

INFLUENZA VACCINE PRODUCTION USING CELL CULTURE WITH MICROCARRIERS

Michael McGlothlen, EMD Millipore, USA
michael.mcglathlen@emdmillipore.com
Lori Mullen , Paul Hatch, EMD Millipore, USA

Key Words: Cell based flu, vaccine production, stir tank bioreactor, single use technologies.

Cell culture production processes are being developed as an alternative to the egg-based influenza vaccine production. Traditional egg-based production methods are slow and risk supply chain disruptions due to external influences, e.g., avian influenza outbreaks, which can leave the human population at risk. Cell culture methods have several advantages over the egg-based production, including robust production at large volumes addressing the risk of supply shortages and rapid response to local pandemic outbreaks. This presentation focuses on the development of an upstream MDCK adherent cell-based influenza A/WS/33 flu production process in 3 L **Mobius**® Single-Use stirred tank bioreactors. Initial development of the cell based process was performed in 250 mL baffled shake flasks to evaluate microcarrier concentration, inoculation densities and cell growth. The process was then optimized for cell growth and virus production by evaluating process control parameters such as cell attachment, dissolved oxygen and agitation in the 3 L **Mobius**® bioreactor system. The developed process improved cell densities from 1.5×10^6 to 3×10^6 cells/mL and virus titer were improved from less than 10,000 HAU/mL to greater than 30,000 HAU/mL.

EVALUATION AND SCALE-UP OF SINGLE-USE BIOREACTORS FOR THE PRODUCTION AND HARVESTING OF A HEPATITIS C VACCINE CANDIDATE

Alex Xenopoulos, EMD Millipore
Alex.Xenopoulos@EMDMillipore.com
Lénaïg Savary, Merck Millipore
Ricardo Silva, iBET
João Clemente, iBET
Rute Castro, iBET
Sofia B. Carvalho, iBET
Mafalda Moleirinho, iBET
Paula Alves, ITQB-UNL/iBET
Manuel Carrondo, FCT-UNL/iBET
Cristina Peixoto, ITQB-UNL/iBET

Key Words: Virus-like particles, Upstream Processing, Scale-up, Harvesting, Clarification, Depth Filtration

The present work examines the suitability of single-use bioreactors for production of a Hepatitis C Virus-Like Particle (VLP) vaccine candidate using the baculovirus expression system with Sf9 cells. It can be shown that a Mobius® 3L bioreactor results in viable cell concentration, viability, growth kinetics, stability and VLP production that are comparable to standard glass bioreactors. A simple translation of hydrodynamic working parameters between the two systems is adequate to match performance. Furthermore, we report on the successful scale-up of this disposable alternative from a 3L to a 50L scale using minimal optimization. These results demonstrate the potential and ease of use of this technology for the production of complex biopharmaceutical products. Using the 50 liters harvested from the run, we evaluated depth filtration and compared the results to centrifugation. Multiple filter trains with different properties were tested and the results on recovery, turbidity and impurity reduction will be presented and discussed.

COMPUTATIONAL FLUID DYNAMICS MODELING FOR HPV FERMENTATION BIOREACTORS

Tracie Spangler, Merck, USA
tracie_saunders@merck.com
Matthew Flamm, Merck, USA

Key Words: Computational Fluid Dynamics, Fermentation, Case Study in Process Development/Scale-up

The fermentation processes for the manufacture of HPV (GARDASIL AND GARDASIL 9) are currently conducted at multiple manufacturing scales. The goal of the bioreactor modeling project for HPV is to generate data and models to support robust manufacturing and process understanding initiatives across the multiple scales. Additionally, this knowledge can be utilized to aid in future process transfers. The modeling work is performed utilizing computer models (Computational Fluid Dynamics via Fluent) and historical data to predict metabolic behavior based on bioreactor configurations and processing conditions.

DEVELOPMENT OF A STABILIZED TRIMER PRE-FUSION RSV F RECOMBINANT VIRAL GLYCOPROTEIN VACCINE

Richard M. Schwartz, Vaccine Research Center, NIAID, NIH
Schwartzri@mail.nih.gov

Althaf I. Hussain, Vaccine Research Center, NIAID, NIH

Mingzhong Chen, Vaccine Research Center, NIAID, NIH

Pefieng Chen, Vaccine Research Center, NIAID, NIH

Mridul Ghosh, Vaccine Research Center, NIAID, NIH

Jonathan W. Cooper, Vaccine Research Center, NIAID, NIH

Sarah E. O'Connell, Vaccine Research Center, NIAID, NIH

Lisa A. Kuiltzo, Vaccine Research Center, NIAID, NIH

Key Words: RSV, trimer, pre-fusion.

It has been known that the RSV fusion protein F is a target vaccine protein to produce a protective immune response. The VRC has shown (Ngwuta, et.al.) through binding competition assays that the amount of pre-fusion site \emptyset -specific antibodies correlates with neutralizing (NT) activity, whereas the pre/post-fusion site II mAbs does not correlate with neutralization. Our results indicate that RSV NT activity in human sera is primarily derived from pre-F-specific antibodies, and therefore, inducing or boosting NT activity by vaccination will be facilitated by using pre-F antigens that preserve site \emptyset . Therefore, the instability of the RSV pre-fusion conformation has limited the potential of this as a vaccine antigen. Therefore, the VRC has designed a structurally stabilized glycoprotein pre-fusion RSV F trimer vaccine antigen and has shown it to be highly immunogenic in preclinical studies. A description of challenges in the development of a high productivity CHO cell line, production process and product quality and antigenic characterization assays for Phase I clinical material will be presented along with comparison of pre-clinical results of research to development material.

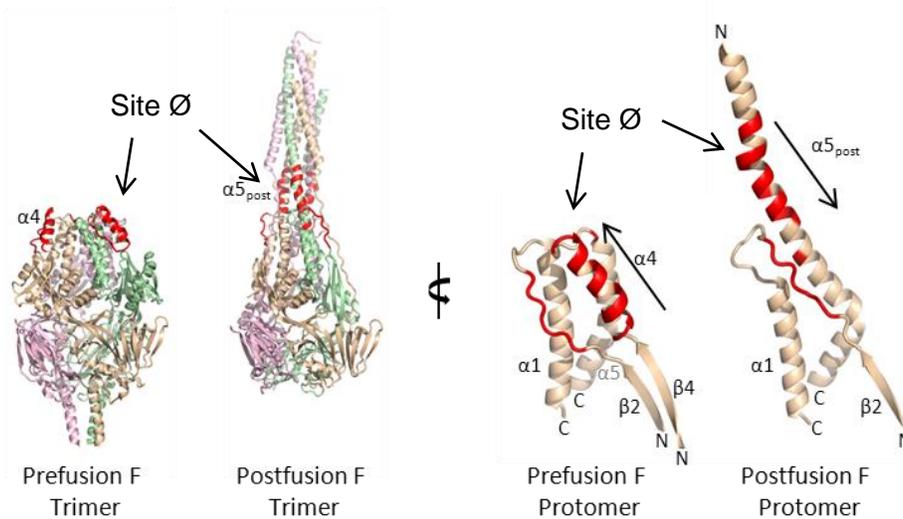


Figure 1. Antigenic Site \emptyset on pre-fusion and post-fusion RSV-F. Trimer on the left and monomer on the right. (McLellan et al. Science 2013).

OPTIMIZATION OF SULFATED CELLULOSE MEMBRANE ADSORBERS FOR THE PURIFICATION OF INFLUENZA VIRUS

A. Raquel Fortuna, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
fortuna@mpi-magdeburg.mpg.de

Sebastian van Teeffelen, Otto-von-Guericke University Magdeburg, Germany

Florian Taft, Sartorius Stedim Biotech GmbH, Germany

Louis Villain, Sartorius Stedim Biotech GmbH, Germany

Michael M. Wolff, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Key Words: influenza vaccine, downstream processing, membrane chromatography, sulfated cellulose

The impact of influenza virus worldwide drives significant efforts and resources into R&D of vaccine manufacturing processes. A major challenge is to improve the flexibility of these processes, without substantially compromising productivity. As in other biotechnological processes, implementation of a chromatographic capturing step is favored since it removes the majority of the impurities (host cell proteins and DNA) and concentrates the product before polishing. Over the last years, significant efforts towards the development of sulfated membrane adsorbers were made. An improved membrane structure and the direct sulfation of a cellulose matrix resulted in a membrane adsorber (SCMA) with pseudo-affinity characteristics which can be used in the main chromatographic separation step for influenza virus.

Pore size and ligand density are structural characteristics critical for the performance of the SCMA. For the best combination tested, the dynamic binding capacity (DBC) of the SCMA was shown to be 5.6×10^6 HAU/ml membrane, which corresponds to an approximately 5.5 times higher capacity than bead-based media. However, both SCMA and bead-based media show a similar performance in terms of product recovery (86%-96%) and contaminant removal.

Experimental results revealed the importance of operational parameters like virus concentration, flow rate, conductivity and elution salt concentration. Therefore, a DoE was used to determine the optimal process conditions in terms of product losses in the flow through as well as overall product yield and purity for an optimized SCMA using an influenza H1N1 virus strain. Currently ongoing experiments aim the identification of the best operating conditions, reevaluation of the DBC and purification performance of the optimized SCMA for the same virus strain. The robustness of the process for other virus (e.g. influenza H3N2 and B) is also being considered.

Combining the advantages of a membrane chromatographic support with a chemical modification that is shown to be specific for the purification of influenza virus is a significant technological advance. After optimization of the SCMA and the respective operating conditions significant improvements in the influenza vaccine production process are to be expected.

PURIFICATION OF CELL CULTURE-DERIVED INFLUENZA VIRUS VIA CONTINUOUS CHROMATOGRAPHY

Laura M. Fischer, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
fischerl@mpi-magdeburg.mpg.de

Sibel Ilhan, University of Stuttgart, Germany

Michael W. Wolff, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Key Words: Downstream processing, influenza vaccine, continuous chromatography, SMB.

In vaccine production downstream processing often constitutes a bottleneck in terms of process productivity and economy. One way to design more efficient purification trains could be the implementation of continuous chromatographic methods.

The aim of this study was the purification of cell culture-derived influenza virus using continuous chromatography. Therefore, two chromatographic modes, flow through with CaptoCore (CC) beads and bind and elute with anion exchange (AEX) monoliths, were characterized for their ability to separate the virus from contaminating host cell protein and DNA. The starting material for the CC was treated with nuclease to decrease the DNA content and fragment size. Further, regeneration conditions for the chromatographic media, a prerequisite for successful continuous implementation, were identified and verified in sequential batch experiments.

Simulated moving bed chromatography (SMB) was performed in an open loop configuration using constant switching times. In case of the CC material, two columns were located in the separation zones and two additional columns were regenerated and equilibrated in detached zones. For the AEX runs, on the other hand, monoliths were used in a three zone configuration with detached high salt zone for regeneration. Results in batch chromatography (BC) and SMB showed similar product yields in the range 60 to 100%. Contaminant depletion was >98% DNA and >58% protein for the AEX monoliths. Both the CC SMB and the BC resulted in comparable impurity levels (33.2 µg protein and 25.6 ng DNA per estimated 15 µg HA) but for BC a higher product yield (89% vs 72%) was achieved. In addition, the virus dilution during the flow through chromatography could be reduced in the cyclic steady state of the SMB by a factor of 1.8.

Overall, the separation performance of the BC has been successfully transferred to the continuous process.

OPTIMIZATION AND SCALE-UP OF CELL CULTURE AND PURIFICATION PROCESSES FOR PRODUCTION OF AN ADENOVIRUS-VECTORED TUBERCULOSIS VACCINE CANDIDATE

Chun Fang Shen, National Research Council of Canada
chunfang.shen@cnrc-nrc.gc.ca
Danielle Jacob, National Research Council of Canada
Tao Zhu, Tianjin CanSino Biotechnology Inc., China
Alice Bernier, National Research Council of Canada
Zhongqi Shao, Tianjin CanSino Biotechnology Inc., China
Xuefeng Yu, Tianjin CanSino Biotechnology Inc., China
Mehul Patel, National Research Council of Canada
Stephane Lanthier, National Research Council of Canada
Sven Ansorge, National Research Council of Canada
Amine Kamen, National Research Council of Canada & McGill University

Key Words: Adenovirus-vectored vaccine, tuberculosis vaccine, cell culture, process development, purification

Tuberculosis (TB) is the second leading cause of death by infectious disease worldwide. The only available TB vaccine is the Bacille Calmette-Guerin (BCG). However, parenterally administered *Mycobacterium bovis* BCG vaccine confers only limited immune protection from pulmonary tuberculosis in humans. There is a need for developing effective boosting vaccination strategies. AdAg85A, an adenoviral vector expressing the mycobacterial protein Ag85A, is a new tuberculosis vaccine candidate, and has shown promising results in pre-clinical studies and phase I trial. This adenovirus vectored vaccine is produced using HEK 293 cell culture.

Here we report on the optimization of cell culture conditions, scale-up of production and purification of the AdAg85A at different scales. Four commercial serum-free media were evaluated under various conditions for supporting the growth of HEK293 cell and production of AdAg85A. A culturing strategy was employed to take advantages of two culture media with respective strengths in supporting the cell growth and virus production, which enabled to maintain virus productivity at higher cell densities and resulted in more than two folds of increases in culture titer. The production of AdAg85A was successfully scaled up and validated at 60L bioreactor under the optimal conditions.

The AdAg85A generated from the 3L and 60L bioreactor runs was purified through several purification steps. More than 98% of total cellular proteins was removed, over 60% of viral particles was recovered after the purification process, and purity of AdAg85A was similar to that of the ATCC VR-1516 Ad5 standard. Vaccination of mice with the purified AdAg85A demonstrated a very good level of Ag85A-specific antibody responses. The optimized production and purification conditions were transferred to a GMP facility for manufacturing of AdAg85A for generation of clinical grade material to support clinical trials.

PAN-HA ANTIBODIES FOR INFLUENZA DETECTION AND QUANTIFICATION

Aziza P. Manceur, National Research Council Canada
aziza.manceur@nrc.ca
Anne Marcil, National Research Council Canada
Wei Zou, National Research Council Canada
Wangxue Chen, National Research Council Canada
Christine Gadoury, National Research Council Canada
Emma Petiot, Université Claude Bernard Lyon 1
Amine A. Kamen, McGill University
Xuguang (Sean) Li, Health Canada
Bozena Jaentschke, Health Canada
Sven Ansorge, National Research Council Canada

Key Words: Influenza, hemagglutinin, universal antibody, quantification, dot blot

The influenza virus imposes a heavy burden for society in terms of health and economy. Influenza is an elusive enveloped virus due to antigenic shift and drift of two surface proteins: neuraminidase (NA) and hemagglutinin (HA). As a result, new strains emerge every year which require seasonal vaccination for protection. Furthermore, large vaccine quantities are urgently needed in case of pandemics. Theoretically, vaccines against a new strain can be manufactured in as little as three weeks with certain platforms and technologies. However, vaccine quantification and release are still relying on the use of the Single Radial Immunodiffusion (SRID) assay using a strain-specific antibody to calculate HA concentration. This is a major limitation because it can take up to three months to generate the reagents necessary to run the SRID assay, including the strain-specific antibody. Hence, one of the major hurdles in the process of influenza vaccine production is the quantification of HA which is critical to establish proper dosing.

To circumvent the need for strain-specific antibodies, we have produced two monoclonal antibodies (F211-11H12-3 and F211-10A9-2) against a highly conserved peptide sequence found within the HA molecule (1). Multiple strains belonging to 13 different influenza A subtypes, as well as 6 strains belonging to B lineages were detected by Western blot and dot blot. Overall, mAb F211-11H12-3 recognizes preferentially influenza A subtype 1, while the mAb F211-10A9-2 has a higher affinity for influenza A subtype 2. Therefore, all strains tested could be detected when both mAb are combined and used as a cocktail. Next, we performed quantitative dot blots by generating a standard curve ranging from 160ng/ml to 20µg/ml HA. This method is simple, easy to implement and highly reproducible. In-process samples as well as purified material can be quantified by dot blot after denaturation with urea. Even though the SRID is the only assay approved by regulatory agencies, quantitative dot blots can be used during manufacturing to optimize and monitor the production process. Finally, ELISA is widely used for quantification and preliminary data demonstrates that samples can be quantified with the pan-HA mAbs.

In conclusion, a pan-HA antibody cocktail was generated against a highly conserved peptide sequence of influenza. Viruses produced in eggs and mammalian cells from 40 different strains were detected by Western blot. Reproducible quantification was achieved by dot blot using the two mAbs and an appropriate calibrating standard. The combination of pan-HA antibodies with an immunoassay such as the dot blot assay could accelerate process development and help establish new generation quantification methods for influenza. As the field is looking for flexible and versatile solutions to shift away from the SRID assay and strain-specific antibodies, the development of broad-spectrum antibodies offers a long-awaited alternative.

1) Chun et al, Universal antibodies and their applications to the quantitative determination of virtually all subtypes of the influenza A viral hemagglutinins, *Vaccine* (26), pp 6068-6076, 2008.

HIGH TITER PRODUCTION OF HIV-1 VIRUS-LIKE PARTICLES BY CAP-T CELLS

Sonia Gutiérrez-Granados, Departament d'Enginyeria Química, Biològica i Ambiental, Universitat Autònoma de Barcelona
sonia.gutierrez@uab.cat

Laura Cervera, Departament d'Enginyeria Química, Biològica i Ambiental, Universitat Autònoma de Barcelona
Mercedes Segura¹, Departament d'Enginyeria Química, Biològica i Ambiental, Universitat Autònoma de Barcelona

Jens Wölfel, CEVEC Pharmaceuticals GmbH

Francesc Gòdia, Departament d'Enginyeria Química, Biològica i Ambiental, Universitat Autònoma de Barcelona
¹Present address: Bluebird Bio Pharmaceutical Sciences, Process Development group

Key Words: Transient gene expression, HIV-1, virus-like particles, CAP-T, Design of experiments

Novel vaccine approaches are moving towards recombinant technology. Upon these, virus-like particles are promising candidates because they have been demonstrated to efficiently elicit both humoral and cellular immune responses. Moreover, they are non-infectious as they are formed basically by the structural viral proteins, mimicking the native virus but without containing the viral genome. CAP-T is a novel human cell line that has been previously demonstrated to be superior to other common cell lines in the production of recombinant proteins and viruses. They grow in suspension in serum-free, chemical defined media and they are easily transfectable with PEI, so they were evaluated for the production of HIV-1 virus-like particles by PEI-mediated transient transfection. Upon transfection of the HIV-1 Gag-GFP protein using the standard conditions, spherical particles with a size consistent with immature HIV virions (130 nm) were observed by TEM and NTA and supernatants containing 3×10^{10} VLPs/mL were harvested in batch culture 72 hours post-transfection. Several key steps of the production protocol were studied to establish the best transfection conditions both in terms of VLP yield and protocol simplicity. It was determined that for optimal production cells need to be growing at mid-exponential phase and can be transfected by independent addition of DNA and PEI with no prior complexation. Noticeably, a medium exchange step from PEM to FreeStyle is required before transfection since the former is not compatible with PEI-transfection while the latter is not compatible with high-density cell growth. A Box-Behnken experimental design was used to optimize cell density at time of transfection and DNA/cell and PEI/cell ratios. For optimal production, cells were transfected at a density of 3.3×10^6 cells/mL with 0.5 µg of DNA/cell and 3 µg of PEI/cell. Using the optimized protocol titers of 6×10^{10} VLP/mL were achieved, 20-fold higher than optimized production with HEK293 cells, making CAP-T a suitable and promising cell line for the production of HIV-1 VLPs and potentially other complex viral-based biotherapeutics. Scale-up of the optimized transfection protocol to 1L bioreactor was evaluated. The medium exchange required before transfection is trivial at Erlenmeyer scale, but becomes cumbersome when working with a bioreactor. For this very reason, transfection media development as well as optimizing transfection protocol are in progress.

CHARACTERIZATION OF HA AND NA-CONTAINING VLPS PRODUCED IN SUSPENSION CULTURES OF HEK 293 CELLS

Amine Kamen, McGill University, Canada
amine.kamen@mcgill.ca
Alina Venereo-Sanchez, McGill University, Canada
Rénald Gilbert, McGill University, Canada
Aziza Manceur, McGill University, Canada
Parminder Chahal, McGill University, Canada
Sven Ansorge, McGill University, Canada
Wangxue Chen, McGill University, Canada

Key Words: Influenza vaccine; Virus Like Particles (VLP); HEK-293 cells, Suspension culture; hemagglutinin, neuraminidase, Gag protein

Virus like particles (VLPs) can be formulated into promising vaccines to prevent influenza infection. In addition of having a structure and composition that mimic the wild type virus, VLPs are safe since they are devoid of viral genes and consequently are not infectious. One approach to scale up the manufacturing of VLPs is to produce them in a serum-free suspension culture using a stable mammalian cell line. Importantly, with VLPs synthesized by mammalian cells, the post-translational modifications of the surface antigens should be similar to the wild type virus, and therefore should trigger a potent and specific immune response for the pathogen. As a proof of concept, we first established a cell line that was stably expressing hemagglutinin (HA) and neuraminidase (NA) proteins of influenza (subtype H1N1) using our patented cGMP human embryonic kidney (HEK293) cell line (clone 293SF-3F6). Transcription of the genes for these two glycoproteins was regulated by the inducible cumate transcription gene-switch. Next, to establish our capability to produce VLPs, we compared the formation of VLPs using these cells after forced expression of two scaffold proteins: Gag from the human immunodeficiency virus and M1 protein from influenza A (H1N1). In addition, monitoring of the VLPs was facilitated by fusing the Gag protein to the green fluorescent protein (GFP). VLP production was therefore initiated by transient transfection of plasmid encoding Gag or M1 and by addition of cumate to the culture medium. The VLPs secreted in the culture medium were recovered by ultracentrifugation on a sucrose cushion. The presence of HA and NA within the VLP fraction was demonstrated by western blot and quantified by dot blot. Interestingly, VLPs were produced more efficiently in the presence of Gag, indicating that Gag is a better scaffolding protein than M1 in this context. Under the electron microscope, the Gag-VLPs appeared as vesicles of 100 to 150 nm of diameter, containing a denser internal proteinous ring, which is a typical morphology for VLPs produced through Gag expression. The production of Gag-VLPs was also validated in a 3-L stirred tank bioreactor in serum-free medium. The immunogenicity of the VLPs is currently under investigation in a murine model for influenza. In conclusion, VLPs containing HA and NA can be manufactured in serum free suspension culture of HEK293 cells through forced expression of Gag. The efficacy of these VLPs for vaccination remains to be demonstrated.

NOVEL AVIAN DuckCeltTM-T17 CELL LINE FOR PRODUCTION OF VIRAL VACCINES: APPLICATION TO INFLUENZA VIRUSES PRODUCTION

Emma Petiot , VirPath Laboratory EA4610, Université Claude Bernard Lyon 1, Lyon, France
emma.petiot@univ-lyon1.fr

Anais Proust , VirPath Laboratory EA4610, Université Claude Bernard Lyon 1, Lyon, France
Aurélien Traversier, VirPath Laboratory EA4610, Université Claude Bernard Lyon 1, Lyon, France
Manuel Rosa-Calatrava, VirPath Laboratory EA4610, Université Claude Bernard Lyon 1, Lyon, France
Jean-Marc Balloul, Research & Development Department, Transgene, 400 Boulevard Gonthier d'Andernach -
Parc d'Innovation - CS8016667405 Illkirch Graffenstaden Cedex – France

Key Words: Influenza vaccine, Viral production processes, Avian cells, Process optimization.

For the last 15 years, the viral vaccine manufacturing sector is looking for new producer cell lines, easily scalable, highly permissive to various viruses, and more effective in term of viral productivity. One critical characteristic for such cell lines is their ability to grow in suspension in serum free conditions at high cell densities. Regarding the pathogens under focus, influenza virus causing severe epidemics both in human and veterinary field is an important threat for world healthcare. The manufacturing sector is still demanding effective production processes to replace/supplement embryonated egg-based process and to provide efficient response to such threats. Cell-based production, with a focus on avian cell lines, is one of the promising solutions. Indeed, three avian cell lines ; namely duck EB66®cells (Vivalis), duck AGE.CR® cells (Probiogen) and quail QOR/2E11 cells (Baxter), are now competing with traditional mammalian cell platforms used for influenza vaccine productions (Vero and MDCK cells) and are currently at advance stage of commercial development for the manufacture of vaccine and biologicals [1].

The DuckCeltTM-T17 derived line presented here is a novel avian cell line developed by Transgene SA[2]. To generate immortalized duck cell lines, Transgene has used its proprietary DuckCelT technology which consisted in constitutively expressing the duck telomerase reverse transcriptase (dTERT) in primary embryo duck cells from spf eggs.

DuckCeltTM-T17 cells were able to grow in batch suspension cultures and serum-free conditions up to 7×10^6 cell/ml and such growth was easily scalable in bioreactors up to 3L. Permissivity for different viruses including influenza has been evaluated. In the present study, DuckCeltTM-T17 cell line was tested for its abilities to produce various influenza strains from different origins; human, avian and porcine. All strains were satisfyingly produced with titres higher than 5.8 log TCID₅₀/ml. H1N1 human strains and H5N2 and H7N1 avian strains were the most efficiently produced with highest titres reached of 8 log TCID₅₀/ml. Porcine strains were also greatly rescued with titres of 4 to 7 log TCID₅₀/ml depending of the subtypes. Interestingly, maximal titres are reached at 24h post-infection, allowing to have early harvest time. Process optimization on H1N1 2009 Human Pandemic strain allowed to identify best operating conditions for production (MOI, trypsin concentration, medium and density at infection) allowing to improve the production level by 2 log.

1. Meyer H-P, Schmidhalter DR: Industrial Scale Suspension Culture of Living Cells. 2014.
2. Balloul Jean-Marc, Duck cell line dedicated to the production of virus-based vaccines and therapeutic products BioProduction Optimization Workshop, September 22 & 23 2010 Frankfurt Germany

PSEUDO-AFFINITY PURIFICATION AND FORMULATION OF A CELL-CULTURE DERIVED WHOLE INFLUENZA VIRUS VACCINE USING MAGNETIC SULFATED CELLULOSE PARTICLES

Michael M. Pieler, Max Planck Institute for Dynamics of Complex Technical Systems, Germany,
pieler@mpi-magdeburg.mpg.de

Sarah Frentzel, Otto von Guericke University, Germany

Dunja Bruder, Otto von Guericke University, Magdeburg & Helmholtz Centre for Infection Research, Germany

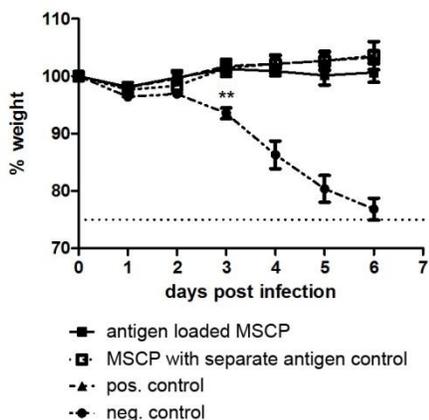
Michael W. Wolff, Max Planck Institute for Dynamics of Complex Technical Systems & Otto von Guericke University, Germany

Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems & Otto von Guericke University, Germany

Key Words: virus, vaccines, gene therapy, purification, high throughput screening (HTS).

The production of viral vaccines usually employs different unit operations where formulation and filling are the final steps of downstream processing (DSP). However, complex DSP is often hard to realize in research laboratories focusing on novel vaccine candidates. Moreover, there are no real ready-to-use tools for high-throughput DSP of whole virus particles that can speed up development. Because of these needs we developed a new platform for easy and straightforward whole virus particle purification and formulation based on magnetic sulfated cellulose particles (MSCP)^{1,2}.

Proof of concept was carried out with an influenza A/Puerto Rico/8/34 (H1N1) whole virus vaccine for the immunization of mice. The virus particles were produced in suspension MDCK cells, clarified, inactivated, and concentrated using a standard protocol. After diafiltration to low salt buffer, the virus particles were bound to the MSCP and the virus loaded MSCP were washed and resuspended in formulation buffer.



The immunization experiment included four groups: immunization with antigen-loaded MSCP, MSCP with separate antigen control, positive control, and negative control. The injection scheme involved a first injection followed by a booster injection. After immunization, the mice were challenged with a lethal virus dose.

The results obtained showed similar high anti-influenza antibody titers in mice immunized with antigen-loaded MSCP and antigen-containing controls. All three groups did not show any weight loss after the challenge. The untreated mice showed no antibody titers and a significant weight loss after challenge (Figure 1). Additionally, the mice's lungs of the negative controls showed a 400-fold increase of influenza nucleoprotein-gene copies, indicating high virus load, when compared to mice immunized with antigen-loaded MSCP.

Figure 1 – Relative body weight loss after lethal virus challenge

In summary, the use of MSCP for purification and formulation of influenza vaccines proved to be practicable and showed excellent protection after a lethal virus challenge. Besides, such a process has the potential to be implemented directly after virus production to realize a single step purification and formulation DSP². Because of these advantages possible applications range from studies in research and development to manufacturing of veterinary vaccines. In addition, optimized MSCP systems could be of interest for future applications in the medical field including vaccine delivery and gene therapy.

- (1) Wolff, M.; Pieler, M. M.; Reichl, U. Process for the preparation of magnetic sulfated cellulose particles, magnetic sulfated cellulose particles and its use. EP14175925.
- (2) Serve, A.; Pieler, M. M.; Benndorf, D.; Rapp, E.; Wolff, M. W.; Reichl, U. *Anal. Chem.* 2015.

TRUMENBA: A CASE STUDY FOR DEVELOPMENT OF A DRUG SUBSTANCE MANUFACTURING PROCESS THROUGH COMMERCIALIZATION

Khurram Sunasara, Pfizer, BioProcess R&D, 700 Chesterfield Parkway, USA
khurram.sunasara@pfizer.com

Key words: TRUMENBA, vaccine, process development, process characterization, breakthrough designation

The phrase "Process equals Product" is often applied to biologicals such as multicomponent vaccines implying that because they are composed of complex and often not well characterized entities, the process needs to be defined and locked early in the development process to ensure consistent quality of the vaccine all the way through scale-up and commercialization. In this presentation, we propose an alternative view of this phrase to mean that the product and process is well characterized to ensure consistent target molecule through development and licensure. This is highlighted with the TRUMENBA® case study, which is composed of a complex dual antigen membrane protein vaccine. For the journey to commercialization, the operating model used to manage this highly accelerated program led to a framework that ensured right first time execution and proactive monitoring of the process. This enabled quick issue identification and proactive resolution, resulting in a robust control strategy for the commercial process.

A STABILIZED SUBUNIT VACCINE FOR EBOLA VIRUS

Keith Chappell, School of Chemistry and Molecular Bioscience, University of Queensland, Australia
k.chappell@uq.edu.au

Daniel Watterson, School of Chemistry and Molecular Bioscience, University of Queensland, Australia
David Muller, Australian Institute for Biotechnology and Nanotechnology, University of Queensland, Australia
Alexandra Depelsenaire, Australian Institute for Biotechnology and Nanotechnology, University of Queensland,
Australia

Andrew Young, School of Chemistry and Molecular Bioscience, University of Queensland, Australia
Glenn Marsh, Australian Animal Health Laboratory, CSIRO, Australia

Mark Kendal, Australian Institute for Biotechnology and Nanotechnology, University of Queensland, Australia
Paul Young, School of Chemistry and Molecular Bioscience, University of Queensland, Australia

Key Words: Ebola, glycoprotein, subunit, vaccine.

The ongoing Ebola epidemic in West Africa has claimed over eleven thousand lives and has highlighted our unpreparedness to counter emerging viral epidemics. While two recombinant vaccines have shown promising results in clinical trials, we have developed an alternate subunit vaccine candidate that could be called upon in the event that problems are encountered with regard to safety or protection efficacy. Our subunit vaccine candidate is based on a soluble version of the recombinant Ebola glycoprotein (GP) stabilized in its pre-fusion conformation. This protein is recognized by the neutralizing monoclonal antibody KZ52 and all three ZMapp antibodies (currently employed as a therapeutic for clinical treatment), indicating both GP1/2 and glycan cap domains are available and are presented in the desired conformation. Immunization via Nanopatch™ (NP) microneedle delivery and intradermal injection were compared in C57 black mice. We assessed the antibody response elicited in immunized mice against Ebola virus (Zaire strain) using facilities at CSIRO's Australian Animal Health Laboratories in Geelong (AAHL). Promising plaque reduction neutralization titers (PRNT₅₀ = 1/80 sera dilution) were demonstrated. Furthermore, we have shown this vaccine is thermostable, retaining significant antigenicity after extended incubation at 37°C, indicating this vaccine strategy may not require cold chain delivery. In addition, the absence of any replicative elements ensures that it is likely to have a safer profile than live recombinant vaccines.

IMPROVED SEED TRAIN STRATEGY APPLIED TO PER.C6[®] CELLS FOR MANUFACTURING READINESS IN VACCINES PRODUCTION

Piergiuseppe Nestola, Janssen Vaccines AG, Bern, Switzerland
pnestola@its.jnj.com

Sabine Kuratli, Janssen Vaccines AG, Bern, Switzerland
Claudio Thomasin, Janssen Vaccines AG, Bern, Switzerland.

Key Words: PER.C6[®] cells, seed train, Adeno vector.

The prevention of infectious diseases is a major goal for global healthcare and vaccines are still among the best preventive tools.

The recent Ebola virus outbreak has challenged the vaccine industry to boost the speed to market and process development activities for new vaccines. To be able to cope with infectious disease outbreaks, our company developed an intensified cell and virus culture process using PER.C6[®] cells and AdVac™ platform. In our intensified process, perfusion bioreactors are used to increase Adeno vector titers while minimizing capital investments. To further increase readiness and flexibility for manufacturability, we improved the current process by substituting the classical cell culture seed train with an innovative concept called LVHD (Large volume high density) where the seed train can be generated from a single bag of cells at high density reducing significantly the time needed for the final cell culture.

In the current study the LVHD expansion train in comparison to the classic roller bottle expansion was evaluated in regard to Cost of Goods, facility footprint and operational flexibility. Moreover, the Adeno vector drug substances obtained from the two processes were compared.

Finally, the robustness and consistency of our LVHD seed train will be shown by using Monte Carlo simulation approach.

HISTORY AND DEVELOPMENT OF A LIQUID FORMULATION FOR ADENOVIRAL VACCINES

Lara Babich, Janssen Infectious Diseases & Vaccines, Netherlands

lbabich@its.jnj.com

Els van Deventer, Janssen Infectious Diseases & Vaccines, The Netherlands

Janik Adriaansen, Janssen Infectious Diseases & Vaccines, The Netherlands

Key Words: Adenovirus, Formulation development, Liquid Formulation, Vaccine Stabilization

The road to developing a stable liquid formulation for a live virus is burdened with challenges requiring a tailored methodology while complying with the target product profile, especially regarding storage temperature. The approach described here yielded a stable Adenovirus-based vaccine stored in frozen state for early stage development, and an improved formulation that allows storage in the liquid state at 2-8°C for late stage development.

In short, formulation components were initially screened and selected according to their stabilizing potential. Subsequently, according to Quality by Design (QbD) principles, a Design of Experiments (DOE) screening was used to define the specifications for each component of the formulation (excipients, pH, API). The resulting early stage formulation was implemented in CTM manufacturing of Adenoviral vaccines with storage and distribution at $\leq -65^{\circ}\text{C}$. This formulation guarantees stability during storage and at accelerated conditions and allows great flexibility outside the normal temperature range of a standard clinical trial.

For late stage development of a vaccine a storage temperature above zero is highly recommended. A more unorthodox methodology was applied to develop the first formulation for Adenoviruses that allows storage in the liquid state. With this formulation it is now possible to stabilize the Adenovirus-based vaccines for at least 2 years at 2-8°C. Results are so encouraging that this new formulation will be implemented in CTM manufacturing. This means a significant step in developing temperature-stable and affordable vaccines that can now be stored in the vast quantities required to prevent and eradicate infectious diseases across the world.

TETRASPANINS DISPLAYED IN RETROVIRUS-DERIVED VIRUS-LIKE PARTICLES AND THEIR IMPACT IN VACCINE DEVELOPMENT

Hugo Soares, iBET - 1-Instituto de Biologia Experimental e Tecnológica, 2-Instituto de Tecnologia Química e Biológica, UNL, Portugal.

hsoares@itqb.unl.pt

Patricia Gomes-Alves, iBET - 1-Instituto de Biologia Experimental e Tecnológica, 2-Instituto de Tecnologia Química e Biológica, UNL, Portugal.

Paula Alves, iBET - 1-Instituto de Biologia Experimental e Tecnológica, 2-Instituto de Tecnologia Química e Biológica, UNL, Portugal.

Manuel Carrondo, iBET - Instituto de Biologia Experimental e Tecnológica, Portugal ; Faculdade de Ciências e Tecnologia/Universidade Nova de Lisboa, Portugal

Ana Sofia Coroadinha, iBET - 1-Instituto de Biologia Experimental e Tecnológica, 2-Instituto de Tecnologia Química e Biológica, UNL, Portugal.

Key-words: Virus-like particles, Tetraspanins, Hepatitis C virus, Host-derived proteins, Retrovirus

Virus-like particles (VLPs) are a particular subset of subunit vaccines which are currently explored as safer alternatives to live attenuated or inactivated vaccines. Retroviruses have been widely explored as vectors for gene therapy and as scaffolds for vaccine candidates. One of retrovirus-like particles (retroVLPs) most attractive characteristic is their ability to incorporate heterologous envelope proteins, known as pseudotyping, as a mean to manipulate tropism or to present foreign antigens. As pseudotyping is a non-selective process, host cellular proteins are also included in retroVLPs membrane [1]. Many studies have addressed the identity of these host-proteins for particle characterization nevertheless, the contribution of host-proteins in retrovirus immunogenicity remains unclear. Moreover, patients infected with HIV and HCV are known to develop autoantibodies targeting self-proteins. As the origin of these autoantibodies is still unclear, the adventitious nature of host-derived proteins present in retroVLPs cannot be discarded.

Tetraspanins, in particular CD81, are amongst the most abundant host-proteins present in retrovirus membrane. Here we analyzed (i) the immunogenicity of tetraspanins in retroVLPs produced in xenogeneic cells (ii) the influence of CD81 in the diversity of host-proteins incorporated in retroVLPs and (iii) the impact of CD81-depletion in developing a retroVLPs-based HCV vaccine candidate.

Our results suggest tetraspanins are major immunogens present in retroVLPs. We show that CD81 is highly incorporated in retroVLPs produced in HEK293 cells inducing strong antibody and T-cell immune response in mice. Our results also show an increased diversity of tetraspanins in retroVLPs after CD81 depletion from producer cells with preservation of the overall immunogenic profile of retrovirus particles [2]. We further explore the impact of CD81 depletion on the development of a retroVLPs-based HCV vaccine candidate and we observe that HCV E2 incorporation on retroVLPs is directly affected by CD81 expression, thus diminished in CD81-negative retroVLPs.

These results highlight the dynamic and non-innocuous nature of host-derived proteins present in retroVLPs membrane. We consider this dynamic nature and its impact in product quality as an additional feature to be considered when developing retrovirus-based biopharmaceuticals.

References:

[1] Segura, M.M., et al., (2008) *J Virol.* 82(3): p. 1107-17

[2] H. R. Soares, *et al.* (2016) *Vaccine.* (in press)

Acknowledgements:

The authors acknowledge the financial support received from Fundação para a Ciência e Tecnologia, Portugal (PTDC/EBB-BIO/118621/2010 and EXPL/BBB-BIO/1129/2013). H.R. Soares, and P. Gomes-Alves acknowledge FCT for the individual grants SFRH/BD/81598/2011 and SFRH/BPD/86513/2012 respectively.

A MODULAR APPROACH FOR EFFICIENT PRODUCTION OF MULTI-HA INFLUENZA VLP-BASED VACCINES

António Roldão, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal
aroldao@itqb.unl.pt

Daniela Sequeira, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

Ricardo Correia, iBET, Instituto de Biologia Experimental e Tecnológica, Portugal

Manuel JT Carrondo, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

Ana P Teixeira, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

Paula M Alves, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

Key words: Virus-like particles; baculovirus-expression vector system; universal influenza vaccine; stable insect cell line.

Safer and broadly protective vaccines are needed to cope with the continuous evolution of circulating influenza virus strains. Promising approaches based on the expression of multiple hemagglutinins (HA) (alone or in combination with neuraminidase and *matrix M1* proteins), in a single vector or virus-like particle (VLP) have been proposed. However, expression of multiple genes in the same vector can be an issue due to tandem repetition of promoter sequences leading to its instability. By combining stable with transient expression we can rationally distribute the number of genes to be expressed by each system and thus mitigate this risk. Therefore, we developed a modular system using stable and baculovirus-mediated expression of HA in insect High Five cells for production of multi-HA influenza enveloped VLPs.

First, a stable pool of High Five cells expressing two HA was established by random integration and intracellular HA expression confirmed by immunofluorescence microscopy. This cell pool was then infected at CCI of 2 or 3×10^6 cells/mL with M1-encoding baculovirus to evaluate the incorporation of stable expressed HA in the M1 core, thus generating Influenza VLPs. Similar levels of Influenza VLPs could be detected in culture medium by hemagglutination assay regardless of the CCI used. Aiming to increase HA production, infections at a higher CCI were attempted by implementing a feeding strategy designed based on the exhaustion of key nutrients, analyzed by $^1\text{H-NMR}$ spectroscopy. Noteworthy, the shake flask cultures that were supplemented and infected at a CCI of 4×10^6 cells/mL showed a 8-fold increase in HA levels when compared to above tested conditions. The robustness of our modular system was then challenged by infecting the stable High Five cell pool with a baculovirus encoding M1 plus three HA proteins. Results obtained at CCI of 4×10^6 cells/mL with supplementation showed a 4-fold increase in HA levels when compared to standard infection conditions (CCI of 2 and 3×10^6 cells/mL). Finally, to demonstrate the scalability of the strategy herein designed, cultures in fully controlled 2L stirred tank bioreactors were performed, and a 1.5-fold improvement in HA levels was obtained when compared to shake flask cultures.

Overall, this work demonstrates the suitability of combining a stable insect cell line with baculovirus-mediated expression as a faster platform for production of multi-HA Influenza VLPs surpassing standard methods such as coinfections or the use of larger, unstable vectors.

Acknowledgements

This work was supported by EU-funded project EDUFLUVAC (FP7-HEALTH-2013-INNOVATION).

IMPROVING DOWNSTREAM PROCESSING OF ENVELOPED VIRUS-LIKE PARTICLES WITH MULTI-COLUMN CHROMATOGRAPHY

Ricardo Silva, iBET
rsilva@ibet.pt

Sofia B. Carvalho, iBET
Alex Xenopoulos, EMD Millipore
Paula Alves, ITQB-UNL/iBET
Manuel Carrondo, FCT-UNL/iBET
Cristina Peixoto, ITQB-UNL/iBET

Key Words: Multi-column chromatography, Virus-like particles, Downstream Processing.

The interest in continuous downstream purification processes is rapidly growing as industry pursues the establishment of continuous manufacturing. Continuous multi-column chromatography is therefore looked as an enabling technology, capable of improving purification yields whilst improving product quality and lowering costs.

We report on the development and comparison of two types of multi-column chromatographic systems aimed at the purification of enveloped VLPs, produced using insect cell-based expression with recombinant baculovirus. By subjecting an array of chromatographic devices to a temporal sequence of operations steps, suchlike column equilibration, product application, production and regeneration, one is able to overcome the limits of dynamic binding capacity characteristic of single-column batch processes. This will enable the increase of volumetric productivity, column capacity utilization and subsequently a decrease on processing costs.

The first process described herein is based on direct product capture using an anion exchange chromatographic media and subsequent elution with the modulation of ionic strength. The second process reported is based on negative chromatographic purification. In this approach, elution conditions are such that impurities should adsorb on the chromatographic media whereas the product of interest flows through the column.

The proposed strategies will be compared in terms of their volumetric productivity, resin capacity utilization, equipment footprint and skid complexity. We will also demonstrate that the optimal design is not only a balance between the manufacturing scale, complexity and imposed product quality requirements, but depends also upon factors such as media capacity for the product and related impurities, operational flow-rates, and mechanical limitations of the systems used.

A CLICK CHEMISTRY STRATEGY TO SPECIFICALLY MONITOR AND IMPROVE PURIFICATION OF INFLUENZA VIRUS-LIKE PARTICLES

Sofia Carvalho, iBET/ITQB-UNL, Portugal
sofiacarvalho@itqb.unl.pt
João Freire, IMM, Portugal
Mafalda Moleirinho, iBET, Portugal
Francisca Monteiro, iBET/ITQB-UNL, Portugal
Diana Gaspar, IMM, Portugal
Miguel Castanho, IMM, Portugal
Paula Alves, iBET/ITQB-UNL, Portugal
Gonçalo Bernardes, IMM, Portugal; University of Cambridge, United Kingdom
Cristina Peixoto, iBET/ITQB-UNL, Portugal
Manuel Carrondo, iBET/ITQB-UNL/FCT-UNL, Portugal

Key Words: Click-chemistry, Influenza VLP fluorescent tag, on-line/at-line fluorescent monitoring, Aha labeling, FACS virus detection.

Virus-like particles (VLPs) constitute a promising platform in vaccine development and targeted drug-delivery. However, most applications use simple, non-enveloped VLPs that present less technical challenges, not only to produce and purify, but also in terms of characterization, compared to enveloped VLPs. Recent advances in upstream processing, new product quality requirements and other regulatory issues, as well as the search for more cost-effective processes, led to the need to develop more efficient downstream processes for biopharmaceuticals.^[1] In that sense, new monitoring and product characterization methods, which can be applied at all stages of downstream processing, are needed.

Here is reported a valuable platform for the downstream processing and monitoring of the *in vivo* production of site-specifically functionalized enveloped Influenza VLPs. This strategy involves a two-step procedure that consists of residue-specific replacement of methionine by an analog (azidohomolanine) that enabled for post-expression functionalization with a fluorophore.^[2]

Importantly, this platform does not impact VLP production or purification processes, and allows functionalization without deleterious effect on hemagglutinin biological function. As a proof of concept a complete downstream processing was performed, including clarification, capture and polishing steps. A flow cytometry analysis (FACS) step was added to achieve a refined discrimination and separation between VLPs and baculovirus - the major impurity of the process.^[3] This was further confirmed using atomic force microscopy (AFM). This tool allowed to accurately monitor our product, achieve higher product recovery yields and higher impurity removal levels. The versatile system presented here is broadly applicable to the production of functionalized enveloped VLPs, for vaccine design, targeted drug delivery and molecular imaging.

[1] CRAMER et al., *Curr Opin Chem Eng*, 1, 27-37, 2011.

[2] BANERJEE et al., *J. Virol.*, 85, 7546-7554, 2011.

[3] VAN DER VLIST et al., *Nature Protoc*, 7.7, 1311-1326, 2012.

ENVELOPED VIRUS-LIKE PARTICLES PURIFICATION USING AN ALL-FILTRATION TECHNOLOGY PLATFORM

Sofia Carvalho, iBET/ITQB-UNL, Portugal
sofiacarvalho@itqb.unl.pt
Ricardo Silva, iBET/ITQB-UNL, Portugal
Bárbara Cunha, iBET/ITQB-UNL, Portugal
Ana Sofia Moreira, iBET/ITQB-UNL, Portugal
Mafalda Moleirinho, iBET/ITQB-UNL, Portugal
Alex Xenopoulos, EMD Millipore, United States of America
Paula Alves, iBET/ITQB-UNL, Portugal
Cristina Peixoto, iBET/ITQB-UNL, Portugal
Manuel Carrondo, iBET/ITQB-UNL/FCT-UNL, Portugal

Key Words: VLPs, membrane purification, platform process, downstream processing

Virus-like particles (VLPs) have become widely used as vaccine candidates because of their versatility, immunogenicity and safety profile. The diversity of surface epitopes contributes, however, to a variability in downstream purification that could ultimately affect manufacturability. For baculovirus expression systems in particular, the similarity between residual baculovirus and VLP particles causes significant problems. For that purpose, we have undertaken an effort to develop platform processes for purification of VLPs. Our initial approach focus on size exclusion as the key mechanism of separation, with the ultimate goal of an all filtration purification process, inserted in the “anything but chromatography” concept. The first step was to evaluate a legacy purification that was not robust or efficient and replace the ion exchange chromatography step with size exclusion chromatography (SEC). Performance of the SEC step will be described and the shortcomings of such a method for a scaled up, GMP process will be discussed.

The proposed all-filtration process employs either normal or tangential flow filtration for the clarification stage, followed by a cascade of ultrafiltration steps with different pore sizes and a sterile filtration step to achieve the needed concentration and purity specifications. Efforts to clear nucleic acid without the use of an endonuclease digestion step and the impact on the downstream unitary operations will also be described. By optimizing the filtration mode of operation we were able to achieve product recoveries above 85%. Globally, we have about 90% of DNA and total protein removal and a baculovirus’ log reduction value of 6. Using this all-filtration platform we are able to speed up the process, to improve the scale-up and to reduce costs due to the removal of chromatographic steps.

To show the potential for a universal, platform process, two cell systems producing two different VLPs were studied and preliminary results will be presented.

THE PAPAYA MOSAIC VIRUS (PAPMV) NANOPARTICLES; A PROMISING TOOL IN VACCINE DEVELOPMENT.

Denis Leclerc, Infectious Diseases Research Center, Laval University, Canada
denis.leclerc@crchul.ulaval.ca

Marilène Bolduc, Infectious Diseases Research Center, Laval University, Canada
Marie-Eve Laliberté, Infectious Diseases Research Center, Laval University, Canada
Alain Lamarre, Infectious Diseases Research Center, Laval University, Canada
Pierre Savard, Infectious Diseases Research Center, Laval University, Canada

Keywords: Nanoparticles, Virus Like Particles, TLR7 agonist, Vaccine platform

There is a major need for the development of new technologies that will facilitate the speed of development of vaccine and show a very high safety profile. In the last 10 years, we have developed a new toll like receptor agonist (TLR) that can trigger innate immunity followed by a strong adaptive immune response. This new agonist targets specifically the TLR7/8 in the endosome of the immune cells. It is made of the coat protein (CP) of a plant virus self-assembled around an RNA that forms flexible rod-shaped nanoparticles of 15x100nm. The highly repetitive and crystalline nature of the nanoparticles are attractive to immune cells leading to its internalization into the endosome where the nanoparticles are broken down by the harsh conditions of this compartment which liberate the RNA that trigger TLR7/8 to induce innate immunity. Therefore, we can use those nanoparticles as an adjuvant and improve the immune response to an antigen or as an immune modulator through the trigger of innate immunity that can induce protection to viral infection or improve the immune response to tumour. Finally, we have showed that we can engineer the nanoparticles into a vaccine platform through fusion of B or T cell epitope at its surface and elicits an efficient and protective immune response to the fused epitope. We will discuss the advantage of using this platform in vaccine development or cancer immunotherapy and show several examples where it has been shown to be efficient and promising.

NOVEL PULSATILE-RELEASE MICROPARTICLES FOR SINGLE-INJECTION VACCINATION

Kevin J. McHugh, Massachusetts Institute of Technology
kjmchugh@mit.edu

Thanh D. Nguyen, Massachusetts Institute of Technology

David Yang, Massachusetts Institute of Technology

Allison R. Linehan, Massachusetts Institute of Technology

Jennifer Lu, Massachusetts Institute of Technology

Stephany Y. Tzeng, Massachusetts Institute of Technology

Adam M. Behrens, Massachusetts Institute of Technology

Robert S. Langer, Massachusetts Institute of Technology

Ana Jaklenec, Massachusetts Institute of Technology

Key Words: Controlled release, vaccine delivery, pulsatile release kinetics, microparticles

Many controlled release devices are designed to achieve near zero-order release kinetics, however for some applications, such as vaccination, non-continuous or pulsatile release is desired. Such pulsatile release systems may enable the creation of single-injection vaccines that eliminate the need for subsequent booster immunizations by spontaneously releasing antigen at time points that correspond to normal vaccination regimens. This would be especially important in the developing world where a lack of consistent access to healthcare contributes to approximately 1.5 million vaccine-preventable deaths each year.¹ Here we present the fabrication and characterization of biodegradable core-shell microparticles that exhibit pulsatile release kinetics due to their unique structure. These particles are produced using a novel fabrication process that combines soft lithography, picoliter dispensing, optical alignment, and a gentle heat-based sintering step to generate microparticles with a biodegradable polymeric shell surrounding an antigen-filled core. By altering the composition (e.g. copolymer ratio or molecular weight) of the poly(lactic-co-glycolic acid) shell, particles can be tuned to release discrete pulses of a model antigen at times ranging from four days to two months. This fabrication method is also compatible with sensitive biologics, such as the inactivated polio virus, which retains >80% of its antigenicity after encapsulation. Further, because the shell of the particle is physically separated from the core, these particles can be filled with any aqueous vaccine solution without affecting release kinetics and be easily scaled via massively parallel fabrication. As a result, these particles have exciting potential as single-injection vaccines that fully mimic the antigen presentation profile of traditional bolus injections administered over the course of months or years.

¹World Health Organization. Global Immunization Data, 2014.

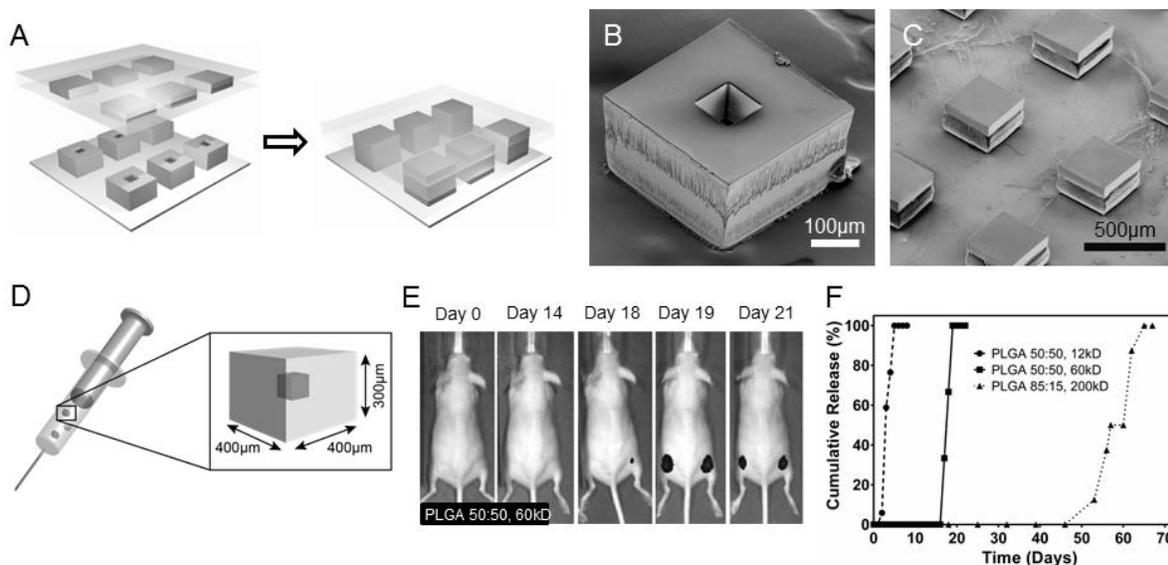


Figure 1 - Microfabricated particles exhibit delayed burst release kinetics. A) Diagram depicting particle sealing by heat and pressure-assisted sintering of PLGA bases and caps. B) One particle base prior to filling and sealing. C) Particle array after sealing. D) Diagram of multiple particles being injected with varying composition and therefore release timing. E) Representative *in vivo* release of fluorescently-labeled dextran from microfabricated particles. F) *In vivo* release kinetics ($n = 6-9$).

STRUCTURALLY CONFINED INFLUENZA SUBUNIT VACCINES IN THE PREFUSION CONFORMATION ELICIT A POTENT NEUTRALIZING ANTIBODY RESPONSE

Daniel Watterson, University of Queensland, Australia
d.watterson@uq.edu.au
Keith J Chappell, University of Queensland, Australia
Christopher McMillan, University of Queensland, Australia
Stacey Cheung, University of Queensland, Australia
Paul R Young, University of Queensland, Australia

Key Words: Influenza, vaccine, structure, epitope, neutralization, fusion, glycoprotein

Effective vaccination against influenza viruses remains a significant global challenge. Despite ongoing efforts, continual antigenic changes in circulating viruses requires constant update of existing vaccine approaches. Furthermore, the majority of current licensed vaccines are derivatives of live virus and are inherently time consuming to produce and limit the potential response time to counter a new virus strain. However, the combined advances in subunit vaccine production and structural determination of critical neutralizing epitopes within influenza hemagglutinin (HA) provide the groundwork for the next generation of influenza vaccines which have the potential to overcome these limitations. In an effort to expand on these findings we have compared the effectiveness of both prefusion and postfusion forms of recombinant influenza hemagglutinin (rHA) as subunit vaccines. Using a novel stabilization tag to confine rHA in the prefusion conformation we demonstrated that while both HA conformations elicit anti-HA responses in mice, a neutralizing response (PRNT₅₀ 1:36000) is only observed for prefusion rHA. Using rHAs from a range of influenza subtypes and domain specific constructs together with a large panel of structurally defined antibodies we also examined the epitope specificity and cross-reactivity of the prefusion specific neutralizing response. Interestingly, a similar conformation dependence has been reported for respiratory syncytial virus^{1, 2}, suggesting a universal strategy for the generation of potent subunit vaccines to target enveloped viruses.

1. Magro, M. et al. Neutralizing antibodies against the preactive form of respiratory syncytial virus fusion protein offer unique possibilities for clinical intervention. *Proc Natl Acad Sci U S A* 109, 3089-3094 (2012).
2. McLellan, J.S. et al. Structure-Based Design of a Fusion Glycoprotein Vaccine for Respiratory Syncytial Virus. *Science* 342, 592-598 (2013).

VACCINATION WITH RECOMBINANT NEURAMINIDASE PROTECTS AGAINST INFLUENZA VIRUS INFECTION IN MICE

Teddy John Wohlbold, Department of Microbiology, Icahn School of Medicine at Mount Sinai; Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, USA

teddyjohn.wohlbold@mssm.edu

Veronika Chromikova, Department of Microbiology, Icahn School of Medicine at Mount Sinai

Philip Meade, Department of Microbiology, Icahn School of Medicine at Mount Sinai; Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai

Fatima Amanat, Department of Microbiology, Icahn School of Medicine at Mount Sinai

Ariana Hirsh, Department of Microbiology, Icahn School of Medicine at Mount Sinai

Raffael Nachbagauer, Department of Microbiology, Icahn School of Medicine at Mount Sinai; Faculty of Life Sciences, University of Vienna, Austria

Gene S. Tan, Department of Microbiology, Icahn School of Medicine at Mount Sinai

Peter Palese, Department of Microbiology, Icahn School of Medicine at Mount Sinai; Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Florian Krammer, Department of Microbiology, Icahn School of Medicine at Mount Sinai

Key Words: influenza, neuraminidase, recombinant, vaccine, antibodies

While the efficacy of most influenza virus vaccines is measured by the ability to induce antibodies against the hemagglutinin (HA), antibodies against the viral neuraminidase (NA) are also correlated with less severe disease in humans and animal models. Yet, neither the amount nor the enzymatic activity of NA is standardized in current seasonal vaccines, and the breadth of NA-based protection is unknown. In the present study, different subtypes of recombinant NA were expressed in a baculovirus system and used to vaccinate mice prior to homologous, heterologous, or heterosubtypic virus challenge. Additionally, pre- and post-vaccination human serum samples from vaccinees that received TIV were studied to compare induction of antibodies against the HA and NA. Finally, the amounts of NA in 4 different vaccine formulations from 2013-2014 were quantified using ELISA. Mice immunized with N2 were 100% protected from morbidity and mortality in a homologous challenge and displayed significantly reduced viral lung titers. Heterologous challenge with a drifted strain resulted in morbidity but no mortality. Mice immunized with B/Yamagata/16/88 NA were 100% protected from morbidity and mortality when lethally challenged with a recent Victoria lineage strain. In our human cohorts, the increase in endpoint titers against N1 NA post-vaccination was less robust than that against HA and, as our quantification data suggests, the N1 NA amounts in seasonal vaccine formulations is quite variable. To confirm the broad protective effects of anti-influenza B NA antibodies on a monoclonal level, a panel of mouse monoclonal antibodies was generated against influenza B virus NA; several of these displayed broad reactivity in ELISA to whole virus and recombinant NA and protected against lethal influenza B virus challenge in mice when delivered at a dose of 5 mg/kg prophylactically, or therapeutically, 48 hours post-infection. Analysis of the protective epitopes is currently in progress. The demonstrated protective capacity of anti-NA antibodies suggests that targeting the NA through vaccination may offer increased protection against influenza virus infection.

RESIDUAL DNA ANALYSIS IN INFLUENZA VACCINE PROCESSING

Camilla Estmer Nilsson, GE Healthcare
camilla.estmernilsson@ge.com
Hanna Kraft, GE Healthcare
Christine Sund Lundström, GE Healthcare
Anna Mattson, GE Healthcare
Elisabeth Wallby, GE Healthcare

Key Words: Residual DNA, sample prep, qPCR, vaccine purity.

In cell-based influenza vaccine production, the European Pharmacopoeia demands a host cell residual DNA concentration of less than 10 ng per dose. To reliably measure residual DNA in both process samples and final vaccine using quantitative PCR, DNA preparation prior to analysis is a necessity. Samples from the vaccine purification process contain different buffers, salts, and cell-based compounds, and vary 3–4 logs in DNA concentration from harvest to the final product, which all put strain on the DNA preparation. For accurate determination of DNA concentration, recovery is of high importance. There are many commercially available DNA preparation kits that use different techniques to bind DNA, from spin columns with a DNA-binding membrane or medium (resin) to magnetic beads. However, these kits are mainly developed to purify DNA fragments from gel electrophoresis or genomic DNA from tissues such as blood or cultured cells, and do not have recovery as a priority. Few kits are intended for residual DNA determination in samples with high concentration of a protein or virus product. In this study, prototype media for DNA preparation, in bind-elute and batch mode, were evaluated for recovery, hands-on time, and throughput. In batch mode, recoveries of > 80% were achieved, but the technique exhibited matrix effects on real process samples. In bind-elute mode, recoveries of 40%–60% were achieved after elution. However, recovery could be improved by determination of DNA concentration, while keeping DNA bound to the medium.

STUDY OF RABIES VLPs EXPRESSION IN BHK-21 CELL LINE FOR VACCINE APPLICATIONS

Claudio Prieto, Biotechnological Development Laboratory ; Cell Culture Laboratory, Biochemistry and Biological Science School, UNIVERSIDAD NACIONAL DEL LITORAL, Argentina
cprieto@fbc.unl.edu.ar

Ernesto Garay, Biotechnological Development Laboratory ; Cell Culture Laboratory, Biochemistry and Biological Science School, UNIVERSIDAD NACIONAL DEL LITORAL, Argentina

Diego Fontana, Biotechnological Development Laboratory ; Cell Culture Laboratory, Biochemistry and Biological Science School, UNIVERSIDAD NACIONAL DEL LITORAL, Argentina

Marina Etcheverrigaray, Ricardo Kratje, Cell Culture Laboratory – Biochemistry and Biological Science School – UNIVERSIDAD NACIONAL DEL LITORAL, Argentina

Key words: Rabies, BHK-21, virus-like particles.

In the last decades, virus-like particles (VLPs) have played an essential role in the development of novel vaccines due the fact that they trigger robust and balanced immune responses and, as they lack viral genome, are biosafe. Nowadays, several VLPs are commercially available for human use and one veterinary product was licensed. Besides, other VLP-based vaccine candidates are in the stages of clinical trials or preclinical evaluation.

Our group had previously developed a rabies glycoprotein based-VLP (RV-VLPs) expressed in HEK293 cells. These RV-VLPs were fully characterized and their capacity to induce a protective response and neutralizing antibodies production was confirmed. As inactivated veterinary vaccines for rabies are usually produced using BHK-21, the goal of the present work was to develop a RV-VLPs expressing BHK-21 cell line to analyze the characteristics of the VLPs produced using this cell substrate.

Therefore, by lentivirus vector-mediated transduction, we generated a rabies virus glycoprotein expressing a stable cell line. The cellular expression of the recombinant protein was analyzed by flow cytometry and the membrane localization was confirmed by fluorescent microscopy. Later, RV-VLPs budding to the supernatant was analyzed by sandwich ELISA. After that, VLPs were purified by density gradient ultracentrifugation and the hydrodynamic diameter of the particles was analyzed by DLS. In a western blot assay, the particles were recognized by specific antibodies present in a rabies polyclonal serum. Finally, the recombinant cell line was cultured in 850 cm² roller bottles producing RV-VLPs continuously during 25 days of culture. Thus, these results encourage further studies to confirm if BHK-21 is a good cell substrate for the production of RV-VLPs as a veterinary rabies vaccine candidate.

EXPRESSION OF RABIES VLPs IN ADHERENCE AND SUSPENSION CONDITIONS: A FLEXIBLE PLATFORM FOR RABIES VACCINE PRODUCTION

Diego Fontana, Biotechnological Development Laboratory ; Cell Culture Laboratory, Biochemistry and Biological Sciences School, UNIVERSIDAD NACIONAL DEL LITORAL, Argentina

dfontana@fbc.unl.edu.ar

Ernesto Garay, Biotechnological Development Laboratory ; Cell Culture Laboratory, Biochemistry and Biological Sciences School, UNIVERSIDAD NACIONAL DEL LITORAL, Argentina

Claudio Priet, Biotechnological Development Laboratory ; Cell Culture Laboratory, Biochemistry and Biological Sciences School, UNIVERSIDAD NACIONAL DEL LITORAL, Argentina

Ricardo Kratje, Marina Etcheverrigaray, Cell Culture Laboratory, Biochemistry and Biological Sciences School, UNIVERSIDAD NACIONAL DEL LITORAL, Argentina

Key words: RABIES, VACCINE, MAMMALIAN CELLS

Rabies is a zoonotic viral disease with a mortality by close to 100%. As there is not an efficacious treatment available, post-exposure vaccination is recommended for individuals in contact with the virus. On the other hand, the most common source of virus transmission is saliva of infected animals, mostly dogs, whereby mass vaccination of pets is the most cost-effective way to reduce human infections. In this context, availability of both human and veterinary vaccines is critical.

In previous works¹⁻², our group developed immunogenic rabies VLPs, expressing the virus glycoprotein in HEK293 cells. We obtained a producer clone capable of growing in adherence (adhP2E5) and then adapted to suspension conditions (sP2E5). In this work, we analyzed the production of VLPs in both conditions, using two different platforms.

On the one hand, adhP2E5 was cultured in 850 cm² roller bottles (GBO) using medium with 5% FCS, that was exchanged every 48 h during the first 10 days and every 24 h during the last 5 days. RV-VLPs were continuously produced and the harvest obtained (2.5 L per bottle) was analyzed by sandwich ELISA, using the 6th International Standard for rabies vaccine that quantify the glycoprotein content (NIBSC), presenting a value of 19 IU.ml⁻¹ in average. On the other hand, we cultured sP2E5 in a 5 L bioreactor during 15 days, using EX-CELL293 SFM (SAFC). The culture reached densities of 2x10⁷ cel.ml⁻¹ and VLPs were continuously secreted to the supernatant. The obtained harvest (28.5 L) presented a glycoprotein content of 28 IU.ml⁻¹, a results that is comparable with the previous one taking into account the number of cells presents in both conditions.

These results showed that our clone could be cultured in both platforms depending on the objectives and characteristics of the desired product. For the production of the rabies veterinary vaccine, RV-VLPs can be produced in adherent conditions using medium supplemented with FCS and, for human vaccine production, RV-VLPs can be produced in bioreactors using SFM.

1 Fontana et al. Rabies virus-like particles expressed in HEK293 cells. *Vaccine* 32 (2014) 2799-2804.

2 Fontana et al. Immunogenic virus-like particles continuously expressed in mammalian cells as a veterinary rabies vaccine candidate. *Vaccine* 33 (2015) 4238-4246.

PROCESS ECONOMY EFFECTS OF MODERNIZATIONS IN VACCINE PURIFICATION

Mia Bennemo, GE Healthcare Life Sciences, Sweden
mia.bennemo@ge.com

Key Words: Process economy, Single-use technology, process optimization, core bead chromatography

The vaccine industry is currently modernizing old legacy processes. Old purification steps are being made obsolete by introducing modern purification techniques, aiming for increased quality and production efficiency at the same time as reducing cost. One approach to increase safety and productivity is to use closed single-use (SU) processing systems, preferably in combination with more modern separation principles.

Size exclusion chromatography (SEC) is a technique traditionally used for reduction of contaminant levels in vaccine processes and is suitable for purification of large molecules such as viruses. However, as the sample load in SEC is typically only 5% of the column volume, a built-in disadvantage is that very large columns will be required in production scale which is both challenging and expensive. Additionally, today such column size requirements are not compatible with a closed SU system approach.

For a more efficient size separation of biomolecules, a different type of bioprocessing chromatography media called core beads has been developed. The core bead technology allows for dual functionality combining size separation with bind/elute chromatography. Viruses and other large entities pass outside the beads while contaminants ($< M_r 700\ 000$) penetrate the inert outer shell and bind to the ligands in the inner core.

To investigate the impact on the process economy of shifting from SEC to single-use core bead chromatography, laboratory experiments were performed and a calculation tool for the total cost of ownership was developed. As model system influenza virus produced in MDCK cells were used, although this strategy could also be applied to other viruses. Small scale experiments showed that performance in terms of hemagglutinin yield and host cell protein removal with both approaches were comparable. In terms of process economy, core bead chromatography in a SU approach presented significantly higher productivity, smaller footprint and reduced WFI consumption than SEC in all investigated scenarios.

PROPAGATION OF INFLUENZA AND MVA VIRUS IN CASCADES OF CONTINUOUS STIRRED TANK BIOREACTORS: CHALLENGING THE “VON MAGNUS EFFECT”

Felipe Tapia, International Max Planck Research School for Advanced Methods in Process and Systems Engineering, Germany

tapia@mpi-magdeburg.mpg.de

Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Ingo Jordan, ProBioGen AG, Germany

Volker Sandig, ProBioGen AG, Germany

Udo Reichl, Chair of Bioprocess Engineering, Otto von Guericke University Magdeburg, Universitätsplatz 2; Max Planck Institute for Dynamics of Complex Technical Systems, Germany.

Key Words: two-stage, MVA, influenza, AGE1.CR.pIX, continuous, semi-continuous

Moving from batch to fully continuously operated upstream processes is one of the big challenges for the coming decades in cell culture-based viral vaccine manufacturing. Continuous processes are known to be more efficient than batch systems for production of large volumes of product, and can therefore be an interesting option for production of highly demanded viral vaccines. One example is the seasonal influenza virus that causes annual epidemics in human populations worldwide and is currently produced in batch processes. Another virus of clinical interest is Modified Vaccinia Ankara (MVA) virus which is a potential platform for recombinant vaccines and can be used as a vector in gene therapy [1]. Continuous propagation of MVA virus seems to be feasible using a new MVA virus strain that can propagate at high yields in non-aggregated avian suspension cells [2]. Because both influenza and MVA are lytic viruses a continuous production strategy was employed that involves cascades of two stirred tank bioreactors, where cell growth and virus propagation occur in separated vessels [3]. However, a possible drawback for continuous virus production is the presence of defective interfering particles among the virus population that cause oscillations in virus levels and low production yields [3], known as Von Magnus effect.

In this work, a small scale two-stage cultivation system (two 100 mL shaker flasks; semi-continuous; SSC) was established as screening tool for influenza and MVA virus propagation before scaling to a 1 L continuous two-stage bioreactor system (two 1 L stirred tank bioreactors; TSB). The MVA virus strains MVA-CR19 and MVA-CR19.GFP were used, and propagated 14 days in the duck cell line AGE1.CR.pIX (all three from ProBioGen, Berlin) using the SSC system. Similarly, the influenza virus strain A/PR/8/34 H1N1 (RKI) was propagated 14 days using two different cell lines (MDCK.SUS2 and AGE1.CR.pIX) in the SSC system. From the best screening result, scale-up to the 1 liter TSB was performed with successful virus production in continuous mode for three weeks. PCR analysis was used to monitor the stability of the viruses in continuous culture.

The SSC system resulted in stable production of cells, and influenza virus titers that approached the oscillatory behavior observed in previous experiments [3]. Interestingly, MVA virus cultivated in the SSC system did not show oscillations in the virus titer. Additional cultivations of MVA virus in the SSC system showed that different residence times in the virus bioreactor could influence virus titers. Subsequently, production of MVA-CR19 was scaled to the TSB system and maintained for 18 days in continuous mode. MVA virus titers showed 7 days of a transient phase, followed by stable titers that confirmed the absence of a Von Magnus effect over 18 days. A yield comparison between an eight days batch-cycle process and the TSB showed that the space-time yield of the TSB cultivation approached that of two parallel batches at 11 days of virus production. PCR analysis indicated that the reporter gene in MVA-CR19.GFP was maintained stably for the complete cultivation period.

Overall, it was demonstrated that production of influenza and MVA viruses in a SSC system is feasible and can be used as a fast and cost-efficient tool for optimizing continuous virus production. Finally, MVA virus is a very promising candidate for production of viral vaccines in cascades of continuous stirred tank bioreactors.

[1] Verheust et al. 2012, *Vaccine* 30(16):2623–32.

[2] Jordan et al. 2013, *Viruses* 5(1):321–39.

[3] Frensing et al. 2013, *PLOS ONE* 8(9):e72288.

[4] Westgate and Emery 1990, *Biotech & Bioeng* 35(5):437-53.

INTENSIFICATION OF MVA AND INFLUENZA VIRUS PRODUCTION THROUGH HIGH-CELL-DENSITY CULTIVATION APPROACHES

Daniel Vázquez, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
vazquez_ramirez@mpi-magdeburg.mpg.de

Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
Michael M. Pieler, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Ingo Jordan, ProBioGen AG, Germany
Volker Sandig, ProBioGen AG, Germany

Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems, Otto-von-Guericke University
Magdeburg, Chair of Bioprocess Engineering

Key Words: scale-down, scale-up, perfusion, modified vaccinia virus Ankara (MVA), influenza virus A/PR/8/34 (H1N1)

Background. Unlike production of recombinant proteins, continuous production of viral vaccines at high cell densities (HCD) is often constrained by a decrease in cell-specific virus yields, early host cell lysis during virus propagation and limited virus recovery from culture broth. Nevertheless, advanced fed-batch [1] and perfusion strategies can be applied to achieve high-yield virus production processes. In this study, the development of a semi-continuous process for the production of the modified vaccinia Ankara virus isolate MVA-CR19 and influenza virus A/PR/8/34 (H1N1) in HCD cultivations of the suspension cell line AGE1.CR.pIX (ProBioGen AG, Berlin) is presented.

Methods. Depending on the required scale, high cell concentrations ($\sim 50 \times 10^6$ cells/mL) were achieved either through medium renewal by periodic centrifugation (semi-perfusion) in 50 mL cultivations or using an alternating tangential flow (ATF) perfusion system for 1 L bioreactors. Process development and optimization comprised three phases: 1) assessment of different fed-batch and medium exchange strategies for the propagation of MVA-CR19 or influenza A/PR/8/34 viruses in 50 mL cultivations; 2) scale-up and process optimization of the selected high-yield process strategy to a 1 L bioreactor with the ATF system, and 3) integration of a one-step purification process using magnetic sulfated cellulose particles (MSCP). For both viruses, conventional batch cultivation (no addition/medium exchange after infection) was compared with processes applying fed-batch, periodic medium exchange and the combination of both during virus propagation.

Results. Perfusion and semi-perfusion at a feeding rate of 0.05 nL/cell \times d was suitable to propagate AGE1.CR.pIX cells above 60×10^6 cells/mL with neither limitation nor overload of nutrients. For infections at 50 mL scale, the application of a combined strategy comprising an initial fed-batch phase followed by a periodic virus harvest phase resulted in the highest product yield with a more than 10-fold increase in virus particles concentration compared to the conventional batch processes operated at 4 to 8×10^6 cells/mL [2]. Additionally, a 3-fold increase in both cell-specific yield (virus particles/cell) and volumetric productivity (virus particles/L \times d) could be obtained. Comparable yields were observed when up-scaling to a 1 L bioreactor using an ATF-system, even when virus particles were retained within the bioreactor. Further selection of the optimal pore size of the ATF membrane allowed semi-continuous harvesting of the produced viruses and its purification with MSCPs with a recovery from 30 to 50%. In all cases, cell-specific yields and volumetric productivities reached their maxima at 72 h post-infection, indicating that the process should be stopped at that time point.

Conclusion. Compared to conventional batch processes, the developed HCD process offers significantly higher productivities including the option to integrate a one-step purification process in a semi-continuous mode. Overall, the results show that there is a great potential for semi-continuous HCD processes for the production of viral vaccines in larger scales, which could support efforts towards the establishment of continuous vaccine manufacturing.

References.

1. Pohlscheidt, M., et al., Development and optimisation of a procedure for the production of Parapoxvirus ovis by large-scale microcarrier cell culture in a non-animal, non-human and non-plant-derived medium. *Vaccine*, 2008. 26(12): p. 1552-65.
2. Lohr, V., et al., New avian suspension cell lines provide production of influenza virus and MVA in serum-free media: studies on growth, metabolism and virus propagation. *Vaccine*, 2009. 27(36): p. 4975-82.

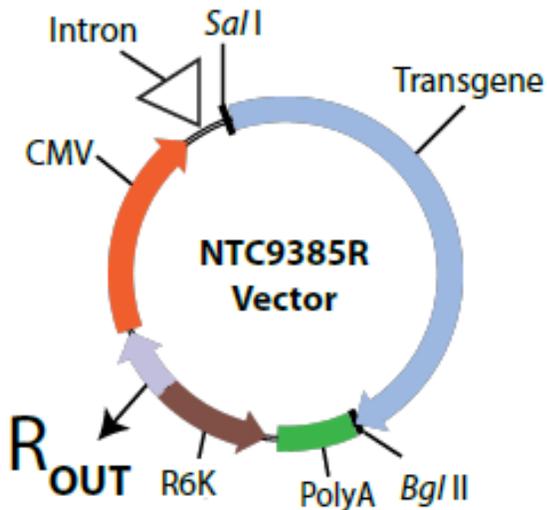
PRODUCTION OF A NANOPLASMID™ WITH A LARGE GENE INSERT USING THE HYPERGRO™ FERMENTATION PROCESS

Aaron Carnes, Nature Technology Corp.
acarnes@natx.com
Neha Tiwari, VGXI Inc.
Jill Beilowitz, VGXI Inc.
Carlos Sampson, VGXI Inc.
Dorothy Peterson, VGXI Inc.
Jim Williams, Nature Technology Corp.

Key Words: DNA vaccination, Nanoplasmid, Plasmid, Antibiotic-free, Fermentation

Plasmid based DNA vaccines are emerging as a promising alternative to traditional vaccines due to several advantages, including faster production of DNA plasmids using *E. coli*. However, further increases in transgene expression are needed to meet efficacy requirements for various non-viral gene therapy and DNA vaccination applications. While existing minicircle DNA technology has been shown to offer improved levels and durations of transgene expression by removal of the bacterial region from the plasmid, low manufacturing yields may be a barrier to widespread use of minicircle DNA for vaccination.

NTC's minimized Nanoplasmid™ vectors utilize RNA-OUT (R_{OUT}) antibiotic-free selection and replace the large 1000 bp pUC replication origin with a novel, 300 bp, R6K-derived mini-origin (Figure 1). Reduction of the spacer region linking the 5' and 3' ends of the transgene expression cassette to <500 bp remarkably increases plasmid-mediated transgene expression. Host strains expressing heat-inducible, high copy R6K replication (Rep) proteins have been developed for selection and propagation of Nanoplasmid. This is an additional Nanoplasmid safety factor since mini-origin vectors can only replicate within the engineered Rep protein-expressing *E. coli* host strain.



With years of expertise in plasmid production, NTC and VGXI have successfully implemented the HyperGRO™ fed-batch fermentation process for traditional plasmid and Nanoplasmid production at yields >1 g/L. However, production of plasmids containing large antigen-coding inserts may have various challenges. Bacterial cell machinery may not be able to produce high cell growth during fermentation due to a large gene insert in the plasmid. Plasmid pNano1, a Nanoplasmid with a gene insert of 3762 bp, was successfully produced using a modified HyperGRO process, with high end cell density of OD_{600} 90.1 and volumetric yield of 0.696 g/L.

VIRUS-LIKE PARTICLES ADSORPTION IN ANION EXCHANGE CHROMATOGRAPHY MEDIA

Patricia Pereira Aguilar, University of Natural Resources and Life Sciences Vienna, Austria
patricia.aguilar@boku.ac.at

Tobias Schneider, Austrian Centre of Industrial Biotechnology, Austria

Alois Jungbauer, University of Natural Resources and Life Sciences Vienna and Austrian Centre of Industrial Biotechnology, Austria

Key Words: Virus-Like Particles; Anion Exchange Chromatography; Chromatography Beads; Charged Hydrogels.

Biotechnological and pharmaceutical industries have the development of modern vaccines and novel drug delivery systems as one of their main focus. At this point, Virus-Like Particles (VLPs) are key candidates once they have the ability to stimulate humoral and cellular immune responses combined with the inability to replicate or proliferate. VLPs are non-infectious self-assembled protein structures which mimic native viruses (lacking any viral genetic material). However great developments in VLPs manufacturing have already been achieved, their purification is still a complex process, usually slow and with low productivity. Accordingly, there is a demand for new purification strategies and unit operations. Anion exchange chromatography is well established and widely used in industry for the purification all sorts of biomolecules. It is already known that polymer-grafted media in form of charged hydrogels and/or chromatography beads have a very high protein binding capacity and they also bind large biomolecules such as plasmids and viruses. However, the separation mechanism of large biomolecules is still not well understood and this lack of knowledge hinders the development and optimization of the purification processes. To overcome this, our aim is to elucidate the adsorption mechanisms of VLPs, large proteins and protein superstructures into different types of anion exchange chromatography media including highly charged hydrogels and polymer-grafted media. The binding kinetics and equilibria of HIV-1 VLPs expressed in CHO cells and Influenza VLPs expressed in Baculovirus-Insect cell system have been measured for polymer grafted media to elucidate the effect of the charged polymer. Adsorption isotherms were measured in microtiter plates and kinetics in batch mode.

Gerster, P., Kopecky, E.-M., Hammerschmidt, N., Klausberger, M., Krammer, F., Grabherr, R., Mersich, C., Urbas, L., Kramberger, P., Paril, T., Schreiner, M., Nöbauer, K., Razzazi-Fazeli, E., Jungbauer, A.

Purification of infective baculoviruses by monoliths (2013) *Journal of Chromatography A*, 1290, 36-45.

Jungbauer, A., Hahn, R. Polymethacrylate monoliths for preparative and industrial separation of biomolecular assemblies (2008) *Journal of Chromatography A*, 1184 (1-2), 62-79.

Jungbauer, A. Chromatographic media for bioseparation (2005) *Journal of Chromatography A*, 1065 (1), 3-12.

READY TO USE GAMMA IRRADIATED MICROCARRIERS FOR VIRUS PRODUCTION IN SINGLE USE BIOREACTOR SYSTEMS

Gustaf Ahlén, GE Healthcare Biosciences, Sweden
gustaf.ahlen@ge.com

Eva Blanck, Margareta Berg and Ann-Christin Magnusson, GE Healthcare Biosciences, Sweden

Key Words: Influenza, microcarrier, cytodex, single use

Since the 80's, microcarriers have been used to provide growth support for adherent cells in vaccine biomanufacturing processes at scales up to 6000 L. Today, as yields are increasing, most newly developed vaccines are manufactured at smaller scales, making single-use technologies suitable. Since many anchorage dependent cell lines grown on microcarriers are sensitive to shear stress, some of the remaining challenges are to have homogenous well-suspended microcarriers and efficient oxygenation of the medium. These prerequisites can limit operating conditions and volumes of the bioreactor process. We show that by carefully selecting operating parameters, good cell growth and virus yields can be achieved in a single-use bioreactor system. Furthermore, the time consuming process of preparation and sterilization of the microcarriers prior to culture can be simplified significantly by using Cytodex™ Gamma.

In this study we describe different approaches for the cultivation of Vero cells to high cell densities using Cytodex and/or Cytodex Gamma microcarriers in serum-free medium using ReadyToProcess WAVE™ 25 and/or Xcellerex™ XDR-10 single-use bioreactor system. Vero cells were grown in the bioreactors and infected with influenza virus during exponential growth phase. The concentration of infectious virus was determined by 50% tissue culture infective dose measurement (TCID₅₀). Viral titers of 10⁷ or higher were observed in all cultures. No significant differences in cell growth were observed between the different Cytodex microcarriers, and similar cell concentrations were reached in both bioreactor systems.

Our results offer valuable information to facilitate design-in of single-use bioreactor systems and ready-to-use Cytodex Gamma microcarriers, enabling flexible future vaccine productions at smaller scales.

DEVELOPMENT OF A VERSATILE VACCINATION PLATFORM BASED ON PAPAYA MOSAIC VIRUS (PAPMV) NANOPARTICLES

Ariane Thérien, Université Laval, Canada.
ariane.therien.2@ulaval.ca
Damien Carignan, Université Laval, Canada.
Gervais Rioux, Université Laval, Canada.
Denis Leclerc, Université Laval, Canada.

Key words: Papaya mosaic virus, virus like particles, sortase A, transpeptidation, vaccine platform.

Over the past years, virus-like particles (VLPs) have shown great potential as highly immunogenic subunit vaccines. These non-infectious viral structures mimic the native pathogen's organisation and conformation. VLPs contain highly repetitive and ordered viral epitopes leading to B cell activation through receptor cross-linking. By displaying heterologous epitopes on VLPs, one can mount an immune response against a different pathogen. These chimeric VLPs serve as presentation scaffold and can sometimes act as adjuvant to boost the immune response. However, VLP assembly can be affected by large epitope insertions altering intra or extra protein interactions impacting its conformation. Even if the insertion is successful, the epitopes have to be exposed at the particle surface to induce an immune response. To circumvent this problem, we have developed a new vaccine platform based on PapMV nanoparticules and sortase A (SrtA) transpeptidase. SrtA catalyzes the covalent conjugation of target antigenic epitopes to already assembled PapMV VLPs harbouring the SrtA recognition motif LPETG. Successful SrtA conjugations were achieved with peptides derived from Influenza (M2e) and HIV (T20). SrtA conjugated PapMV nanoparticles induce strong humoral responses in mice against both M2e and T20 peptides. PapMV-M2e vaccinated mice were protected against a lethal dose of Influenza H1N1 (A/WSN/33). Sera from PapMV-T20 vaccinated mice did not reduce *in vitro* HIV infection even with the high presence of specific antibodies. This new PapMV-SrtA platform eliminates the need for genetic fusion of the coat protein that can be difficult, time consuming and, sometime, unrealizable. The modification of PapMV VLP post-assembly facilitates its use in the rapid development of new vaccines by changing the nature of the target epitopes conjugated. This could be particularly useful when developing a pandemic vaccine or personalised vaccine for cancer therapy.

EVALUATION OF PRODUCER CELL LINES FOR YELLOW FEVER VIRUS PRODUCTION IN UP TO 1 L BIOREACTOR SCALE

Alexander Nikolay, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
nikolay@mpi-magdeburg.mpg.de

Katharina Hermann, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems & Otto-von-Guercke University,
Chair of Bioprocess Engineering, Germany

Key Words: Vero, BHK-21, yellow fever virus, process optimization.

Yellow fever virus (YFV) vaccine is currently produced in embryonated chicken eggs. Following recent outbreaks of flavivirus-related diseases, such as Zika fever, significant efforts are needed towards fast establishment of cell culture-based production processes for attenuated or inactivated virus vaccines.

To support the development of such processes, we have screened various cell lines, including adherent and suspension cells, for permissiveness and productivity of YFV. In particular, the parental adherent Vero cell line possesses a reasonable cell-specific productivity of about 13 PFU/cell. However, surface-dependend scale-up restricts production processes to roller bottles, microcarrier-based or fixed-bed bioreactors with limited monitoring and excessive efforts for large-scale production. A preferential alternative is the cultivation of single-cells in stirred-tank bioreactors, which can be operated in perfusion mode to achieve higher cell-densities. Towards this process intensification, we have adapted the parental WHO Vero cell line to grow in suspension. However, infection studies of Vero suspension cells with YFV in spinner flasks using chemically defined medium showed a reduced cell-specific titer (2 PFU/cell).

Another option might be the use of BHK-21 cells reaching cell-densities above 5×10^6 cells/mL in shake flasks. Infection studies with YFV in small-scale have resulted in a cell-specific productivity of 10 PFU/cell. Thus, infection parameters (time of infection, MOI = ratio of virus to cell) were optimized and subsequently transferred into 1 L bioreactors. Final titer of 5×10^7 PFU/mL could be reached. As a reference, adherent Vero cells were cultivated on Cytodex-1 microcarriers in 1 L scale resulting in a final titer of 2×10^7 PFU/mL. In both cultivations, cell-specific yields were comparable but due to the adjusted MOI of 10^{-4} in the BHK-21 cultivation, the overall virus production was 50 x higher than for the Vero cultivation on microcarriers.

Although BHK-21 cells and their application for human vaccines are controversial with respect to tumorigenicity and oncogenicity, our results show that it may be worth to reconsider this cell line for future production processes.

PROPAGATION OF BRAZILIAN ZIKA VIRUS STRAINS IN STATIC, MICROCARRIER-BASED AND SUSPENSION CULTURES USING BHK AND VERO CELLS

Alexander Nikolay, Max Planck Institute for Dynamics of Complex Technical Systems, Germany
nikolay@mpi-magdeburg.mpg.de

Leda Castilho, Federal University of Rio de Janeiro, Cell Culture Engineering Laboratory, Rio de Janeiro, Brazil

Amilcar Tanuri, Federal University of Rio de Janeiro, Genetics Department, Rio de Janeiro, Brazil

Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems & Otto-von-Guericke University,
Chair of Bioprocess Engineering, Germany

Yvonne Genzel, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Key Words: Brazilian Zika virus, microcarrier, suspension cells, high-cell density cultivation, virus adaptation

The spread of Zika virus (ZIKV) in the Americas results in an urgent need for the development of a ZIKV vaccine. Current strategies for ZIKV propagation in animal cells rely mainly on adherent Vero and C6/36 cells. This work focused on understanding ZIKV replication in animal cell culture to develop an inactivated or live-attenuated ZIKV vaccine in microcarrier culture or, preferably, in suspension cells, so that low cell-specific yields can be overcome by the establishment of high-cell density processes.

First, adherent cells (Vero and BHK-21) were infected with different Brazilian ZIKV isolates. Comparing both cell lines, maximum infectious titers and cell-specific yields (1–48 PFU/cell) of respective virus strains were similar, whereas process yields across different strains strongly varied by two log-scales.

Scale-up of Vero cells in bioreactors using 6 g/L Cytodex 1 resulted in maximum cell concentrations of 5.3×10^6 cells/mL. However, low cell-specific yields of 0.0002 PFU/cell indicated poor virus replication. Using suspension-adapted BHK-21 cells grown in a chemically-defined medium, higher virus titers were achieved when infections were initiated at the mid/late exponential growth phase at MOI 0.001. Nevertheless, cell-specific yields did not exceed 0.0002 PFU/cell. Subsequent RT-qPCR data indicated a poor virus release as intracellular viral RNA levels were 20-fold higher than extracellular levels.

At small-scale, centrifugal spinoculation was evaluated to enhance ZIKV infection in suspension BHK-21 cells, with no significant improvements. In a further investigation with these cells in a perfusion bioreactor using an ATF-2 filtration system, a maximum cell concentration of 14×10^6 cells/mL was achieved with a final titer of 4.6×10^6 PFU/mL and an increased cell-specific yield of 0.09 PFU/cell.

Overall, the present results demonstrate that ZIKV propagation in microcarrier- and suspension-based systems is challenging regarding virus yields. Future investigations will focus on improving cell-specific yields by adapting Zika virus isolates to suspension cell lines, and on increasing maximum titers by process intensification.

ELECTRON MICROSCOPY AS AN EMERGING ANALYTICAL TOOL FOR CHARACTERIZING VACCINES

Anette Schneemann, Nanolmaging Services Inc., USA
aschneem@nanolmaging.com
Joyce J Sung, Nanolmaging Services Inc., USA
Jeffrey A. Speir, Nanolmaging Services Inc., USA
Sean K. Mulligan, Nanolmaging Services, Inc., USA
Kat On, Nanolmaging Services Inc., USA
Joel Quispe, Nanolmaging Services Inc., USA
Bridget Carragher, Nanolmaging Services Inc., USA
Clinton S. Potter, Nanolmaging Services, Inc., USA

Key Words: TEM, structural characterization, VLP, adjuvant, antibody

Characterization of nanoparticles and biologics is a critical step in the development of important new pharmaceutical products and biosimilars. Biologics pose unique characterization challenges that require an interdisciplinary approach in which several orthogonal methods are used to provide a complete picture. The physical characteristics of a biological product include properties such as the size, shape, morphology and aggregation state of the particles. These properties are often dependent on the specific environment of the particles and thus ideally must be assessed under conditions that reflect the final formulation of the pharmaceutical. Electron microscopy (EM) and in particular cryo-electron microscopy (cryoEM), has a unique advantage in that it provides a direct means of observing the individual particles in a sample, preserved in their natural hydrated state (cryoEM), simultaneously providing information on homogeneity, size distribution, titer, morphology, preservation state, flexibility, and aggregation state. For particles with a regular size and shape, particle averaging methods can provide 3D structural information, complementing X-ray crystallography analysis.

We will demonstrate the use of EM as an analytical and structural characterization tool by presenting a number of case studies as highlights. Specifically, we will discuss the characterization of Human Papilloma Virus (HPV) VLPs in GARDASIL®, including the structure of the VLPs alone, on adjuvants, and when interacting with neutralizing antibodies [1]. We will also show how TEM was used as a non-intrusive tool to understand the structure and function of Hepatitis B surface antigen (rHBsAg) VLPs, the active component in the HBV vaccine [2]. We will furthermore demonstrate how TEM can be used to provide supporting information for characterization of a biosimilar drug delivery nanoparticle, a recombinant tuberculosis vaccine antigen, interacting with a lipid-based adjuvant [3], and a bi-specific, tetravalent immunoglobulin G-like molecule [4].

References:

- [1] Zhao Q, et al. 2013. Characterization of virus-like particles in GARDASIL(R) by cryo transmission electron microscopy. *Hum Vaccin Immunother.* 10:1-6.
- [2] Mulder AM, et al. 2012. Toolbox for non-intrusive structural and functional analysis of recombinant VLP based vaccines: a case study with hepatitis B vaccine. *PLoS One* 7:e33235.
- [3] Fox CB, et al. 2014. Cryogenic transmission electron microscopy of recombinant tuberculosis vaccine antigen with anionic liposomes reveals formation of flattened liposomes. *Int J Nanomedicine* 9:1367-77.
- [4] Correia I, et al. 2013. The structure of dual-variable-domain immunoglobulin molecules alone and bound to antigen. *MAbs.* 5:364-72.

OPTIMIZING SCALE-UP OF VERO CELLS CULTURED ON MICROCARRIERS IN SERUM-FREE MEDIUM FOR VACCINE PRODUCTION

Anna-Barbara Hachmann, Thermo Fisher Scientific, Grand Island, NY 14072
anna-barbara.hachmann@thermofisher.com

Andrew M. Campbell, Thermo Fisher Scientific, Grand Island, NY 14072
Stephen F. Gorfien, Thermo Fisher Scientific, Grand Island, NY 14072

Key Words: Vero, microcarriers, scale-up, vesicular stomatitis virus, serum-free

Vaccine production with adherent cell lines faces multiple challenges which include selection of a suitable vessel, detachment of cells for scale up, optimization of infection, as well as harvest of virus particles. Microcarriers greatly increase the surface area for adherent cells and offer flexibility for expansion to bioreactors, but scale-up methods require optimization of bead-to-bead transfer. Even though the majority of cell culture based vaccines are produced with adherent cell lines, literature provides limited information in regards to optimization of adherent cell line processes. Some process improvements have been achieved; for example, recent advances in serum free media which no longer require medium exchange prior to virus infection. In this study we focus on the production of the rabies virus surrogate, vesicular stomatitis virus, in Vero cells. Using Cytodex-1 microcarriers in spinner flasks, we evaluated effects of intermittent and continuous stirring, detachment of cells, variation in the addition of new microcarriers on the growth of Vero cells, and effects on vesicular stomatitis virus production. Viable cell density measurements revealed that initial intermittent stirring resulted in increased cell densities compared to continuous stirring after microcarrier addition. In an effort to further simplify the process, we demonstrate that detachment of cells was not required to facilitate bead-to-bead transfer on Cytodex-1 microcarriers.

CUSTOM OPEN POLYETHERSULFONE ULTRAFILTRATION MEMBRANES FOR VACCINES

Clifton Ngan, MilliporeSigma, USA
clifton.ngan@emdmillipore.com

Key Words: polysaccharide, conjugate, ultrafiltration (UF), yield, purity

Vaccines save millions of lives every year, and improve the quality of life for countless others. Ultrafiltration (UF) is a size-based separation that is used in nearly all vaccine processes for product concentration, impurity clearance, and buffer adjustment. Polyethersulfone (PES) UF membranes, such as Biomax® from MilliporeSigma, are widely used in the industry. An advancement in the PES membrane manufacturing process can enable better uniformity, improved control of the membrane pore size, and the capability to produce custom variants that could enhance yield, purity, efficiency, and economics in new or existing vaccine processes.

The poster highlights the new capability by showing the relative retention of various pneumococcal polysaccharides across a range of membrane pore sizes or nominal molecular weight cutoffs. A custom membrane would allow the vaccine manufacturer to maximize the yield while maintaining acceptable purity. In conjugated polysaccharide (CPS) vaccines like those for pneumonia and meningitis, bacterial polysaccharides (PS) are chemically linked or conjugated to carrier proteins (CP), e.g. diphtheria or tetanus toxoid to enhance the immune response beyond that of the PS alone. UF membranes are used for the concentration and purification of each component, and are critical for the supply of vaccines worldwide.

One method to characterize the pore size distribution of UF membranes is through the rejection of mixed dextrans (Tkacik and Michaels¹). In Figure 1, dextran rejection curves (rejection vs molecular weight) are shown for a number of membrane variants. In Figure 2, the molecular weight of the dextran rejected at 90% (R90) and the normalized water flux, are shown for a number of membrane variants along with boxes denoting the performance of standard commercial membranes.

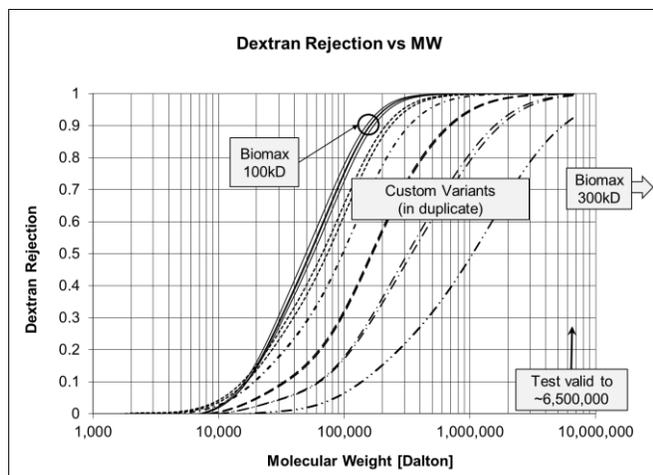


Figure 3 – Dextran Rejection Curves

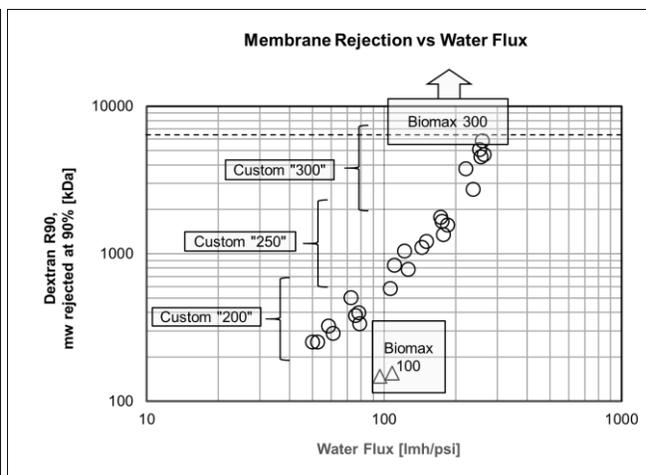


Figure 2 – Rejection vs Water Flux

Vaccine manufacturers may be able to improve yields in existing processes or to select a custom membrane to maximize yields in new processes. Please stop by the poster to find out more.

¹Tkacik, G. and Michaels, S., (1991) Bio/Technology 9, 941-946

HIGHLY CROSS-CONSERVED BURKHOLDERIA T CELL EPITOPES GENERATE EFFECTOR T CELL RESPONSES *IN VITRO*

Annie De Groot, EpiVax, Inc., Institute for Immunology and Informatics, University of Rhode Island
Rui Liu, Institute for Immunology and Informatics, University of Rhode Island
Lenny Moise, EpiVax, Inc., Institute for Immunology and Informatics, University of Rhode Island
Ryan Tassone, Institute for Immunology and Informatics, University of Rhode Island
Mohab Ali, Institute for Immunology and Informatics, University of Rhode Island
Matthew Ardito, EpiVax, Inc.
Frances Terry, EpiVax, Inc.
William Martin, EpiVax, Inc.

Key Words: Burkholderia, immunoinformatic, T cell epitope, effector immunity, vaccine

Burkholderia pseudomallei and Burkholderia mallei cause glanders and melioidosis, respectively. Both of them are classified as Category B biothreat agents due to their high infectivity and potential use as a bioweapon. The related species Burkholderia cepaciae causes fatal 'cepacia syndrome' in cystic fibrosis patients, which is characterized by rapid deterioration, bacteremia and necrotizing pneumonia. Clinical eradication of Burkholderia infection often fails due to antimicrobial resistance. Effective vaccination against Burkholderia infection is critically important to protect populations living in endemic areas worldwide and against bioterror threats. No vaccines or other prophylactics for these pathogens are available. Vaccines against Burkholderia should target cell-mediated immune response, which is believed to be essential to successfully clear Burkholderia infection. We hypothesize that a single vaccine comprising highly cross-conserved Burkholderia T cell epitopes might generate protective cell-mediated immune response against all the three species. Immunoinformatics tools were used to identify immunogenic consensus sequences (ICS) that are enriched for promiscuous and highly conserved CD4+ T cell epitopes in all three Burkholderia species. The ICS peptides were validated in peripheral blood mononuclear cells (PBMCs) derived from healthy donors [1].

All of the peptides (100%) bound to at least two HLA alleles, 98% bound to at least three HLA alleles, 98% bound to at least four HLA alleles and 92% bound to all seven HLA alleles. The overall predictive accuracy was 81% (both positive and negative) [2]. Significant IFN γ response was induced by all peptides in at least one human donor as measured by IFN γ ELISpot assay. 86% of the peptide-specific IFN γ ELISpot responses were completely inhibited by antibody block of HLA-DR, indicating that these peptides are HLA-DR-restricted. Significant peptide-specific proliferation and Th1 cytokine production (IFN γ , TNF α and IL-2) in CD4+ T cells from healthy donors were observed in flow cytometry analysis. Immunoinformatics predictions, coupled with in vitro validation, can accelerate the selection of highly conserved T cell epitopes from genome sequence databases. The approach can be used for rapid selection of vaccine candidates for a wide array of emerging infectious diseases and biodefense targets.

References:

1. Wullner D, Zhou L, Bramhall E, Kuck A, Goletz TJ, Swanson S, Chirmule N and Jawa V. Considerations for optimization and validation of an in vitro PBMC derived T cell assay for immunogenicity prediction of biotherapeutics. *Clinical Immunology*. 2010,137, 5-14.
2. De Groot AS, Ardito M, Moise L, Gustafson EA, Spero D, Tejada G and Martin W. Immunogenic Consensus Sequence T helper Epitopes for a Pan-Burkholderia Biodefense Vaccine. *Immunome Res*. 2011, 7(2). pii: e7.

PREDICTING TOLERANCE IN VACCINE ANTIGENS: APPLICATION TO INFLUENZA, HCV AND HIV

Annie De Groot, EpiVax, Inc., Institute for Immunology and Informatics, University of Rhode Island
Rui Liu, Institute for Immunology and Informatics, University of Rhode Island
Lenny Moise, EpiVax, Inc., Institute for Immunology and Informatics, University of Rhode Island
Ryan Tassone, Institute for Immunology and Informatics, University of Rhode Island
Frances Terry, EpiVax, Inc.
William Martin, EpiVax, Inc.

Key Words: JanusMatrix, immunoinformatic, T cell epitope, cross-conservation, regulatory T cell

JanusMatrix, a newly developed algorithm by EpiVax, Inc, identifies cross-reactive T cell epitopes by matching the T cell receptor (TCR)-accessible amino acids (the TCR face) of peptides that bind the same human leukocyte antigen (HLA) [1]. Taking into both HLA binding and TCR contact account, JanusMatrix is uniquely able to compare T cell epitope conservation between protein sequences from bacterial and viral organisms that make up the human gut microbiome, autologous proteins from the human genome, and human viral and bacterial pathogens.

We recently discovered that pathogens might escape human immune response by mutating their epitopes to present “human-like” amino acid sequences to TCR when displayed on antigen-presenting cells [2]. We hypothesized that such “human-like” T cell epitopes contained in pathogens may trigger autologous regulatory T cells (Tregs), actively suppress immune response to themselves, thus improving their ability to survive in the host. We have used JanusMatrix to identify such “human-like” pathogen sequences that both bind HLA and share the same TCR-face patterns as human proteins.

In a large scale analysis of viruses that infect humans, we found that chronic viruses that establish persistent infection in human (such as Epstein-Barr, Herpes Simplex Virus and Cytomegalovirus) contain a significantly higher number of T cell epitopes that are cross-conserved (at the TCR face) with human proteins than viruses that do not establish chronic infection (such as Ebola and Marburg) [3]. Using JanusMatrix, we identified human-like T cell epitopes in H7N9 influenza hemagglutinin (HA) protein [4]. A promiscuous T cell epitope from H7N9 HA expanded Tregs and suppressed responses to other H7N9 peptides. This may explain the low titer of H7N9 HA inhibiting antibody responses and diminished seroconversion rates. Depletion the Treg-activating epitope increased antibody titers by 5 fold and B cell response by 20 fold (Unpublished). We also identified a “human-like” HCV T cell epitope, HCV_G1_p7_794, induced a marked increase of Tregs in PBMC derived from HCV-infected patients [5]. In HIV-1 Env, we found a human-like epitope that shares a TCR-face with a large number of human leukocyte antigen (HLA) class I molecule sequences. This highly conserved epitope is found in both the HIV-1 E and B Env antigens used in the ‘moderately effective’ HIV RV144 trial. It was found that this HIV Env-derived T cell epitope trigger functional Treg responses in HIV negative individuals. All these results suggest that JanusMatrix tool opens up a new window on the networks of cross-reactive T cell epitopes in human immune response, which may lead to significant improvements in the efficacy of vaccines.

References:

1. Moise L, Gutierrez AH, Bailey-kellogg C, et al. The two-faced T cell epitope: examining the host-microbe interface with JanusMatrix. *Hum Vaccin Immunother.* 2013;9(7):1577-86.
2. Moise L, Liu R, Gutierrez AH, Tassone R, Bailey-Kellogg C, et al. Immune Camouflage: Relevance to Vaccine Design and Human Immunology. *Hum Vaccin. Immunother.* 2014;1:e36134.
3. He L, De Groot AS, Gutierrez AH, Martin WD, Moise L, et al. Integrated assessment of predicted MHC binding and cross-conservation with self reveals patterns of viral camouflage. *BMC Bioinformatics.* 2014; 15:S1.
4. Liu R, Moise L, Tassone R, Gutierrez AH, Terry FE, et al. H7N9 T-cell epitopes that mimic human sequences are less immunogenic and may induce Treg-mediated tolerance. *Hum Vaccin Immunother.* 2015;11(9):2241-2252.
5. Losikoff PT, Mishra S, Terry F, et al. HCV epitope, homologous to multiple human protein sequences, induces a regulatory T cell response in infected patients. *J Hepatol.* 2015;62(1):48-55.

IN VIVO VALIDATION OF PREDICTED AND CONSERVED T CELL EPITOPES IN A SWINE INFLUENZA MODEL

Andres H. Gutiérrez, Institute for Immunology and Informatics, University of Rhode Island, USA
anunez.uri@gmail.com

Crystal Loving, Virus and Prion Diseases Research Unit, NADC, USDA ARS, USA
Susan L. Brockmeier, Virus and Prion Diseases Research Unit, NADC, USDA ARS, USA

Holly R. Hughes, Virus and Prion Diseases Research Unit, NADC, USDA ARS, USA

Leonard Moise, EpiVax Inc., USA
Frances E. Terry, EpiVax Inc., USA
William D. Martin, EpiVax Inc., USA
Anne S. De Groot, EpiVax Inc., USA

Key Words: T cell epitope prediction, immunoinformatics, swine vaccine, immunogenicity, influenza, PigMatrix

Swine influenza is a highly contagious respiratory viral infection in pigs that is responsible for significant financial losses to pig farmers annually. Current measures to protect herds from infection include: inactivated whole-virus vaccines, subunit vaccines, and alpha replicon-based vaccines. As is true for influenza vaccines for humans, these strategies do not provide broad protection against the diverse strains of influenza A virus (IAV) currently circulating in U.S. swine. Improved approaches to developing swine influenza vaccines are needed. Here, we used immunoinformatics tools to identify class I and II T cell epitopes highly conserved in seven representative strains of IAV in U.S. swine and predicted to bind to Swine Leukocyte Antigen (SLA) alleles prevalent in commercial swine. Epitope-specific interferon-gamma (IFN γ) recall responses to pooled peptides and whole virus were detected in pigs immunized with multi-epitope plasmid DNA vaccines encoding strings of class I and II putative epitopes. In a retrospective analysis of the IFN γ responses to individual peptides compared to predictions specific to the SLA alleles of cohort pigs, we evaluated the predictive performance of PigMatrix and demonstrated its ability to distinguish non-immunogenic from immunogenic peptides. Overall, this study confirms the capacity of PigMatrix to predict immunogenic T cell epitopes and demonstrate its potential for use in the design of more broadly cross-protective influenza and other vaccines for swine.

DEVELOPMENT OF INSECT CELL PLATFORMS FOR FAST PRODUCTION OF PSEUDO-TYPED VLPs FOR DRUG AND VACCINE DEVELOPMENT

João Vidigal, IBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal
jvidigal@ibet.pt

Bárbara Fernandes, IBET, Instituto de Biologia Experimental e Tecnológica/ Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa

Mafalda M Dias, IBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal.

Marco Patrone, Biocrystallography Unit, San Raffaele Scientific Institute, Italy.

Manuel JT Carrondo, IBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal.

Paula M Alves, IBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal.

Ana P Teixeira, IBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal.

Key Words: Stable insect cell platform; Recombinase-mediated cassette exchange; HIV Gag-VLPs; GPCRs; Vaccines.

Production technologies providing high concentrations of membrane proteins in their native structure are essential in the vaccine field, as well as to support the drug discovery pipeline. In this work, we took advantage of insect cell expression and site specific gene integration based on flipase-mediated cassette exchange (FMCE) technology to develop cell platforms for efficient production of membrane proteins on the surface of enveloped virus-like particles (VLPs). The co-expression of membrane proteins with capsid proteins of enveloped viruses (such as HIV Gag) will enable their capturing in lipid rafts of the cellular plasma membrane and displaying on the surface of budding VLPs, thus providing a native conformation for downstream assays. Parental Sf9 and Hi5 insect cells were randomly tagged with a GFP-fused Gag protein and FACS enriched with cells tagged in genomic “hot-spots” supporting high expression. A linker including a Flp recognition target (FRT) site was used to allow posterior removal of the marker gene from the particle through cassette exchange. By confocal microscopy we could see that Gag localizes preferentially at the plasma membrane, whereas by electron microscopy we could detect correctly assembled Gag-VLPs in the culture supernatant of both cell hosts. Upon promoting Flp-mediated recombination in the tagging populations, cassette exchange was well-succeeded (showing that the FRT site composing the linker fusing the two genes does not impact recombination), allowing to recover cells tagged in loci supporting FMCE. We are currently evaluating the capability of the Gag-VLPs as scaffolds to display GPCRs (e.g. beta-2 adrenergic receptor) and Influenza HA proteins. Overall, modular insect platforms are being constructed to be readily adaptable to produce a broad range of VLP-based vaccines and receptor display particles for drug screening or antibody discovery.

Acknowledgments: Funding from European Commission (Project EDUFLUVAC; Grant nr. 602640) and Fundação para a Ciência e a Tecnologia through the project EXPL/BBB-BIO/1541/2013 and PhD fellowships SFRH/BD/86744/2012 and SFRH/BD/90564/2012.

SAFE AND GREEN, THE HYPERBAR INACTIVATION

Fabien Lux, Industrial Operations / Manufacturing Technology Sanofi Pasteur, France
fabien.lux@sanofipasteur.com

Key Words: Inactivation, high hydrostatic pressure, effective vaccine

To make a safe, effective vaccine, the process must be able to completely inactivate the pathogenic microorganism while retaining the organism's immunogenic potential. The classic method, using toxic chemicals such as thiomersal or formaldehyde, has many disadvantages: the safety constraints needed to handle these products, the potential for a residual presence in the final product and a possible change in the antigen structure impacting the immune response. A new technology assessed in collaboration with Merial and TOP Industry makes it possible to design a patented new type of equipment dedicated for the inactivation of any kind of bacteria (*Bordetella pertussis*, *Erysipelothrix rhusiopathiae*, *Escherichia coli*, *Salmonella*...), while maintaining the bacterial cell structure. This new process consists in applying high hydrostatic pressure to the pathogenic microorganism. The inactivation of the microorganism is completed after several minutes under high pressure, whereas the classical method can require several days. This new technology dramatically reduces the duration of this critical step, improves the safety of the patient by avoiding the use of toxic chemicals and potentially reduces the dose amount needed for the vaccination while improving the quality of the antigens. The poster will present the technology, some of the trials performed to inactivate these bacterias and the results obtained.

INFLUENZA A VIRUS PROPAGATION IN MDCK: INTRACELLULAR VIRUS REPLICATION, VIRUS RELEASE AND CELL-CYCLE PREFERENTIAL INFECTION ANALYSIS

Gallo-Ramírez, L.E, Max Planck Institute for Dynamics of Complex Technical Systems, Germany.
Frensing, T, Reichl, U, Max Planck Institute for Dynamics of Complex Technical Systems ; Otto-von-Guericke-Universität, Germany.
Kupke, S, Bachmann, M, Fritzsche, S, Max Planck Institute for Dynamics of Complex Technical Systems, Germany.

Key Words: Influenza A virus, MDCK cells, replication dynamics.

Cell culture-based processes for vaccine manufacturing offer advantages over egg-based processes in terms of product uniformity and sterility, production time and scaling up capacity^{1,2}. Regarding influenza vaccines, MDCK cells are one of the host cell lines currently used to manufacture licensed products; however, virus titers remain lower compared to those obtained in eggs and further increase of specific and volumetric yields is required. To identify bottlenecks in influenza A virus (IAV) production, we thoroughly studied IAV replication in MDCK cells. For this, we analyzed different features of the infection process such as viral RNA replication, intracellular localization of viral components, virus release and morphology of the particles, and the preferential infection in different cell-cycle phases.

Using synchronous infections, we found that production of infectious particles dropped much earlier than the production of total particles. Furthermore, we found that the maximum virus release rate was reached when all viral RNA species attained their maximum intracellular concentration. Using qPCR we determined that the vRNA maximum concentration per cell was 10-fold higher than the specific viral titers obtained, indicating that vRNA concentration does not limit IAV particle assembly. When we evaluated the morphology of particles released using electron microscopy, we observed that a higher fraction of the viral particles produced at late times possess an abnormal morphology, concurring with the increased production of non-infectious viruses.

Using imaging flow cytometry, we determined that the export of influenza viral genome segments (ribonucleoprotein complexes, vRNPs) from the nucleus to the cytoplasm strongly correlated with the onset of virus release. However, our results also suggest that the induction of apoptosis caused that virus assembly became deficient producing more non-infectious particles at late infection times.

Lastly, using low MOI infections and imaging flow cytometry, we found that -in contrast to previous publications- IAV did not preferentially infect a specific cell cycle phase and no cell cycle arrest induction was observed during the time frame of the experiment (9 hpi).

In summary, the data presented here offers a comprehensive overview of the dynamics of IAV infection in MDCK and might contribute to the development of molecular or cell culture-based strategies to improve IAV production in MDCK cells.

¹ Gallo-Ramírez et al, 2015. *Exp. Rev. Vaccines* 14 (9).

² Pardue et al. 2011. *Exp. Rev. Vaccines* 10 (8).

VP2 POTENTIATES THE PROTECCION INDUCED BY VP6 AGAINST THE ROTAVIRUS INFECTION IN A DNA VACCINE MODEL

López-Guerrero DV, Facultad de Medicina, UAEM
vannylpz@yahoo.com

Gutiérrez-Xicotencatl L, Pedroza A, Chiu L, Plett T, Centro de Investigaciones sobre Enfermedades Infecciosas, SSA.

Villegas J, Rosas G, Arias N, Esquivel-Guadarrama F, Facultad de Medicina, UAEM.
López S, Arias C, Fernández G, Instituto de Biotecnología, UNAM. Cuernavaca, Morelos, México.

Key Words: VP2, VP6, DNA vaccine, rotavirus infection.

Viruses like particles (VLPs) composed of VP2/VP6 are very effective in inducing protection against the rotavirus infection in animal models. Individually, VP6 also can induce protection against the infection; however, there is no information about the immunogenicity of VP2. The aim of this work was to evaluate the efficacy of DNA vaccines that codify for VP2 and VP6 alone or combined to induce protection against the rotavirus infection. Murine rotavirus VP2 and VP6 genes were cloned into the pCDNA-3 vector. Adult BALB/c mice were inoculated 3 times by intramuscular injections with 100 or 200 μ g of pCDNA-3_{VP2} and pCDNA-3_{VP6}, alone or combined. Two weeks after the last inoculation, mice were challenged with the murine rotavirus EDIM. We found that both plasmids pCDNA-3_{VP2} and pCDNA-3_{VP6} were able to induce rotavirus-specific serum antibodies, but not intestinal rotavirus-specific IgA. Only pCDNA-3_{VP6} at 200 μ g could induce 30 % protection against the infection. Co-administration of 100 μ g of pCDNA-3_{VP2} with 100 μ g of pCDNA-3_{VP6} induced 35 % protection. When different ratios of pCDNA-3_{VP2}/pCDNA-3_{VP6} were used, it was found that the co-administration of 10 μ g pCDNA-3_{VP2}/ 100 μ g pCDNA-3_{VP6} gave the best result with up to 55 % protection. These results indicate that the DNA plasmid expressing VP6 is a better vaccine candidate than the one expressing VP2 but co-administration of both plasmids is a good alternative to potentiate the protection induced by VP6, probably by the formation of VLPs VP2/VP6 *in vivo*.

DIFFERENTIAL ANTIBODY RESPONSE AGAINST CONFORMATIONAL AND LINEAR EPITOPES OF THE L1 PROTEIN FROM HUMAN PAPILLOMAVIRUS TYPES 16/18 IS GENERATED IN VACCINATED WOMAN OR WITH DIFFERENT EXPOSURES TO THE VIRUS.

Gutierrez-Xicotencatl L. Research Center for Infection Diseases, & Center for Research in Population Health, National Institute of Public Health, Cuernavaca, Morelos, Mexico;
Pedroza-Saavedra A, Chihu-Amparan L, Maldonado-Gama M, Salazar-Piña DA, Research Center for Infection Diseases, & Center for Research in Population Health, National Institute of Public Health, Cuernavaca, Morelos, Mexico;
Rodríguez-Ocampo AN, Cruz-Valdez A, Academic Unit of Chemical Biology and Pharmaceutical Sciences, Autonomous University of Nayarit, Mexico;
Carmona-Balandrano E, Esquivel-Guadarrama F, Faculty of Medicine, Autonomous University of Morelos Estate, Mexico.

Keywords: Cervical cancer, human papillomavirus, L1 protein, VLPs, linear and conformational epitopes, HPV vaccine.

Antibodies against human papillomavirus (HPV) L1 protein are associated to past infections and related to the evolution of the disease, while antibodies against L1 virus like particles (VLPs-L1) are used to follow the neutralizing immune response in vaccinated women. In this study, sera antibodies against conformational and linear epitopes of L1 protein from HPV16/18 were evaluated to discriminate HPV vaccinated women from those naturally infected or with uterine cervical lesions. The VLPs-L1 from HPV16/18 generated in baculovirus were purified by CsCl gradient and linear L1 protein obtained by denature VLPs. Serum antibodies against VLPs-L1 and L1 from vaccinated women or with different exposures to the virus were measured by ELISA. Regression and ROC analysis were carried out to evaluate the test performance to discriminate the different women populations. The results showed that antibodies against VLPs-L1-16/18 highly associated with vaccinated women (OR=2.11e+08 and 57.74, respectively), but not associations were observed with natural infected women or with cervical lesions. However, antibodies against L1-16/18 showed high associations with vaccinated women (OR= 101.33 and 37.91, respectively) ($p \leq 0.05$), but also associations of these antibodies were observed with the cervical cancer (CC) group (OR= 3.33 and 5.65, respectively). The ROC analysis showed that antibodies against VLPs-L1 and L1 16/18 were highly effective to detect vaccinated women (AUC= 0.96, 0.91, 0.80 and 0.78, respectively), with high sensitivity for HPV16 (100% and 86.3%), and moderate for HPV18 (63.6%). However, anti-L1 antibodies had the best test performance to discriminate the cervical intraepithelial neoplasia grade 3 (CIN3)/CC group from controls (AUC= 0.67 HPV16 and 0.63 HPV18), with low sensitivity (15% to 20%, respectively) and high specificity (96% and 92%, respectively). In conclusion, our results suggested that anti-VLPs-L1 16/18 antibodies are highly efficient to detect vaccinated women, but anti-L1 antibodies are better to discriminate CIN3/CC among the general population.

N-GLYCOSYLATION AFFECTS HUMORAL IMMUNE RESPONSE OF HER1 CANCER VACCINE

Katia Garcia Duardo, Development Process Direction, Center of Molecular Immunology
katiag@cim.sld.cu

Yanet Cueto Cabañas, Institute of Pharmacy and Food Sciences, Havana University
Gretchen Bergado Baez, Tumoral Biology Direction, Center of Molecular Immunology
Judith Raymond Pous, System Biology Direction, Center of Molecular Immunology
Estela Yamilet Rabasa Legón, Development Process Direction, Center of Molecular Immunology
Adolfo Castillo Vitloch, Development Process Direction, Center of Molecular Immunology
Kathya R. de la Luz Hernández, Development Process Direction, Center of Molecular Immunology

Key words: Cancer vaccine, glycosilation, Her-1, immunotherapy

Vaccine preparations based on the extracellular domain of Her1 protein (Her1-ECD) have demonstrated, in vitro and in vivo, a potent antimetastatic effect on EGFR⁺ Lewis lung carcinoma model, while associated side effects were absent. The Her1-ECD is a glycoprotein with a molecular weight of 105 kDa and has 11 potencial sites for N-glycosylation. Glycosylation is a post-translational modification that can affect the protein folding, stability, regulates protein half-life, immunogenecity, biological activity and other functions. In this work, the N-glycosylation Her1-ECD was preliminarily characterized by SDS-PAGE, glycan differentiation by lectin and normal phase chromatography. Finally, the biological activity of the glycosylated and totally deglycosylated Her1-ECD protein was compared. As results were obtained that N-glycosylation profile of Her1-ECD is composed of high mannose, hybrid and complex N-glycans types, and Her1-ECD glycosylation modifies the humoral immune response, measured as antibody titers, recognition of EGFR in A431 cell line and cell cycle arrest.

IL-17A AND *STREPTOCOCCUS PNEUMONIAE* RESPIRATORY INFECTION: PROSPECTS FOR THE DEVELOPMENT OF NEW IMMUNOTHERAPIES

Rial A., Céspedes M.P., Comas V., Marqués J.M., Muñoz N., Iwakura Y, and J.A. Chabalgoity

Depto. Desarrollo Biotecnológico, Instituto de Higiene, Facultad de Medicina, Universidad de la República.
Montevideo, Uruguay

Keywords: Th17, IL-17A, *Streptococcus pneumoniae*, pneumonia, colonization.

Nasopharyngeal colonization by *Streptococcus pneumoniae* constitutes a pre-requisite for development of pneumonia and invasive pneumococcal diseases. Colonization is typically asymptomatic and is resolved due to a dynamic and complex interplay between microbiota, host immune system and environmental factors. Working with a murine model of pneumococcal nasopharyngeal colonization, we have shown that IL-17A is a key cytokine in this process, since *Il17a*^{-/-} mice were persistently colonized for up to 6 months whereas wild type mice cleared colonization in 10 days. We are currently trying to elucidate the downstream mechanisms that may account for the phenotype showed in *Il17a*^{-/-} mice, including the production of specific antibodies, as well as the recruitment of innate cells and the expression of immune mediators in WT and *Il17a*^{-/-} mice. On the other hand, we have studied the role of IL-17A in the development of protective immunity against acute pneumococcal pneumonia. Previously, we showed that prior sublethal infection resulted in solid protection against invasive pneumonia which is associated with over expression of IL-17A together with the presence of Th17 cells in the lungs. However, *Il17a*^{-/-} mice showed same level of protection than WT, demonstrating that IL-17A by itself is not essential for protective immunity. Interestingly *Il17a*^{-/-} mice showed overexpression of other IL-17 related genes suggesting a complex network where compensatory effects may be occurring. Finally, we have developed and tested alternative immunotherapies against pneumococcal pneumonia, and have evaluated the role of IL17A in the protection afforded. Overall, we believe that deciphering the molecular basis of protective immunity will result in the development of new cost-effective immunotherapies against pneumococcal pneumonia.

TRANSFERRING METHODS FOR VACCINE RELEASE BETWEEN THE INDUSTRY, ACADEMY AND A REGULATORY AGENCY: LESSONS LEARNED

Elizabeth Carrasco-Caballero, Laboratorio Nacional para la Producción y Análisis de Moléculas y Medicamentos Biotecnológicos (LAMMB), Instituto de Biotecnología (IBT), Universidad Nacional Autónoma de México (UNAM), México.
carrasco@ibt.unam.mx

Momena Choudhury, Katia Esqueda, Manon Cox, Elena Semenova, Protein Sciences Corporation (PSC), USA.
Andrea Téllez-Galicia, LAMMB, IBT-UNAM, México
Sergio Valentinotti, Manuel Cardoso, Laboratorios Liomont, México.

A. Ruth Pastor, Laura A. Palomares. Laboratorio de Ingeniería de Bioprocesos y Nanobiotecnología. IBT-UNAM, México.

Key Words: Flublok, Regulatory Agency, SRID, Analytical methods.

Flublok, developed and manufactured by Protein Sciences Corporation (PSC), is the first recombinant influenza vaccine in the market, which was approved by the FDA in 2013.. In August 2014, Flublok was licensed to Laboratorios Liomont for the Mexican market and, potentially, other Latin American countries. In order to obtain approval in Mexico and begin commercialization, a joint team of PSC, Liomont and LAMMB formed an alliance for registering Flublok in Mexico and transferring the analytical methods needed for vaccine testing and release by CCAYAC and COFEPRIS, respectively,, which are the Mexican agencies responsible for vaccine commercialization control. Flublok was approved in Mexico in October 2015, and method transfers from PSC to LAMMB and CCAYAC began soon afterwards.. Several analytical methods are compendial methods or are routinely performed by CCAYAC, who also releases the traditional influenza vaccines for the Mexican market, but two methods -SRID and DNA- were identified as critical for vaccine release, and thus method transfer protocols were set in place. In this work, an account of the challenges and lessons learned during method and technology transfer between institutions from distinct fields -industry, academy and regulatory- , will be presented. After intense multi-institutional and multidisciplinary team work, method transfer was successfully performed between PSC and both Mexican organizations. This work set the basis for the commercialization of Flublok in Mexico for the 2016-2017 winter season.

UNMASKING STEM-SPECIFIC BROADLY NEUTRALIZING EPITOPES BY ABOLISHING N-LINKED GLYCOSYLATION SITES FOR VACCINE DESIGN

Suh-Chin Wu, Institute of Biotechnology, Department of Medical Science, National Tsing Hua University, Taiwan
scwu@life.nthu.edu.tw

Wen-Chun Liu, Institute of Biotechnology, National Tsing Hua University, Taiwan

Yun-Ju Huang, Institute of Biotechnology, National Tsing Hua University, Taiwan

Jia-Tsong Jan, Genomics Research Center, Academia Sinica, Taiwan

Key words: influenza vaccine, stem, broadly protective

Targeting highly conserved HA stem regions has been proposed as a useful strategy for designing universal influenza vaccines. The influenza virus HA stem region, consisting of a HA1 N-terminal part and full HA2 part, contains several potential sites for the addition of N-glycans. We expressed a series of recombinant HA (rHA) mutant proteins with deleted N-linked glycosylation sites in the HA1-stem and HA2-stem regions of H5N1 and pH1N1 viruses. Unmasking N-glycans in the HA2-stem region (rH5HA N484A and rH1HA N503A) did not affect the trimeric structure of HA. Immunizations using rH5HA N484A and rH1HA N503A elicited more potent neutralizing antibody titers against homologous, heterologous and heterosubtypic viruses. Unmasking the HA2-stem N-glycans of rH5HA N484A induced higher levels of stem-specific CR6261-like and FI6v3-like antibodies, improved the ability of stem-specific anti-fusion antibodies, enhanced H5 stem helix A epitope-specific B and T cell responses in splenocytes, and provided better protection against both homologous and heterosubtypic virus challenges. These findings suggest that HA2-stem N-glycan unmasking holds potential as a useful design strategy for developing more broadly protective influenza vaccines.

IMPROVED DIAGNOSTICS AND SURVEILLANCE IDENTIFY NOVEL REASSORTANT SWINE INFLUENZA A VIRUSES IN CHILE

Rafael A. Medina, Pontificia Universidad Católica (PUC), Facultad de Medicina, Chile
rmedinas@med.puc.cl

Barbara Brito, Universidad de Chile, Facultad de Cs. Veterinarias y Pecuarias, Chile

Rodrigo Tapia, Universidad de Chile, Facultad de Cs. Veterinarias y Pecuarias, Chile

Marco Saavedra, Pontificia Universidad Católica (PUC), Facultad de Medicina, Chile

Karla Tapia, Pontificia Universidad Católica (PUC), Facultad de Medicina, Chile

Montserrat Torremorell, College of Veterinary Medicine, University of Minnesota, USA

Victor M. Neira, Universidad de Chile, Facultad de Cs. Veterinarias y Pecuarias, Chile

Key Words: Swine Influenza virus, molecular epidemiology, influenza virus surveillance

Influenza A Virus (IAV) circulates endemically in nature, representing a constant concern to public health and animal production systems worldwide. The emergence of the 2009 H1N1 influenza A pandemic (A(H1N1)pdm09) highlighted the need of conducting systematic surveillance studies, and evidenced the significant gap of knowledge of the swine IAVs (SwIAV) circulating in pigs worldwide at the time of the outbreak. This also highlighted the role of swine as important reservoirs for the generation of endemic strains with zoonotic and pandemic potential. Despite this, comprehensive information of SwIAV circulating in Latin America is still lacking. While Chile has strong ecological barriers and high stringency controls regarding livestock trading, IAV is endemic and has been consistently detected in pigs. This has caused great concerns for the swine industry, because of the on-going clinical and production losses. Nevertheless, few surveillance efforts have been conducted in Chile and there is limited information regarding the genetic diversity and origin of swIAVs. Determining the viral subtypes and genotypes circulating in swine is key for the development of appropriate vaccine approaches and control measures for the swine industry, and it is crucial for identifying novel strains with pandemic potential. We developed improved diagnostic tools to detect endemic viruses and characterized the diversity and origin of SwIAV and its prevalence in Chilean swine production farms. From December 2013 to June 2015, we sampled 27 farms representative of intensive swine production systems located between the Valparaiso and Araucania administrative regions. Animals were mainly found to be susceptible to IAV infection at 50 to 110 days of age. Serological analysis of 718 serum samples obtained from 3 different farms showed 91-59% prevalence to SwIAV, with 18-67% positivity to A(H1N1)pdm09-like strains and 29-58% positivity to a novel SwH1N2 virus. We also obtained a total of 1016 nasal swabs (NS, 85%) and 176 oral fluids (OF, 15%) that were tested by Real-time RT-PCR (qPCR) and virus isolation. Of these, 295 samples (25%) were positive to swIAV (23% of NS and 38% of OF). A large number of farms (21 out of 27, 78%) were positive in at least one visit, and most farms showed temporal co-circulation of at least 2 viruses or mixed infections. We subtyped selected positive samples and sequenced 51 complete viral genomes using the Illumina platform. Nineteen additional hemagglutinins (HA) genes were sequenced by Sanger. We performed Bayesian Evolutionary Analyses to reconstruct the phylogenies of the viral segments. This identified the circulation of 2 predominant swIAV genotypes in Chile, the A(H1N1)pdm09-like (35%) strain and a novel SwH1N2 virus (45%). This H1N2 virus is unique to Chile since it is genetically distinct from the H1 virus clusters seen in North America and is not related to any previously reported IAV. The genome of this swH1N1 virus contains genes from 3 different human contemporary viruses. Its H1 and N2 genes are derived from human H1N1 and H3N2 viruses from the mid 90's, respectively, suggesting that both of these human viruses were likely introduced into the Chilean swine population during that time. All the internal genes are from the A(H1N1)pdm09 strain, indicating that multiple and recent reassortment events gave rise to this novel reassortant virus. Of interest, we identified additional reassortant viruses that also contain the internal genes derived from the A(H1N1)pdm09 strain. These included an H1N1 containing the novel SwH1 and NA gene derived from the A(H1N1)pdm09-like strain, an H1N2 virus containing an H1 derived from the A(H1N1)pdm09 strain and the novel SwN2, a SwH3N2 virus and a human-like H3N2 virus, among others. Additionally, our analyses also revealed at least 3 independent human-to-swine introductions of the A(H1N1)pdm09 strain within the last 4 years in Chile. Overall this indicates that close human-swine interactions greatly contribute to the genetic diversity and emergence of IAVs in Chilean swine. This is the first comprehensive molecular epidemiological study of swIAV in Chile demonstrating the co-circulation of multiple viral strains in intensive swine production systems. Our data emphasizes the value of conducting long-term SwIAV surveillance in Latin America, a poorly studied region of the world.

Acknowledgements: CEIRS program: HHSN266200700010C, HHSN272201400008C from NIH-NIAID, FONDEF IDeA Grant ID14I10201 and Proyecto Anillo de Investigación en Ciencia y Tecnología - PIA ACT1408, both from CONICYT Chile.

FIRST CHARACTERIZATION OF IMMUNOGENIC CONJUGATES OF VI NEGATIVE SALMONELLA TYPHI O-SPECIFIC POLYSACCHARIDES WITH REPA PROTEIN FOR VACCINE DEVELOPMENT

Salman, M, Vaccine Program, Human Health Therapeutics Portfolio, National Research Council, Canada ; Health Biotechnology Division, National Institute for Biotechnology, Pakistan ; Department of Biotechnology, Abdul Wali Khan University, Pakistan
s.amazai@yahoo.com

Ali, A., Health Biotechnology Division, National Institute for Biotechnology, Pakistan

Rahman, M., Health Biotechnology Division, National Institute for Biotechnology, Pakistan

Iqbal, M., Health Biotechnology Division, National Institute for Biotechnology, Pakistan

Haque, A, Department of Pathology, The University of Faisalabad, Pakistan

Cox, A. D, Vaccine Program, Human Health Therapeutics Portfolio, National Research Council, Canada

Keywords: Salmonella Typhi Vi negative; OSP antigen; Conjugate vaccines; rEPA; typhoid; immunogenicity

Efficacious typhoid vaccines for young children will significantly reduce the disease burden in developing world. The Vi polysaccharide based conjugate vaccines (Vi-rEPA) against Salmonella Typhi Vi positive strains has shown high efficacy but may be ineffective against Vi negative S. Typhi. In this study, for the first time, we report the synthesis and evaluation of polysaccharide-protein conjugates of Vi negative S. Typhi as potential vaccine candidates. Four different conjugates were synthesized using recombinant exoprotein A of *Pseudomonas aeruginosa* (rEPA) and human serum albumin (HSA) as the carrier proteins, using either direct reductive amination or an intermediate linker molecule, adipic acid dihydrazide (ADH). Upon injection into mice, a significantly higher antibody titer was observed in mice injected with conjugate-1 (OSP-HSA) ($P = 0.0001$) and conjugate 2 (OSP-rEPA) ($P = <0.0001$) as compared to OSP alone. In contrast, the antibody titers elicited by conjugate 3 (OSP_{ADH}-HSA) and conjugate 4 (OSP_{ADH}-rEPA) were insignificant ($P = 0.1684$ and $P = 0.3794$, respectively). We conclude that reductive amination is the superior method to prepare the S. Typhi OSP glycoconjugate. Moreover, rEPA was a better carrier protein than HSA. Thus OSP-rEPA conjugate seems to be efficacious typhoid vaccines candidate, it may be evaluated further and recommended for the clinical trials.

DEVELOPMENT OF A PRODUCTION PROCESS FOR A RECOMBINANT PROTEIN PNEUMOCOCCAL VACCINE

Ana Maria P. Santos; Bio-Manguinhos, Oswaldo Cruz Foundation (FIOCRUZ)
anamaria@bio.fiocruz.br

Mariana Miguez; Bio-Manguinhos, Oswaldo Cruz Foundation (FIOCRUZ)

Ana Paula Argondizzo; Bio-Manguinhos, Oswaldo Cruz Foundation (FIOCRUZ)

Maria Helena Rocha-Leão, Escola de Química, Federal University of Rio de Janeiro (UFRJ)

Key Words: PsaA, recombinant vaccine, purification, pneumococcal vaccine, chitosan

Pneumonia represents important infection in young children (under five years old). Even with efficient vaccines, this disease was responsible for 992,000 deaths in 2015 and it was globally equivalent to 15% of all deaths in children. Bio-Manguinhos/Oswaldo Cruz Foundation (Brazil), supplies in their National Immunization Program the 10-valent pneumococcal vaccine. This vaccine contains 10 of the 92 serotypes of this pathogen, and it's obtained through fermentations and purifications of the capsular polysaccharides of each serotype, followed by chemical couplings to specific carrier proteins. This work presents a proposal for industrial process of only one recombinant protein antigen, the PsaA (pneumococcal surface adhesion A with 37 kDa), potentially capable to act against most of all prevalent serotypes of *streptococcus pneumoniae*. Based on protein expression in prokaryotic system and purification, this process reached high purity levels and yield superior to conventional fermentation systems. Biomass was obtained in bench scale reactor and studies of expression times verified that it could be reduced in 60% from presented previously. Moreover, in purification steps was observed good resolution using anion exchange (DEAE Sepharose FF®). For desalting, it was observed that gel filtration technique could be replaced for tangential cross flow filtration, better to scale-up, and using 10 kDa membranes. The developed process resulted in overall yield of 35-40%, based in BCA analysis. Electrophoretic analysis (SDS-PAGE) of the purified sample showed a band with molecular weight of 37 kDa corresponding to 80% of identified proteins. As formulation proposal, chitosan in different concentrations were used to prepare capsules of the antigen resulting in encapsulation efficiency between 26 and 36% that can be evaluated as an intranasal delivery system. This data show that the methodology developed is promising for use in industrial process aimed at obtain a recombinant vaccine.

DEVELOPMENT OF A HIGH-YIELD PURIFICATION PROCESS FOR THE PRODUCTION OF INFLUENZA VIRUS VACCINES

Hyung-Jin Jeon, Purification Process Team 3, Central Research Center, Green Cross. Corp., Korea
intiin@greencross.com

Mi-Hyang Cho, Purification Process Team 3, Central Research Center, Green Cross. Corp., Korea
Mi-Suk Kim, Purification Process Team 3, Central Research Center, Green Cross. Corp., Korea
Eun-Young Cho, Purification Process Team 3, Central Research Center, Green Cross. Corp., Korea
Soo-In Kim, Cell Culture Process Team 2, Central Research Center, Green Cross. Corp., Korea
Jae-Hoon Moon, Purification Process Team 3, Central Research Center, Green Cross. Corp., Korea
Dong-Ho Ahn, Vice president, Central Research Center, Green Cross. Corp., Korea

Key Words: Influenza virus vaccine, Animal cells, Hemagglutinin (HA), Benzonase, Column chromatography

Production of influenza virus in animal cells has emerged as an alternative to conventional platforms such as egg-based production system. Animal cells, especially MDCK and VERO cell lines, are widely used as the primary production cell for influenza virus vaccine because of their high susceptibility to infection with various influenza viruses. Recently, a robust and reliable purification process was successfully developed for the production of quadri-valent HA proteins (from two strains of the type A virus and two strains of the type B virus) by using animal cell-based production system in Green Cross Corp., Korea. The UF/DF process, Benzonase treatment at high temperature as well as column chromatography strategy was optimized to maximize the final HA production yields. Benzonase treatment was conducted to reduce in hcDNA (host cell DNA) because hcDNA was main impurity for cell-based influenza virus vaccine. A simple and stable UF/DF process has been tested with membrane molecular weight cutoffs of 100 and 300 kDa as well as 0.2 and 0.45 μm microfiltration membrane. Anion exchange chromatography (AEC) and size exclusion chromatography (SEC) were selected for acceptable reduction in hcDNA and HCP. AEC was used to separate hcDNA from virus at a salt concentration of 0.5 M sodium chloride. The HA yield through AEC & SEC combination process was sufficiently achieved under specific purification process condition. Overall, the amount of residual hcDNA was reduced to an acceptable level (10ng/dose) and the increased HA yield was maintained throughout the whole process. The performance, productivity and scalability of the purification process were successfully demonstrated in over 30 GMP batches using 4 different influenza virus strains.

SIMPLE AND ROBUST DOWNSTREAM PURIFICATION PROCESS FOR CELL-DERIVED INFLUENZA VACCINES

Yu-Fen Tseng, National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Taiwan

yufentseng@nhri.org.tw

Tsai-Chuan Weng, National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Taiwan

Chia-Chum Lai, National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Taiwan

Alan Yung-Chih Hu, National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Taiwan

Key Words: Influenza, vaccine, cell culture, purification, chromatography

New emerging influenza viruses with pandemic potentials were occurred in recent years, e.g. H5N1 in 1997, H1N1 in 2009, and H7N9 in 2013. The demand of producing pandemic influenza vaccines for human use with quick supply is high. For the cell-based pandemic influenza vaccines, we proposed a flow-through chromatography purification process. This process has only involved few purification steps and is easy to operate. Vero- and MDCK- cell derived avian influenza viruses including H5N1 and H7N9 were purified efficiently by the process proposed.

The presented purification process consisted of clarification, inactivation, concentration, anion exchange chromatography (Capto Q), size exclusion and adsorption chromatography (Capto Core 700), diafiltration and sterile filtration. In the chromatography steps, cell DNA and protein were removed remarkably, and the virus were flowed through these columns. The flow rate was set as fast as 250 cm/min. The loading volume of virus solution was up to 50 times of column volume (CV). The DNA was removed over 90% after using Capto Q column, and was further removed by Capto Core 700 column. The overall removal rate of cellular DNA was more than 99%. The HA recovery rates of H5N1 and H7N9 influenza virus from Vero and MDCK cells were 20 to 40%. The DNA concentration of all purified bulks met the regulatory requirement of 10ng per dose. The developed purification process is simple and efficient, and it is suitable for purification of various influenza virus strains and can be used for the pandemic influenza vaccine production.

SYNGEM: AN INTRANASAL PREFUSION-LIKE RSV F SUBUNIT VACCINE

Maarten van Roosmalen, Mucosis BV, Groningen, Netherlands
roosmalen@mucosis.com

Bert Jan Haijema, Mucosis BV, Groningen, Netherlands

Kees Leenhouts, Mucosis BV, Groningen, Netherlands

Peter J.M. Rottier, Utrecht University, Netherlands

Cornelis A.M. de Haan, Utrecht University, Netherlands

Respiratory syncytial virus (RSV) is an important cause of respiratory tract disease in (naive) young infants, older infants, the elderly and immune-compromised. Despite the medical need and the market potential, no licensed vaccine is available. Mucosis B.V. is a Dutch biotech company developing innovative mucosal vaccines, based on the Bacterium-Like Particles (BLP) technology. Such vaccines can be administered needle-free, e.g. through the nasal mucosa. In support of the mucosal approach, there is accumulating evidence that RSV F-specific local S-IgA antibodies secreted in the upper respiratory tract of humans correlate well with protection.

Because of its ability to induce broadly neutralizing antibodies the RSV F protein is the most attractive antigen. The current view is that in particular serum antibodies directed against the prefusion form of RSV F belong to the most potent neutralizing antibodies and the ability to elicit these is a pivotal attribute for a successful RSV vaccine. We studied different variants of F with respect to their conformation using neutralizing monoclonal antibodies (mAbs), following the view that F proteins mimicking the meta-stable prefusion form of F expose a more extensive and relevant epitope repertoire than F proteins corresponding to the postfusion F structure. Both addition of a trimerization motif and mutation of the furin cleavage sites increased the reactivity of F with the prefusion-specific mAb D25, with the highest reactivity being observed for F proteins in which both these features were combined. This candidate antigen, called Flys-GCN, is suitable in the development of mucosal as well as intramuscular RSV vaccines.

Here we describe the development of our intranasal candidate RSV vaccine, SynGEM, which is based on the validated BLP technology. The non-living BLPs allow for presentation of stable, trimeric prefusion-like RSV F proteins bound to the particle surface. Intranasal vaccination of naïve and convalescent mice with SynGEM induced long-lasting virus neutralizing RSV-specific serum IgG and robust levels of local IgA. Cotton rats immunized intranasally with SynGEM were protected upon RSV challenge, as represented by a low viral load in the lungs. Enhanced levels of pre- versus postfusion specific antibodies were observed in individual animals, confirming the ability of the SynGEM vaccine to induce potent neutralizing antibodies. A clinical Phase I study with intranasal SynGEM is planned for 2016.

CHALLENGES IN THE CONSTRUCTION OF A MULTI-PRODUCT VACCINE FACILITY

Rachel Appetiti, Sanofi Pasteur, France
rachel.appetiti@sanofipasteur.com
Fabien Barbirato, Sanofi Pasteur, France
Gaëlle Bernard, Sanofi Pasteur, France
Vincent Dalbès, Sanofi Pasteur, France
Catherine Jourdat, Sanofi Pasteur, France
Nadège Leray, Sanofi Pasteur, France
Grégory Piquet, Sanofi Pasteur, France
Christelle Plantard, Sanofi Pasteur, France
Nadège Poisson, Sanofi Pasteur, France

Key words: building, fermentation, process, regulatory affairs, troubleshooting, validation.

A new multi-product vaccines facility in Sanofi Pasteur was designed and equipped with new state-of-the-art technologies. The construction of this building aims to increase capacity of producing pediatric vaccines and allowed to implement manufacturing process improvements, increase quality compliance level and addressed environmental, and safety concern. This new facility harbors three antigens processes from fermentation to purification and detoxification.

The project has been challenging on different points: new equipment, process transfer issues, new qualification/validation strategy and regulatory registration. Apart from global feedbacks on costs, organization, resources, performance and authorities communication strategies, focus was also directed towards the resolution of a process issue during validation steps. A trouble-shooting group has been mobilized to work on the different axes with a specific method.

Resolution of all the issues permitted the building registration, and therefore the vaccination of millions of children.

LONGITUDINAL LANDSCAPES OF SERUM ANTIBODY REPERTOIRES AFTER INFLUENZA INFECTION AND VACCINATION

Jiwon Lee, Depart. of Chemical Engineering, University of Texas at Austin, Austin
g1lee@utexas.edu

Daniel R. Boutz, Center of Systems and Synthetic Biology, University of Texas at Austin, Austin
Andrew P. Horton, Center of Systems and Synthetic Biology, University of Texas at Austin, Austin
Jonathan R. McDaniel, Depart. of Chemical Engineering, University of Texas at Austin, Austin
Erik L. Johnson, Depart. of Chemical Engineering, University of Texas at Austin, Austin
Alexander Frühwirth, Institute for Research in Biomedicine, Switzerland.
Leontios Pappas, Institute for Research in Biomedicine, Switzerland.
Davide Corti, Institute for Research in Biomedicine, Switzerland.

Edward M. Marcotte, Center of Systems and Synthetic Biology, University of Texas at Austin, Austin
Gregory C. Ippolito, Depart. of Molecular Biosciences, University of Texas at Austin, Austin
Antonio Lanzavecchia, Institute for Research in Biomedicine, Switzerland.
George Georgiou, Depart. of Chemical Engineering, University of Texas at Austin, Austin

Key Words: serological repertoire, proteomics, bNAbs, longitudinal analysis

Vaccination is the most effective means of infectious disease prevention. Despite its success, however, we still lack a clear understanding of vaccine responses in humans. For example, influenza vaccines still leave a large fraction of population vulnerable. Over the past decade, single B-cell analysis and next-generation sequencing (NGS) technologies have become invaluable tools for studying the antibody repertoire to influenza. Such studies have led to discoveries of broadly-neutralizing antibodies (bNAbs), which can neutralize across multiple strains of influenza virus, promoting the notion of designing a universal vaccine that will elicit such antibodies. One of such isolated bNAbs, called FI6, showed remarkable ability to neutralize all of the influenza A virus strains through targeting the conserved epitope in the stem of hemagglutinin (HA). However, it remains unclear whether such bNAbs actually play a role in conferring protection against influenza since antibody proteins (not B-cells) need to circulate at physiologically relevant concentrations in serum to have implications in protection. Using high-resolution proteomics coupled with NGS, we quantitatively determined the serological antibody repertoire to CA09 HA (H1) at the individual clonotype-level in a donor (whom FI6 was isolated from) following influenza infection (in 2010 with pandemic CA09) and vaccination across five years (2010-2014 with seasonal flu vaccine). We analyzed the temporal changes of head-targeting and stem-binding antibodies, illustrating the gradual increase of stem-targeting antibodies following repeated exposures to CA09 HA. Following vaccination in 2014, >60% of the repertoire consisted of one single clonotype of stem-binding antibody that was present at very low abundance in 2010. Our data demonstrate that the repetitive exposure to influenza skews the serological repertoire toward antibodies that target conserved epitopes, and these antibodies continue to be boosted every time the same epitopes are encountered. Once elicited, stem-binding antibodies displayed a tendency to persist in serum across multiple years while head-specific antibodies decayed quicker. The differential longevity of stem-binding and head-specific antibodies presented here has direct implications for the design of the future universal vaccine.

MICROCARRIER-BASED PRODUCTION OF DENGUE VIRUS IN AN OPTIMIZED ANIMAL-FREE VIRUS PRODUCTION MEDIUM

Grishma Patel, Pall Corporation, Ann Arbor, MI
mark_szczyпка@pall.com;

Mark Szczyпка, Pall Corporation, Ann Arbor, MI
Steve Pettit, InVitria, Fort Collins CO

Claire Y. H Huang, US Center for Disease Control (CDC-DVBD), Fort Collins, CO

Key Words: Microcarriers, dengue, vaccine, animal component free

Microcarriers have been a mainstay production platform for vaccine production for decades. They are used for production of both human and animal vaccines. However, many processes still use animal derived components in their manufacturing processes. The use of undefined components in animal product-free (APF) cell culture media is one source of variability in cell culture processes. Therefore a production medium optimized for use with microcarrier-based production systems would be desirable. In these studies, a hydrolysate-free and APF, Vero cell production medium that is ideal for use in stirred-tank vessels with Pall SoloHill® microcarriers was developed. During development, medium formulations were optimized through an iterative process in concert with various microcarrier types to support robust cell growth and virus production in small-scale spinners. Further optimization then occurred in larger bioreactors.

The medium supports sustained, high-density cell growth on multiple types of APF SoloHill® microcarriers over a seven day expansion cycle without need for medium exchange. Virus production has been demonstrated to be equal to or greater than DMEM containing FBS and two different commercially-available serum-free media. The peak of wild type dengue 2 virus production advances up to 3 days earlier in microcarrier culture when compared to static conditions, and cumulative titer is increased.

Vero cells achieve cell densities of >3 M cells/mL without a medium exchange (batch culture) and maintain cell growth for 8-9 days. In mock infections, the medium enables cell densities of up to 6 M cells/mL in bioreactors.

INCREASING PROCESS PRODUCTIVITY FOR AN ANTIBODY-BASED CANCER VACCINE

Gabriel E. Rivera, Center of Molecular Immunology, Cuba
gabrielr@cim.sld.cu

Maydel Castillo, Center of Molecular Immunology, Cuba

Dayana Aguilar, Center of Molecular Immunology, Cuba

Idelmis Gonzalez, Center of Molecular Immunology, Cuba

Ernesto Chico, Center of Molecular Immunology, Cuba

Keywords: hybridoma cells, sodium acetate, cancer vaccine, apoptosis, perfusion

Racotumumab is a monoclonal antibody mimicking tumor associated antigens that has been used to treat lung cancer patients in a therapeutic vaccination approach. This antibody has proved the capacity to elicit an immune response against the tumor cells, when injected together with an adjuvant. The antibody is obtained from hybridoma cells using a perfusion process. In this work we explore the use of sodium acetate to increase the productivity of racotumumab perfusion process. Increasing concentrations of sodium acetate (5 – 15 mM) were evaluated first in batch cultivation to understand the effect on the physiology and secretion rates of the hybridoma cells. Further evaluation was carried on perfusion runs performed at small scale. Very low toxic effect was found for sodium acetate concentrations below 15 mM in batch culture. The negative effect of sodium acetate was even lower at perfusion culture, when no signal of apoptosis induction was found. Overall increase on specific production rate (qp) reached 2 – 4X compared to control experiments, but not a clear trend was found supporting the idea of the higher the sodium acetate concentration the higher the qp-increase. In order to understand the stimulatory effect of sodium acetate we speculate over the need of a ratio of sodium acetate availability in the culture broth and the cell concentration. By controlling this ratio we may enhance the stimulatory effect of sodium acetate without inducing significant cell death.

PATHOGEN GENETIC DIVERSITY A CHALLENGE FOR VACCINE DEVELOPMENT: LOOKING FOR THE PATHOGEN'S ACHILLES' HEEL

Ousmane A. Koita, Laboratory of Applied Molecular Biology, University of Science, Techniques and Technologies of Bamako, Mali

oakoita@yahoo.com

Annie S. DeGroot, Epivax, Inc. Providence ; Institute for Immunology & Informatics, University of Rhode Island, USA

Creation of variant forms has serious consequences in diagnostic, treatment strategies and the future vaccine development. Thus, the actual and future roles of the altered or emergent pathogens in the global pandemic of AIDS, Malaria, Flu and Ebola must be monitored in new molecular epidemiological studies. During the last 5 years, we studied the genetic structure of several pathogens such as 1] malaria parasite showing that gene deletion, recombination can occur and lead to false RDT negative and to the creation of new antigens (hybrid parasites); 2] in HIV, our findings indicate a shift in the virus population circulating over time in Mali. Those observations are suggesting that a vaccine development against those pathogens such *Plasmodium falciparum* parasite and HIV will be a challenge. Our approach that is to target pieces of antigens within a genome which must be well conserved across the specie and immunogenic enough in boosting the immune response. Four steps were identified in that approach which are: 1] Genome mining using computational and experimental tools to identify genes that encode proteins with promising vaccine antigens properties, 2] use of the Immunoinformatics tools to map protein sequences for short, linear putative T-cell epitopes CTL/ T helper, 3] then candidates are synthesized as peptides and evaluated for HLA binding and antigenicity (*in vitro* evaluation) and then 4] Prototype epitope-based vaccines are evaluated for immunogenicity in human Host (*in vivo* evaluation). Our laboratory has partnered with the GAIA foundation to test this approach in Mali.

**FINDING THE MISSING LINK IN COMPLETE SINGLE USE FERMENTATION LINE:
EVALUATION OF SINGLE USE LARGE SCALE BIOREACTORS FOR AEROBIC BACTERIA**

M.Monnet, Sanofi Pasteur, France
marjorie.monnet@sanofipasteur.com
Marie Izac N.Chaudet, Sanofi Pasteur, France
G.Exbrayat, Sanofi Pasteur, France
JM.Guillaume, Sanofi Pasteur, France

Key Words: Disposable, new technology, fermentation.

The application of single use technologies for the bioproduction represents a major technology innovation advance. Single use bioreactors for cell culture have been largely implemented for manufacturing, providing significant flexibility, costs reduction and fast implementations. In contrast, few technologies are available for aerobic bacterial fermentation mainly due to limitations for oxygen and heat transfer. Like clinical products derived from bacterial expression system still represent a significant number of bio therapeutics/vaccines, some investigations have been performed in Sanofi Pasteur to evaluate new single use fermenters suitable for aerobic process and matching to clinical manufacturing scale. The 300L HyPerforma SUF (ThermoFisher) has been evaluated with an *E.coli* strain to produce a recombinant protein and with yeast.

METABOLIC DRIVERS OF IC-BEVS PRODUCTIVITY: TACKLING THE PRODUCTION OF ENVELOPED VIRAL PARTICLES

Francisca Monteiro, iBET, Oeiras, Portugal; Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa , Oeiras, Portugal
fmonteiro@itqb.unl.pt

Vicente Bernal, Universidad de Murcia, Murcia, Spain; Current address: Centro de Tecnología de REPSOL, Móstoles-Madrid, Spain

Paula M. Alves, iBET, Oeiras, Portugal; Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa , Oeiras, Portugal

Key Words: Baculovirus expression vector system; insect cells; metabolic flux analysis; metabolomics; bioprocess optimization; influenza VLPs.

The Insect Cell-Baculovirus Expression System (IC-BEVS) has a major track record for the production of recombinant proteins and vaccines. Although its widespread use, the physiological aspects that contribute to systems productivity are still to be fully disclosed.

In the present work, the metabolic features of the two main insect host cell lines, Sf9 and High Five, were analyzed during cellular growth and after baculovirus infection for the production of enveloped Influenza VLPs (Inf-VLPs). The gathered data were contextualized in a metabolic network representative of central carbon and nitrogen metabolism. Metabolic Flux Analysis (MFA) was performed to have a quantitative overview of the cellular fluxome dynamics that followed infection. In addition, the main carbon sources that contributed most to flux activity were identified. The impact of baculovirus infection on the physiology of High Five and Sf9 host cell lines was assessed by metabolomics, aiming at the identification of metabolic markers of productivity. The information herein generated was used to design tailored supplementation schemes that could boost IC-BEVS production yields of two enveloped viral particles: influenza VLPs (Inf-VLP) as a vaccine candidate and the recombinant baculovirus (BV).

The strong correlation observed between the metabolic state of the host cell and baculovirus infection highlights the capacity of this virus to act as a metabolic engineer, re-directing the cellular fluxome to support virus replication and production. The results also show that the viral load influence the cellular responsiveness to the supplements, with lower MOIs retrieving higher improvements in specific productivity. The careful selection of the MOI, along with the supplementation of culture medium with compounds altering cellular redox state and cholesterol metabolism, yielded a 6-fold improvement of specific productivity. These results pave the way to deepen our knowledge on the relationship between host cell and virus, contributing to the disclosure of the metabolic determinants that contribute to productivity.

IMMUNIZATION WITH SURFACE IMMUNOGENIC PROTEIN INDUCES A DECREASE OF VAGINAL COLONIZATION BY GROUP B *STREPTOCOCCUS* IN AN EXPERIMENTAL MOUSE MODEL

Jorge A. Soto^{1,2,3}, jorge.sotoramirez@gmail.com Diego Diaz-Dinamarca², Daniel A. Soto¹, Robert Rojas⁴, Alexis M. Kalergis^{2,3} and Abel E. Vasquez^{1,2*} avasquez@ispch.cl

¹Sección de Biotecnología. Instituto de Salud Pública de Chile, Santiago, Chile.

²Millennium Institute on Immunology and Immunotherapy.

³Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.

⁴Centro de Genómica y Bioinformática. Facultad de Ciencias, Universidad Mayor, Santiago, Chile

Keywords: *S. agalactiae*, SIP, Vaccine, Vaginal colonization.

The Group B streptococcus (GBS) is the leading cause of neonatal sepsis and meningitis in developed countries and an emerging pathogen in adults. A neonatal infection occurs predominantly during the delivery by either inhalation or ingestion of contaminated secretions of the mother's vagina. Maternal screening by rectovaginal GBS colonization at 35–37 weeks of gestation, with subsequent intra-partum antibiotic prophylaxis (IAP) at the onset of labor, is implemented in some countries to prevent newborn invasive by GBS. Currently, there are not vaccines to prevent the devastating consequences of GBS and a glycoconjugate vaccine is under clinical experimentation (Clinical Trials Phase III).

The Surface Immunological Protein (SIP) of GBS is highly immunogenic and conserved between different serotypes of this bacterium. The SIP had been described to induce antibodies type IgG that induces protective immunity in animal model challenged intraperitoneally with GBS.

Here we describe the immunization with SIP mixed with an AbISCO-100 adjuvant in mice model challenged to GBS vaginal infection. The vaccine has demonstrated to decrease the GBS colonization in infected mouse. The SIP immunization has also increased the circulating IgA, IgG and IgG2a levels against SIP and antigen-specific circulating levels of IFN- γ and IL-2. Moreover, transfer of serum and T cells from a vaccinated animal into a non-immunized animal induced immune protection to the animals from challenged GBS colonization of the vaginal tract. In conclusion, we have demonstrated that a simple and effective vaccine is able to prevent GBS colonization, where cellular immunity plays an important role. To our knowledge, is the first report the SIP-based vaccine reduces the vaginal GBS colonization in an animal model.