floating in suspension, in aggregates of various target sizes, and also on porous or solid carriers of various sizes. It should work well with cells and multi-cellular structures that are particularly shear sensitive. Ideally, it should be a well-mixed vessel with a homogenous culture environment. To achieve these low shear and mixing objectives, the bioreactor should be capable of suspending aggregates and solid carriers, as well as provide good mixing, at low power inputs per volume. Furthermore, in order to harvest attached cells when needed, it should also be capable of rapidly applying a uniform target high shear environment to viably remove cells from solid carriers.

To achieve these objectives, a single-use bioreactor system using an innovative Vertical-Wheel technology has been developed. The first design hypothesis was that a large vertical wheel, when rotated at relatively close clearance to a circular tank bottom, could provide homogeneous liquid mixing, as well as uniform suspension of solid carriers or cellular aggregates, at lower power input per volume than traditional stirred tanks. The second design hypothesis was that the same vertical wheel design could be used to viably harvest cells from solid carriers by simply turning up the rotation speed to a target level for a short period of time.

In this talk, we present data directly testing both of these hypotheses. Experimental data will be presented showing the measured power curves for the novel vertical wheel design, as well as the resulting power levels required to achieve uniform fluid mixing and suspension of solid microcarriers across a range of scales, from 0.5 liters to 80 liters. Data will also be shown regarding the ability of this system to viably harvest cells from solid microcarriers. Data regarding the performance of this system for the culture of several different cell types is presented as part of other talks and posters at this conference. In summary, clear evidence will be presented on whether Vertical-Wheel technology provides the most ideal, versatile bioreactor for cell and gene therapy applications.

A NOVEL SCALABLE MANUFACTURING PLATFORM
FOR T-CELL ACTIVATION AND EXPANSION IN ADOPTIVE T-CELL THERAPY

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Key Words: Bioreactor, CAR T-cell manufacturing, Upstream processing technology, automate, closed loop

Adoptive T cell therapy (ACT) is growing rapidly, representing the revolution in cancer treatment. However, the current manufacturing platforms are largely based on magnetic microbeads surface coated with agonist antibodies to T-cell receptors CD3 and CD28. These manufacturing platforms use expensive reagents including the viral transduction vectors, and also require multiple discrete stages and open processes with significant human interaction, contributing to the high-cost for cGMP manufacturing of these therapies.

We developed a single-use, beads-free bioreactor system (*Figure 1a*), which provides a closed-loop T-cell activation and expansion. The perfusion-based platform also facilitates the development of the bioreactor system into a fully automated, turn-key system used in both centralized and decentralized (e.g., hospitals) manufacturing settings. The bioreactor has a unique internal structure (*Figure 1b*), formed by a large number of interconnected hollow spheres tightly packed in a 3D space, which yields large surface areas to increase the reactivity between the bioreactor and the T cells flowing through the bioreactor. The surfaces of the bioreactor are coated with anti-CD3 and CD28 antibodies to mimic the antigens for T activation and expansion. The feasibility of using the perfusion-based bioreactor for T-cell activation and expansion is demonstrated in *Figure 2*. Briefly, $20x10^6$ PBMCs were seeded into the bioreactor system and perfused for two days during the T-cell activation phase, with the medium containing no cytokine IL2. After two-day of activation, human IL-2 was added to the system so that the total IL-2 concentration was 20 IU/mL. Then the T-cell expansion phase was carried out for three-days. On Day 5, T cells were achieved a three-time expansion after activation, which is similar to the magnetic beads-based system. However, the perfusion based bioreactor has the potential to offer multiple advantages over the current beads-based system as discussed above.

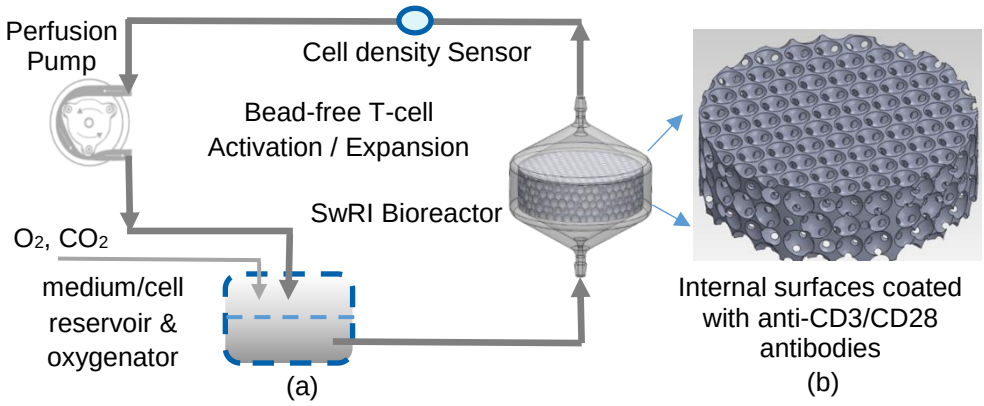

Figure 1: (a) Bioreactor for T-cell expansion; (b) internal structure of the bioreactor.

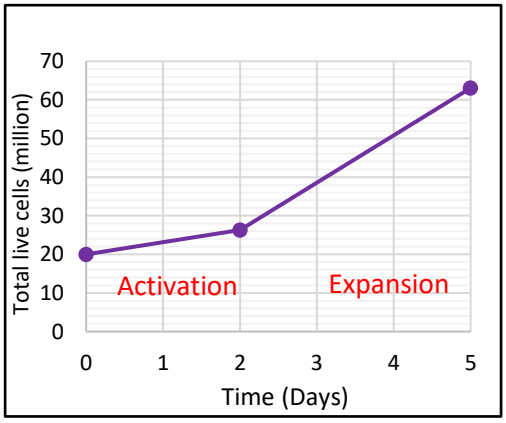

Figure 2: T-cell expansion in the perfusion bioreactor.

OPTIMISING HEK293T CULTURE FOR THE IMPROVED MANUFACTURE OF GENE THERAPIES

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Key Words: Gene Therapy, HEK293T, Manufacturing, Bioprocess, Standardisation

The development of gene therapies into effective tools for molecular medicine can be contributed to the successful outcomes of clinical trials followed by therapeutic product approval. Despite this, there are still many challenges to product development, including the scalability of the process and translating laboratory research into viable clinical applications. Cell expansion is currently labour and time intensive, and the processes lack the reproducibility and standardisation for regulatory and commercial compliant production.

This research aims to improve current gene therapy manufacturing processes by developing a standardised process for manufacturing to a clinical grade standard. One avenue to achieve this is by increasing the overall cell yield and lentivirus titre. Initial experiments showed that implementing standardisation techniques and set seeding densities produced more favourable results than generic 1 in 3 splits. Overall applying the set seeding densities led to stable growth and routine passages, thereby reducing the reliance on visual confluency estimations. This resulted in a more predictable and reliable process that also had set culture periods, consistent harvest densities and fold expansions that were maintained over long culture periods, ranging between $3x10^5$ and $4x10^5$ cells/cm^2 and 15-20 fold expansions per passage. Thereby creating a more suitable clinical grade manufacturing process which could be applied to both gene therapy and CAR-T cell therapy vector production.

Future work aims investigate what factors influence transfection, discern what differences in approach are required for the different types of gene therapy and if a standard methodology can be developed for each approach.

A SCALABLE AND PHYSIOLOGICALLY RELEVANT SYSTEM FOR HUMAN INDUCED PLURIPOTENT STEM CELL EXPANSION AND DIFFERENTIATION

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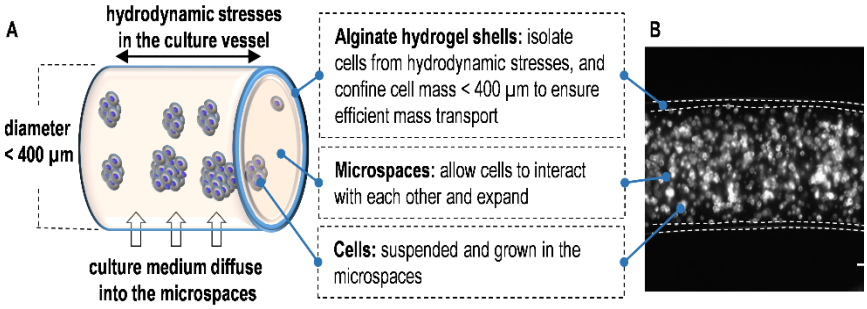
Key Words: human pluripotent stem cells, expansion and differentiation, manufacturing, alginate hydrogel tubes, SFIT

Human induced pluripotent stem cells (iPSCs) and their derivatives are needed in large numbers for various biomedical applications. However, scalable and cost-effective manufacturing of high quality iPSCs and their derivatives remains a challenge. In vivo, cells reside in a 3D microenvironment that has plenty of cell-cell and cell-ECM (extracellular matrix) interactions, sufficient supply of nutrients and oxygen, and minimal hydrodynamic stresses. The current iPSC culturing methods, however, provide highly-stressed culturing microenvironments, leading to low culture efficiency. For instance, we and others showed iPSCs typically expanded 4-fold/4 days to yield ~2.0x10^6 cells/mL with current 3D suspension culturing. These cells occupy ~0.4% of the bioreactor volume. To our best knowledge, the largest culture volume demonstrated to date for iPSCs is less than 10 liters. There is a critical need to develop new culture technologies to achieve the iPSCs' potential.

We here report a novel technology that can overcome all the limitations of current methods and provide a physiologically-relevant culture microenvironment. With this technology, iPSCs are processed into and cultured in microscale alginate hydrogel tubes (termed SFIT or stress-free intratubular cell culture) that are suspended in the cell culture medium in a culture vessel (Figs. 1A and B). The hydrogel tubes create free microspaces that allow cells to interact with each other and expand. Meanwhile, they protect cells from hydrodynamic stresses in the culture vessel and confine the cell mass <400 µm (in radial diameter) to ensure efficient mass transport during the entire culture (Figs. 1A and B). This technology is simple, scalable, defined and cGMP-compliant that make it commercially viable. We showed that, under optimized culture conditions, SFIT offered paradigm-shifting improvements in cell viability, growth, yield, culture consistency and scalability over current methods. We demonstrated long-term culturing (>10 passages) of iPSCs without uncontrolled differentiation and chromosomal abnormalities. Cultures between batches and cell lines were very consistent. iPSCs in SFIT had high viability, growth rate (1000-fold/10 days/passage in general) and yield (~5x10^8 cells/mL microspace). The expansion per passage (e.g. up to 4200-fold/passage was achieved) and volumetric yield are much higher than current methods. The high yield and high expansion fold significantly reduce the culture volume and time, numbers of passaging operations, and the production cost, making large-scale cell production technically and conically feasible.



iPSCs could be efficiently differentiated into various tissues cells in SFIT. Additionally, we have shown other human cells, such as T cells, could also be efficiently cultured in this technology. Two SFIT-based automated bioreactors for producing autologous and allogenic iPSCs and their derivatives are under developing. This technology has high potential to address the cell manufacturing challenge. Details of the method can be found in very-recent publications: Biofabrication. doi: 10.1088/1758-5090/aaa6b5; Sci Rep. doi: 10.1038/s41598-018-21927-4; ACS Appl Mater Interfaces. doi: 10.1021/acsami.8b05780; Adv Healthc Mater. doi: 10.1002/adhm.201701297.

TOWARDS AN ALLOGENEIC THERAPY FOR NEURAL REGENERATION

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Key Words: Olfactory ensheathing cells, regenerative medicine, co-culture, spinal cord repair, neural regeneration

Injuries to the central nervous system (CNS) can be devastating. CNS injuries include those to the spinal cord, where there can be a complete loss of function below the point of injury. Spinal cord injuries impact up to 500,000 people worldwide every year and where function is lost, quality of life can be severely limited. Olfactory ensheathing cells (OECs) are a promising cell therapy candidate for treatment of neurologic injury as they have been shown to promote neuronal survival and facilitate regeneration of severed axons. Despite their unique properties, OECs are very challenging cells to work with since they are difficult to isolate, difficult to sustain in culture for prolonged periods and there is still controversy around how to characterize their identity and potency.

Due to the inherent variability of OEC yield in biopsies and difficulties in growing these cells, producing an autologous therapy is not currently a viable option. Therefore, we sought to develop allogeniec OEC lines using a conditional immortalization tool. We undertook characterization work to ensure they expressed key putative OEC markers (such as p75NTR and S100β) and were able to support neuron extension in *in vitro models*. Subsequently, extensive bioprocess development was undertaken to investigate parameters such as:

- Cell culture conditions, e.g. effect of different culture media, initial seeding densities and microcarrier-based expansion

- Characterization process parameters, e.g. effect of culture time and pre-selection on identity marker expression, impact of detergents on identity marker measurement, and finally their impact in functional assays (including neuron co-culture to identify the impact of processing on neuronal support activity).

A number of important insights have been gained from this work including: identity markers are transient making characterization challenging; and analytics methodologies employed themselves affect what is measurable. However, these insights are informing our future approach to creating candidate cell lines for treatment of neurologic injury.

ENGINEERING AND MANUFACTURING OF PROBIOTIC *E. COLI* TO TREAT METABOLIC DISORDER

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Key Words: Synthetic biology, cell therapy, metabolic, microbiome

The fields of synthetic biology and microbiome research developed greatly over the last decade. The convergence of those two disciplines is now enabling the development of new therapeutic strategies, using engineered microbes that operate from within the gut as living medicines. Inborn errors of metabolism represent candidate diseases for these therapeutics, particularly those disorders where a toxic metabolite causing a syndrome is also present in the intestinal lumen. Phenylketonuria (PKU), a rare inherited disease caused by a defect in phenylalanine hydroxylase (PAH) activity, is one such disease and is characterized by the accumulation of systemic phenylalanine (Phe) that can lead to severe neurological deficits unless patients are placed on a strict low-Phe diet. As an alternative treatment, *Escherichia coli* Nissle (EcN), a well-characterized probiotic, was genetically modified to efficiently import and degrade Phe (SYNB1618). The coupled expression of a Phe transporter with a Phe ammonia lyase (PAL) allows rapid conversion of Phe into trans-cinnamic acid (TCA) in vitro, which is then further metabolized by the host to hippuric acid (HA) and excreted in the urine. Experiments conducted in the *enu2-/-* PKU mouse model showed that the oral administration of SYNB1618 is able to significantly reduce blood Phe levels triggered by subcutaneous Phe injection. Decreases in circulating Phe levels were associated with proportional increases in urinary HA, confirming that Phe metabolism was caused by the engineered pathway in SYNB1618. Subsequent studies have shown that SYNB1618 is similarly operative in a non-human primate model, providing a translational link to inform future human clinical studies. Consistent with preclinical studies, recent Phase 1/2a clinical data demonstrate that oral administration of SYNB1618 resulted in significant dose-dependent production of biomarkers specifically associated with SYNB1618 activity, demonstrating proof-of-mechanism of this cell therapy.

DEVELOPMENT OF A CLOSED CAR-T MANUFACTURING PROCESS

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Key Words: chimeric antigen receptor, immunotherapy, T lymphocytes, manufacturing, lentiviral vector

The field of immunotherapy has emerged as a promising new type of treatment for cancer with the approval of the first two CAR-T therapies. The clinical success of T-cell based immunotherapies necessitates a robust manufacturing process for these products to be consistently produced at commercial scale. Our CAR-T workflow combines unit operation specific solutions for thaw of an apheresis unit, wash, CD3 selection, T-cell activation, lentiviral transduction, incubator- and reactor-based expansion culture, harvest, formulation, cryopreservation and thaw of CAR-T product. We have evaluated the impact of both serum-containing and xeno-free culture media, commercially available T-cell selection and activation reagents, closed small-scale culture vessel options, alternative solutions to enhance transduction, and the specific timing of process steps to develop a modular platform process that is robust and flexible for the varied needs of CAR-T developers. Frozen apheresis units are processed using the SmartWash protocol on the Sepax™ 2 and T-cells are isolated with EasySep™ Release CD3 Positive Selection Kit. The cells are then activated with ImmunoCult CD3/CD28/CD2 T-cell activator before being transduced 24 hours later using the Sepax™ 2. Expansion of T-cells are carried out in two stages: incubator-based culture before going into the Xuri™ Cell Expansion System W25 with a perfusion feeding regime. Cultured cells are then harvested and washed in Plasmalyte-A with human serum albumin and formulated with CryoStor® CS10 using the FlexCell protocol on the Sefia™ Cell Processing System. The final cell products are cryopreserved using the VIA Freeze controlled-rate freezer. We have also accessed a point-of-care thawing strategy using the VIA Thaw. Our CAR-T process achieves greater than 1.0E10 expanded T-cells with >80% eGFP transduction efficiency across an 8-day manufacturing process.

LEVERAGING BIOPROCESS PLATFORM TECHNOLOGY FOR THE DEVELOPMENT OF A ROBUST, SCALABLE, AND ECONOMIC MANUFACTURING PROCESS OF ALLOGENEIC CAR-T CELL THERAPY PRODUCTS

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The current autologous chimeric antigen receptor (CAR)-T cell therapy products, both commercially approved and in clinical development, have been instrumental in changing treatment paradigm to benefit cancer patients. The therapeutic success of these treatment options has, to some extent, been tempered by the challenges of consistently manufacturing patient-specific products and by the introduction of a new manufacturing model involving scaling-out and subsequent releasing 100-1000s of patient-specific lots.

Adicetbio is building on the therapeutic success of patient-specific CAR-T cell therapies by employing gamma delta (gd) T cells and engineered T-cell receptor-like tumor recognition to improve the safety and efficacy of CAR-T cell therapies against both liquid and solid tumors. Additionally, we are optimizing the manufacture allogeneic CAR gd-T cells to facilitate off-the-shelf treatment of hundreds of patients per manufacturing run. . This approach greatly simplifies the complexity of manufacturing associated with the autologous model through minimizing the variation of processing patient material and eliminating the need to support large product release testing infrastructure. Results will be presented outlining results of ongoing process development and demonstrating that, by leveraging established bioprocess platform technologies, we can efficiently engineer, expand, harvest, and cryopreserve up to $2x10^{11}$ CAR-T cells from a single healthy donor in a cGMP-compliant manner. Figure 1 outlines the CAR gd-T cell manufacturing process. This approach is scalable to both support the needs of internal development as well as clinical trials, achieving substantial economies of scale while maintaining product quality consistency.

CHARACTERIZATION OF CAR-T TRANSDUCTION PARAMETERS USING A LENTIVIRAL VECTOR

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Key Words: CAR-T, transduction, lentivirus, cell process development

Production of chimeric antigen receptor T cells (CAR-T) can be accomplished using a viral vector to deliver the transgene. Lentiviral vectors (LVV) are commonly used for transduction due to their safety profile, stable transduction, ease of use, and ability to transduce both dividing and nondividing cells. Characterization studies were conducted to identify parameters within the manufacturing process that could impact transduction efficiency, such as the multiplicity of infection (MOI), application time after T cell activation, and LVV degradation profile. Optimizing these parameters will result in improved reproducibility within the manufacturing process and the efficient use of LVV material. Response curves for transduction efficiency, vector copy number and potency exhibited similar patterns to one another; signals increased with increasing MOI until reaching a plateau.

PLATELET LYSATE BOOSTS TRANSGENE LEVELS AND MAINTAINS UNDIFFERENTIATED T CELL SUBTYPES FOLLOWING LENTIVIRAL DELIVERY TO HUMAN PRIMARY T CELLS

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Key Words: Human platelet lysate, T cells, lentiviral transduction

Adoptive immunotherapy with T lymphocytes (T cells) modified *ex vivo* has emerged as a promising therapeutic strategy to treat various cancer and autoimmune diseases. T cells engineered to express chimeric antigen receptors (CARs) have shown high rates of clinical response in patients with hematological malignancies and even early indications of clinical activity in solid tumors. Key steps in the manufacture of CAR T cell therapies are the *ex vivo* transduction of autologous cells with a viral vector and expansion of the genetically modified cells. Patient T cells respond inconsistently when cultured in chemically defined media; therefore, basal media are typically supplemented with serum. AB serum (ABS) has supply limitations and may not be sufficient to meet the expected demand for immunotherapies while fetal bovine serum (FBS) carries a risk of pathogen transmission as well as xeno-immunization against bovine antigens. Human platelet lysate (hPL) obtained from transfusable donor platelets is widely recognized as a valuable alternative to both FBS and ABS for production of clinical cellular therapies. The goal of the present study was to evaluate the feasibility of using a new pathogen-reduced human platelet lysate during lentiviral transduction of primary T cells. PR hPL is produced with a method that has been demonstrated to reduce pathogens, including enveloped and non-enveloped viruses. Cryopreserved T cells enriched from peripheral blood mononuclear cells of normal donors were thawed, activated and cultured in media containing interleukin-2 and different concentrations of FBS, ABS or PR hPL. After four days of culture, cells were transduced with a lentiviral vector to deliver a green fluorescent protein (GFP) reporter transgene. We found that two days after transduction with CMV-GFP lentivirus the percentage of GFP positive cells was generally comparable among the various conditions suggesting that the transduction process itself was similarly efficient. Interestingly, cells transduced/cultured in PR hPL consistently exhibited brighter GFP compared to cells in ABS with a mean fluorescence intensity (MFI) on average 2.5-fold higher (range 1.9 – 3.5-fold, depending on donor). PGK-GFP and EF1A-GFP lentiviruses were similarly tested and expression of the PGK-GFP reporter was also found to be higher with PR hPL. We additionally examined the phenotype of cells transduced and cultured in PR hPL using CCR7 and CD62L, markers for less differentiated T cells including T_{naive} (T_n) and $T_{central\ memory}$ (T_{cm}). Cells in PR hPL exhibited a higher fraction of T_n/T_{cm} T cells than cells in ABS. Emerging *in vivo* and clinical data in the CAR T field predict that the presence of less differentiated T cells is associated with improved persistence following transfer. Our study demonstrates the feasibility of using PR hPL for primary T cell modification and expansion. Furthermore, the data predict that one may be able to use less lentivirus for a given therapeutic dose of transgene expression, thereby allowing a reduction in cost of producing the therapy.

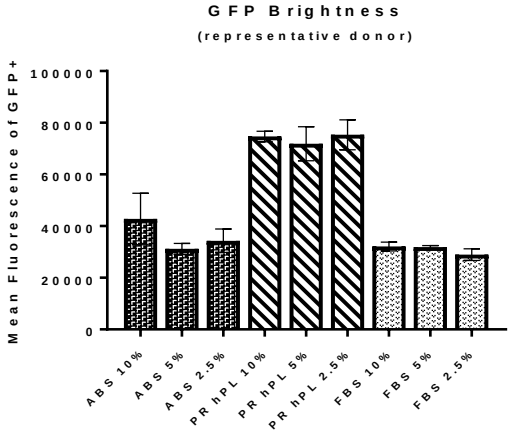

Figure 1 – GFP brightness 2 days

NEW VIRAL AND NON-VIRAL PLATFORMS FOR T-CELL ENGINEERING

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FDA approval of the first Chimeric Antigen Receptor T cell (CAR-T) therapy offers cancer patients more promise than ever for curative effects. However, many technical challenges in T cell gene delivery still remain in order for this therapy to become a standard of care practice. In this webinar, we will highlight the different viral and non-viral delivery approaches used in T cell engineering for cell and gene therapy applications including:

- New solution for small-to-large scale serum-free, suspension lentiviral production – LV-MAX™ Lentiviral Production System
  - Platform development process using Design of Experiment (DOE) methodologies
  - High-throughput to large scale bioreactor protocols
  - Cost benefits of this system over current methods

- Novel gene editing tools for primary T cells
  - New potent gene editing tools to increase knock-in and knock-out efficiency
  - Addressing non-viral delivery barriers through protocol optimization

Learning Objectives:
- Current industry trends and challenges of cell and gene therapy manufacturing
- Benefits of innovative new upstream technologies for virus generation in suspension

DEVELOPMENT OF FEEDER-FREE PSC CULTURE SYSTEM ENABLING TRANSLATIONAL & CLINICAL RESEARCH

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Pluripotent stem cell (PSC) culture using the xeno-free Essential 8™ Medium/truncated recombinant human Vitronectin system has been shown to support normal PSC properties and provide a large pool of cells for disease modeling and drug development. As research moves from translational to clinical research, general regulatory guidance from the US Food and Drug Administration (FDA) indicates that, cGMP manufactured, or clinical grade reagents should be used whenever available as ancillary reagents to minimize downstream risk to patients. Thus, we sought to identify regulatory compliant, animal-originfree alternatives for growth factors contained within the Essential 8™ Medium and incorporate ISO13485 manufacturing for the recombinantly expressed, truncated human Vitronectin (rhVTN-N), producing a qualified ancillary system for PSC expansion. Here we present data to support a seamless transition from the xeno-free Essential 8™ Medium system to the Cell Therapy Systems (CTS™) animal-origin free system. Compatibility is shown with existing cGMP-manufactured passaging reagents: Versene Solution for clumped cell passaging and CTS™ TrypLE™ Select combined with RevitaCell™ Supplement for single cell passaging. Upon expansion, PSCs are shown to maintain normal PSC properties, including morphology, pluripotency, karyotype, and trilineage differentiation potential. Together this system provides a consistent, feeder-free PSC culture medium for translational and clinical research.

XENO-FREE EXPANSION OF LATE-ADHERENT HUMAN OLFACTORY MUCOSA CELLS: TOWARDS AN ALLOGENEIC THERAPY FOR NEURAL REGENERATION

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Key Words: xeno-free, human olfactory mucosa cells, microcarriers, allogeneic cell therapy

Human olfactory mucosa cells (hOMCs) are anchorage dependent cells that have potential for treatment of spinal cord injury. However, current hOMC therapies relied on autologous transplantation and it is not feasible to prepare and characterize sufficient quantities of cells (in the order of 10^7 - 10^8 cells) within a timeframe to treat acute injury. Thus an allogeneic (universal) "off-the-shelf" approach would offer an alternative for this case.

We incorporated the regulator-approved c-MycER^{TAM} gene (ReNeuron) into primary late-adherent hOMCs to extend their *ex vivo* proliferation in the presence of the synthetic drug 4-hydroxytamoxifen (4-OHT). Polyclonal populations of hOMCs were generated and characterized, with an ultimate goal of developing a potential cell therapy product for application in spinal cord injury. Due to the lack of scalability, the availability of labour intensive manual processes and fetal bovine serum (FBS) supplementation, we aimed to develop a xeno-free process for the expansion of a these cells.

An initial issue for the manufacture of hOMCs is that key bioprocess parameters have not been established. In this work, we performed cell growth characterization to provide information about their growth i.e. effect of initial cell seeding density, long-term culture, and metabolite profiles to ultimately define the expansion process window.

Although widely used, FBS is a finite resource that raises concerns about the presence of adventitious agents. Alternative human-derived (xeno-free) or chemically-defined (serum-free) supplements were assessed for their ability to sustain cell growth. From these studies, human platelet lysate supplementation at 2-5% (% v/v) was found to be a viable xeno-free option to sustain growth of hOMCs with no adverse effects on their phenotype.

Finally, we sought to replace the current manually intensive monolayer expansion process with a more flexible and scalable platform such as suspension culture on animal-free microcarriers. Successful expansion of c-MycER^{TAM}-derived late-adherent hOMCs on plastic microcarriers at 80-mL scale was achieved to establish a suspension culture expansion platform for the translation of a potential candidate cell therapy for neural regeneration.

In summary, we show a systematic approach to address main hOMC bioprocessing challenges for an allogeneic therapy to treat patients suffering from spinal cord injury.

DECODING HUMAN CARDIAC STEM CELLS REGENERATIVE POTENTIAL IN ACUTE MYOCARDIAL INFARCTION

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Key Words: Myocardial Infarction, Human Cardiac Stem/Progenitor Cells, Stem Cell Characterization, Stirred Tank Bioreactors, Proteomics.

Acute Myocardial Infarction (AMI) remains a leading cause of death worldwide. After AMI, clinical restauration of blood flow aggravates tissue damage (Ischemia/Reperfusion, I/R injury), critically decreasing the number of viable cardiomyocytes (CMs). Human myocardium harbors a population of endogenous cardiac stem/progenitor cells (CSCs) that is activated upon I/R injury, contributing to myocardial repair through the establishment of an auto/paracrine molecular crosstalk between CSCs and CMs in stress. Transplantation of CSCs is currently being tested in several clinical trials, and although some improvements have been reported regarding decrease of the infarcted area, it is still not enough to show benefit over pharmacological standard-of-care.

Our work aims at combining the development of relevant I/R *in vitro* human cell models with implementation of advanced mass spectrometry (MS)-based proteomic tools to further characterize hCSC and unveil associated regenerative mechanisms upon AMI. hCSCs employed in the phase I/II clinical trial CARE-MI (NCT02439398) were used (allogeneic therapy).

Different strategies were explored to recapitulate both phases of I/R injury in the human adult heart, including: the use of human adult/mature cells, 3D culture system and stirred-tank bioreactor technology. Firstly, we developed a transwell co-culture cell based I/R model, with human CSCs and human induced pluripotent stem cell derived CMs (hiPSC-CMs). Following this work, and aiming at further improving the relevance of the I/R injury *in vitro* setup, 3D hiPSC-CM aggregate cultures and bioreactors were combined, allowing the control/monitoring of environmental parameters such as pH and dissolved oxygen, critical in the context of I/R physiology.

Important features of I/R injury were successfully captured in the two models, including hiPSC-CM death upon reperfusion, disruption of cell ultra-structure organization, as well as increased release of angiogenic and inflammatory cytokines, consistent with the described pathophysiology of AMI. hCSCs response to I/R was further probed using whole proteome analysis (including quantitative SWATH methodology), allowing us to propose new pathways in the hCSCs-mediated regenerative process along the different phases of I/R injury through the identification of more than 3800 proteins and quantification of 714 proteins. Our data shows that our AMI-setup up-regulates hCSC proteins associated with several pro-migratory, proliferation and stress response-related pathways. Moreover, our results reinforce the idea that paracrine-mediated mechanisms are a central response in hCSC activation, with the enrichment of several paracrine signaling and pro-angiogenic pathways. We also show for the first time increased CXCL6 secretion by hCSCs upon injury, suggesting a relevant role of this angiogenic cytokine in hCSC mediated myocardial regeneration.

Overall, multiple strategies were used to develop novel and robust I/R injury *in vitro* models, recapitulating several features of the human adult myocardium. The systems established allowed to better characterize hCSC mechanisms of action in response to AMI contexts. The knowledge generated has the potential to be used in the development of novel strategies excelling endogenous and transplanted hCSCs regenerative potential.

ADVANCING THE KNOWLEDGE ON IMMUNOMODULATORY PROPERTIES OF HUMAN CARDIAC STEM CELLS

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Key Words: cardiac stem/progenitor cells; allogeneic stem cell therapy, tryptophan metabolism, immunosuppression, T lymphocytes.

Transplantation of allogeneic human cardiac/stem progenitor cells (hCSC) is currently being tested in several phase I/II clinical trials as a novel and promising therapy for restauration of myocardial tissue function in acute myocardial infarction (AMI) patients. Previous findings demonstrate that these cells have an immune suppressive profile, interacting with different populations from the immune system, resulting in overall attenuation of myocardium inflammation. However, transplanted hCSCs are still recognized and cleared from the injured site impairing long retention times in the tissue that could be translated into a higher clinical benefit. In this work, different models of allogeneic hCSC/ T-lymphocyte interaction *in vitro* were explored, using the same hCSCs employed in the allogenous hCSCs transplantation phase I/II clinical trial CARE-MI, NCT02439398. T lymphocytes were cultured either in direct contact with hCSCs, or using transwell inserts or with hCSC conditioned medium.

In our results, we show that IFN-γ activation is correlated with an increase in hCSC indoleamine 2,3-dioxygenase (IDO) enzyme expression. We also show a significant inhibition of T lymphocyte inhibition when cultivating human peripheral blood mononuclear cells (hPBMCs) in direct cell-cell contact, using transwells or with activated hCSC conditioned medium, combined with tryptophan depletion and kyurenine (a tryptophan metabolite) accumulation in activated hCSCs conditioned medium.

These findings provide evidence, that although playing a role in the process, PDL-1 cell contact dependent T-regulatory cell modulation is not the exclusive neither the central mechanism involved in T-lymphocyte proliferation inhibition. This finding further supports the prominent paracrine-based beneficial CSC activities in the host tissue.

Our results demonstrate for the first time that hCSCs exert an immune-suppressive effect on T lymphocyte proliferation through a paracrine mechanism associated with IDO enzyme mediated tryptophan metabolism. The knowledge generated contributes not only to a better understanding on hCSC immunomodulatory mechanisms, but also open new avenues in the development of new hCSC transplantation strategies in allogeneic settings.

CRYOPRESERVATION CRITICAL PROCESS PARAMETERS: IMPACT ON POST-THAW RECOVERY OF CELLULAR PRODUCT

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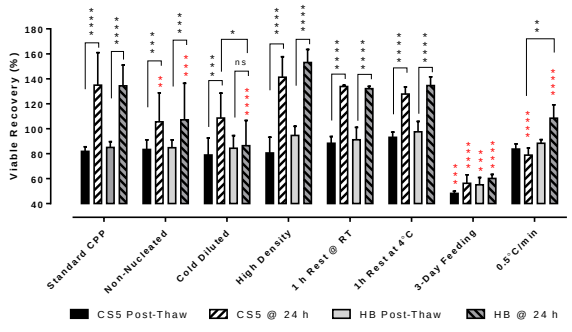
Key Words: Cryopreservation, Critical Process Parameters

Technological advances have transformed cells from mere drug targets into potent 'living drugs' with the potential to cure formerly incurable diseases such as cancer. Such Regenerative Medicine Advanced Therapies (RMATs) require stringent and complex vein-to-vein support to deliver their intended function. Cold Chain pertains to strategies designed to ensure the product potency and efficacy during ex-vivo transition, and includes various components starting from the source material collection, to culture and expansion process, formulation, fill-finish and packaging, storage, transportation, chain of custody, and delivery. Biopreservation is the overarching theme of the cold chain and refers to strategies to slow down or fully suspend the biological clock to allow logistical considerations. The two main modes of biopreservation are hypothermic storage and cryopreservation. This presentation aims to map the connection between a specific biopreservation strategy, namely cryopreservation, and formulation and fill-finish, and how implementation of Biopreservation Best Practices can improve the outcome of Cold Chain.

There is more to Biopreservation than storage on ice or freezing at a rate of -1°C/min in 10% DMSO. To comprehend the rationale behind Biopreservation Best Practices, a basic understanding of cellular response to cold and freezing is essential. In this study, we highlight critical process parameters (CPPs) of cryopreservation, such as freezing and thawing rates, storage and post-thaw stability, and container type, among others. Using a Jurkat T-cell model, we will discuss the impact of these CPPs on critical quality attributes (CQAs) such as viability, yield, proliferation rate, and return to function. We will also discuss the connection between variability in CPPs and characterization assay results. In general, implementation of best practices in formulation can directly address multiple process bottlenecks, including GMP compliance, minimizing freezing damage, support stability during storage and against transient warming events, support post-thaw stability, and excipient use. The CQAs may also be significantly improved by adjusting a few parameters in the freezing profile. For example, a missed or improper nucleation step during freezing may result in decreased recovery and increased variability in post-thaw proliferation rates. We have also found that the feeding timeline prior to freezing can have a profound impact on post-thaw viability and recovery in Jurkat T cells. While discussing these results, we will also review the underlying biophysics of such phenomena. The basic knowledge of designing a freezing profile may introduce degrees of freedom to process engineers to minimize the DMSO concentration in the formulation, and improve the CQAs of "hard-to-freeze" cells such as Natural Killer (NK) cells. We will also discuss the interplay between the cryopreservation CPPs and the choice of container format and how it may impact the CQAs.



Cryopreservation CPPs and their impact on post-thaw recovery of Jurkat T cells

Incorporation of Biopreservation Best Practices conveys important advantages upstream and downstream of cell manufacturing, including: (1) Assisting in the selection of the right delivery model, i.e. fresh vs. frozen, and the specific infrastructure and personnel requirements of each, based on which a commercial model is structured, (2) Reducing the Quality/Regulatory footprint of Cold Chain, and eliminating the burden of process changes when advancing to clinical trials, and (3) Improving the product CQAs, all of which could potentially improve safety and efficacy of RMAT-based clinical trials. As such, early incorporation of Biopreservation Best Practices in cell manufacturing is highly recommended.

OPTIMIZED MEDIA AND WORKFLOW FOR THE EXPANSION OF HUMAN PLURIPOTENT STEM CELLS AS AGGREGATES IN SUSPENSION CULTURES

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Key Words: PSC Culture Scale-up, Fed-batch Culture, Media Optimization

3D suspension culture enables scale-up of human pluripotent stem cell (hPSC) manufacturing. However, media and methods optimized for 2D adherent cultures can lead to low volumetric productivity and laborious workflow in suspension cultures. To overcome these limitations we developed fed-batch media based on either mTeSR™1 (BSA-containing) or TeSR™-E8™ (animal component-free) for hPSC expansion as aggregates in suspension cultures. Fed-batch feeding protocols are more efficient and cost-effective than batch media changes because only exhausted components are replenished. Optimization studies were performed using human embryonic (H7 and H9), and human induced pluripotent (WLS-1C and STiPS-M001) stem cell lines. Suspension cultures were fed daily using either 50% medium exchanges of standard 2D media, or fed-batch optimized media and protocols. hPSC aggregate diameter must be kept below 350 µm to maintain cell viability and phenotype. With observed growth rates, aggregates required passaging every 3 or 4 days into clumps of 5-10 cells with Gentle Cell Dissociation Reagent. Clumps were re-seeded into fresh test medium plus 10 µM Y-27632. Passaging and feeding cycles were repeated for at least 5 passages. Optimization was performed by iteratively modifying the feed solution to maintain consistent nutrient levels and maximal growth rate while maintaining cell quality. Control and optimized fed-batch formulations demonstrated between 1.4 and 1.8-fold expansion per day, >90% viability, Oct4 and TRA-1-60 expression >90%, *in vitro* trilineage differentiation, and normal karyotype (n=8 independent cultures). Suspension culture optimized mTeSR™-3D or TeSR™-E8™3D fed-batch media enables the cost-effective production of hPSCs as aggregates with efficient workflow and high cell quality.

REFINING iPSC-BASED 3D NEURAL CELL MODELS AND CHARACTERIZATION TOOLS TO ADRESS BRAIN MICROENVIRONMENT RELATED DISEASES

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Key Words: neurospheroids, brain microenvironment, perfusion bioreactor, proteomic, transcriptomic, neuronal connectivity

Brain microenvironment plays important roles in neurodevelopment and pathology and can affect therapy efficacy. Neural cell culture typically relies on the use of heterologous matrices that poorly resemble brain extracellular matrix (ECM) or reflect its pathological features. We have shown that perfusion bioreactor-based 3D differentiation of iPSC-derived human neural stem cells (hiPSC-NSC) sustains the concomitant differentiation of the three neural lineages (neurospheroids). If this neurospheroid culture strategy also allows deposition of native neural ECM it would be possible to (i) mimic cellular and microenvironment remodeling during neural differentiation, without the confounding effects of exogenous matrices and (ii) recapitulate pathological phenotypic features of diseases in which homotypic/ heterotypic cell-cell interactions and ECM are relevant. To characterize the neural extracellular space we employed quantitative transcriptomic (NGS) and proteome (SWATH-MS) analysis. Neurogenic developmental pathways were recapitulated in neurospheroids, with significant changes in cell membrane and ECM composition along differentiation; a significant enrichment in structural proteoglycans, typical of brain ECM, a downregulation of basement membrane proteins constituents and a higher expression of synaptic and ion transport machinery were observed. Neurospheroids were generated using hiPSC-NSC derived from Mucopolysaccharidosis type VII (MPS VII) patients. MPS VII is a rare neuronopathic lysosomal storage disease caused by deficient β-glucuronidase (β-gluc) activity, leading to glycosaminoglycan (GAGs) accumulation in the brain. The main MPS VII molecular hallmarks were recapitulated, e.g. accumulation og GAGs. By combining the neurospheroid culture with a 3D neuronal connectivity assay based on calcium imaging analysis we refined a new analytical strategy to characterize neuronal connectivity defects in a more predictive setting. We showed that MPS VII neurospheroids presented reduced neuronal activity and disturbances in network functionality, with alterations in connectivity and synchronization. These data provide insights into the interplay between reduced β-gluc activity, GAGs accumulation, alterations in neuronal network and its impact on MPS VII-associated cognitive defects Applying the characterization tools refined in this work to cope with 3D neurospheroid cultures, namely the neuronal connectivity assay, we provide a new platform to unveil the cellular processes responsible for brain dysfunction in neurological disorders and to test and optimize new therapies.

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MS data were obtained by UniMS – Mass Spectrometry Unit, ITQB and iBET, Oeiras, Portugal.

METABOLITE-BASED MODEL PREDICTIVE CONTROL OF CELL GROWTH

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Key Words: Model predictive control, bio-process, cell growth, metabolites

Batch-to-batch variation causes significant challenges in the production of cell and gene therapies in terms of manufacturing planning and process comparability. Since the processes deal with living systems that are complex and time-varying, remediating process variability requires dynamic control over the Critical Process Parameters.

In this work, the aim was to control the length of the culture time while optimizing medium consumption by applying Model Predictive Control (MPC) to the medium feed-rate for a cell culture vessel (Figure 1). First, a dynamic predictive model was created, that links nutrient consumption or waste production (lactate production in this specific case) to the growth kinetics of the cells ($Y(k)$). With the aid of this model, MPC is then used to optimize, based on measurement data from the batch under consideration, the amount of nutrients that should be provided to the cells ($C_2(k+1)$) (i.e. controlling the medium refreshment frequency and volume $U(k)$) in order for the cells to follow an *a priory* determined reference growth trajectory ($Y_R(k)$). Adjustable constraints are applied to the controller in a way that the maximum and minimum refreshable volume and frequency can be tuned to the culture system in use. Furthermore, the MPC cost function can be defined to include economical optimization of the process by optimizing not only the cell growth rate, but also minimizing the cost of goods via active controlling of the medium refreshment volume.

Simulated results of the MPC optimizer has shown that by using a predictive controlling of the cell growth we were able to save up to 49.5% of media in comparison to conventional methodology (i.e., 6.1 ml instead of 12 ml in a small scale experiment).

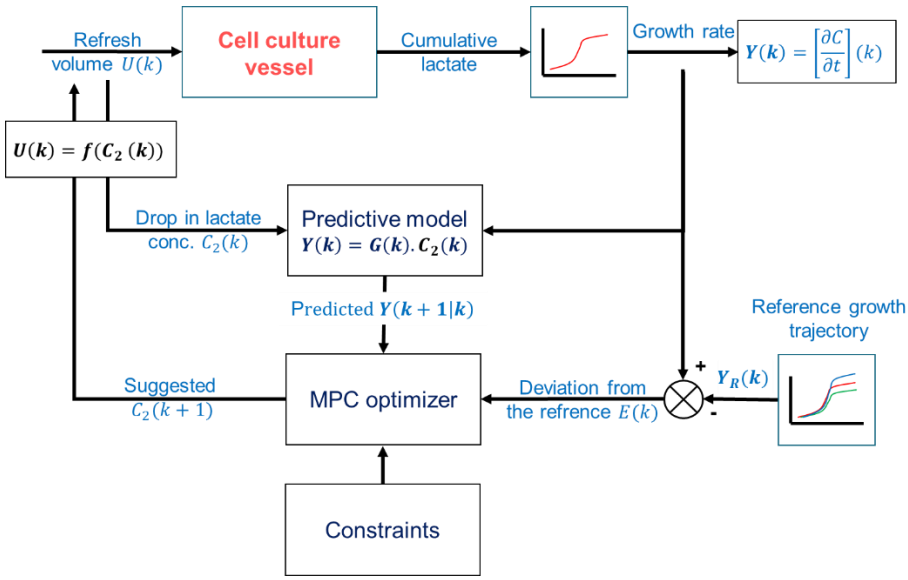

Figure 8 – Schematic process control scheme

STREAMLINING CELL THERAPY MANUFACTURING: AUTOMATED PRODUCTION AND INTEGRATED DATA MANAGEMENT

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Key Words: Bioprocessing, Automation, Cell therapy, Analytics, Data management

The manufacturing of cell therapy is a complex sequence of different unit operations. The substantial amount of manual and/or (semi-)automated manufacturing steps in a strictly regulated environment raise the need for scalable manufacturing and data management tools. Bioreactors have long been advocated as enabling technology for manufacturing of cell-based products. Automated bioreactors are not only able to substitute manual culture operations, but provide real-time monitoring and control of the culture environment, ultimately leading to improved process robustness. Additionally, they can be more easily integrated in an 'Industry 4.0' framework where data integration is required in real-time for process planning, regulatory compliance, etc.

This work presents the integration of new a new bioreactor and a software platform, allowing more automation and efficient streamlining of the cell therapy manufacturing processes. On one hand, a closed benchtop perfusion bioreactor system with a reduced footprint was developed that is suitable for point-of-care use. Precise monitoring of the culture environment (pH, DO_2 and T°) was achieved thanks to flexible sensor ports. The perfusion circuit was designed as a closed, single-use silicone tubing circuit linking a culture medium reservoir to a perfusion chamber via a peristaltic pump (cfr. Figure 1). A reusable cassette enclosed the disposable circuit and contained all the electronics required for bioreactor operation and environment control. A proof of concept study provided validation of the system on the perfusion culture of skeletal progenitor cells seeded onto 3D scaffolds. Regular sampling of the medium was performed during culture to measure glucose and lactate concentrations. Metabolic profiles, Live/Dead staining and DNA quantification of the constructs indicated similar cell growth kinetics compared to previously validated perfusion cultures of identical constructs.

Additionally, an online platform for centralised management of cell production process data was developed, named MyCellHub. The software enables efficient and regulatory compliant logging and management of all data related to cell manufacturing. Role-specific profiles in the software were made for operators, QA/QC units and process managers allowing to both log, access and visualize data relevant to their role. Every step of the manufacturing processes can be overseen in real-time with the aid of a digital audit trail. Based on this data, process analytics are provided and automated activity logs and batch records can be generated.

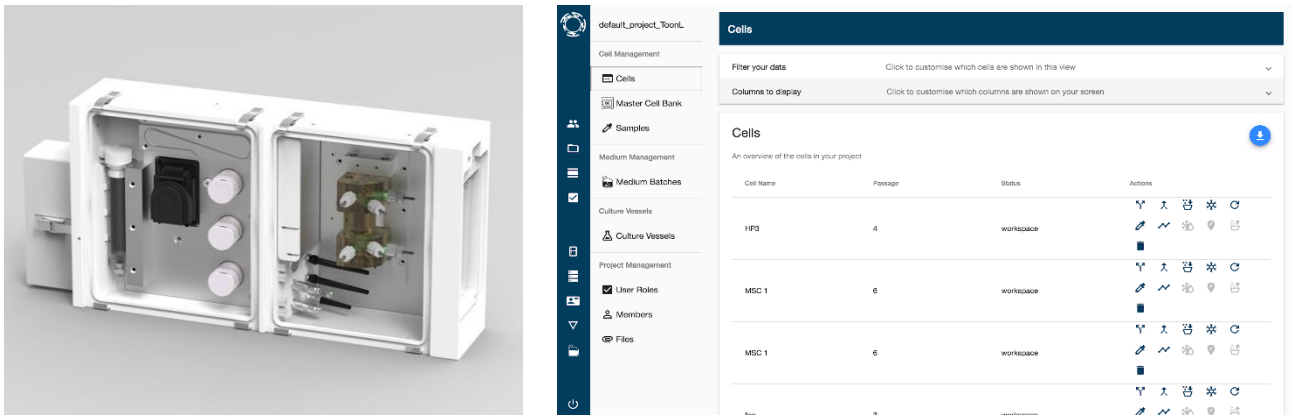

Figure 1: (left) Bioreactor cassette containing the perfusion circuit and all the electronics required for bioreactor operation and environment control. (right) Data management interface on the MyCellHub platform.

APPLICATION OF QUALITY BY DESIGN TOOLS TO UPSTREAM PROCESSING OF PLATELET PRECURSOR CELLS TO ENABLE IN VITRO MANUFACTURE OF BLOOD PRODUCTS

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Key Words: Platelet, Megakaryocyte, Manufacture, Upstream processing, Mechanistic modelling.

Annually 4.5 million platelet units are transfused in Europe and the United States. These are obtained solely from allogeneic donations and have a shelf life of 5-7 days. To address the corresponding supply challenge, Moreau et al.1 devised a novel process for producing megakaryocytes (MKs, the platelet precursor cell) in vitro. A transcription-factor driven, forward-programming (FOP) approach converts human pluripotent stem cells into MKs. This strategy has the unique advantage of generating high yields of pure MKs in chemically defined medium which could lead to the production of a consistent, reliable supply of platelets which overcomes the logistical, financial and biosafety challenges for health organisations worldwide.

Here we follow a Quality by Design (QbD) approach to enable improvements to the upstream processing of FOPMKs. Firstly, we created a process flow diagram for production of in vitro platelets for transfusion, which segregated processes into individual unit operations for control and optimisation. Next, we developed a Quality Target Product Profile (QTPP) and identified Critical Quality Attributes (CQAs) for each stage.

We conducted a range of experiments utilising Design of Experiments (DOE) and mechanistic modelling2 tools to link Critical Process Parameters (CPPs) to CQAs. For adherent culture, we identified a productivity limit related to surface area available for growth and a cell loss phase which was dependent on cell seeding density, RhoK inhibitor usage and seed density. Using suspension cultures of FOPMK. We noted that TPO and Doxycycline concentration were CPPs as these impacted cell net growth rate and phenotype trajectory. Furthermore, we noted that medium exhaustion led to a 30% loss of viable cells over 8 hours. Proof of concept studies also showed that FOPMKs can be cultured in scaled-down suspension systems (ambr-15 and spinner flask culture) whilst retaining CQAs.

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DIELECTRIC SPECTROSCOPY MONITORING OF A BIOREACTOR PROCESS FOR hiPSC EXPANSION AND DIFFERENTIATION

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Key Words: dielectric spectroscopy; bioprocess monitoring; human pluripotent stem cells; stirred tank bioreactors; cell aggregates

Bioprocessing strategies using 3D cell culturing approaches, such as cell aggregates, are promising solutions to achieve efficient and scalable bioprocesses for stem cell expansion and differentiation. However, tracking viable and total cell numbers in such culture systems is not straightforward. It requires cell detachment, disaggregation or disruption, which results in measurements that are laborious, biased and with high variability.

In this work, we used a commercially available capacitance probe to explore the applicability of dielectric spectroscopy for in situ monitoring of a multistep process for expansion and differentiation of human induced pluripotent stem cells (hiPSC) cultivated as cell aggregates. After 5 days of cell expansion in a bioreactor, the hepatic differentiation step was integrated by addition of different levels of specific soluble factors at various stages of the process to promote growth and generate populations successively enriched for definitive endoderm, hepatoblasts, hepatocyte progenitors and mature hepatocytes. While this differentiation procedure has been previously validated for monolayer cultures, this was the first time it was carried out in a stirred tank bioreactor operated in perfusion mode. Phenotype analysis confirmed a marked increase in key hepatic differentiation markers culminating at day 21 of differentiation.

Our data shows a good correlation between total volume of the cell aggregates and permittivity measured by the probe ($R^2 = 0.84$). However, there was a delay between changes in cell concentration and the permittivity signal. This suggests that cell expansion requires a few days to result in increased volume of the cell aggregates and that each aggregate behaves as one overall inducible dipole. The β-dispersion curve shape also appears to change over culture time and could eventually be used as an indicator for differentiation progression.

Dielectric spectroscopy has been used successfully to monitor viable cell concentration in different single-cell suspension cultures, but there are few published applications to 3D cultures. Our results demonstrate the potential of dielectric spectroscopy to monitor complex bioprocesses for human stem cell aggregates in stirred cultures.

Acknowledgements: Funding provided by ERA-NET/E-Rare3 programme through research project ERAdicatPH (E-Rare3/0002/2015). The authors acknowledge Dr Juan Rodriguez-Madoz (University of Navarra, Spain) and Dr Anders Aspegren (Takara Bio Europe – Cellartis AB, Sweden) for helpful discussions on hepatic differentiation of hiPSC.

SCALING UP AND INDUSTRIALIZATION THE PRODUCTION AND PURIFICATION OF VIRAL VECTORS FOR THERAPEUTIC USE: CHALLENGES AND PROGRESS

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Key Words: Virus production, scale up, adherent cell culture, bioreactor, manufacturing.

With several recent FDA approvals and a strong drug pipeline, gene therapy is coming of age. With this comes the requirement to ensure that there are robust manufacturing processes in place in order to scale with demand and to make these therapies readily accessible to those who need them. However, current manufacturing processes for gene therapies have often been developed with limited scalability in mind and large shifts in technology have to take place to enable industrialization. This also has to be done while keeping costs in mind. Here, we will present a case study which illustrates the challenges and solutions to scale both up and downstream process steps required to manufacture adenovirus. After implementation of a bioreactor, the bioreactor scale increased 125 fold, from 0.53m^2 to 66 m^2. With the implementation of several scalable unit operations on the downstream, this took 1 day as opposed to 3 – 4 days required for the entire optimized process generating purified viral vector for the successfully completion of a global in vivo toxicology study. Altogether, the practicalities around manufacturing virus to industrial scale.

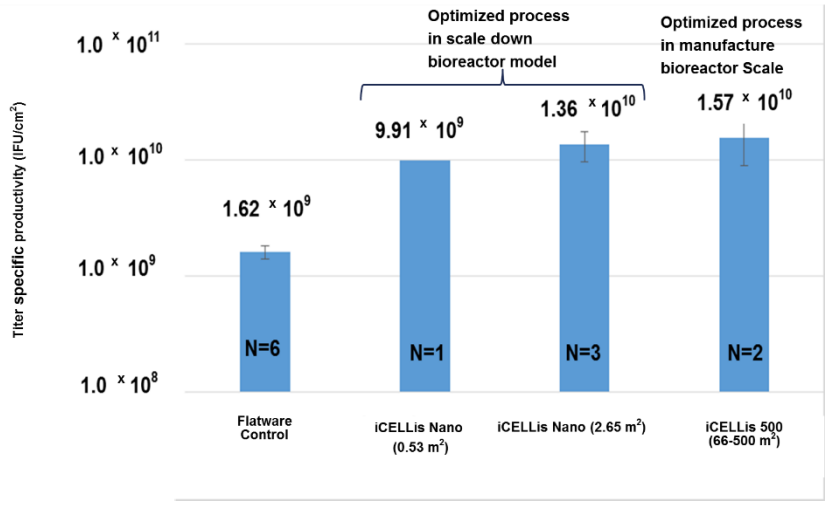

Figure 9 – Linear titer scalability: Process development from flatware to Manufacturing Scale

OPTIMIZATION OF HEK293T SUSPENSION CULTIVATION WITH A DoE-APPROACH IN ambr®15 MICROBIOREACTOR

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Key Words: lentiviral vector, bioprocess modelling, ambr15, DoE, optimization, high throughput

Cell and gene therapies present a new treatment paradigm that have the potential to address clinical needs that are unmet by current small molecule and biotherapeutic approaches.

Viral vectors such as adenoviruses, adeno-associated viruses and retroviruses are effective delivery systems for genetic material used in cell and gene therapies. Especially lentiviruses are used for example for the transfer of genetic information for novel cellular immunotherapy like CAR-T cell therapy. These novel approaches promise to be a substantial part of next-generation therapies with the potential to cure devastating diseases.
HEK293T cells are a workhorse cell line for lentiviral vector production for both cell and gene therapy applications. A significant challenge for the cell and gene therapy industry is to develop a HEK293T suspension cell culture processes that is well characterized and can be scaled-up for production whilst ensuring clinical and commercial success.

Ambr® 15 is an automated micro-scale bioreactor system that mimics the features and process control (pH, DO, temperature, stirring rate) provided by much larger scale bioreactors, in a volume of 10 - 15 ml. Parallel processing capability and excellent reproducibility enable rapid, high throughput process improvement and optimization, including DoE studies.

High-throughput tools with parallel processing, such as ambr® 15, help to address a major manufacturing bottleneck. They can be used as a scale-down model for process screening, clone selection and effective media optimization in less time with reduced reagents use and labour savings.

In the study presented we used ambr® 15 for the optimization of the HEK293T culture in suspension. We identified optimal stirring speed, DO and pH value by performing a DoE approach with the use of MODDE® software for experiment planning. Viable Cell Concentration (VCC), viability and generation time have been monitored and compared to standard shake flask culture.

We observed that cultivation of HEK293T cells in the ambr® 15 microbioreactor yields improved cell growth and viability as compared to standard shake flask culture. We identified that pH was the most significant factor - besides stirring speed - which has a lesser significant impact on cell health and growth. By using the MODDE® software we were able to determine an optimal set-point for improved cell growth that can be used for scaling-up studies in stirred tank reactors.

This study demonstrates that the ambr® 15 micro bioreactor system in combination with the DoE MODDE® software enables a systematic investigation of critical process parameters and rapid, high throughput process improvement and optimization. The results prove that the transition from shake flask to a scalable stirred bioreactor system can be accomplished in a timely manner. A key next step is to use the identified HEK293T culture conditions to perform a DoE study with the ambr® 15 to optimize viral vector production for cell and gene therapy applications.

Figure 10 – ambr® 15 microbioreactor and disposable vessels

DETERMINING THE ROLE OF LACTATE IN INDUCED PLURIPOTENT STEM CELL METABOLISM

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Key words: metabolism, Warburg Effect, pluripotency

Induced pluripotent stem (iPS) cells hold the potential to dramatically improve cell-based therapies and *in vitro* drug screening applications in the near future. Yet, for iPS cells to have a clinical impact, these cells must be generated in sufficient quantity and quality that currently exceeds today's capabilities. To meet these cell needs, a comprehensive understanding of how environmental conditions affect iPS cell metabolism and pluripotency is essential. Rapidly proliferating cells, including cancer and iPS cells, catabolize glucose and secrete lactate at elevated rates, even in the presence of sufficient oxygen, a process referred to as the Warburg effect (Vander Heiden, Cantley, Thompson 2009; Varum et al. 2011; WARBURG 1956). In cancer cell metabolism, lactate accumulation is associated with cancer stem cell-like gene expression, drug-resistance, metastasis, and poor prognosis in breast cancer patients (Martinez-Outschoorn et al. 2011). In addition, lactate has previously been shown to stabilize hypoxia inducible factors and induce a hypoxic response for cells cultured in normoxic environments (Pérez-Escuredo et al. 2016). However, there remains an incomplete understanding of the metabolic role of lactate for iPS cells and its effects on pluripotency. This study examined the impact of extracellular lactate on cellular metabolism and pluripotency of iPS K3 cells grown with sufficient glucose. Extracellular glucose, lactate, and amino acid concentrations were monitored throughout the experiment to determine the extracellular consumption or production fluxes. In addition, [1,2-^{13}C] glucose, [U-^{13}C] glutamine, and [U-^{13}C] lactate isotope tracers were used in parallel labeling experiments to determine the intracellular metabolic contribution of each carbon source to iPS cell metabolism. High extracellular lactate resulted in altered cell metabolism, including a decrease in lactate production and glucose consumption. This was coupled with a decrease in glucose contribution to the TCA cycle. Also, lactate was catabolized to pyruvate, alanine, and TCA intermediate metabolites in the high-lactate condition. Furthermore, high extracellular lactate did not affect iPS cell pluripotency. These results suggest that lactate partially serves as a metabolic substrate for iPS even as it continues to accumulate in the extracellular media. The implications of these findings towards understanding iPS cell metabolism and improving future cell culture conditions will be discussed.

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COMPUTATIONAL FLUID DYNAMICS (CFD) MODELING OF SINGLE-USE, VERTICAL-WHEEL BIOREACTORS AS A PREDICTIVE SCALE-UP TOOL FOR LARGE SCALE STEM CELL CULTURE

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Key Words: CFD Modeling, Vertical-Wheel Bioreactor, Scale-up, Induced Pluripotent Stem Cells

Hydrodynamic variables in bioreactors such as velocity, shear rate, and energy dissipation rate have been shown to affect stem cell properties including: aggregate size, growth, plenotype, and differentiation potential. Unlike traditional bioreactor scale-up equations, CFD modeling allows the user to customize geometry so that scale-up equations can be derived between reactors of any given shape and size. We have recently published data that suggests maintaining the volume average energy dissipation rate, derived from CFD simulations, provides a robust method for scale-up of aggregate culture in stirred suspension bioreactors. Turbulent flow consists of eddies formed when kinetic energy is transferred. Energy dissipation rate is the parameter that determines the amount of energy lost by viscous forces in the flow, and interactions with turbulent eddies influence aggregate size. Aggregates in the culture that are smaller than eddies are engulfed and aggregates that are larger are sheared apart.

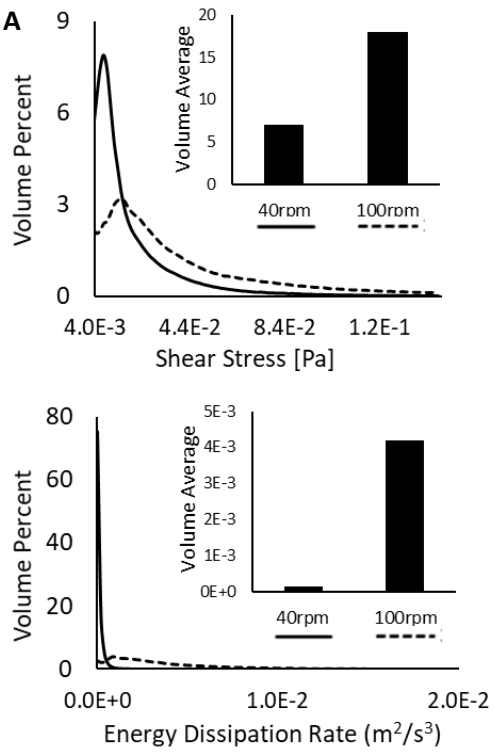

Figure 11: Shear stress [A] and energy dissipation rate [B] distributions and volume average values in a 100mL vertical-wheel reactor

In this study, PBS Biotech's 100mL Vertical-Wheel bioreactor was modeled at various agitation rates using CFD software, Fluent. Results were compared to models generated for traditional horizontal impeller 100mL NDS and 500mL DasGip bioreactors (modeled with the addition of control probes). It was discovered that volume average hydrodynamic values between the vertical-wheel and horizontal blade 100mL reactors were nearly identical, with velocity and shear stress scaling linearly, and energy dissipation rate scaling exponentially relative to agitation rate. While volume average shear rates between the 100mL and the 500mL reactors remained similar at set agitation rates, the average energy dissipation rates varied greatly. At 100rpm, the volume average energy dissipation rate in the 500mL DasGip was nearly 10 times higher than in the 100mL vertical-wheel reactor. This increase can likely be attributed to the addition of control probes that occupy a large portion of the liquid headspace in the DasGip. These results suggest that a drastic decrease in agitation rate the between the 100mL and 500mL reactors would be required for aggregate size to be maintained through scale-up.

Another important finding comes from evaluating the 100mL vertical-wheel hydrodynamic variable distributions graphs. Typical distribution graphs highlight the variance in hydrodynamic variables throughout the bioreactor, resulting in small volume percentages occupying a single value, as was the case for the shear stress distributions, Figure 1A. Unlike traditional horizontal impeller bioreactors, the energy dissipation rate proved relatively constant throughout the volume of the vertical-wheel reactor, Figure 1B. This was particularly evident at a lower agitation rate (40rpm), where nearly 80% of the bioreactor volume was at a single energy dissipation rate. Given the relationship between energy dissipation and aggregate size, this should result in increased aggregate size control and a decrease in size distribution. Data generated from the CFD models was used to test the predicted theories outlined and further optimize bioreactor operating conditions to culture human induced pluripotent stem cells as aggregates in the vertical-wheel reactor. Future experiments will use CFD modeling for larger sized vertical-wheel bioreactors to evaluate the consistency of the hydrodynamic characteristics during scale-up of cell culture processes.

A COST/QUALITY ANALYSIS OF PRIMARY HUMAN T-CELLS IN DIFFERENT EXPANSION SYSTEMS

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Key Words: Bioreactors, T-Cells, CAR-T, Scale-up, Process Development.

Recent developments in cell and gene therapy, in particular the emergence of Chimeric Antigen Receptor (CAR) T-cell immunotherapies, have demonstrated a new therapeutic modality to combat life-threatening diseases. Kymriah® (Novartis) and Yescarta® (Gilead) are the first CAR-T therapies that have been approved by both FDA and EMA. They are autologous therapies, where T-cells are isolated from the patient and engineered in order to express a specific CAR which recognizes and targets cancer cells.

The clinical success of these products is driving the need for a consistent and cost effective manufacturing process to meet the lot-sizes required for commercial production. Current T-cell manufacturing and production capabilities are greatly lagging and will not be able to meet the predicted surge in demand (£6.8 bn by 2028, CAGR of 46%) unless new technologies and processes are developed. These technologies need to be highly regulated and consistently achieve high yield, without compromising the quality of the cells, irrespective of donor.

A number of systems have been used for CAR-T cell expansion, namely flasks, bags (static or dynamic), G-Rex® (WolfonWilson) and CliniMACS Prodigy® (Miltenyi Biotec). All these technologies have many advantages, but they generally lack in scalability, suffer from manufacturing bottlenecks or incur in high suppliers costs and manual intervention.

The aim of this work is to understand the relation between reduction of production costs and cell growth quality. We compared the growth of human primary T-cells from multiple healthy donors in the aforementioned culture platforms, characterizing cell growth and phenotype. Cells were expanded for 7 days in each of the platforms with the aim of comparing the different systems with respect to scalability, productivity and efficiency. The phenotype composition of the final product was determined by flow cytometry.

A cost analysis for the different platforms related to the final product, as well as the cost related to the media and supplements consumption, has been performed. The different platforms have been compared with automated systems on the market or those in final development stage. Additional work includes bioprocess development for T-cell production with batch, fed-batch, and continuous systems to improve consistency and to provide a greater understanding of how selected process parameters influence T-cells critical-to-quality attributes.

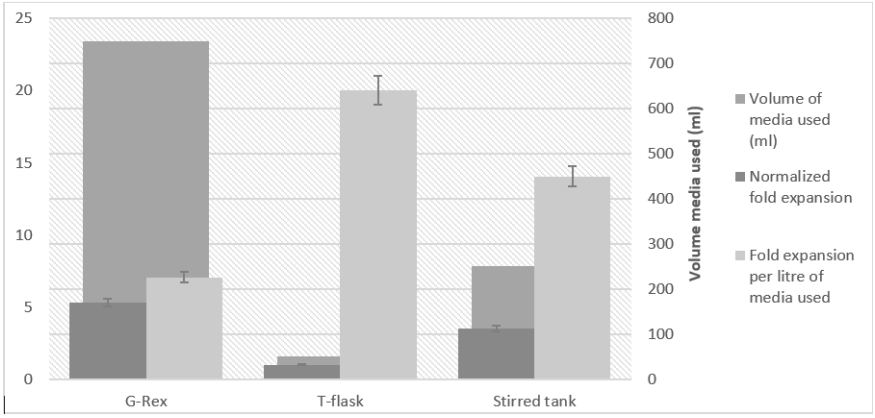

Figure 12 – Preliminary results on fold expansion and volume used to grow T-Cells in cRPMI with IL-2 addition in different expansion platform.

HIGH SHEAR STRESS FROM A RESONANCE PHENOMENON IN WAVE BIOREACTOR REVEALED BY COMPUTATIONAL FLUID DYNAMICS SIMULATION

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Keywords: Wave bioreactor, CFD, microcarriers, cell aggregates

Wave bioreactors are getting a wide acceptance for the culture of human cells. These bioreactors are attractive for sensitive cells such as stem cells or immune cells, in suspension, aggregates or adherently growing on microcarriers. The optimization of the mixing, the oxygen transfer rate and the shear stress require a deep understanding of the hydrodynamics taking place in the Wave bioreactor bag.

In the present study, we perform numerical simulations (Ansys-FLUENT) to characterize the flow conditions in a 10L cellbag [1]. The numerical simulations were carried out to investigate the fluid structures for nine different operating conditions of rocking speed and angle. The mixing and the shear stress induced by the liquid motion were studied. We observed that these increased with the cellbag angle from 4˚ to 7˚ but that the highest rocking velocities were not systematically associated with the highest mixing and shear stress. As a matter of fact, the lowest studied rocking speed, 15 rpm, generated the highest fluid velocity, mixing and shear stress compared to the higher speeds of 22 and 30 rpm. It was concluded that a resonance phenomenon was responsible for this behavior.

These observations were theoretically benchmarked against shear stress levels reported in the literature. Although the obtained shear stress levels in a Wave bioreactor were lower compared to stirred tank bioreactor, their magnitude was such that they could have an influence on the cell metabolism or could lead to cell lysis of cells adhering on microcarrier. On another hand, the studied operating parameters of speed and angle generated a shear stress too low to obtain the formation of aggregates or spheroids. Therefore a system generating harsher shear stress than the Wave bioreactor would be more suitable for the formation of cell aggregates.

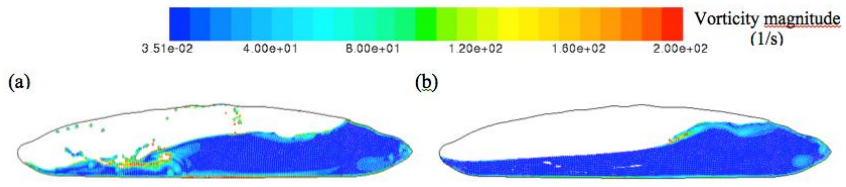

Figure 1. Vorticity magnitude in the liquid phase for angle 7° and speed (a) 15 rpm or (b) 30 rpm by 2D simulation in 5 L volume.

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DEFINING CELL CULTURE DYNAMICS IN RESPONSE TO GROWTH FACTOR PROVISION FOR EFFICIENT OPTIMIZATION OF CELL BASED THERAPIES

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Key Words: Bioprocess, Growth factor, Optimize, Dynamic Model, Manufacture.

The design of processes to manufacture Cell Based Therapies (CBTs) is particularly challenging due to the dynamic cell culture environment and the complex response of the cell product. An approach to process design needs to achieve a balance between resource spent and process knowledge gained to meet manufacturing goals around cost, quality and risk. In order to address this, we developed a parsimonious mathematical modelling framework designed to allow representation of common mechanisms in cell culture, and associated process operations, that drive cell culture outcomes[(1)]. We propose that this framework can be applied to generate widely applicable 'template models' and experimental strategies for process optimization. In this case we have demonstrated the application to define provision rates of elements that support cell growth in a hematopoietic culture system (erythroblasts). Building on a model that defined the influence of environmental change (incorporated as a single non-specific variable linked to cell growth and density) on population growth[(2)], we have defined the cell culture dynamics in response to specific elements such as growth factor provision. We have shown how achieving the correct combination of delivery rates of growth factors with other factors (e.g. metabolic) can ensure that each is used with maximal efficiency, significantly driving down volume use (more than 3-fold) and process supplementation costs. The models also define the corollary of such intensification in terms of increasingly demanding process control with respect to volume handling and process timing; however, an advantage of the level of intensification achieved is that the system oxygen demand increases to a level that oxygen input rates can be used to identify cell growth-inhibition in advance of reduced viability. This work demonstrates that models that appropriately represent common process dynamics will be a key tool to achieve the risk and cost reduction required for CBT manufacture.

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DEVELOPMENT OF MEDIA PRODUCTION PROCESSES FOR CAR-T THERAPIES

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Many of the standard cell culturing unit operations utilized by early stage CAR-T manufacturing processes have been derived from benchtop scale academic processes and require further development to become commercially viable. Critical unit operations, such as isolation, activation, transduction, and expansion are often the focus of next generation or automation technologies. Development of ancillary processes such as medium production, however, should not be overlooked and can take advantage of economies of scale and technologies that have been proven in other pharmaceutical industries like biologics. Special consideration should be taken when developing these medium scale-up processes since cell therapies are complex and can be highly sensitive to medium composition changes. In addition, significant changes may be needed to update medium production processes from a process suited for an academic setting to one suited for a commercialized product. This poster discusses Celgene's approach for developing a commercially sustainable media preparation process by applying available filtration and bulk solution preparation technologies and the unique challenges associated with applying these technologies to CAR-T therapies.

ECONOMICS OF LENTIVIRAL VECTOR PROCESSES

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Key Words: lentiviral vectors, CAR T-cell therapies, haematopoietic stem cell gene therapies, adherent and suspension HEK293T cell culture, transient transfection.

With the recent market approvals of autologous CAR T-cell therapies, lentiviral vectors (LVs) have been in the spotlight as a potential bottleneck to their already hindered scalability. Unstable at room temperature, LVs are routinely manufactured in multi-layered vessels using transient transfection methods. However these traditional processes are not sufficiently scalable or cost-effective for future anticipated demands. This poster discusses five different cell culture platforms that have been reported to deliver LVs: the 10-layer vessels, hollow fibre bioreactors, fixed bed bioreactors, rocking motion bioreactors in microcarrier mode and single-use bioreactors in suspension mode. These are compared from a process economics perspective across a range of scenarios that include different titre and dose size scenarios. The use of LVs for two therapeutic approaches are explored, namely for CAR T-cell therapies and haematopoietic stem cell/gene therapies. Costs of goods (COG) trends are described for a range of demands, target process parameters are identified and uncertainty analysis is carried out to capture the impact of variations in titre on the performance of each type of process.

CMC STRATEGY FOR AAV GENE THERAPIES IN THE AGE OF RMAT DESIGNATION

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Key Words: AAV, Gene therapy, RMAT, CMC Strategy

The Regenerative Medicine and Advanced Therapy (RMAT) designation by the FDA allows for faster, and more streamlined approvals of cell and gene therapies for unmet medicinal needs. Advantages of RMAT designation include all of the benefits of the fast-track and breakthrough designation programs, including early interactions with the FDA. Voyager's VY-AADC program, an Adeno Associated Virus (AAV) gene therapy approach for advanced Parkinson's Disease (PD), was one of the therapies granted RMAT designation in 2018.

Owing to the durable nature of gene therapy, clinical development may entail just two trials before approval, a Phase 1 Safety/Dose escalation in patients, followed by a Phase 2/3 Efficacy trial. While this strategy is expected to greatly accelerate the path to licensure, it also requires a much earlier (and in some cases, heavier) investment on CMC Development and Manufacturing as compared to regular biopharmaceutical development. Also, an RMAT designation by the FDA does not reduce quality and CMC expectations for a BLA submission, further underscoring the need for robust CMC strategies.

This presentation discusses Voyager's overall development strategy for AAV-gene therapy, focusing on a two-stage CMC approach: one encompassing development activities leading to drug product manufacture for Phase 1/IND filing studies, and the other directed to late-stage/commercial manufacture for approval/launch-enabling pivotal studies. Ways to balance early CMC investment and optimize resources, while still enabling an aggressive fast-to-licensure timeline to bring urgent therapies to patients, are also covered.

CAR T-CELL THERAPIES: THE CONCEPT OF A DYNAMIC SUPPLY CHAIN

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Novarti's Kymriah and Kite's Yescarta, are the first Chimeric Antigen Receptor (CAR) T-cell therapies to receive regulatory approval both in the United States and in Europe. They are suggested as new face in cancer treatment and in particular in B-cell acute lymphoblastic leukaemia (ALL). Their promising results have encouraged numerous research groups and manufacturers to explore the potential of those therapies in the treatment of various cancer types, resulting into 317 clinical trials globally (based on a recent search on ClinicalTrials.gov (2018)). Today, in the UK CAR T cells are only available through clinical trial schemes (approximately 250 patients per trial), thus being produced and delivered at a small scale. However, their recent European Medicines Agency Approval will allow them to become available to a wider patient population (approximately 40,000 eligible patients by 2031 (Figure 1), based on research performed on the UK patient population), requiring, therefore significant scale up/out both in the manufacturing line as well as in the logistics/supply chain model. In this work we focus on the design of a modelling tool to assist the decision making in the design of the supply chain model of CAR T cell therapies. Expanding our previous work (Wang et al., 2018), we demonstrate the design of a Resource Technology Network (RTN) for the identification of the key steps/decisions in the CAR T supply chain model. Based on previous qualitative results (Papathanasiou, 2018), we present a comparison between three supply chain model structures and we introduce the concept of the "dynamic" supply chain model. The latter refers to a versatile supply chain network that is tailored to the varying therapy demand. Based on the demand profiles, the modelling tool decides on: (a) number and location of clinical sites, (b) number and location of manufacturing sites and (c) best means of transport for the therapy. Lastly, the model considers time restrictions related to product shelf life, as well as different business decisions (e.g. in-house versus outsourcing quality control).

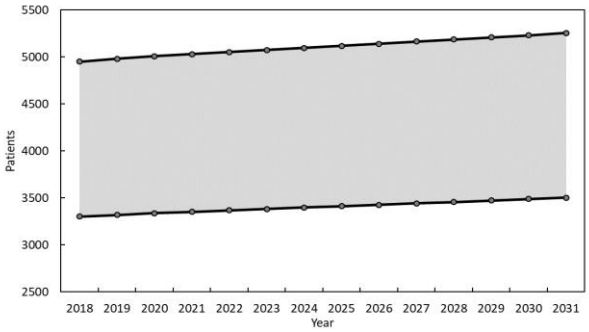

Figure 13 Forecast scenario of patient population eligible to receive CAR T cell therapies by 2031. Calculations are based on the assumption that only 10% of the patients with B-cell malignancies in the UK will be able to receive the therapy.

Acknowledgments
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ADVANCING THE ROBUST MANUFACTURE OF T-CELL THERAPIES THROUGH THE APPLICATION OF STIRRED TANK BIOREACTORS

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Key Words: CAR-T, TCR, Cell Culture, Bioreactor, Scale-up/Scale-out

A growing pool of efficacy has ballooned the landscape of engineered T-cell therapy clinical trials to over 90 active trials in the United States with many more in pre-clinical phases. This group of therapies consisting of Chimeric Antigen Receptor T-cells (CAR-T), T-cell Receptors (TCRs), Regulatory T-cells (T-regs), and Tumor Infiltrating Lymphocytes (TILs) could provide a breadth of solutions in the oncologic space and elsewhere, however a robust manufacturing process is required to realize these solutions in the clinic. A main hurdle in establishing a robust manufacturing process for these therapies is that a lengthy expansion period is typically necessary to reach a therapeutic dose of cells. The issues with current culture methods present in the forms of a high cost of goods, increased production timelines, and highly variant expansion rates.

At Hitachi Chemical Advanced Therapeutic Solutions, we have investigated stirred tank bioreactors as an alternative to the standard static and rocking motion culture methods using the ambr15 microbioreactor system. Stirred tank bioreactors pose a unique solution for the manufacture of both autologous and allogeneic T-cell therapies with high rates of mass and gas transfer and established process control methods. Given these advantages, a comparison study of T-cells expanded in the ambr15 microbioreactor system and batch fed static cultures resulted in a three-fold increase in total cell number with the bioreactors. Further development of stirred tank culture methods has demonstrated 64-fold expansion over an eight-day culture period. These outcomes indicate at a T-cell expansion method that could expand the potential of the revolutionary therapies through reduced media requirements, shortened culture periods, and high yields. By further utilizing established techniques in cell culture, T-cell therapies become increasingly viable treatments for a range of indications.

AUTOMATED FILTRATION SCREENING OF LENTIVIRAL VECTORS WITH MULTIPLE ENVELOPE PROTEINS

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Key Words: Lentiviral Vectors, Membrane Filtration, Automation, Gene Therapy, Cocal-G, VSV-G, RD-Pro

Lentiviral Vectors (LV) have been shown to successfully transfer therapeutic genes into dividing and non-dividing cells in laboratory and clinical environments for the benefit of cell and gene therapies. Current LV production features an initial clarification stage to remove cellular debris in addition to viral and serum protein aggregates prior to further downstream processing. Such filtration tasks have illustrated decreases in titer of vectors potentially via damage to external envelope proteins or the unwanted retention of particles [1]. LV production is generally characterized by its fragility and careful downstream processing design is required to ensure high recovery and purity of vectors. Evidence suggests that the selection of salt concentration and pH affects the aggregation propensity of proteins and the binding of vectors and contaminants to filters such as that seen with adeno-associated virus processing [2] whilst also negatively impacting the infectivity of the vector [3]. Such conditions need to be evaluated to ensure effective processing if vector development is to proceed to meet future demands.

A design of experiment definitive scree model was implemented in a Tecan liquid handling platform to rapidly screen various filters under different salt concentrations and pH ranges. Vectors containing the viral envelope proteins VSV-G, Cocal-G and RDPro was filtered across four membrane filter types. The vector transmission was measured by reverse transcriptase activity as a % of unfiltered product, and total protein transmission by Bradford assay. Data has shown vector and total protein transmission is not strongly affected by salt concentration, whereas pH 9 shows improved LV transmission across all envelopes and filters tested. RDPro enveloped LV report strongest filter transmission, whilst Cocal-G and VSV-G remain similar in efficiency. The highest reported LV transmission was found in filters with PVDF chemistry, whereas the best performer for protein removal was PES microwells. Positive correlation between LV and protein transmission was also seen. The work increases our understanding of how filtration affects initial clarification of vectors of differing envelope proteins harvested from cell culture and attempts to characterize the impact of salt concentration and pH value. In identifying the impact of such conditions on vectors, work can continue to improve LV processing, leading to ideal and scalable solutions to address demand for vectors in cell and gene therapies.

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PRODUCER CELL LINE ENGINEERING FOR LARGE VOLUME MANUFACTURING OF THERAPEUTIC AAV

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Key Words: rAAV, producer cell line

Recombinant adeno-associated viruses (rAAV) are among the most promising gene therapy delivery vectors for treating patients with genetic abnormalities. rAAV can safely deliver long-lasting expression of a therapeutic transgene to target cells. Multiple studies using rAAV have demonstrated sustained transgene expression in cultured cells and pre-clinical models, suggesting that rAAV could provide a cure for certain diseases. Moreover, bioengineering advancements have expanded the viral tropism beyond the constraints of naturally occurring AAV capsids, increasing the cell types that can be thought of as targets. Taken together, rAAV therapies have attractive qualities to safely address the needs of patients where other modalities may fall short.

One challenge with therapeutic rAAV is the ability to generate enough virus for clinical trials and commercial supply. This challenge is particularly true with neuromuscular or hemophilia patients in which doses can exceed 1×10^{14} viral genomes per patient. Typical yields from a rAAV production are around 10^4 viral genomes per cell, meaning batch cell numbers would need to exceed 10^{10} for a single dose. These doses require a robust, scalable platform to generate quantities of rAAV to meet patient demand. Biogen has selected the producer cell line (PCL) platform to meet the large demand for therapeutic rAAV. A PCL is a stable cell line engineered to contain the ITR flanked transgene of interest and AAV sequences needed to produce rAAV upon addition of helper virus. We will present our rationale for selecting the PCL platform as a cost-effective manufacturing strategy for gene therapy programs as well as current technical improvements and our vision for the next generation PCL platform.

VOLUME REDUCTION, CELL WASHING AND AFFINITY CELL SELECTION USING MULTI-DIMENSIONAL ACOUSTIC STANDING WAVE TECHNOLOGY

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Key Words: Cell wash, Cell concentration, Affinity Cell Selection, Acoustic Processing, Low shear

Acoustic Cell Processing is a unique acousto-fluidics platform technology for shear-free manipulation of cells using ultrasonic standing waves. The platform has broad applications in the field of cell and gene therapy, e.g., cell concentration and washing, cell culturing, microcarrier/cell separation, acoustic affinity cell selection and label-free cell selection. The acoustic radiation force exerted by the ultrasonic standing wave on the suspended cells in combination with fluid drag forces and gravitational forces is used to manipulate the cells and achieve a certain cell processing unit operation, e.g., separate, concentrate, or wash. The technology is single-use, continuous, and can be scaled up, down or out. It therefore allows for a flexible and modular approach that can be customized to process a desired cell count, cell culture volume or cell concentration within a given required process time. Utilizing its proprietary multi-dimensional standing wave platform, FloDesign Sonics (FD Sonics) has been developing two applications for cell and gene therapy manufacturing, an Acoustic Concentrate-Wash (ACW) and Acoustic Affinity Cell Selection (AACS) system for closed and shear free Cell and Gene Therapy manufacturing, namely CAR-T immunocellular therapies. The ACW technology has been applied to Jurkat T-cells and primary cultures of T-cells of 1-2 Liters (L) with cell concentrations ranging from 1 million cells per milliliter (ml) to 40 million cells per ml. The process flow rate varies from 2-3 L/hour with average cell recoveries of more than 80% in 60 to 90 minutes. The efficiency of the cell washing process ranges from 95-99% depletion of a model protein (BSA), depending on the wash methodology. The AACS technology is a scalable acoustic affinity cell selection method using acoustic (non-paramagnetic) affinity beads for positive or negative cell selection. A multi-dimensional acoustic standing wave is then used to separate the affinity bead-cell complexes from the unbound cells, thereby completing the process of a negative or positive cell selection. A population of 1 billion CAR-T cells containing 30% T-Cell Receptor positive (TCR+) and 70% T-cell Receptor Negative (TCR-) cells has been depleted of 99% of its TCR+ population. The TCR- cell recovery for this process was above 70% and the full process took less than 2 hours. When used for positive selection of CD3+ cells, AACS allowed for an enrichment of 2.5-fold in CD3+ population. ACW and AACS are powerful acoustic-based cell processing technologies that lower cost and risk while enabling a modular, automation-friendly manufacturing process for cell and gene therapy manufacturing.

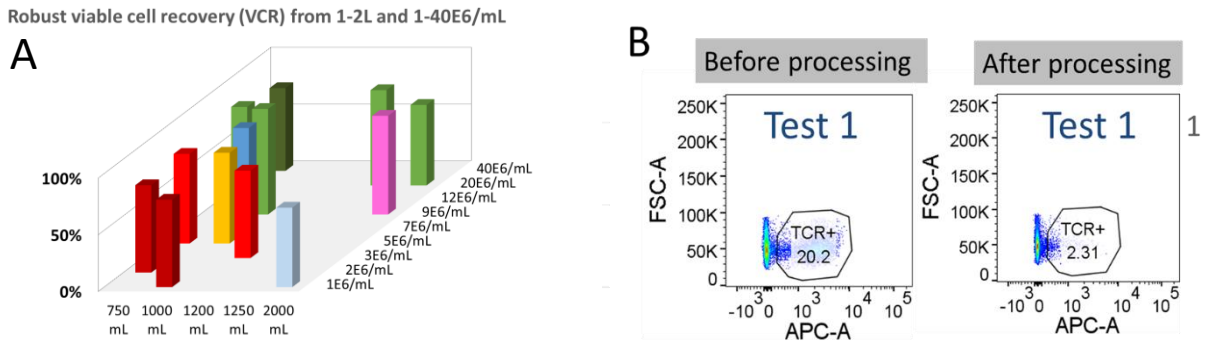

Figure 1 – Acoustic Concentrate Wash (ACW, A) and Acoustic Affinity Cell Selection performance (AACS, B). A depicts the cell recoveries obtained using ACW in the input space from 1-2L and 1-40 million cells per mL., using Jurkat T-cells; these results were confirmed with primary human T-cells. B shows a typical AACS TCR+ cell depletion result using one of FD Sonics proprietary acoustic beads, where 100 million primary human T-cells with 20% TCR+ were 10-fold depleted.