X SIMPOSIO LATINOAMERICANO DE TECNOLOGÍAS EN CULTIVOS CELULARES

LIBRO DIGITAL

13-16 Noviembre
Viña del Mar
Chile

TABLA DE CONTENIDOS

BIENVENIDA

Estimados colegas, investigadora/es y amiga/os,

Nos complace darles la bienvenida al **X Simposio Latinoamericano de Tecnología de Cultivos Celulares (SLATCC)**, que se desarrollará entre el 13 y el 16 de noviembre del 2024 en la ciudad de Viña del Mar, Chile.

El SLATCC ha venido realizándose en forma bianual desde 2004, acumulando una trayectoria de 20 años enfocada en alentar el intercambio científico entre investigadores vinculados tanto al ámbito académico, empresarial y público.

El SLATCC también busca generar las condiciones para un encuentro propicio de experiencias de trabajo y resultados relacionados con las diversas esferas de aplicación de los cultivos celulares. De esta forma, el SLATCC ha representado una instancia de excelencia para la difusión de conocimiento y la formación de personas altamente capacitadas en este ámbito de la biotecnología.

La primera edición del Simposio tuvo lugar en la Universidad Federal do Rio de Janeiro, Brasil, en la que se realzó la necesidad de contar con un espacio para discusión de proyectos afines, así como la consolidación del intercambio científico-técnico. De manera acorde a los intereses académicos que motivaron al SLATCC desde sus inicios, las siguientes ediciones se realizaron en São Paulo (Brasil, 2006), La Habana (Cuba, 2008), Montevideo (Uruguay, 2010), Santa Fe (Argentina, 2012 y 2020), Valparaíso (Chile, 2014), Coyoc (México, 2016), Río de Janeiro (Brasil, 2018), y ha reunido a la comunidad, principalmente, hispana que trabaja en tecnología de células animales, biofarmacéuticos, vacunas, terapias celulares y genéticas y áreas relacionadas.

En esta oportunidad, las presentaciones orales, conferencias y exhibiciones de póster estarán estructuradas en torno a las siguientes áreas temáticas:

1.Desarrollo de líneas celulares para la producción de proteínas recombinantes.
2.Metabolismo celular y optimización de medios de cultivo.
3.Bioprocesos: Upstream – Downstream.
4.Desarrollo de bioterapéuticos y control de calidad.
5.Terapia génica y terapia celular.
6.Acelular: secretoma, microvesículas, partículas y vacunas.

De esta manera se abordan temáticas complejas e interrelacionadas, cuya relevancia es clave para el incremento de los procesos de formación, investigación y desarrollo vinculados al campo de los cultivos celulares y producción de bioterapéuticos.

BIENVENIDA

El comité organizador ha dispuesto grandes esfuerzos en la realización del Simposio, para garantizar la continuidad del alto nivel académico que caracteriza al evento y brindar a todos los participantes una agradable estadía en la ciudad de Viña del Mar. Una ciudad definida por el buen gusto, las actividades al aire libre, la recreación y la gastronomía de alto nivel. Su extensa costanera y herencia arquitectónica patrimonial monumental la han convertido en una de las capitales turísticas más atractivas del litoral central de Chile.

A nombre del comité organizador del X SLATCC, queremos expresar nuestro agradecimiento a las entidades que brindaron patrocinio y auspicio al evento: **Agencia Nacional de Investigación y Desarrollo de Chile, a través de diverso fondos concursables de I+D, Pontificia Universidad Católica de Valparaíso, ESACT (European Society for Animal Cell Technology), Biotech Academy in Rome, Centro Basal IMPACT, Centro de Biotecnología SOFOFA HUB, InES Género e InES I+D PUCV, Ingeniería PUCV 2030, Science UP y Electronic Journal of Biotechnology. Asimismo, agradecemos a nuestros destacados auspiciadores en categoría Perfusión: AP-Bioprocess, Arquimed, GeneX-Press, GrupoBios, MERCK, Platech, Sartorius-Dilaco, Solventum y Thermofisher; Categoria Fed-bach: CellZion, Galenica y VectorBuilder; y Categoria batch: BioSystem, Izon Science y Veterquímica.**

Reconocemos que el X SLATCC representa una instancia única para el fortalecimiento de los lazos de la comunidad biotecnológica de América Latina, lo que nos ha permitido desarrollar una red de colaboración potente y solidaria a nivel regional. El desafío que enfrentamos hoy, es la formalización de esta red mediante la implementación una propuesta institucionalizada que consolide y posicione a nivel LATAM el quehacer de la comunidad SLATCC.

Por último, deseamos que este evento sea una experiencia memorable, sobre todo en el intercambio científico, y les animamos a disfrutar de una agradable estadía en esta hermosa Ciudad Jardín.

Dra. Claudia Altamirano Gómez

Organizadora X SLATCC 2024

Profesora Titular, Escuela de Ingeniería Bioquímica,

Pontificia Universidad Católica de Valparaíso

COMITÉ CIENTÍFICO

Dr. Julio Berríos
Pontificia Universidad Católica de Valparaíso

Dra. Ziomara Gerdtzen
Universidad de Chile

Dra. María Carmen Molina
Universidad de Chile

Dr. Jorge Toledo
Universidad de Concepción

COMITÉ EJECUTIVO

Dra. Alejandra Arancibia
Pontificia Universidad Católica de Valparaíso

Dra. Paloma Fuentes
Pontificia Universidad Católica de Valparaíso

Dra. Carla Gutierrez
Pontificia Universidad Católica de Valparaíso

Dra.(c) Constanza Núñez
Pontificia Universidad Católica de Valparaíso

Dra. Ana María Vega
Pontificia Universidad Católica de Valparaíso

Dr. Mauricio Vergara
Pontificia Universidad Católica de Valparaíso

EXPOSITORES INTERNACIONALES









Dra. Paula Alves

Universidad NOVA de Lisboa. Portugal.

La Dra. Paula Alves, CEO de iBET, es miembro de la Iniciativa de Medicamentos Innovadores (IMI por sus siglas en ingles) y profesora en la Universidad NOVA de Lisboa. Con un Ph.D. en Ingeniería Bioquímica y una Licenciatura en Bioquímica, la Dra. Paula Alves fue elegida miembro de la Academia Nacional de Ingeniería de EE.UU. en 2021.

Desde 1990, la Doctora Paula trabaja en Tecnología de Células Animales, integrando el metabolismo celular con la ingeniería bioquímica para mejorar la eficiencia de los bioprocesos. Su experiencia abarca la producción de biofármacos, como vectores virales y vacunas, y el desarrollo de sistemas de cultivo 3D para terapia celular y medicina regenerativa.

Fue presidenta de ESACT (2017-2022) y actualmente es miembro de comités asesores internacionales y europeos. Paula ha publicado más de 310 artículos y ha recibido varios premios, incluyendo el Premio al Mérito Científico y la Medalla de Oro de Oeiras.

Dra. Renata Alvim

Universidad Federal do Rio de Janeiro. Brasil.

La Dra. Renata Alvim posee una maestría en Ingeniería de Procesos Químicos y Bioquímicos y un Doctorado en Ingeniería Química, ambos obtenidos en la Universidad Federal de Río de Janeiro (UFRJ).

Actualmente se desempeña como Profesora Titular en la UFRJ y desde el 2012 trabaja como investigadora en el Laboratorio de Cultivo Celulares (COPPE/UFRJ).

Su investigación se centra en los procesos previos a la producción de proteínas recombinantes, incluyendo la generación de líneas celulares recombinantes y el desarrollo de procesos de alimentación por lotes y perfusión utilizando biorreactores de tanque agitado.

Dra. Mariela Bollati

Institut Pasteur de Montevideo. Uruguay.

La Dra. Mariela Bollati es Bioquímica y Doctora en Ciencias Biológicas de la Universidad Nacional del Litoral, situada en Santa Fe, Argentina. Realizó su formación postdoctoral en el grupo de Inmunología Experimental del Centre for Infection Research en Braunschweig, en Alemania.

Desde el 2006, se desempeña como responsable de la Unidad de Biología Celular en el Institut Pasteur de Montevideo, Uruguay.

Su principal área de investigación ha sido el diseño y generación de líneas celular recombinantes de interés en la biomedicina y en la biotecnología. Entre ellas se encuentran una gran variedad de líneas celulares reporteras como NF-κB, IFN de tipo I y biosensores REDOX.

Dr. Ernesto Chico

Universidad Tecnológica de La Habana. Cuba.

El Dr. Ernesto Chico es Ingeniero Químico y miembro del grupo fundador del Centro de Inmunología Molecular de La Habana, Cuba. Durante una década en este Centro fue Director Técnico y de operaciones.

Obtuvo su Doctorado en Ciencias Técnicas y es Profesor titular adjunto en la Facultad de Ingeniería Química de la Universidad Tecnológica de La Habana (CUJAE) donde imparte materias de pregrado y postgrado.

Ha desarrollado y escalado procesos productivon que han resultado en la obtención de medicamentos de alto impacto clínico y económico, como la eritropoyetina humana recombinante, el anticuerpo nimotuzumab y la vacuna contra el cáncer CIMAvax. Parte de su actividad profesional ha incluído el diseño, construcción y puesta en marcha de plantas biotecnológicas en varios países incluyendo Cuba, China, India, Brasil y Tailandia.

Dr. Alan Dickson

University of Manchester. Reino Unido.

El Dr. Alan Dickson es parte del comité directivo de **BioProNET2**, una iniciativa que fomenta de manera activa la colaboración entre la academia y la industria en el Reino Unido. Además, es profesor de Biotecnología en el prestigioso Manchester Institute of Biotechnology (MIB) de la University of Manchester (UoM) en Reino Unido (UK) y dirige el Centre of Excellence in Biopharmaceuticals de la misma Universidad.

Su investigación se enfoca en la producción de proteínas recombinantes y vectores virales, con fines terapéuticos, a partir del manejo y cultivo de células de mamíferos. Junto a su grupo, ha sido pionero en la adopción de enfoques 'ómicos' en el estudio y mejoramiento de células CHO, lo cual ha permitido dirigir la ingeniería celular hacia el desarrollo de procesos de manufactura más eficientes.

Dada su importante contribución al sector biofarmacéutico, el 2017 fue reconocido con el premio Peter Dunnill, otorgado por la UoM.

Dr. Francesc Gòdia

Universitat Autònoma de Barcelona. España.

El Dr. Francesc Gòdia es **Director General de la planta piloto MELiSSA**, un proyecto pionero en sistemas de soporte de vida biológicos para misiones espaciales de larga duración.

Es profesor de Ingeniería Química en la UAB, licenciado y Doctor en Ciencias Químicas por la Universitat Autònoma de Barcelona (UAB), es una figura destacada en el campo de la biotecnología y la ingeniería bioquímica .

Desde 1999, Francesc ha sido miembro del Comité Ejecutivo de la Sociedad Europea de Tecnología Celular Animal y desde 2005, del Consejo Ejecutivo de la Federación Europea de Biotecnología.

Su especialización en Biotecnología y Ingeniería Bioquímica se enfoca en el cultivo de células animales para la producción de biofármacos, vacunas y tecnologías de soporte de vida en el espacio. Su investigación ha impulsado innovaciones cruciales en biomedicina e ingeniería celular.

Dr. Marcos Oggero-Eberhardt

Universidad Nacional del Litoral, Santa Fe. Argentina.

El Dr. Marcos Oggero-Eberhardt es Bioquímico y Doctor en Ciencias Biológicas, egresado de la Facultad de Bioquímica y Ciencias Biológicas (FBCB) de la Universidad Nacional del Litoral (UNL), situada en Santa Fe, Argentina.

Actualmente, se desempeña en el Centro Biotecnológico del Litoral de la FBCB-UNL, donde desarrolla actividades como Profesor Adjunto e Investigador Principal del CONICET.

Sus actividades de investigación se centran en la innovación de proteínas mediante técnicas de glicoingeniería, el desarrollo de novedosos derivados de la eritropoyetina humana con actividad en neuroprotección y neuroplasticidad, y la generación de anticuerpos monoclonales quiméricos y humanizados a partir de fragmentos de cadena única.

Dra. Laura Palomares

Universidad Autónoma de México. México.

La Dra. Laura Palomares es una destacada Ingeniera Bioquímica, egresada del ITESM, con maestría y doctorado en Biotecnología por la UNAM, y un posdoctorado en la Universidad de Cornell. Desde 1999 es investigadora en el Instituto de Biotecnología de la UNAM y actualmente es Investigadora Titular C, PRIDE D, y SNI nivel III.

Su trabajo se enfoca en la biotecnología médico-farmacéutica, destacándose en la caracterización de proteínas recombinantes y el desarrollo de tecnologías basadas en proteínas virales, con aplicaciones en nanobiomateriales, vacunas y vectores para terapia génica.

Ha participado en el desarrollo de la primera vacuna recombinante contra influenza en México y colabora con diversas empresas en la producción de vacunas virales.

Se encuentra galardonada, con los Premio Universidad Nacional en Innovación Tecnológica y Diseño Industrial (2018, UNAM), Premio Sor Juana Inés de la Cruz, (UNAM, 2012), Premio Canifarma Veterinaria. 1er lugar en la modalidad de desarrollo tecnológico (2010), entre otros.

Dra. Norma Adriana Valdez-Cruz

Universidad Autónoma de México. México.

La Dra. Norma Adriana Valdez-Cruz, Investigadora principal del Instituto de Investigaciones Biomédicas y Profesora Titular de la Universidad Nacional Autónoma de México (UNAM).

Es una destacada Químico Farmacobióloga, egresada de la Universidad Autónoma de Baja California, con un Doctorado en Ciencias Bioquímicas otorgado por el Instituto de Biotecnología de la UNAM. Actualmente es Líder Académico e Investigadora Titular B de Tiempo Completo en el Departamento de Biología Molecular y Biotecnología del Instituto de Investigaciones Biomédicas.

A lo largo de su carrera, ha realizado importantes contribuciones en su campo, incluyendo estudios sobre gradientes de glucosa y oxígeno en cultivos de *Escherichia coli*, y el desarrollo de vesículas extracelulares bacteriana para mejorar la productividad biotecnológica. También ha participado en investigaciones sobre métodos de extracción de terpenos de fuentes naturales y en el desarrollo de un antígeno RBD modificado de SARS-CoV-2 como posible inmunoestimulante oral para el cuidado de la enfermedad COVID-19

EXPOSITORES NACIONALES





Dra. Claudia Altamirano

Pontificia Universidad Católica de Valparaíso. Chile

La Dra. Claudia Altamirano es una destacada académica de la Pontificia Universidad Católica de Valparaíso (PUCV). La Dra. Altamirano es Ingeniera Civil Bioquímica, con un Magister en Ciencias de la ingeniería con mención en Ingeniería Bioquímica de la misma casa de estudios. Posteriormente, se trasladó a España, donde obtuvo el grado de Doctora en Biotecnología por la Universidad Autónoma de Barcelona.

Su investigación se centra en la Ingeniería metabólica y el cultivo celular, áreas en las que ha contribuido al mejoramiento de procesos de cultivo tanto en células mamíferas como en microorganismos.

Actualmente, es Directora del Proyecto Anillo ACT 210068 e Investigadora Principal del Centro de Medicina Intervencionista de Precisión y Terapia Celular Avanzada (IMPACT), donde ha podido fortalecer sus líneas de trabajo enfocadas en el escalamiento de procesos para la manufactura de terapias avanzadas.

Q.F. MSc. José Crisóstomo

Instituto de Salud Pública. Chile

El Msc. Crisóstomo es Farmacéutico de la Universidad de Chile, con un Magister en Biopharmaceuticals en King's College London. En su trayectoria, se ha dedicado a la fabricación de productos farmacéuticos, además de ser parte de la Agencia Nacional Reguladora de Chile, donde ha abordado áreas clave como Química, Manufactura y Control, Buenas Prácticas de Manufactura (GMP) y asuntos regulatorios.

José es un experto en el desarrollo de productos farmacéuticos, escalado de procesos y validación. Se ha especializado en la revisión del documento técnico común (CTD), centrándose principalmente en el Módulo 3 para el proceso de autorización de comercialización de biofarmacéuticos en el mercado chileno, incluyendo vacunas y anticuerpos monoclonales.

Durante 2020 y 2021, se dedicó exclusivamente a coordinar la evaluación de la calidad, eficacia y seguridad de las vacunas contra la COVID-19, analizando documentación de empresas como Pfizer, Sinovac, Covaxin, Sputnik, AstraZeneca y Janssen, y colaborando con otras agencias reguladoras de la región y el mundo, como ANVISA, ANMAT, COFEPRIS, INVIMA y EMA.

Dra. Ziomara Gerdtzen.

Universidad de Chile. Chile.

La Dra. Ziomara Gerdtzen es una destacada académica de la Facultad de Ciencias Físicas y Matemáticas de la Universidad de Chile. La Dra. Gerdtzen es Ingeniera Civil en Biotecnología de la misma casa de estudio, y obtuvo su Doctorado en Ingeniería Química en la University of Minnesota. A lo largo de su carrera, se ha enfocado en la investigación y docencia en biotecnología aplicada, en el área de modelamiento y cultivo celular.

Actualmente lidera el Laboratorio de Cultivo de Células Mamíferas (Mammalian Cell Culture Lab- MCCL) en el CeBIB-FCFM, dedicado al cultivo de células mamíferas, ingeniería celular y modelamiento matemático aplicado a sistemas biológicos, con el objetivo de diseñar y optimizar bioprocesos. Además, ha invertido gran parte de su carrera en la formación de estudiantes de pregrado y postgrado en programas de ingeniería.

Entre sus investigaciones más recientes, destaca el desarrollo y validación de la plataforma Cell Culture Media Optimization (CELIA)

Dr. Maroun Khoury

Universidad de Los Andes. Chile.

El Dr. Khoury es un destacado Doctor en Salud y Biología por la Universidad de Montpellier, Francia. Su formación académica también incluye un postdoc en Ciencias Biológicas y Biomédicas en el Massachusetts Institute of Technology (MIT), donde profundizó en temas de vanguardia en biotecnología.

Su investigación se centra en la terapia génica para enfermedades articulares, utilizando estrategias avanzadas como ARN de interferencia y vectores basados en liposomas y virus. El Dr. Khoury también ha trabajado en la alianza MIT-Singapur para la Investigación y Tecnología (SMART), donde se especializó en células madre hematopoyéticas y mesenquimatosas. Esta experiencia enriquecedora le permitió establecer su propio laboratorio de medicina nano-regenerativa en la Universidad de los Andes, donde dirige un equipo dinámico y altamente motivado.

Desde 2021, ocupa el cargo de Director Ejecutivo del Centro de Medicina Intervencionista de Precisión y Terapia Celular Avanzada (IMPACT). Su dedicación y contribuciones al campo le han valido varios reconocimientos, incluido el Young Investigator Award en 2019.

Dr. Flavio Salazar

Universidad de Chile. Chile.

El Dr. Salazar es un destacado Inmunólogo y Biólogo argentino-chileno, es Doctor en Filosofía en el Instituto Karolinska de Suecia (1998), donde también se especializó en inmunología. Desde 1999, el Dr. Flavio Salazar ha sido profesor titular en la Facultad de Medicina de la Universidad de Chile, donde ha desempeñado un papel fundamental en el ámbito de la investigación y la educación.

El Dr. Salazar se ha destacado en la investigación sobre inmunología tumoral, centrándose en el melanoma y la identificación de nuevos antígenos relacionados. Ha liderado ensayos clínicos de fase I y II en Chile para inmunoterapia contra el melanoma y el cáncer de próstata. Parte de su investigación ha incluido el desarrollo de nuevas vacunas, como la TRIMELVax, y el estudio de la interacciones celulares, mediada por uniones gap, que puede facilitar la destrucción de células tumorales.

.Como un impulsor del ecosistema biotecnológico en Chile, ha cofundado dos empresas de biotecnología: Oncobiomed, dedicada a la transferencia tecnológica en inmunoterapia celular, y Bionex, que apoya a científicos en el diseño de proyectos de I+D aplicados.

RESÚMENES DE CONFERENCIAS

13
NOVIEMBRE

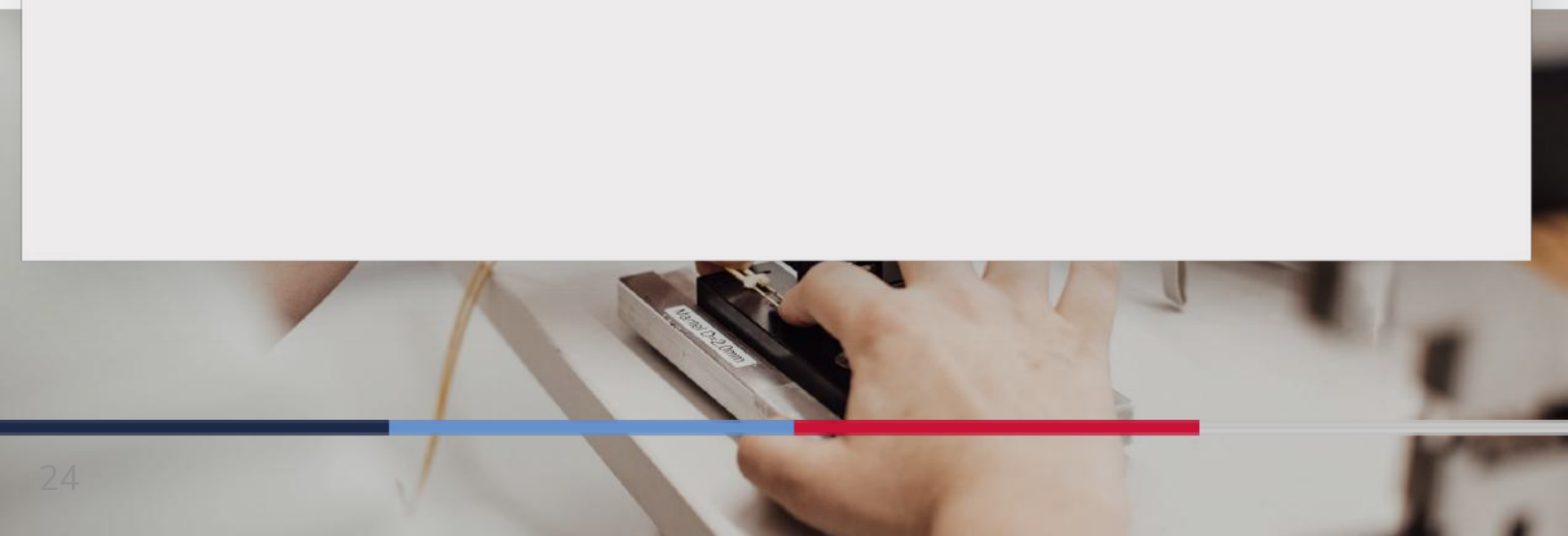
Charla Inaugural
Dr. Francesc Gòdia
18:00 - 19:00 horas

Mammalian cell-based technologies enabling the production of biopharmaceuticals and advanced therapy medicinal products

Francesc Gòdia

In about four decades mammalian cell-based technologies have matured dramatically to become an efficient platform delivering a myriad of biopharmaceuticals that have changed healthcare all over the World. The impact of an ever-increasing knowledge at molecular and cellular level in combination with advanced bioprocessing technologies has enabled to develop more intensive and efficient manufacturing processes delivering complex molecules with the necessary quantity and quality to protect the health of millions of persons.

Today, these consolidated capacities are setting the basis for the flourishment of a new generation of products, the so-called advanced therapy medicinal products, including gene, cell therapies and their combination, such as cancer immunotherapies. Although still with relevant challenges, such as the need to develop these therapies at lower costs facilitating patient access, Precision Medicine will continue developing in the new future hand in hand of advanced mammalian cell-based technologies.



14
NOVIEMBRE

Desarrollo de líneas celulares para la producción de proteínas recombinantes

Dr. Alan Dickson.
Bloque: 8:00 - 10:20 horas

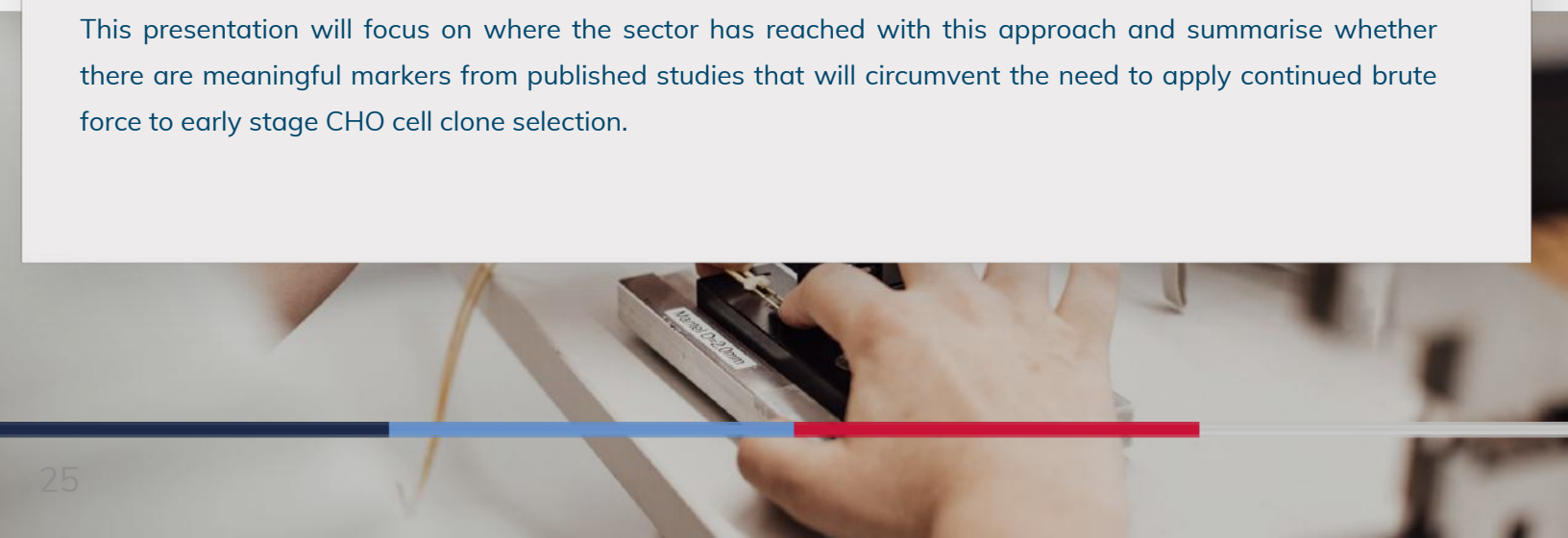
Cell line development in the age of 'Omics

Alan Dickson

This presentation will focus predominantly on the generation of stable Chinese hamster ovary cell lines for the manufacture of secreted recombinant proteins. At the start of the process of cell line development we seek to "find" a clone within the large number of individual cells arising from transfection that will (a) grow rapidly to high density, (b) secrete significant amounts of the desired recombinant product, (c) generate product with the functional structural features and (d) retain stability in all desired properties during scale up and prolonged periods of culture.

In relation to the latter aspect, cells will move from initial culture conditions to others that may involve a change of environment (physical culture condition, medium changes and feed regimes). Whilst high throughput approaches and automation have been introduced to expand the number of clones that can be handled following transfection, this does not address the capacity to find the "holy grail" within the clones. Indeed, it may further add to the challenge by imposing a greater number of clones to be handled, carrying the same fundamental unknowable – is this the one that will be the best of all potential clones? As an alternative to increasing the application of "brute force" to cell handling and experimental work, the sector has sought to utilise the potential power of 'omics characterisation of CHO cell populations and derived clones towards a predictive identification of clone properties associated with understanding the molecular foundation of desirable cell properties.

This presentation will focus on where the sector has reached with this approach and summarise whether there are meaningful markers from published studies that will circumvent the need to apply continued brute force to early stage CHO cell clone selection.



14
NOVIEMBRE

Desarrollo de líneas celulares para la producción de proteínas recombinantes

Dra. Claudia Altamirano
Bloque: 8:00 - 10:20 horas

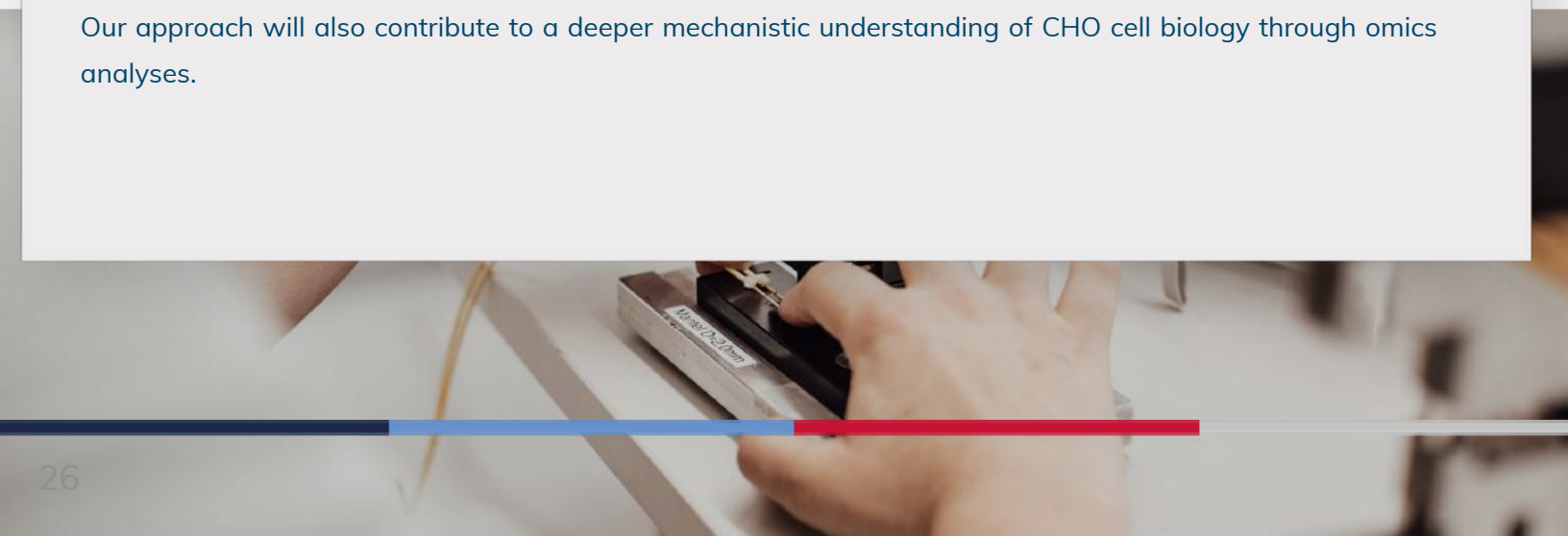
Integrating continuous culture and Omics approaches to deepen understanding on CHO cell metabolism and identify optimization targets

Claudia Altamirano

Biopharmaceuticals have revolutionized the treatment of life-threatening diseases, delivering substantial therapeutic benefits and offering unique commercial opportunities. However, the growing demand for novel therapeutic modalities necessitates innovation in biopharmaceutical manufacturing to enhance access and cost-effectiveness. This study aims to advance Chinese Hamster Ovary (CHO) cell factories, equipping them with enhanced capabilities for continuous biopharmaceutical production.

Although CHO cells are extensively used in traditional batch processing, their efficiency in continuous production remains limited. Over the past 15 years, our research has focused on understanding CHO cell biology in continuous cultures, identifying key areas for cellular-level optimization. We found that controlling cell proliferation—particularly by arresting the cell cycle in the G1 phase—significantly boosts CHO cell productivity. Moreover, we demonstrated the importance of increasing cellular resistance to apoptosis. With these insights, our primary goal is to establish a strategy for recombinant IgG production in perfusion culture, using a custom-designed CHO cell line capable of regulating cell growth and apoptosis as needed.

Our initial efforts have concentrated on developing an inducible CHO cell platform that can control cell growth arrest and reduce apoptosis on demand. The outcomes of this research are expected to substantially impact continuous CHO cell manufacturing by enhancing cell productivity while preserving product quality. Our approach will also contribute to a deeper mechanistic understanding of CHO cell biology through omics analyses.



14
NOVIEMBRE

Metabolismo celular y optimización de medios de cultivo

Dra. Norma Adriana Valdez-Cruz
Bloque: 10:50 - 13:00 horas

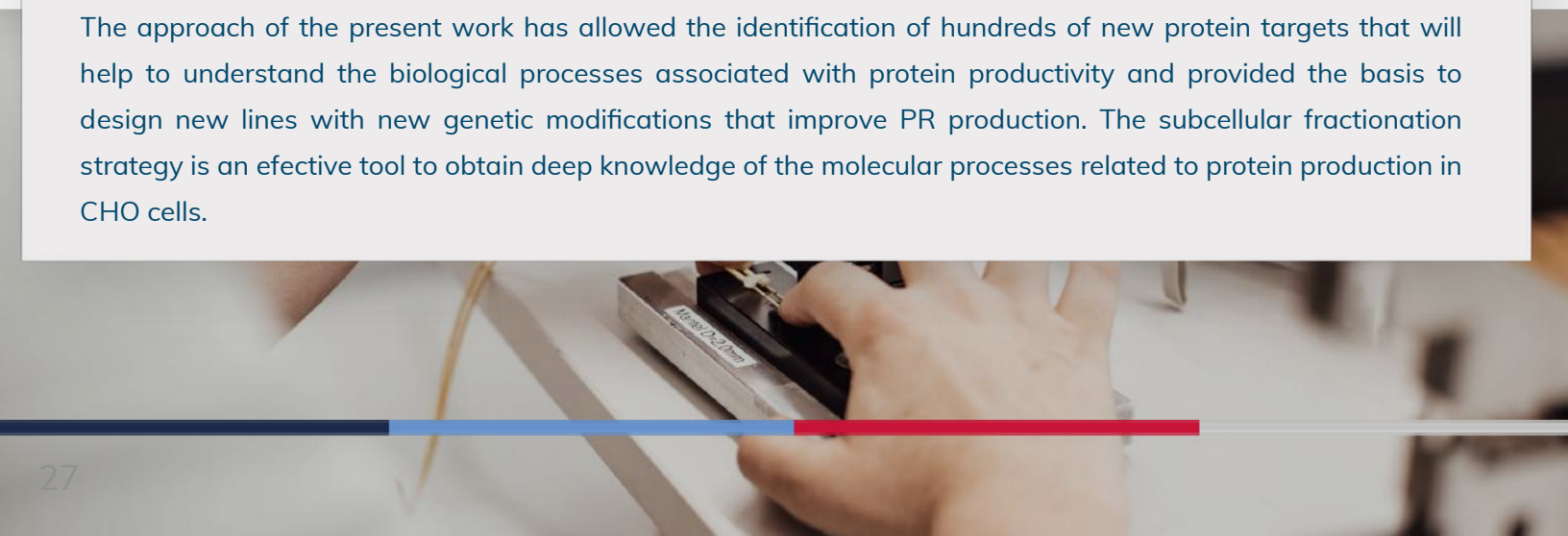
Comparative omic studies of CHO cells producing biopharmaceuticals

Norma Adriana Valdez-Cruz

The production of therapeutic recombinant proteins (RP) is critical for treating diseases like cancer and diabetes, though their high cost limits accessibility. CHO cells are widely used for RP production, comprising 70% of biopharmaceuticals due to their ability to perform human-like post-translational modifications and ensure viral safety. However, CHO cells have low productivity, slow growth, and are prone to apoptosis. Omics studies aim to understand CHO cell biology and the mechanisms behind RP production, with a focus on overcoming bottlenecks in the classical secretion pathway (CSP), which involves modifications in the endoplasmic reticulum, Golgi apparatus, and transport vesicles. Despite advances, CSP-related transcripts and proteins remain undercharacterized.

CHO cell lines were cultured in CDM4CHO medium supplemented with glutamine, insulin and methotrexate and maintained at 37°C, 5% CO2 in humidity. Cell concentration, viability, metabolites, ions, pH, specific productivity were determined. We performed transcriptomic (Illumina Hiseq) and differential proteomic (MS/MS) assays. We enriched subcellular fractions, sucrose gradients analyzed by MS/MS (Q-Exactive HF-X). Results: Our projects have identified transcripts and proteins related to VSC in CHO cells, related to the increase in PR production. We have contributed with the identification of protein targets, many of them associated with VSC related to the increase in biopharmaceutical production in CHO cells. We implemented a subcellular fractionation method, one of precipitation and recovery of proteins from CHO cells to make depth studies.

The approach of the present work has allowed the identification of hundreds of new protein targets that will help to understand the biological processes associated with protein productivity and provided the basis to design new lines with new genetic modifications that improve PR production. The subcellular fractionation strategy is an efective tool to obtain deep knowledge of the molecular processes related to protein production in CHO cells.



14
NOVIEMBRE

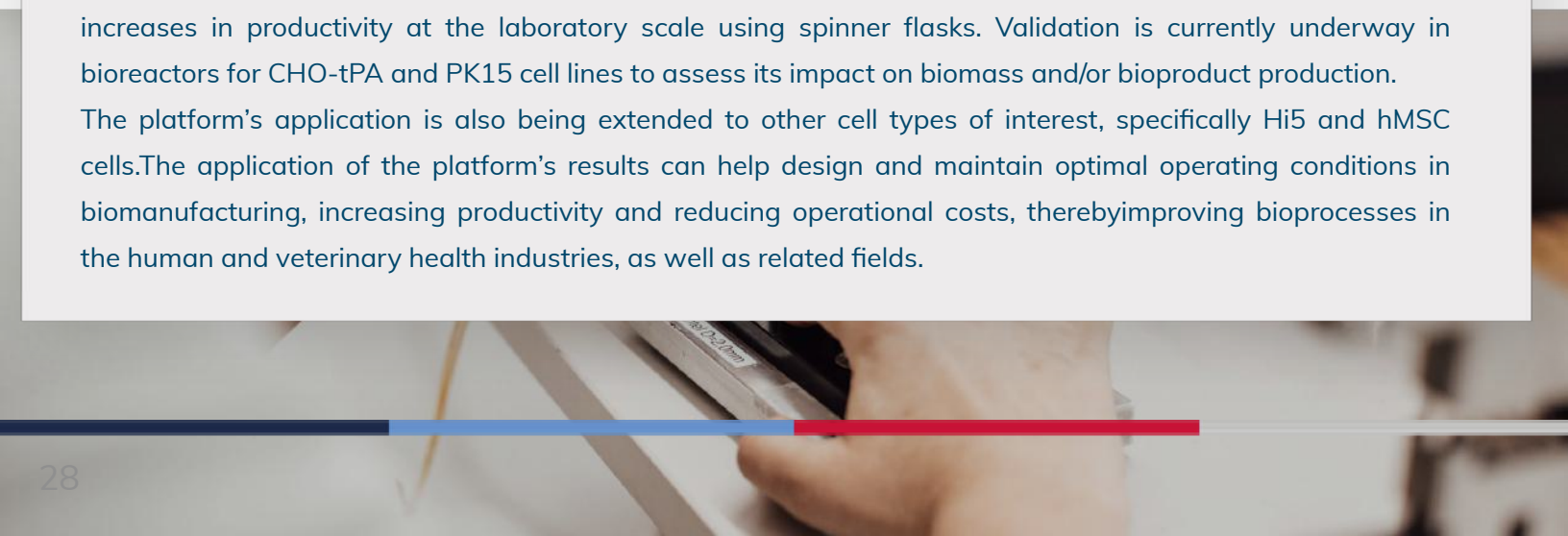
Metabolismo celular y optimización de medios de cultivo

Dra. Ziomara Gerdtzen
Bloque: 10:50 - 13:00 horas

Validation of the CELIA Platform: Design and Optimization of Culture Media and Feeding Strategies for Mammalian Cell-Based Biomanufacturing Processes

Ziomara Gerdtzen

The biomanufacturing industry requires new tools to facilitate the scaling up and increase the productivity of production systems. This is especially important for the development of local biopharmaceutical manufacturing processes, particularly those involving mammalian cell cultures to produce biomolecules for therapeutic applications. One key to achieving optimal performance is maintaining the proper operating conditions for cell growth. Defining the appropriate culture and feeding media to meet the needs of the production system is essential. A significant challenge for the industry is ensuring these conditions throughout the entire process, from initial small-scale development to large-scale clinical and commercial manufacturing, while maintaining consistent performance. To address this challenge, the cell culture media design platform CELIA (Cell Culture Media Optimization) was developed. This tool, based on a stoichiometric, dynamic, and predictive mathematical model, integrates the nutritional needs of the cells under batch and fed-batch culture conditions, along with the cellular and product composition, to estimate the stoichiometric requirements for biomass production. The characteristic parameters of the basal process operation are fed into a mathematical model specific to the production system, which generates tailored feeding strategies and culture medium compositions for the system. Results obtained with the CELIA platform have demonstrated significant increases in productivity at the laboratory scale using spinner flasks. Validation is currently underway in bioreactors for CHO-tPA and PK15 cell lines to assess its impact on biomass and/or bioproduct production.
The platform's application is also being extended to other cell types of interest, specifically Hi5 and hMSC cells.The application of the platform's results can help design and maintain optimal operating conditions in biomanufacturing, increasing productivity and reducing operational costs, therebyimproving bioprocesses in the human and veterinary health industries, as well as related fields.



14
NOVIEMBRE

Metabolismo celular y optimización de medios de cultivo

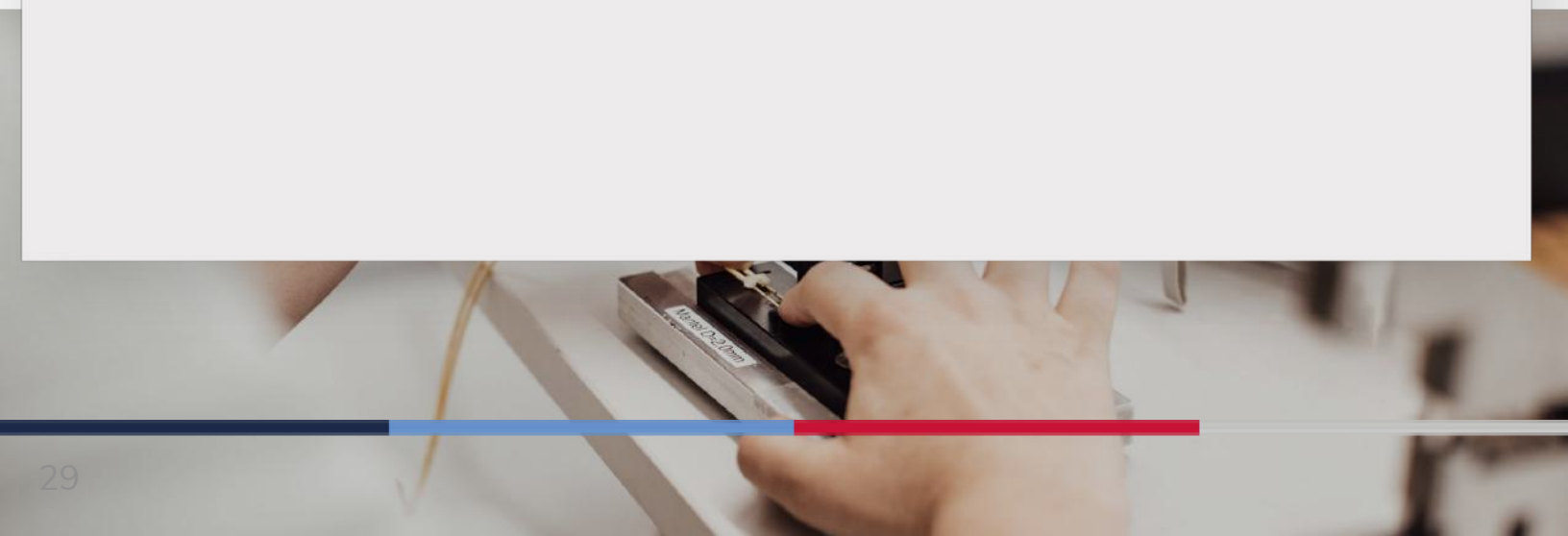
Invitado Especial: Dr. Mauro Torres. Reino Unido.
Bloque: 10:50 - 13:00 horas

Deciphering molecular drivers of lactate metabolic shift in mammalian cell cultures

Mauro Torres

Lactate metabolism plays a pivotal role in mammalian cell bioprocessing, where it influences culture longevity and productivity. The lactate metabolic shift, a transition from lactate production to consumption, is advantageous for enhancing culture performance, yet the molecular mechanisms driving this shift remain poorly understood.

This talk will explore the factors that influence the lactate metabolic shift by examining both environmental and genetic drivers in CHO cells. Through an integrated approach involving process, metabolome, and transcriptome analyses, we elucidated key molecular regulators of lactate metabolism and discuss their relevance for optimizing bioprocesses and advancing applications in biotechnology.



14
NOVIEMBRE

Bioterapéuticos: Desarrollo y control de calidad

Dr. Marcos Oggero
Bloque: 17:00 - 19:00 horas

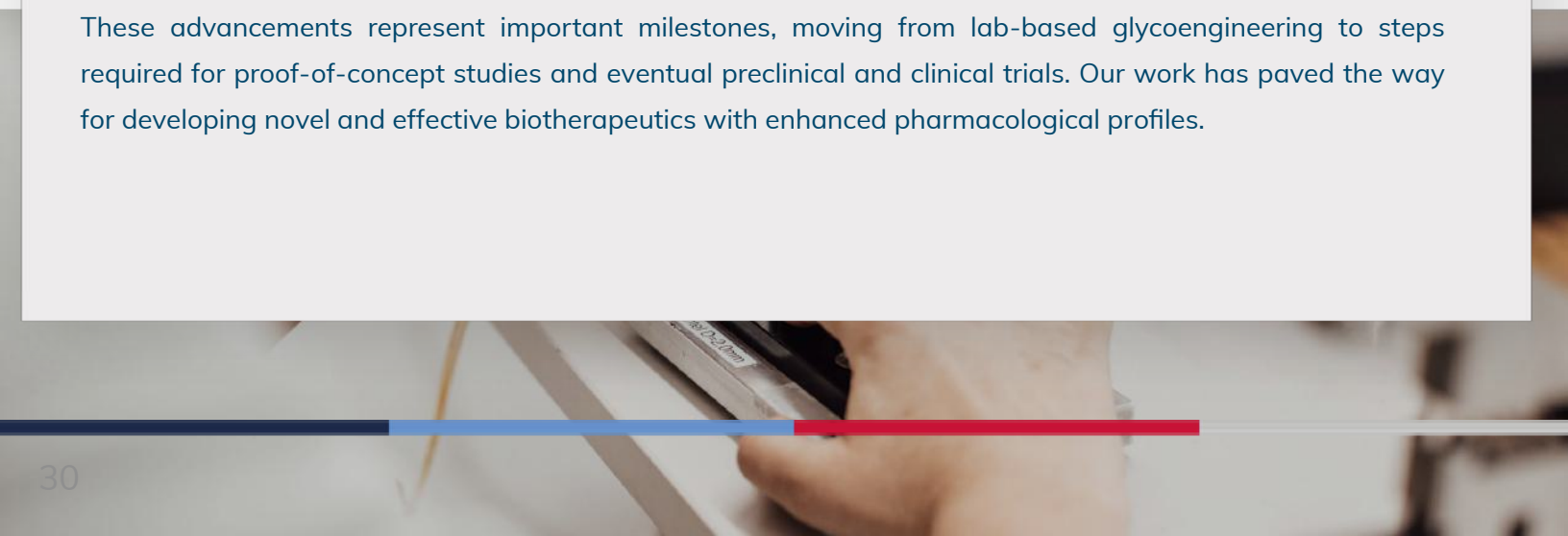
Glycoengineering by hyperglycosylation: A single approach to tailor biotherapeutic properties

Marcos Oggero

Eukaryotic proteins typically undergo co- and post-translational modifications during or after synthesis, with N- and O-glycosylation being among the most common and complex. These modifications are crucial, as they affect key biological characteristics like solubility, stability against proteases and temperature, immunogenicity, bioactivity, and pharmacokinetics. Glycosylation, in particular, plays a critical role in the efficacy and potency of therapeutic proteins, as it alters protein size and charge, making it a powerful tool for enhancing biotherapeutic properties.

Our lab has explored glycoengineering to develop advanced biotherapeutics using animal cell cultures. We focused on two notable glycoproteins: interferon-α2b and erythropoietin. Interferon-α2b, a cytokine with antiproliferative, immunomodulatory, and antiviral effects, was engineered through hyper N- or O-glycosylation to improve its pharmacokinetic profile, making it potentially more effective as a therapeutic agent. Erythropoietin, on the other hand, was modified through N-glycoengineering to reduce its erythropoietic activity while preserving its neurobiological functions. This modified erythropoietin variant has been foundational in establishing a start-up and progressing to national patent filings.

These advancements represent important milestones, moving from lab-based glycoengineering to steps required for proof-of-concept studies and eventual preclinical and clinical trials. Our work has paved the way for developing novel and effective biotherapeutics with enhanced pharmacological profiles.



14
NOVIEMBRE

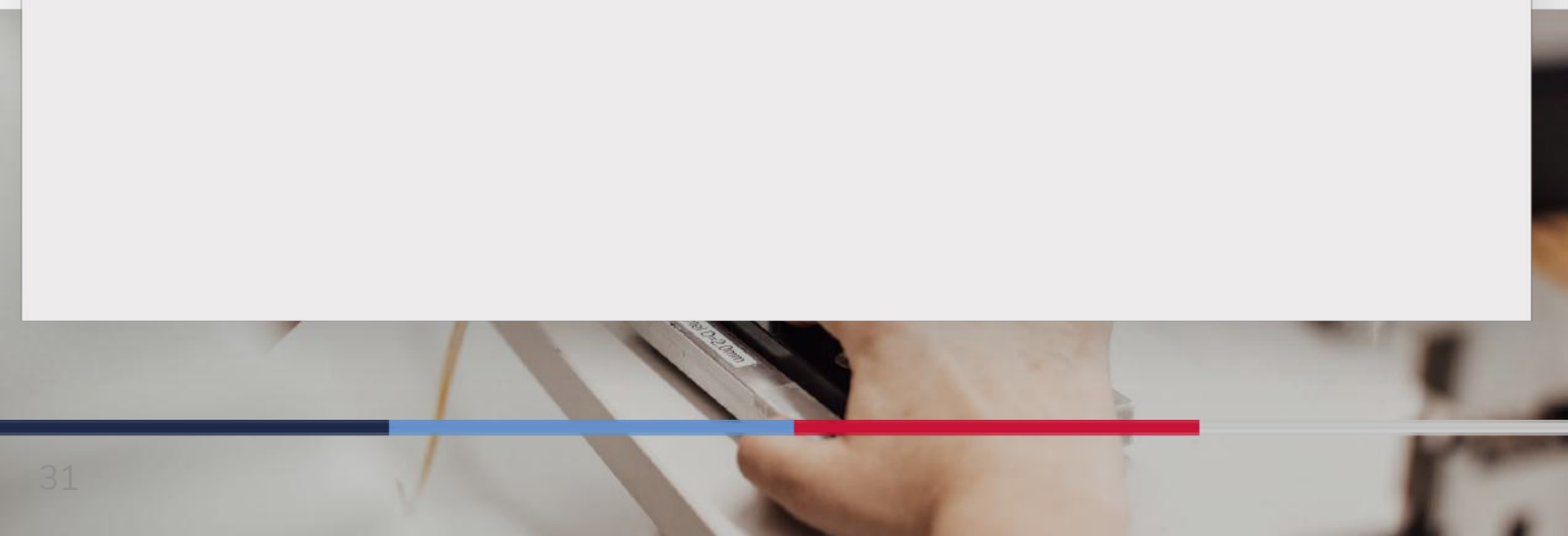
Bioterapéuticos: Desarrollo y control de calidad
Dra. Mariela Bollati
Bloque: 17:00 - 19:00 horas

Leishmania tarentolae as recombinant protein production platform: Expression of chimeric recombinant bovine follicle-stimulating hormone as case study

Mariela Bollati

This presentation explores the use of Leishmania tarentolae (L. tarentolae) as a heterologous expression system for the recombinant production of bovine follicle-stimulating hormone (rFSH). L. tarentolae, a unicellular eukaryote non-pathogenic to mammals, presents significant advantages as a host for recombinant protein production: it can be cultured in bioreactors at 26°C using simple and cost-effective semisynthetic media, without requiring CO2 or bovine serum.

In our lab, we have produced a single chain rFSH (scbFSH), with fused α and β subunits, and characterized it both biochemically and biologically. Our results demonstrated that L. *tarentolae* can generate a homogeneous, glycosylated, and bioactive rFSH with an adequate yield, making it a promising and cost-effective expression system for animal reproduction applications.



14
NOVIEMBRE

Bioterapéuticos: Desarrollo y control de calidad
Invitado Especial: Dr. Ioscani Jimenez. Irlanda
Bloque: 17:00 - 19:00 horas

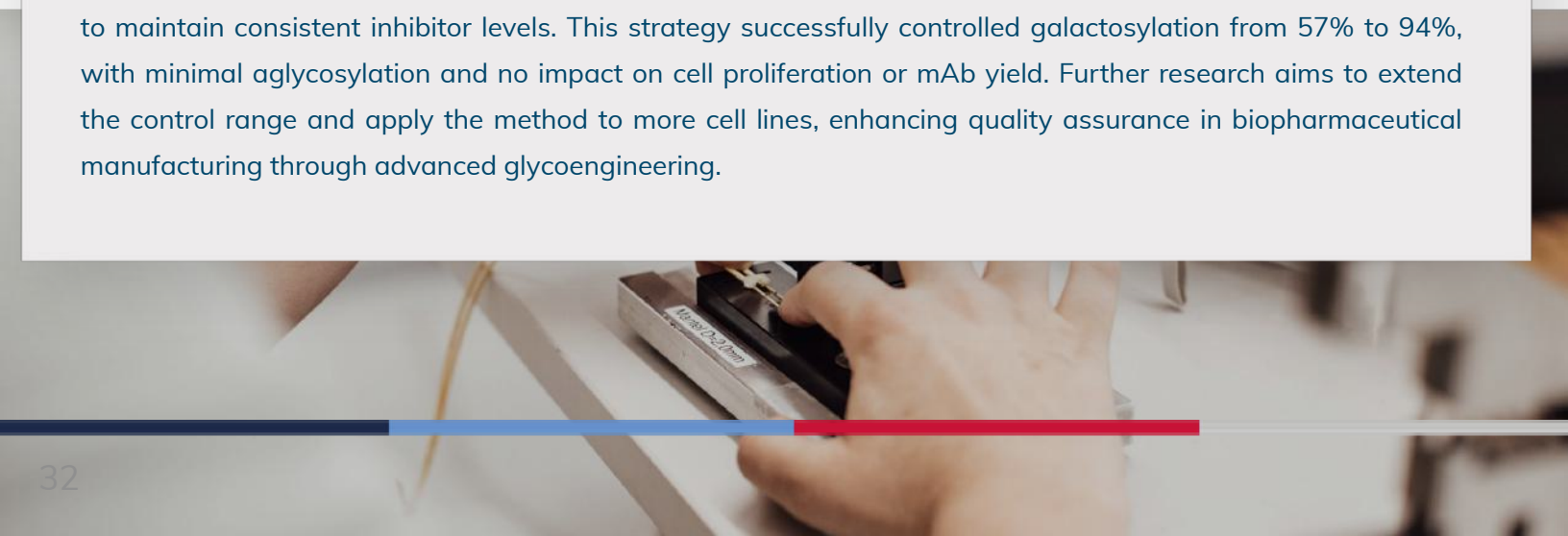
Next-gen glycoengineering: Combining cellular and metabolic engineering to fine-tune mAb β1,4-galactosylation

Ioscani Jimenez

N-linked galactosylation significantly affects the heterogeneity of monoclonal antibody (mAb) products and enhances their effector functions, including CDC, ADCC, and ADCP. Despite the importance of controlling mAb galactosylation, existing strategies are inadequate: standard cell engineering does not allow real-time control, advanced genetic engineering is slow and complex, and metabolic glycoengineering compromises cell growth and product yield.

We developed a novel method for real-time galactosylation control using 2-deoxy-2-fluoro-d-galactose (2FG) as a decoy substrate in hypergalactosylating CHO cells (GalMAX cells). CHO-DP12 and CHO-VRC01 cell lines, capable of up to 98% β4-galactosylation, were treated with 2FG concentrations between 0.1mM and 2mM. Daily monitoring showed that 2FG dosing allowed precise, dose-dependent galactosylation control from 45% to 93% without affecting cell growth or mAb yield. However, higher 2FG levels led to unwanted aglycosylated mAb products. Analysis indicated that the 45% galactosylation level was due to timing rather than 2FG effectiveness, as a significant portion of highly galactosylated mAbs was produced before the first 2FG feed.

The approach was validated with VRC01-GalMAX cells, using 2FG doses of 10pg to 80pg on days 3, 4, and 5 to maintain consistent inhibitor levels. This strategy successfully controlled galactosylation from 57% to 94%, with minimal aglycosylation and no impact on cell proliferation or mAb yield. Further research aims to extend the control range and apply the method to more cell lines, enhancing quality assurance in biopharmaceutical manufacturing through advanced glycoengineering.



15
NOVIEMBRE

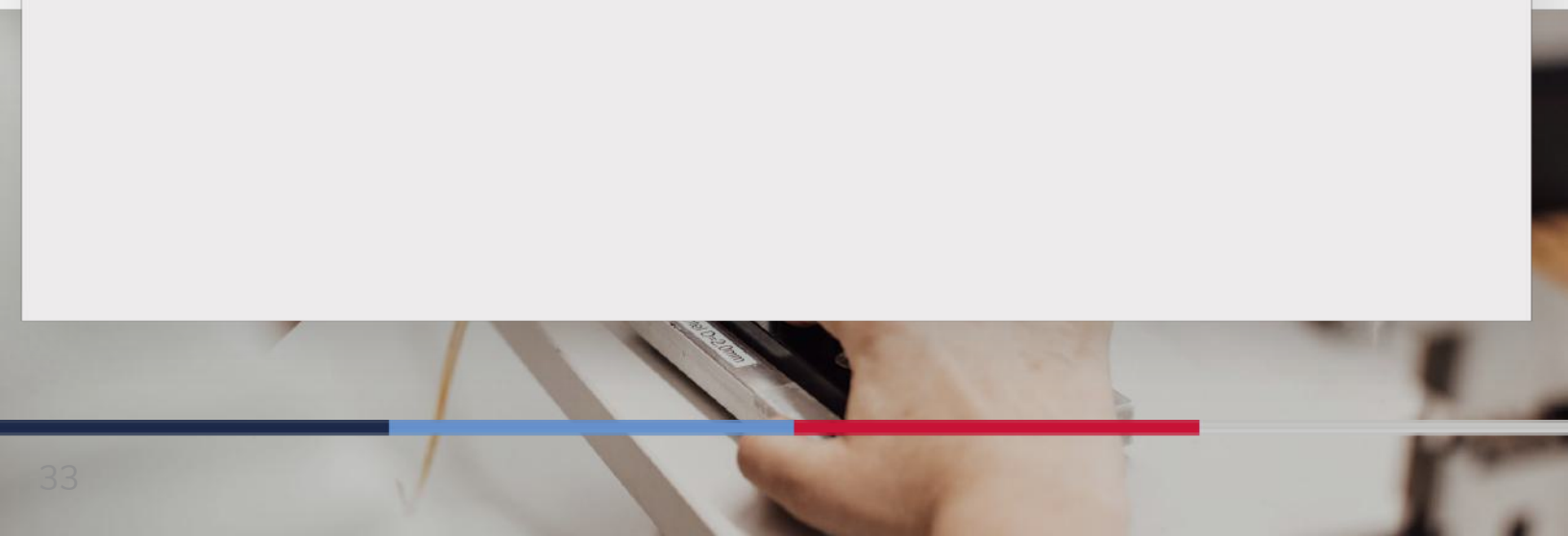
Bioprocesos: upstream and downstream
Dr. Ernesto Chico
Bloque: 8:00 - 10:20 horas

Industrial response under time pressure: lessons learned from Cuban COVID vaccines

Ernesto Chico

Animal cell culture technology has expanded significantly in the last decades, mainly driven by the opportunity derived from the market of innovative and biosimilar drugs. The industrial infrastructure created in different regions represented an opportunity to face the COVID-19 pandemic by producing effective vaccines. However, product development and scale-up with animal cell technology usually require longer than needed to develop vaccines under pandemic conditions.

In this work, we discuss some of the key challenges of developing and scaling COVID Soberana vaccine using animal cell technology in Cuba. Despite the significant industrial constraints, Soberana vaccine production made it possible to carry out the first reported massive vaccination campaign in the pediatric population. This proved a critical step in controlling the COVID pandemic in Cuba, especially against the Omicron variant. Lessons learned from the use of animal cell technology under time-pressure conditions are discussed.



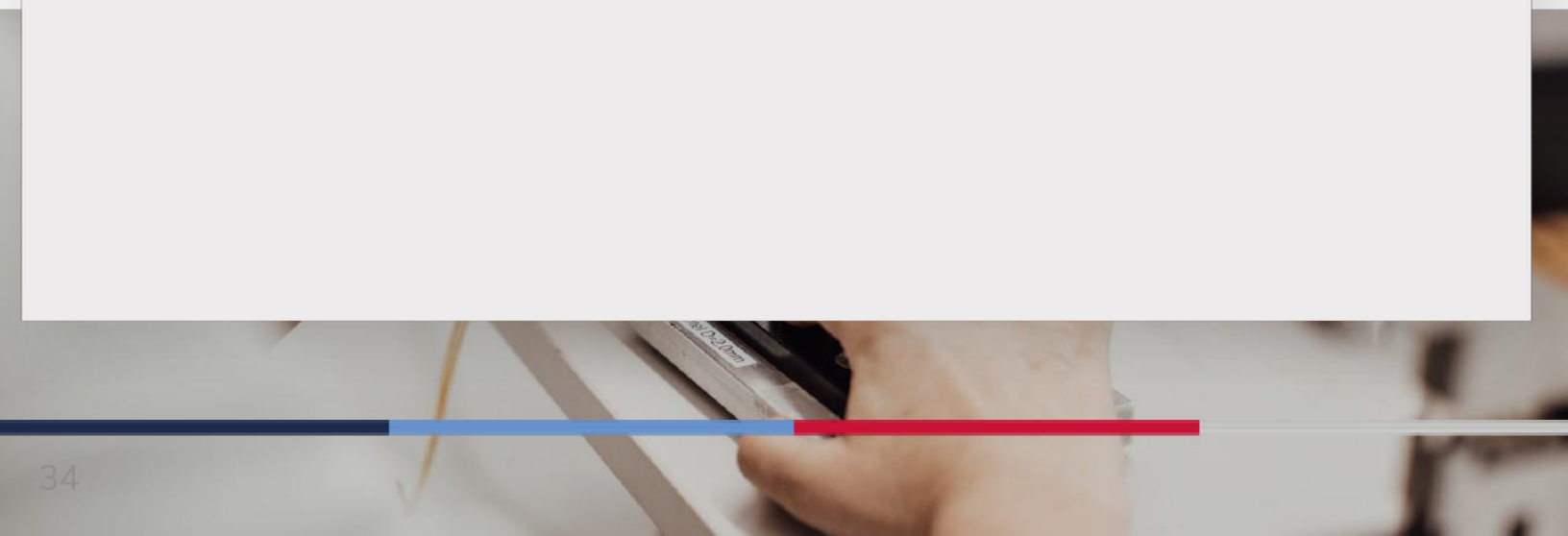
15
NOVIEMBRE

Bioprocesos: upstream and downstream
Dra. Renata Alvim
Bloque: 8:00 - 10:20 horas

Recombinant proteins as viral vaccine candidates

Renata Alvim

The presentation Recombinant proteins as viral vaccine candidates will approach relevant topics related to the production of recombinant proteins and their importance in the context of the development of vaccines against viral diseases. Additionally, results generated by the Cell Culture Engineering Lab and its collaborators will be briefly discussed, including latest advances in the development of a trivalent recombinant vaccine for SARS-CoV-2 variants.



15
NOVIEMBRE

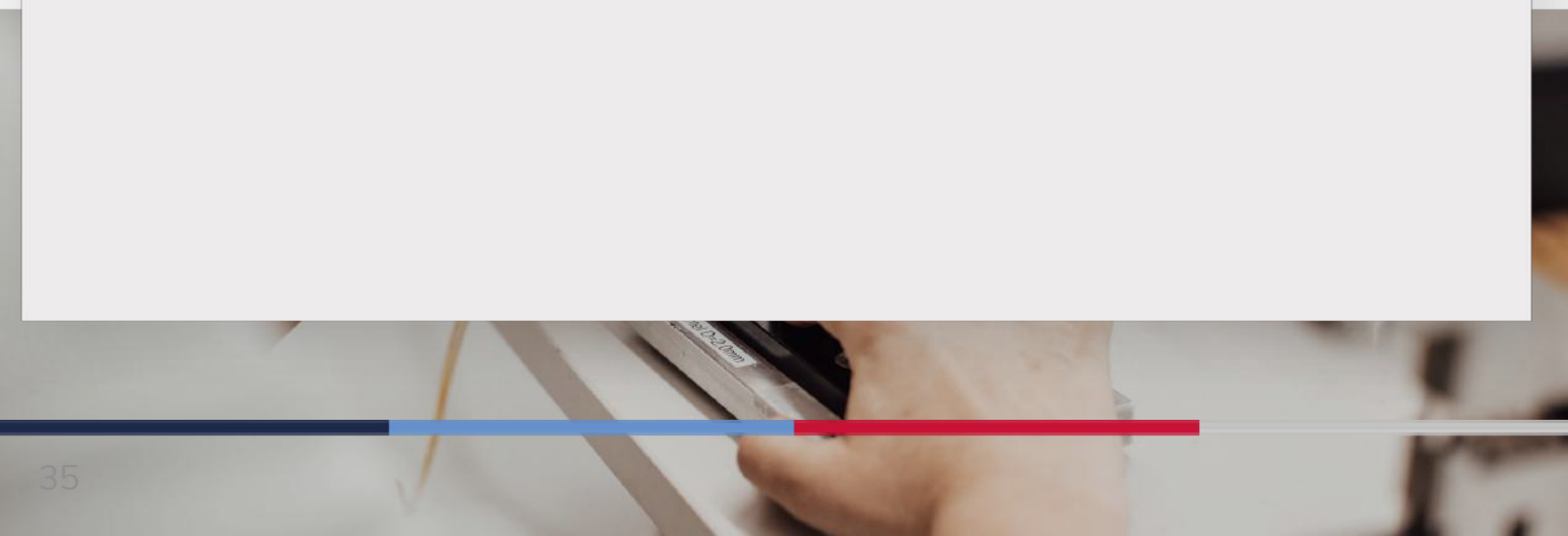
Terapia génica y terapia celular
Dra. Paula Alves
Bloque: 10:50 - 13:00 horas

Bioengineering Approaches for Gene and Cell Based Products

Paula Alves

The last decades witnessed major changes in medical care for human health. Several diseases considered chronic until recently can now be cured. A better understanding of the mechanisms of disease at the cellular and molecular level, driven by rapidly evolving technological advancements, propelled targeted, and more effective treatments. A new era of personalized and precise medicine started and, with it, a demand for new generation of medicinal products where gene and cell based biotherapeutics have been playing a major role.

I will present an overview on bioengineering challenges we face to develop scalable upstream and downstream processes yielding relevant quantities of gene and cell-based products with the required quality attributes. The development of analytics to assist bioprocess development and how the integration with -omics data can impact quality will also be emphasised. I also will show how some of these tools and technologies can be used to develop advanced 3D in vitro cell models, mimicking the behaviour of cells in tissues, while maintaining the system as simple as possible, to allow in-depth cellular and molecular interrogation, thus providing powerful tools for pre-clinical research.



15
NOVIEMBRE

Terapia génica y terapia celular

Dr. Maroun Khoury

Bloque: 10:20 - 13:00 horas

From Cell Culture Speakeasy to Cell Therapy Cabaret: Illuminating the Cellular Mechanisms Powering Advanced Therapies

Maroun Khoury

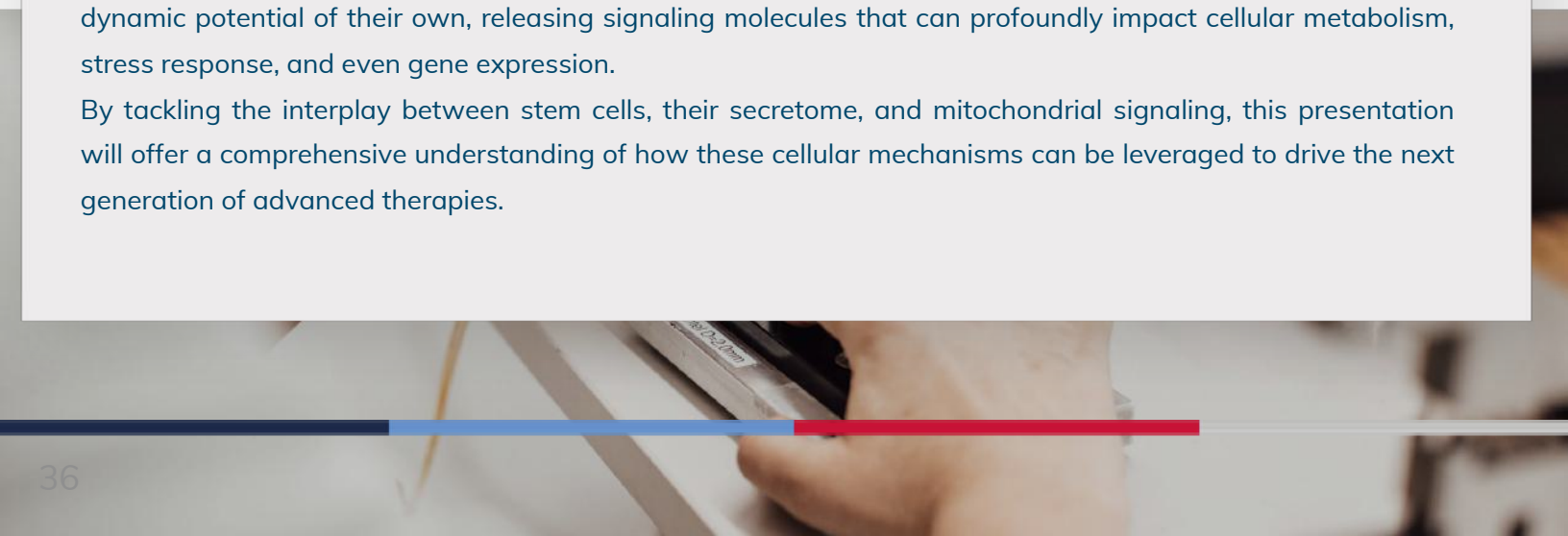
Cell and gene therapies hold immense promise for treating a wide range of diseases. More than 100 therapies have been approved worldwide, but fully harnessing their potential requires a deep understanding of the underlying cellular mechanisms at play. Beside the scientific challenge also lays a huge access gap, especially in Chile.

In the term Secretome lies the word "secret". A speakeasy was a secret establishment during Prohibition where people could discuss politics, arts and illicitly purchase and consume drinks. This presentation will take the audience on a journey from the "speakeasy" of cell culture, hints at the secretive or hidden aspects of the cellular processes involved research to the "cabaret" of clinical cell therapy applications, shining a spotlight on the crucial roles of stem cells, their secretome including extracellular vesicles and mitochondria.

Stem cells possess an innate ability to self-renew and differentiate into diverse cell types, offering a complete panel of regenerative potential. Meanwhile, their secretome - a rich mix of growth factors, cytokines, and extracellular vesicles - has emerged as a powerful mediator of paracrine effects, influencing the function and fate of surrounding cells.

Equally vital are the mitochondria, the cellular "powerhouses" that fuel the energy-intensive processes of cell growth, division, and adaptation. Recent research from our group has revealed that mitochondria possess a dynamic potential of their own, releasing signaling molecules that can profoundly impact cellular metabolism, stress response, and even gene expression.

By tackling the interplay between stem cells, their secretome, and mitochondrial signaling, this presentation will offer a comprehensive understanding of how these cellular mechanisms can be leveraged to drive the next generation of advanced therapies.



15
NOVIEMBRE

Acelular: Secretoma, microvesículas, vacunas y partículas
Dra. Laura Palomares
Bloque: 17:00 - 19:00 horas

Developing plataforms for vaccine design

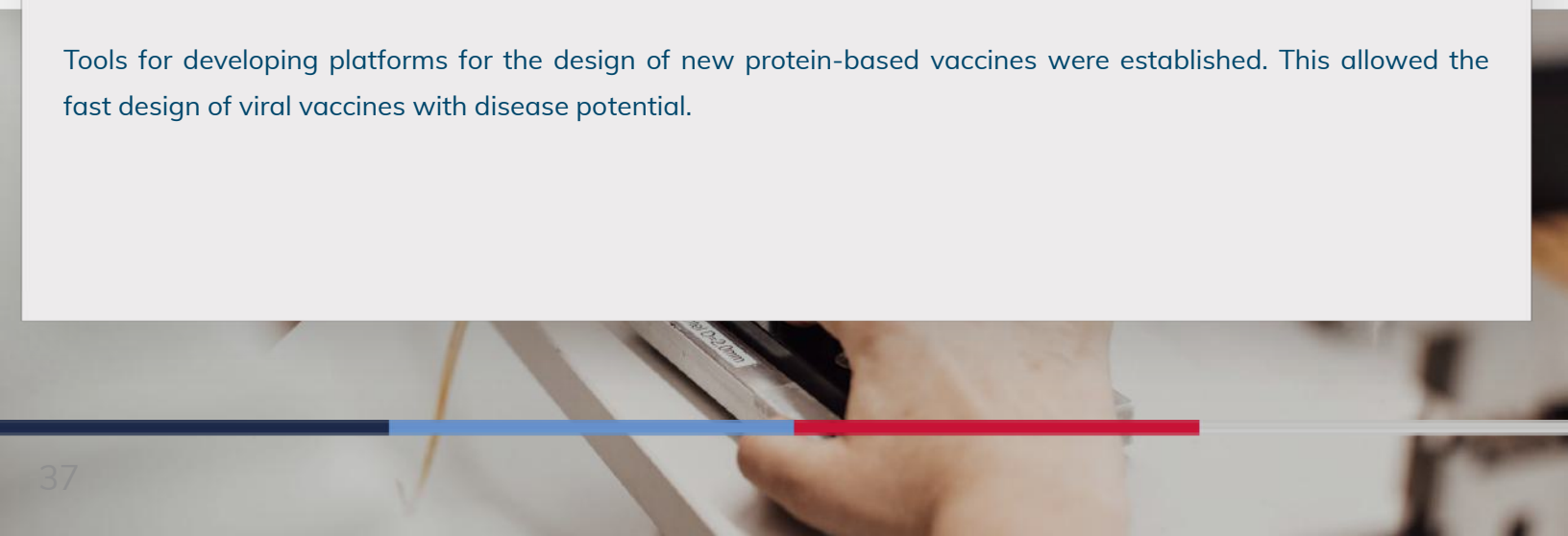
Laura A. Palomares

Vaccines are one of the most effective health interventions. As the field progresses, vaccine design has become more challenging as pathogens of interest increase in complexity. Of particular interest are viruses with pandemic potential, which can be unknown. A rapid response is required that can only be obtained using platforms that share manufacturing processes already vetted by regulatory agencies. Protein-based vaccines remain as a safe and cost-effective alternative with the possibility of multivalent antigen display and the capability of nucleic acid delivery.

Structural proteins of adeno-associated virus (AAV) and rotavirus were recombinantly produced using the insect cell baculovirus expression vector system, as described in Cuevas-Juárez et al. (2023) and Rodríguez et al. (2021). Mimotopes were characterized and selected as described in Cuevas-Juárez et al. (2023). Mice were immunized and their immune response was measured as described in Cuevas-Juárez et al. (2023).

Platforms for the standardized and fast production of novel vaccines using AAV capsids and rotavirus VP6 assemblies as scaffolds for antigen display were developed. Antigen display on virus-like particles (VLP) resulted in a high immune response in mice. Standardized processes for vaccine production were developed for fast and streamlined production of protein-based vaccines.

Tools for developing platforms for the design of new protein-based vaccines were established. This allowed the fast design of viral vaccines with disease potential.



15
NOVIEMBRE

Acelular: Secretoma, microvesículas, vacunas y partículas
Dr. Flavio Salazar
Bloque: 17:00 - 19:00 horas

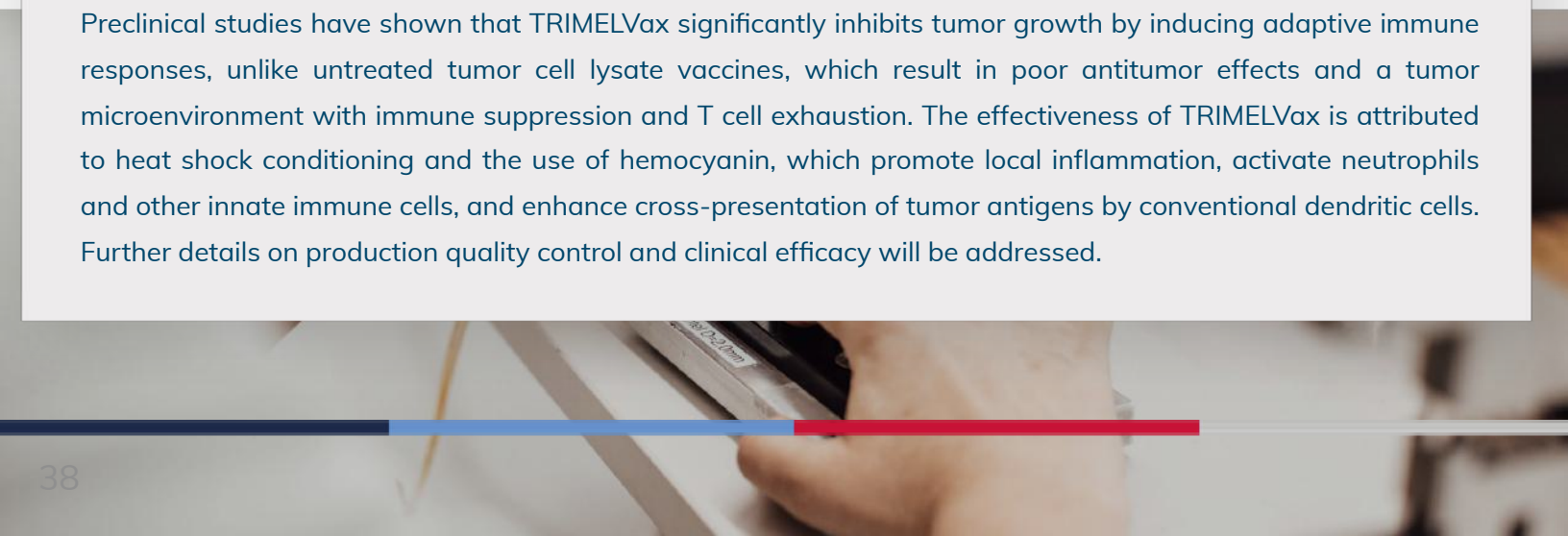
Immunological essentials for optimizing the clinical effect of tumor cell-based vaccines.

Flavio Salazar

Whole tumor cell (WTC) vaccines are a form of cancer immunotherapy that use whole or lysed tumor cells, either unaltered or modified, as sources of antigens to stimulate a robust immune response. They provide a wide array of tumor antigens that elicit diverse CD8+ and CD4+ T cell reactions. WTC vaccines can be derived from either autologous or allogeneic tumor cells. Allogeneic vaccines offer the advantage of being pre-manufactured and readily available, avoiding the need for patient-specific preparations and providing a shared source of tumor-associated antigens for cases where tumor sampling is difficult.

Recent advancements have focused on enhancing the immunogenicity of WTC vaccines through strategies like inducing immunogenic cell death (ICD) and using effective adjuvants. Our team has developed two innovative technologies: TAPCells and LycellVax. TAPCells, an autologous dendritic cell-like vaccine, has been tested in over 150 patients for melanoma and prostate cancer. TRIMELVax is a polyvalent WTC vaccine formulation designed to stimulate protective immune responses against murine B16F10 melanoma and MC38 colorectal cancer. It combines heat shock-conditioned tumor cell lysates with a mollusk-derived glycosylated hemocyanin as an adjuvant.

Preclinical studies have shown that TRIMELVax significantly inhibits tumor growth by inducing adaptive immune responses, unlike untreated tumor cell lysate vaccines, which result in poor antitumor effects and a tumor microenvironment with immune suppression and T cell exhaustion. The effectiveness of TRIMELVax is attributed to heat shock conditioning and the use of hemocyanin, which promote local inflammation, activate neutrophils and other innate immune cells, and enhance cross-presentation of tumor antigens by conventional dendritic cells. Further details on production quality control and clinical efficacy will be addressed.



16
NOVIEMBRE

Normativas y Políticas Públicas ISP

Q.F. MSc. José Crisóstomo
Bloque: 8:00 - 9:45 horas

Pharmaceutical Products in Chile with a Specific Focus on Biological Products, Biopharmaceuticals

José Crisóstomo

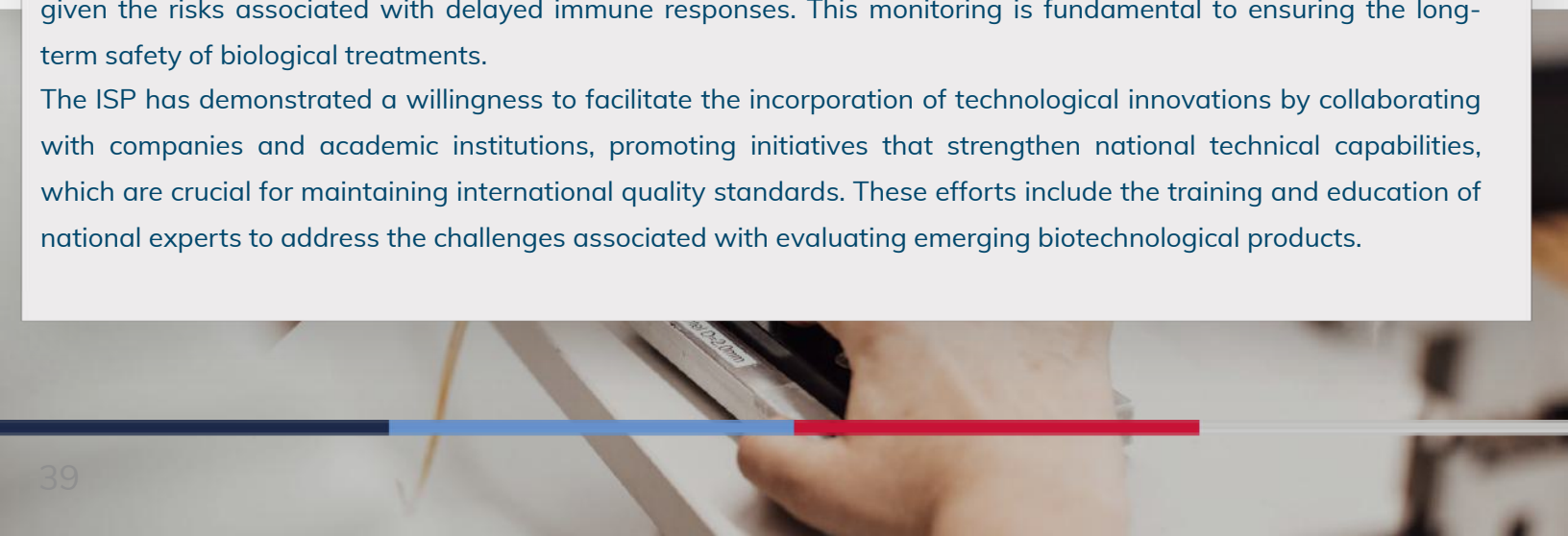
The registration of pharmaceutical products in Chile, regulated by the Institute of Public Health (ISP), ensures the quality, safety, and efficacy of medications marketed in the country. In this context, biological products, also known as biopharmaceuticals, represent a highly complex area due to their origin from living sources and sophisticated molecular structures, which impose strict regulatory requirements.

For the registration of biological products, the ISP requires detailed documentation that supports the quality of the biological material and the manufacturing processes. The safety and efficacy requirements include preclinical and clinical trials conducted under international standards, such as Good Clinical Practices (GCP) and Good Laboratory Practices (GLP). Preclinical studies focus on immunogenicity and toxicity, while clinical trials are conducted in progressive phases to evaluate safety and efficacy in humans.

The introduction of new technologies, such as advanced therapies, adds complexity to the regulatory framework. These therapies include gene, cell, and tissue-engineering treatments with significant therapeutic potential. The ISP has begun to adapt its regulations to evaluate these technologies, implementing accelerated review processes and compassionate use authorizations when no alternatives are available. These measures aim to balance innovation with patient safety.

Traceability and pharmacovigilance are essential for the regulation of biological and advanced products. The Chilean system requires a robust post-marketing monitoring plan to detect and mitigate adverse events, especially given the risks associated with delayed immune responses. This monitoring is fundamental to ensuring the long-term safety of biological treatments.

The ISP has demonstrated a willingness to facilitate the incorporation of technological innovations by collaborating with companies and academic institutions, promoting initiatives that strengthen national technical capabilities, which are crucial for maintaining international quality standards. These efforts include the training and education of national experts to address the challenges associated with evaluating emerging biotechnological products.



RESÚMENES DE EXPOSICIONES ORALES

DESARROLLO DE LÍNEAS CELULARES

IDENTIFICATION AND CHARACTERIZATION OF MACROPHAGE GALACTOSE LECTIN 2 (MGL-2) LIGANDS IN TUMOR PROGRESSION AND ANGIOGENESIS

Valeria da Costa 1, Eugenia Cedres 1, Matias Chalkling 1, Teresa Freire 1.

1. Laboratorio de Inmunomodulación y Vacunas. Unidad Académica de Inmunobiología. Facultad de Medicina, UdelaR. Gral Flores 2125, Montevideo, Uruguay

Background: The Tn antigen (GalNAc-O-Ser/Thr) stands out as a critical marker in 90% of adenocarcinomas, not only serving as a diagnostic tool but also a prime target for immunotherapies. It originates from disruptions in the mucin O-glycosylation pathway, often linked to deficiencies in core 1 synthase activity which is highly dependent on the Cosmc chaperone. MGL, a C-type lectin receptor found on dendritic cells and macrophages, recognizes Tn, steering immune responses crucial in anti-tumoral response. Our team has engineered a murine model of Tn+ lung cancer with incomplete glycosylation triggered by mutations in the cosmc gene. Our findings reveal that these Tn-expressing cells give rise to more aggressive and vascularized tumors, characterized by infiltrating IL-10+ T cells. Remarkably, this immunosuppressive environment hinges on MGL2+ cell presence, as their depletion halts tumor progression, angiogenesis, and the recruitment of IL-10+ T cells. Considering that MGL interacts with Tn on cancer cells, our study aims to identify and characterize MGL2 ligands that facilitate tumor progression within this model.

Methodology: MGL2 ligands were isolated from the cell membrane and conditioned medium of Tn+ cells by affinity chromatography, with a recombinant MGL2 Lectin. Through mass spectrometry analysis, the eluted proteins were identified, and potential ligands were categorized using O glycosylation prediction software (Net-O-glyc). Additionally, recombinant VEGF-A and SPARC were synthesized in the lung cancer cell line, both with normal and blocked glycosylation, using a lentiviral induction system.

Results: We have identified 95 glycoproteins from our study, spanning categories like extracellular matrix, metabolic enzymes, membrane trafficking proteins, and cytoskeleton components. Notably, among these, we emphasize the secreted protein acidic and rich in cysteine (SPARC), known for its role in extracellular matrix-cell interactions. Previous research indicates SPARC's involvement in promoting angiogenesis by enhancing vascular endothelial growth factor-A (VEGF-A) signaling. Currently, our focus is on investigating the angiogenic properties of recombinant VEGF-A and SPARC, each produced by the engineered lung cancer cell lines with normal and blocked glycosylation. Additionally, our study has revealed several extracellular matrix proteins among the ligands of MGL2, including Fibronectin and Nidogen-1. We are exploring their contributions to extracellular matrix assembly, structure, and collagen binding mechanisms.

Conclusions: Altering the O-glycosylation pathway of tumor cells resulted in the production of multiple MGL2 ligands present on both the cell surface and in the secretome, that might mediate tumor associate processes. The MGL2-protein interaction is an indicator of altered glycosylation in the protein, potentially influencing protein stability and biological function within the tumor microenvironment.

DESARROLLO DE LÍNEAS CELULARES

DEVELOPMENT OF A STABLE CELL LINE PRODUCER OF A FULLY HUMAN ANTIBODY USING THE EXPICHO-S SYSTEM

José A. Rodríguez-Siza 1, Karen Toledo-Stuardo 2, María J. Garrido 2, Sebastián Vergara 1, María C. Molina 2, Claudia Altamirano 1,3.

1. Biochemical Engineer School, Faculty of Engineer, Pontifical Catholic University of Valparaiso, Valparaiso, Chile.
2. Program of Immunology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Independencia, Chile.
3. Center of interventional medicine for precision on advanced cellular therapy (IMPACT), Las Condes, Chile.

Background: The development of a stable cell line is one of the most critical points in the development of a biopharmaceutical. CHO cells are the most widely used cell system to produce recombinant proteins for therapeutic purposes. In the development of stable cell lines, CHO K1, CHO DG44, and CHO-GS cell lines are routinely used, where the gene of interest is randomly integrated and can be amplified using selection pressure markers. ThermoFisher developed a cell line based on CHO-s cells for transient gene expression, named ExpiCHO-s, which have the property of growing in suspension without the need for fetal bovine serum at high cell densities. Despite this, ExpiCHO-s cells also allow the development of stable cell lines and even genetic amplification using methotrexate (MTX), as the manufacturer mentions, the cell line has reduced expression of the dhfr gene. However, there are not many reports on the development of this. The objective of this study was to develop a stable cell line producing a fully human antibody using the ExpiCHO-s system.

Methods: To achieve this, a stable transfection of two vectors was performed: one containing the genes for the light chain and the dihydrofolate reductase (dhfr) gene, and another with the genes for the heavy chain and neomycin (G418) resistance. The cell line was amplified and tested in different culture media, then subsequently cloned and characterized.

Results: The results showed that four clones were selected post-transfection, highlighting that clone B7 showed a qp of ~0.08 pg/cell/day. Gene amplification was performed up to concentrations of 0.8 µM MTX and 1.6 mg/mL G418, resulting in an increased qp of around 0.6 pg/cell/day. Subsequently, several culture media were tested, and it was found that using FortiCHO media, the maximum cell concentration could be optimized up to 11x106 cells/mL, achieving titers of 6 mg/L in batch culture. Finally, clones displaying similar qp were selected from this gene amplification.

Conclusion: Based on these results, we concluded that a stable cell line susceptible to gene amplification was successfully developed.

DESARROLLO DE LÍNEAS CELULARES

GENERATION OF A HETEROZYGOUS SLC18A1-BFP DOUBLE REPORTER HUMAN IPSC LINE (HMGUI001-A-8-36)

Freire-Flores Danton 1, Llanovarced-Kawles Nyna1, Rogers Nicole 2, Burtscher Ingo 2.

1. Centre for biotechnology and bioengineering, Department of chemical engineering, biotechnology, and materials, Universidad de Chile, Santiago, Chile.
2. Institute of Diabetes and Regeneration Research, Helmholtz Munich, Neuherberg, Germany.

Background: Human induced pluripotent stem cells (hiPSCs) can be differentiated with a stepwise protocol towards insulin-expressing pancreatic β-like cells, which have a broad range of applications in diabetes research. However, current differentiation protocols towards β-like cells result in heterogeneous cell populations. Besides functionally relevant insulin-expressing β-like cells, other hormone-secreting cell types as well as non-hormone-producing progenitors and cells of otherlineages are generated, among which are enterochromaffin cells (ECs), a subpopulation of serotonin-producing gut cells. In order to eliminate ECs from the desired cellular product, it is proposed to mark these cells with a fluorescent protein to separate these populations by fluorescence sorting. SLC18A1 protein can be used to identify ECs, as this protein is an integral membrane protein, embedded in synaptic vesicles, and serves to transfer monoamines. The SLC18A1-BFP hiPSC double reporter line (HMGUi001-A-8-36) was generated by heterozygous insertion of the 2A-H2B-BFP sequence between the translated and untranslated regions of exon 16 in the SLC18A1 gene. Genome editing was performed by CRISPR/ Cas9 targeting of the previously described iPSC line HMGUi001-A-8. Correct insertion was verified by PCR. Subsequent Sanger sequencing of the single guide RNA (sgRNA) targeted region revealed correct insertion in one allele and no unwanted mutation in the targeted allele or wild type allele. The generated line showed typical hiPSC colony formation. It was positive for the nuclear pluripotency markers SOX2 and OCT3/4 as well as the cell surface pluripotency markers SSEA-4 and TRA-1-60. Furthermore, pluripotency was confirmed by successful differentiation towards all three germ layers, which was demonstrated by immunostaining for endoderm, mesoderm and ectoderm specific markers. After differentiation of the line, mCherry expression could be detected in β-like cells by live imaging as well as by flow cytometry analysis. Furthermore, BFP expression could be detected by flow cytometry and immunostaining in enterochromaffin cells, confirming that BFP was co-expressed and co-localized with SLC18A1 protein. In summary, the HMGUi001-A-8-36 line allows for the monitoring of expression of SLC18A1 during differentiation towards pancreatic β-like cells. Beyond that, it can be used to obtain a homogeneous population of enterochromaffin cells.

DESARROLLO DE LÍNEAS CELULARES

EVALUATION AND CHARACTERIZATION OF ENDOGENOUS EUKARYOTIC PROMOTERS FROM CHO-K1 CELLS BY TRANSIENT AND STABLE TRANSFECTION ASSAYS AS TOOLS TO IMPROVE THE PRODUCTION OF DIFFICULT-TO-EXPRESS RECOMBINANT PROTEINS.

María Belén Ardusso 1, Agustina Gugliotta 1, Victoria Gastaldi 1, Claudio Prieto 2,3.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Ciudad Universitaria, Ruta Nacional No 168, km 472, CC 242 (CPA S3000ZAA), Santa Fe, Argentina.
2. UNL, FBCB, Centro Biotecnológico del Litoral, Ciudad Universitaria, Ruta Nacional No 168, km 472, CC 242 (CPA S3000ZAA), Santa Fe, Argentina.
3. Cellargen Biotech S.R.L., Antonia Godoy 6369 (S3000), Santa Fe, Argentina.

Background: Most recombinant proteins with applications in human and veterinary medicine are mainly produced using animal cell cultures, which have the disadvantage of low specific yields. Currently, strong viral-derived promoters are the most widely used in production processes, as they allow a high rate of expression. However, they can be susceptible to gene silencing. In this regard, endogenous promoters from eukaryotic cells are an interesting alternative since they are able to respond to cell culture conditions, avoiding problems of expression instability. In this work, we aimed to evaluate the activity of eukaryotic endogenous promoters, previously identified and isolated in our laboratory from genes with high expression levels in CHO-K1 cells cultured in bioreactors. For this purpose, the Receptor Binding Domain (RBD) of SARS-Cov2 was chosen as an example of a secretion protein that is difficult to produce.
Methods: Plasmid vectors containing the sequences of the endogenous promoters PromA, PromB, and PromC, were designed to drive RBD expression. The CMV plus enhancer promoter (CMV+E) was used as a control. Transient and stable gene expression were analysed in CHO-K1 cells by RT-qPCR and protein expression level by SDS-PAGE/western blots and competitive ELISA. Finally, RBD protein expression stability was assessed by competitive ELISA over approximately 40 generations
Results: Studies revealed that the endogenous PromA and PromB promoters allow equal or higher RBD expression compared to the CMV+E promoter in transient transfections and stable cell lines. Analysis by RT-qPCR, SDS-PAGE/western blot and competitive ELISA revealed that the higher levels of mRNA achieved with PromA and PromB correlate with the amount of RBD in the culture supernatant, supporting the direct relationship between transcription and translation in the gene expression process. Long-term stability studies showed that RBD expression under the control of these two promoters was higher in contrast to CMV+E. Particularly, the results obtained with the PromB promoter are promising, given that it is a sequence scarcely described in the literature.
Conclusions: The notable effectiveness of PromA and promB in driving RBD expression suggests that they can be a starting point for the design of synthetic promoters, as well as for the development of industrial cell lines, offering an alternative and potentially more effective way to overcome the challenges associated with the production of complex proteins in eukaryotic cell systems.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

CHARACTERIZATION OF MELANOMA CELL LINE CULTURES AND THEIR ADAPTATION IN THREE-DIMENSIONAL CULTURES FOR THE PRODUCTION OF IMMUNOGENIC LYSATES USED IN VACCINES AGAINST HUMAN MELANOMA

Ignacia Segovia 1, Paulina Soto 1, Iván Flores 2,3, Cristian Pereda 2,3, Flavio Salazar 2,3, Ziomara P. Gerdtzen 1.

1. Mammalian Cell Culture Lab MCCL, Center for Biotechnology and Bioengineering CeBiB, Department of Chemical Engineering, Biotechnology and Materials, University of Chile.
2. Oncobiomed Advanced Cells Technologies.
3. Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile.

Background: Malignant melanoma is the most aggressive form of skin cancer. Although it comprises less than 5% of skin cancer diagnoses, melanoma is responsible for 65% of deaths related to this type of cancer. Therefore, there is a pressing need to develop effective immunotherapies and establish a scalable production methodology to meet demand and be accessible to patients. Oncobiomed, a biotechnology company focused on technologies related to the prevention, diagnosis, and treatment of cancer, has developed a biologic vaccine for melanoma immunotherapy that requires the use of lysates from three Melanoma Cell Lines: MCL 1, MCL 2, and MCL 3. The production of this vaccine faces limitations due to the complexity and laboriousness of the lysate generation protocol. Consequently, our ongoing work focuses on implementing different strategies for improving the production of these cell lines. This includes the characterization of the culture, implementation of a suspension culture-based process to enhance scalability, optimization of media composition and feeding to increase productivity and setting the basis for a biomanufacturer process design.
In the first stage, batch adherent cell culture was carried out for each melanoma cell line, monitoring the cell density, nutrient profile, and viability. These results were analyzed to calculate the key culture parameters, which provide information regarding their metabolic requirements and cell growth. Regarding batch adherent cultures, doubling times of 18 hours were obtained for the three lines. In the second stage, repeated batch cultures in suspension were conducted under specific cell density and culture conditions to favor the formation of self-assembled cell structures or spheroids. In these experiments, MCL 1 reaches a cell density similar to that of the batch adherent culture, and MCL 2 shows a constant cell density. Ongoing experiments are being conducted to determine the ideal conditions for suspension cultivation, including agitation speed, medium composition, and a feeding strategy to increase biomass production. These results provide insights into the specific characteristics and requirements of each melanoma cell line under suspension conditions and allow for the design of a suitable production process for each particular cell line.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

CELLULAR BIOTECHNOLOGY STRATEGIES TO ENHANCE BOVINE FOLLICLE STIMULATING HORMONE PRODUCTION IN CHINESE HAMSTER OVARY CELLS

Daniela Araya 1, Rita Cerro 2, Constanza Núñez 1, Yesenia Latorre 1, Camila Aros 1, Constanza Collarte 1, Mauricio Vergara 1, Claudia Altamirano 1, Jorge Toledo 2.

1. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.
2. Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

Background: The biopharmaceutical industry is constantly growing, facing the challenge of increasing production without raising costs. One of the main issues is that super-producing cell lines often experience endoplasmic reticulum stress, creating a "bottleneck" that limits production. This thesis explores strategies to overcome this problem, focusing on the relationship between lipid metabolism and the endoplasmic reticulum stress response.
Methodology: A super-producing cell line of recombinant bovine FSH protein, essential for synchronizing reproduction in the livestock industry, was generated. Using the recombinase-mediated homologous recombination technique, the EPO protein was replaced with the bFSH protein. Subsequently, the cells were cultured under moderate hypothermia conditions (33°C) and the medium was supplemented with lipid concentrate, hypothesizing that lipids would improve production through the UPR response.
Results: Techniques for measuring viability, cell growth, and metabolite quantification revealed that lipids improve cell viability at 33°C and reduce lactate concentration in the medium. Additionally, molecular analyses through real-time PCR and Western blotting showed that lipids decrease endoplasmic reticulum stress response both under standard (37°C) and moderate hypothermia conditions.
Conclusions: Supplementing the medium with lipid concentrate not only enhances cell viability and reduces endoplasmic reticulum stress but also increases recombinant protein production in both culture conditions. These findings are particularly novel under moderate hypothermia conditions, offering new perspectives for improving production in the biopharmaceutical industry.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

BINDING CAPACITY OF A FULLY HUMAN ANTI-MICA ANTIBODY TO THE MICA PROTEIN IN GASTRIC CELL LINES

Yuneisy Guerra Peña 1, Carla Diaz 1, Karen Toledo-Stuardo 1, Fabiola González-Herrera 1, Samantha Tello 1, María José Garrido 1, Douglas J. Matthies 1,2, Mauricio González-Olivares 1, Ivo Campos 1, Nicolás Fehring 1, Claudia Altamirano 3,María Carmen Molina 1.

1. Programa de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile
2. Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile
3. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

Background: MICA is a highly polymorphic transmembrane protein overexpressed in damaged cells and it has been described in different types of cancer, including gastric cancer. This protein is a ligand of the NK cell activating receptor, NKG2D. Upon interaction, it generates a cytotoxic response, facilitating the early elimination of damaged cells. This immune mechanism is evaded by proteolytic cleavage of the MICA protein, where released MICA generates a low expression of NKG2D on the NK cell membrane, thereby attenuating the cytotoxic response. In this context, a fully human antibody (AcHu) against the MICA protein was developed in our laboratory to block the interaction with its receptor and prevent its internalization. In tests carried out on AcHu, it was shown that it was able to bind to the recombinant MICA protein, but it was necessary to demonstrate that this occurred with the native protein present in the cell membrane. Therefore, the aim of this study was to evaluate the binding capacity of AcHu anti-MICA to the MICA protein present in gastric cell lines.
Methods: Gastric cell lines GES-1, MKN45, and AGS were used, and the allelic variant of MICA expressed was determined by sequencing. Subsequently, the binding capacity of the AcHu anti-MICA to the different allelic variants expressed by the gastric cell lines was evaluated by flow cytometry. A commercial murine anti-MICA antibody was used as a positive control.
Results: The results showed that the gastric cell lines presented different allelic variants of MICA (GES-1: MICA*008, MKN45: MICA*009, and AGS: MICA*010). On the other hand, it was demonstrated that the AcHu anti-MICA is able to bind to MICA present in GES-1 and MKN45 cell lines, which correspond to different MICA allelic variants of epidemiological interest. In contrast, the commercial anti-MICA antibody only showed recognition by GES-1.
Conclusion: In conclusion, the AcHu anti-MICA is able to bind to different allelic variants of MICA present in different gastric cell lines, demonstrating its potential as a future anti-cancer therapy.
Acknowledgments: Proyecto ANILLO ACT210068, FONDECYT N° 1221031.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

DEVELOPMENT AND CHARACTERIZATION OF A NOVEL HYPERGLYCOSYLATED RECOMBINANT BOVINE FOLLICLE-STIMULATING HORMONE WITH IMPROVED PHARMACOKINETIC PROPERTIES AND ENHANCED BIOPOTENCY: A POTENTIAL CANDIDATE FOR USE IN VETERINARY INDUSTRY

Javier Villarraza 1, Sebastián Antuña 2, María Belén Tardivo 2, María Celeste Rodríguez 1,2, Lucas Etchevers 4, María Emilia Odriozola 4, Pablo Díaz 4, Hugo Ortega 4, Claudio Prieto 3,5, Natalia Ceaglio 1.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral (CBL), Santa Fe, Argentina.
2. Biotecnofe S.A. Parque Tecnológico Litoral Centro (PTLC), Santa Fe, Argentina.
3. UNL, FBCB, Cátedra de Química Analítica II, Santa Fe, Argentina.
4. UNL, CONICET, ICiVet-Litoral, Centro de Medicina Comparada (CMC), Esperanza, Santa Fe, Argentina.
5. UNL, FBCB, Centro Biotecnológico del Litoral (CBL), Santa Fe, Argentina.

Background: Bovine ovarian hyperstimulation is a process currently based on porcine follicle-stimulating hormone (pFSH) preparations partially purified from pituitary glands. This comprises many disadvantages, including batch-to-batch variations, possible contamination with luteinizing hormone, and an extremely short circulating half-life. Several attempts to produce bovine FSH (bFSH) in recombinant systems have been reported; nonetheless, up to date, the most commonly used products are partially purified preparations derived from porcine or ovine (pFSH or oFSH) pituitaries. For these reasons, the development of a recombinant bovine FSH (rbFSH) with improved pharmacokinetic parameters represents a powerful strategy to substitute the animal-derived preparations.

Methods: LA-rbFSH was designed by fusing three copies of a highly O-glycosylated peptide derived from hGM-CSF (named mGMOP) to the C-terminus. Cell lines were generated through lentiviral transgenesis of suspension CHO-K1 cells. Cells were cultured in a one-liter bioreactor for 17 days. rbFSH and LA-rbFSH proteins were purified from culture supernatants by immobilized metal affinity chromatography (IMAC). Physicochemical characterization of purified proteins was performed following SDS-PAGE, western-blot, isoelectric focusing and sialic acid quantification by HPAEC-PAD. Biopotency was evaluated in female rats using the Steelman and Pohley bioassay. Pharmacokinetic studies were conducted in female rats.

Results: rbFSH and LA-rbFSH were obtained with high purity levels (>95%), with traces of free α and β subunits as main contaminants. LA-rbFSH presented a higher apparent molecular mass with more acidic isoforms and a 4-fold increase in sialic acid content compared to rbFSH. It also demonstrated a notable improvement in pharmacokinetic properties after administration to rats, including higher concentration in plasma and a significant (7-fold) reduction in apparent clearance (CLapp). In addition, the in vivo specific bioactivity of LA-rbFSH in rats was 2.4-fold higher compared to rbFSH.

Conclusion: The strategy reported in this work allows the production of a novel recombinant hyperglycosylated rbFSH with enhanced bioactivity and pharmacokinetic parameters, and lack of LH activity, representing an attractive alternative to the use of pituitary-derived FSH. Further experiments must be conducted to evaluate the bioactivity of the rbFSH variants in cattle and to elucidate the dosing regimen of both recombinant variants.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

IMPROVEMENT OF IGG PRODUCTION IN CHEMICALLY DEFINED SYSTEMS VIA PROTEIN HYDROLYSATE SUPPLEMENTATION

Brandon Wrage 1, Brittany A. Patterson 1, Salewa Akande 1, Any Goncalves 1, Angel Varela-Rohena, PhD 1.

1. Kerry Pharma RD&A - Cell Nutrition 3400 Millington Road, Beloit, WI 53511, USA

Background: A growing focus for the biopharma industry is the implementation of process intensification as a more efficient and cost-effective approach to the production of biologic drugs. Media development and optimization are important strategies for bioprocess optimization during the production of biotherapeutics. Each process and cell lineage can exhibit different nutritional requirements. While most CHO-based processes utilize chemically-defined (CD) media and feeds, plant-derived protein hydrolysates can serve as an easy to implement, nutritionally dense and sustainable tool to increase production yields.
Methods: This study focuses on two CHO cell lines expressing industrially relevant IgG. Each cell line was grown in three chemically defined media and feed systems according to each manufacturer's recommendations. Sheff-CHO supplements were supplemented once the cells reached peak viable cell density. The performance of the new experimental conditions was evaluated in terms of cell proliferation, culture viability, IgG titer and glycosylation profiles.
Results: These case studies demonstrate that Sheff-CHO supplements and ultrafiltered protein hydrolysates can increase IgG titers up to 25% over standard fed-batch processes. The developed methods enable use of these supplements and hydrolysates within existing processes, without the need for cell adaptation or changes to medium and feed. The individual effect of each supplement or hydrolysate can be dependent on the medium, feed or cell line. Thus, screening and dose optimization of Sheff-CHO supplements and HyPepTM protein hydrolysates should be considered. Cost reductions of 20-30% on a per gram basis of IgG were achieved by supplementation with plant hydrolysates.
Conclusion: Impact of supplementation on product quality will be discussed, but basal media on its own imparted a greater effect on glycosylation than supplementation with plant hydrolysates. Future work will explore the effect of these supplements and hydrolysates as tools for process intensification.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

LARGE SCALE MANUFACTURING OF ANTIBODIES: USING PROCESS INDICATORS TO IDENTIFY PRODUCTIONS RESERVES

Maivy Martínez 1, Melissa Bravo 1, Roberto Rafael Núñez 1, Osvaldo Mora 1, José Antonio Fernández1, María Caridad Duartes 1, David Curbelo1, Miguel Arias 1, Danais Alfonso 1, Yaritza Galvez 1, Ernesto Chico 1,2.

1. Centro de Inmunología Molecular (CIM), Calle 15 esq. 216 S/N, Siboney, Playa, La Habana, Cuba. A.P 16040, La Habana 11600.
2. Facultad de Ingeniería Química, Universidad Tecnológica de la Habana (CUJAE), Calle 114 #11901 / Ciclovía y Rotonda CP 19390, Marianao, La Habana, Cuba.

Background: The antibody industry has experienced significant growth in recent years, and it is crucial to improve manufacturing operations to ensure large-scale production and delivery of high-quality products. The Center of Molecular Immunology (CIM) is a Cuban biotechnological institution that has been dedicated for several decades to the production of monoclonal antibodies and recombinant proteins for the treatment of cancer and other chronic diseases. At the Antyter plant of CIM, the monoclonal antibody nimotuzumab used for the treatment of different types of cancer, has been manufactured to reach an accumulated output of more than 150kg. In this report, key bioprocess aspects such as downstream yields, cell densities, production costs, and quality of the final product will be discussed along production history. It was found that the isolated analysis of process variables and current production indicators makes it difficult to identify capacity reserves and opportunities for improvement. A model was developed to estimate the Overall Equipment Effectiveness (OEE) of the antibody plant. This is a key process performance indicator, originally developed for other industries, which was here adapted to monitor and improve biopharmaceutical operations and estimate the global efficacy of production campaigns, by combining the effect of time availability, process performance and quality. The capability of the proposed new indicator (OEE) to estimate production output was explored by correlation analysis along historical data, demonstrating it is a useful tool in production planning and the identification of potential reserves for improved productivity. The aim of this paper is to share the CIM's experience in measuring process performance with a holistic view, in order to identify specific areas that require attention and optimization in industrial cell culture operations.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

COMPARISON OF ZIKA VIRUS PRODUCTION IN VERO CELLS IN TWO DIFFERENT FIXED-BED CULTIVATION SYSTEMS

Flávia Ferreira Barbosa 1, Júlio Cesar Lima Alves Cassoli 1, Diogo Santos de Sousa 1, Renato Mancini Astray 1.

1. Laboratório Multipropósito, Instituto Butantan, Brazil.

Background: Virus production for vaccine products many times is performed in Vero cells growing adherent to plastic or polymer supports. The use of fixed-bed technologies allows cultivation to be carried out using a process intensification strategy. In this way, a greater number of cells can be cultivated at a higher concentration compared to conventional technologies.
Methods: Vero cells were grown in two fixed bed culture systems, iCellis (Cytiva) and CellCradle (Esco). The cell growth was evaluated in kinetic experiments with samples taken at least daily. Serum-free culture medium VP (Thermo) was used and replaced when necessary to sustain cell growth in appropriate condition. Cells were infected with Zika virus in the time of infection defined previously based in DoE studies and growth kinetics.
Results: In these systems, cells were able to reach cellular concentrations per area similar to those obtained in T culture flasks (1.3 to 1.8x10^5 cells/cm2). Although there was an initial process of adaptation of the cells to the new surfaces (showing lag phase), the culture showed normal growth dynamics after starting growth. The doubling times, growth speed, metabolite consumption and maximum cell concentration are discussed in the work and compared between the systems and between them and the cells cultured in T flasks. The best time for viral infection in each of the systems under study was determined and the results demonstrated the potential of fixed bed systems to generate products with higher viral concentration at the end of the process.
Conclusion: Fixed-bed systems are valuable tools for Vero cell process intensification for vaccine production.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

CELLULAR RESPONSE AND HYDRODYNAMIC STRESS ADAPTATION OF CHO CELL LINES BY EXPOSURE TO ACOUSTIC RESONANCE MIXING.

Fernando David Montalvo-Sandoval 1,2, Norma A. Valdez-Cruz 2, Mauricio A. Trujillo-Roldán 2.

1. Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, México. Unidad de Posgrado, Edificio B, 1° Piso, Circuito de Posgrados, Ciudad Universitaria, Coyoacán, C.P. 04510, CDMX, México.
2. Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, C.P. 04510, Ciudad de México, México.

Background: Mammalian cells cultivated in bioreactors are pivotal to produce biopharmaceuticals. However, scalability is hindered by hydrodynamic stress, as increased agitation improves mixing but harms cell viability. Several scaling-down approaches have been explored to investigate hydrodynamic stress. Nonetheless, a comprehensive model that encompasses all aspects of cellular response remains to be established. Particularly in the context of acoustic resonance mixing (RAM), increasing oxygen transfer rates in bacterial shake flask cultures has shown potential for improving productivity. Hence, reducing hydrodynamic stress with low-frequency acoustic energy, coupled with potential cell adaptation to agitation conditions, is anticipated to have a beneficial effect on CHO cells. Here, we evaluated the impact of RAM on the production of a monoclonal antibody (mAb) alongside the adaptation of industrially relevant recombinant CHO DG44 cell lines. Cells were adapted to growth under RAM in 250 mL Corning polycarbonate Erlenmeyer flasks with 50 mL of culture media. The CHO cell's lethal and sub-lethal effects were assessed in cultures with lower hydrodynamic stress (orbitally agitated T25 flasks at 60 rpm) through kinetic and stoichiometric analysis, specific mAb productivity, and lactate dehydrogenase release. Additionally, glucose consumption and ammonia and lactic acid release were monitored throughout the cultures.

Cells grown using RAM at 3 x g seem to experience a significant increase in stress, resulting in a specific growth rate of 0.023 h-1 compared to the same cell line evaluated under lower stress conditions of 0.029 h-1 for the T25 flask system. No significant differences in mAb production were found (around 90 ng/mL). Direct adaptation, involving a continuous change in culture medium, maintained cell viability under RAM for almost 500 hours. Obtaining five cell lines at different exposure times of the culture revealed an increase in maximum viable cell density of around 50% when cells were grown without stress in T25 flasks. Additionally, a differential response was observed following the exposure of cells to acute stress in the acoustic mixer, measured by LDH release.

RAM has been shown to be a robust method for adapting CHO cells to hydrodynamic stress. Understanding cell response to hydrodynamic stress is crucial for optimizing cell culture processes in biopharmaceutical production. This study presents a hydrodynamic stress-adapted CHO cell line, marking a significant advancement in understanding hydrodynamic stress in mammalian cell culture.
Acknowledgments to the financial support provided by the PAPIIT-UNAM (IN210822, IV201220), CONAHCYT (CF-2023-I-1248, CF-2023-I-1549), and PAPIIT Sabbatical stay granted to Dr. NAV-C and MAT-R.

TERAPIA GÉNICA Y TERAPIA CELULAR

IMPACT OF GALACTOSE/LACTATE SUPPLEMENTATION T CELLS DIFFERENTIATION, PROLIFERATION AND THERAPEUTIC POTENTIAL

Víctor Cerpa 1,2, Mauricio Vergara 1, Paloma Fuentes 2, Charlotte Hill2, Maroun Khoury 2, Claudia Altamirano 1,2.

1. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2085, Valparaíso 2362803, Chile.
2. IMPACT-Center of Interventional Medicine for Precision and Advanced Cellular Therapy. Concurso Centro Científico y Tecnológico de Excelencia Basal FB210024 (2022-2026).

Background: CAR-T cell therapy is an autologous therapy and use genetically modified T cells with a recombinant receptor that recognizes specific tumor-associated antigen to target and eliminate the tumor cells. It is currently approved for the treatment of acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma and multiple myeloma. During the manufacturing process, the T cells are differentiated into different subsets, where effector T cells predominate, this subset is characterized by a high cytotoxic activity but limited self-renewal. Promoting the differentiation into stem cell memory T cells with superior self-renewal capacity is critical for enhancing therapeutic efficacy. And this level of differentiation will be given mainly by the metabolic state in which the cells are in, where effector cells will have a highly glycolytic metabolism and memory stem cells rely on oxidative phosphorylation and fatty acid oxidation. The conventional manufacturing process is characterized by rapidly inducing this glycolytic metabolism to the cells, which is why the effector phenotype is favored. To change this, the need has been established to change the carbon source during lymphocyte culture, opting for one that is slowly metabolizable by the cell, as opposed to glucose, thus decreasing glycolysis. For this purpose, the culture medium has been modified by replacing glucose by a combination of galactose and lactate, since it has been reported in different mammalian cell lines that these are slowly metabolizable carbon sources, nutritional conditions are systematically altered to observe changes in metabolic pathways, particularly focusing on glycolysis and oxidative phosphorylation (OXPHOS). Flow cytometry is used to analyze various markers to determine the differentiation status of the culture. Initial results indicate that galactose/lactate supplementation can down-regulate glycolytic metabolism while enhancing oxidative capacity. These metabolic shifts are associated with increased potential for TSCM formation, suggesting a promising strategy for improving CAR-T cell profiles. By defining the effects of galactose/lactate supplementation on CD8+ T cell metabolism, this study provides insights into optimizing CAR-T cell differentiation. The findings are expected to contribute to the development of CAR-T cells with enhanced self-renewal and persistence, improving therapeutic outcomes in R/R aggressive B-cell lymphomas.

TERAPIA GÉNICA Y TERAPIA CELULAR

ADIPOSE MESENCHYMAL STROMAL CELLS: ISOLATION, CHARACTERIZATION, AND APPLICATION IN ANTITUMOR CELL THERAPY EMPLOYING LIGHT-RESPONSIVE NANOPARTICLES

Eugenia Bühler 1, Lucía Beaugé 2, Paolo Lippi 3, Luis Sommariva Odone 3, Matías Fernández Viña 4, Simona Camorani 5, Rodrigo Palacios 2, Laura Cerchia 5, Luis Ibarra 1.

1. Instituto de Biotecnología Ambiental y Salud (INBIAS), National Council for Scientific and Technical Research (CONICET) and National University of Río Cuarto (UNRC), Río Cuarto, Argentina.
2. Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA), National Council for Scientific and Technical Research (CONICET) and National University of Río Cuarto (UNRC), Río Cuarto, Argentina.
3. Centro de Traumatología y Artroscopía Privado S.R.L., Río Cuarto, Argentina.
4. Stem Cell Therapy Argentina, San Nicolás de los Arroyos, Argentina.
5. Institute of Experimental Endocrinology and Oncology Gaetano Salvatore, Italian National Research Council (CNR), Napoli, Italy.

Background: The targeting of therapeutic nanoparticles (NPs) to solid tumors, including those in the central nervous system (CNS), is an emerging area of research focused on improving diagnostic and therapeutic precision. Coating NPs with tumor-specific ligands enhances cancer selectivity, a process known as active NP targeting. Despite extensive research, few formulations have advanced to clinical trials due to numerous biological barriers. Thus, novel strategies are essential to improve NP transport and selectivity within tumors. An integrated cell therapy strategy proposes using mesenchymal stromal cells (MSCs) derived from adipose tissue as delivery vectors for actively targeted photoactivatable polymeric nanoparticles to treat CNS tumors like glioblastoma (GBM). This approach leverages the biological recruitment of circulating immune and non-immune cells to solid tumors, aiming to enhance light-mediated therapeutic efficacy.

Methods: MSCs were isolated from subcutaneous adipose tissue samples from 52 human patients at the Centro de Traumatología y Artroscopía Privado S.R.L. in Río Cuarto. Both tissue explants and micronized fat (MFAT) obtained via mechanical disaggregation with cannulas of 2.4, 2, and 1.2 mm diameters were cultured in different growth media, including DMEM, RPMI, and Hybri-Care, supplemented with fetal bovine serum. MSCs were identified using antibodies against CD44, CD73, CD90, and CD45. Nanoparticles based on conjugated polymers (NPC) were synthesized from PCPDTBT and PFOTBT, showing cytotoxic action post-irradiation. Aptamers Gint4.T, which specifically bind to the human Platelet-Derived Growth Factor Receptor β (PDGFRβ) ectodomain overexpressed in MSCs, and a scrambled sequence (SCR) were developed for conjugation to the surface of NPCs using an EDC/NHS reaction.

Results: Various conditions, including the volume ratio of tissue to support, were tested to establish and standardize an MSC isolation protocol. DMEM with 20% FBS was more effective for MSC proliferation, achieving 80-100% confluence faster than with 10% FBS and other media tested. Flow cytometry revealed that both thawed and cultured samples, as well as freshly proliferated samples from patients, expressed CD44, CD73, and CD90 (≥ 95% of the cell population) and lacked CD45 (≥ 95% of the cell population), confirming their identity as MSCs. Gint4.T aptamer enhanced NPC uptake by MSCs without affecting cell viability, demonstrating endosomal trafficking behavior.

Conclusions: Many biological cells inherently target brain tumors, are less immunogenic, have longer circulation lifespans, can be bioengineered, and naturally cross biological barriers. MSCs are promising NP delivery vectors due to their ease of ex vivo expansion and ability to deliver extracellular vesicles loaded with therapeutic NPs.

TERAPIA GÉNICA Y TERAPIA CELULAR

DETERMINATION OF SUSPENSION CULTURE STRATEGIES USING MICROCARRIERS TO INCREASE THE PRODUCTION OF MELANOMA TUMOR LYSATES FOR THE DEVELOPMENT OF THERAPEUTIC CANCER VACCINES

Paulina Soto 1, Ignacia Segovia 1, Anamaría Daza 1, Iván Flores b¿2,3, Cristian Pereda 2,3, Flavio Salazar 2,3, Ziomara Gerdtzen 1.

1. Mammalian Cell Culture Lab MCCL, Centre for Biotechnology and Engineering CeBiB, Department of Chemical Engineering, Biotechnology and Materials, University of Chile.
2. Oncobiomed Advanced Cells Technologies.
3. Institute of Biomedical Sciences ICBM, School of Medicine, University of Chile

Background: Cutaneous melanoma is the most malignant skin tumor due to its high capacity to metastasize, being responsible for 75% of skin cancer deaths. A successful treatment alternative developed by Oncobiomed is the TAPCells technology: antigen-presenting cells that, after being injected into the patient, generate an immune response against melanoma. TAPCells are previously trained with tumor lysates from melanoma cell lines; however, the production of these lysates is limited by their low scalability, as these lines only grow adherent to surfaces. Therefore, to increase yield, it is necessary to adapt them to suspension culture.
This study aims to optimize the culture conditions of the melanoma cell line (Melanoma Cell Line) MCL 2 using suspension culture methodologies with microcarriers. The goal is to characterize the most suitable cell concentration and cell-to-microcarrier ratio during the seeding stage, as well as to explore agitation conditions during suspension. For this purpose, a test was conducted in a 12-well plate where CultiSpher G microcarriers were seeded at 1 g/L and 3 g/L, with ratios of 0:1, 5:1, 10:1, and 15:1 cells per microcarrier sphere. The conditions that allowed the best cell adherence were qualitatively determined. Subsequently, suspension tests were carried out using the seeding conditions found, and the cells were cultured under different agitation parameters. Once the most suitable parameters are established, it is proposed to quantify cell growth in this configuration and evaluate the functionality of the generated cells in relation to the activation capacity of TAPCells. From these studies, it is expected to advance the development of a scalable process for the culture of MCL2.

TERAPIA GÉNICA Y TERAPIA CELULAR

ADVANCING STEM CELL-DERIVED ISLET-LIKE CLUSTERS FOR DIABETES MELLITUS THERAPY: EVALUATING ENRICHED ISLET FUNCTION, MICROCARRIER-MEDIATED PANCREATIC PROGENITOR EXPANSION, AND IPS DIFFERENTIATION

Nyna Llanovarced Kawles 1,2, Danton Freire 1,2, Bárbara Andrews 1,2, Juan Asenjo 1,2.

1. Centre for biotechnology and bioengineering, CeBiB, Beauchef 851, 8370448 Santiago, Chile.
2. Department of Chemical Engineering, Biotechnology and Materials, University of Chile, Beauchef 851, 8370448 Santiago, Chile.

Background: A promising treatment for type 1 diabetes involves replacing dysfunctional insulin-producing β-cells with stem cell-derived (SC-) β-cells. The islets of Langerhans have a complex endocrine regulation involving different cell types, with predominant cell types being the beta and alpha cells. To create a functional tissue capable of sensing and responding to surrounding glucose variation, a more complex structure with more than one cell type is essential. Several differentiation protocols to β-like cells via pancreatic progenitors have been developed, but unwanted cells are also produced during the process, impairing the overall function of the tissue and keeping the production of desired cells low. Added to this, due to the high demand for functional cells (around one billion cells per patient), new differentiation and expansion methods need to be studied to improve production. This study aims to enhance the generation of islet-like clusters using two approaches. First, by creating an improved islet consisting exclusively of alpha and beta cells to assess their performance compared to those obtained from traditional differentiation protocols. Second, by increasing the production of cells of interest using microcarrier scaffolds for SC-β-cell production from iPS and for the expansion of pancreatic progenitors. This is due to the high surface area-to-volume ratio and scalability potential to bioreactors offered by microcarriers. The results show that an islet produced exclusively by beta and alpha cells performs better compared to those produced by the currently used protocols; however, its generation increases production costs. Regarding the use of microcarriers, initial results show the feasibility of the process, allowing the seeding and expansion of pancreatic progenitors, while retaining characteristic markers such as PDX1 and NKX-6.1, with a higher fold expansion than the control. As for SC-β-cells production on microcarriers, the results showed no percentage of cells maintaining characteristic differentiation markers during the process, suggesting microcarriers are a better platform for expanding already differentiated cells than for the differentiation process itself. This initial analysis suggests the need for further investigations to establish process parameters as an opportunity to improve the process, providing flexibility in research and therapeutic production.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

MUCOSAL DELIVERY AND IMMUNOGENICITY OF A PHAGE-BASED MULTISTATE VACCINE CANDIDATE FOR *MYCOBACTERIUM TUBERCULOSIS.*

Angela Hidalgo 1,2, Evaldo Rivas 2, Cristian Vargas 2, Fátima Reyes 2, Lionel Zapata 2, Rafael Maura1, Roxana Zúñiga 2, Yaneysis Lamothe 2, Óscar Otero 2, Oliberto Sánchez 2, Jorge R. Toledo 1, Norazmi Mohd Nor 3, Frank Camacho 2.

1. Laboratorio de Biotecnología y Biofármacos, Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
2. Laboratorio de Biofármacos Recombinantes, Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
3. School of Health Sciences, Universiti Sains Malaysia, Malaysia.

Background: Tuberculosis (TB) is one of the 15 leading causes of death worldwide. In 2020, there will be 10 million new TB cases and 1.5 million deaths. There are two TB-related conditions: latent TB infection (LTBI) and TB disease. LTBI is the result of an infection with M. tuberculosis that was controlled by the immune system and occurs in a quarter of the world's population. When the immune response fails to control the infection, disease develops and people with TB are highly infectious. Vaccination is the most effective way to prevent and control infectious diseases. The first and only licensed vaccine against TB is Bacillus Calmette-Guérin, but its effectiveness varies from 0% to 80%. The high incidence and mortality of TB, the lack of early diagnosis, the poor adherence to treatment and the absence of an effective vaccine all point to the need to develop new strategies to prevent TB.
Methods: We developed a novel mucosal vaccine candidate against M. tuberculosis based on a recombinant phage targeting the mucosa and expressing a multistage TB antigen. We administered the vaccine candidate by different routes: intranasal, oral and subcutaneous, and evaluated the systemic and mucosal immune responses in comparison with the control group in mice.
Results: The phage-based multistate vaccine candidate against M. tuberculosis promotes IgA and IgG antibody production at the mucosal and systemic levels, respectively. There are differences in antibody production between groups vaccinated with the phage-based vaccine candidate with and without mucosal targeting. Subcutaneous administration appears to induce an enhanced immune response in mice when comparing antibody production in different fluids.
Conclusions: Our phage-based vaccine candidate acts as a vaccine carrier for the TB antigen and the mucosal targeting has a direct effect on the levels of antibodies produced. This suggests that our vaccine candidate elicits a targeted immune response against the M. tuberculosis antigen at the mucosal and systemic levels. Further analysis will include evaluation of the antigen-specific cellular response and determination of protection in a challenge with the pathogen.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

DYNAMIC MODELING OF THE BULK PROTEIN TRANSPORT IN THE MAMMALIAN SECRETORY PATHWAY

Santiago Benavides-López 1,2, Laura M. Muñoz-Echeverri 3,2, Mauricio A. Trujillo-Roldán 2, Norma A. Valdez-Cruz 2.

1. Posgrado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, Unidad de Posgrado, Edificio B, 1° Piso, Circuito de Posgrados, Ciudad Universitaria, Coyoacán, C.P. 04510, CDMX, México.
2. Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, AP. 70228, Ciudad de México. CP. 04510, México
3. Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Unidad de Posgrado, Edificio D, 1° Piso, Circuito de Posgrados, Ciudad Universitaria,Coyoacán, C.P. 04510, CDMX, México.

Background: Tuberculosis The secretory pathway is a cellular process that delivers newly synthesized proteins and other molecules packaged into transport vesicles to various destinations within and outside the cell. This pathway is essential for producing recombinant glycoproteins of biopharmaceutical interest in mammalian expression systems and has been identified as an intracellular bottleneck for their production. Therefore, understanding the events involved in this pathway is crucial for developing strategies to maximize the productivity of these biotechnological products. In this study, we developed a dynamic model for protein transport across the secretory pathway.
Methods: The model was created using the Phenomenological Based Semi-Physical Model (PBSM) methodology. PBSM offers significant advantages in studying biological systems, providing mechanistic insights without excessive mathematical complexity. To validate the model, we compared simulations against literature data under various conditions.
Results: The proposed model represents the bulk flow of proteins along the organelles of the mammalian secretory pathway. It considers the role of the Ras GTPase family cycles in controlling vesicle coat assembly, the recycling of v-SNARE proteins between organelles, and the dynamic equilibrium between the endoplasmic reticulum and Golgi apparatus volumes due to the constant vesicle flow between organelles. The model is structured using formal units rather than arbitrary units or normalized measures. We integrated kinetic data from human, hamster, and monkey cells for model validation. Following the well-mixing assumption and the mass action law principle, the model describes the bulk flow of luminal and membrane proteins at the intra-organelle level. It reveals a fine equilibrium between anterograde and retrograde transport to maintain a constant volume in the pathway's organelles. Nonetheless, the system is robust to changes in the intracellular concentrations of vesiculation machinery proteins. The model predicts a close relationship between vesicle volume and cargo of v-SNARE proteins, identifying them as potential gene targeting candidates.
Conclusions: This study presents a dynamic model that represents the bulk flow of proteins along the organelles of the secretory pathway. The model provides a gray-box description of the vesiculation processes between the pathway's organelles, revealing quantitative information that enhances the understanding of vesiculation processes in mammalian cells. Acknowledgments to the financial support provided by the PAPIIT- UNAM (IN210822, IV201220), CONAHCYT (CF-2023-I-1248, CF-2023-I-1549), and PAPIIT Sabbatical stay granted to Dr. NAV-C and MAT-R.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

SMALL EXTRACELLULAR VESICLES FROM METABOLICALLY REPROGRAMMED MESENCHYMAL STEM/STROMAL CELL AS A POTENTIAL IMMUNOSUPPRESSIVE MECHANISM FOR INFLAMMATORY DISEASES

Eliana Lara-Barba 1,3, Yesenia Flores-Elías 1,2,3, Felipe Bustamante Barrientos1,3, María Jesús Araya,1,3, Yeimi Herrera-Luna, 1,3 Noymar Luque-Campos, 1,3 Ana María Vega-Letter4 , Patricia Luz-Crawford1,3

1. Laboratorio de Inmunología Celular y Molecular, Centro de Investigación Biomédica, Facultad de Medicina, Universidad de Los Andes, Santiago, Chile.
2. Facultad de Ciencias, Universidad de Chile, Santiago, Chile.
3. IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.
4. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaiso, Valparaiso,Chile.

Background: MSCs are multipotent fibroblast-like cells that exert different biologic functions, including immunosuppressive activity, making them attractive for autoimmune disease treatment. Their immunomodulatory activity is mediated mainly by paracrine factors. However, the release of small extracellular vesicles (sEV) by these cells has been demonstrated as a principal mechanism by which MSCs perform their biological effects. Our studies in human umbilical cord MSCs showed that metabolic reprogramming to glycolysis significantly improves their immunoregulatory capacity over proinflammatory T cells (Th1, Th17) through inducing T regulatory cells (Treg). Therefore, in the present study we evaluated the role of sEVs from glycolytic and non-glycolytic UC-MSCs on their immunosuppressive properties in vitro and in vivo in a murine model of delayed type hypersensitivity type I (DTH).

Material and methods: First, we obtain the different fractions of glycolytic or non-glycolytic MSCs secretome including their sEVs, that were characterized by NTA and FACS of specific sEVs markers, and evaluate their immunosuppressive activity over PBMCs, proinflammatory T cells and induction of T regulatory cells were evaluated. Furthermore, sEVs internalization in T cells was evaluated by FACS and confocal microscopy techniques. Moreover, we assessed the sEV effect on memory T-CD4 cells that were culture in the presence or absence of sEVs, the phenotype of proinflammatory and anti-inflammatory cells in memory T-CD4 cells was evaluated by FACS and the production of IL-10 by ELISA. Finally, we evaluated sEVs immunosuppressive activity in vivo in a mouse model of DTH.

Results: We found that the MSC glycolytic conditioned medium and their sEVs, significantly decreased the proliferation of CD4+T, reduced Th1 cells and induced Treg cells in vitro. Our internalization experiments showed that sEVs are incorporated into memory T-CD4+ cells and decrease the percentage of Th1 or Th17 cells, while no effects on the percentage of Treg cells, with increase in IL 10 production. In vivo assays show that glycolytic sEVs-MSC significantly decrease the inflammation response, decreasing proinflammatory T cells.

Conclusion: Glycolytic MSC - sEVs are able to internalized and specifically modulate activated T cell, regulating the frequency of T cells proliferation and phenotype, exhibited in vitro and in vivo immunomodulatory effect on T cells, showing the potential of this immunosuppressive tool for inflammatory diseases.

RESÚMENES DE PÓSTERES

DESARROLLO DE LÍNEAS CELULARES

DEVELOPMENT OF AN APOPTOSIS-RESISTANT CHO CELL LINE FOR THE PRODUCTION OF MONOCLONAL ANTIBODIES

Sheila C. Valdés 1, Camila A. Orellana 1.

1. Department of Chemical and Bioprocess Engineering, School of Engineering, Pontificia Universidad Católica de Chile. Avenida Vicuña Mackena 4860, Macul, Santiago de Chile, Chile.

Background: Chinese hamster ovary (CHO) cells are the preferred manufacturing system for biopharmaceutical production, however, one of the main limitations of CHO cell culture is that the cells undergo apoptosis. Apoptosis is a suicidal response of cells exposed to different types of stress, leading to decreased cell density and shorter culture duration, ultimately resulting in decreased product quantity and quality. Although several genetic engineering strategies have been successfully employed to reduce apoptosis, there remains room to further improve the performance of CHO cell lines. Through a comprehensive review of omics studies in CHO and comparison with results in cancer cells, three novel targets were selected with the aim of attenuating apoptosis in CHO cells.

Methods: A monoclonal antibody (mAb) will be randomly inserted into a CHO cell line containing a RMCE-landing pad using chemical transfection. Individual clones will be isolated by limiting dilution and a high producer clone will be selected. Then, three novel anti-apoptotic targets will be independently overexpressed in the CHO-mAb cell line by inserting the gene in the RMCE-landing pad. The modified cell lines will be characterized and compared to the parental line with respect to apoptosis-related caspases activity, culture duration and monoclonal antibody production. A proteomic study will also be performed to understand the main factors contributing to the attenuation of apoptosis.

Expected results: Selected anti-apoptotic genes have multifaceted functions with a wider range of action, affecting more than one enzyme, which we expect to lead to greater effects on the apoptosis pathway, and perhaps even productivity. Overexpression of these genes has been successful in reducing apoptosis in various cancer cells and, consequently, they are promising alternatives for CHO cell engineering. This study will not only show which of the proposed genes is the best for attenuating apoptosis and its effect on product titer and quality, but the proteomic study will also answer why it works and what are the mechanisms behind it, something that has not yet been fully answered.

Conclusion: Here, CHO cell lines with high resistance to apoptosis will be generated. Longer culture duration due to extended cell line life will make achieving a greater quantity of high quality product possible, which would ultimately help reducing costs for patients and the health care system.

DESARROLLO DE LÍNEAS CELULARES

PRODUCTION OF A SINGLE-CHAIN RECOMBINANT BOVINE FOLLICLE-STIMULATING HORMONE

Marilla Amaranto 1, Agustina Godino 1, Javier Nicolás Garay-Novillo 1, José Luis Barra 1.

1. Centro de Investigaciones en Química Biológica de Córdoba - CONICET; Departamento de Química Biológica Ranwel Caputto - Facultad de Ciencias Químicas – Universidad Nacional de Córdoba. X5000HUA, Córdoba, Argentina.

Background: Currently, several glycoproteins that are not species specific are used in animal reproduction, with follicle-stimulating hormone (FSH) extracted from porcine pituitary being the most widely used at present. However, this porcine FSH has several limitations. Among them, relying on animal-derived materials, carries the potential risk of hormone contamination and pathogen transmission, short circulating half-life, contamination of luteinizing hormone and other proteins in the extract, high application frequency, which generates stress, affecting the number of transferable embryos. The use of recombinant hormones opens up new possibilities for improving reproductive efficiency and success rates in the field of animal reproduction. In this work we developed an expression vector to produce a single-chain recombinant bovine FSH (bFSH) using transient gene expression.

Metodology: Recombinant bFSH was expressed by polyethylenimine (PEI)-mediated transfection and further transient gene expression (TGE) in the suspension-adapted human embryonic kidney (HEK) cell line FreeStyle 293-F cultured in serum-free media. The single-chain recombinant bFSH was designed by considering the amino acid sequences of the alpha and beta chains of native Bos taurus FSH covalently linked by a spacer. To express this variant, the vector pTriEx2 was used. In this expression vector, bFSH production is under the control of CMV immediate-early enhancer fused to the chicken β-actin promoter, and contains a His-tag and an HA-tag fused to its C-terminus.

Results: Recombinant bFSH was successfully produced using an easy and fast expression and purification system. A large amount of the single-chain recombinant bFSH was obtained with the expected molecular weight for an heterogeneous glycosylation pattern. Maximum expression was observed at day 4 after transfection and remained stable until day 8, when the supernatant was collected for purification. The recombinant protein purification was carried out in a single step by IMAC.

Conclusions: Successful expression of both recombinant fused subunits by a suitable spacer ensures the 1:1 molar ratio necessary for the heterodimer formation. This successful preliminary result may contribute to solve some current limitations in the field of animal reproduction. This is the first step towards the development of a cell line for the production of recombinant bFSH.

DESARROLLO DE LÍNEAS CELULARES

ENGINEERING AND PRODUCTION OF A NOVEL CYTOKINE TRAP FOR EFFECTIVE TREATMENT OF CANINE ATOPIC DERMATITIS USING ENHANCED BIOTHERAPEUTIC FUSION PROTEIN TECHNOLOGY

Pablo Esteban Mussio 1,2, María Celeste Rodríguez 1,2, Claudio César Prieto 2.

1. Laboratorio de Desarrollo Biotecnológico (LDB), Centro Biotecnológico del Litoral (CBL), Facultad de Bioquímica y Ciencias Biológicas (FBCB), Universidad Nacional del Litoral (UNL), Ciudad Universitaria, Ruta Nacional 168 - Km 472,4 - C.C. 242 - (S3000ZAA) Santa Fe, Pcia. de Santa Fe, Argentina.
2. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

Background: Canine atopic dermatitis arises from a complex interplay between genetic and environmental factors, which leads to immune dysregulation and skin barrier dysfunction. This creates a vicious cycle known as the itch-scratch cycle. Interleukin 31 (IL-31) is a critical cytokine responsible for inducing pruritus, disrupting the epithelial barrier, and promoting inflammation. Given its critical role, inhibiting IL-31 provides an effective treatment. Soluble receptors can block cytokines, but they have limitations due to their short half-lives and reduced affinity. This is because cytokine-receptor binding involves multiple receptors. By the contrary, cytokine traps, which are fusion proteins comprising receptor cytokine binding domains (CBDs) and IgG constant regions (Fc), overcome these limitations. The use of CBDs from multiple receptors extends half-life and enhances affinity.

Methods: Two fusion proteins, R-Fc and R-His, were designed using CBDs from canine IL-31 receptors. For R-Fc, the Fc region of canine IgG1 was incorporated, while R-His was linked to a histidine tag. HEK 293 recombinant cell lines were generated through four rounds of lentiviral transduction and limiting dilution cloning. Cells were adapted from adherent growth in 10% (v/v) fetal bovine serum to suspension growth in a serum-free medium and cultured in batch mode. Purification of R-Fc and R-His was accomplished using protein A chromatography and immobilised metal affinity chromatography (IMAC), respectively. Purity and identity were confirmed via SDS-PAGE Coomassie staining and Western blot analysis. Protein characterization was performed using intrinsic fluorescent spectroscopy and thermal shift assay.

Results: R-Fc and R-His were designed with distinctive structural features. Stable cell lines were successfully generated and adapted to suspension growth, ensuring high production levels. A single purification procedure yielded high purity levels for both proteins. Characterization revealed the unique structural features and provided insights into the thermal stability of each protein.

Conclusion: This comprehensive methodology establishes a robust foundation for the efficient design and production of fusion protein, representing a significant advancement in developing effective therapies for canine atopic dermatitis.

DESARROLLO DE LÍNEAS CELULARES

ROLE OF JERDOSTATIN, A SNAKE-VENOM-DERIVED DISINTEGRIN, IN MODULATING TUMOR ANGIOGENESIS

Ignacio García 1, Martin Flo 2, Teresa Freire 1.

1. Laboratorio de Inmunomodulación y Vacunas. Unidad Académica de Inmunobiología. Facultad de Medicina, Udela R. Gral Flores 2125, Montevideo, Uruguay
2. Laboratorio de Inmunovirología. Institut Pasteur de Montevideo. Mataojo 2020, Montevideo, 11400, Uruguay

Background: Integrins are a family of transmembrane receptors that mediate cell-cell and cell-extracellular matrix interactions and trigger signaling cascades, which regulate diverse responses including cell adhesion, migration, proliferation, differentiation, and death. Integrin α1β1 is one of the primary collagen-binding receptors expressed in many cell types, including cancer cells and vascular endothelium. There are very few specific inhibitors of the α1β1 receptor, among which a series of peptides derived from snake venom stand out as unique and specific natural inhibitors of integrin α1β1, such as Jerdostatin. The anti-angiogenic effect of Jerdostatin has been studied in smooth muscle cells but not in cancer angiogenesis models. Previously, we have developed a murine lung cancer model with truncated O-glycosylationand Tn antigen expression. Tn+ cells induce larger and more angiogenic tumors than wild type tumor cells. Our aim is to evaluate the anti-angiogenic potential of Jerdostatin in the context of aberrant glycosylation.

Methods: We are currently working on producing recombinant Jerdostatin in insect cells. To achieve this, we have designed a Drosophila expression vector (DroExp) with the nucleotide sequence of Jerdostain from Protobothrops jerdonii, incorporating a Strep-tag and a Tobacco Etch Virus protease cleavage site. Our strategy consists in transforming S2 insect cells, followed by the isolation of Jerdostatin using affinity chromatography targeting the Streptactin tag. Subsequently, this tag will be removed by proteolysis with Tobacco Etch Virus protease. Once we obtain the purified protein, we will evaluate its anti-angiogenic potential on a lung cancer murine model with incomplete glycosylation. Firstly, we will assess Jerdostatin capacity to bind Human Umbilical Vein Endothelial Cells. We will further evaluate proliferative, migratory and tube formation capacity on these cells when incubated with Jerdostatin. Finally, if our initial experiments provide promising results, we plan to explore the anti-angiogenic properties of Jerdostatin by injecting mice with murine lung cancer cells exhibiting aberrant glycosylation, which promote highly vascularized tumors.

Conclusions: To sum up, our investigation will provide a robust model to assess Jerdostatin's therapeutic potential in angiogenesis inhibition. This will shed light on future anti-angiogenic therapies for cancer treatment.

DESARROLLO DE LÍNEAS CELULARES

ENHANCING MONOCLONAL ANTIBODY YIELD IN CHO CELLS FOR GASTRIC CANCER: ASSESSING RATIOS OF GLOBAL REGULATORS

Ms. Isis Araya 2,3, Dr. Yesenia Latorre 1,2, Dr. Karen Toledo 1, Dr. Carla Gutierrez2, Mr. José Rodriguez2, Dr. María Carmen Molina1, Dr. Claudia Altamirano2,4,5,6

1. Facultad de Medicina, Universidad De Chile, Santiago, Chile,
2. Escuela de Ingeniería Bioquímica, PUCV, Valparaíso, Chile,
3. Universidad Adolfo Ibañez, Santiago, Chile.
4. Centro Regional de Estudios de Alimentos y Salud, CREAS, Valparaíso, Chile.
5. Center of Interventional Medicine for Precision and Advanced Cellular Therapy, IMPACT, Santiago, Chile.
6. Centro de Investigación interdisciplinar en Biomedicina, Biotecnología y Bienestar, C3B, Valparaíso, Chile

Background: The biopharmaceutical industry, centered around monoclonal antibodies, grapples with challenges in CHO cells, characterized by toxic metabolite accumulation and inefficiencies in protein secretion. Previous attempts to enhance CHO cell production through genetic modifications targeting protein transporter and enzyme genes have fallen short of optimal outcomes. Consequently, attention has shifted to global regulators such as c-Myc and Xbp1s, known for their positive influence on CHO cell metabolism and protein secretion. Earlier studies have showcased increased productivity and growth rates in EPO CHO cell producers through the co-overexpression of c-Myc and Xbp1s. This study aims to enhance human monoclonal antibody (mAb) productivity by transiently transfecting CHO cells with varying ratios of these regulators.

Methods: The experimental design employs response surface methodology, utilizing Statgraphics 19 software. CHO-S cells that produced the mAb were cultured under standard conditions (37°C, 5% CO2, humid conditions. The vectors, including pCDH-puro-cMyc (addgene, #46970) and pLHCX-XBP1-mNeonGreen-NLS (addgene, #115971), were transiently transfected in different proportions using the ExpiCHO transient transfection kit, following the manufacturer's instructions. Each transfection was conducted in triplicate.

Results: Ongoing transfections prevent conclusive results at this stage.

Conclusion: Based on prior findings, it is anticipated that a higher concentration of regulators may not necessarily yield better results. Additionally, it is hypothesized that an optimal proportion between regulators may exist, negatively impacting productivity with an increase in cell viability over time, and viceversa — suggesting a trade-off between productivity and cell viability.

DESARROLLO DE LÍNEAS CELULARES

STABLE C-MYC EXPRESSION CONTROLS MAMMALIAN TARGET OF RAPAMYCINPATHWAY IN CHINESE HAMSTER OVARY CELLS PRODUCER OF RECOMBINANT PROTEIN

Constanza Núñez 1, Constanza Collarte 1, Sebastián Vergara 1, Ana María Vega 1,4, Claudia Altamirano1,2,3,4.

1. Escuela de Ingeniería Bioquímica, PUCV, Valparaíso, Chile,
2. Centro Regional de Estudios de Alimentos y Salud, CREAS, Valparaíso, Chile.
3. Center of Interventional Medicine for Precision and Advanced Cellular Therapy, IMPACT, Santiago, Chile.
4.Centro de Investigación interdisciplinar en Biomedicina, Biotecnología y Bienestar, C3B, Valparaíso, Chile

Background: Successful and acceptance of recombinant proteins (r-proteins) as biopharmaceuticals led researchers to develop strategies to improve the production of r-proteins. Understanding the regulatory mechanisms governing cellular growth and protein production is crucial for optimizing biotechnological processes such as recombinant protein expression in mammalian cells. Chinese hamster ovary (CHO) cell line is the most used systems in the expression of mammalian proteins because its ability to grow up in suspension and manufacture of human-like PTM proteins. To improve the production of r-proteins, cell lines have been genetically modified with a gene of interest. Among the studied genes, the human c-myc gene caught the attention of researchers, due to proven great potential. However, just a few articles have described the effects of c-myc overexpression in CHO cells and the pathway which c-Myc could increase the production of r-proteins is still unknown. One of the most cellular metabolism and proteins synthesis-related signaling pathway, is the mTOR pathway. In this study, CHO cells, producer of recombinant human erythropoietin, were cultured under controlled conditions to investigate the impact of c-Myc overexpression on cellular metabolism, cellular proliferation, and the mTOR pathway under a r-protein production context.

Methodology: CHO cells were cultured in 50 mL of Hyclone SFM4CHO medium (+40 mM glucose, +6 mM glutamine), under 5% CO2, 50 rpm, and 37oC for 7-8 days. Cell concentration and viability were monitored every 24 hours using the trypan blue exclusion test. At 24 hours of culture, cells were exposed to 100 nM of 10058-F4 or rapamycin to assess the influence of c-Myc overexpression on the mTOR pathway. Culture samples were collected for metabolite measurement, h-EPO production by ELISA, and c-Myc and mTOR pathway expressions by Western blot.

Results: Compared to CHO-EPO cells, CHO-EPO-c-Myc cells exhibited a 16% decrease in μmax and a 67% increase in qEPO. These effects were restored when cells were treated with 100 nM of 10058-F4 or rapamycin. Furthermore, there were increased rates of qglc by 58%, qgln by 112%, and qamm by 89%, along with a 74% decrease in qlac. The expression peak of c-Myc occurred at 72 h in CHO-EPO cells, while high and constant levels were observed in CHO-EPO-c-Myc cells. Notably, a significant increase in the expression of mTOR pathway proteins was observed at 72 h in CHO-EPO-c-Myc cells.

Conclusion: Stable c-Myc expression controls the mTOR pathway in CHO cells, inducing changes in r-protein production and cell growth.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

CAN WE PREDICT TUMOR GROWTH FROM CELL CULTURE KINETICS?

Maivy Martínez 1, Ernesto Chico 1,2.

1. Centro de Inmunología Molecular (CIM), Calle 15 esq. 216 S/N, Siboney, Playa, La Habana, Cuba. A.P 16040, La Habana 11600.
2. Facultad de Ingeniería Química, Universidad Tecnológica de la Habana (CUJAE), Calle 114 #11901 / Ciclovía y Rotonda CP 19390, Marianao, La Habana, Cuba.

Brackground: Decades of experience using immortal tumor cell lines for protein manufacturing in cell culture technology have allowed us to understand and control cell´s behavior under in vitro conditions. Interestingly, cells in culture share most of the dynamic patterns of bacteria and yeast, behaving like single cell organisms. Based on these observations several math models have been developed for animal cell culture dynamics, recycling most of the math formulation originally used to describe exponential growth in bacteria fermentation. Tumor cell lines grow in vitro following an exponential pattern until the population reaches certain physical or nutrient limits.

This phenomenon has been historically described using logistic or Monod-type models. Based on the common metabolic patterns observed in industrial cell lines and tumor cells, the following questions could be relevant to formulate a working hypothesis:

- Once implanted in mice, cells forming a solid tumor follow an exponential or logistic growth pattern?
- Is the growth limited by host tissue physical or metabolic constraints?
- Experimental tumors in mice share common kinetic parameters with spontaneous tumor growth in human patients?

In the present work we explore this hypothesis comparing cell culture models with in vivo growth of tumor in mice and humans. The growth pattern found can be described as an exponential growth that is continuously exponentially retarded. We estimate the kinetic parameters from tumor growth in mice and compare with specific growth rate of animal cells *in vitro*. Finally, we discuss the underlying common cellular processes that could explain the difference in growth patterns in a search of a unified growth model.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

GALACTOSE/LACTATE CONSUMPTION IMPROVE SPECIFIC REPO PRODUCTIVITY IN PERFUSION CULTURE OF C-MYC OVEREXPRESSOR CHO CELLS

Mauricio Vergara 1, Sebastián Vergara 1, Óscar Gatica 1, Constanza Collarte 1, Constanza Nuñez 1, Mauro Torres 2, Yesenia Latorre 1,3, Ana Vega-Letter 1, N. Adriana Valdez 4, Claudia Altamirano 1,5,6.

1. Pontificia Universidad Católica de Valparaíso. Chile. Código Postal: 2362803.
2. University of Manchester, United Kingdom. Código Postal: M1 7DN
3. Universidad de Chile, Chile. Código Postal: 8380453
4. Universidad Nacional Autónoma de México, México. Código Postal: 04510
5. Centro Regional en Alimentos Saludables (CREAS), Chile. Código Postal: 2373223
6. Center of Interventional Medicine for Precision and Advanced Cellular Therapy (IMPACT), Chile.Código Postal: 762008

Background: The overexpression of c-Myc, and the replacement of the carbon source, glucose (Glc) by galactose/lactate (Gal/Lac) have previously demonstrated to increase the specific productivity of Erythropoietin recombinant (rEPO) protein in batch culture of CHO cells. However, this strategy has not been evaluated before in perfusion culture at high cell density. First research that addresses perfusion culture of high cell density to improve the productivity of the biopharmaceutical EPO recombinant protein, through Cell Engineering (overexpression of C-MYC) and design of the culture medium (use of slowly metabolizable carbon sources).

Methods: Two clones of CHO cells, EPO producers (CHO-EPO) and one of them also c-Myc overexpressor (CHO-EPO-CMYC) were cultured in triplicate in perfusion mode. CHO-EPO using Glc as carbon source at 20mM was cultured as standard reference, and CHO-EPO-CMYC using a mixture of Gal/Lac (32/16mM) as carbon source was also cultured. The response of each condition evaluated was analyzed through cell growth and cell cycle progression, r-protein production, and metabolic behavior. Statistical analysis of the results was performed by ANOVA.

Results: The overexpression of c-MYC and the replacement of Glc by Gal/Lac mixture in perfusion culture did not have a significant effect on the specific cell growth rate, in both cases it was of the order of 0.018 h-1, however, the maximum density of viable cells reached was reduced from 3.7x106 cells/mL in standard conditions to 2.5x106 cells/mL in productive conditions. Regarding the production of the recombinant EPO protein, the productive conditions allowed a significant increase in the specific productivity of the recombinant protein by 3 times. Furthermore, this increase in productivity is related to a significant accumulation of cells in the G1/G0 phase of the cell cycle. Meanwhile, in relation to metabolic behavior, the productive conditions significantly reduced the consumption of the hexose used by 40% and simultaneously allowed the re-metabolization of lactate.

Conclusion: The use of the proposed variables achieved a more efficient metabolism of carbon sources, directed towards the production of the recombinant EPO protein.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

NON-CYTOTOXIC CISPLATIN EXPOSURE INDUCES MITOCHONDRIAL DAMAGE AND TRIGGERS OVER-DIFFERENTIATION OF NEURAL STEM/PROGENITOR CELLS VIA MITOCHONDRIA-DERIVED REACTIVE OXYGEN SPECIES

Felipe A. Bustamante-Barrientos 1,2, Eliana Lara-Barba 1,2, Yeimi Herrera-Luna 1,2, Cynthia García 1,2, Claudia Altamirano 3, Ana María Vega-Letter 3, and Patricia Luz-Crawford 1.

1. Immunology Program, Center for Biomedical Research and Innovation (CIIB), Universidad de Los Andes, Chile. San Carlos de Apoquindo 2500; Postal Code: 7620001.
2. Centro de Investigación e Innovación Biomédica (CiiB), Universidad de Los Andes. San Carlos de Apoquindo 2500; Postal Code: 7620001.
3. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile. Avenida Brasil 2085, Valparaiso; Postal Code: 2362803.

Background: Neural stem and progenitor cells are pivotal in the development and maintenance of the nervous system, persisting in the adult brain and activated by specific stimuli that enhance neuroplasticity through differentiation. While neuroplasticity naturally declines with age, chemotherapeutic regimens can expedite this decline by disrupting various cellular processes, linked to increased oxidative stress and reduced neurogenic potential. Cisplatin, an FDA-approved platinum-based chemotherapeutic agent commonly used for solid tumors and hematologic malignancies, induces cytotoxic effects on neural progenitors in the nervous system, impairing endogenous brain repair mechanisms and causing cognitive decline.

Methods: Neural stem/progenitor cells derived from postnatal mice were exposed to increasing doses of cisplatin and, in certain instances, co-treated with MitoTempo, a mitochondria-targeted antioxidant. Mitochondrial function and progenitor cell differentiation were assessed using techniques such as flow cytometry and confocal microscopy.

Results: Our findings reveal that even sub-cytotoxic concentrations of cisplatin disrupt mitochondrial homeostasis, resulting in excessive differentiation of neural stem cells in vitro. Perturbations in mitochondrial function impacted oxidoreductase activity, ATP synthesis, mitochondrial mass, membrane potential, and reactive oxygen species production. These alterations led to exacerbated differentiation of neural stem cells into neuronal and astroglial lineages, a process mitigated by a mitochondria-specific antioxidant. Conclusion. Mechanistically, our results suggest that cisplatin-induced over-differentiation of neural stem cells is driven by reactive oxygen species from mitochondria, leading to non-cytotoxic depletion of neural progenitors. Thus, combining cisplatin with mitochondria-targeted antioxidants may help prevent neural stem cell depletion and sustain neuroplasticity following chemotherapy treatments.

Funding: FONDECYT Postdoctorado 3220204, FONDECYT Regular 1211353.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

ADAPTATION OF CHINOOK SALMON EMBRYO CELLS TO SUSPENSION CULTURE TO INCREASE INFECTIOUS PANCREATIC NECROSIS VIRUS PRODUCTION

Karen Jara 1,2,3, Kurt Pohlhammer 3, Tomás Cancino 3, Iván Valdés 3, Ziomara Gerdtzen 1,2.

1. Mammalian Cell Culture Lab, Department of Chemical Engineering, Biotechnology, and Materials, University of Chile, Beaucheff 851, Santiago Chile.
2. Centre for Biotechnology and Bioengineering, University of Chile.
3. Veterquimica S.A. Chile

Background: Salmonid aquaculture is a crucial sector of the Chilean economy, contributing 1.5% to the GDP. The species produced in Chile are Rainbow Trout, Atlantic Salmon, and Coho Salmon, with 23% of their mortality attributed to infectious diseases, including Infectious Pancreatic Necrosis (IPN) caused by the Infectious Pancreatic Necrosis Virus (IPNv). Controlling this disease is essential to ensure the sustainable growth of salmonid aquaculture, the most common solution involves inactivated vaccines produced by infecting Chinook Salmon Embryo cells (CHSE-214) with IPNv.

Traditionally, CHSE-214 cells are cultured in adherence using cell stack plates that provide a surface for cell adhesion. However, suspension culture can offer numerous advantages for cell propagation, such as a larger cell population, a faster growth rate, and the potential for culture in a stirred bioreactor. Therefore, this research aims to evaluate the impact of the adaptation of CHSE-214 cells to suspension culture for IPNv production, as the basis of a scalable process design. The potential of CHSE cells to be adapted to suspension culture in at least three culture medium formulations for reduced adherence is studied. Adapted cells will be infected with IPNv to determine conditions that provide the highest yield in virus production. Cell culture parameters and characteristics will be compared to those of adherent cultures.

Preliminary tests have been carried out in suspension and a potential for adaptation has been observed. It is expected that low adherence media formulations will allow the adaptation of CHSE-214 to suspension increasing cell concentration and IPNv production compared to adherence culture. Selected conditions will be implemented in a bioreactor setting.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

MODEL-BASED OPTIMIZATION OF HUMAN MESENCHYMAL STEM CELL CULTURE UNDER LOW AND HIGH OXYGEN LEVELS CONDITIONS

Temistocles Molina 1,2, Aaron Canales 2,3, Bastián Herrera 2,3, Francisca Pizarro 2,3, Isidora Prat 1,2, Iván Paredes 1,2, Ziomara Gerdtzen 1,2,4, J. Cristián Salgado 2,3, Valentina Jarur 5, Rodrigo Arancibia 5,6, Francisco Aulestia 5,6.

1. Mammalian Cell Culture Lab MCCL, Centre for Biotechnology and Bioengineering CeBiB, Universidad de Chile, Av. Beauchef 851, Santiago, Chile.
2 .Centre for Biotechnology and Bioengineering CeBiB, Universidad de Chile, Santiago, Chile.
3. Process Modeling Distributed Computing Lab PMDC, Departamento de Ingeniería Química, Biotecnología y Materiales, Universidad de Chile, Santiago, Chile.
4.Núcleo Milenio MASH, Santiago, Chile.
5. Cellus Medicina Regenerativa S.A., Santiago, Chile.
6. Cellus Biomédica, Parque Tecnológico de León, León, España.

Background: The global stem cell-based therapies market will grow to USD 2,452.8 million by 2032 offering treatments for chronic and cardiovascular disorders, wounds and injuries. The most commonly used cells are human mesenchymal stem cells (MSCs). Therapeutic doses of up to 8 million MSC/kg body weight are required, quantities that can only be obtained by in vitro expansion. The conventional way to grow MSCs is 2D cultures using a repeated-batch (rBc) feeding regimen by changing the spent culture media with fresh media periodically. However, this strategy generates drastic changes in culture conditions, is laborious and involves a high use of culture media, a good that according to some experts would represent 20% or more of the manufacturing costs of cell therapies. A bioprocess strategy is then required that allows reducing the use of culture media and increasing cell production simultaneously.

Methods: Our team has proposed a strategy for an efficient expansion of MSCs in intensified fed-batch (iFBc) cultures under low and high oxygen conditions. The strategy is based on the calculation of stoichiometric ratios between nutrients for the design of a feeding media, and the use of the CELIA (Cell Culture Media Optimization) platform that provides a feeding profile for the culture over time by mathematical model that predicts nutrient requirements.

Results: In the first application of the CELIA platform, a significant reduction in the use of media in iFBc cultures and maximum numbers of cells comparable to the rBc cultures were obtained, increasing the expansion efficiency (ratio between integral of viable cells and total usage media volume) by 116 and 215% compared to the rBc cultures in low and high levels of oxygen, respectively.

Conclusions: The mathematical model-based CELIA platform allows optimizing MSC cultures increasing efficiency in the use of the culture media. A second application of the CELIA platform will be performed, aiming to further enhance the expansion efficiency of the culture.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

EVALUATION OF DIFFERENT CELLULAR MATRICES AS ALTERNATIVES TO MATRIGEL FOR THE CULTIVATION OF COLORECTAL CANCER ORGANOIDS

Villavicencio, C.1, Sandoval, F. 1, Leiva, MJ. 1, Saldivia, P. 1, Hidalgo, A. 1, Contreras, M. 1, Benavides, C. 2, Toledo, J.R. 1.

1. Laboratorio de Biotecnología y Biofármacos, Departamento de Fisiopatología, Facultad de Ciencia Biológicas, Universidad de Concepción, Víctor Lamas 1290, 4070386 Concepción, Bío Bío, Chile.
2. Unidad de Coloproctología, Hospital Guillermo Grant Benavente, San Martín 1436, 4070022 Concepción, Bío Bío, Chile.

Background: Colorectal cancer (CRC) represents a significant public health challenge in Chile, ranking fifth in terms of mortality and being the third most common cancer in the country, with a prevalence rate that places it as the second. In this context, three-dimensional (3D) cell culture in the form of organoids has emerged as an essential tool in cancer research, although its implementation entails high costs. In response to this need, the search for economical and sustainable alternatives to Matrigel, the commercially used extracellular matrix in organoid culture, becomes crucial. Two promising candidates have emerged: Gelatin/Sodium Alginate (GelAlg) and Gelatin/Methacrylate (GelMa). These matrices offer potential advantages in terms of cost, availability, and biocompatibility, making them attractive options for organoid culture in colorectal cancer studies and other areas of biomedical research. Objectives: To evaluate the suitability of GelAlg and GelMa as viable alternatives to Matrigel for the cultivation of CRC organoids, with the goal of optimizing 3D culture methods and advancing the understanding of the disease.

Methods: Initially, proliferation assays were conducted using the SW620 cell line in Matrigel, GelAlg, GelMa matrices. Cells were seeded in 24-well plates with drops of 30 µL per well. Matrigel polymerization was performed at 37°C for 30 minutes, GelAlg with 100mM calcium chloride for one minute, and GelMa using UV radiation at 365 nm for 30 seconds. Cell viability was assessed using the AlamarBlue reagent on days 1, 7, and 14. Subsequently, CRC tumor tissue organoids obtained from patients were cultured in the same matrices. Organoids were incubated for up to 72 hours at 37°C with 5% CO2 and cell viability was evaluated using AlamarBlue at 48 and 72 hours.

Results: In the CRC SW620 cell line, both matrices (GelAlg and GelMa) showed favorable growth up to 14 days of culture, supporting cell development and adaptation to the new scaffold. When culturing CRC tissue organoids, preliminary results indicated a favorable response in both alternative matrices, with an increase in cell viability after 72 hours. This demonstrates that the matrices can keep the organoids viable. Although variation was observed in the replica results, the trend was consistent over time.

Conclusions: Initial assays suggest that GelAlg and GelMa are viable alternatives to Matrigel for CRC organoid culture, with potential for use in bioprinting and other biomedical research. Additional trials are necessary to confirm these findings.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

EFFECT OF HYDROXICINAMIC ACID ON PROLIFERATION, CELL VIABILITY, OXIDATIVE STRESS, AND METABOLISM OF HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELLS IN PROLONGED CULTURE

Sofia Trujillo 1, Nelson Osses 1,3, Alejandra Arancibia 2,3, Claudia Altamirano 2,3.

1. Instituto de Química, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.
2. Escuela de Ingeniería Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.
3. IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.

Background: Mesenchymal stromal cells (MSCs) are promising candidates for various therapeutic applications due to their differentiation potential, immunomodulatory effects, and tissue regeneration capabilities. However, their clinical use is hampered by challenges associated with in vitro expansion and replicative senescence. Oxidative stress is a key inducer of senescence, negatively affecting MSCs viability and functions. The use of antioxidants has emerged as a rational strategy to improve MSCs culture aimed to therapeutic applications. Hidroxycinamic Acid(HA) has potent antioxidant properties. However, limited research exists on its effect on MSCs, particularly its impact on prolonged cultures of human umbilical cord- derived MSCs (hUC-MSCs). This study aims to evaluate the impact of CGA on the proliferation, cell viability, oxidative stress, and cellular metabolism hUC-MSCs in prolonged cultures.

Methods: The first phase involves a concentration screening of HA to determine the optimal dose that does not induce cytotoxicity on hUC-MSCs cultured during short and long exposures. This dose will then be used in the second phase, which involves a prolonged culture of hUC-MSCs for 10 days. Monitoring cell cytotoxicity, cell proliferation, reactive oxygen species production, and metabolite quantification (glucose and glutamine consumption; lactate release).

Results: To standardize our assays and minimize variability associated with MSCs (e.g., patient origin, isolation method, growth phase), we initially used a fibroblast cell line. Short-term cultures (48 hours) with HA (300 or 500 µM) did not exhibit cytotoxicity. However, after a prolonged culture period (7 days), only one-third of the cells remained compared to the control. An initial screening in hUC-MSCs cultured with HA for 48 h (250, 500, 1000, and 1500 µM) did not exhibit any apparent cytotoxic effects.

Conclusions: Our in vitro study provides preliminary insights into the effect of chlorogenic acid on MSCs. While short-term exposure (48 h) to chlorogenic acid did not induce evident cytotoxicity in hUC-MSCs or fibroblast cells, long-term exposure (7 days) in fibroblast cells resulted in decreased cell numbers. These findings suggest that chlorogenic acid may be useful for hUC-MSCs in short-term cultures, but further research is needed to determine its long-term effects and optimal concentrations for extended culture applications.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

MILD HYPOTHERMIA IN MAMMALIAN AND INSECT CELL CULTURES: A REVIEW

Dayana Morales Borrell 1, 2, Ziomara P. Gerdtzen 1, 2, 3.

1. Mammalian Cell Culture Lab - MCCL, Department of Chemical Engineering, Biotechnology and Materials, University of Chile, Beauchef 861, Santiago, Chile.
2. Center for Biochemical Engineering and Biotechnology, Department of Chemical Engineering, Biotechnology and Materials, University of Chile, Beauchef 861, Santiago, Chile.
3. Center of Interventional Medicine for Precision and Advanced Cellular Therapy – IMPACT, Santiago, Chile.

Background: Mammalian cells constitute the preferred expression system for obtaining recombinant proteins (r-proteins), due to their ability of generating glycosylation patterns very similar to those of humans. The slow growth and low cellular productivity of mammalian cells compared to that of prokaryotic host cells, added to the increasing of the biopharmaceutical market based on complex therapeutic proteins, has meant that for some decades alternatives have been sought to increase the productivity of these cultures. In this context, mild hypothermia (MH) has become a tool for improving the viability and productivity of mammalian cell cultures and some insect-derived cell lines. However, the effect of MH is not only specific to protein and cell-line, it is also influenced by the promoter, the method of reducing temperature, supplementation, pH, and operation mode. MH also can cause changes in the synthesis of substrates and the production of toxic metabolites. Post-translational modifications of r-proteins, specifically glycosylation, can undergo changes due to MH. In terms of molecular mechanisms, MH intervenes in the cell cycle arrest, apoptosis delay, production and/or stability of r-protein mRNA, protein synthesis, reorganization of the cytoskeleton and induction of endogenous transcription factors, improving cell cultures productivity and viability. The present review provides important considerations related to the application of MH in mammalian and insect cell cultures. In addition, we provide a detailed global and integrated overview of the molecular mechanisms that underlie MH identifying potential targets for cell engineering that could enhance r-protein production.

Keywords: mild hypothermia, temperature shift, mammalian cell cultures, insect cell cultures, r-proteins, glycosylation, apoptosis, cell cycle arrest, cytoskeleton, transcription factors, cold shock proteins.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

ENHANCING PRODUCTIVITY OF AN ANTI-VIRAL PROTEIN FOR SALMON VACCINE PRODUCTION IN HI5 CELLS USING A CUSTOMIZED MODEL-BASED FEEDING STRATEGY

Isidora Prat 1,2, Temístocles Molina 1,2, Francisca Pizarro 2,3, Aaron Canales 2,3, Iván Paredes 1,2, J. Cristian Salgado 2,3, Ziomara Gerdtzen 1,2,4,5.

1. Mammalian Cell Culture Lab, Department of Chemical, Biotechnology and Materials Engineering, University of Chile, Beauchef 851, Santiago, Chile.
2. Centre for Biotechnology and Bioengineering, University of Chile,
3. Process Modeling and Distributed Computing Lab, Department of Chemical Engineering, Biotechnology and Materials, University of Chile
4. Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.
5. Milenium Nucleus Marine Agronomy for Seaweed Holobionts, Santiago, Chile, Santiago, Chile.

Background: By 2027, the veterinary biopharmaceutical industry market is expected to exceed 10 billion USD. To contribute to this industry's development in the country and meet the demand, we applied the CELIA platform developed by our group to a salmon fish vaccine production system using the Hi5 insect cell line culture. The platform provides a media composition and feeding strategy designed to maximize culture productivity in terms of biomass and/or product synthesis. It relies on a dynamic metabolic model with optimizable parameters considering cell and product composition, culture conditions, and nutritional requirements. Currently, the platform is being validated in productive systems and operational conditions of industrial interest with commercially relevant cells for the country. The protein productivity of the Hi5 system is limited by the cell density achievable in culture. To improve the system's yield, we aim to increase Hi5 cell concentration in culture by applying the optimized feeding strategy determined by CELIA.

Methods: The culture system's base parameters, metabolic profiles, and performance were characterized in batch mode. Cell composition was determined by quantifying the main macromolecular components of cells in their exponential growth phase, and this data served to determine a stoichiometric equation for Hi5 biomass synthesis. This equation, along with culture parameters, metabolic consumption, and production rates of key metabolites, was input into the platform to define feeding media composition and a feeding strategy specifically designed for Hi5 cells under our culture conditions to maximize biomass production.

Results: A stoichiometric equation for Hi5 biomass production in culture was obtained. In the first iteration, the use of the CELIA strategy led to a 27% increase in biomass production compared to the base case. This yield is anticipated to improve further with a second iteration that incorporates fed-batch data. Additionally, we expect to obtain results on the production of the anti-viral protein using the strategy that optimizes biomass production.

Conclusion: This strategy allows for an increase in biomass concentration in cultures with minimal intervention to existing processes. This scalable and effective approach provides an alternative to enhance the productivity of biomanufacturing systems of veterinary interest. By improving the competitiveness of national production processes, it facilitates their scaling and expansion into other markets. The application of the CELIA platform in Hi5 cell cultures has demonstrated significant improvements in biomass production and holds promise for further optimization and production of anti-viral proteins, contributing to the advancement of the veterinary biopharmaceutical industry.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

STUDY AND CONTROL OF OXIDATIVE STATE IN THE PROLIFERATION OF HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS

Cristian Torres 1,2,3, Alejandra Arancibia 3,4, Paloma Fuentes 3,4, Valeria De María 1,2,3, Claudia Altamirano 3,4,5, Ziomara P. Gerdtzen 1,2,3,6.

1. Mammalian Cell Culture Lab (MCCL), University of Chile, Santiago, Chile.
2. Centre of Biotechnology and Bioengineering (CeBiB), University of Chile, Santiago, Chile.
3. IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.
4. School of Biochemical Engineering, Pontifical Catholic University of Valparaíso, Valparaíso, Chile.
5. Regional Center for Studies in Healthy Foods (CREAS), Valparaíso, Chile.
6. Millennium Nucleus Marine Agronomy of Algal Holobionts (MASH), Santiago, Chile.

Background: Mesenchymal stem cells (MSCs) are highly valued for their ability to differentiate, regulate inflammation, promote growth, and repair tissues, as well as their easy acquisition from multiple tissues without ethical implications. This makes them ideal candidates for regenerative therapies and clinical applications. However, they face significant challenges in in vitro culture, particularly related to oxidative stress, which can compromise their proliferation and functionality. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cells. This imbalance can induce cellular senescence and aging,reduce differentiation capacity, and compromise the therapeutic properties of the cells. MSCs have been shown to possess inherent antioxidant defenses, but these can be overwhelmed under in vitro conditions, necessitating external antioxidant support. Therefore, it is crucial to control the oxidative state, observable even from the culture medium, during the cultivation period to maintain their viability and therapeutic effectiveness. The proposed strategy to mitigate oxidative stress is periodic supplementation with antioxidants ascorbic acid 2-phosphate and N-acetylcysteine to restore oxidative state levels, which would improve the proliferation and functionality of the culture.
Methods: Nine antioxidant supplementation combinations are proposed, using two concentrations of each antioxidant in addition to a negative control, in a D-MEM/F12 medium supplemented with 5% hPL v/v and 0.1% v/v Lipid Concentrate. MSCs from a single donor are cultured in 6-well plates at 37°C, with 21% O2 and 5% CO2. The culture media are partially renewed (50%) every 48 hours. Every 24 hours medium supernatant samples are collected for analysis, and cell viability is counted in duplicate using trypan blue exclusion. Growth curves are created to analyze cell proliferation and viability. Cellular metabolism is characterized by analyzing the consumption and/or production rates of glucose, lactate, pyruvate. These concentrations are measured through enzymatic reactions and spectrometry. To quantify the oxidative state, the antioxidant capacity of the culture medium is analyzed throughout the study period using ORAC, DPPH, and FRAP methods.
Expected results: These analyses allow monitoring the oxidative state of the culture and evaluating the effectiveness of antioxidant supplementation. Furthermore, the study examines how different antioxidant combinations impact MSC growth and oxidative stress markers over time. The findings from these evaluations are expected to provide insights into optimizing MSC culture conditions for improved therapeutic applications.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

IMPACT OF GLYCOSYLATION PRECURSORS SUPPLEMENTATION ON QUALITY ATTRIBUTES OF AN INNOVATIVE ANTI-SST2 IGG PRODUCED IN CHO CELLS

Javier Bravo-Venegas 1, Marcela Hermoso 2, Julio Berríos 1 and Claudia Altamirano 1,3,4.

1. School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.
2. Laboratory of Innate Immunity, Program of Immunology, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Santiago, Chile.
3. Regional Center of Healthy Food Studies (CREAS) R17A10001, CONICYT REGIONAL, GORE, Valparaíso, Chile.
4. Center of Interventional Medicine for Precision and Advanced Cellular Therapy (IMPACT), Santiago, Chile.

Background: Controlling glycosylation, a Critical Quality Attribute(CQA) of several biopharmaceuticals as monoclonal antibodies (mAbs), is key for its role modulating the biological activity and therapeutic efficacy. For the innovative anti-sST2, therapeutic for ulcerative colitis and other autoimmune diseases characterised by high levels of sST2 protein, it has been hypothesised that glycosylation ant the affinity with the FcRn receptors would play a significant role on its mechanism of action. Although the impact of the different glycosylation patterns on the FcRn affinity is still unclear. The glycosylation precursors supplementation is one of the most successful strategies to enrich the glycan distribution with complex patterns, however the synergetic action of adding these components remains unexplained. The purpose of this work is to approximate the scientific development associated with the anti-sST2 to productive conditions using serum-free media in high cell density suspended cultures, therefore, it is expected to improve the mAb quality (glycosylation, FcRn affinity) in a high productive scenario.

Methods: Firstly, an anti-sST2 expressing CHO cell line was developed employing standard electroporation techniques. Cloning was carried out by limit dilution. Employing the highest-producing clone and through response surface methodology the nutritional conditions that improve the mab CQAs were determined without compromising mAb cell-specific productivity. Finally, the consistency and stability of the strategy in time under optimised culture conditions was evaluated.

Results: The amplification cycles significantly increased the specific productivity of the anti-sST2 expressing pools. Several clones were obtained and characterized. Interestingly the supplementation had an impact on different response variables improving not only the quality but also productivity. Among these, the impact of galactose and manganese were the most significant.

Conclusions: The rational strategy employed proved to be consistently improve the anti-sST2 CQAs. From a general perspective, our findings provide a better quantitative and objective understanding of the relationship between environmental culture conditions and glycosylation, one of the key quality attributes for mAbs, that can be used to further improve decision making for the selection of production strategies to increase product quality.

METABOLISMO CELULAR Y OPTIMIZACIÓN DE MEDIOS DE CULTIVO

MEASURING GOLGI'S REDOX POTENTIAL IN MAMMALIAN CELLS USING A FLUORESCENT REDOX-SENSING PROTEIN

Stfanny W. Meza 1, Octavio T. Ramírez 1, and Laura A. Palomares 1.

1. Instituto de Biotecnología. Universidad Nacional Autónoma de México. Av. Universidad #2001, Col. Chamilpa C.P. 62210, Cuernavaca, Morelos, México.

Background: The cellular redox homeostasis contributes to the correct cell function through participating in the regulation of metabolism, cellular signalling and others. It is known that the redox environment of the Golgi complex is oxidant, which is important for disulfide bond formation, but it has not been studied as much as of other cell compartments despite the role of this organelle in the cell. A recent study showed the importance of Golgi's redox in the activity of alpha-2,6-sialyltransferase (ST6Gal-I), which is a key enzyme in the N-glycosylation of proteins. On the other hand, the cytoplasm's redox environment in cancer cells is more reducing than normal cells. There is not much information about Golgi's redox environment in cancer cells, but it is known that the deregulation of glycosylation helps the progression of cancer. Because of this, we wanted to study more deeply the Golgi's redox potential of non-cancerous CHO and cancerous HeLa cells, which are used to produce therapeutic N-glycosylated proteins and for medical research, respectively.

Methodology: We used the roGFP1-iL protein for measuring theGolgi's redox potential. This redox-sensing protein allows the measurement of redox potential independently of its concentration inside the cell. To direct the sensor protein to the Golgi, it was fused with the transmembrane domain of the α-2,3-sialyltransferase (ST3Gal IV) from Cricetulus griseus. CHO and HeLa cell cultures expressing the redox protein sensor were incubated in normal incubator conditions (37°C and 5%CO2) and the Golgi's redox potential was evaluated 48 hours after seeding. First, we obtained the oxidation percentage of protein and then we calculated the redox potential with the Nernst equation, and considering that E°′ roGFP1-iL = 229 mVolts (Lohman and Remington, 2008). Results: The localization of the redox roGFP1-iL protein sensor was confirmed. The Golgi's roGFP1-iL in CHO cells was oxidized 76.7 ± 4.1%, while in HeLa cells, it was 53.5 ± 3.3 %., corresponding to basal redox potentials of -212.9 ± 3 mV for CHO cells and -227 ± 4.1 mV for HeLa cells.

Conclusion: We found different Golgi's basal redox potentials for CHO and HeLa cells. This can be related to the different origin of each cell line and to differences in their metabolism. This discovery provides information for the better comprehension about how Golgi works, and it can be useful to improve therapeutic protein quality, as well as in search of diagnostic and therapeutic tools for cancer.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

A NEW ALTERNATIVE SINGLE USE CULTIVATION SYSTEM COVERING THE SCALE RANGE FROM ML TO 2500L WORKING VOLUME

Tibor Anderlei 1, Adolf Kühner AG 1, Sven Goebel 2,, Yvonne Genzel 2, Axel T. Lehrer 3.

1. Switzerland.
2. Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany .
3. Department of Tropical Medicine, Medical Microbiology & Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, USA

Background: Since more than 60 years the shake flask is the standard shaken bioreactor in biotechnology. Regarding cell cultivation the shake flask has been used intensively since more than 15 years. The presentation gives an overview of the variety of shaken bioreactors from mL to 2500L scale (microtiter plate, TubeSpin, Erlenmeyer flask, Orbital Shaken Bioreactor, ...) focusing on engineering parameters such as mixing time, kLa, etc.

In addition to discussing key engineering parameters, application examples (CHO, HEK cells) will be presented. A highlighted case study will demonstrate process intensification via perfusion mode for MARV-GP production in Drosophila S2 cells using disposable orbital shaken bioreactors (OSB) coupled to an alternating tangential flow system (ATF2, Repligen).

Key Words: High cell density, ATF perfusion, insect cells, orbital shaken bioreactor.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

MONITORING CELL STRESS IN INDUSTRIAL CELL CULTURE IN PERFUSION BY FLOW CYTOMETRY

Jennifer de la Caridad Quesada 1,2, Roberto Rafael Núñez 1,2, María Caridad Duartes 1,2, José Antonio Fernández 1 and Ernesto Chico 1,2.

1. Center of Molecular Immunology, CIM, 216 Street, corner to 15 street, Havana, Cuba. Postal Address 11600.
2. Technological University of Havana José Antonio Echeverría, CUJAE, Street 114 # 11901 / Ciclovía and Rotonda, Havana, Cuba. Postal Address 19390.

Background: The analysis of cell populations by flow cytometry allows to evaluate the behavior of structural and morphological properties of cells under different culture conditions. The characterization of size and granularity are directly related to the forward scattering coefficient (FSC) and side scattering coefficient (SSC) of light and has been reported as a potential indicator of cellular stress.

Methods: In the present investigation, cultures of CHO-K1 cell line were characterized by flow cytometry for the production of a recombinant protein in continuous mode with biomass recirculation in two fermentation systems of 500 L (intermediate scale) and 2000 L (industrial scale) effective volume. The working flows in the biomass recirculation systems were 120 L/h and 500 L/h in the intermediate and industrial scale, respectively, with the same residence time in both systems. To study the performance of the morphological characteristics of CHO-K1 cells, a culture mode with low mechanical stress was chosen
as a reference, so the cells were cultured in roller bottles in discontinuous mode, with the aim of evaluating the behavior of FSC and SSC factors in the different growth stages.

Results: Two indicators were established for the study of cell cultures: relative size and relative granularity, since FSC and SSC factors are absolute values. Cell size showed a similar behavior in all the systems studied, depending on the growth stage. It increased during the growth phase, and at the end of the growth phase a tendency to decrease was observed as a possible transition of the cells to G0/G1 phase. The relative size did not show significant statistical differences. Relative granularity increased under cell culture stress conditions at all scales studied, at the death stage in batch culture and at the start of perfusion in intermediate and commercial scale cultures. This indicator was 1.5 times higher in the large-scale culture compared to the other perfusion system, suggesting a higher stress of the cell population, which may be due to shear stress from mechanical damage because of the differences in perfusion systems.

Conclusions: Indicators capable of detecting differences in the culture of CHO-K1 cells, under different stress conditions have been developed using flow cytometry. This represents a valuable tool for the analysis and optimization of industrial processes in perfusion and must be evaluated for other experimental conditions.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

MATHEMATICAL MODELING AND 3D DESIGN OF A TANGENTIAL FLOW FILTRATION SYSTEM FOR MESENCHYMAL STEM CELLS HARVEST

Vicente Mérida 1, Pilar Vera 1, Barbara A. Andrews 1,2,3, Pablo Caviedes 2,4, Juan A. Asenjo 1,2,3,Ana maría Daza 2,3.

1. Department of Chemical Engineering, Biotechnology and Materials (DIQBM), Faculty of Physical and Mathematical Sciences (FCFM), University of Chile, Santiago, Chile.
2. Institute for Cell Dynamics and Biotechnology (ICDB), FCFM, University of Chile, Santiago, Chile.
3. Centre of Biotechnology and Bioengineering (CeBiB), University of Chile, Santiago, Chile.
4. Program of Molecular Clinical Pharmacology (ICBM), Faculty of Medicine, University of Chile, Santiago, Chile

Background: Mesenchymal stem cells (hMSCs) are pivotal in current regenerative medicine, serving as the primary resource in cell therapies. In this context, hundreds of millions of cells are often required per dose. To meet this demand, various strategies for cultivating hMSCs have been developed. Suspension cultures with microcarriers as anchoring surfaces provide an effective and scalable system for producing large quantities of cells. However, there are bottlenecks in the process. For recovering, the hMSCs must be harvested and separated from the microcarriers. Very few cells are recovered from this step, making it difficult to scale. Issues such as the stacking of microcarriers, cell adherence to microcarriers, and aggregation are common and are heavily dependent on the scale of the culture. In this study, a filtration system was developed to achieve a high recovery rate of viable cells, considering a mathematical model of mass transfer.

Methodology: First, a supporting mathematical model was developed and evaluated using the COMSOL software. This model aims to represent the real phenomenon by incorporating mass transfer equations, and finally, to simulate the filtration. The equations considered in the model were: 1. Diffusion through the membrane, 2. Cell-microcarrier attachment, 3. Detachment influenced by flow rate. Using the functional model, various parameters were defined that would later serve as initial values for the real system, including flow rate and membrane length. For the next part, 3D models of the filtering devices were sketched and modeled using the Fusion 360 program, for subsequent printing, the polymer used was PLA. For the design, a commercial nylon membrane is placed inside these devices. A peristaltic pump and an Arduino system for flow control were also integrated, the Arduino controls the stepper motor inside the pump.

Results: Using the program simulations was possible to approach the mass transfer in the filtration section of the system. Here, we can observe the number of recovered and lost cells. Considering the advantages observed in modeling, according to sizing and layout, the system was sketched in the fusion 360 program and ultimately built with an Ender 3 Pro printer and assembled.

Conclusions: It is important to analyze the mass transfer in depth, in order to include all the phenomena that occur at the same time. This detailed simulation provides valuable data for optimizing the instrument. The use of multiphysics simulation software is beneficial for designing specialized devices to separate cells.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF HUMAN CHORIONIC GONADOTROPIN FUSED TO HIS TAG (RHCG-HIS): A PROMISING PRODUCT TO POTENTIATE LIVESTOCK INDUSTRY

Agustina Gugliotta 1, Victoria Gastaldi 2,3, Natalia Ceaglio 2, Celeste Rodriguez 2, David Saucedo 3, Diego Fontana 1,3 and Claudio Prieto 1,3.

1. UNL, FBCB, CBL, Santa Fe, Argentina.
2. UNL, CONICET, FBCB , CBL, Santa Fe, Argentina.
3. Biotecnofe S.A. PTLC, Santa Fe, Argentina

Background: Human Chorionic Gonadotropin (hCG) is a heterodimeric glycoprotein that has an important role in reproduction since it induces progesterone production that is essential for early pregnancy survival. In the veterinary industry, hCG is used in different livestock species such as cattle, pork, equine, goat and sheep, to promote ovulation. Thus, it is widely used for animal production. The hCG available in the veterinary industry is obtained by purification from natural source (post-menopausal women urine). As it is known, this product exhibits poor batch-to-batch consistency, low purity and low specific activity. Thus, the development of a new product based on rhCG-HIS using animal cell culture technology is of great interest.

Methods: rhCG alpha and beta subunits sequences were obtained by chemical synthesis. The addition of the 6xHIS tag sequence was performed by PCR at the N-terminus of each subunit. CHO producer clones were obtained by lentiviral transduction, puromycin selection and cloning using the limit dilution method. Production was carried out in batch conditions. rhCG-HIS was purified by IMAC. Physicochemical, glycosidic and biological characterizations were performed.

Results: Sequences of alpha and beta rhCG subunits with and without 6xHIS tag were used for cell line generation by transduction and selection. As a result, the best producer cell line was the one generated with both subunits bearing 6xHIS tag, since it exhibited the highest productivity and the expected molecular weight. Individual clones of the above mentioned cell line, were screened by specific productivity assays and SDS-PAGE/western blot. The best 4 clones were suspension adapted to the production medium. Growth curves were built in order to determine the growth and productivity of each clone under this condition. Clone 4E10 was used for production in batch culture since it presented the best characteristics in terms of specific productivity and specific growth rate. Purification was assessed by IMAC obtaining more than 10 mg of protein with a high purity, being the main related impurities alfa and beta free subunits. rhCG-HIS was further characterized in terms of glycosylation, physicochemical and biological activity, obtaining promising results.

Conclusions: rhCG-HIS produced in CHO cells and purified by IMAC represents an interesting therapeutic candidate for the veterinary industry. This novel product offers significant advantages over naturally derived options, claiming superior purity and batch to batch consistency. rhCG-HIS constitutes a novel candidate that can be used alone or in combination with other hormones to improve animal reproduction and thus, potentiate the livestock industry.

DEVELOPMENT OF AN INTEIN-BASED SELF-CLEAVING AFFINITY TAG FOR PURIFICATION OF THERAPEUTIC RECOMBINANT PROTEINS PRODUCED IN MAMMALIAN CELLS

Javier Nicolás Garay-Novillo 1, Marilla Amaranto 1, José Luis Barra 1, Agustina Godino 1.

1. Centro de Investigaciones en Química Biológica de Córdoba - CONICET; Departamento de Química Biológica Ranwel Caputto - Facultad de Ciencias Químicas – Universidad Nacional de Córdoba. X5000HUA, Córdoba, Argentina.

Background: Biopharmaceuticals require high purity levels and stringent downstream processing. Affinity tags are essential tools in protein purification, however, removing these tags is costly and often inefficient. Inteins are self-splicing polypeptides capable of excising themselves from proteins under mild conditions with high precision. This study aimed to develop a new one-step purification method using DnaB or DnaX inteins to produce human α-galactosidase A (GLA) and Deoxyribonuclease I (DNaseI) in mammalian cells. These proteins serve as biotherapeutics for Fabry disease and cystic fibrosis, respectively.

Methods: CHOK1 adherent cells and HEK293F suspension cells were used for recombinant protein production. Four plasmid vectors were constructed to produce proteins of interest (POI) fused to either DnaB or DnaX inteins at their N-terminal (DnaB-GLA, DnaB-DNaseI) or C-terminal (GLA-DnaB, GLA-DnaX) ends. Each fusion included HA and CBD tags for Western blot detection and affinity chromatography purification, respectively. We evaluated in vivo spontaneous cleavage (unwanted) and in vitro controlled cleavage by analyzing total protein samples with Western blot before (spontaneous) and after (controlled) incubation in a cleavage buffer (Tris-HCl, pH 6.5) at room temperature for 24 or 48 hours.

Results: Previous results indicated successful production of GLA and DNaseI in CHOK1 and HEK293F cells. However, when fusing DnaB to the POI N-terminal end no GLA production was detected, and DNaseI levels were low and showed no intein cleavage could be obtained (spontaneous or controlled). This prompted the use of a different intein (as DnaX, already used in our lab) or fusions of inteins to POI C-terminal end. C-terminal fusion showed positive expression for GLA-DnaB and GLA-DnaX. Nevertheless, GLA-DnaB did not undergo cleavage. While GLA-DnaX showed significant cleavage but it was due to in vivo cleavage, with no increase after in vitro treatment. Consequently, a split-intein strategy was designed using DnaX divided into two fragments (DnaXN and DnaXC), which could only cleave when reassembled in vitro. Two expression vectors were constructed to produce DnaXN fused to the C-terminal end of POI (GLA-DnaXN and DNaseI-DnaXN). These vectors will be tested expecting no spontaneous cleavage and a successful controlled cleavage using the chemically synthesized DnaXC peptide.

Conclusions: Current results suggest that the C-terminally fused DnaX is a promising self-cleaving candidate. Future experiments will focus on utilizing the split-intein strategy with DnaXN and the DnaXC peptide for recombinant tag-free therapeutic protein purification in a single chromatography step.

EXCITATION-EMISSION MATRIX COUPLED WITH CHEMOMETRIC MODELLING AS A STRATEGY FOR MONITORING THE UPSTREAM BIOPROCESS OF SARS-COV-2 SPIKE PROTEIN

María Celeste Rodríguez 1, Denise Kimey Mendoza 2, Javier Villarraza 1, Agustina Gugliotta 1, Claudio Prieto 2,3, Marina Etcheverrigaray 1.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral (CBL), Santa Fe, Pcia. Santa Fe S3000ZAA, Argentina.
2. UNL, FBCB, Centro Biotecnológico del Litoral (CBL), Santa Fe, Pcia. Santa Fe S3000ZAA, Argentina.
3. Biotecnofe S.A. Parque Tecnológico Litoral Centro (PTLC), Ruta 168 Santa Fe, Pcia. Santa Fe S3000ZAA, Argentina.

Background: Upstream bioprocess monitoring and characterization are time and resource-intensive tasks. Process Analytical Technology (PAT) approach allows for efficient bioprocess monitoring, ensuring product quality in a cost-effective manner. This work reports the development of an analytical strategy based on fluorescence excitation-emission matrices (EEMs) combined with second-order chemometric models to monitor variations of the SARS-CoV-2 Spike ectodomain glycoprotein (S-ED), a COVID-19 subunit vaccine candidate, in HEK293 perfusion bioreactor cultures.
Methods: The univariate reference method (sandwich enzyme-linked immunosorbent assay, sELISA) was optimized using response surface methodology (RSM) and validated following ICH guidelines to ensure result accuracy and consistency. A qualitative chemometric analysis was then performed through extended multivariate curve resolution-alternating least squares (MCR-ALS) modelling to characterize two independent S-ED perfusion processes (BioR-1 and BioR-2) and initially inspect the entire dataset. To address potential energy transfer, inner-filter effect (IFE), and quenching due to sample matrix complexity, we studied the dilution effect on prediction accuracy using parallel factor analysis (PARAFAC) modelling. Thereafter, an outlier inspection of the spectral data was performed using unsupervised principal component analysis (PCA). Subsequently, PARAFAC was implemented for S-ED prediction in bioreactor samples.
Results: We optimized the sELISA using design of experiments (DoE) and RSM approaches. This method enhanced sELISA performance and accelerated its validation process compared to traditional one-variable-at-a-time strategies. The PARAFAC model from bioreactor samples diluted at 1:50 provided the best results compared to undiluted or lesser-diluted samples, ensuring cleaner and more accurate spectra for multivariate calibration modelling. Thus, samples were diluted 1:50 for multivariate calibration. The calibration model obtained through PARAFAC proved to be an accurate and precise predictive method for monitoring the S-ED production process. This variable is a critical process parameter (CPP) as it significantly influences subsequent unit operations, such as downstream purification. Validation samples showed adequate predictions with a relative error of prediction (REP) of 12.90%, considering the standard error of the reference method. Further analysis using elliptical joint confidence region (EJCR) test and bivariate least square (BLS) regression confirmed the method's precision and accuracy (slope: 1.18 ± 0.47, SD: 0.20; intercept: -0.18 ± 0.47, SD: 0.20), with the ideal point (1,0) contained within the elliptical EJCR domain.
Conclusions: The developed multivariate calibration method was successfully implemented to quantify S-ED evolution in perfusion bioreactor samples, aligning with PAT guidelines. Overall, this approach enhances efficiency across the entire S-ED production process.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

MAMMALIAN CELL PLATFORM FOR PORCINE CIRCOVIRUS 2: A NEW ADAPTABLE VACCINE PROTOTYPE

Florencia Fadel 1, Sergio Pantano 3, Natalia Olivero-Deibe 2, Claudia Ortega 4.

1. Programa de Tecnología Molecular, Celular y Animal (ProTeMCA), Institut Pasteur de Montevideo, Mataojo 2020, 11400 Montevideo, Uruguay.
2. Laboratorio de Simulaciones Biomoleculares, Institut Pasteur de Montevideo, Mataojo 2020, 11400 Montevideo, Uruguay.
3. Laboratorio de Inmunovirología, Institut Pasteur de Montevideo, Mataojo 2020, 11400 Montevideo, Uruguay.
4. Programa de Tecnología Molecular, Celular y Animal (ProTeMCA), Institut Pasteur de Montevideo, Mataojo 2020, 11400 Montevideo, Uruguay.

Background: Porcine circovirus type 2 (PCV2) is a significant pathogen that affects the swine population, leading to clinical signs such as diarrhea, abortion, and respiratory disorders, collectively known as Post Weaning Multisystem Wasting Syndrome (PMWS). The economic losses caused by PCV2 infection to the industry worldwide are substantial. Our research, aimed at developing updated vaccines, could potentially mitigate these losses. The high mutation rate of this virus contributes to the emergence of new genotypes and variations in its prevalence over time, further underscoring the importance of our work. Virus-like particles (VLPs) mimic the structure of viruses but lack genetic material and are, therefore, unable to infect host cells. This inherent safety, combined with their ability to activate the immune system at both the humoral and cellular levels, makes them an attractive and safe alternative for vaccine platforms.
Experimental strategy: The project aims to implement a high-yield viral particle production protocol based on mammalian cell technology. We characterized PCV2 VLPs and some mutants biochemically, biophysically, and immunologically to find the best possible candidate and obtain super-stable VLPs for vaccine prototypes. EXPI293F cells were transfected to monitor protein production. We explored optimizing DNA concentrations for transient transfections, which showed potential for reduced DNA usage without yield loss. We monitored cell viability, glucose consumption, and lactic acid production. We evaluate different lysis methods, the use of DNases, and clarification through filtration. In addition to classical ultracentrifugation VLP purification, we implemented chromatography methods to scale up the bioprocess.
Results: We have successfully expressed and purified wild-type and mutant VLPs in EXPI293F cells. From a biochemical point of view, we found one VLP mutant that was more stable than the wild type. The optimization of cell culture duration after transient transfection with PEI was achieved, showing that maintaining the cells in culture for six days and adding glucose yields three times higher than that obtained with three days of culture, which was previously reported. Additionally, we evaluated different DNA concentrations to be used during the six-day transfection of the cells. Two purification methods were compared: discontinuous gradient with CsCl by ultracentrifugation and ion exchange chromatography, followed by gel filtration. VLPs obtained by both purification methods were confirmed by Western and Dot Blot, DLS, and MET analysis. This initial purification by ion exchange is of particular interest for future scaling of the bioprocess since VLP purification by ultracentrifugation is not applicable at the veterinary industry level.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

EPITOPE TAGGING: DEVELOPMENT OF AN INNOVATIVE IONIC-STRENGTH MODULATED SYSTEM FOR ENHANCED DETECTION, QUANTIFICATION AND PURIFICATION OF RECOMBINANT PROTEINS

María Jesús Leopold 1, Ricardo Kratje 1, Marcos Oggero 1, Natalia Ceaglio 1.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral Santa Fe, Pcia. Santa Fe S3000ZAA, Argentina.

Background: Epitope tagging is a well-established and a very useful tool for tracking recombinant proteins. These highly specific systems use peptide tags and anti-peptide monoclonal antibodies (mAbs). In particular, in our lab, we developed a mAb called CC1H7 which recognizes an epitope present in a novel O-glycosylated tag named mGMOP and exhibits ionic strength-modulated binding. This characteristic has turned this system into a versatile strategy to set up methods such as ELISA, chromatography, and western blot.
Methods: To begin with this study, two new molecules were obtained: one with a single copy and another with four copies of mGMOP tag fused to human interferon-α2b (hIFN-α2b). These proteins were produced in suspension CHO-K1 cells. mAb-CC1H7 producing hybridoma was adapted to suspension in vitro culture. For this, FBS was progressively reduced from 5 to 1.25% in adherence culture medium, and then cells were directly subcultured into serum-free medium with agitation. The mAb specificity was assessed through a specific indirect ELISA and western blot. Protein-A purified mAb facilitated techniques for detecting, quantifying, and purifying proteins labeled with mGMOP peptide. An experimental design explored the impact of antichaotropic salts and pH on CC1H7-mGMOP interaction. Optimal conditions were applied in an immunoaffinity chromatography. Another experimental design was performed to find optimal conditions for quantifying tagged proteins using a competition ELISA.
Results: Interferon variants with different number of mGMOP tags were successfully obtained for development of detection, quantification, and purification assays. Hybridoma mAb productivity increased concomitantly with serum reduction in culture media. The mAb specificity after adaptation was demonstrated by indirect ELISA and by western blot assay, showing a detection limit of 10 ng for both proteins. Using an experimental design, a competition ELISA with appropriate sensitivity and detection limit to quantify variants containing different copies of mGMOP peptide could be developed. Also, another experimental design allowed to identify optimal high ionic strength conditions that enhanced epitope paratope interaction. In this way, incubation of the complex in the presence of Na2SO4 1 M (pH 8) increased the apparent affinity about 15 times compared to the control (without salt). The use of salts allowed the improvement of the dynamic capacity of the resin. Under these conditions, purification achieved 80% yield and 96% purity.
Conclusion: mAb-CC1H7 was successfully obtained through in vitro, serum-free, suspension culture, and used to develop a mAb-mGMOP system useful for detection, quantification and purification of proteins tagged with the O-glycosylated peptide.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

OPTIMIZATION OF A PURIFICATION PROCESS OF PEGYLATED RECOMBINANT HUMAN A-GALACTOSIDASE A (RHA-GAL) FOR FABRY DISEASE TREATMENT

Malen Menegon 1, Gabriel I. Amadeo 2, Natalia A. Ceaglio 2, Santiago E. Vaillard 1.

1. Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), UNL-CONICET, Santa Fe, 3000, Argentina.
2. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Pcia. Santa Fe S3000ZAA, Argentina

Background: PEGylation is becoming increasingly important for improving the efficacy of therapeutic proteins. This is achieved by improving their half-life, reducing renal clearance, enhancing stability against proteolytic enzymes, increasing solubility in biological fluids, and reducing immunogenicity. Although these effects lead to lower dosage requirements and administration frequency, the challenge of low product yield and purification of the desired product still remains.
Methods: To improve the pharmacological efficacy of recombinant human α-Galactosidase A (rhα-GAL) used for Fabry disease treatment, chemical modification by PEGylation was carried out. rhα-GAL, previously produced and purified in sCHO cells in our lab, was pegylated under mild conditions using a 30 kDa linear mono-functional methoxypolyethylene glycol-N-hydroxysuccinimidyl ester (mPEG-NHS ester). The pegylated enzyme, mPEG-rhα-GAL, was purified in one-single step employing MacroCap Q® anionic exchange chromatography specifically designed to purify PEGylated and other large biomolecules. The elution condition consisted in a 10 CV linear gradient from 0 to 0.5 M NaCl. Fractions were buffer-exchanged and concentrated by tangential flow ultrafiltration, and then analyzed by non-reducing SDS PAGE. Gels were stained with Coomassie blue and BaCl2/I2. Only those fractions containing >80% monoPEGylated rhα-GAL monomer were pooled. Product purity was determined by densitometry using Image-J® software. Specific enzymatic activities (EA) of rhα-GAL and purified mPEG-rhα-GAL were measured with 4MU-α-Gal assay.
Results: The analysis of pegylation reactions indicated that 40% of the protein was monoPEGylated after 20 min, and several PEGylated dimers or multimers were observed as the reaction progressed. In that way, the optimal monoPEGylated/diPEGylated protein ratio was achieved at 10 min. Mono-PEGylated rhα-GAL was successfully recovered in a one-step purification process using a 0.2 M to 0.3 M NaCl gradient. Most of the unreacted mPEG-NHS ester was removed during the washing step, and the elution condition resulted in the recovery of the monoPEGylated enzyme with 80% purity. Further analysis of purified mPEG-rhα-GAL revealed that PEGylation partially affected EA. Several reports have shown that PEGylation deeply affects therapeutic protein activity; however, mPEG-rhα-GAL retained 50% of native specific EA.
Conclusion: PEGylation of rhα-GAL revealed high reproducibility, as similar degrees of conjugation were achieved in different reaction experiments performed under similar conditions. The PEGylated protein was obtained with 80% purity in a single-step purification. Besides, although a decrease in activity was observed, mPEG-rhα-GAL preserved near 50% of residual AE. These results are very encouraging in terms of PEGylated products, some of which, despite retaining only 7 % of in vitro activity, still show an improved efficacy in vivo compared their unmodified counterparts.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

USING MATHEMATICAL MODEL TO ESTIMATE LOSS OF CELL DENSITY DUE TO MECHANICAL STRESS IN INDUSTRIAL PERFUSION SYSTEMS

Roberto Rafael Núñez 1,2, Jennifer de la Caridad Quesada 1,2, María Caridad Duartes 1,2, José Antonio Fernández 1, Julio César Dustet 2, Ernesto Chico 1,2.

1. Center of Molecular Immunology, CIM, 216 Street, corner to 15 street, Havana, Cuba. Postal Address 11600.
2. Technological University of Havana José Antonio Echeverría, CUJAE, Street 114 # 11901 / Ciclovía and Rotonda, Havana, Cuba. Postal Address 19390.

Background: Continuous culture with biomass recirculation is a system of high operational complexity that allows high productivity. In perfusion processes, previous research shows that the mechanical stress generated in the biomass recirculation system significantly affects the cell concentration due to the adverse environment present in this system, where different variables that favor cell survival and proliferation are not controlled.

Methods: In the present investigation, the impairment of cell growth was studied in two systems in perfusion mode, with different recirculation system volumes and recirculation flows, but with the same residence times. The first system is a 500 L effective volume bioreactor (intermediate scale) in which three cell lines, NS0-1, CHO and CHO-K1, were used. In the second system, CHO-K1 and NS0/H cell lines were cultivated in 2000 L effective volume (large scale).

Results: A mathematical model was obtained including Monod and mass balance equations for substrate and biomass, the latter introducing a specific death rate term representing the effect of mechanical stress on cell growth in the recirculation system. This model was able to describe the kinetic behavior of the four cell lines without the need to identify the limiting substrate, and has been validated in low mechanical stress systems. The death rate associated with the retention system was determined; at the intermediate scale it was 0.04 h-1 for the three cell lines, four times lower than the death rate in large-scale culture for the cell lines under study.

Conclusions: The use, for the first time, of a specific death rate term associated with the retention system made it possible to explain the impairment of growth for all the cell lines studied. It was observed that this term was higher at higher retention volumes and was associated with the cell recirculation system independently of the cell line used. The research carried out shows that the perfusion processes have the potential to achieve higher cell concentrations, which is of great importance for the optimization of processes on an industrial scale.

USE OF THE PLATECH HUMAN MEGAKARYOBLASTIC PLATFORM FOR THE PRODUCTION OF RECOMBINANT PROTEINS WITH HIGH COMMERCIAL IMPACT, USING HUMAN TGF-B AS A MODEL.

Ignacia Möller 1,2, Pía Viera 2, Ignacio Valencia 2, Cristobal Curkovic 2.

1. Universidad Andres Bello, Chile.
2. Platec , Chile.

Background: The recombinant protein market is experiencing significant growth and is projected to reach USD$5.09 billion by 2030. Leading companies like Merck and Pfizer are employing cutting-edge technologies for recombinant protein production. However, challenges and costs associated with expression systems, including bacteria, yeast, insect, and mammalian cells, persist. Careful selection of the biotechnological platform and along with post-translational modifications are crucial to ensure the proper structure and function of the proteins. While human expression systems exhibit lower yields and cell growth, they are indispensable for producing complex proteins. In light of these considerations, a novel human megakaryoblastic platform is proposed for the generation of recombinant human TGF-β, a highly complex protein of considerable commercial interest. TGF-beta's therapeutic potential and market demand drive commercial interest, while its structural complexity and expression system limitations pose technical challenges. The Platech platform offers a promising solution for efficient production.

Methods: Within the methodological framework, activities related to the scaling up of the transduced cell line for the overexpression and production of recombinant TGF-β using cell culture in T-flask, spinner flask, and bioreactor formats will be undertaken. These activities will involve analyzing growth rates, viability, and cell morphology (compared to their wild type counterpart). For the characterization of the recombinant protein, cell lysis will be performed to obtain proteins and RNA for subsequent molecular assays such as Western Blot and qPCR. Protein and transcript levels will be compared between the transformed clone and the WT version.

Results: Transformed TGF- β overexpressing cells are capable of adapting and growing in the different scaling formats, maintaining growth rates similar to their wild type counterpart. Moreover, the cells maintain high viability and do not exhibit significant morphological changes when comparing the different growth formats. The presence of the recombinant protein can be detected, both intracellularly and in the conditioned medium. Its expression, at the protein and transcript level, is remarkably higher than its wild type counterpart.

Conclusion: The utilization of the Platech platform enables human megakaryoblast cell growth and the production of recombinant human TGF-β, indicating the potential application of this technology for the production of other proteins of commercial interest. As a projection, it is expected in the short term to test its biological activity by treating HepG2 cells with the recombinant protein, to analyze the activation of the SMAD pathway.

IMAGE ANALYSIS METHOD TO DETERMINE MIXING TIME IN BIOREACTORS

Fernando A. Cecatto 1, Yago G. S. Silva 1, Elisabeth F. P. Augusto 1.

1. Science and Technology Institute, Federal University of São Paulo, São José dos Campos, São Paulo, Brazil.

Background: Stirred tank bioreactors are widely used for animal cells cultivation, and the characterization of their homogeneity are relevant during process developments carry out at lab-scale, or scale-up studies. Several techniques are based on the application of a step-type perturbation of a certain variable (pH, conductivity, temperature, etc.), and following its evolution over time until a final value, when mixing time is defined. Among available techniques, the one that monitors the evolution of the fluid color corresponding to a pH variation has clear advantages: it is non-invasive, as it does not alter the geometry of the bioreactor with the introduction of sensors, and provides a global response of mixing time, as opposed to the local response of a sensor. In this context, the present work sought to adapt this method, from experimental setup to image processing treatment, aiming to increase the reproducibility of mixing time measurements in a benchtop bioreactor.

Materials and methods: Runs were carried out in a 2.5 L stirred tank benchtop bioreactor (1.5 L used volume), setting up different geometries from the selection of an impeller type (1 or 2 flat blades, or 1 pitched blade, or 1 marine propeller) and a gas sparger type (perforated ring or microporous). Water (reverse osmosis grade) was used as fluid, and HCl/NaOH as tracers, and the pH variation was measured by the color evolution (phenolphthalein indicator) through a video image capture (30fps). The influences of the following aspects were evaluated: area of analysis, lighting, color system (RGB; gray scale; HSV), type of step (ascending or descending pH value), tracer volume (0.125 – 3.75 mL), mixing time defining criterion (mean or standard deviation).

Results: The area of analysis were defined to guarantee the same number of pixels regardless of the bioreactor configuration. A Python software was developed for processing video images and being capable of identifying the initial time of the test using the HSV color system (hue, saturation, and brightness). It also calculates mixing times based on: i) normalized average of the green component (RGB system); ii) normalized average of the gray scale; and iii) standard deviation of the normalized green color. The method proved to be more accurate for the latter treatment. Higher tracer volumes disturbed the fluid flow and the response was more reproducible for 1mL.

Conclusions: The study made it possible to establish test conditions that ensure good reproducibility of pH measurements through a image analysis method.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

VALIDATION OF A MODEL-BASED PLATFORM FOR THE OPTIMIZATION OF MAMMALIAN CELL CULTURE STRATEGIES

Iván Paredes 1,2, Temistocles Molina 1,2, Isidora Prat 1,2, Francisca Pizarro 1,3, Aaron Canales 1,3, Bastián Herrera 1,3, J. Cristian Salgado 1,3, Ziomara P. Gerdtzen 1,2,4.

1. Centre of Biotechnology and Bioengineering, University of Chile.
2. Mammalian Cell Culture Lab, Department of Chemical Engineering, Biotechnology and Materials, University of Chile.
3. Process Modeling and Distributed Computing Lab, Department of Chemical Engineering, Biotechnology and Materials, University of Chile.
4. Center of Interventional Medicine for Precision and AdvancedCellular Therapy, Santiago, Chile.

Background: The current growth of the biopharmaceutical industry emphasizes the need to improve production strategies, prioritizing effectiveness and efficiency in bioprocess design. To address this challenge, our efforts focus on optimizing bioproduct production through the establishment and implementation of protocols to evaluate culture requirements using the CELIA platform (CEll cuLture medIA optimization). This platform is based on specific models of cell lines to determine a composition and feeding strategy that improves the productivity of the production system of interest. By integrating cellular composition, product, and characteristic process parameters, we can predict the specific nutritional requirements of a given cell line in a specific production process to design a medium composition and a customized feeding strategy for each production system. Using CELIA, an optimized Feed-Batch process design is obtained where the feeding medium is based on the nutritional requirements of the cells for their maintenance, growth, and production.

Methods: To validate the use of the platform, the CHO tPA and PK15 cell lines were selected. The first for being widely used to produce biopharmaceuticals and the second as a platform for veterinary vaccines. CHO and PK15 cells were cultured in duplicate, in a 200 mL bioreactor in DMEM/F12 medium, under control of pH, dO2, and To with a setpoint of 7.4 and 30% and 37°C, respectively. Monitoring biomass, nutrient consumption, and production to determine cellular requirements for growth and product synthesis

Results: In the case of PK15 the optimized Fed-Batch cultures showed a 69% increase in biomass compared to Batch cultures and a 35% increase compared to the reference Fed-Batch culture which uses concentrated feed. Lactate concentration achieved was much lower in the optimized Fed-Batch versus the reference Fed-Batch, which allowed the culture time to be prolonged and a higher cell concentration. For CHO-tPA cells, Fed-Batch cultures with CELIA strategy, recorded a 61% increase in biomass compared to Batch cultures and a 36% increase compared to reference Fed-Batch cultures. In addition to the increase in biomass, a lower lactate production and an increase in tPA concentration were obtained.

Conclusions: The results show that the implementation of culture strategies that consider the difference in cellular composition and specific metabolic requirements for cell lines through the CELIA platform leads to a significant increase in culture productivity and efficiency in the use of media. This model-based strategy opens the possibility of improving biopharmaceutical process by controlling critical culture variables.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

CHO-K1 EXPRESSING E2 PROTEIN FROM BOVINE VIRAL DIARRHEA VIRAL SUBGENOTYPE 1E

Erick Bonilla 1, Verónica Avello 2, Eduardo Ramos 2, Raquel Montesino 2.

1. Departamento de Ciencias de la Vida y de la Agricultura, Universidad de las Fuerzas Armadas ESPE, Av. General Rumiñahui s/n y Ambato 171103, Quito, Ecuador.
2. Biotechnology and Biopharmaceuticals Laboratory, Pathophysiology Department; School of Biological Sciences. Universidad de Concepción. Victor Lamas 1290, P.O. Box 160C, Concepción, Chile.

Background: Bovine Viral Diarrhea is a disease with a significant reproductive and economic impact on the livestock sector. Spontaneous abortions and persistently infected individuals are the major problems associated with this disease. Recombinant vaccines that generate neutralizing antibodies in the vaccinated animal could be successfully used for BVDV control.

Methodology: The CHO-k1 line was used as a host for obtaining recombinant E2 protein of Bovine Viral Diarrhea viral subgenotype 1e. The CHO-k1 expressed E2-1e was adapted to suspension growth in agitated vessel systems, increasing the concentrations of EX -CELL medium in RPMI medium, which also led to a decrease of Fetal Bovine Serum in the medium. E2-1e glycoprotein was purified from the harvested supernatants by metal ion binding chromatography, and the volumetric yield was analyzed. Additionally, the glycosylation profile was studied using normal-phase chromatography. The N-glycan pool was derivatized with 2-amino benzamide, and the presence of sialylated structures was determined by ion exchange chromatography and neuraminidase digestion, followed by NP-HPLC.

Results: E2-1e obtained in adherent conditions reached a volumetric yield of 7.6 ug/mL; meanwhile, the highest value (18,5 µg/mL) was obtained when the CHO-K1 was cultured in suspension. In this culture condition, a cell density of 1.8 x10^6 cells/mL and a replication rate of less than 50 hours were obtained. IMAC liquid chromatography for protein purification obtained a purity of 96.71%. Post-transductional modification was addressed by the addition of N-glycan composed mainly of complex sialylated structures.

Conclusions: CHO-K1 cells are an efficient platform for producing recombinant proteins for biopharmaceutical purposes. Increasing concentrations of EXCELL medium in RPMI medium using the spinner shaking system allowed the successful adaptation to suspension conditions, which was beneficial for E2-1e protein production, improving the purity and volumetric yield. In addition, using CHO-K1 cells as a platform for recombinant protein production ensured the expression of E2-1e protein with post-translational modifications, including the synthesis of N-glycan sialylated complex structures.

EVALUATION OF THE DYNAMIC BINDING CAPACITY OF THE IMAC MATRIX USED IN THE PURIFICATION PROCESS OF THE RBD-HIS

Ivis Regalado 1,2, Patricia León 1,2, Lourdes Hernández de la Rosa 1, Lourdes M. Zumalacárregui 2.

1. Center of Molecular Immunology, CIM, 216 Street, corner to 15 street, Havana, Cuba. Postal Address 11600.
2. Technological University of Havana José Antonio Echeverría, CUJAE, Street 114 # 11901 / Ciclovía and Rotonda, Havana, Cuba. Postal Address 19390.

Background: The recombinant protein RBD-His (aa319-541), is the antigen of the Soberanas® vaccines. The dynamics of the global health crisis imposed by the new coronavirus known as SARS-CoV-2 led to development and production of new vaccine candidates. The production of RBD is based on the culture of Chinese Hamster Ovary (CHO) cells in stirrer tank bioreactors, using culture media free of serum and components of animal origin. The technological process at industrial scale shows several stages: preparation of culture media and solutions, fermentation, supernatant clarification and purification process. This product is used to face the current epidemiological situation of Covid-19. The use of adequate technologies for the large-scale production of this product in accordance with good manufacturing practices and reasonable production costs, becomes a necessity to introduce these products in high demand markets with high quality requirements. The dynamic binding capacity (DBC) of the Immobilized Metal Affinity Chromatography matrix (IMAC) used in the purification step of the RBD-His protein (aa319-541) are currently unknown, so it will be the objective of the present investigation.

Methods: A column packed with 5 mL of IMAC matrix and an elution of the affinity step with immobilised metals, obtained in the production process, with concentration 2.49 g/L, dimer purity 64.63 % and monomer purity 30.14 %, diluted to concentration 0.20 mg/mL, were used for the experimentation. The independent variables were: residence time, pH and conductivity of the equilibrium buffer; while the dependent variable was the dynamic binding capacity of the IMAC matrix. For the study of the dynamic binding capacity of the IMAC matrix, a central composite design was carried out with two replicates at the central point and six star points, with three levels for each variable analysed.

Results: The operating range that maximises the dynamic binding capacity and residence time 3 minutes. With the use of filtered supernatant, an adsorption capacity of 5 mg protein/mL gel was achieved and it is proposed to pack the column with 25 L of the studied matrix.

Conclusions: With the use of filtered supernatant, the dynamic binding capacity of the IMAC matrix is 7.7 times lower than that obtained with purified protein. The volume can be decreased from 33 L to 25 L of packed gel. The operation time of the affinity step for immobilised metals is reduced by 3.47 h.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

METHODOLOGY FOR INTEGRATING PERFUSION CULTURES OF MAMMALIAN CELL LINES FOR THE DESIGN OF OPTIMISED FEEDING STRATEGIES USING THE CELIA PLATFORM

Frank Delgado 1,2, Iván Paredes 1,2, J. Cristian Salgado 2,3, Ziomara Gerdtzen 1,2,4,5.

1. Mammalian Cell Culture Lab, Department of Chemical Engineering, Biotechnology and Materials, University of Chile.
2. Centre for Biotechnology and Bioengineering, University of Chile
3. Process Modeling and Distributed Computing Lab, Department of Chemical Engineering, Biotechnology and Materials, University of Chile
4. Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.
5. Milenium Nucleus Marine Agronomy for Seaweed Holobionts, Santiago, Chile, Santiago, Chile.

Background: Biopharmaceuticals are in high demand in the pharmaceutical market, mainly associated with their applications to treat cancer and autoimmune diseases with increased efficacy and fewer side effects. Conventional production processes are complex and time-consuming, so to keep pace with growing demand, manufacturing processes must be optimised to increase production flexibility and reduce costs. Optimisation ranges from modifications of cell lines to adjustment of culture media, operational conditions and process configurations. In recent years, the industry has moved towards continuous manufacturing instead of traditional batch processes. Perfusion culture has been the focus of interest for upstream processes in continuous operation. In this context, the CELIA platform offers a tool based on a stoichiometric, dynamic and predictive mathematical model that allows the development of a specific feeding strategy and culture medium composition for different cell lines and has enabled significant productivity increases in batch processes. It is highly attractive to define a model system that combines this tool with kinetic models describing perfusion operation to maximise productivity under these conditions.
Methods: The first step for the implementation of this framework is the characterisation of the culture parameters of the cell line of interest in a stirred tank bioreactor operating under batch and fed-batch conditions to determine key parameters and cell requirements under conditions that maximise productivity. At this stage, the best environmental conditions should be defined, including temperature, pH, oxygen transfer rate and agitation speed. The parameters to be determined are the concentration of viable cells, viability, product titres, substrate concentration and secondary metabolites. From the mass balance for biomass, metabolites and key nutrients and product in a perfusion operation system and considering a feed composition based on cellular requirements, a model describing the process is proposed and adjusted based on experimental data. The defined structure will be incorporated into the current CELIA culture media design platform to extend the scope of its operation to perfusion systems, and its experimental validation will be carried out.
Expected results: With this proposal it is expected to obtain a culture media design platform capable of delivering strategies for the different modes of operation in a specific way for each manufacturing line and process, which will increase the productivity of these processes.
Conclusion: This work extends the scope of the CELIA platform applications to new modes of operation and is conducive to process optimisation in order to meet market demands.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

OPTIMIZATION OF CONDITIONS FOR MULTIDISPLAY OF PROTEINS ON THE SURFACE OF FILAMENTOUS PHAGE M13

Cristian Vargas 1, Evaldo Rivas 1, Angela Hidalgo 1,2, Fátima Reyes 1, Lionel Zapata 1, Rafael Maura 2, Roxana Zúñiga 1, Yaneysis Lamothe 1, Óscar Otero 1, Oliberto Sánchez 1, Norazmi Mohd Nor 3, Frank Camacho 1.

1. Laboratorio de Biofármacos Recombinantes, Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
2. Laboratorio de Biotecnología y Biofármacos, Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
3. School of Health Sciences, Universiti Sains Malaysia, Malaysia.

Background: M13 filamentous phages are a type of virus characterized by circular single-stranded DNA that infects many gram-negative bacteria, such as E. coli. The structure of the phage virion is shaped like a tube consisting of an average of 2700 copies of the major capsid protein, pVIII, with the minor capsid proteins, pVII and pIX, at one end and pIII and pVI at the other. This structure makes M13 phages excellent cloning vehicles because their replication and assembly are not limited by their genetic material and dimensions. In addition, their efficient adaptation to modifications gives them the most important properties for their use in peptide and protein display. This has led to the development of the phage display technique, which consists in displaying peptides or proteins on the bacteriophage surface by cloning a gene encoding a capsid protein. This technique is widely used to obtain, identify, isolate and analyze peptide sequences with high affinity and specificity for a target of interest. Usually only one protein is present in each phage, here we present the optimization of a new system for protein mutidisplay in filamentous phages that can have multiple applications in the field of applied biotechnology.

Methods: A DNA sequence was designed that encodes three fusion proteins under the control of the Lac promoter. For the optimization of the conditions for obtaining the phage, multiple parameters were evaluated, such as growth media, temperature and induction agent. The best growth conditions were selected to establish the phage production process.

Results: The best growth conditions were selected: phage titers and relative presentation values of proteins A and B. Regarding the growth process, the best culture medium, temperature, additive, time and agitation were selected, in a 1.5 bioreactor, achieving titers of at least 1012 CFU/mL.

Conclusions: A production process was established that guarantees obtaining a phage that correctly multidisplay proteins A and B with viral titers higher than 1012 CFU/mL. In the context of vaccine development, this technology is a great tool. These results suggest that multidisplay of proteins in a phage can be used as an approach to develop new vaccines candidates capable of carrying multiple antigens, allowing to generate multiepitope or multistage vaccines against complex pathogens.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

IMPROVING ZIKA ONCOLYTIC THERAPY PRODUCTION: SUCCESSFUL PROOF-OF-CONCEPT TRANSFER TO ICELLIS NANO BIOREACTOR PLATFORM

Carla Longo 1, Thais Peron 1, Giovanna Olberg 1, Diego Modolo 1, Lívia Ramos 1, Fabiane Honório 1, Pedro Machado 1 and Letícia Parizotto 2.

1. Vyro Bio Inc., 2242 Av Prof. Lineu Prestes, Sao Paulo, 05508000, Brazil.
2. Cytiva, 20 Walkup Dr, Westborough, MA 01581, USA.

Background: In 2015, an epidemic of Zika virus affected the population of North, Central, and South America, leading to over 1.5 million infections and causing severe brain abnormalities in infants. The Zika virus targets the neural stem and progenitor cells, resulting in cell death and the development of tumors in the central nervous system (CNS), contributing to this tragic situation. However, researchers have harnessed the virus's brain-targeting ability to create an innovative therapy for treating CNS tumors. The Zika virus has been modified to be safe, selective, and effective as an oncolytic therapy by employing microRNA-sensitive technology. This Zika oncolytic therapy is in the initial stages of clinical studies. However, the production process using Vero cells in 2D flasks presents challenges in scalability and batch-to-batch consistency. To overcome these limitations and support the expansion of clinical studies, this work aimed to test the proof-of- concept (PoC) transfer of the production process to the iCELLis Nano Bioreactor.

Methods: The iCELLis bioreactor is an automated, single-use, fixed-bed bioreactor designed to provide a controlled environment for adherent cells. The scaling up process was successfully achieved by maintaining the same media volume per surface area ratio, seed density, and multiplicity of infection (MOI), while testing different cell culture media compositions and pH control strategies.

Results: As a result, the functional viral production significantly increased in the bioreactor, reaching up to 8 x 10^5 pfu/mL, compared to 1.90 ± 0.50 x103 pfu/mL in flasks.

Conclusions: Therefore, the iCELLis Nano bioreactor has been demonstrated to be successful in the PoC transfer from flasks, can support the expansion of clinical studies, and has the potential for further optimization and scale-up to the manufacturing scale.

BIOPROCESOS: UPSTREAM – DOWNSTREAM

MOUSE HYBRIDOMA CELL CONTINUOUS CULTURE DURING 100 DAYS IN THE LAMBDA-MINIFOR BIOREACTOR

Marcos González 1, Daily Hernández 2 , Airela Llamo 1 , Mayté Quintana 1, Maylín LaO 3 , Zoila Velázquez 3 , José García 1 , Rodolfo Valdés 1.

1. Department of Monoclonal Antibody Production. Center for Genetic Engineering and Biotechnology, Ave 31 be/158 and 190, Cubanacán, POBox 6162, Havana 10600, Cuba.
2. Direction of Agriculture Research. Center for Genetic Engineering and Biotechnology, Ave 31 be/158 and 190, Cubanacán, POBox 6162, Havana 10600, Cuba.
3. Direction of Quality Control. Center for Genetic Engineering and Biotechnology, Ave 31 be/158 and 190, Cubanacán, POBox 6162, Havana 10600, Cuba

Background: this report focused on demonstrating capacity of a mouse hybridoma cultivated during 100 days in protein free medium in the Lambda-MINIFOR bioreactor to produce CB.Hep-1 monoclonal antibody (mAb) without modifications in biochemistry properties and target antigen immunopurification capacity.

Results: results corroborated a CB.Hep-1 mAb stable production during 100 days [1.1 ± 0.2 x 106 cell/mL, SPR: 25.7± 9.8 µg/106 cell/day (cell specific production rate), 82.5 ± 6.7 % (viability), 122.7 ± 43.6 mg/L (mAb concentration), 125.7 ± 43.6 mg/harvest/day, 12.5 g of harvested mAb and 10.1 g of purified mAb] renovating up to 19 % of bioreactor vessel volume per day. The mAb purity was over 98 % and no aggregation and modification of specificity and affinity constant (CKTCTT, 0.9 ± 0.6 x 1010 M-1) were detected. Target antigen immunopurification efficiency was comparable to those measured when mAb was isolated from other biological sources (adsorption capacity 81.4 ± 21.8 µg/mg, adsorption efficiency 52.6 ± 14.1 %, elution capacity 60.0 ± 20.8 µg/mg, elution efficiency 83.2 ± 11.60 %, antigen purity 88.5 ± 1.0 %, released-coupled mAb ratio 0.03 ± 0.015 %) and leakage mAb-antigen ratio (0.1 - 4.8 ng mAb/µg Ag). Considering these bench top-scale evidences, CB.Hep-1 mouse hybridoma culture could be scale-up to a 600 L-bioreactor (continuous culture without cell retention) or to a 50 L-bioreactor (perfusion at 25 - 30 x 106 cell/mL) to produce approximately 1400 g of purified mAb per year.

Conclusions: CB.Hep-1 hybridoma produces mAb without modifications in biochemistry properties and target antigen immunopurification capacity in the Lambda-MINIFOR bioreactor during 100 days and this study confirms Lambda-MINIFOR bioreactor robustness to proof long-term cultivation of mammalian cells at bench-scale.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

IN VITRO ANALYSIS OF THE SAFETY PROFILE OF ADJUVANT U-OMP19 FORMULATION: CYTOTOXIC AND GENOTOXIC EVALUATION

Fátima Gasser 1, María Sol Renna 1, Belkis Marelli 1, Laura Bruno 2, Hugo Ortega 1, Juliana Cassataro 2, Karina Pasquevich 2, María Eugenia Baravalle 1.

1. Centro de Medicina Comparada, Instituto de Ciencias Veterinarias del Litoral (ICIVET Litoral UNL-CONICET), Universidad Nacional del Litoral (UNL), Esperanza, Santa Fe, Argentina.
2. Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín (UNSAM), San Martín, Buenos Aires, Argentina.

Background: U-Omp19, is a new protein adjuvant currently in preclinical development with several vaccine candidates, primarily intended for use in orally administered vaccine formulations. In this study, we investigated the effect of U-Omp19 adjuvant on in vitro cytotoxic and genotoxic activity in CHO-K1 cell line (Chinese hamster ovary cells).

Methods: The cytotoxic activity of U-Omp19 (500, 400, 300, 200, 150, 100, 50, 25, 10, 5 µg/ml) was evaluated by the MTT assay. Doxorubicin was used positive control. Cell cycle arrest and induction of cell death were also analyzed by using Propidium Iodide and FITC- Annexin V kit by flow cytometry. Doxorubicin and mitomycin C were used as positive controls. Intracellular production of ROS was evaluated using staining with dihydroethidium and analyzed by flow cytometry. Hydrogen peroxide and Doxorubicin were used as positive controls. The genotoxic activity of U-Omp19 was evaluated using micronucleus and comet assays. Bleomycin and hydrogen peroxide were used as positive controls.

Results: U-Omp19 did not inhibit the proliferation of the CHO-K1 cell at any of the concentrations evaluated, while a dose-dependent decreasing cell viability curve was obtained with Doxorubicin. U-Omp19 showed low levels of early apoptosis, late apoptosis and necrosis similar to the basal control ($p>0.05$), while Doxorubicin induced apoptosis and mainly necrosis in CHO-K1 cells. U-Omp19 does not produce ROS compared to the positive controls used ($p>0.05$). The percentage of cells in G0/G1, S and G2/M phase for U-Omp19 did not show a significant difference compared to the basal control ($p>0.05$), but did show a significant difference compared to the positive controls ($p<0.05$). Finally, the frequency of micronuclei in binucleated cells for U-Omp19 showed no significant difference compared to the basal control ($p>0.05$), but did show a significant difference compared to the positive control ($p<0.05$). The U-Omp19 adjuvant is a molecule that does not produce cytotoxic or genotoxic activity in the CHO-K1 cell line. This evidence suggests that this adjuvant has a promising safety profile, which positions it as a suitable candidate for integration into vaccine formulations.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

IN VITRO ASSESSMENT OF THE SAFETY PROFILE AND INHIBITORY ACTIVITY AGAINST *CANDIDA ALBICANS* INVASION IN VERO CELL LINE BY A NEW FORMULATION OF LIPOSOMAL AMPHOTERICIN B

Baravalle M.E. 1,2, Berengeno A 1, Gasser F. 1, Renna M.S. 1, Perelló A. 2, Nicastro A. 3, Ortega H.H.1.

1. Centro de Medicina Comparada, Instituto de Ciencias Veterinarias del Litoral (ICIVET Litoral UNL-CONICET), Universidad Nacional del Litoral (UNL), Esperanza, Santa Fe, Argentina.
2. Centro Universitario Gálvez, Universidad Nacional del Litoral (UNL), Gálvez, Santa Fe, Argentina.
3. Lipomize SRL, PTLC, Santa Fe, Argentina.

Background: Among available antifungals, Amphotericin B (AmB) stands out as the preferred treatment for systemic fungal infections. However, its use is often constrained by toxicity-related issues, notably nephrotoxicity. To circumvent these limitations, various lipid-based delivery systems have been developed. One such formulation, AmBisome®,utilizes liposomes to facilitate the administration of higher doses of the drug while significantly reducing side effects and toxicity. A new formulation similar in composition to Ambisome, Amb-Lip, was checked about its in vitro performance. The main difference between Ambisome and Amb-Lip is in the manufacturing
Methods: this last one is made using the nanoprecipitation concepts. The aim of this study was to assess the in vitro safety profile and activity of AmB-Lip, compared to AmBisome, in inhibiting the infection of the Vero cell line by the *Candida albicans* strain SC5314. The antifungal efficacy of formulations was evaluated using the broth microdilution method for minimum inhibitory concentrations (MIC) determination. The cytotoxic effect was examined in triplicate on Vero cell monolayers over a concentration range from 0.1 to 50 µg/mL by the MTT assay. Furthermore, Vero cell viability after exposure to C. albicans (1x106 UFC/mL) and simultaneous treatment with different concentrations of formulations (5, 1, and 0.25 µg/mL) was assessed using the MTT assay. Co-cultured cells were incubated for 48 hours, washed twice with PBS to remove *C. albicans*, and then subjected to the MTT assay.
Results: Both AmB-Lip and AmBisome demonstrated in vitro effectiveness against *C. albicans*, with MIC of 0.125 µg/mL and 0.25 µg/mL at 24 and 48 hours, respectively. The Vero cell viabilities when exposed to several concentrations of AmB-Lip and AmBisome were greater than 70% for both compounds, confirming lower-toxic properties in accordance with international references guidelines. In addition to adhesion, the invasion of C. albicans plays a crucial role in the pathophysiology of candidiasis. Our preliminary results indicate that as the concentration of AmB-Lip and Ambisome decreases, Vero cell viability decreases at the same concentration of *C. albicans* ($p<0.05$). Furthermore, greater cell viability was observed in Vero cells for AmB-Lip compared to AmBisome at the concentration of 0.25 µg/mL of both antifungals ($p<0.05$).
Conclusions: These findings collectively highlight the ability of AmB-Lip to inhibit *C. albicans* invasion in these cells at non-toxic concentrations on Vero cells. Therefore, we propose that this novel formulation represents a significant advancement in addressing the growing demand for an effective and less toxic alternative to AmB-based formulations.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

STRUCTURAL AND STABILITY STUDY OF IFNB-1A VARIANTS WITH ENHANCED THERAPEUTIC PROPERTIES AND REDUCED IMMUNOGENICITY

Julieta Pioli 1,2, Maia Lisa 1,2, Sergio Garay 2, Eduardo Mufarrege 1.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Pcia. Santa Fe S3000ZAA, Argentina.
2. UNL, FBCB, Departamento de Física, Área de Modelado Molecular, Santa Fe, Pcia. Santa Fe S3000ZAA.

Background: Interferon beta-1a (IFNβ-1a) is a biological drug widely used for treating Multiple Sclerosis and viral infections. Despite its therapeutic benefits, its application is often limited due to immunogenicity risks. To address this, we developed two de-immunized IFNβ-1a variants, designated as IFNβ-1a-VAR1 and IFNβ-1a-VAR2. VAR1's exhibits increased biological activity and thermal stability. This study aims to structurally characterize these variants and understand the structural differences, ensuring they retain the properties of the native protein while offering enhanced therapeutic benefits.

Methods: IFNβ-1a variants were produced in CHO cells, followed by purification through two successive chromatographic steps: Blue Sepharose and C4-HPLC. We also conducted Molecular Dynamics (MD) simulations over 3 microseconds for IFNβ-1a-WT (the native molecule), IFNβ-1a-VAR1, and IFNβ-1a- VAR2 in the presence of mannitol, the primary excipient used in the drug's formulation. Root-Mean- Square Deviation (RMSD), radius of gyration, and secondary structure analysis were used to determine equilibrium time. Additionally, we performed in vitro antiviral activity assays using WISH cells to assess protein thermal stability.

Results: Mannitol did not exert any change in Root Mean Square Fluctuation (RMSF). However, MD simulations indicated that mannitol is more excluded from the surface of the de-immunized variants than the native IFNβ-1a. Mannitol typically acts as a stabilizer by excluding itself from the protein surface, stabilizing the protein structure by altering the hydration shell and reducing water's ability to compete for intramolecular hydrogen bonds. This suggests that IFNβ-1a variants could have increased their stabilities due to this phenomenon. In addition, in vitro assays revealed that IFNβ-1a-VAR1 displayed residual activity even after exposure to 95°C, whereas the original protein exhibited no antiviral function after exposure to 85°C.

Conclusions: The structural characterization and stability assessment of IFNβ-1a-VAR1 and IFNβ-1a-VAR2 demonstrate their potential as enhanced therapeutic options with reduced immunogenicity. Further research will be needed to fully elucidate the molecular mechanisms behind the improved thermal stability and antiviral activity of IFNβ-1a-VAR1.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

DEVELOPMENT OF A TECHNOLOGICAL PLATFORM TO ASSESS THE POTENTIAL IMMUNOGENICITY OF BIOTHERAPEUTIC CONTAMINANTS

Florencia Rivarosa 1, Lucia C. Peña 1, Marina Etcheverrigaray 1, Eduardo F. Mufarrege 1.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Santa Fe S3000ZAA, Argentina.

Background: Over 90% of biotherapeutics under development are unsuccessful in clinical trials I and II because they do not fulfill safety and efficacy standards. Thus, one of the main concerns for biologic manufacturers is the occurrence of unwanted immune responses in patients. Despite efforts in the downstream processing of biotherapeutics, the presence of impurities from the process can compromise therapy performance and safety. After microbial contaminants, host cell proteins (HCPs) are the second source of contamination among innate immune response-modulating impurities (IIRMIs). These IIRMIs are therefore regarded as crucial quality attributes. In the development of biotherapeutics, monitoring and
characterizing HCPs is a challenge because of the heterogeneity of the proteins, the difficulties of profiling, and host cell proteomes that range from thousands to hundreds of thousands of proteins. The use of in silico tools is suggested for evaluating the immunogenicity of HCPs; however, these are restricted to T-cell epitope prediction. Currently, there are no strategies to predict the capacity of HCPs to modulate the innate immune system.
Methods: Our work focuses on the development of a platform that allows the identification and characterization of the immunogenic profile of HCPs accompanying biotherapeutics. For this, we propose the use of mass spectrometry technology for the identification of HCPs, followed by their recombinant expression in Escherichia coli, and purification through affinity chromatography. For immunogenicity characterization, we have employed in vitro and ex vivo assays.
Results: As a case study, we identified nine HCPs in five batches of a biotherapeutic produced in *E. coli* used to treat multiple sclerosis. The total number of HCPs found per batch (between four and five) is consistent with what has been published in the literature when analyzing high-purity biotherapeutic products. Three ubiquitous proteins were found in all batches, and others were present only in some specific samples. To date, we have produced four of the identified HCPs with high purity degree. To evaluate the potential of the HCPs to activate innate immune cells, the detection of pro-inflammatory genes by RT-qPCR in the human cell line THP1 was optimized. Furthermore, the capacity to activate murine macrophages was evaluated using the RAW-Blue reporter cell line.
Conclusions: Developing a platform to identify and characterize the immunogenic profile of HCPs in biotherapeutics is crucial for improving therapy safety and efficacy. Utilizing an experimental platform that combines mass spectrometry, recombinant protein expression, purification, and immunogenicity assays provides a comprehensive approach to assessing HCP contamination.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

COMPARISON OF MAMMALIAN EXPRESSION PLATFORMS FOR THE PRODUCTION OF HUMAN SERUM ALBUMIN-FUSION PROTEINS

Lucía Peña 1, Florencia Rivarosa 1, Marina Etcheverrigaray 1, Eduardo Mufarrege 1.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Provincia. Santa Fe S3000ZAA, Argentina.

Background: Human serum albumin (HSA) is the most abundant protein in the blood, recognized for its ability to transport various endogenous and exogenous molecules. Its stability, prolonged half-life, and capacity to evade renal filtration make it an ideal candidate for fusion engineering with therapeutic peptides and drugs. Previous investigations have shown that the fusion of proteins to HSA is a promising strategy to enhance their therapeutic potential by improving the pharmacokinetic profiles, stability, and efficacy. In fact, several HSA-fused proteins have already been approved for clinical use by the FDA. These DNA constructions have been successfully generated in bacterial, yeast, and mammalian cells. Each platform offers unique advantages; nevertheless, mammalian cell-based strategies are preferred for their ability to perform post-translational modifications, which are critical for protein function. Among the mammalian cell lines, Chinese Hamster Ovary (CHO) and Human Embryonic Kidney (HEK) cells have been utilized to produce HSA fusion proteins. However, there are no studies comparing the performance of these two platforms for HSA-peptide/protein fusion production. The aim of this work was to compare the expression of HSA-peptide fusions in CHO-K1 and HEK293 cells to identify the more suitable platform.

Methods: To achieve this goal, we generated two DNA constructs: one with a peptide fused to HSA and another with HSA alone as a control. Stable protein-expressing cell lines were generated for both DNA constructs in CHO and HEK cells by lentiviral transduction. To produce the proteins, the recombinant cell lines were first adapted to growth in suspension (serum-free cell culture). Afterward, HSA-peptide fusions were purified using immobilized metal affinity chromatography (IMAC).

Results: We found that the protein purity level was significantly dependent on the cell line used for protein production. For instance, HSA-peptide fusions produced in CHO cells exhibited a number of impurities that persisted through multiple attempts to optimize the purification process. In contrast, protein expression in HEK cells followed by IMAC purification led to HSA-peptide fusions with high purity levels. These findings indicate that the host cell proteins (HCPs) derived from CHO cells exhibit a higher affinity for the pseudo-affinity matrix, or they interact with the HSA accompanying it in the purified product.

Conclusions: This work highlights the significance of selecting the appropriate expression system for HSA-peptide/protein fusions at the early stages of therapeutic product development.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

INNOVATIVE ANALYTICAL SOLUTIONS AND QUALITY BY DESIGN APPROACHES IN PREFORMULATION STUDIES OF A RECOMBINANT PROTEIN AS A BIOTHERAPEUTIC CANDIDATE

María Celeste Rodríguez 1, María De Los Milagros Bürgi 1, Ricardo Kratje 1, Marcos Oggero Eberhardt1.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral (CBL), Santa Fe, Pcia. Santa Fe, S3000ZAA, Argentina.

Background: The preformulation and formulation stages of biotherapeutic proteins are complex and time-intensive due to their intricate nature. Critical material attributes (CMAs) such as pH, buffering systems, and the selection of compatible excipients under stress conditions are commonly studied. High-throughput (HT) technologies and Quality by Design (QbD) approaches contribute significantly to the rational design of formulations, providing a clear path for drug product development. The aim of this work was to rapidly and effectively identify CMAs to ensure the quality of a recombinant molecule for the treatment of neurological disorders, combining HTP strategies such as differential scanning fluorimetry (DSF) and QbD approaches.
Methods: For proof of concept, two variants of the biotherapeutic candidate from alternative purification processes (n=6) were analysed using DSF. Among them, a promising variant was selected, and the impact of CMAs was studied under stress conditions like thermal stress. Additionally, fluorescence excitation-emission matrix (EEM) coupled with chemometric modelling (MCR-ALS) was used to monitor pH-induced changes in the protein's tertiary structure. Finally, a multivariate formulation procedure through design of experiment (DoE) was implemented to evaluate potential excipients and their interaction effects on protein stability. Independent assays (at least n=2) were performed to ensure the reproducibility and consistency of all the results.
Results: At least 95%-pure variants from the alternative purification procedures were used for assessing. Tm values ranging from 48 to 55 °C were determined for the six batches evaluated by DSF analysis. Statistical significance between samples (p-value: 0.0092) was observed through a non-parametric test. The promising variant was then assayed for conformational stability under different pH conditions (3.0 9.0), performing DSF studies. At acidic pHs (3.0-4.0), melting curves indicated protein unfolding, with the highest Tm at pH 6.0 (47.5 ± 0.7 °C). This result was confirmed using EEM coupled with MCR-ALS modelling, providing spectral and abundance distribution profiles for monitoring pH-induced changes. Different excipients, either alone or in combination with buffer agents, were evaluated for their ability to stabilize the protein through DSF assays. The presence of NaCl and Pluronic® F-68 increased the Tm value, indicating higher thermal stability. A screening fractional factorial DoE was performed in accelerated stability studies to determine the effects of different excipients, identifying combinations that improve the molecule's stability.
Conclusions: This comprehensive and systematic approach represents a practical procedure that helps elucidating CMAs, and facilitates the development of the final dosage form of the biotherapeutic, for a better performance of its stability, efficacy, and safety.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

AN ALL-IN-ONE BIOTHERAPEUTIC CANDIDATE FOR THE TREATMENT OF NEOVASCULAR RETINOPATHIES: NEUROPROTECTION, NEUROPLASTICITY AND ANTIANGIOGENIC EFFECTS

Milagros Bürgi 1,2,Victoria Vaglienti 3,Cecilia Sanchez 3, Matías Depetris 2,4 Marcos Oggero 1,2.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Pcia. de Santa Fe, Argentina.
2. BioSynaptica SA, Santa Fe, Pcia. de Santa Fe, Argentina.
3. UNC, CIBICI-CONICET, FCQ, Departamento de Bioquímica Clínica, Córdoba, Pcia de Córdoba, Argentina .
4. UNL, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Pcia. de Santa Fe, Argentina.

Background: Background: Neovascular retinopathies (NR) are leading causes of blindness, with diabetic retinopathy (DR), retinopathy of prematurity (ROP), and age-related macular degeneration (AMD) being the most common forms. Hypoxic or ischemic events trigger these pathologies, causing an imbalance between pro- and anti-angiogenic molecules. This imbalance leads to inflammation, apoptosis, oxidative stress, and gliosis in the retina. Current treatments primarily involve anti- VEGF antibodies, which inhibit neovascularization but do not address other pathological processes of NR. Human erythropoietin (hEPO) has shown positive effects on these processes; however, its erythropoietic activity (EA) increases thrombosis risk. This study aimed to develop an innovative hEPO-derived biotherapeutic that lacks EA while retaining its neuroplastic and cytoprotective functions.

Methods: A glycoengineered hEPO variant, named BSY02, was developed. BSY02 was produced in CHO.K1 cells, immunoaffinity purified, and extensively characterized. The protein's structure and stability were assessed using circular dichroism and thermal shift analysis. EA was evaluated by measuring} hematocrit in normocytemic BALB/c mice. The BSY02 antiapoptotic and neuroplastic activities were studied in primary hippocampal neuron cultures. Also, the neuroprotective regulation was studied in vitro in Müller cells (retinal macroglial cells). BSY02's efficacy was further studied in the oxygen-induced retinopathy (OIR) mouse model, a widely validated model of ROP, analyzing its effects on vasculature, glial, and neuronal activity.

Results: BSY02 exhibited a properly folded structure and blocked EA while preserving its neuroprotective activity (NA). In vitro, BSY02 reduced staurosporine-induced apoptosis in hippocampal neurons, promoted neurite outgrowth, increased filopodia density, and enhanced synapse formation ($p<0.001$). BSY02 also demonstrated neuroprotective and neuroplastic activity, increasing pro-BDNF (brain-derived neurotrophic factor) protein expression ($p<0.05$) in Muller glial cells. In the ROP model, BSY02 protected retinal neurons by increasing pro-BDNF production and completely reducing apoptosis ($p<0.05$). It showed a trend to reduce gliosis. Additionally, a significant 1.6-fold and 5-fold decrease in vase-obliteration and neovascularization was respectively observed.

Conclusions: Despite additional studies are needed, BSY02 emerges as a promising biotherapeutic candidate for treating NR. It demonstrates highly desirable effects on both the neuronal and vascular components of retinopathy damage, distinguishing it from those treatments only existing for the vascular contributor of the disease.

ANTIVIRAL ACTION OF AQUEOUS EXTRACTS OF PROPOLIS FROM *SCAPTOTRIGONA AFF. POSTICA* (HYMENOPTERA; APIDAE) AGAINST ZICA, CHIKUNGUNYA, AND MAYARO VIRUS

Mendonça R.Z.1, Nascimento R.M.1, Fernandes A.C.O. 1, Silva Junior P.I.2.

1. Laboratory of Parasitology, Butantan Institute.
2. Laboratory for Applied Toxinology (LETA), Center of Toxins, Immune-response and cell signaling (CeTICS/CEPID), Butantan Institute,

Background: The limited availability of antivirals for new highly pathogenic strains of virus has become a serious public health. Among the multiple sources for news antivirals, insect exudates or their products has become an increasingly frequent option. Insects emerged 350 million years ago and have showed a high adaptability and resistance to the most varied biomes. Their survival for so long, in such different environments, is an indication that they have a very efficient protection against environmental infections. Since the ancient civilizations, the products obtained from the bee have been of great pharmacological importance. Investigations of biological activity of propolis have been carried out, mainly in the species *Apis mellifera*. However, for the Meliponini species, known as stingless bees, there are few studies, either on their chemical composition or on their biological activities. The importance of studying these bees is because they come from regions with native forests, and therefore with many species of plants not yet studied, in addition to which they are regions still free of pesticides, which guarantees a greater fidelity of the obtained data. Previous studies by our group with propolis demonstrated an intense antiviral activity against Herpes, influenza, and rubella viruses. In this work, we use aqueous extracts, which can be extracte substances different from those obtained in alcoholic extracts. Objective. This study aimed to isolate and characterize compounds with antiviral effects from aqueous propolis extracts from *Scaptotrigona aff. postica*, against arbovirus.

Methods: The evaluation of the antiviral activity of the crude and purified material was performed by reducing infectious foci in VERO cell cultures. The chemical characterization of the compounds present in the extracts was performed by high-pressure liquid chromatography. Results. The results obtained with crude propolis, indicate a high reduction of zica virus (64x) and mayaro (128x). The reduction of chikungunya virus was of 256 fold. Through the purification of propolis by HPLC and mass spectrometry, it was possible to identify and isolate a peak with antiviral activity. This purified fraction showed activity against all viruses tested. When purified fraction was used, the reduction observed was of 16 fold for zicavirus, 32 fold for mayaro virus and 512 fold for chikungunya virus. Likewise, it was observed that the antiviral response was concentration dependent, being more intense when propolis was added 2 hours after the viral infection. Now we are carrying out the chemical characterization of the purified compounds that showed antiviral action.

Keywords: *Scaptotrigona aff. postica*, propolis, antiviral activity, zica virus, chikungunya, mayaro virus.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

HEK293: CELL PLATFORM FOR UNVEILING NEUROPROTECTION OF NOVELGLYCOENGINEERED ERYTHROPOIETIN VARIANTS AND THERAPEUTIC APPLICATIONS

Delfina Gagliardi 1,2, Marcos Oggero-Eberhardt 1,2, María de los Milagros Bürgia 2.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral (CBL), Santa Fe, Argentina.
2. BioSynaptica SA. Santa Fe, Argentina.

Background: hEPO, initially recognized as a hematopoietic growth factor, exhibits direct effects on the nervous system, positioning it as a promising drug for neurodegenerative diseases. While contributing to neuroprotection, it also fosters neuroplasticity. To retain its neurobiological action, three hyperglycosylated hEPO muteins were obtained by glycoengineering and produced using CHO.K1 cells. In this study, a straightforward HEK293 cell line-based model was evaluated for investigating cytoprotective effects of hEPO muteins, with the aim of stablishing a platform for biological quality control of potential biotherapeutics.

Methods: HEK293 cells underwent treatments with hEPO or its hEPO-derived variants, followed by apoptosis induction with glucose (125 mM) or staurosporine (0.125 µM). Cell viability was assessed through the MTS/PMS cell proliferation assay. Additionally, the mRNA levels of BAX and BCL-2 (pro and anti-apoptotic genes) were quantified using Real-Time PCR.

Results: The optimal apoptotic agent concentration was initially defined as the concentration which promotes approximately 30% of apoptosis. Subsequently, several hEPO and variants concentrations were assessed to identify the maximum protective impact. Observations revealed heightened cell viability in hEPO and hEPO variants-treated cells after apoptotic agent exposure compared to control. RNA isolation and cDNA synthesis unveiled elevated BAX levels in untreated versus hEPO or hEPO variants-pretreated cells, contrasting with BCL-2 expression.

Conclusion: This study successfully highlights the use of the HEK293 cell line as an outstanding platform for investigating neuroprotective effects, opening an innovative pathway for the screening or quality control of drugs like human erythropoietin (hEPO) or its derivatives, which are targeted at neurodegenerative disorders.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

CHO.K1 AND HEK-293 CELL PLATFORMS: TOWARDS THE PRODUCTION OF ERYTHROPOIETIN ANALOGS AS NEUROTHERAPEUTIC CANDIDATES

Francisco Iturraspe 1, María de los Milagros Bürgi 1,2, Ricado Kratje 1,2, Camila Scorticati 3,4, Marcos Oggero 1,2.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Pcia. Santa Fe S3000ZAA, Argentina.
2. BioSynaptica SA, Santa Fe, Pcia. de Santa Fe, Argentina.
3. Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín (UNSAM) – CONICET, San Martín, Buenos Aires, Argentina.
4. Escuela de Bio y Nanotecnologías (EByN), Universidad Nacional de San Martín. San Martín, Buenos Aires, Argentina.

Background: Human erythropoietin (hEPO), a biotherapeutic used for treating anemia, has been proposed as a neurotherapeutic considering its neuroprotective and neuroplastic properties. Aiming to block the erythropoietic activity (EA) but preserving its neurobiological action (NA), two hyperglycosylated hEPO muteins were designed through glycoengineering and produced using CHO.K1 cells. To obtain hEPO variants with properties that resemble the naturally brain-derived EPO, HEK-293 cells were chosen as host cells due to their ability to produce simpler glycosidic structures, lacking residues considered immunogenic. Aiming to compare CHO vs. HEK-hEPO derived muteins in terms of their biological performance, an extensively characterization was carried out.

Methods: EPO derivatives were produced in both cell lines, immunoaffinity purified and characterized using SDS-PAGE, isoelectric focusing, thermal shift, circular dichroism, partial deglycosylation and glycan analysis. Pharmacokinetic (PK) parameters were studied in Wistar rats and NA was evaluated in vitro by assessing neuritogenesis and filopodia formation and in vivo by studying the dendritic tree of hippocampal pyramidal neurons.

Results: N-deglycosylation procedure demonstrated a maximum expected occupancy degree of four N-glycosylation sites of variants produced in both cell lines. HEK-293 variants showed lower molecular mass and lesser acidic isoforms than those produced by CHO.K1 cells. These differences are probably due to less complex glycan structures with lower antennarity and a lesser sialic acid content. Also, the increase in glycosidic content of hEPO muteins or the amino acid mutations practically did not affect the temperature of thermal denaturation compared to hEPO, confirming that tertiary structure was preserved after glycoengineering. Disparities in the glycosylation profiles resulted in significant differences in the PK parameters. Thus, the plasmatic clearance and half-life of CHO-derived muteins were 14-fold lower and 2-fold higher, respectively than the corresponding HEK-293-derived molecules. In terms of biological activity, all variants preserved in vitro neuritogenesis and filopodia formation, similar to hEPO. Remarkably, the in vivo NA was significantly improved regards to hEPO using both muteins produced in both cell lines. As a result, 42%-higher dendrite length and a 41%-higher number of intersections per neuron were observed.

Conclusion: Despite the fact that variants produced in HEK-293 cells displayed less favorable PK parameters than their CHO.K1 counterparts, they enhanced in vivo neuroplasticity as regards to EPO with no statistical differences between muteins. The contribution of the PK and receptor interaction to the in vivo biological activity should be compared in the future in order to propose them as candidates for treatment of neurological disorders.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

THERMO STABILITY ANALYSIS OF MONOCLONAL ANTIBODIES

Juan Paulo Pinto 1,2, Nicolás Fehring 1, Karen Toledo-Stuardo 1, FabiolaGonzález-Herrera 1, Mauricio González-Olivares 1, María José Garrido 1, Yuneisy Guerra 1, Marcela Hermoso 3, Claudia Altamirano 4, María Carmen Molina 1.

1. Programa de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile.
2. Facultad de Ciencias Naturales, Matemática y del Medio Ambiente, Escuela de Industria Alimentaria y Biotecnología, Universidad Tecnológica Metropolitana.
3. Laboratorio de Inmunidad Innata, Programa disciplinario de Inmunología, ICBM Facultad de Medicina, Universidad de Chile.
4. Facultad de Ingeniería, Escuela De Ingeniería Bioquímica, Pontificio Universidad Católica de Valparaíso.

Background: Recombinant proteins are artificially synthesized by cells that do not naturally produce them. Their stability is a crucial parameter for their functionality and can be affected by various factors, including temperature, pH, and ionic strength. These factors can influence protein aggregation, which can be deleterious for their biological activity.

Methods: The objective of this study was to evaluate different buffer formulations maintaining the stability of an IgG1 isotype monoclonal antibody at various temperatures. Methodologically, we propose a list of buffers based on the available information of commercial IgG1 antibodies, which describe their buffers, stabilizers, and pH used to maintain their stability during the storage. Based on this information, we tested four different buffers (Sodium Phosphate, Sodium Citrate, Sodium Acetate and L-Histidine) with four distinct pH conditions. The melting temperatures in the different buffers were measured using thermofluor assays, in a RT-PCR equipment, exposing the samples to an increase in temperature from 25 °C to 95°C, with an increment of 0.3 °C per step, measuring the fluorescence of the SYPRO Orange dye to determine the level of denaturation. Subsequently, we tested different stabilizers prepared in the selected buffer at the optimal pH. The samples were stored at -20 °C, 4 °C and 37 °C with a concentration of 2 mg/mL of stabilizer for periods of one week, one month and three months, and parallel freeze-thaw cycle studies were performed. To evaluate the efficiency of the excipients and see the level of aggregation, the samples were analyzed by SEC-FPLC and SDS-PAGE.

Results: Thermofluor assays demonstrated that the recombinant protein exhibited a higher melting temperature in the sodium phosphate buffer within the pH range of 6.0 to 6.3, with an approximate value of 66 °C.

Conclusions: In conclusion, the results show the importance of selecting the buffer, stabilizers and pH for the storage of an antibody, thus better ensuring its stability for future assays.

Acknowledgments: Proyecto ANILLO ACT210068, FONDECYT N° 1221031, FONDEF ID23I10018.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

AN INNOVATIVE BIOASSAY APPROACH FOR ASSESSING BIOLOGICS IMMUNOGENICITY IN EARLY DEVELOPMENT STAGES

María Jesús Leopold 1, Agustina Gugliotta 1, Marina Etcheverrigaray 1, Eduardo Mufarrege 1.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral Santa Fe, Pcia. Santa Fe S3000ZAA, Argentina

Background: Immune tolerance typically dictates that therapeutic proteins identical to autologous proteins should not provoke immune responses. However, some therapeutic proteins, even when the protein sequence is similar to the endogenous one, can be immunogenic in patients. Factors influencing protein immunogenicity include administration route, formulation, dosage, treatment duration, and protein characteristics such as aggregates and glycosylation. Understanding these factors, especially for highly immunogenic proteins such as recombinant human interferon-alpha (rhIFN-α), is crucial for developing safe and effective biotherapeutics. Regarding this, developing ex vivo protocols to assess protein immunogenicity using peripheral blood mononuclear cells, represents significant progress in ensuring product safety during pre-clinical stages.
Methods: IFN variants were obtained from IFN-producing CHO-K1 cell supernatants and purified using immunoaffinity chromatography. For ex vivo assays, blood samples were collected from 12 healthy donors and peripheral blood mononuclear cells (PBMC) were isolated using a Ficoll-Hypaque density gradient. HLA-DR1 allotypes were determined using Luminex technology. Monocytes were isolated through plastic adherence, differentiated into immature dendritic cells (iDCs), and then incubated with each IFN variant. After maturation with recombinant TNF-α, antigen-pulsed DCs were incubated with autologous T-cells. After 48-72 hours, the supernatants were collected and assayed for IFN-gamma and IL-4 production using sandwich ELISA. A stimulation index (SI) criterion was defined as the ratio of cytokine concentrations from protein-challenged PBMCs and unchallenged PMBCs (culture media). A response was considered positive when SI was equal to or greater than 2.
Results: T-cell proliferation assays showed that all tested proteins exclusively induced IFN-gamma production in a protein and donor-dependent manner. In particular, HLA-DRB1*08, HLA- DRB1*09, HLA-DRB1*13, and HLA-DRB1*16 alleles were directly involved in IFN- derived peptide presentation. Additionally, a comparative analysis revealed that 3NM47/95 was the variant that exhibited higher immunogenicity with 42% of positive responses. In contrast, IFN-3NM47-Nter was less immunogenic than the other IFN variants (25% of responders). Furthermore, a similar proportion of responders (33%) was observed for IFN-2NM47/95, IFN-3NM47, and IFN-WT. It is important to highlight that IFN-3NM47-Nter is the most glycosylated IFN version. Therefore, these results suggest that higher glycan contents played a role in antigen recognition, processing, and presentation.
Conclusion: Based on these results, IFN-3NM47-Nter emerges as a promising candidate for antiviral therapy. Additionally, the strong correlation between the findings presented here and those previously reported from clinical studies emphasizes the usefulness of this experimental platform as a screening tool to predict the potential susceptibility of patients to develop undesired immune responses to biologics.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

ANALYSIS OF MACROPHAGE-MEDIATED PHAGOCYTOSIS OF TUMOR CELLS FOR FUNCTIONAL EVALUATION OF A NOVEL FULLY HUMAN ANTI-MICA ANTIBODY

Fabiola González-Herrera 1, Javiera Landaeta 1, Lucas Gutiérrez-Moscoso 1, Camila Jiménez 1, Karen Toledo-Stuardo 1, Mauricio González-Olivares 1,Yuneisy Guerra 1, María José Garrido 1, María Carmen Molina 1.

1. Laboratorio de Anticuerpos Recombinantes e Inmunoterapia, Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.

Background: Cancer continues to represent a significant global health burden. Thus, biotechnological innovations in cellular therapies offer novel avenues for treatment. In the field of immuno-oncology, the potential of leveraging the innate immune system's capacity for targeted action against tumor cells is particularly promising. Macrophages are essential players in immune defense, capable of executing foreign cell clearance via phagocytosis. The protein MICA, which is overexpressed in a wide range of tumor types, including gastric cancer, represents a promising target for therapeutic intervention. Our biotechnological approach involves the use of a fully human IgG1 anti-MICA antibody, which was developed through rational design, to harness macrophages for targeted tumor cell elimination via antibody-dependent cellular phagocytosis (ADCP).
Methods: In this study, we engineered U937 monocytic cells to stably express EGFP by lentiviral transduction. Differentiation of the cell line into macrophages was achieved through the addition of phorbol 12-myristate 13-acetate (PMA). Gastric cell lines MKN-45, GES1, and AGS, which are known to express MICA, were labeled with a fluorescent probe and subsequently incubated with our anti-MICA antibody, PBS (vehicle), or rituximab (isotype control) for one hour at 37°C. These cancer cells were then co-incubated with macrophages at a 2:1 ratio for another hour. The phagocytic efficacy of macrophages was quantitatively assessed using flow cytometry to measure double-positive cells, indicative of successful macrophage ingestion of tumor cells, and confocal microscopy for cellular interaction visualization.
Results: Our results distinctly show an enhancement in macrophage-mediated phagocytosis of gastric cancer cells with the anti-MICA antibody compared to vehicle and isotype controls. These findings validate the biotechnological potential of the anti-MICA antibody as a powerful antitumor biopharmaceutical. Furthermore, this study exemplifies the critical role of cell culture techniques in evaluating and enhancing the functional capabilities of biopharmaceuticals, potentially extending this strategy to a broader range of therapeutic applications in oncology and other diseases. This research not only paves the way for innovative cancer treatment strategies but also illustrates the integral role of biotechnological advancements in the development and characterization of next-generation biotherapeutics.
Acknowledgments: Proyecto FONDECYT N°3240175, Proyecto ANILLO ACT210068, Proyecto FONDECYT N° 1221031.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

A PARTIALLY-FOLDED HYPERGLYCOSYLATED ERYTHROPOIETIN WITH NO ERYTHROPOIETIC ACTIVITY WHICH PROTECTS NEURONS FROM DAMAGE AND PROMOTES NEUROPLASTICITY

Milagros Bürgi 1,2, Camila Scorticati 3,4, Victoria Vaglienti 5, Cecilia Sanchez 5, Matías Depetris 2,6, Ricardo Kratje 1,2, Marcos Oggero 1,2.

1. UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Pcia. de Santa Fe, Argentina.
2. BioSynaptica SA, Santa Fe, Pcia. de Santa Fe, Argentina.
3. Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín (UNSAM) – CONICET, San Martín, Buenos Aires, Argentina.
4. Escuela de Bio y Nanotecnologías (EByN), Universidad Nacional de San Martín. San Martín, Buenos Aires, Argentina.
5. UNC, CIBICI-CONICET, FCQ, Departamento de bioquímica Clínica, Córdoba, Pcia de Córdoba, Argentina.
6. UNL, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Pcia. de Santa Fe, Argentina.

Background: Neurodegenerative diseases (ND) are characterized by their chronicity and progressive evolution impacting significantly in the quality of life of patients. These diseases compromise the viability and functionality of neurons. Despite their increasing incidence, effective treatments are often lacking or insufficient. Erythropoietin (EPO) is a promising candidate due to its neurobiological actions (NA); however, its erythropoietic activity (EA) causes undesirable effects. This study aimed to describe an innovative EPO-derived neurotherapeutic candidate. It was obtained through glycoengenieering hEPO by hyperglycosylation to block its EA while preserving its NA.

Methods: A glycoengineered EPO variant, named BSY12, was developed. BSY12 was produced in CHO.K1 cells, immunoaffinity purified, and extensively characterized. The protein's structure and stability were assessed using circular dichroism and thermal shift analysis. EA was evaluated by measuring hematocrit in normocytemic BALB/c mice. The BSY12 antiapoptotic and neuroplastic activities were studied in primary hippocampal neuron cultures. Additionally, Sholl analysis was performed on CA1 pyramidal neurons from BSY12-treated CF1 mice. The ability to cross the blood-brain barrier (BBB) was investigated in CF1, C57Bl/6N, and SOD1G93A mice. In vivo neurobiological performance was evaluated using the Complex Running Wheels test in healthy C57Bl/6N mice and in an amyotrophic lateral sclerosis model with SOD1G93A mice. Finally, its neuroprotective and antigliotic effects were studied in Müller cells (retinal ganglion cells).

Results: BSY12 exhibited a partially-folded structure in comparison with hEPO; nevertheless, the EA was blocked while its NA was preserved. BSY12 reduced staurosporine-induced apoptosis in vitro in hippocampal neurons and promoted neurite outgrowth, the augment of filopodia density, and synapse formation ($p<0.001$). In vivo, BSY12 increased total dendrite extension and dendritic branch intersections ($p<0.05$). BSY12 improved motor-cognitive performance in healthy animals ($p<0.0001$) and motor abilities in SOD1G93A mice until advanced pathology stages ($p<0.1$). BSY12 also demonstrated neuroprotective/neuroplastic and antigliotic activities in Müller cells, increasing pro-BDNF (Brain-derived neurotrophic factor) protein expression and reducing GFAP (Glial fibrillary acidic protein) levels, respectively ($p<0.05$).

Conclusions: Despite the conformational changes of BSY12, it represents a promising molecule to continue studying how these modifications allow conserving or inclusively improving its performance as a biotherapeutic for treating various neurodegenerative diseases, including degenerative retinopathies.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

RATIONAL DESIGN AND EXPERIMENTAL VALIDATION OF ENHANCED AFFINITY ANTIBODIES TARGETING MICA AND SST2

Douglas J. Matthies 1,2, Mauricio González-Olivares 1, María José Garrido 1, Fabiola González-Herrera 1, Nicolás Fehring 1, Karen Toledo-Stuardo 1, María Carmen Molina 1, Gerald Zapata-Torres 2.

1. Centro de InmunoBiotecnología (Centro IBT), Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile
2. Centro de Modelamiento Molecular, Biofísica y Bioinformática (CM2B2), Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile

Background: Affinity maturation represents a crucial stage in the development of antibodies, with the objective of enhancing their binding affinity to their targets. While traditional methods of affinity maturation are effective, they are often limited by time-consuming and resource-intensive experimental processes. To address these limitations, in silico affinity maturation methods have emerged as a means of accelerating and enhancing the antibody development process.
In this study we employed an affinity maturation process utilizing both molecular modeling techniques and subsequent experimental validation with the specific goal of enhancing the binding affinity far a fully human anti-MICA antibody and a fully human anti-sST2 antibody to their target epitope, MICA and sST2, respectively.
Methods: Computational methods were employed to model and optimize the structure of both antibodies and their interaction with their antigen. The framework and CDR regions were modeled using RosettaAntibody. The structure of the immune complexes was refined by global and local docking using ClusPro2.0 and SnugDock, respectively. Furthermore, molecular dynamics simulations were conducted to evaluate the Gbinding using MMGBSA. Far the purpose of experimental production, both antibodies and their mutants were expressed in ExpiCHO cells. Finally, the binding affinity of the native and mutant antibodies was assessed by ELISA assays.
Results: Molecular dynamics simulations identified 30 favorable sites within the complementarity-determining regions of the anti-MICA antibody, and 10 favorable sites within the CDRs of the anti-sST2 antibody, which were deemed to be suitable far potential mutations. The afarementioned sites were subjected to exhaustive mutation by 10 amino acids. Subsequently, the resulting Fv mutants, comprising 300 mutants far the anti-MICA antibody and 100 mutants far the anti-sST2 antibody, underwent further evaluation through free energy binding calculations. Six Fv mutants of each antibody that demonstrated enhanced in silico binding affinities were expressed as full antibodies in CHO-S cells in arder to validate their experimental binding affinity. The Elisa assays demonstrated that the mutant 129D of the anti-MICA antibody and mutants ESOH, S57F and V33A of the anti-sST2 antibody showed an enhanced affinity compared to their wild-type amino acid sequence.
Conclusions: The molecular interactions between the anti-MICA antibody and the anti-sST2 antibody and their respective antigens have been characterized. Furthermore, mutants with enhanced affinity were obtained far both antibodies, thereby demonstrating that this integrative approach provides a comprehensive understanding of the antibody-antigen interaction and establishes a salid basis far the rational design of antibodies.
Acknowledgements: ANILLO ACT210068, FONDECYT GRANTS 1221031;

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

DEVELOPMENT AND CHARACTERIZATION OF A FULLY HUMAN MONOCLONAL ANTIBODY TARGETING MICA FOR CANCER IMMUNOTHERAPY

María José Garrido 1, Karen Toledo-Stuardo 1, Fabiola González-Herrera 1, José Rodríguez 3, Samantha Tello 1, Douglas J. Matthies 1,2, Mauricio González-Olivares 1, Nicolás Fehring 1, Yuneisy Guerra 1, María Carmen Molina 1.

1. Programa de Inmunología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile.
2. Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile.
3. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.

Background: Cancer is one of the leading causes of death worldwide. During the last few years, immunotherapy has made significant advances, which has allowed the development of novel targeted therapies using monoclonal antibodies (mAbs). MICA is a cell surface protein overexpressed under cellular stress in different types of cancer. It is recognized by the NKG2D receptor, which triggers cytolytic activation of Natural Killer (NK) cells. However, tumors have developed several strategies to evade the immune response, such as proteolytic elimination of MICA, which induces down-regulation of the NKG2D receptor on NK cells, thus compromising their function. This study focused on generating a fully human anti-MICA antibody and analyzing its binding capacity to soluble MICA and its ability to block the interaction of the MICA receptor and NKG2D.

Methods: The antibody was constructed from an antibody fragment (scFv) employing phage presentation technology to the α1 non-polymorphic region of MICA. It was produced in CHO-S cells in transient cultures and purified on high-performance chromatography coupled to a protein G column. The affinity of the antibody for MICA and its ability to block the MICA-NKG2D interaction was assessed by enzyme-linked immunosorbent assay (ELISA).

Results: The results demonstrated that the anti-MICA antibody effectively neutralizes the soluble form of MICA and avoids its interaction with NKG2D. This broad binding capacity can be attributed to the fact that the antibody targets a nonpolymorphic site in the α1 domain of MICA. These findings support the therapeutic potential of the antibody in cancer immunotherapy and in other MICA-expressing tumors.

Conclusions: In conclusion, our monoclonal antibody holds promise as a valuable tool in the treatment of MICA-expressing cancers, particularly in the context of immunotherapy, by offering a strategic approach to enhance NK cell-mediated cytotoxicity against tumor cells, potentially overcoming one of the key immune evasion mechanisms employed by cancer cells.

Acknowledgments: Proyecto ANILLO ACT210068, FONDECYT N° 1221031.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

UNRAVELING THE MECHANISMS OF ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC) MEDIATED BY A FULLY HUMAN IGG1 ANTIBODY TARGETING THE MICA PROTEIN

Karen Toledo-Stuardo 1, Fabiola González 1, Carla Díaz 1, Nicolás Fehring 1, María José Garrido 1, Mauricio González 1, Douglas J. Matthies 1, Valentina Álvarez 1, Nicolás Cristi 1, José Rodríguez 2, Samantha Tello 2, Carolina Ribeiro 1, Claudia Altamirano 2, María Carmen Molina 1.

1. Program of Immunology, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Independencia, Chile (Av. Independencia 1027, postcode: 8380453).
2. School of Biochemical Engineering, Faculty of Engineering, Pontifical Catholic University of Valparaiso, Valparaiso, Chile (Av. Brasil 2085, postcode: 2362803).

Background: Antibody-dependent cellular cytotoxicity (ADCC) is one of the major mechanisms by which monoclonal antibodies exert their effects in oncology. Upon binding to its target, the antibody's crystallizable fragment (Fc) interacts with Fc gamma receptors on immune cells, initiating a cytotoxic response. Natural killer (NK) cells are commonly studied for their role in ADCC due to their release of perforin and granzyme, a process known as degranulation. However, the effect of antitumor antibodies on other cytotoxic immune cells remains less explored. The main objective of this work was to evaluate the role of NK cells and other immune cell types in the ADCC mechanisms of a novel fully human IgG1 anti-MICA antibody.
Methods: We produced a fully human anti-MICA antibody in a transient expression system using ExpiCHO-S cells by co-transfection with optimized promoter vectors for light and heavy chains. The antibody was purified by affinity chromatography and then dialyzed. PBMCs enriched for NK cells (effectors) were isolated from blood samples of healthy volunteers. To evaluate the ADCC mechanism, these cells were co-cultured with K562 cells (targets) in the presence of the anti-MICA antibody. ADCC was evaluated by measuring Immune cell degranulation and IFN-γ production by flow cytometry.
Results: The anti-MICA antibody significantly increased CD107a, a marker of degranulation, on NK cells (CD3-CD56+ cells) co-cultured with K562 cells at an effector: target ratio of 10:1. This also led to increased IFN-γ production compared to an IgG1 human isotype antibody. Notably, cytotoxic activity was observed in the CD3+CD56+ immune cell population, indicated by enhanced CD107a expression. This suggests that these cells could contribute to the overall ADCC activity of the anti-MICA antibody.
Conclusion: These findings elucidate the ADCC mechanisms of the novel anti-MICA antibody in vitro and, for the first time, highlight the involvement of CD3+CD56+ immune cells in this process. This suggests that evaluating ADCC across different immune cell types with cytotoxic potential may provide a more comprehensive assessment of the efficacy of antitumoral monoclonal antibodies.
Acknowledgment: FONDECYT Postdoctorado ANID 3230454, ANILLO ACT210068, FONDECYT N° 1221031.

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

OPTIMIZATION OF EXPRESSION, REFOLDING AND STORAGE CONDITIONS OF SINGLE-CHAIN VARIABLE FRAGMENTS IN *ESCHERICHIA COLI*

Nicolás Fehring 1, Karen Toledo-Stuardo 1, Camila Jiménez 1, Nicolas Cristi 1, Javiera Landaeta 1, Mauricio González-Olivares 1, Gonzalo Vásquez 2, Douglas J. Matthies 1, María Carmen Molina 1.

1. Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.
2. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso

Background: Single-chain variable fragments (scFv) are small antibody fragments that are fusion proteins of the variable regions of the heavy and light chains linked together by a flexible linker. As small and non-glycosylated proteins, scFvs can be overexpressed in prokaryotic hosts such as Escherichia coli. However, high-level expression of scFvs usually accumulates as unfolded protein aggregates known as inclusion bodies. In vitro refolding conditions must be determined empirically for each protein, and the storage buffer composition has to be optimized to maintain full activity and homogeneity for downstream applications. In this study, we optimized the expression conditions for three different scFvs to obtain the maximum amount of soluble and insoluble protein based on tuning of three cultivation variables: temperature, isopropyl-beta-D-thiogalactopyranoside (IPTG) concentration and post-induction time. Finally, we identified and optimize refolding and storage buffers to maintain maximum stability of the scFvs through Thermofluor assays.
Methods: All scFvs were expressed in *E. coli* SHuffle T7 in Luria-Bertani broth. Cultures supplemented with ampicillin were incubated at 30 oC under shaking and grown to mid-log phase. Bacterial culture was then separated in parallel cultures and induced with 0.5, 0.2 and 0.1 mM IPTG at 30, 25 and 20°C. Samples were taken at 3, 6 and 18 hours after induction and were centrifuged and sonicated. The soluble fraction was harvested, and the insoluble fraction was solubilized with guanidinium. Protein concentration was determined using the Bradford method and the amount of expressed scFv were assessed by SDS PAGE via ImageJ analysis. Refolding conditions were screened trough Thermofluor assays, including a pH range from 6.0 to 9.0 with the presence and absence of glutathione redox pair. Best storage buffer was identified by screening pH, buffer composition and salt concentration.
Results: All three scFv were highly expressed as inclusion bodies. Maximum amount was obtained at 30°C after 4 hours with a yield up to 10 mg/L. No soluble protein was observed. The best refolding condition found was buffers with arginine and redox pair. All storage buffers differed between scFv.
Conclusions: This study optimized the expression and refolding conditions for three scFvs, achieving maximum protein production in the form of inclusion bodies. The optimal refolding conditions and storage buffers were identified to maintain the stability of the scFvs, providing an efficient framework for their production and application in research and therapies.
Acknowledgments: Proyecto ANILLO ACT210068, FONDECYT N° 122103 and FONDEF ID23I10018

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

DEVELOPMENT OF SCFV ANTI-MICA*002, A VARIANT PRESENT IN GASTRIC CANCER

Mauricio González-Olivares 1, Karen Toledo-Stuardo 1, Fabiola González-Herrera 1, Ivo Campos1, José Rodríguez 2, Samantha Tello 1, Douglas J. Matthies 1, María José Garrido 1, Nicolás Fehring 1, Carla Díaz 1, Claudia Altamirano 2, María Carmen Molina 1.

1. Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile.
2. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso

Background: MICA is a polymorphic protein whose soluble form is recognized as an oncological target, due to its potential to promote immune evasion by downregulating the NKG2D receptor on natural killer (NK) cells, a key component of antitumor cytotoxicity. Additionally, a predominance of the MICA *002 allele has been reported in patients with gastric adenocarcinoma. In this context, the neutralization of soluble MICA through the use of antibodies represents an intriguing strategy to counteract this mechanism, which has been shown to favour tumor progression. The aim of this study was to demonstrate that a single-chain variable fragment (scFv) can bind to the α2 domain of the MICA *002 allele.

Methods: The genetic sequence of the scFv, selected from a phage display library, is part of the IGKV2-3001 and IGHV1-4601 germline within antibody families. The recombinant production of the scFv was carried out in E. coli BL21, with refolding permitted in a buffer containing L-arginine. The yield of the scFv was assessed using size exclusion chromatography (SEC). To ascertain its specific interaction capacity with the MICA *002 variant, a cytometry assay was conducted utilizing CHO-K1 cells stably expressing MICA *002.

Results: The recombinant scFvs achieved a yield of 1 mg/mL with a purity exceeding 90%, and the buffer employed permitted the refolding of more than 70% of the total purified protein. The cytometry assay demonstrated a specific interaction of the scFv with MICA *002 compared to parental cells.

Conclusion: In conclusion, phage display has proven to be an effective method for generating scFv antibodies against MICA. This innovative technology is very promising for the development of highly specific antibodies that can target multiple molecular targets, representing an advancement in the field of therapeutic antibody research.

Acknowledgment: Fondef IDeA ID20I10106, PROYECTO FONDECYT Nº 1221031, Anillo Regular de Investigación en Ciencia y/o Tecnología 2021 (ACT210068)

BIOTERAPÉUTICOS: DESARROLLO Y CONTROL DE CALIDAD

DEVELOPMENT OF A FULLY HUMAN ANTIBODY FROM AN SCFV TARGETING THE SST2 PROTEIN: A THERAPEUTIC APPROACH AGAINST ULCERATIVE COLITIS

Gonzalo Vásquez M. 1,3, Pedro Novoa 3, Douglas Matthies 2,9, Karen Toledo-Stuardo 1,3, Karen Dubois 3, Marjorie de la fuente 4, Glauben Landskron 4, Rodrigo Quera 6, Gerald Zapata 9, María Carmen Molina 2, Claudia Altamirano 1,7,8, Marcela Hermoso 3,5.

1. Animal Cell Culture Laboratory, School of Biochemical Engineering, Pontificia Universidad Católica de Valparaiso, Valparaiso, Chile.
2. Laboratory of recombinant antibodies and immunotherapy, immunology program, institute of biomedical sciences, university of Chile, Santiago, Chile.
3. Innate Immunity Laboratory, Immunology Program, School of Medicine, Universidad de Chile, Santiago, Chile.
4. Biomedicine Research Laboratory, Medical School, Universidad Finis Terrae, Santiago, Chile
5. Gastroenterology and Hepatology Department, University Medical Center Groningen, Groningen.
6. Center for Digestive Diseases, Inflammatory Bowel Disease Program, Clínica Universidad de Los Andes, Universidad de los Andes, Santiago, Chile.
7. Center of interventional medicine for precision and advanced cellular therapy (IMPACT)
8. Regional Center for Food and Health Studies, CREAS
9. Molecular Graphics Unit-Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.

Background: Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by an aberrant immune response that leads to sustained colon inflammation. The ST2 protein, particularly its soluble form (sST2), plays a significant role in UC pathogenesis by modulating the IL-33/ST2L signaling pathway, which is responsible for the release of pro-inflammatory cytokines that exacerbate the disease. Elevated levels of sST2 in UC patients interfere with IL-33 binding to its receptor, ST2L, hindering the normal regulatory functions of this pathway. To address this issue, we have developed a fully human monoclonal antibody, AcHu-αsST2, specifically targeting sST2. The goal is to neutralize the excess sST2, restore the functionality of the IL-33/ST2L pathway, and reduce inflammation.
Methods: The AcHu-αsST2 antibody was produced in CHO-K1 cells. The antibody was purified using protein G affinity chromatography. To evaluate its therapeutic potential, biopsy samples from UC patients and healthy controls were treated with various stimuli in the presence or absence of AcHu-αsST2. The levels of cytokines IL-10 and TNFα were quantified using flow cytometry-based assays. Additionally, the role of glycosylations in the Fv region of the antibody was investigated. The anti-sST2 antibody was treated with PNGase F to remove glycosylations, and differences in molecular weight were analyzed by Western blot. The impact of glycosylation on the binding affinity to sST2 was evaluated by ELISA.
Results: Treatment with AcHu-αsST2 significantly increased IL-10 levels and decreased TNFα levels in UC patient samples compared to vehicle-treated controls, indicating a shift towards an antiinflammatory environment. Western blot analysis confirmed a lower molecular weight for the deglycosylated antibody, consistent with the removal of glycosylations. Additionally, ELISA results showed that glycosylations in the Fab region generate a change in the binding affinity of AcHu-αsST2.
Conclusions: The fully human AcHu-αsST2 antibody has shown promise in modulating the inflammatory response in UC by targeting the soluble ST2 protein. The presence of N-glycosylations plays a crucial role in the antibody's binding affinity and therapeutic efficacy. These findings support the continued development of AcHu-αsST2 as a potential therapeutic agent for UC.
Acknowledgments: Anillo ACT_210068_2021, FONDEF ID23i10018, Fondecyt 1220702, and USM Doctoral Scholarship No. 041 / 2024.

TERAPIA GÉNICA Y TERAPIA CELULAR

EVALUATING CONDITIONS FOR CULTURING HUMAN WHARTON'S JELLY STEM CELLS IN SUSPENSION WITH MICROCARRIERS UNDER XENO-FREE CONDITIONS

Pilar Vera Moncada 1,2, Jorge Alvarado 3, Jaime Plane 4, Barbara A. Andrews 1,2,5, Pablo Caviedes 2,6, Juan A. Asenjo 1,2,5, Anamaría Daza 2,5.

1. Faculty of Physical and Mathematical Sciences (FCFM), University of Chile, Santiago, Chile.
2. Institute for Cell Dynamics and Biotechnology (ICDB), FCFM, University of Chile, Santiago, Chile.
3. Clinical Hospital San Borja Arriarán, Santiago, Chile.
4. Faculty of Medicine, University of Chile, Santiago, Chile.
5. Centre of Biotechnology and Bioengineering (CeBiB), Department of Chemical Engineering, Biotechnology and Materials, FCFM, University of Chile, Santiago, Chile.
6. Program of Molecular Clinical Pharmacology, (ICBM), Faculty of Medicine, University of Chile, Santiago, Chile.

Background: Mesenchymal stem cells (MSCs) are crucial for the development of regenerative medicine and cell therapy. MSCs can be obtained from various tissues, including Wharton's jelly from the umbilical cord (hWJ-MSC), which is easily accessible. For their use in cell therapy, they must be expanded in the laboratory on a surface for adherent growth. Traditional cultivation methods are not suitable for producing the required number of cells due to their laboriousness, risk of contamination, and poor scalability. Microcarriers (MCs) provide an anchorage surface for suspension cultures, allowing for scalable homogeneous cultures.

Methods: This work optimizes the cultivation of hWJ-MSCs in suspension using MCs under xeno-freem conditions for human cell therapy, characterizes their metabolic profile through glucose and lactate consumption/production, and demonstrates through trilineage differentiation that the cells maintain their multipotency after harvesting. The suspension culture with MCs was seeded with 4500 cells/cm2 in 50 mL of αMEM Antiox medium supplemented with fetal bovine serum (FBS) or human platelet lysate (hPL) and agitated in a 125 mL spinner flask. Glucose was added and the volume was increased during the culture.

Results: With this methodology, an efficient cultivation strategy using FBS as a supplement is obtained, reaching 118 million cells. The cells were adapted to culture with hPL (xeno-free conditions), but 58% less biomass was produced due to improper cell adhesion to the MCs.

Conclusions: It is essential to continue experimenting with cultivation strategies to achieve higher cell biomass under xeno-free conditions. Alternatives to improve adhesion, such as coatings for MCs or culture medium supplements, will be evaluated.

TERAPIA GÉNICA Y TERAPIA CELULAR

METABOLIC CHARACTERISATION OF HIGH-DENSITY MICROCARRIER SUSPENSION CULTURE OF HUMAN ADIPOSE AND WHARTON'S JELLY MESENCHYMAL STEM CELLS

Anamaría Daza 1,2, Pilar Vera Moncada 1,3, Jorge Alvarado 4, David Vantman 5, Barbara A. Andrews1,2,3, Pablo Caviedes 2,6, Juan A. Asenjo 1,2,3.

1. Institute for Cell Dynamics and Biotechnology (ICDB), Santiago, Chile
2. Centre of Biotechnology and Bioengineering (CeBiB), University of Chile, Santiago, Chile
3. Department of Chemical Engineering, Biotechnology and Materials (DIQBM), Faculty of Physical and Mathematical Sciences (FCFM), University of Chile, Santiago, Chile.
4. Clinical Hospital San Borja Arriarán, Santiago, Chile
5. Centro de Estudios Reproductivos, Santiago, Chile
6. Program of Molecular Clinical Pharmacology, (ICBM), Faculty of Medicine, University of Chile, Santiago,Chile.

Background: Mesenchymal stem cells (MSCs), renowned for their immunomodulation, bioactive molecule secretion, and multi-differentiation potential, have significantly advanced regenerative and cell therapy. Their application extends to treating a spectrum of conditions, including heart failures, bone/cartilage-related issues, diabetes, cancer, premature ovarian insufficiency, etc. Despite being a small proportion within tissues, MSCs are self-renewable and are expandable in vitro. However, conventional plate culture, involving labour-intensive procedures, extensive handling, contamination risk, and scalability issues, is inadequate for producing cells needed for cell therapy. Microcarrier culture addresses these challenges by providing an anchoring surface for cells while are homogeneously suspended in an agitated system. This culture system is scalable to various bioreactor dimensions.

Methods: Cells isolated from Wharton's Jelly (WJ-MSC) or adipose tissue (ASC) were expanded in an agitated suspension culture. It was performed in a spinner flask with microcarriers at high concentrations in αMEM-Antiox medium. Media was replaced as needed, glucose was added to prevent growth limitations, and volume was increased during the culture. Metabolite (glucose, lactate and amino acid) concentration was monitored during the culture by enzymatic reactions and HPLC detection.

Results: Human WJ-MSCs and ASCs were successfully grown in a microcarrier suspension culture. Over the culture, up to 250 million cells were obtained in a single flask, resulting in a 30-fold increase in cell number. Metabolite concentrations were successfully quantified during the culture

Conclusion: Our developed protocol allows high-density MSC expansion. A single 125 mL spinner flask is equivalent to at least forty T175-dishes. This presents a user-friendly, contamination-resistant, and scalable system adaptable to controlled bioreactors.

TERAPIA GÉNICA Y TERAPIA CELULAR

IMPLEMENTATION OF A 3D CULTURE OF HUMAN UMBILICAL CORD MESENCHYMAL STEM/STROMAL CELLS ON MICROCARRIERS UNDER AGITATION CONDITIONS AND DIFFERENT OXYGEN AVAILABILITY

Constanza Collarte 1,2, Paloma Fuentes 1,2, Alejandra Arancibia-Díaz 1,2, Claudia Altamirano 1,2,3.

1. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2085, Valparaíso, Chile.
2. IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Av. Mons. Álvaro del Portillo 12455, Las Condes, Santiago, Chile
3. Centro Regional de Estudios en Alimentos Saludables, Av. Universidad 330, Curauma-Placilla, Valparaíso, Chile

Background: Tissue engineering and regenerative therapy, crucial in modern medicine, rely on mesenchymal stem/stromal cells (MSCs) due to their differentiation and regenerative capabilities. However, their isolation and expansion present critical challenges, such as inefficient scaling in 2D cultures and the use of animal-derived components, which introduce variability and clinical limitations. This study proposes using 3D suspension cultures with microcarriers and xeno-free conditions through human platelet lysate to overcome these limitations. Maintaining an appropriate balance between agitation and oxygen availability is crucial for promoting cell proliferation and functionality, thereby improving the quality of produced mesenchymal cells and enhancing their clinical applicability in regenerative therapies.
Methods: Mesenchymal stem/stromal cells from umbilical cord (UC-MSCs) from three donors (A, B, C) at passage 5 were expanded in 2D at 37°C, 21% O2, and 5% CO2. Upon reaching 80% confluence, the UC-MSCs were assessed on four microcarriers (MC1, MC2, MC3, MC4). Cells were seeded in low-adherence 6-well plates, and viability, cell concentration, and microcarrier occupancy were analyzed via microscopy to select the best donor-microcarrier combination. The selected donor-microcarrier pair was then used for 3D cultivation, studying two agitation speeds under normoxic and hypoxic conditions. Key metabolites were analyzed to establish a metabolic profile using a biochemical analyzer. Immunophenotyping was performed by flow cytometry, identifying specific markers. The cellular differentiation capacity was evaluated using osteogenesis, adipogenesis, and chondrogenesis kits.
Results: After evaluating the microcarriers with the three donors, it was observed that donor A, using MC4, achieved the best results under the studied conditions. 100% of MC4 analyzed had one or more cells adhered to their surface, while, on average, 70% of microcarriers in other groups had only one or two cells adhered after one hour of incubation. Furthermore, donor A with MC4 reached a total of 1.3×10^5 adhered cells with 98% viability, surpassing MC1, MC2, and MC3, which did not exceed 0.6×10^5 cells and had cell viability between 85% and 94%.
Conclusions: The study confirms that specific combinations of donors and microcarriers enhance the adhesion and expansion of mesenchymal cells, demonstrating greater adherence, cell concentration, and viability, thus paving the way for advancing to agitation-based cultures.
Funding: Financiamiento Basal para Centros Científicos y Tecnológicos de Excelencia - ANID (IMPACT#FB210024), Fondo Anillos de Investigación en Ciencia y Tecnología - ANID (ACT210068), Proyecto FONDECYT Regular (1241453).

TERAPIA GÉNICA Y TERAPIA CELULAR

TECHNICAL ECONOMIC PREFEASABILITY STUDY OF DESCENTRALIZED CAR-T CELL MANUFACTURING FACILITIES UNDER GOOD MANUFACTURING PRACTICES GUIDELINES IN CHILE

Pablo Salgado 1,4, Cristóbal Silva 1,4, Sofía Soto 1,4, Claudia Altamirano 1,2,3 and Víctor Cerpa 1,2.

1. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2085, Valparaíso 2362803, Chile.
2. IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Av. Monseñor Álvaro del Portillo 12455, Las Condes, Santiago 7550000, Chile.
3. Centro Regional de Estudios en Alimentos Saludables, Av. Universidad 330, Curauma-Placilla, Valparaíso 2340000, Chile.
4. These authors contributed equally.

Background: In response to the increasing number of refractory/relapse cases of certain hematological cancers worldwide, immunotherapy with Chimeric Antigen Receptor (CAR) expressed in T lymphocytes has proven to be an effective treatment for achieving disease remission. Nevertheless, the elevated pricing of this therapy modality, bordering $450.000 USD, can limit its accessibility. It has been suggested that one way to make CAR-T more affordable is to bring the production closer to the patients, by building the manufacturing facilities within their country. Hence, the aim of this study is to evaluate the technical economical prefeasibility of the construction of decentralized manufacturing facilities in the north, center and south of Chile in terms of a decade period. This research integrates an examination of the prevalence and mortality ofspecific hematological cancers in Chile, identifying the potential patient population and market demand. Based on the assumption that primarily the wealthiest 10% can afford the therapy and an extra number of individuals could access it through a state financial protection system, it is established that the production capacity required should supply at least 307 doses annually at the end of the project.

Furthermore, by assessing local healthcare infrastructure, technological and logistical capabilities, three high complexity hospitals were selected as potential hosts of these facilities. Since this therapy modality is autologous, this measure ultimately seeks to reduce costs and the “vein to vein” time. A screening of the processes and equipment required for each task is carried out, verifying their compatibility with the regulatory landscape framed by Good Manufacturing Practices (GMP). As a result, it is concluded that a fully automated manufacturing process would be more suitable to improve the cost-benefit of the project.

Additionally, a mass and energy balance of the manufacturing process is studied to estimate the quantities and costs associated to the electrical consumption, supplies, reagents and fungible items. Lastly, a standardized diagram of the manufacturing area and the distribution of the cleanrooms is provided, considering the necessary airlocks, passive transfer hatches and the personal, material, waste and air flows.

This study intends to explore the economic implications, including cost analysis and potential return on investment, to provide a comprehensive overview of the feasibility of this venture. The findings aim to guide healthcare stakeholders and investors in making informed decisions about the development of a high-complexity CAR-T cell manufacturing facility in Chile, ultimately contributing to the advancement of personalized cancer treatment in the region.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

BIVALENT CHIMERIC VIRUS-LIKE PARTICLES FOR PORCINE CIRCOVIRUS PROTECTION AND IMMUNOCASTRATION

Ernesto Garay 1,2, Victoria Gastaldi 1, Diego Fontana 1,2, Hugo Ortega 3, Belkis Marelli 3 and Claudio Prieto 1,2.

1. UNL, FBCB, Centro Biotecnológico del Litoral, S3000ZAA, Santa Fe, Pcia. de Santa Fe, Argentina
2. CEVA Biotecnofe. Parque Tecnológico del Litoral Centro, Ruta 168 - Paraje El Pozo - Lote B1, S3000, Santa Fe, Pcia. de Santa Fe, Argentina
3. UNL, Instituto de Ciencias Veterinarias del Litoral - CONICET. R.P Kreder 2805- S3080HOF,Esperanza, Pcia. de Santa Fe, Argentina

Background: Porcine Circovirus 2 (PCV-2) is the etiologic agent of the Postweaning Multisystemic Wasting Syndrome, a pig disease that produces great economic impact worldwide. Its capsid protein (Cap) is able to self-assemble forming virus-like particles (VLPs), resembling the structure of native virus but without its genome, which makes them biosafe. On the other hand, chirurgical castration is a normally applied process in the swine production industry to reduce aggressivity and to impede changes in smell and flavor of animal meat, being a traumatic process for the animal. An alternative and less applied strategy relies on neutralizing gonadotropin releasing hormone (GnRH) by active immunization, a process defined as immunocastration. In this work we aimed to design and characterize a bivalent vaccine candidate for immunocastration and PCV-2 prevention, by fusing tandem GnRH peptides to Cap, forming chimeric self-assembling VLPs.

Methods: Fusion proteins that contain three (Cap-3) or six (Cap-6) copies of GnRH peptide variants fused to the C-terminal end of Cap, were designed. These sequences were cloned on pET plasmids, along with a wt Cap construct, and then used to transform BL-21 pLys E. coli strain. Isolated clones were induced with IPTG for five hours, and soluble and insoluble fractions were analyzed by SDS-PAGE and western blot. VLP formation was assessed by iodixanol gradient ultracentrifugation and transmission electron microscopy. VLP immunogenicity was assessed on Balb/c mice, using a cage-like particle product as adjuvant (Liposap), and antibodies were detected in plasma samples by indirect ELISA.

Results: Cap-3 and Cap-6 were found on both fractions, proving that fusion proteins were able to remain partially soluble. Moreover, fusion proteins were detected by western blot using anti-cap and anti-GnRH antibodies, showing a differential molecular weight that can be attributed to the different number of copies of the peptide fused to the Cap protein. TEM micrographs of Cap-3 and Cap-6 samples evidenced the presence of round particles with an approximate size of 17 nm, as expected for Cap VLPs. Thus, peptide insertions did not affect correct VLP assembly. Finally, VLPs were able to trigger anti-Cap and anti-GnRH antibodies in Balb/c mice, the last ones in similar levels to those obtained by a commercial immunocastration vaccine (CEVA Valora).

Conclusions: We were able to obtain PCV-2 chimeric VLPs that could be used as a bivalent vaccine for PCV-2 protection and immunocastration, and that can be obtained through a cost-effective expression system without requiring any refolding or chromatographic purification step.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

DESIGN OF A SYSTEM OF POLYMERIC PARTICLES, SYNTHESIZED BY ELECTROSPRAYING METHOD, ENCAPSULATING NUCLEIC ACIDS FOR A VACCINE APPLICATION.

Carlos A. Villacis-Aguirre 1, Thelvia I. Ramos 1,2, Nicole Paredes 2, Angy Ñacato 2, Carolina Gómez Gaete 3, Jorge R. Toledo 1.

1. Laboratorio de Biotecnología y Biofármacos, Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Víctor Lamas 1290, Concepción 4070386, Chile.
2. Grupo de Investigación en Sanidad Animal y Humana (GISAH), Carrera Ingeniería en Biotecnología, Departamento de Ciencias de la Vida y la Agricultura, Universidad de las Fuerzas Armadas—ESPE, Sangolquí 171103, Ecuador.
3. Departamento de Farmacia, Facultad de Farmacia, Universidad de Concepción, Concepción, Chile

Background: Emerging and re-emerging diseases have arisen over the past few decades and represent a global health challenge. COVID-19 pandemic was a global emergency, forcing a redoubling of efforts to contain and prevent the spread of infection, and vaccines emerged as the most effective public health mechanism to combat the disease.

Nucleic acid vaccine technology was validated in mass vaccination campaigns between 2020 and 2022, demonstrating efficacy, safety, and rapid implementation. Due to the susceptibility to degradation by endonucleases, nucleic acids must be transported and delivered through a system that protects it, and although lipid nanoparticles are currently used for this purpose, this system still has several drawbacks that can be solved with the use of polymeric nanoparticles. Using cationic polymers, such as polyethylenimine (PEI), and biodegradable and biocompatible polymers, such as poly lactic-co-glycolic acid (PLGA), it is possible to generate an effective encapsulating matrix. Additionally, the synthesis of nanoparticles by electrospray method, allows to have a global control of the process, as well as to achieve a high encapsulation efficiency.

With this background, we designed a system of polymeric particles synthesized by electrospray method, which encapsulates nucleic acids for a possible subsequent vaccine application. We developed a PLGA and PEI formulation that encapsulated plasmid DNA with 89% efficiency. These particles had an average size of 1014 nm, a transfection efficiency of 7.9%, and did not generate toxicity in vitro. This formulation represents a starting point, which can be optimized to increase transfection efficiency and thus achieve the defined application.

Keywords: Nucleic acid vaccines, polymeric encapsulation, electrohydrodynamics.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

CHARACTERIZATION OF VIRAL PSEUDO-PARTICLES OF PORCINE CIRCOVIRUS TYPE 4

Gabriela Paradeda 1, Florencia Fadel 1, Natalia Olivero-Deibe 2, Sergio Pantano 3, Claudia Ortega 1.

1. Programa de Tecnología Molecular, Celular y Animal (ProTeMCA), Institut Pasteur de Montevideo,Mataojo 2020, 11400 Montevideo, Uruguay,
2. Laboratorio de Inmunovirología, Institut Pasteur de Montevideo, Mataojo 2020, 11400 Montevideo, Uruguay,.
3. Laboratorio de Simulaciones Biomoleculares, Institut Pasteur de Montevideo, Mataojo 2020, 11400

Background: Members of the Circoviridae family are the smallest viruses capable of infecting mammalian cells. These naked viruses, with an icosahedral structure and a dimension close to 20 nm in diameter, are named Circovirus due to the circular nature of their single-stranded DNA genome. The viral capsid comprises 60 repetitions of the Cap protein, responsible for the immune response. Within the Circovirus genus, the porcine type 2 variant (designated PCV2, Porcine Circovirus Type 2) is essential for pig farming. PCV2 is the causative agent of post-weaning multisystemic wasting syndrome (PMWS). Its global incidence confirms its status as one of the central expanding diseases in pig populations, significantly impacting the economy. Virus-like particles (VLPs) present a promising alternative to traditional vaccines. Their resemblance to wild virions, safety due to the absence of viral genetic material, and high immunogenicity make them a compelling option. Their ability to stimulate the immune response efficiently, without the need for artificial adjuvants, further enhances their potential in vaccine development. A new viral genotype called PCV4, with a sequence identity of ~50%, has been recently identified in China, Spain, and the United States. This pathogen is more related to Circoviruses that infect other species, and its role in developing diseases in pigs is still unclear. However, it has raised significant concern due to its ability to infect cattle.

Methods: The main objective of this project is to obtain a proof of concept on the possibility of adapting the production protocol of VLPs developed for PCV2 and to leverage the acquired experience to produce VLPs of Porcine Circovirus Type 4. We propose increasing response capability with adaptable technologies that allow for rapid, simple, and robust action when new viral variants emerge. As an experimental strategy, we follow these steps: Cloning and expression of the Cap4 protein in the EXPI293F cell line using suspension cultures, purification of the VLPs through ultracentrifugation in a discontinuous CsCl gradient, and Biophysical/Biochemical characterization. We also focus on understanding and enhancing the immune response of the VLPs, a crucial aspect of vaccine development.

Results: We successfully expressed and purified PCV4 VLPs in EXPI293F cells. The VLPs exhibited the expected size and morphology, confirming their structure through DLS and TEM. We continue tocharacterize the VLPS immunogenicity. These preliminary results could contribute to studying PCV4 as an emerging pathogen.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

DEVELOPMENT OF A GOLD NANOPARTICLE-BASED NANOSYSTEM FOR DETECTION OF SOLUBLE ST2 IN ULCERATIVE COLITIS.

Ivo Campos 1,2,3, Ana Riveros 2, Marcelo Kogan 2, María Carmen Molina 1, Marcela Hermoso 3, 4.

1. Laboratorio de Anticuerpos Recombinantes e Inmunoterapia, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile, 8380453, Santiago, Chile.
2. Laboratorio de Nanomedicina y Nanoteranóstica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, 8380494, Santiago, Chile.
3. Laboratorio de Inmunidad Innata, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile, 8380453, Santiago, Chile
4. Gastroenterology and Hepatology Department, University Medical Center Groningen, 9713 GZ, Groningen, Netherlands.

Background: Ulcerative colitis (UC) is a chronic inflammatory disease affecting the colon and rectum, with high prevalence and incidence worldwide. The IL-33/ST2 axis is crucial for tissue repair, but its function is inhibited by soluble ST2 (sST2), a potential biomarker for UC progression. Our team developed an innovative antibody targeting sST2 and explored its integration with gold nanoparticles to create a new detection system. Aim: To detect sST2 in complex matrices using an innovative anti-sST2 antibody conjugated to gold nanospheres, aiming to develop a novel detection kit for monitoring ulcerative colitis progression.

Methods: We employed a cooperative adsorption technique to develop a nanosystem comprising gold nanospheres functionalized with the anti-sST2 antibody and blocked with BSA. The system was characterized using hydrodynamic diameter, Z-potential, electron microscopy (STEM), and UV-Vis spectrophotometry. Antigen recognition capability was evaluated through Western blot assays.

Results: The synthesized gold nanospheres had a hydrodynamic diameter of 59.8 ± 26.9 nm, Z-potential of -32.6 ± 13.5 mV, and a STEM diameter of 41.1 ± 4.1 nm. Successful conjugation of anti-sST2 and blocking with BSA were confirmed by an increased hydrodynamic diameter (78.53 ± 32 nm before BSA, and 107.2 ± 50 nm after BSA), UV-Vis plasmon red shift (3-6 nm), and immunodot blot. The nanosystem demonstrated specific antigen recognition in Western blot assays.

Conclusion: We developed a functional nanosystem for detecting sST2, combining gold nanoparticles with an innovative antibody. While further optimization is required, our results suggest this system could be the basis for a new detection kit for ulcerative colitis biomarkers.

Acknowledgments: Proyecto ANILLO ACT210068, FONDEF ID23I10018 and ANID scholarship program DOCTORADO NACIONAL N°21221729.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

DETECTION OF THE BOVINE VIRAL DIARRHEA VIRUS ERNS PROTEIN USING A LATERAL FLOW ASSAY BASED ON MONOCLONAL GOLD LABELED AND AVIAN IGY ANTIBODIES.

Nicolás Gutiérrez 1, Viana Manrique-Suárez 2, Angela Hidalgo 1, Natalie Parra 1, Fátima Reyes 2, María Angélica Contreras 1, Camila Quezada 3, Carla Villavicencio 1, Frank Camacho 2, Jorge R. Toledo 1.

1. Laboratorio de Biotecnología y Biofármacos, Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
2. Laboratorio de Biofármacos Recombinantes, Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.
3. Departamento de Ingeniería en Materiales (DIMAT), Facultad de Ingeniería, Universidad de Concepción, Concepción, 4070409, VIII región, Chile.

Background: Bovine Viral Diarrhea Virus (BVDV) is a pestivirus belonging to the Flaviviridae family which affects the global cattle industry in different ways, reducing the reproductive capacity of animals, the weight gain, and the milk production considering it a sanitary problem for the cattle industry worldwide. The BVDV is classify in 2 subtypes, Cytopathic and No- Cytopathic (NCP). If BVDV infect a pregnant animal and is of the NCP subtype, the virus can infect the fetus and birth a persistently infected (PI) animal, which are considered the principal reservoir of the virus in nature. Efforts to control this disease are based on control and eradication programs focusing on the identification PI animals. The BVDV Erns structural protein is one of the most immunogenic proteins and is also genetically conserved among the different sub-genotypes of the virus, which makes it a good marker for the identification of IP animals. Currently the development of new pathogen detection platforms focuses on the ASSURED criteria. This concept describes a device which is Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free and Deliverable and one of the devices that fits within this concept are lateral flow devices. The need for new methodologies that allow the detection of PI animals in field work for rapid identification and separation of PI animals makes lateral flow assays a good option as a detection system.
Methods: Recombinant Erns (rErns) protein was produced in insect cells to immunize mice and chickens to obtain monoclonal and polyclonal antibodies respectively. IgG monoclonal antibodies were labelled with nanogold particles and used as capture antibody and the avian IgY anti-rErns and anti-mouse IgG polyclonal antibodies were printed on the nitrocellulose membrane to finally assemble the lateral flow system.
Results: The obtained monoclonal and IgY antibodies recognize rErns protein by ELISA and Western blot immunoassays and do not recognize any protein present in bovine serum. The established prototype lateral flow assay recognizes rErns protein up to 25 ng of protein with the naked eye.
Conclusions: The proposed lateral flow assay prototype can become a tool for the identification of PI animals for BVDV, helping to carry out field trials, reducing the time taken to identify and isolate PI animals and thus reducing the infections and economic losses caused by BVDV in cattle herds

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

IGY ANTIBODY PRODUCTION IN LAYING HENS: PREVENTION OF RESPIRATORY INFECTIONS, HANTAVIRUS AS A STUDY MODEL..

Natalie Parra 1, Estefania Escobar 1, Thelvia Ramos 2, Martha Enríquez 2, Estefany Mendoza 2, Ma Francisca Starck 2, Oliberto Sánchez 2, Emilio Lamazares 1, Eduardo Ramos 1, Carolina Muñoz 4, Raquel Montesino 1.

1. Laboratorio de Biotecnología y Biofarmacos, Departamento de Fisiopatología. Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, 4030000, Chile.
2. Grupo de Investigación en Sanidad Animal y Humana (GISAH), Departamento de Ciencias de la Vida y la Agricultura, Universidad de las Fuerzas Armadas ESPE, Sangolquí 171103, Ecuador.
3. Laboratorio de Biofármacos Recombinantes, Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, 4030000, Chile..
4. Laboratorio de Microbiología de Alimentos, Departamento de Patología y Medicina Preventiva, Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán 3780000, Chile.

Background: Infectious diseases of the respiratory tract have been diverse and recurrent in recent history, with reemergence of new viral strains exhibiting increased infectivity and dissemination, leading to public health crises with severe economic and social implications. Examples include the outbreak of a new strain of Influenza H1N1 that began in 2009 in Central America and the recent COVID-19 pandemic. In our country, we have a particular interest in the Cardiopulmonary Syndrome caused by Hantavirus infection. The Andes strain is endemic to Chile and Argentina, presenting a high mortality rate. In this context, our proposal focuses on developing and validating the production of IgY antibodies in eggs from laying hens as an effective, low-cost, and rapidly implementable platform to address infectious outbreaks affecting both human and animal health.

Method: For the immunization of the hens, recombinant Gn and Gc proteins from the Hantavirus capsid were expressed in a Pichia pastoris clone, solubilized, purified, and renatured. Three groups of hens were immunized with the protein antigen and the nucleotide sequence of the GnGc proteins. IgY antibodies were purified by polyethylene glycol (PEG) precipitation and analyzed by SDS-PAGE and western blot. Antigen recognition was performed by indirect ELISA, and additionally, a pseudotyped lentiviral vector was designed to evaluate the neutralizing effect of IgY antibodies.

Results: Gn and Gc proteins were purified with a purity greater than 90%, and 100 µg were used for hen immunization. Purification of IgY with PEG yielded a yield between 5 to 15 mg/ml of total protein from the yolk. Antibody titers against GnGc ranged from 1:16,000 to 1:64,000 over 30 days.

Conclusions: It was demonstrated that the IgY platform can produce antibodies against the antigen of interest with a stable yield for thirty days, i.e. thirty eggs per hens. In this production platform, it is possible to optimize the immunization processes of the animals, with the objective of increasing the number of eggs producing the antibody of interest.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

DEVELOPMENT OF GOLD NANORODS FUNCTIONALIZED WITH ANTI-MICA ANTIBODIES THROUGH FC PORTION CONJUGATION

Benjamín Riquelme 1,2, Marcelo Kogan 2, Ivo Campos 2,3, María Carmen Molina 3, Claudia Altamirano 1,4,5

1. Animal Cell Culture Laboratory, School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.
2. Nanomedicine and Nanotheranostics Laboratory, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Santiago, Chile.
3. Recombinant Antibodies and Immunotherapy Laboratory, Immunology Program, Institute of Biomedical Sciences, Universidad de Chile, Santiago, Chile.
4. Regional Center for Healthy Food Studies, Av. Universidad 330, Curauma-Placilla, Valparaíso, Chile.
5. IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.

Background: A platform that has gained relevance in cancer therapy is the use of gold nanorods (GNR) due to their unique optical and electronic properties and potential applications in biological imaging, molecular detection, and drug delivery, especially in photothermal therapy (PTT) for cancer and other diseases. Conjugating them with monoclonal antibodies (mAb) that recognize a biomarker, such as a cancer antigen, generates a nanoconstruct with specific targeting (active targeting). Directional conjugation can be achieved through the carbohydrate fraction found on one of the heavy chains of the Fc portion of most antibodies. This study aims to develop a nanoconstruct that conjugates the glycosylation of the Fc portion of anti-MICA IgG onto GNR using boronic acid (BA), seeking an antibody orientation that promotes high antigen capture.
Methodology: GNR will be synthesized by a seed-mediated method and pegylated with carboxylated polyethylene glycol (PEG-COOH) to be conjugated with anti-MICA through the bifunctional linker molecule 3-aminophenylboronic acid (APBA), where the amino group reacts with the carboxyl groups of PEG-COOH, and the boronic group reacts with the glycosylation at Asp 297 of the Fc portion of anti-MICA. A non-oriented conjugation methodology will be developed for comparison purposes. These nanoconstructs will be characterized by dynamic light scattering, zeta potential, UV-Visible spectrophotometry, and STEM. The recognition capacity of the nanoconstruct will be evaluated through direct ELISA on MICA-coated plates, measuring the optical density (O.D) of the gold by spectrophotometry.
Expected Results: It is expected to develop a reproducible synthesis of the GNR-anti-MICA nanoconstruct using BA, with increased antigenic recognition compared to a non- oriented conjugation method.
Conclusions: A gold-based nanoconstruct, conjugated with directionally oriented mAb via its Fc portion, shows up to a 30% improvement in antigen capture immunoassays compared to non-oriented methods. In this case, the anti-MICA mAb conjugated to GNR will allow selective recognition of MICA with high antigen capture values.
Acknowledgments: This thesis is funded by FONDECYT 1241453, ANILLO C&T ACT210068, and National Doctoral Scholarship 2023 2123202.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

PREFEASIBILITY STUDY ON LARGE-SCALE PRODUCTION OF SMALL EXTRACELLULAR VESICLES FROM GLYCOLITIC MESENCHYMAL STEM CELLS: POTENTIAL INJECTABLE THERAPY FOR OSTEOARTHRITIS

Daniela Canessa 1, María Fernanda González 1, Danae Aguirre 1, Patricia Luz-Crawford 2, Ana María Vega-Letter 1, Claudia Altamirano 1,2,3.

1. Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Avenida Brasil 2085, Valparaíso 2340000, Chile.
2. IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Av. Monseñor Álvaro del Portillo 12455, Las Condes, Santiago 7550000, Chile.
3. Centro Regional de Estudios en Alimentos Saludables, Av. Universidad 330, Curauma-Placilla, Valparaíso 2340000, Chile

Background: Osteoarthritis is characterized by chronic pain and reduced joint function due to cartilage degradation leading to disability and chronic pain. Current treatments, including physiotherapy, medications, and surgeries, are limited in their ability to regenerate tissue and are often accompanied by side effects and high costs. Mesenchymal stem cells (MSCs) offer a promising alternative due to their regenerative and chondroprotective properties. However, because cell therapy can have potential adverse effects, there is growing interest in cell-free therapies based on the cellular secretome. Among these, small extracellular vesicles (sEVs) derived from MSCs show significant promise. These vesicles can transport proteins and microRNAs, potentially replicating or even exceeding the therapeutic effects of MSCs. Recent studies have shown that inducing glycolysis in MSCs with oligomycin enhances the immunosuppressive and chondroprotective properties of their derived sEVs, improving their therapeutic efficacy for osteoarthritis. Despite this, challenges remain, including the lack of standardized processes and scalable production strategies. This study evaluates the technical and economic feasibility of producing sEVs from glycolytic MSCs for osteoarthritis treatment in Chile.

Methods: Through a market analysis, we estimate the future treatment demand, and evaluate competition, suppliers and possible institutions where this project could take place. Additionally, we assess production costs and select the necessary equipment. The process is designed following Food and Drug Administration guidelines and Good Manufacturing Practices regulations. We analyze and compare studies on the production of these extracellular derivatives to define the necessary operations to ensure safety, quality, and reproducibility.

Results: We propose mobile production units at hospitals like Hospital Universidad de Chile and Clínica Alemana for efficient product distribution. Our streamlined process is divided in: 1. Collection and expansion of mesenchymal stem cells; 2. Propagation in 3D culture systems, such as stirred tank bioreactors, to increase cell concentration and small extracellular vesicle production; 3. Separation and concentration using ultrafiltration and tangential flow filtration; 4. Formulation into soluble preparations with final 21 operations. Our approximation of GMP-standard cryovials, we can produce 21,274 doses of glycolytic sEVs, covering 75.8% of the projected 2035 demand for 28,053 patients.

Conclusions: The production of small extracellular vesicles from glycolytic mesenchymal stem cells is technologically feasible, with standardized procedures to guarantee the final product's quality and effectiveness. This method shows significant promise as an alternative treatment for osteoarthritis, potentially substantially impacting public health and the therapeutic market.

Founding: ANID Fondecyt Iniciación 11220549 and Regular 1211353, FONDEF ID 21I10194.

ACELULAR: SECRETOMA, MICROVESÍCULAS, VACUNAS Y PARTÍCULAS

EVALUATION OF FUNCTIONAL PARAMETERS IN A MICROREACTOR FOR LIPOSOME PRODUCTION

Víctor Cano 1, Aldonza Jaques 1, Monica Selva 2, Diego Benavente 3, Cristian Acevedo 2,3 and Yusser Olguín 2,3,4.

1. Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María
2. Centro de Biotecnología Daniel Alkalay (CBDAL), Universidad Técnica Federico Santa María
3. Centro Científico Tecnológico de Valparaíso (CCTVaL), Universidad Técnica Federico Santa María
4. Departamento de Química y Medio Ambiente, Universidad Técnica Federico Santa María

Background: The study explores the use of microfluidics for generating liposomes, which are vital for encapsulating various therapeutic agents such as drugs, proteins, cells, and genetic material. Traditional methods for liposome formation are divided into high-energy and low-energy techniques, with high-energy methods often damaging encapsulated elements. Therefore, there's significant interest in developing efficient low-energy methods, where microfluidic systems stand out due to their precision, versatility, efficiency, and safety. This study aims to evaluate different experimental conditions for producing liposomes using microfluidic chips. The study focuses on five variables: system geometry, total flow, flow ratios, phospholipid concentrations, and types of phospholipids.

Methods: The study utilized microfluidic chips with geometric configurations based on intersection angles of 45° (Y chips), 90° (T chips), and co-flow configurations. These chips were fabricated using PMDS and photolithography on silicon plates. The total flow in the systems was set at 200 µL/min with flow ratios ranging from 2.5 to 50. Phospholipid concentrations were fixed at 0.27 mM, with ratios of 2:1 and 3:1 for POPE and POPG. The study employed a model to relate liposome size with operational variables for potential industrial scaling.

Results: The study found that liposome sizes decreased with higher flow ratios between the phases, maintaining a constant total flow. The proportion of phospholipids significantly impacted the final liposome size, with a 3:1 ratio of POPE producing smaller liposomes due to intermolecular interactions. The COMSOL simulations helped optimize the experimental phase, providing a basis for understanding the relationship between flow dynamics and liposome formation.

Conclusions: The study successfully demonstrated that microfluidic systems could produce liposomes with controlled sizes by modulating flow ratios and phospholipid proportions. These findings suggest the potential for scaling up the production process for industrial applications, offering a low-energy, efficient method for generating liposomes suitable for various biomedical applications. Further research could focus on refining these techniques and exploring additional variables to enhance liposome production.

Acknowledgments: Fondecyt 1240757.

PATROCINADORES Y AUSPICIADORES

Agradecemos la participación de todos nuestros auspiciadores que apoyaron en la realización de X SLATCC 2024.

PATROCINADORES

PONTIFICIA UNIVERSIDAD CATÓLICA DE VALPARAÍSO

Agencia Nacional de Investigación y Desarrollo
Ministerio de Ciencia, Tecnología, Conocimiento e Innovación
Gobierno de Chile

UNIVERSIDAD DE CHILE

Universidad de Concepción

IMPACT
Center of Interventional Medicine for Precision and Advanced Cellular Therapy

InES I+D PUCV

FACULTAD DE INGENIERÍA

PONTIFICIA UNIVERSIDAD CATÓLICA DE VALPARAÍSO

PATROCINADORES

INVESTIGADORAS PUCV
PROYECTO InES GÉNERO

D&P
Anticuerpos
Monoclonales

ESCUELA DE
INGENIERÍA BIOQUÍMICA
PUCV

SOFOFA HUB

SCIENCE UP
CONSORCIO CIENCIA 2030 PUCV | USACH | UCN

ESACT

Biotech
Academy
In Rome

AUSPICIADORES

Categoría Perfusión

ARQUIMED INNOVACION

solventum

AP-Bioprocess
Biotechnology products and services

SARTORIUS

GENEXPRESS
Life Science Business

ThermoFisher SCIENTIFIC

GRUPOBIOS
Lo llevamos en nuestro ADN

MERCK

Platech
Platelet Technologies ®

AUSPICIADORES

Categoria Fed Batch

Cell Zion
SOLUCIONES EN INVESTIGACIÓN Y MEDICINA

GALENICA
SOMOS CIENCIA & TECNOLOGÍA

VectorBuilder

AUSPICIADORES

Categoria Batch

BioSystems

IZON

VETERQUIMICA®
CREANDO SALUD ANIMAL

DÉCIMO 2024

SLaTCC

SIMPOSIO LATINOAMERICANO DE TECNOLOGÍA DE CULTIVOS CELULARES