FBCB

UNL • FACULTAD DE BIOQUÍMICA Y CIENCIAS BIOLÓGICAS

IX SLATCC 2022

SIMPOSIO LATINOAMERICANO DE TECNOLOGÍA DE CULTIVOS CELULARES

IX Simposio Latinoamericano de Tecnología de Cultivos Celulares (IX SLATCC)

October 27-29, 2022
Santa Fe, Santa Fe Province

Centro Biotecnológico del Litoral

CONTENTS

IX SLATCC

SIMPOSIO LATINOAMERICANO DE
TECNOLOGÍA DE CULTIVOS CELULARES

BIENVENIDOS AL IX SLATCC

Estimados participantes:

Diez años han transcurrido desde el último SLATCC en Santa Fe y el 2022 se ha convertido en un año especial, que nos permite con gran placer darles la bienvenida a una nueva edición del Simposio organizado por el Centro Biotecnológico del Litoral (CBL) de la Facultad de Bioquímica y Ciencias Biológicas de la Universidad Nacional del Litoral.

Allá por 2019, comenzamos esta tarea con mucho entusiasmo, y luego un escenario mundial con una “larga sesión de virus”, hizo que tengamos que enlentecer esta iniciativa, pero nunca detenerla. Afortunadamente, las vacunas le pusieron límite a la pandemia y este año decidimos impulsar la IX edición de esta reunión latinoamericana (e iberoamericana) de colegas y de amigos.

En efecto, estamos alcanzando este anhelo a través de un grupo increíble, procedente de distintos países, que ha colaborado para que lleguemos a tiempo con esta propuesta que pronto tendremos el orgullo de compartir.

Estamos muy satisfechos de recibirlos en nuestra sede, donde realizaremos un intenso encuentro inmerso en diversas sesiones científicas con presentaciones orales, pósteres y una novedad, que consiste en una propuesta de participación colectiva que deseamos puedan aprovechar al máximo.

Agradecemos a todo el equipo del CBL, que ha puesto y pone todo el empeño y la mejor predisposición para garantizar el éxito del evento y para recibirlos con calidez.

Una Santa Fe primaveral, con el entorno de un paisaje fluvial dominado por la laguna Setúbal, nos dará el marco para un encuentro en el que también podamos brindar por un futuro cada vez más promisorio en nuestras latitudes.

¡Les damos la bienvenida!

Dra. Natalia Ceaglio **Dra. Marina Etcheverrigaray** **Dra. Guillermina Forno** **Dr. Marcos R. Oggero Eberhardt**

WELCOME TO IX SLATCC

Dear participants:

Ten years have passed since last SLATCC was held in Santa Fe. Year 2022 has turned into a special year, which allows us to welcome you with great pleasure to a new edition of the Symposium organized by the Biotechnological Center of Litoral from the School of Biochemistry and Biological Sciences from the Universidad Nacional del Litoral.

Back in year 2019 we started this task with great enthusiasm, and after a world scenario crossed by a long “virus session”, we were forced to slow down this initiative, but never stop it. Luckily, vaccines could restrain this pandemic, and this year we decided to give impulse to the IX edition of the Latin-American (and Ibero-American) meeting of colleagues and friends.

Indeed, we are achieving this dream through an amazing group from different countries, which has collaborated with us to get in time with this proposal that we will proudly share with all participants soon.

We are delighted to welcome you to our city venue, where we will develop an intense meeting designed around seven scientific topics with oral presentations, posters, and a novel proposal consisting in a collective construction of future that we wish you can all make the most of it.

Our deepest gratitude goes also to the complete CBL team for their continuous effort and willingness to guarantee the success of this event.

A spring Santa Fe city, embraced by a landscape dominated by the Laguna Setubal, will give us the perfect scenario for a great SLATCC meeting where our community will celebrate for a more promising future in our latitudes.

On behalf of the Organizing Committee, we welcome you and wish you all an outstanding SLATCC meeting!




Dra. Natalia Ceaglio **Dra. Marina Etcheverrigaray** **Dra. Guillermina Forno** **Dr. Marcos R. Oggero Eberhardt**

IX SLATCC COMMITTEES

ORGANIZING COMMITTEE

Natalia Ceaglio

Marina Etcheverrigaray

Guillermina Forno

Marcos Oggero-Eberhardt

ABSTRACT EVALUATION COMMITTEE

María de los Milagros Bürgi

Marina Etcheverrigaray

Diego Fontana

Agustina Gugliotta

Ricardo Kratje

Eduardo Mufarrege

Claudio Prieto

POSTER PRIZE COMMITTEE

Eduardo Mufarrege (Coordinator)

Mariela Bollati-Fogolín

María de los Milagros Burgüi

Agustina Gugliotta

Andrea Maranhão

Marianela Masin

María Carmen Molina

Claudia Ortega

Oliberto Sánchez

Aldo Tonso

DESIGN COMMITTEE

Leopold, María Jesús

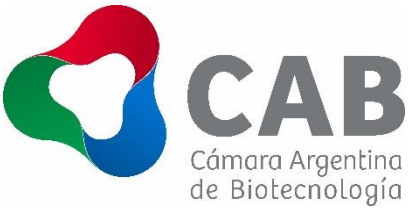
Wandel Petersen, Valentina

SPONSORS


CONICET


Agencia I+D+i
Agencia Nacional de Promoción
de la Investigación, el Desarrollo
Tecnológico y la Innovación


COLEGIO
SANTAFESINO
DE BIOTECNÓLOGOS


CAB
Cámara Argentina
de Biotecnología


Santa Fe
Provincia


ESACT


ThermoFisher
SCIENTIFIC


SARTORIUS


cytiva


MERCK

SPONSORS


PALL
PALL CORPORATION


VIGENIUS
BIOTECH


lobov
científica


INFORS HT


EmbioTec
EMPRESA DE BIO TECNOLOGÍA
info@embiotec.com.ar | www.embiotec.com.ar


Roche


TOSOH
TOSOH BIOSCIENCE

INVITED SPEAKERS

Francesc Gòdia

Full Professor of Chemical Engineering at Universitat Autònoma de Barcelona (UAB) since 1993. Teaching activities in Biotechnology and Chemical Engineering. Coordinator of the Biotechnology Doctoral Program at UAB. His research activity is focused on Animal Cell Technology for the production of biopharmaceuticals, recombinant vaccines and vectors for gene therapy and Bio-Regenerative Life Support Systems in Space. Overall Manager of the MELiSSA Pilot Plant a joint facility of UAB and ESA (European Space Agency). Author of more than 125 papers in JCR journals, 5 patents, 9 book chapters and advisor of 37 completed PhD thesis. Member of the Scientific and Organizing Committee of several International Congresses. Coordinator of the ESACT International Courses on Animal Cell Technology and Cell-based Viral Vaccines. Vice-President of the European Federation of Biotechnology. Member of the Executive Board of the European Society of Animal Cell Technology.

Oliberto Sánchez Ramos

Microbiologist and doctor in biological sciences from the University of Havana. His professional life has been dedicated to developing biopharmaceuticals, vaccines, and diagnostic systems, as well as the establishment of processes for their production. He is currently a Full Professor at the Universidad de Concepción and directs its Recombinant Biopharmaceuticals Laboratory. Dr. Sánchez is the author of more than 56 scientific publications and 16 invention patents in recombinant protein production.

Octavio Tonatiuh Ramírez Reivich

Professor at the Biotechnology Institute of the National Autonomous University of Mexico, serving as its director from 2013 to 2021. His research interests include applications of bioengineering principles to improve bioprocesses through better understanding of fundamental phenomena; cellular and molecular responses to environmental gradients in industrial reactors; metabolic engineering of bacterial and animal cells; and production of glycoproteins and virus-like particles for use as therapeutics, vaccines and bio-nanomaterials. He has established milestones producing and characterizing biopharmaceuticals through close collaborations and technology transfers with many companies and organizations. He has more than 150 publications and 7 patents and has graduated 53 students.

Lucas Filgueira Risso

Biochemist (UBA). He worked in development and production of biopharmaceuticals in national companies: PC-Gen SA (production manager) and GEMA Biotech (development manager). In 2008, he co-founded pharmADN, today mAbxience, a pioneer company in the production of biosimilar monoclonal antibodies by single use systems in the region. Currently, he works as Site Manager of the two manufacturing facilities of mAbxience in Buenos Aires. He directed and participated in projects in the field of process development, scale-up, process engineering and capacity expansion. Also he was involved in technology transfer projects with Germany, China, Turkey, Russia, Denmark, Brazil, Paraguay, Mexico, EU and USA.

Leda Castilho

Professor for Chemical Engineering and Pharmaceutical Biotechnology and coordinates the Cell Culture Engineering Laboratory of COPPE, at the Federal University of Rio de Janeiro (UFRJ). Her research focuses on the development of efficient technologies for the production of recombinant proteins by animal cell culture, with the aim of developing biotherapeutics, diagnostic tools and vaccines. She has organized two books and published many scientific articles, book chapters and one book. She has been an affiliated member of the Brazilian Academy of Sciences (2008-2013). Her research group has been heavily involved in the development of Covid-19 countermeasures.

Andrea Queiroz Maranhao

PhD in Molecular Biology. Fields of study: antibody engineering, including humanization and expression in bacteria, yeast and mammalian cells. Has experience in Phage Display library construction and selection aiming new human monoclonal antibodies against cell antigens and pathogens for therapeutic purposes in cancer, autoimmune and infection diseases. Recently, joined a CAR-T Cell project where new scFvs are being developed to treat leukemia.

Juliana Cassataro

Dr. Juliana Cassataro focuses her research on vaccine development against infectious diseases. She holds a degree in Biological Sciences from the National University of Mar del Plata and a PhD in Immunology from the University of Buenos. She is at present Principal Researcher from the National Council for Scientific and Technical Research (CONICET-ARGENTINA) and Associate Professor at UNSAM.

Catarina Brito

Principal Investigator, heading the Advanced Cell Models Lab of the Animal Cell Technology Unit of iBET and ITQB-NOVA since 2014. Her Lab develops human cell models, employing 3D co-culture strategies, induced pluripotent stem cells and other patient-derived cells, to depict the deregulation of the cellular microenvironment in disease progression and treatment response. Key areas of interest include the innate immune microenvironment in carcinomas and neurological disorders, and immunotherapies.

Marcelo Grabois

Professor of Intellectual Property, Strategic Intelligence and Innovation Management. ISO Expert in ISO TC 279 - Innovation Management Director of the Strategic and Technological Intelligence Program at UNL. CEO of ITERA, that offers services to technology-based companies in the management of R+D+i and the valorization of inventions. Chemical Engineer and Patent Agent.

Diego Fontana

Biotechnologist and PhD in Biological Sciences for the Universidad Nacional del Litoral (UNL), Argentina. Currently, Assistant Researcher in the National Council for Scientific and Technical Research (CONICET) and teacher at the School of Biochemistry and Biological Sciences, UNL. Main research focuses on the development of recombinant viral vaccines and other novel biotech products for animal health, using animal cells as a platform to produce recombinant proteins.

IX SLATCC SCHEDULE

	THURSDAY 27	FRIDAY 28	SATURDAY 29
08:30 – 09:00	**REGISTRATION**	**SESSION 3: BIOTHERAPEUTICS: DEVELOPMENT AND QUALITY CONTROL** Chair: Ricardo Kratje (Universidad Nacional del Litoral-CONICET, Argentina) **Plenary lectures:** Andrea Queiroz Maranhão (Universidade de Brasília, Brasil) Lucas Filgueira Risso (mAbxience, Argentina) **Oral presentation of selected abstracts**	**SESSION 7: INNOVATIONS AND CHALLENGES** Chair: Andrea Queiroz Maranhão (Universidade de Brasília, Brasil) **Oral presentation of selected abstracts**
09:00 – 09:30	**OPENING SESSION** Welcome from IX SLATCC organizers Dean of Facultad de Bioquímica y Ciencias Biológicas - Universidad Nacional del Litoral (FBCB-UNL)		
09:30 – 10:30	**KEYNOTE LECTURE** Francesc Gòdia (Universitat Autònoma de Barcelona, Spain)		**"Future of cell technologies". Invitation to participate in a coffee space and collective construction of possible future scenarios.** Coordinator: Marcelo Grabois (Universidad Nacional del Litoral/ ITERA SRL, Argentina)
10:30 – 11:00	**COFFEE BREAK** **EXHIBITOR/SPONSOR STANDS**	**COFFEE BREAK** **POSTER SESSION (ODD NUMBERS)** **EXHIBITOR/SPONSOR STANDS**	
11:00 – 11:30			**COFFEE BREAK**
11:30 – 12:00	**SESSION 1: RECOMBINANT CELL LINE DEVELOPMENT - GENOME EDITION** Chair: Mariela Bollati-Fogolín (Instituto Pasteur de Montevideo, Uruguay) **Plenary lecture:** Oliberto Sánchez Ramos (Universidad de Concepción, Chile) **Oral presentation of selected abstracts**	**SESSION 4: VACCINES I: COVID19** Chair: Francesc Gòdia (Universitat Autònoma de Barcelona, Spain) **Plenary lectures:** Juliana Cassataro (Universidad Nacional de San Martín-CONICET, Argentina) Leda Castilho (Universidade Federal do Rio de Janeiro, Brasil) **Oral presentations of selected abstracts**	**NEW TRENDS IN CELL TECHNOLOGIES** Gustavo Lorca - Thermo Fisher Scientific. Juliana Groba - Pall Technologies
12:00 – 12:30			
12:30 – 13:00			**POSTER PRIZE AWARD** **CLOSING SESSION** IX SLATCC organizers
13:00 – 14:30	**LUNCH**	**LUNCH**	**LUNCH**
14:30 – 16:00	**UPDATES IN UPSTREAM AND BIOANALYTICS APPLIED TO BIOTECHNOLOGY PROCESSES** Fernando Carmona - Cytiva Lucía Cragnaz - Sartorius Esteban Salvatore - Sartorius	**SESSION 5: VACCINES II** Chair: Leda Castilho (Universidade Federal do Rio de Janeiro, Brasil) **Plenary lecture:** Diego Fontana (Universidad Nacional del Litoral-CONICET, Argentina) **Oral presentations of selected abstracts**	
16:00 – 17:00	**COFFEE BREAK** **POSTER SESSION (EVEN NUMBERS)** **EXHIBITOR/SPONSOR STANDS**	**COFFEE BREAK** **POSTER SESSION (EVEN AND ODD NUMBERS)** **EXHIBITOR/SPONSOR STANDS**	
17:00 – 17:30	**MONOCLONAL ANTIBODY PURIFICATION FROM GRAM TO KILOGRAM USING MULTI-COLUMN CHROMATOGRAPHY.** Kevin J. ODonnell - Tosoh Biosciences	**IMPROVE CELL LINE TITER FOR MONOCLONAL ANTIBODY PRODUCTION.** Mariana Curcio - Thermo Fisher Scientific	
17:30 – 19:00	**SESSION 2: CELL METABOLISM AND OPTIMIZATION OF CELL CULTURE CONDITIONS** Chair: Claudia Altamirano (Pontificia Universidad Católica de Valparaíso, Chile) **Plenary lecture:** Octavio Ramírez (Universidad Nacional Autónoma de México, México) **Oral presentations of selected abstracts**	**SESSION 6: DEVELOPMENT OF CELL-BASED TECHNOLOGIES AND THERAPIES** Chair: Eduardo Mufarrege (Universidad Nacional del Litoral-CONICET, Argentina) **Plenary lecture:** Catarina Brito (iBET/ITQB NOVA, Portugal) **Oral presentations of selected abstracts**	
19:30	**WELCOME COCKTAIL**		

SCIENTIFIC PROGRAM

THURSDAY 27TH OCTOBER

09:00 – 09:30 **IX SLATCC Opening session**

Welcome from IX SLATCC organizers and from the Dean of Facultad de Bioquímica y Ciencias Biológicas (FBCB), Universidad Nacional del Litoral (UNL).

9:30 – 10:30 **Keynote lecture**

Cell Culture Technology, Biopharmaceuticals and Biomedicine
Francesc Gòdia
Universitat Autònoma de Barcelona, Spain

11:00 – 13:00 **Session 1: Recombinant cell line development. Genome edition.**

Chair: Mariela Bollati-Fogolín
Unidad de Biología Celular, IPMont, Montevideo, Uruguay

Plenary lecture:

Direct transduction of the mammary glandular epithelium: An approach to produce recombinant proteins in milk
Oliberto Sánchez Ramos
Universidad de Concepción, Chile

Oral presentation of selected abstracts:

New endogenous promoters for recombinant expression discovered in CHO cells by RNA-seq. Transient and stable characterization
Agustina Gugliotta
Centro Biotecnológico del Litoral (CBL), FBCB, UNL / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Effects of human growth factors TGF-β1 and PDGF-BB stable heterologous expression and exogenous addition over the growth performance of CHO and HEK cell lines in adherent culture
Aldo Tonso
Universidade de São Paulo, Brazil

Development of a CHO cell line for production of an anti-MICA human monoclonal antibody by RMCE technique
José Rodríguez
Laboratorio de Anticuerpos Monoclonales Recombinantes e Inmunoterapia, Facultad de Medicina, Universidad de Chile, Chile

Increasing transduction efficiency in mammals: development of a stable sf9 insect cell line for the production of G-VSV pseudotyped baculoviruses
María del Pilar Plastine
Instituto de Agrobiotecnología y Biología Molecular (IABIMO), Instituto Nacional de Tecnología Agropecuaria (INTA), CONICET, Argentina

14:30 – 16:00 **Updates in upstream and bioanalytics applied to biotechnology processes**

Specialized media for cell culture
Fernando Carmona - MsC | Especialista de Productos
Cytiva

Latest developments in culture media and cutting-edge technologies for their evaluation
Lucía Cragnaz
Sartorius

Bioanalytic characterization of molecules throughout the production process
Esteban Salvatore
Sartorius

16:00 – 17:00 **Poster session (even numbers)**
Exhibitor/Sponsor stands

17:00 – 17:30 *Monoclonal antibody purification from gram to kilogram using multi-column chromatography*
Kevin J. ODonnell - PhD | R&D Manager -Tosoh Biosciences

17:30 – 19:00 **<u>Session 2</u>: Cell metabolism and optimization of cell culture conditions**
Chair: Claudia Altamirano
Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Chile

Plenary lecture:
Overexpression of the mitochondrial pyruvate carrier for increased metabolic efficiency and recombinant protein productivity in CHO cell cultures
Octavio Ramírez
Instituto de Biotecnología (IBt), Universidad Nacional Autónoma de México (UNAM), México

Oral presentation of selected abstracts:
Potential use of fish collagen matrix to evaluate tubulogenesis in vitro
Daiana Medina
Laboratorio de Investigación en Proteínas (LabInPro), IQUIBA-NEA CONICET, Universidad Nacional del Nordeste, Argentina

Development of a dynamic SH-SY5Y 3d culture model for biological evaluation of Alzheimer-induced pathology
Macarena Fernández
Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Chile

Development of a model-based cell culture media design platform for biomass and product optimization: current results and future perspective

Ziomara Gerdtzen
Centro de Biotecnología y Bioingeniería (CeBiB), Departamento de Ingeniería Química, Biotecnología y Materiales, Universidad de Chile, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Núcleo Milenio de Agronomía Marina, Chile

FRIDAY 28TH OCTOBER

08:30 – 10:30 **Session 3: Biotherapeutics: development and quality control**

Chair: Ricardo Kratje
Centro Biotecnológico del Litoral (CBL), FBCB, UNL / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Plenary lectures:

Isolation of therapeutic antibodies
Andrea Queiroz Maranhão
Universidade de Brasília, Brazil

Manufacturing of Biological Products in Argentina nowadays: Challenges and Possibilities
Lucas Filgueira Risso
mAbxience, Argentina

Oral presentation of selected abstracts:

Bioactivity characterization of IFN-β biosimilar candidates through a multiplexed gene expression platform
Lucía Peña
Centro Biotecnológico del Litoral (CBL), FBCB, UNL / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

In vitro and in vivo characterization of the binding capacity of a fully human anti-MICA antibody to the α1 non-polymorphic region of MICA
Samantha Tello Aguayo
Laboratorio de Anticuerpos Recombinantes e Inmunoterapia, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile, Chile

From erythropoiesis to neuroprotection: on the way of a new erythropoietin-based biotherapeutic
Milagros Bürgi
Centro Biotecnológico del Litoral (CBL), FBCB, UNL / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

10:30 – 11:30 **Poster session (odd numbers)**
Exhibitor/Sponsor stands

11:30 – 13:00 **Session 4: Vaccines I: COVID19**

Chair: Francesc Gòdia
Universitat Autònoma de Barcelona, Spain

Plenary lectures:

Development of a vaccine for boosters and adapted to the new variants of SARS-CoV-2 that can be produced in Argentina
Juliana Cassataro
Instituto de Investigaciones Biotecnológicas (IIBIO) - CONICET, Argentina

Development of a trivalent vaccine based on the recombinant spike protein of Covid-19 variants
Leda Castilho
Universidad Federal de Río de Janeiro, Brazil

Oral presentation of selected abstracts:

Production and purification of SARS-CoV-2 soluble antigens, potential tools for the development of diagnostic tests and vaccine candidates
Javier Villarraza
Centro Biotecnológico del Litoral (CBL), FBCB, UNL / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

14:30 – 16:00 **Session 5: Vaccines II**

Chair: Leda Castilho
Universidad Federal de Río de Janeiro, Brazil

Plenary lecture:

From the academy toward the industry: Rhabdo-like recombinant (VLPs) a novel recombinant rabies vaccine for animal health
Diego Fontana
Centro Biotecnológico del Litoral (CBL), FBCB, UNL / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Oral presentation of selected abstracts:

Biological recombinant adjuvant for viral diseases in aquaculture
Leonardo Alexis Ortega
Laboratorio de Biotecnología y Biofármacos, Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Chile

Optimization of the production of GaHV-1 in LMH using T-flask and scaling to Cellcube-Bioreactor System
Juana Quispe
Laboratorio de Virología Molecular. QUIMTIA S.A., Perú

In-process controls for the evaluation of harvest point of Zika virus in a fixed-bed bioreactor
Renato Mancini Astray
Laboratório Multipropósito, Instituto Butantan, Brazil

16:00 – 17:00 **Poster session (even and odd numbers)**
Exhibitor/Sponsor stands

17:00 – 17:30 *Improve cell line titer for monoclonal antibody production*
Mariana Curcio - PhD | Cell Culture Specialist
Thermo Fisher Scientific Latam

17:30 – 19:00 **Session 6: Development of cell-based technologies and therapies**

Chair: Eduardo Mufarrege
Centro Biotecnológico del Litoral (CBL), FBCB, UNL / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Plenary lecture:

3D Culture Strategies to Model Cell Microenvironment in Disease and Therapeutic Response
Catarina Brito
Instituto de Biología Experimental e Tecnológica (iBET)/ ITQB NOVA, Portugal

Oral presentation of selected abstracts:

Conditioned medium derived from murine BM-MSCs cultured as spheroids exhibit in vitro immunomodulatory capacity

Paloma Fuentes
Escuela de Ingeniería Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso, Chile

Mammal intestinal organoids for studying zoonotic pathogens

Saira Cancela
Unidad de Biología Celular, Programa de Tecnología Molecular, Celular y Animal (ProTeMCA), Instituto Pasteur de Montevideo, Uruguay

Preliminary evaluation of cell-based carriers of novel conjugated polymer nanoparticles with antitumoral action

Lucía Beaugé
Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA), Universidad Nacional de Río Cuarto (UNRC), CONICET, Argentina

SATURDAY 29TH OCTOBER

08:30 – 09:00 **<u>Session 7</u>: Innovations and challenges**

Chair: Andrea Queiroz Maranhão
Universidade de Brasília, Brazil

Oral presentation of selected abstracts:

Development of a platform based on the cooperative adsorption of monoclonal antibodies on gold nanoparticles
Ivo Campos
Laboratorio de Anticuerpos Recombinantes e Inmunoterapia, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile, Chile

Engineering PCV2's VLP as a new and adaptable biotechnological platform for vaccine development
Natalia Olivero
Laboratorio de Inmunovirología, Instituto Pasteur de Montevideo, Uruguay

09:00 – 11:00 *"Future of cell technologies". Invitation to participate in a coffee space and collective construction of possible future scenarios*
Marcelo Grabois
Facultad de Ingeniería Química (FIQ), UNL, ITERA SRL, Argentina

11:30 – 12:30 **New trends in cell technologies**

How to generate physiologically relevant cell models? New technologies and trends for the future
Gustavo Lorca
Thermo Fisher Scientific

The iCELLis® Nano Bioreactor Provides a Reliable Method to Produce Viral Vaccines with High Titer and High Potency
Juliana Groba
Pall Technologies

12:30 – 13:00 **Poster Prize Award**

Closing session
IX SLATCC organizers
Vice-dean FBCB UNL

POSTER PRESENTATIONS

S1- Recombinant cell line development. Genome Edition

S1-1 **New endogenous promoters for recombinant expression discovered in CHO cells by RNA-seq. Transient and stable characterization**
Gugliotta, A.; Tossolini, I.; Lopez Díaz, F.; Kratje, R.; Prieto, C.

S1-2 **Applicated bioengineering for improvement the specific and volumetric productivity in CHO cells, difficult to expres protein (DTEP) producer**
Latorre Y.; Molina M.C.; Altamirano C.

S1-3 **Characterization of CHO cells overexpressor of c-Myc and its relationship with mTOR pathway in the production of recombinant protein**
Núñez-Soto, C., Altamirano, C.

S1-4 **Effects of human growth factors TGF-β1 and PDGF-BB stable heterologous expression and exogenous addition over the growth performance of CHO and HEK cell lines in adherent culture**
Reinhardt Tagliani, M.; Sogayar, M.C.; Oliveira Carreira, A.C.; Tonso, A.

S1-5 **Direct transduction of the mammary glandular epithelium: An approach to produce recombinant proteins in milk**
Sanchez Ramos, O.; Toledo Alonso, J.R.; Camacho Casanova F.; Parra Pereira, N.C.; Reyes López, F.; Acosta Alba, J.

S1-6 **Selection of MDCK cell clone for Influenza Virus multiplication**
Pereira, N.A.; Ferreira Barbosa, F.; Perini de Araújo, A.P.; de Lima Paoli, R.; Mancini Astray, R.

S1-7 **Mammalian cell line development strategies for recombinant protein expression using reporter genes "in cis"**
Mussio, P.; Gastaldi, V.; Gugliotta, A.; Rodríguez, M.C.; Prieto, C.

S1-8 **Development a producer line cho cell of a monoclonal human antibody anti mica by rmce technique**
Latorre-Aguirre, Y.; Rodriguez-Siza, J.; Toledo-Stuardo, K.; Dubois-Camacho, K.; Tello-Aguayo, S.; Vergara-Castro, M.; Molina, M.C.; Altamirano, C.

S1-9 **Insect cell lines capable of endowing chitinase ChiA to AcMNPV baculovirus occlusion bodies for improving their bioinsecticidal properties**
López, M.G.; Barros, G.; Victoria Alfonso A.; Oscar, T.

S1-10 **Trans-complementation of ac109 knock out baculoviruses by trangenic insect cell lines for the production of virion-free recombinant proteins**
Amalfi, S.; Mattera, R. ; Plastine, M.P.; Lopéz, M.G.; Taboga, O.; Alfonso, V.

S1-11 **Increasing transduction efficiency in mammals: development of a stable sf9 insect cell line for the production of G-VSV pseudotyped baculoviruses**
Plastine, M. del P.; Amalfi, S.; López, M.G.; Taboga, O.; Alfonso, V.

S1-12 **Expression of a recombinant cytokine in Escherichia coli**
Villavicencio, C.; Gutiérrez, N.; Hidalgo, A.; Llamazares, E.; Ramos T.; Toledo, J.R.

S2- Cell metabolism and optimization of cell culture conditions

S2-13 **Potential use of fish collagen matrix to evaluate tubulogenesis in vitro**
Medina, D.; Acevedo Gomez, A.; Leiva, L.; Pellegrini Malpiedi, L.; Bustillo, S.

S2-14 **Development of a rh-EPO production strategy in c-Myc overexpressing CHO cells in batch culture, based on lactate re-metabolization**
Gatica, O.; Vergara, M.; Nuñez, C.; Collarte, C.; Vergara, S.; Torres, M.; Latorre, Y.; Valdez-Cruz, N.; Altamirano, C.

S2-15 **Mixture-design optimization of culture medium: towards a more economical, reproducible and scalable in vitro mAb production process**
Wandel-Petersen, V.; Kratje, R.; Bürgi, M.; Oggero-Eberhardt M.

S2-16 **Toxicity evaluation of pecan nutshell bioactives against cho-k1 and mda-mb 231 cell lines: cytotoxicity, cell cycle arrest and apoptosis**
Gasser, F.B.; Ribas, L.E.; Renna, M.S.; Marelli, B.; Ortega, H.H.; Hein, G.J.; Baravalle, M.E.

S2-17 **Development of a dynamic SH-SY5Y 3D culture model for biological evaluation of alzheimer-induced pathology**
Fernández, M.; Arancibia, A.; Núñez, C.; Torres, M.; Vergara, M.; Altamirano, C.

S2-18 **Modification of the assimilative metabolism of Pichia pastoris for the improvement of tolerance to methanol and the productivity of recombinant protein**
Guerrero, K.; Quezada, J.; Veas, R.; Altamirano, C.; Fickers, P.; Berrios, J.

S2-19 **Optimization and characterization of the production of a fully human anti-MICA antibody in Chinese hamster ovary (CHO) cells**
Garrido, M. J.; Latorre, Y.; Toledo, K.; Tello, S.; Gatica, O.; Altamirano, C.; Molina, M.C.

S2-20 **Cellular biotechnology strategies to improve the production of bovine follicle stimulating hormone in Chinese hamster ovary cells**
Araya, D.; Latorre, Y.; Gödecke, N.; Vergara, M.; Toledo, J.; Altamirano, C.

S2-21 **Metabolic profile characterization of mesenchymal stem cells in a microcarrier suspension culture**
Sánchez-Daza, A.; Gonzalez, E.; Gerdtzen, Z.P.; Andrews, B.A.; Caviedes, P.; Asenjo, J.A.

S2-22 **Mimicking dynamic in vivo environment by 3D cell laden culture: in house model**
Martinez Marignac, V.L.; Vidal, J; Oertlin, G.; Mostoslavsky, G.; Richard Silvina, M.

S2-23 **Enhancement of Human Adipose derived Stem Cells proliferation by pyruvate and nicotinamide supplementation**
Molina Burgos, T.; Sánchez-Daza, A.; Gerdtzen, Z.P.; Andrews, B.A.; Caviedes, P.; Asenjo, J.A.

S2-24 **Electro-fermentation of sugars to organic acids and alcohols using Clostridium autoethanogenum**
Martínez Ruano, J.A.; Suazo, A.I.; Veliz de la Vega, F.A.; Otálora, F.C.; Conejeros Risco, R.; González Romo, E.; Aroca, G.A.

S2-25 **Development of a model-based cell culture media design platform for biomass and produ optimization: current results and future perspective**
Gerdtzen, Z.; Villanueva, A.; Narváez, D.; Morales, D.; Carvajal, B.; Quiroga-Campano, A.L.; Cristian Salgado, J.

S3- Biotherapeutics: development and quality control

S3-26 **Development and characterization of interleukin 2 chimeric mutein, with high antineoplastic activity and reduced systemic toxicity**
Lagos, P.; Sánchez, O.

S3-27 **Tools for determining the binding affinity of therapeutic antibodies in solution**
Toledo-Stuardo, K.; Gómez, H.; Garcia, E.; Valdés, A.; Guerra, D.; Fuentealba, D.; Matties, D.; Zapata, G.; Altamirano, C.; Molina, M.C.

S3-28 **CHO vs. HEK cell lines: impact on antigen-binding and neutralizing activity of engineered scFv-Fc antibodies**
Aguilar, M.F.; Attallah, C.; Etcheverrigaray, M.; Oggero, M.

S3-29 **Development of funcional de-immunized versions of interferon alpha for the treatment of emerging viral infections**
Giorgetti, S.; Etcheverrigaray, M.; Terry, F.; Martin, W.; DeGroot, A.; Ceaglio, N.; Oggero Eberhardt, M.; Mufarrege, E.

S3-30 **Biotherapeutic potencial of a biosurfactant extract obtained from *Pseudomonas syringae pv tabaci***
Martini, G.; Nerli, B.; Bustillo, S.; Pellegrini Malpiedi, L.

S3-31 **The Production of Biopharmaceuticals in the Public Laboratories of Argentina**
Burgardt, N. I.

S3-32 **Galectin expression in mammalian cell system**
Estruch, B.; Prato, C.A.; Tribulatti, M.V.

S3-33 **Two novel hGM-CSF-derived peptides to improve both the properties and the production processes of biotherapeutics**
Leopold, M. J.; Kratje, R.; Oggero Eberhardt, M.; Ceaglio, N.

S3-34 **Bioactivity characterization of IFN-β biosimilar candidates through a multiplexed gene expression platform**
Peña, L.; Ricotti, S.; Haile, L.; Etcheverrigaray, M.; Verthelyi, D.; Mufarrege, E.

S3-35 **Selection of a mini antibody against the alfa 2-domain of the MICA protein by Phage Library**
González, M.; Tello, S.; Rodríguez, J.; Molina, M.C.

S3-36 ***In vitro* and *in vivo* characterization of the binding capacity of a fully human anti-MICA antibody to the α1 non-polymorphic region of MICA**
Tello, S.; Farías, C.; Gutiérrez, M.; Toledo, K.; Siza, J.R.; Jerez, B.; Ribeiro, C.H.; Molina, M.C.

S3-37 **Production of complex recombinant proteins in *Komagataella phaffii*: Presence of de pro-domain and its influence on secretion of BMP2 and tPA**
Veas, R.; Quezada, J.; Guerrero, K.; Altamirano, C.; Fickers, P.; Osses, N.; Berrios, J.

S3-38 **From erythropoiesis to neuroprotection: on the way of a new erythropoietin-based biotherapeutic**
Bürgi, M.; Wandel-Petersen, V.; Depetris, M.; Aparicio G.; Scorticati C.; Arinrad S.; Ehrenreich H.; Kratje R.; Oggero-Eberhardt M.

S3-39 **Production of new hyperglycosylated-EPO analogs using CHO.K1 and HEK-293 cell lines**
Iturraspe, F.; Bürgi, M.; Kratje, R.; Oggero Eberhardt, M.

S3-40 **Development Of a Novel Cell Line Producing Recombinant Human Chorionic Gonadotropin (rhCG) For The Veterinary Industry**
Cervetti, J.; Gugliotta, A.; Antuña, S.; Fontana, D.; Prieto, C.

S3-41 **The neuroprotective effects of purified spent coffee ground extracts obtained of the SSF in a culture of SH-Sy5y induced Alzheimer disease model**
Arancibia-Diaz, A.; Vergara-Castro, M.; Astudillo-Castro, C.; Soto-Maldonado C., Altamirano-Gómez C., Zúñiga-Hansen M.E.

S3-42 **Cell culture production of a recombinant bovine follicle-stimulating hormone (rbFSH) and a hyperglycosylated variant**
Villarraza, J.; Tardivo, M.B.; Antuña, S.; Díaz, P.; Ortega, H.; Prieto, C.; Ceaglio, N.

S3-43 **Viral safety in cell banks used to produce biological products**
Franco, L.; Becerra, M.L.; Pérez López, J.; Salazar, D.; Politzki, R.; Ruiz, V.; Alvarez, I.

S3-44 **Characterization of a ScFV a-ST2: a new perspective for Inflammatory Bowel Disease study**
Vásquez G.; Dubois-Camacho, K.; De la Fuente, M.; Novoa, P.; Gutiérrez, M.; Sánchez, O.; Molina, M.; Hermoso, M.A.

S4- Vaccines I: COVID-19

S4-45 **Development of a quantitative sandwich ELISA as a tool for RBD bioprocess optimization**
Acuña Intrieri, M.E.; Deriane, M.A.; Rey Serantes, D.A.; Czibener, C.; Goldbaum, F.A.; Comerci, D.J.; Cerutti, M.L.

S4-46 **Development of clonally-derived HEK293 cell lines expressing trimeric spike protein of SARS-COV-2 variants for the development of a multivalent COVID-19 vaccine candidate**
Marsili, F.F.; Alvim, R.G.F.; Silva, T.G.; Castilho, L.R.

S4-47 **Design, optimization and validation of a RBD indirect ELISA for detecting antibodies against SARS-CoV-2**
Rodríguez, M.C.; Ceaglio, N.; Fuselli, A.; Villarraza, J.; Garay, E.; Antuña, S.; Gastaldi, V.; Fontana, D., Gugliotta, A.; Prieto, C.

S4-48 **Development, characterization and validation of a sensitive an low-cost method to detect antibodies anti SARS-Cov-2**
Soldano, N.; Raimondi, A.; Vogt, L.; Shaurli, D.; Bosco, B.; Amadeo, I.; Forno, G.

S4-49 **Analysis of the concentration step of SARS-CoV-2 produced in Vero cells in serum-free medium for antigen production**
Perini de Arújo, A.P.; Rodrigues Ueoka, A.; Ferreira Barbosa, F.; Mancini Astray, R.

S4-50 **Production and purification of SARS-CoV-2 soluble antigens, potential tools for the development of diagnostic tests and vaccine candidate**
Villarraza, J.; Fuselli, A.; Gugliotta, A.; Garay, E.; Antuña, S.; Gastaldi, V.; Tardivo, B.; Rodríguez, M. C.; Alvarez, D.; Castro, E.; Cassataro, J.; Fontana, D.; Ceaglio, N.; Prieto, C

S4-51 **A novel Rabies/SARS-CoV-2 fusion glycoprotein for vlps expression: a potential vaccine candidate for COVID19**
Garay, E.; Fontana, D.; Villarraza, J., Fuselli, A.; Gugliotta, A.; Antuña, S.; Gastaldi, V.; Tardivo, B., Rodríguez, M.C.; Ceaglio, N.; Prieto, C.

S5- Vaccines II

S5-52 **Biological recombinant adjuvant for viral diseases in aquaculture**
Ortega Rivas, L.A.

S5-53 **Early and Solid Protection Afforded by the Thiverval Vaccine Provides Novel Vaccination Alternatives Against Classical Swine Fever Virus**
Lamothe-Reyes, Y.; Bohórquez, J.A.; Wang, M.; Alberch, M.; Pérez-Simó,M., Rosell, R.; Ganges,L.

S5-54 **A novel display platform for vaccine applications based on chimeric vlps produced in cell suspension mode and serum free medium**
Garay, E.; Fontana, D.; Leschiutta, L., Kratje, R.; Prieto, C.

S5-55 **Optimization of the production of GaHV-1 in LMH using T-flask and scaling to Cellcube-Bioreactor System**
Quispe, J.; Flores-Santos, J.C.; Caballero, M.; Mendoza-Espinoza, A.

S5-56 **In-process controls for the evaluation of harvest point of Zika virus in a fixed-bed bioreactor**
Barbosa, F.F.; Perini de Araújo, A.P.; Fongaro Botosso, V.; Mancini Astray, R.

S5-57 **The iCELLis® Nano Bioreactor Provides a Reliable Method to Produce Rabies Vaccines with High Titer and High Potency**
Groba, J.; Lundeen, T.

S5-58 **Baculovirus AcMNPV genome modification with the GPC fragment of the Andes virus to generate a pseudotyped baculovirus as a new biotechnology tool against HCPS**
Jerez, B.; Camacho, F.; Sánchez, O.

S5-59 **Development of a biotechnological platform for vaccine production based on VLP using porcine circoviruses recombinant protein**
Gastaldi, V.; Garay, E.; Fontana, D.; Prieto, C.

S5-60 **Analysis of the performance of working cell bank in the production of Candid #1, a live attenuated Junin Virus Vaccine**
Mogetta, M.H.; Maiza, A.; Arias, A.; Abalo, M.; Riera, L.; Saavedra, C.

S6- Development of cell-based technologies and therapies

S6-61 **Cytotoxic analysis of in vitro pulverized endodontic files – clinical correlation with the occurrence of endodontic file fracture**
Soimu, G.; Carvalho da Silva, G.C.; Oliveira Freitas Lione, V.; Voight, D.; Heggendorn, F.L.

S6-62 **Gene and cell therapies in Chile: a regulatory challenge**
Hechavarría Núñez, Y.; Salas Duguet, V.; Crisóstomo Landeros J.; Carmona Sepúlveda P.

S6-63 **Conditioned medium derived from murine BM-MSCs cultured as spheroids exhibit in vitro immunomodulatory capacity**
Fuentes, P.; Torres, M.J.; Vergara, M.; Altamirano, C.

S6-64 **Mammal intestinal organoids for studying zoonotic pathogens**
Cancela, S.; Pagotto, R.; Francia, M.E.; Crispo, M.; Yim, L.; Bentancor, L.; Bollati-Fogolín, M.

S6-65 **Preliminary evaluation of cell-based carriers of novel conjugated polymer nanoparticles with antitumoral action**
Beaugé, L.; Palacios, R.E.; Ibarra, L.E.

S7- Innovations and challenges

S7-66 **Precipitation using polyethylene glycol and zinc chloride as a purification step for monoclonal antibodies capture**
Moreno Pássaro, A.C.; Orban de Souza, M.P.; Rocha Antunes Pereira Bresolin, I.; Peroni, L.A.; Lazzarotto Bresolin, I.T.

S7-67 **Application of QbD concepts to the development of a recombinant veterinary product liquid formulation**
Rodríguez, M.C.; Villarraza, J.; Antuña, S.; Tardivo, B.; Fontana, D.; Ceaglio, N.; Prieto, C

S7-68 **Development of a platform based on the cooperative adsorption of monoclonal antibodies on gold nanoparticles**
Campos, I.; Riveros, A.; Altamirano, C.; Kogan, M.; Hermoso, M.; Molina, M.C.

S7-69 **Engineering PCV2's VLP as a new and adaptable biotechnological platform for vaccine development**
Olivero, N.; Frigini, E.; Ramos, N.; Carrión, F.; Arbiza, L.; Pritsch, O.; Pantano, S.; Ortega, C.

S7-70 **Humanized mouse model to evaluate an immunotherapeutic nanomedicine**
Mitarotonda, R.; Arévalo, A.P.; Pagotto, R.; Bollati-Fogolín, M.; Crispo, M.

S7-71 **Production of IgY avian antibodies against the structural protein Erns of the Bovine viral diarrhea virus**
Gutierrez, N.; Hidalgo, A.; González, E.; Villavicencio, C.; Manríquez, V.; Camacho, F.; Toledo, J.

S7-72 **CO_2 conversion into Polyhydroxybutyrate (PHB) through a bioelectrochemical system by Cupriavidus necator**
Travali Barriga, M.; Martínez Ruano, J. A.; González Romo, E.

Session 1: Recombinant cell line development. Genome edition

New endogenous promoters for recombinant protein expression discovered in CHO cells by RNA-seq. Transient and stable characterization

Gugliotta, A.[1 +]; Tossolini, I.[1 +]; Lopez Díaz, F.[5], Kratje, R.[1]; Prieto, C. [2,3,4]
[1] UNL, CONICET, FBCB, Santa Fe, Argentina
[2] UNL, FBCB, Santa Fe, Argentina
[3] Cellargen Biotech S.R.L., FBCB, UNL, Santa Fe, Argentina
[4] Biotecnofe S.A. PTLC, Argentina
[5] Regulatory Biology Laboratory, Salk Institute for Biological Studies, 10010 N. Torrey Pines Road, La Jolla, CA 92037, USA
[+] These authors should be considered joint first author.
Contact to: agugliotta@fbcb.unl.edu.ar

The generation of recombinant cell lines for the production of therapeutic proteins requires the appropriate host selection. Chinese Hamster Ovary (CHO) cells are the most widely used platform for the production of biotherapeutics. Also, the design of the transgene expression cassette is crucial, being the promoter a key element. Strong viral-derived promoters are commonly used since they allow a high rate of expression. However, they usually induce cellular stress and can be susceptible to epigenetic silencing. Endogenous promoters that are active during the production stages in animal cell culture constitute a good alternative and may be useful to avoid such drawbacks. In this work, new endogenous promoters were discovered based on high gene expression levels in RNA-seq data of CHO-K1 cells cultured in bioreactors. The promoters of Actb, Ctsz, Hmox1, Hspa5, Vim and Rps18 genes were selected for generating new expression vectors. The reporter protein ZsGreen1 was used to speed up the analysis. Experiments were performed using CMV plus enhancer (CMV+E) promoter as a control. Transient expression analysis revealed that the endogenous promoters (excluding Rps18) evidenced high levels of ZsGreen1 not only in CHO but also in BHK-21 and HEK293 cells. Stability studies showed that ZsGreen1 expression driven by the newly discovered endogenous promoters was markedly higher compared to CMV+E. Temperature shift from 37 to 32°C was beneficial for endogenous promoter activity. The new promoters constitute a promising alternative to CMV+E since they can be engineered for the design of synthetic promoters relevant for industrial cell line development.

Applicated bioengineering for improvement the specific and volumetric productivity in CHO cells, difficult to express protein (DTEP) producer

Latorre Y.[1]; Molina M.C.[1]; Altamirano C.[2]

[1] Laboratorio de Anticuerpos Recombinantes e Inmunoterapia, Programa Disciplinario de Inmunología, Facultad de Medicina, Universidad de Chile. Santiago de Chile, Chile

[2] Laboratorio cultivo de células animales, Escuela de ingeniería Civil Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

Contact to: yesenia.latorre@pucv.cl

The more utilized productive mAb cells are the CHO cells. However, CHO cell has two important problems: toxic metabolites accumulation (lactate and ammonium) and the protein secretor process is not efficient. This last inconvenient show more evidence when the DTEPs, like mAb, are being expressing. For a long time, specific gen modifications were done: protein transporter and enzyme genes were increased or decreased. All of this, for improvement the CHO cells production problems. But, the results of this modification, are not the best. Because of that, the focus was changing, and the global regulators take more importance. These global regulators can activate or inhibited different important cellular processes, for resolved the CHO cells inconvenient. Two global regulators highlight: c-Myc and Xbp1s. Each one has demonstrated has a positive effect on CHO cell metabolism and/or protein secretion. The aim of this work is improvement the human mAb specific and volumetric productivity, overexpressing both regulators. For that, the CHO cells were transient transfected with mAb vector and with or without regulator vectors. The cells were cultivated in batch, at 37°C, 5%CO2 in a humidity atmosphere. The culture medium that we used was Hyclone SFM4CHO with 20mM glucose and 6mM glutamine, serum free. Currently the cells are still in serum free and suspension adaptation process, before transfection. Anyways we hope that the mAb increased when the two regulators are overexpressed.

Characterization of CHO cells overexpressor of c-Myc and its relationship with mTOR pathway in the production of recombinant protein

Núñez-Soto, C.[1], Altamirano, C.[1]
[1] Pontificia Universidad Católica de Valparaíso, Chile
Contact to: cony.ldea@gmail.com

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 post-translational modifications proteins. To improve the production of recombinant-proteins, cell lines have been genetically modified with a gene of interest like the human *c-myc* gene, which caught the attention of researchers, due to proven great potential. However, the pathway which c-Myc could increase the production of recombinant-proteins is still unknown. CHO cells were genetically modified to manufacture human erythropoietin and to overexpress human c-Myc. Thus, this research aims to evaluate the effect of the overexpression of c-Myc in CHO cells over the h-EPO production, the metabolites consumption and production, growth rate, cellular viability, and the expression of both c-Myc and proteins of the mTOR pathway, due it is one of the most cellular metabolism and proteins synthesis-related signaling pathway. CHO cells were cultured in a suspension and agitation (50 rpm) system, with a 5% of CO_2, 95% of humidity, and 37ºC for 7-8 days. The media Hyclone SFM4CHO was supplemented whith glucose (40 mM) and glutamine (6 mM). The cell growth rate and the viability were messured each 24 h by trypan blue exclusion test. Culture samples were taken to measure metabolites, evaluate h-EPO production by ELISA, and evaluate both c-Myc and the mTOR pathway expressions by RT-qPCR and WB. Compared to culture control of CHO-EPO cells, the results show that CHO-c-Myc cells increase q_p (33,3%) and Q_p (53,9%) of h-EPO. Also, increase glucose consumption (41%), ammonium (527%) and lactate production (293%). However, at the end of the culture, the consumption of lactate was observed, which is considered a metabolic efficiency marker. Furthermore, the expression peak of c-Myc was observed at 72 h in control CHO-EPO cells, and high and constant levels of c-Myc were observed in CHO c-Myc cells.

Effects of human growth factors TGF-β1 and PDGF-BB stable heterologous expression and exogenous addition over the growth performance of CHO and HEK cell lines in adherent culture

Reinhardt Tagliani, M.[1]; Sogayar, M.C.[2]; Oliveira Carreira, A.C.[2,3]; Tonso, A.[1]
[1] Departamento de Engenharia Química, Escola Politécnica, Universidade de São Paulo, Brazil
[2] Núcleo de Terapia Celular e Molecular (NUCEL), Faculdade de Medicina, Universidade de São Paulo, Brazil
[3] Departamento de Cirurgia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Brazil
Contacto to: martinotagliani@hotmail.com

Peptide growth factors (GF) are multifunctional proteins with a wide array of clinical and research applications, notably in regenerative medicine and tissue engineering. The mammalian cell lines that produce most commercially available GF are susceptible to signaling and regulation by these molecules which are, in fact, common components of cell culture media, providing crucial mitogenic stimuli. Even so, there are few reports on the effects of heterologous GF expression on the growth performance of mammalian host cells, and there is evidence to support that these genetic manipulations hold the potential to leverage cell proliferation and decrease animal serum dependence in cultivation processes. In the present study, parental CHO-DG44 and HEK293 cells were cultivated along with sublineages featuring the stable expression of human GFs TGF-β1 and PDGF-BB, in Alpha-MEM and DMEM basal media, respectively. The growth curves and kinetic parameters of 24-well plate adherent cultures were assessed and compared between GF producing and parental cells cultivated with three different concentrations of fetal bovine serum (FBS), which naturally contain GF, and with three conditions of exogenous GF application. No stimulatory effect was observed with HEK cells, but the heterologous expression of TGF-β1 by CHO cells led to maximum specific growth rate increases in an inversely proportional linear relation to FBS concentration in media and 2-5 times higher maximum cell concentrations. The effect observed here indicates a good direction of research for the development of serum-free media formulas and bioprocess optimization strategies.

Acknowledgments: FAPESP São Paulo Research Foundation (Thematic Grant No. 2016/05311-2) and CAPES (Scholarship 88887.364018/2019-00)

S1-5

Direct transduction of the mammary glandular epithelium: An approach to produce recombinant proteins in milk

Sanchez Ramos, O.[1,3]; Toledo Alonso, J.R.[2,3]; Camacho Casanova F.[1]; Parra Pereira, N.C.[2]; Reyes López, F.[1]; Acosta Alba, J.[2]

[1] Laboratorio de Biofármacos Recombinantes, Departamento de Farmacología, Facultad de Ciencias Biológicas, Universidad de Concepción, P.O. Box 160C, Concepción, Chile

[2] Laboratorio de Biotecnología y Biofármacos, Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile

[3] Centro de Biotecnología y Biomedicina Spa., Vilumanque 186, Concepción, Chile

Contact to: osanchez@udec.cl

Direct transduction of the mammary glandular epithelium has become a strategy for producing high levels of recombinant proteins in the milk of non-transgenic ruminants. Adenoviral and adeno-associated vectors have been used as gene transfer vehicles to mammary epithelial cells. Adenoviral vectors allow the protein of interest to be produced at levels of up to 3.5 grams per liter of milk. However, this expression only lasts between 7 and 10 days due to the immunogenicity of this type of vector. On the other hand, AAV vectors guarantee expression levels greater than 1 g/L and for up to three months. However, the production of AAVs vectors is expensive and complex. To overcome these limitations, our team has developed a new type of vector based on bacterial viruses. (PhagMam vector). This vector has low immunogenicity, and its production is straightforward and inexpensive. To date, PhagMam vectors have demonstrated their ability to enter mammary epithelial cells, escape from their endosome, concentrate in the cell nucleus, and mediate transgene expression. These preliminary results are promising. Our next trials focus on studying the in vivo performance of PhagMam vectors.

Selection of MDCK cell clone for Influenza Virus multiplication

Pereira, N. A.[1]; Ferreira Barbosa, F.[1]; Perini de Araújo, A. P.[1]; de Lima Paoli, R.[1]; Mancini Astray, R.[1]
[1] Laboratorio Multipropósito, Instituto Butantan, Brazil
Contact to: renato.astray@butantan.gov.br

The MDCK cell line is commonly used for Influenza virus multiplication for the production of Influenza cell-based vaccines. However, the original MDCK NBL-2 (ATCC®) consists of a population of cells showing different morphologic characteristics. The development of a consistent process for cell growth and virus production requires the utilization of a cell clone with stable characteristics. The original MDCK was expanded and cell subpopulations were obtained through the inoculation of 96-well plates with 0.5 cell/well. The observation and analysis of the subpopulations was performed daily starting at 5 hours post-inoculation. Wells containing one cell and single morphologic characteristics were expanded. The cells were then classified into different subtypes, according to the morphological characteristics of the culture, and subsequently frozen. The different cell subtypes were analyzed by imaging cytometry (Amnis, Luminex®) for cell size and complexity. The first cloning cycle lasted approximately 25 days until freezing (4-5 passes from limiting dilution to bank preparation), resulting in 15 subpopulations, with pseudosyncytial and cupular subtypes being predominant. The cytometry data identified three distinct populations with different median size and complexity, corroborating the morphological microscopy observations found in the different cell subtypes. Some subpopulations were submitted to the second round of selection generating individual clones for the analysis of growth rate, virus productivity and adaptation to serum-free medium.

Mammalian cell line development strategies for recombinant protein expression using reporter genes "*in cis*"

Mussio, P.[1]; Gastaldi, V.[1,3]; Gugliotta, A.[1]; Rodríguez, M.C.[1]; Prieto, C.[2, 3, 4]
[1] UNL, CONICET, FBCB, CBL, Santa Fe, Argentina
[2] UNL, FBCB, CBL, Santa Fe, Argentina
[3] Biotecnofe S.A. PTLC, Santa Fe, Argentina
[4] Cellargen Biotech SRL, FBCB, Santa Fe, Argentina
Contact to: pablo.esteban.mussio@gmail.com

Reporter genes encode proteins that can be distinguished from a background of endogenous proteins and lack toxicity. These can be applied to ease the selection of high-producing cell lines, which is usually a time-consuming and labor-intensive stage during cell line process development. Here, we describe two different strategies to select high-producing cells, employing expression vectors encoding fluorescent proteins *in cis* with the gene of interest. In the first case, CHO-K1 cells were transfected, with a vector encoding the fluorescent protein mCherry and a neomycin resistance gene, four sequential times. Thereafter, antibiotic selection was performed with 1 µg/mL of neomycin during one week. The cell population expressing mCherry was further enriched with a double-round of fluorescent activated cell sorting. Following the selection, the percentage of mCherry positive-cells was 11%. Cell sorting resulted in positive cells percentage of up to 50% and 95%, during the first and second round. This process took place in a period of 3 months, with a cell positive enrichment of 8.6 times.In the second case, CHO-K1 cells were transfected, with a vector encoding the fluorescent protein eGFP, only once. Subsequently, fluorescent cells were selected using limiting dilution cloning to prevent non- and low-producing cells from outgrowing high-producing cells. The limiting dilution process took one month, achieving up to 99.5% of eGFP-positive cells. Hence, we propose that these strategies can be applied to isolated stable cell lines with desirable expression of genes of interest.

Development of a CHO cell line for production of an anti-MICA human monoclonal antibody by RMCE technique

Latorre-Aguirre, Y.[1,2]; Rodriguez-Siza, J.[2]; Toledo-Stuardo, K.[1]; Dubois-Camacho, K.[1]; Tello-Aguayo, S.[1]; Vergara-Castro, M.[2]; Molina, M.C.[1]; Altamirano, C.[2]

[1]Laboratorio de Anticuerpos Monoclonales Recombinantes e Inmunoterapia, Programa de Inmunología, Facultad de Medicina, Universidad de Chile, Chile.

[2] Laboratorio de Cultivo de Células Animales, Escuela de Ingeniería Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso, Chile.

Contact to: yesenia.latorre@pucv.cl

The development of a cell line is one of the most important steps for the production of pharmaceutical antibodies. The Chinese hamster ovary (CHO) cell line has become the preferred host for the production of biopharmaceutical proteins. In general, to obtain stable expression, commonly used methods cannot control the chromosomal position of transgenes, and their expression is affected by transgene silencing due to epigenetic regulations. In this work, we use a recombinase-mediated cassette exchange (RMCE) technique to generate stable clones. The vector was designed to be compatible with the RMCE technique, in addition to expressing a new human monoclonal antibody: Anti-MICA, developed for the treatment of gastric cancer. In detail, the vector includes the CAGGS promoter, enhanced with omnipresent chromatin opening elements (UCOE), which inhibit DNA methylation and histone acetylation, decreasing transgenic silencing. After obtaining the vector, transfection was performed. For this, lipofectamine® 2000 was used, adding, in addition to the vector of interest, a vector that codes for flippase (necessary for cassette exchange), on modified CHO cells that express the RFP gene. Once transfected, cells could be selected for negative sorting. Because homologous recombination would occur, that is, the replacement of the RFP gene by the protein of interest, losing its fluorescence. However, the RFP protein, in the RMCE master cell line, is expressed under the CMV promoter, which is rapidly silenced. Due to this, it was necessary to make a cold surface labeling with a conjugated anti-IgG. Subsequently, cells with a positive signal were selected by sorting (flow cytometry). Subsequently, the sorted population was cloned, using limit dilution. When analyzing the production of mAb from the supernatant of a selected clone, by ELISA assay, it was not possible to detect the antibody. Given the above, a cell lysis was performed and subsequent WB where the presence of the antibody was observed. This indicates that the antibody is not being secreted by the cell, which is associated with the deficient secretion machinery that CHO cells have.

Insect cell lines capable of endowing chitinase ChiA to AcMNPV baculovirus occlusion bodies for improving their bioinsecticidal properties

López, M.G.[1]; Barros, G.[1]; Victoria Alfonso A.[1]; Oscar, T.[1]
[1] Laboratorio de Estudios en Baculovirus. IABIMO.INTA Castelar/CONICET, Argentina
Contact to: lopez.mariag@inta.gob.ar

Baculoviruses are insect viruses used mainly for biological pest control and recombinant protein expression. As biopesticides, although they have numerous advantages over chemical pesticides and great potential for application in integrated pest management (IPM), they tend to be slower than conventional synthetic strategies, limiting their widespread use. The objective of this work was to endow AcMNPV occlusion bodies with a disrupting protein of the gut peritrophic membrane of susceptible insects, to enhance the bioinsecticidal properties of AcMNPV. For this purpose, stably transformed Sf9 cell lines were developed with the ChiA gene with and without addition of the nuclear localization signal KRKK, regulated by an infection-inducible POLH promoter and enhancer sequences. The construction of polyclonal Sf9ChiA and Sf9ChiA$_{KRKKK}$ lines was performed by transfecting the transfer vectors into Sf9 cells and exerting selection pressure with the antibiotic blasticidin. After infection with AcMNPV baculovirus, both lines were able to express the proteins at high levels. In addition, it was possible to recognize the presence of ChiA with specific antibodies in polyhedra samples isolated from infections of both transgenic lines with wild-type AcMNPV and recombinant baculoviruses expressing another infectivity enhancer protein, GP37, of interest since it presents synergistic action with ChiA. The nuclear localization signal did not show to play a major role in the incorporation of ChiA into polyhedra. In summary, it was possible to endow wild-type and recombinant baculovirus occlusion bodies with the ChiA protein from stably transformed insect cell lines, opening the way to potential uses in IPM with AcMNPV.

S1-10

Trans-complementation of ac109 knockout baculoviruses by trangenic insect cell lines for the production of virion-free recombinant proteins

Amalfi, S.[1]; Mattera, R. [1]; Plastine, M.P. [1]; Lopéz, M.G. [1]; Taboga, O.[1]; Alfonso, V. [1]
[1] Instituto de Agrobiotecnología y Biología Molecular IABIMO-INTA/CONICET, Argentina
Contact to: amalfi.sabrina@inta.gob.ar

The baculovirus expression system is widely used for the production of recombinant proteins, which in many cases co-purify with viral particles. We previously described *ac109* of *Autographa californica* multiple nucleopolyhedrovirus as an essential gene for the production of infectious viral progeny. This work aimed to use an *ac109* knockout virus (Ac109KO) trans-complemented from transgenic insect cells to produce recombinant proteins with minimal levels of co-produced virions. First, different promoters and *ac109* nucleotide sequences were evaluated in their ability to trans-complement Ac109KO. We also determined the strategies that produced lower levels of regenerated wild type (wt) viruses by recombination. Then, we selected a modified codon sequence of *ac109* driven by its own promoter zone (pac109) or by a synthetic polyhedrin-based promoter as the best genetic constructs and obtained two polyclonal Sf9-derived cell lines that rendered high levels of complemented viruses and less than 1% of wt viral particles. Finally, the production of a reporter protein was evaluated by fluorometry in Sf9 cells infected with complemented or control baculoviruses. Interestingly, Ac109KO viruses obtained by trans-complementation with both developed Sf9 cell lines expressed higher levels of the reporter gene than wt viruses. In conclusion, the use of defective baculoviruses complemented with transgenic Sf9 lines is a promising tool to obtain protein products with very low levels of budded virion contaminations.

S1-11

Increasing transduction efficiency in mammals: development of a stable sf9 insect cell line for the production of G-VSV pseudotyped baculoviruses

Plastine, M. de P.[1]; Amalfi, S. [1]; López, M. G. [1]; Taboga, O. [1]; Alfonso, V. [1]

[1] Instituto de Agrobiotecnología y Biología Molecular, IABIMO-INTA/CONICET, Argentina
Contact to: plastine.maria@inta.gob.ar

Baculoviruses are promising vectors for gene delivery in mammals, although they exhibit distinct levels of transduction according to the cell line. Pseudotyping viral vectors is a widely used strategy to enhance cell tropism and increase transduction efficiency. In this work, we developed a stable Sf9 insect cell line that expresses the G-protein of the vesicular stomatitis virus (G-VSV) under the very late strong and infection-inducible promoter pXXL, previously constructed in our laboratory. First, we cloned the *G-VSV* gene in a plasmid carrying a blasticidin resistance gene and we transfected it into Sf9 cells. The recombinant cells were selected by antibiotic resistance and diluted twice to obtain an oligoclonal cell line. Due to G-VSV is a fusogenic protein, we selected some clones based on the size and number of syncytia obtained after infection with baculovirus. Then, we evaluated the level of G-VSV expression by immunodetection in cell extracts after infection. Finally, we selected the cells that showed the highest incorporation of G-VSV in viral particles. To determine the infection conditions for the best cell-line performance, we obtained a baculovirus that expresses eGFP in mammals using different multiplicities of infection and harvest times and then measured the reporter gene expression by fluorescence microscopy and flow cytometry in mammalian cells. Using different pseudotyped baculoviruses, a consistent increase in the transduction efficiency was detected in all the tested mammalian cell lines. The results highlight the feasibility and convenience of improving the performance of gene delivery without inserting the pseudotyping gene into the baculoviral genomes.

Expression of a recombinant cytokine in *Escherichia coli*

Villavicencio, C.[1]; Gutiérrez, N. [1]; Hidalgo, A. [1]; Llamazares, E. [1]; Ramos T. [2]; Toledo, J.R. [1]

[1] Universidad de Concepción. Laboratorio de Biotecnología y Biofármacos. Departamento de Fisiopatología. Concepción, Chile

[2] Universidad de las Fuerzas Armadas-ESPE. Departamento de Ciencias de la Vida y la Agricultura. Quito, Ecuador

Contacto to: cjohana@hotmail.es

Interferons (IFN) are an important family of cytokines involved in the activation and regulation of innate and adaptive immune responses. They allow the transcription of hundreds of genes with antiviral and antiproliferative activity. IFN-α, a type I IFNs, has multiple therapeutic applications for the treatment of various human and animal diseases of viral origin. It is known that 2.5% of dog deaths are caused by viral infections such as distemper, rabies, and parvovirus. Currently, there is no effective treatments for such diseases, with the available treatments only being able to mitigate the symptoms. In the present work, an IFN-α protein was obtained using *Escherichia coli* BL21 codon plus as the expression system. The protein was obtained in inclusion bodies with a purity of 83.84 %. The biological activity of this cytokine was tested by qPCR *in vitro* assays and showed significative difference in the expression levels of mRNA antiviral markers protein kinase R (PKR) and 2 ', 5'-Oligoadenylate Synthetase 2 (OAS-2). Future projections are based on the scaling up of protein production, and its subsequent design of a prototype for veterinary use.

Session 2: Cell metabolism and optimization of cell culture conditions

Potential use of fish collagen matrix to evaluate tubulogenesis *in vitro*

Medina, D.[1]; Acevedo Gomez, A.[2]; Leiva, L.[1]; Pellegrini Malpiedi, L.[3]; Bustillo, S.[2]

[1] Laboratorio de Investigación en Proteínas (LabInPro), IQUIBA-NEA CONICET, Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina

[2] Grupo de Investigaciones Biológicas y Moleculares (GIBYM), IQUIBA-NEA CONICET, Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina

[3] Instituto de Procesos Biotecnológicos y Químicos Rosario (IPROBYQ), Universidad Nacional de Rosario (UNR)- CONICET, Rosario, Argentina

Contact to: temomb@gmail.com

Formation or sprouting of new blood vessels (angiogenesis) is a complex process that involves the extracellular matrix (ECM) and endothelial cells (EC). A common *in vitro* angiogenesis assay consists of culturing EC on or inside distinct ECM components such as collagen. Recently, collagen from aquatic sources has gained attention in tissue engineering applications. Thus, in this work the potential of fish collagen from *Pygocentrus nattereri* (CP) to induce tubular structures in tEnd.1 (RRID: CVCL_6272) cell line was evaluated. Firstly, collagen from discarded fish skin was extracted by acid solubilization, concentrated by salt precipitation, dialyzed against 100 mM acid acetic and preserve at 8°C. The hydroxyproline analysis revealed a collagen concentration of 6,9 mg/mL (73,8 % of total protein content) and the electrophoretic pattern (SDS-PAGE under non-reducing conditions) showed that extracted CP was type I. To evaluate CP use in angiogenesis assay, 100 µL of a 0.5 mg/mL solution (culture medium-pH 7) was added to each well of 96-well plate and incubated for 30 min at 37°C. Endothelial cells (30.000 cells/well DMEM-5%FBS) were seeded on collagen-coated wells and incubated for 24h at 37°C-5%-CO_2. Controls were performed using untreated wells. Tube formation was monitored by an inverted phase contrast microscope and images were taken with a digital camera. Using ImageJ software, a number of capillary structures was analyzed. EC cultured in collagen matrixes organized rapidly into an irregular network, which was formed by many tubular structures radiating out from cell aggregates. These results suggest the potential use of fish collagen in cell culture.

S2-14

Development of a rh-EPO production strategy in c-Myc overexpressing CHO cells in batch culture, based on lactate re-metabolization

Gatica, O.[1]; Vergara, M.[1]; Nuñez, C.[1]; Collarte, C.[1]; Vergara, S.[1]; Torres, M.[1]; Latorre, Y.[1]; Valdez-Cruz, N.[1]; Altamirano, C.[1].

[1] Laboratorio de cultivo de células animales, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
Contact to: oscarnanuepara@hotmail.com

Aiming to increase the productivity of recombinant proteins (r-proteins) in CHO cells, various studies have pointed to the use of slowly metabolizable carbon sources and the overexpression of global regulators as a strategy to enhance the expression of r-proteins.

This study was carried out in batch culture of CHO cells, where an erythropoietin-producing clone was compared with one which also overexpresses the global regulator c-Myc. Glucose was used as a control at a low and a high concentration, which was compared with a galactose-based feed, also at two concentrations.

In most of the conditions studied, c-Myc overexpression caused a decrease in the specific growth rate of cells. This overexpression, in combination with a diet based on a mixture of galactose and lactate at a high concentration, as a result, a 4-fold increase in the specific productivity of erythropoietin was generated. In addition, a decrease in the expression of the enzyme lactate dehydrogenase A was observed, which triggered a metabolic shift towards lactate consumption, achieving a detoxification of the culture media.

Finally, it is concluded that the use of galactose and the overexpression of c-Myc in CHO cells enhances the specific productivity of r-proteins, decreases the production of toxic metabolites, promotes lactate consumption, and systematically improves culture development.

S2-15

Mixture-design optimization of culture medium: towards a more economical, reproducible and scalable *in vitro* mAb production process

Wandel-Petersen, V.[1]; Kratje, R.[1,2]; Bürgi, M.[1,2]; Oggero-Eberhardt M.[1,2]

[1] Universidad Nacional del Litoral (UNL), CONICET, Facultad de Bioquímica y Ciencias Biológicas (FBCB), Centro Biotecnológico del Litoral (CBL), Santa Fe, Pcia. de Santa Fe, Argentina

[2] BioSynaptica SA, Santa Fe, Pcia. de Santa Fe, Argentina

Contact to: wandelp.valentina@gmail.com

Our laboratory has generated an anti-recombinant human erythropoietin (rhEPO) monoclonal antibodies (mAb2B2) hybridoma. This mAb recognizes rhEPO and hEPO derivatives developed in our laboratory as neurotherapeutic candidates. Hence, it's an essential reagent for a variety of immunochemical approaches. Heretofore, mAb2B2 was obtained *in vivo* from mice ascitic fluid. In this work, the hybridoma was adapted to an optimized serum-free culture medium (SFM) to enable its *in vitro* large-scale production. Sequential and direct protocols were assessed to adapt hybridomas to SFM. Different mixtures of commercial media (DMEM Ham'sF12:EXCELL-620) were evaluated in a D-optimal experimental design (DoE). Hybridoma cultures adapted to DoE conditions were compared by evaluating: growth rate, productivity and mAb2B2 half maximal effective concentration (EC50) as an estimator of its affinity. Sequential adaptation decreased hybridoma productivity by 96%, whereas direct adaptation maintained a growth rate and productivity comparable to the initial condition. This clone yielded 200 mg of antibodies in a 300 ml-single batch culture. DoE revealed two optimal SFM formulations: the 67:33 and 94:6 proportions of DMEM Ham'sF12:EXCELL-620, that reduced SFM cost by 6 and 22 times, respectively. Both conditions maintained the growth rate of the initial condition (0.04 h^{-1}) increasing clone productivity in 1.7 and 1.5-fold. Also, they allowed conserving the EC50 and increased its purity 1.3-fold compared with its *in vivo* production. The optimized process significantly outperformed the *in vivo* production method, allowing us to replace it by an economical, reproducible and scalable process, free from animals and its derivatives.

Toxicity evaluation of pecan nutshell bioactives against CHO-K1 and MDA-MB 231 cell lines: Cytotoxicity, cell cycle arrest and apoptosis.

Gasser, F. B.[1,3]; Ribas, L. E.[1,2]; Renna, M. S.[1,3]; Marelli, B.[1,3]; Ortega, H. H.[1,3]; Hein, G. J.[1,2,3]; Baravalle, M. E.[1,2,3].

[1] Centro de Medicina Comparada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL), Esperanza, Santa Fe, Argentina

[2] CITEDA, Centro Universitario Gálvez, Universidad Nacional del Litoral, Gálvez, Santa Fe, Argentina. [3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Contact to: fatima.gasser@icivet.unl.edu.ar

The pecan nutshells contain high amounts of phenolic compounds which are believed to be responsible for some bioactive properties such as antioxidant and antitumoral activities. We studied the cytoxicity activity against breast cancer and normal cell lines. After aqueous extraction of bioactives from pecan nutshells, the cytotoxic activity after exposure for 48 h on CHO-K1 (hamster ovary cells) and MDA-MB 231 (human breast cancer cells) was examined through the MTT assay. The IC_{50} and the selective index (SI) with respect to the normal cells were determined.

Cell cycle arrest and induction of cell death were also analyzed by using Propidium Iodide and FITC-Annexin V kit and flow cytometry. Hydrogen peroxide and doxorubicin were used as cytotoxicity positive controls.

A dose-dependent decreasing cell viability curve was obtained and the IC_{50} was 63.13mg/L and 26.47mg/L for the CHO-K1 and MDA-MB 231 cell lines, respectively, with a SI>1 (SI= 2.4) indicating less cytotoxic activity in non-cancerous cells. The main cytotoxicity mechanism of the bioactives (IC_{50}) was the induction of apoptosis (33.4% for CHO-K1 and 54.7% for MDA-MB 231), being lower than the % obtained for doxorubicin (58.1% for CHO-K1 and 96.0% for MDA-MB) ($p<0.05$). Treatment with bioactives (IC_{50}) resulted in cell cycle arrest at the G0/G1 and G2/M phase for CHO-K1 and MDA-MB 231 cells, respectively. Besides, treatment with doxorubicin (IC_{50}) resulted in cell cycle arrest at the G2/M phase for both cell lines. These bioactives could be relevant as a complementary biotherapeutic to cancer treatments contributing to overall health.

Development of a dynamic SH-SY5Y 3D culture model for biological evaluation of Alzheimer-induced pathology

Fernández, M.[1]; Arancibia, A.[1]; Núñez, C.[1]; Torres, M.[2]; Vergara, M.[1]; Altamirano, C.[1].
[1] Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Av. Brasil, 2085 Valparaíso, Chile
[2] Manchester Institute of Biotechnology, Faculty of Science and Engineering, University of Manchester, Manchester, UK
Contact to: m.fernandez.linderman@gmail.com

Alzheimer's disease is the neurodegenerative pathology with the highest incidence in the world population. The technological advance for its diagnosis and development of drugs for its treatment goes through the development of cellular models that allow the pathology to be reproduced both *in vitro* and *in vivo*. Currently, the use of bioassays in 2D monolayer culture modality allows carrying out research to evaluate the potential neuroprotective effect of different molecules in early stages. However, the prevalence of cell-support interactions over cell-cell interaction doesn't simulate the behavior exhibited by tissues *in vivo*. The objective of this project is to develop a dynamic 3D culture model to more efficiently simulate cell-cell and cell-matrix interaction in SH-SY5Y neuroblastoma cell culture.

Obtaining cell self-aggregates or spheroids (3D) will be carried out first by the static method with the Hanging drop technique, where the spheroids will be generated. Subsequently, the stability of the spheroids will be evaluated dynamically in shaking cultures (*Techne*). Then, the spheroids will be induced to produce beta-amyloid peptide (disease marker) with 27-hydroxycholesterol, which activates the expression of the peptide, thus finally being able to carry out the biological evaluation of molecules with therapeutic potential.

Initial results indicate that the variables in static culture 72 hours and 1000 cells/drop allow reaching spheroids of 400um with cell viability over 97%. It is proposed to study the variables in a dynamic system: agitation speed, number of spheroids, together with the evaluation of bioactive molecules from the coffee industry.

Modification of the assimilative metabolism of *Pichia pastoris* for the improvement of tolerance to methanol and the productivity of recombinant protein

Guerrero, K.[1]; Quezada, J.[1]; Veas, R.[1]; Altamirano, C.[1]; Fickers, P.[2]; Berrios, J.[1]
[1] Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Chile
[2] Gembloux Agro-Bio Tech, Université Libre de Bruxelles, Bélgica
Contact to: karlo.guerrero.m@mail.pucv.cl

Pichia pastoris is a methanol-inducible expression system for the production of recombinant proteins (rProt). Although it has many advantages, there are aspects of methanol metabolism that still need to be improved. The use of methanol as a carbon source leads to a decrease in growth rate (μ) and the intracellular accumulation of formaldehyde, a toxic intermediary that negatively affects growth and rProt synthesis. A modification of the assimilative metabolism through the overexpression of the di-hydroxyacetone-synthetase (DAS) would contribute to overcome these problems, which could improve the productivity of rProt. A strain of *Pichia pastoris* has been modified to overexpresses DAS and co-express a *Candida Antarctica* lipase B (CalB) as a model protein. Using batch culture with defined culture medium and under controlled conditions of temperature, agitation and initial pH, the strain has been characterized in terms of its growth kinetics, substrate consumption and CalB production, using variable concentrations of methanol (3 to 18 $g \cdot L^{-1}$). The control was a strain that produces CalB but without the DAS overexpression. The results obtained shows a greater tolerance to methanol in the range of concentrations used, presenting significant inhibition only above concentrations above 15 $g\ L^{-1}$, while the control strain is inhibited above 10 $g\ L^{-1}$. In addition, the modified strain shows a growth rate approximately 30% higher compared to the control strain. From the determination of the activity of the CalB in the supernatant an increase of 300% was observed in the modified strain, indicates a greater production capacity of this recombinant protein.

S2-19

Optimization and characterization of the production of a fully human anti-MICA antibody in Chinese hamster ovary (CHO) cells

Garrido, M. J.[1,2]; Latorre, Y.[1,3]; Toledo, K.[1]; Tello, S.[1]; Gatica, O.[3]; Altamirano, C.[3]; Molina, M. C.[1].

[1] Laboratorio de Anticuerpos Recombinantes e Inmunoterapia, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile

[2] Facultad de Ciencias Naturales, Matemática y Medio Ambiente, Escuela de Industria Alimentaria y Biotecnología, Universidad Tecnológica Metropolitana, Chile

[3] Facultad de Ingeniería, Escuela de Ingeniería bioquímica, Pontificia Universidad Católica de Valparaíso, Chile

Contact to: maria.garridog@utem.cl

Monoclonal antibodies are high molecular weight glycoproteins with a complex three-dimensional structure that require essential post-translational modifications and folding to introduce both structural and functional heterogeneity. These characteristics depend on the production platform, where one of the most widely used corresponds to Chinese hamster ovary (CHO) cells, as they produce bioactive and human-compatible modifications and foldings. However, one of the problems it presents is the low yield in the production of the protein of interest. The aim of this work was to optimize and characterize the production of a fully human anti-MICA antibody produced by CHO-K1 cells in a spinner culture system. The optimization of the production was based on two strategies, supplementation of the culture medium with sodium butyrate (3mM) and lowering the temperature (30°C). The concentration of the antibody produced was determined by an antibody light chain capture ELISA assay. Affinity between the antibody and the recombinant MICA protein could be observed by immunoprecipitation. The presence of the light and heavy chain of the anti-MICA antibody was evidenced by western blotting of lysates from antibody-producing CHO cells and conventional PCR. Detection of the anti-MICA antibody on the membrane was analyzed by flow cytometry, where about 1% of the antibody was detected on membrane at 4°C. It is concluded that hypothermia and sodium butyrate conditions increase the productivity (qp) of anti-MICA antibody in CHO-K1 cells.

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

S2-20

Cellular biotechnology strategies to improve the production of bovine follicle stimulating hormone in Chinese hamster ovary cells

Araya, D.[1]; Latorre, Y.[1]; Gödecke, N.[2]; Vergara, M.[3]; Toledo, J.[4]; Altamirano, C.[1,3].
[1] Escuela ingeniería bioquímica, PUCV, Valparaíso, Chile
[2] MSYS, HZI, Braunschweig, Germany
[3] CREAS, Valparaíso, Chile
[4] Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile
Contact to: dvaraya@gmail.com

Different strategies have been used to increase the volumetric productivity (QP) of r-proteins in CHO cell cultures, including cell genetic engineering, low temperature or/and changes in carbon source to improve the inherent limitations in cell proliferation, metabolism and protein synthesis and processing.
In this work we will evaluate the impact of the use the concentrate lipids in protein synthesis and productivity of bovine follicle stimulating hormone (rb-FSH) in the culture medium of CHO cell line, at different temperatures.
To obtain rb-FSH-CHO (CC) cell line the RMCE technique was used. The CHO cells was growth with serum-free medium and incubated at 70 rpm and 5% CO to 96 h or 168 h. Samples were taken every 24 h and batch cultures were performed to four conditions: CC without lipids in culture medium at 37°C (CC-37), CC with lipids in culture medium at 37 °C (CC-L-37), CC without lipids in culture medium at 33°C (CC-33), CC with lipids in culture medium at 33°C (CC-L-33). CC was successfully transformed. Our results showed a significantly lower cell density the CC-33 (0,44x106 cell/ml), than CC-L-33 (0,86x106 cell/ml) and reduce lactate production by glucose consumption. In addition at 37°C lipid concentrate induce a significant increase in the productivity of rb-FSH, unlike 33°C is observed a decrease in production in presence of lipids.
These results suggest that the lipids concentrate supplementation is a good strategy to be used jointly with changes in culture temperature for increases viable cell density, productivity and reduce toxic waste from the culture medium.

Metabolic profile characterization of mesenchymal stem cells in a microcarrier suspension culture

Sánchez-Daza, A.[1,2]; Gonzalez, E.[4]; Gerdtzen, Z. P.[2,3]; Andrews, B. A.[1,2]; Caviedes, P.[2,4]; Asenjo, J. A.[1,2]

[1] Institute for Cell Dynamics and Biotechnology (ICDB), Faculty of Physical and Mathematical Sciences (FCFM), University of Chile

[2] Centre for Biotechnology and Bioengineering (CeBiB), Department of Chemical Engineering, Biotechnology and Materials, University of Chile, Santiago, Chile

[3] Center of Interventional Medicine for Precision and Advanced Cellular Therapy (IMPACT), Santiago, Chile

[4] Program of Molecular Clinical Pharmacology, ICBM, Faculty of Medicine, University of Chile

Contact to: anitaasd@gmail.com

Cell-based therapies are a promising treatment option for several diseases including type 1 diabetes. Adipose tissue contains a significant amount of mesenchymal stem cells (hMSCs) that can be easily harvested using liposuction, yet their necessary expansion to attain sufficient cell mass for cell transplant therapy is still limited. Adipose-derived stem cells can be expanded and differentiated using adherent culture techniques. A scale-out approach is labor-intensive, time-consuming, and susceptible to contamination. On the other hand, suspension culture provides an alternative for generating large amounts of cells and is more easily scalable. Nevertheless, an efficient protocol has yet to be established. The general aim of this work is to establish a general strategy for growing MSCs in a microcarrier suspension culture and characterize their metabolic profile. The hMSCs were characterized by their maximum growth rate (μ_{max}), doubling time, glucose, lactate, and amino acids specific production and consumption rates, among others. 140x 10^6 total cells were achieved in 100 mL culture volume in a spinner flask using 10 g/L of microcarriers. The feeding strategy used prevented the depletion of amino acids during the culture.

Mimicking dynamic in vivo environment by 3D cell laden culture: in house model

Martinez Marignac, V. L.[1]; Vidal, J[1]; Oertlin, G.[1]; Mostoslavsky, G.[2]; Richard Silvina, M.[3]
[1] CICYTTP- CONICET, Provincia de Entre Ríos y UADER, Argentina
[2] Center for regenerative medicine-CREM- Boston University, USA
[3] IMBICE –CIC Provincia de Buenos Aires, Argentina
Contact to: veromm99@gmail.com

One of 6 deaths worldwide is caused by cancer. The development of new treatments has been successful due to 2D cell culture drug screening. Currently the analysis and testing of drugs lack of alternatives that specifically simulate the architectural complexity found in tumors, likewise recent studies in the use of iPSC (induced polipotential stem cells) on matrigel for tissue regeneration do not take into account how 3D architecture affects regenerative processes, making it necessary to develop 3D supports that take into account not only biocompatibility but the influence of the architectural organization on organs and tissues. In consequence, preclinical strategies would be improved by other approaches which create the biological and natural architecture of tumors. We implemented a 3D biocompatible printed matrix as commercial or primary cell lines support for drug testing. We evaluated HCT116 and HEK293 as cell laden on 3D printed matrix by a common syringe or a Bioplotter. We evaluate the architecture of 3D by scanning microscopy, and cell drug response between 2D and 3D models. For 2D cell culture we use SRB assay and Live/Dead viability assay on 3D constructs. Our results showed different architectural organization between cell lines in 3D matrix, as different drug responses between types of culture. Our results demonstrate that dimensional and architectural matrix differences not only affect drug response but as the drugs studied target metabolism we confirmed differential effects on cell organization and metabolism where 3D matrix prone the cell lines to be more or less dependent to catabolic metabolism.

Enhancement of Human Adipose derived Stem Cells proliferation by pyruvate and nicotinamide supplementation

Molina Burgos, T.[1]; Sánchez-Daza, A.[2,3]; Gerdtzen, Z. P.[3,4]; Andrews, B. A.[2,3]; Caviedes, P.[3,5]; Asenjo, J. A.[2,3]

[1] Faculty of Physical and Mathematical Sciences (FCFM), University of Chile

[2] Institute for Cell Dynamics and Biotechnology (ICDB), Faculty of Physical and Mathematical Sciences (FCFM), University of Chile, Santiago, Chile

[3] Centre of Biotechnology and Bioengineering (CeBiB), FCFM, University of Chile, Santiago, Chile

[4] Center of Interventional Medicine for Precision and Advanced Cellular Therapy (IMPACT), Santiago, Chile

[5] Program of Molecular Clinical Pharmacology, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile

Contact to: temomb@gmail.com

Human Adipose derived Stem Cells (hASC) are multipotent adult cells with potential in regenerative medicine and cell therapy. For this purpose, hundreds of millions of cells are required to have therapeutic effects. However, their growth kinetics are slow, with doubling times of 45 h or higher. To increase hASC proliferation rate, the effect of enriching culture media with pyruvate (PYR) and nicotinamide (NAM) was explored. In particular, PYR is an intermediary of 6 metabolic fates and links glycolysis and tricarboxylic cycle. On the other hand, NAM is a precursor of Nicotinamide Adenine Dinucleotide ($NAD^{\cdot}$) that maintains the glycolytic metabolism of stem cells and regulates cellular functions. A Design of Experiments methodology was applied, considering concentrations of NAM and PYR between 1 and 10 mM under hypoxic (5% O_2) and normoxic (~21% O_2) conditions, and the fold change of cell number was estimated. This parameter was fitted to a Response Surface Model (RSM) to find an optimal combination of PYR and NAM concentrations that maximizes the fold change in the experimental domain. After performing 18 experiments, it was observed that PYR and NAM affect growth of hASC. By changing PYR and NAM concentrations in culture media to optimal levels, in normoxic condition, the fold change increased from 4.76 (control) to 6.22 (optimized) while, in hypoxic condition, increased from 4.71 (control) to 5.2 (optimized). It is concluded that optimal combination of PYR and NAM concentrations predicted by RSM allows enhancement of hASC growth.

Electro-fermentation of sugars to organic acids and alcohols using *Clostridium autoethanogenum*

Martínez Ruano, J. A.[1]; Suazo, A. I.[1]; Veliz de la Vega, F. A.[1]; Otálora, F. C.[1]; Conejeros Risco, R.[1]; González Romo, E.[1]; Aroca, G. A.[1]

[1] School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
Contact to: temomb@gmail.com

Clostridium autoethanogenum is a gram-positive anaerobic bacteria able to growth both heterotrophically or autotropically producing organic acids (formic, lactic, and acetic acid) and alcohols (ethanol and 2,3-butanediol). Electro-fermentation (EF) has been reported to be useful for achieving higher production yields with several microorganisms, including *Clostridium* strains. EF involves the manipulation of microbial metabolism through the application of an external potential using solid-state electrodes. This process can enhance substrate uptake and alters product distribution during fermentation. Despite these facts, the effect of EF on microbial growth and product distribution is not completely understood. This work aims to evaluate *C. autoethanogenum* cultures using fructose as carbon source, for both conventional fermentation and EF, regarding growth kinetics and product distribution. Experiments were performed using a H-type reactor which chambers were separated by a cation exchange membrane. The cathodic chamber was filled with 1L of culture medium while the anodic chamber contained 0.25 L of minerals solution. The biolectrochemical culture media was formulated with 10 g L^{-1} of carbon source under pH-control using a fixed potential of -600 mV (*versus* Ag/AgCl). Samples were periodically taken for the measurement of pH, fructose, biomass, organics acids and alcohols. As a result, it was found that the external electron supply decreases the biomass generation and leads to a redistribution of products. The experiments reveals that the application of an electrochemical potential affects mainly the production of lactate, acetate, and ethanol. Lactic acid is the main product of electro-fermentation, which production is significantly increased (4-fold).

S2-25

Development of a model-based cell culture media design platform for biomass and product optimization: current results and future perspective

Gerdtzen, Z.[1,2,4,5*];Villanueva, A.[1,2]; Narváez, D.[1,2]; Morales, D.[3]; Carvajal, B.[1,3]; Quiroga-Campano, A. L.[1,2]; Cristian Salgado, J.[1,3].

[1] Centre of Biotechnology and Bioengineering, University of Chile, Chile

[2] Mammalian Cell Culture Lab, Department of Chemical Engineering, Biotechnology and Materials, University of Chile, Chile

[3] Process Modeling and Distributed Computing Lab, Department of Chemical Engineering, Biotechnology and Materials, University of Chile, Chile

[4] Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile.

[5] Milenium Nucleus Marine Agronomy, Santiago, Chile.

Contact to: zgerdtze@ing.uchile.cl

Recent events have shown the relevance of establishing local biopharmaceutical manufacture processes for human use products. To ensure the competitiveness of these processes, new tools for increased productivity and process scale-up are required.

A key aspect for obtaining a good product yield in mammalian cell-based production processes is to keep operation conditions necessary for cell growth and maintenance throughout the process. In this context, the use of culture and feeding media suitable for the requirements of the production system are essential. Guaranteeing these conditions since process design stages to ensure a stable yield from a small-scale process design stage to a manufacture is still a challenge. To address this problem a model-based cell culture media design platform for biomass and product optimization, CELIA (CEll cuLture medIA optimization), was developed. This tool, based on a detailed dynamic metabolic model with optimizable parameters integrates cell and product composition, and characteristic process parameters to predict the specific nutritional requirements of a given cell line in a specific process. This information is used to design a specific media composition customized for each production system for the design of an optimized fed-batch based on nutritional requirements of the cells for maintenance, growth, and production, as well as a feeding strategy. Results obtained at a laboratory scale showed a significant increase in biomass for the model cells considered PK15, HEK293 CHO- tPA, ranging from 151% to 355% with respect to batch cultures. These findings will be validated in a semi-industrial setting before transferring them to the industrial productive set-up.

The platform CELIA has high potential for contributing the development of biopharmaceutical and biosimilar production processes.

Session 3: Biotherapeutics: development and quality control

Development and characterization of interleukin 2 chimeric mutein, with high antineoplastic activity and reduced systemic toxicity

Lagos, P.[1]; Sánchez, O.[1]

[1] Recombinant Biopharmaceutical Laboratory, Department of Pharmacology, Faculty of Biological Sciences, University of Concepción, Chile

Contact to: osanchez@udec.cl

IL-2 is a 15-kDa cytokine produced primarily by activated $CD4^+$ and $CD8^+$ T lymphocytes. The high-affinity receptor for IL-2 (IL-2R) is composed of three subunits: IL-2Rα (CD25), IL-2Rβ (CD122), and γ-chain (CD132). This trimeric form is expressed constitutively on $CD4^+$ $CD25^+Foxp3^+$ regulatory T cells (Tregs) and on activated $CD4^+$ and $CD8^+$ T cells. In contrast, the intermediate-affinity form composed of β- and γ-chains is also functional and highly expressed in memory $CD8^+$ T cells and NK cells. IL-2 has been used for the treatment of melanoma and renal cell carcinoma. Several clinical trials showed the limitations of this therapy; although some patients experienced complete or long-lasting responses, only 15–20% of treated patients received some clinical benefit. Moreover, the high doses required to obtain such results induce high toxicity, with vascular leak syndrome being the most frequent and severe complication, and the expansion of Tregs responsible for suppressing the antitumor response. To improve cytokine therapy, variants of IL-2, called muteins, have been developed into which mutations have been introduced to modify their function. In this work, the objective is to develop and characterize a mutein that differs from the human wild type IL-2 (wtIL-2) in seven mutations and is linked to the Fc fragment of human IGg1. The designed mutant conserves the capacity to bind to the βγ-chains of the IL-2R, but not to the α-chain.

Tools for determining the binding affinity of therapeutic antibodies

Toledo-Stuardo, K.[1]; Gómez, H.[2]; Garcia, E.[2]; Valdés, A.[3]; Guerra, D.[4]; Fuentealba, D.[4]; Matties, D.[5]; Zapata, G.[5]; Altamirano, C.[6]; Molina, M. C.[1]

[1] Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Universidad de Chile, Santiago, Chile
[2] Instituto de Química, Universidad Autónoma de México, Ciudad de México, México
[3] Instituto de Investigaciones Biomédicas, Universidad Autónoma de México, Ciudad de México, México
[4] Facultad de Química y Farmacia, Pontificia Universidad Católica de Chile, Santiago, Chile
[5] Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile
[6] Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.
Contact to: karentoledostuardo@gmail.com

The development of therapeutic antibodies more effective for the cancer is challenging. The early determination of the affinities between antibodies and antigens is critical for the selection of effective antibodies. The use of fast and cost-effective methods for determining this characteristic should be implemented at the research level to select preliminarily the best candidates of antibodies. These tools should consider the structural aspects both antigen and antibody to demonstrate the minimal changes in their conformation that could modify its interaction. In this context, the isothermal titration calorimetry (ITC) and fluorescence are methods that use low concentrations of unlabeled molecules in solution avoiding possible structural modifications of other methods where these molecules are immobilized on a surface. The main objective of this work was to determine the affinity constants of three mini-antibodies directed to the MICA protein, an immunotarget in cancer, through ITC and fluorescence. We produced a recombinant mini antibody wild type (WT) and variants, which include mutations in their heavy and light chains, called G188W and Beta. These mutations were proposed by an *in-silico* model for improving the affinity against the MICA protein. The mini-antibodies were produced in BL21 (DE3) cells and purified by affinity chromatography with Ni-NTA. The affinity constants were measured by ITC and fluorescence of tryptophane contained in the sequence of these mini-antibodies. The affinity constant of the Beta mini-antibody was higher than the WT mini-antibody by calorimetry and fluorescence. The calorimetry had the advantage of allowing to determine the thermodynamic characteristics such as enthalpy and entropy, which were favorable for the WT mini-antibody and its mutants with a stoichiometry 1:1 in all cases. The fluorescence of tryptophane contained in the sequences of mini-antibodies allowed obtaining affinity constants, which were comparable to ITC method. In conclusion, the determination of the affinity constants of the anti-MICA mini-antibodies is comparable both in ITC and fluorescence. Thus, we suggest that these methods could be implemented at the research level for a fast and cost-effective selection of best candidates of antibodies.

Acknowledgments: FONDEF IDeA ID20I10106, FONDECYT Nº1221031, Anillo Regular de Investigación en Ciencia y/o Tecnología 2021 (ACT210068).

CHO vs. HEK cell lines: impact on antigen-binding and neutralizing activity of engineered scFv-Fc antibodies

Aguilar, M. F.[1]; Attallah, C. [1]; Etcheverrigaray, M. [1]; Oggero, M. [1]
[1] UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Pcia. de Santa Fe, Argentina
Contact to: fernandaaguilar74@gmail.com

Antibodies as therapeutic candidates for autoimmune diseases have gained importance in recent decades. We developed two novel molecules from an anti-human interferon-α2b (hIFN-α2b) single-chain variable fragment (scFv) earlier obtained in our lab: a chimeric fragment between the scFv fused to the human Fcγ1 region (qm.scFv-Fc) and a humanized scFv also fused to the Fcγ1 region (hz.scFv-Fc). Both molecules, were stably expressed in CHO and HEK cells and their affinity constants (K_A) and *in vitro* rhIFN-α2b neutralizing ability were analyzed. HEK-derived hz.scFv-Fc showed a notable decrease in both parameters in comparison with CHO-produced hz.scFv-Fc. In the case of qm.scFv-Fc, differences in neutralizing capacity were only demonstrated. Considering these results and the fact that Fc N-glycosylation is the main posttranslational modification (PTM) in antibodies, the influence of N-glycosylation on antigen-antibody (Ag-Ab) binding was evaluated. In this sense, the same parameters mentioned above were analyzed for qm.scFv-Fc and hz.scFv-Fc molecules previously N-deglycosylated under non-denaturing conditions. Only the neutralizing ability was considerably reduced using both partially-deglycosylated molecules. In addition, N-glycan profile analysis showed that Fc N-glycosylation degree influences the *in vitro* antigen-neutralizing ability of these molecules.
These results give evidence that the producer cell line influences the antigen-binding and neutralizing ability, leading to the conclusion that the host cells should be carefully taken into account in order to develop a new therapeutic antibody.

Development of functional de-immunized versions of interferon alpha for the treatment of emerging viral infections

Giorgetti, S.[1]; Etcheverrigaray, M.[1]; Terry, F.[2]; Martin, W.[2]; DeGroot, A.[2,3]; Ceaglio, N.[1]; Oggero Eberhardt, M.[1]; Mufarrege, E.[1]

[1] Laboratory of Cell Culture, Faculty of Biochemistry and Biological Sciences, Biological Center of Litoral - Universidad Nacional del Litoral, S3000ZAA Santa Fe, Argentina

[2] EpiVax, Inc., Providence, RI 02903, USA

[3] Institute for Immunology and Informatics, University of Rhode Island, Providence, RI 02903, USA

Contact to: mufarrege@fbcb.unl.edu.ar

Human interferon α (hIFN-α) administration constitutes the current FDA approved therapy to treat chronic Hepatitis B and C virus infections. Additionally, numerous studies have highlighted this cytokine as candidate for the treatment of emerging viral diseases such as Zika, Chikungunya or Dengue virus infections. Moreover, the pegylated forms of hIFN-α2a and hIFNα2b have been proposed as therapeutic alternatives to treat infections caused by the acute respiratory syndrome coronavirus2 (SARS-CoV-2). However, a major issue related with hIFN-2b therapy is given by its short plasma half-life. To optimize the cytokine's pharmacokinetic profile, our group has developed a highly O-glycosylated IFN, GMOP-IFN, by fusing the N-terminal end of the cytokine to a peptide containing four potential O-glycosilation sites. Given that hIFN-α is immunogenic, neutralizing antibodies (NAb) induction is frequently observed after repeated administrations of this biologic. For this reason, in order to develop a safer and more efficient IFN therapy, in this study we applied the DeFT (De-immunization of Functional Therapeutics) approach to develop functional, de-immunized versions of GMOP-IFN. Two variants out of four displayed reduced ex vivo immunogenicity, while preserving antiviral function. Moreover, both IFN muteins exhibited null specific antiproliferative activity, which constitutes an additional highly favorable characteristic when considering the dramatic consequences associated to hematologic disorders commonly produced during hIFN-α therapy. Altogether the results obtained in this work indicate that the new de-immunized GMOP-IFN variants constitute promising candidates for antiviral therapy, exhibiting reduced immunogenicity while lacking antiproliferative properties.

Biotherapeutic potencial of a biosurfactant extract obtained from *Pseudomonas syringae pv tabaci*

Martini, G.[1]; Nerli, B.[1]; Bustillo, S.[2]; Pellegrini Malpiedi, L.[1]

[1] Instituto de Procesos Biotecnológicos y Químicos Rosario (IPROBYQ), Universidad Nacional de Rosario (UNR)- CONICET, Rosario, Argentina

[2] Grupo de Investigaciones Biológicas y Moleculares (GIBYM), IQUIBA-NEA CONICET, Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina

Contact to: martini@iprobyq-conicet.gob.ar

The interest in biosurfactants (BS) has increased in recent years due to their biodegradability, biological properties and the possibility of production by fermentation. However, there are few publications on their potential antitumor activity. Thus, in this work we evaluate the effect of a lipopeptides (LP) extract produced by *P. syringae pv. tabaci* on tumoral cell adhesion and migration. Firstly, cytotoxicity of LP (0.625-10 mg/mL) was assessed in a murine tumoral cell line, LM3 (CVCL_D269), grown in DMEM-5%FBS at 37°C-5%-CO2 for 3-24h. Non-cytotoxic concentrations were selected for both assays. For cellular adhesion evaluation, cells (3×10^4/well) were preincubated for 30 min at 37°C with LP (0.625-5 mg/mL) or culture medium (control). Adherent cells were fixed and stained with crystal violet and the percentage of cell adhesion was determined. Cell migration was measured by wound-healing assay. Cells were grown in a 6-well plate and a wound was created by a sterile pipette tip. Culture medium alone or LP (2 mg/mL) were added to the cells and incubated for 24 h. Wound widths were measured and percentage of cell migration was calculated. Results showed that the lowest dose assayed (0.625 mg/mL) evidenced a 17.7% of adhesion inhibition effect and with 5 mg/mL, 58.5% of cells didn't adhere to the substrate. Moreover, the control wound scratch almost closed after 24 h but treatment with 2 mg/mL of LP resulted in the suppression of cell migration in a 37.3 %. Although more studies are needed, these findings demonstrate the therapeutic potential of these biosurfactants.

The Production of Biopharmaceuticals in the Public Laboratories of Argentina

Burgardt, N. I.[1]
[1] Consejo Nacional de Investigaciones Científicas y Técnicas - CONICET, Agencia Nacional de Laboratorios Públicos – ANLAP, Argentina
Contact to: burgardtnoelia@gmail.com

Argentina has four public laboratories that produce vaccines and/or biological medicines: Hemoderivados Laboratory, Tomás Perón Biological Institute, the National Institute for the Production of Biologicals, and the “Julio Maiztegui” Human Viral Diseases Institute. Public laboratories conceive medicines as a social good and guide their production according to health demand. The incorporation of cell culture techniques is essential to meet the current demand in medical treatments involving biotechnological products. The objective of this work was to carry out a survey of the use of cell culture technologies in public laboratories. As a result, we found that two of these laboratories use animal cell cultures for the production of viral vaccines. The Institute of Human Viral Diseases "Julio Maiztegui" produces the Candid-1 vaccine using FRhL-2 cell cultures. The Tomás Perón Biological Institute produces the anti-rabies vaccine for veterinary use through BHK21 cell cultures. In addition, the Tomás Perón Biological Institute and the National Institute for the Production of Biologicals use cultures of mycobacteria for the production of BCG Immunotherapeutic and Tuberculin PPD. On the other hand, there are projects for the development and production of biopharmaceuticals using these technologies. For example, the Blood Products Laboratory is developing the production of recombinant Factor IX by CHO cell culture. The results obtained indicate that these laboratories are in a process of strengthening their capacities for the production of biopharmaceuticals through cell culture technologies.

Galectin expression in mammalian cell system

Estruch, B.[1,2]; Prato, C. A.[1,2]; Tribulatti, M. V.[1,2]

[1] Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín (UNSAM) -Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

[2] Escuela de Bio y Nanotecnologías (EByN), Universidad Nacional de San Martín, Buenos Aires, Argentina

Contact to: beestruch@gmail.com

Galectins (Gals), a family of mammalian lectins, have become key soluble players in the immune system. Despite Gals do not possess a signal peptide, they are readily secreted into the extracellular space by an unconventional mechanism. Galectin-8 (Gal-8), in particular, displays multiple immunostimulatory properties which makes it an interesting candidate to be used as an adjuvant in vaccine formulations. The present work aims to express recombinant Gal-8 in an endotoxin-free system and test its activity in the immune response. For this purpose, we expressed recombinant Gal-8 in the HEK293 cell line by lentiviral transduction with the addition of a heterologous signal peptide (Sec-pLB-Gal-8) to enhance protein secretion and optimize the purification step from the culture supernatant. Despite Gals are not glycosylated, they present potential glycosylation sites in their sequences. Therefore, we also included in our study the expression of recombinant Gal-8 without signal peptide (pLB-Gal-8) to compare protein function with the signal peptide-containing version. As a result, we obtained a high efficiency (> 80%) stable-transduced HEK293 cell line that secretes Gal-8 to the extracellular milieu. The galectin bound to Lactosyl-Sepharose resin and was eluted with lactose, indicating that lectin-activity is preserved. Moreover, secreted Gal-8 possessed an apparent molecular weight higher than the intracellular version without the signal peptide, compatible with glycosylation. Cell culture conditions were optimized to augment recombinant Gal-8 expression and, even at a low-scale lab production, the protein yield was consistent to perform molecular and functional characterization.

Two novel hGM-CSF-derived peptides to improve both the properties and the production processes of biotherapeutics

Leopold, M. J.[1]; Kratje, R.[1]; Oggero Eberhardt, M.[1]; Ceaglio, N.[1]
[1] UNL, CONICET, FBCB, Centro Biotecnológico del Litoral (CBL), Ciudad Universitaria, Ruta Nacional 168- Km 472,4-C.C. 242-(S3000ZAA), Santa Fe, Argentina
Contact to: mleopold@fbcb.unl.edu.ar

Engineering by hyperglycosylation represents a strategy for improving the performance of biotherapeutics, being the use of glycosylated peptide tags an attractive alternative. Accordingly, two novel hGM-CSF-derived peptides, the native GMOP and a modified version of GMOP (mGMOP) with 4 and 6 potential O-glycosylation sites, respectively, were developed. Both tags contain a linear epitope (APAR) recognized by a monoclonal antibody (mAb CC1H7). The fusion of these peptides to hIFN-α2b (as case study), increased the molecular mass due to O-glycan attachment, and improved the pharmacokinetics and stability of GMOP-IFN and mGMOP-IFN chimeras, without significant differences among them. The goal of this work was to study the properties of novel peptide-IFN chimeras in a hyperglycosylated context. Also, the potentiality of both peptides to behave as tags for facilitating analytical techniques was evaluated. Two variants were designed by adding 3 copies of GMOP or mGMOP to the N-terminus and 1 copy to the C-terminus of hIFN-α2b, and expressed in CHO-K1 cells. Online predictors of potential O-glycosylation sites showed molecules with 17 and 27 putative sites, respectively. Both chimeras showed higher molecular masses (~45 and ~65 kDa) compared to wild-type hIFN-α2b, indicating the successful incorporation of O-linked glycans. Interestingly, $GMOP_3$-IFN-GMOP showed around 2-times higher antiviral activity than the corresponding for $mGMOP_3$-IFN-mGMOP. Also, APAR/CC1H7 interaction was useful to develop an affinity tag system, allowing the successful purification of IFN chimeras. In conclusion, both peptides are susceptible to be used in a dual conceptualization: to improve biological properties and to enhance the production processes of therapeutic proteins.

Bioactivity characterization of IFN-β biosimilar candidates through a multiplexed gene expression platform

Peña, L.[1]; Ricotti, S.[1]; Haile, L.[2]; Etcheverrigaray, M.[1]; Verthelyi, D.[2]; Mufarrege, E.[1]

[1] Laboratory of Cell Culture, Faculty of Biochemistry and Biological Sciences, Biological Center of Litoral - Universidad Nacional del Litoral, S3000ZAA Santa Fe, Argentina

[2] US Food and Drug Administration, Silver Spring, Maryland, USA

Contact to: mufarrege@fbcb.unl.edu.ar

Recombinant human interferon β (rhIFN-β) therapy is the first-line treatment in relapsing-remitting forms of multiple sclerosis (MS). The mechanism of action underlying its therapeutic activity is only partially understood, as IFN-βs induce the expression of over 1000 genes modifying multiple immune pathways. Currently, assessment of potency for IFN-β products is based on their antiviral effect, which is not necessarily linked to its therapeutic effect. In this study, we explored the use of a multiplexed gene expression system to more broadly characterize IFN-β bioactivity. We find that MM6 cells stimulated with licensed rhIFN-βs induce a dose dependent and reproducible pattern of gene expression. This pattern of gene expression was used to compare the bioactivity profile of biosimilar candidates with the corresponding innovator rhIFN-β products, Rebif and Betaseron. While the biosimilar candidate for Rebif matched the pattern of gene expression, there were differences in the expression of a subset of interferon-inducible genes including Cxcl-10, Cxcl-11, and Gbp1induced by the biosimilar candidate for Betaseron. Assessment of product impurities in both products showed that the difference was rooted in the presence of innate immune response modulating impurities (IIRMI) in the licensed product. These studies indicate that determining the expression levels for an array of reporter genes that monitor different pathways can be informative as part of the demonstration of biosimilarity or comparability for complex immunomodulatory products such as IFN-β, but the sensitivity of each gene to potential impurities in the product should also be tested to fully interpret the results.

S3-35

Selection of a mini antibody against the alfa 2-domain of the MICA protein by Phage Library

González, M.[1,2]; Tello, S.[1]; Rodríguez, J.[3]; Molina, M. C.[1]

[1] Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Universidad de Chile, Chile

[2] Facultad de Ciencias Naturales, Matemática y del Medio Ambiente, Escuela de Industria Alimentaria y Biotecnología, Universidad Tecnológica Metropolitana, Chile

[3] Facultad de Ingeniería, Escuela de Ingeniería bioquímica, Pontificia Universidad Católica de Valparaíso, Chile

Contac to: mauricio.gonzalezo@utem.cl

Given the need to identify new target molecules for the development of antitumor therapies, the MICA protein was proposed due to its high expression in tumor cells and its participation in immune evasion through the internalization of the activation receptor NKG2D in Natural Killer cells. The aim of this work was to develop a mini antibody (scFv) directed against the Alpha2 domain of MICA (α-Alpha2). To obtain the α-Alpha2 scFv, a phage library was designed from human antibody variable region sequences, which were inserted into the pUCh1 vector and phages carrying the α-Alpha2 scFv were selected from twenty colonies of DH5α bacteria previously infected by the phage library. The affinity of the selected phages was analyzed by enzyme-linked immunoassay (ELISA), analyzing the binding of the phages to the peptide corresponding to the Alpha 2 domain of MICA. Structural characterization of the α-Alpha2 scFv was performed by IgBlast, comparing the identity of the phage DNA with the germline genes of antibody families. The construction of the scFv was performed from IMGT/Collier-de-Perle. Two mini antibodies were obtained from infected colonies with high affinity against the Alpha 2 domain of MICA. The nucleotide sequences of the scFvS reached an identity higher than 98.5% with respect to the antibody families, IGKV2-30*01 and IGHV1-46*01, presenting a unique sequence in the scFv CDRs and a correct amino acid conformation, characteristic of the mini antibody. The developed methodology allowed the selection of a mini α-Alpha2 antibody, which can be applied for the selection of new targets.

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

S3-36

In vitro and *in vivo* characterization of the binding capacity of a fully human anti-MICA antibody to the α1 non-polymorphic region of MICA

Tello, S.[1]; Farías, C.[1]; Gutiérrez, M.[1]; Toledo, K.[1]; Siza, J. R.[1]; Jerez, B.[1]; Ribeiro, C. H.[1]; Molina, M. C.[1]

[1] Laboratorio de Anticuerpos Recombinantes e Inmunoterapia, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile, Chile

Contact to: samantha.tello.a@gmail.com

MICA is involved in tumor immunosurveillance and immune evasion processes. It is expressed in different cancers, including gastric adenocarcinoma, being proposed as a therapeutic target. MICA is highly polymorphic, with protein variants that differ in their secretion characteristics and affinity for the NKG2D receptor in Natural Killer cytotoxic cells. The aim of this work was to characterize the expression of allelic variants of MICA in gastric cell lines to evaluate the *in vitro* and *in vivo* binding capacity of a fully human anti-MICA antibody (AcHu-αMICA) constructed from an antibody fragment (scFv) targeting the non-polymorphic α1 region of MICA. For this purpose, the MICA gene was genotyped from GES-1, AGS and MKN-45 cell lines. The binding of AcHu-αMICA to MICA expressed by different cell lines was assessed by flow cytometry. The *in vivo* binding capacity of AcHu-αMICA was assessed in a murine xenograft model with gastric metastatic cells (MKN-45) by bioluminescence. The presence of the MICA*010:01 allele was identified in the AGS line,MICA*009:01 in MKN-45 cells and MICA*008 in GES-1. AcHu-αMICA was shown to bind to all allelic variants present in the cell lines. Biodistribution of anti-MICA scFv to the tumor site was evidenced in the murine xenograft model with MKN-45 cells. It is concluded that a fully human anti-MICA antibody recognizes different MICA variants *in vitro* and *in vivo* (scFv), being an attractive strategy for the future treatment of tumors expressing this molecule.

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

Production of complex recombinant proteins in *Komagataella phaffii*: Presence of the pro-domain and its influence on secretion of BMP2 and tPA

Veas, R.[1,2] ; Quezada, J.[1] ; Guerrero, K.[1] ; Altamirano, C.[1] ; Fickers, P.[3] ; Osses, N.[2]; Berrios, J.[1]
[1] Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Chile
[2] Instituto química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Chile
[3] Gembloux Agro-Bio Tech, Université Libre de Bruxelles, Bélgica
Contact to: rhondaveas@gmail.com

The methylotrophic yeast *Komagataella phaffii,* known as *Pichia pastoris* is a versatile and economical system for recombinant protein production with pharmacological potential. A group of these recombinant proteins are known as difficult-to-express (DTE) whose inherent characteristics could limit their production. Previous studies indicate that the presence of native pro-domain could play an important role in the folding, expression and secretion of DTE proteins. In this study we propose different modifications in the pro-domain of two complex proteins (human Bone Morphogenetic Protein 2: BMP2; human tissue plasminogen activator: tPA), could determine their secretion level in *K. phaffii*. Different structures of BMP2 and tPA were compared, such as complete protein (native), protein fragment (mature) and modification to the cleavage site. All the nucleotide sequences (BMP2 and tPA gene) were optimised for expression in *K. phaffii*, then synthesised, cloned into the pPICZαA vector and expressed under the control of the AOX promoter in *K. phaffii* X-33. All recombinant proteins were purified using two-step ion-exchange chromatography. The results have shown different yields from the purified culture medium for all variants. The proposed strategy is expected to improve the production of DTE proteins in *K. phaffii*, and other complex proteins with pharmacological potential, in economic systems of production.

From erythropoiesis to neuroprotection: on the way of a new erythropoietin-based biotherapeutic

Bürgi, M.[1,4]; Wandel-Petersen, V.[1]; Depetris, M.[4]; Aparicio G.[2]; Scorticati C.[2]; Arinrad S.[3]; Ehrenreich H.[3]; Kratje R.[1,4]; Oggero-Eberhardt M.[1,4]

[1] UNL, CONICET, FBCB, Centro Biotecnológico del Litoral, Santa Fe, Pcia. de Santa Fe, Argentina

[2] UNSAM, CONICET, IIB-INTECH, Laboratorio de Neurobiología, San Martín, Pcia. de Buenos Aires, Argentina

[3] Clinical Neuroscience, Max Planck Institute of Experimental Medicine Göttingen, Germany

[4] BioSynaptica SA, Santa Fe, Pcia. de Santa Fe, Argentina

Contact to: mburgi@fbcb.unl.edu.ar

Neurodegenerative diseases are pathologies that affects millions of people worldwide. They are characterized by its chronicity and progressive evolution. Despite the alarming statistics, nowadays, there is no effective treatment. In this sense, erythropoietin (EPO) represents a promising candidate to treat these pathologies because, besides its erythropoietic activity (EA), it displays neurobiological actions. However, when recombinant human EPO (rhEPO) pretends to be used as a neurotherapeutic in patients without anemia, its EA produces undesirable effects. To circumvent this condition, new hyperglycosylated EPO-analogs were developed through glycoengineering by hyperglycosylation. Using site-directed mutagenesis, one or two amino acid residues of EPO sequence were replaced to introduce an extra N-glycosylation site aiming to block its EA but preserving its neurobiological properties and extending the molecule half-life. Mut 45_47, Mut 104 and Mut 151_153 were produced in CHO.K1 recombinant cell lines. Then, EPO-analogs were affinity-purified and characterized. They showed higher molecular mass and glycan content than rhEPO which resulted in an improvement of their pharmacokinetic properties. Furthermore, the *in vitro* and *in vivo* EA was successfully blocked while its neuroprotective and neuroplastic activity (analyzed in neuron primary cultures) were preserved. Finally, the *in vivo* effectiveness to improve motor-cognitive performance in mice was studied by testing their behavior in complex running wheels. Only Mut 151-153 was comparable to rhEPO. In summary, Mut 151-153, represents a promising neurotherapeutic candidate to explore in different animal models of neurodegenerative diseases.

Production of new hyperglycosylated EPO-analogs using CHO.K1 and HEK-293 cell lines

Iturraspe, F.[1]; Bürgi, M.[1]; Kratje, R.[1]; Oggero Eberhardt, M.[1]

[1] UNL, CONICET, FBCB, Centro Biotecnológico del Litoral. Ciudad Universitaria UNL. Pje. "El Pozo" - C.C. 242, (S3000ZAA) Santa Fe, Argentina

Contact to: fiturraspe@fbcb.unl.edu.ar

Three hyperglycosylated human erythropoietin (hEPO)-analogs were produced in our laboratory as neurotherapeutic candidates through glycoengeniering by hyperglycosylation to block the erythropoietic activity (EA) but preserving its neurobiological action (NA). Mut 45_47, Mut 104 and Mut 151_153 were produced by CHO.K1 cells, affinity-purified and characterized, evidencing the incorporation of an extra N-glycan chain, the lack of their EA and the preservation of their NA. Furthermore, the HEK-293 cell line produces glycoproteins with simpler glycosidic structures, lower sialic acid content and higher receptor binding. Therefore, HEK-293 cells-derived-hEPO could be similar to the hEPO produced in the brain. Likewise, human cell lines are widely considered to produce glycoproteins as biotherapeutics due to their ability to introduce post-translational modifications comparable to those found in proteins naturally secreted by humans. To obtain hEPO-variants with a similar glycan profile to the hEPO produced in the brain, HEK-293 cell lines were transduced with lentiviral particles. The new proteins were purified by immunoaffinity chromatography showing yields between 43% and 60% and purities higher than 90% in a single purification step. Also, the HEK-produced proteins did not show *in vitro* EA as the corresponding analogs produced in CHO.K1 cells. Besides, HEK-produced variants showed lower molecular mass than those produced by CHO.K1 cells. This difference could be due to less complex glycan structures produced by HEK-293 cells as a consequence of a lower antennarity and/or a lesser sialic acid content, resembling the hEPO produced in the brain.

Development of a novel cell line producing recombinant human chorionic gonadotropin (rhCG) for the veterinary industry

Cervetti, J.[1]; Gugliotta, A.[2]; Antuña, S.[3]; Fontana, D.[2,3]; Prieto, C.[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
Contact to: joaquin.cervetti@gmail.com

Human chorionic gonadotropin (hCG) is a glycoprotein hormone that is produced primarily by syncytiotrophoblastic cells of the placenta during pregnancy, and exists as a heterodimer composed of an α-subunit and a disulfide-linked β-subunit. The hormone stimulates the corpus luteum to produce progesterone to maintain the pregnancy. In the veterinary industry, hCG is used for fertility treatments in different livestock species such as cows, pigs, sheeps, horses, goats, domestic species, among others. Currently, the hCG available in the veterinary industry is obtained by purification from the urine of post-menopausal women. Thus, the development of a new product based on rhCG using animal cell culture technology is of great interest. The sequences of α and β rhCG were chemically synthesized and independently cloned into lentiviral expression plasmids. Next, the assembly of lentiviruses α rhCG, β rhCG and GFP (the latter for the determination of the lentivirus titer by flow cytometry) was carried out, and these were used for the transduction of CHO-K1 cells. Newly generated cell lines were selectively pressed with puromycin and subsequently cloned by the limiting dilution method. The cell line as well as two clones were adapted to suspension growth using a chemically defined serum-free medium. Growth curves and rhCG productivity were determined in order to compare them. The best candidate for production was cultured in 1L stirred tank bioreactor operated in perfusion mode during 7 days. Cell density, viability, glucose consumption, lactate production and rhCG concentration were monitored during the process.

The neuroprotective effects of purified spent coffee ground extracts obtained of the SSF in a culture of SH-Sy5y induced Alzheimer disease model

Arancibia-Diaz, A[1].; Vergara-Castro, M.[1]; Astudillo-Castro, C.[2,3]; Soto-Maldonado C.[3], Altamirano-Gómez C.[1,3], Zúñiga-Hansen M.E.[1,3]

[1] School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2085, Valparaíso 2362803, Chile

[2] School of Food Engineering, Pontificia Universidad Católica de Valparaíso (PUCV), Waddington 716, Playa Ancha, Valparaíso 2360100, Chile

[3] Centro Regional de Estudios en Alimentos Saludables, Av. Universidad 330, Curauma-Placilla, Valparaíso 2373223, Chile

Contact to: carolina.astudillo@pucv.cl

This research aimed to evaluate enriched and purified Spent Coffee Ground extracts (SCGE) with potential neuroprotective properties in an SH-Sy5y cell line 2D model-induced Alzheimer's disease (AD). SCGEs enriched with a solid-state fermentation (10th and 30th days) and purified for ultrafiltration at 3 kDa (SCGE-T103kDa and SCGE-T303kDa) were the most innocuous, observing a viability ≥60% at a dose of 1.5 mg/mL. When oxidative stress was induced with H2O2 in the SH-Sy5y cell line, the effect on ROS reduction showed a 90% with SCGE-T103kDa and an 87% with SCGE-T303kDa in comparison to a control. The mechanism of the action exerted by SCGE-T103kDa and SCGE-T303kDa on signaling pathways associated with AD in a culture of SH-Sy5y induced with 27-hydroxycholesterol (27-OHC) was analyzed. It was shown that 27-OHC causes an increase in ROS intracellularly and that SCGE-T103kDa had the highest efficacy in reducing these ROS by 88.5% compared to the induced control. For its part, 27-OHC showed β-amyloid peptides (1-40) production, the primary biomarker of AD, determining that at a dose of 0.20 mg/mL of SCGE-T103kDa, it managed to reverse the expression of the peptide Aβ by 1.5 times compared to the control. Based on these results, a neuroprotective model of SCGE-T103kDa was proposed, associating its action to the potential antioxidant effect, and silencing the signaling pathway through the cascade regulated by the CHOP protein directly involved in the activation of genes involved in the induction of the amyloidogenic pathway. This research demonstrated that SCGE with possible neuroprotection activities on SH-Sy5y cell line, against the appearance of peptide β-amyloid 1-40 as a biomarker of Alzheimer's disease, suggesting an action on the signaling pathway activated by oxidative stress caused by the inducer 27-OHC, which allows silencing or inactivating at least one of the activation pathways of the amyloidogenic pathway in the above-mentioned cell line.

Cell culture production of a recombinant bovine follicle-stimulating hormone (rbFSH) and a hyperglycosylated variant

Villarraza, J.[1]; Tardivo, M. B.[2]; Antuña, S.[2]; Díaz, P.[3]; Ortega, H.[3]; Prieto, C.[2, 4, 5]; Ceaglio, N.[1]

[1] UNL, CONICET, FBCB, CBL, Santa Fe, Argentina

[2] Biotecnofe S.A. PTLC, Santa Fe, Argentina

[3] Centro de Medicina Comparada, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), UNL / CONICET, Esperanza, Santa Fe, Argentina; Facultad de Ciencias Veterinarias del Litoral, UNL, Esperanza, Santa Fe, Argentina

[4] UNL, FBCB, CBL, Santa Fe, Argentina

[5] Cellargen Biotech SRL, FBCB, Santa Fe, Argentina

Contact to: jvillarraza@fbcb.unl.edu.ar

Porcine follicle-stimulating hormone (pFSH) is a glycoprotein hormone used to induce superovulation in cows. The only product available on the market consists of partially purified pFSH preparations from pituitary glands comprising many disadvantages, including batch to batch variations, possible contamination with luteinizing hormone and an extremely short circulating half-life. For these reasons, the development of a recombinant bovine FSH (rbFSH) represents a powerful strategy to substitute the pituitary-derived preparations. The aim of this work was to develop a biotechnological process for the production of rbFSH and a hyperglycosylated long-lasting variant (rbFSH-LD) both fused to a His-tag and expressed in suspension CHO-K1 (sCHO-K1) cells cultured in serum-free medium (SFM). Cell lines were generated through lentiviral transgenesis of sCHO-K1 cells. After adaptation to SFM, cells were cultured in a one-liter stirred tank bioreactor for 18 days. rbFSH and rbFSH-LD proteins were purified from culture supernatants by immobilized metal affinity chromatography (IMAC). Both proteins eluted with high purity levels (>95%) with traces of free 〈 and ® subunits as main contaminants. The biopotency of purified hormones was evaluated in female rats through the Steelman and Pohley bioassay using a recombinant human FSH (rhFSH) as a reference preparation. These preliminary assays showed that both hormones display FSH activity in experimental animals. Moreover, the specific biological activity of rbFSH-LD was similar to the one obtained for non-modified rbFSH, suggesting that the addition of new glycosylation sites to the molecule did not alter the hormone´s function.

Viral safety in cell banks used to produce biological products

Franco, L.[1]; Becerra, M.L.[1]; Pérez López, J.[1]; Salazar, D.[1]; Politzki, R.[1]; Ruiz, V.[1,2]; Alvarez, I.[1,2]
[1] Laboratorio de Virus Adventicios, Instituto de Virología e innovaciones Tecnológicas (IVIT), INTA-CONICET, Argentina.
[2] CONICET, Argentina
Contact to: alvarez.irene@inta.gob.ar

All biotechnology products derived from cell lines have the potential risk of viral contamination, that could have serious clinical consequences. This contamination can arise from the contamination of the source cell lines used as a cell substrates or from adventitious introduction of virus during production. The viral safety of biotechnological products can be assured by the application of a virus testing program including three main complementary approaches: (1) selecting the source material, (2) testing of source materials and products originated during the manufacturing process for the absence of detectable virus, and (3) testing the ability of the manufacturing process to remove or inactivate viruses. The standard adventitious viral control scheme of a cell bank used to produce biological products should include the inoculation of the sample into susceptible indicator cell lines capable of detecting a wide range of specific-species and relevant viruses. The sample should also be inoculated into laboratory animals (suckling and adult mice) and embryonated eggs, in order to reveal viruses that cannot grow in cell cultures. The specific detection of the viruses should be performed by immunoassays. The Adventitious Laboratory at Virology Institute of INTA has the facilities approved by Argentina´s National Sanitary Authority in animal health (SENASA) required to perform the adventitious contaminant control in biological products. Every study is designed in accordance with national and international regulatory guidelines, and with the sponsor requirements as well.

Characterization of a ScFV α-ST2: a new perspective for Inflammatory Bowel Disease study

Vásquez, G.[1]; Dubois-Camacho, K.[1]; De la Fuente, M.[1]; Novoa, P.[1]; Gutiérrez, M.[2]; Sánchez, O.[3]; Molina, M.[2]; Hermoso, M. A.[1]

[1] Laboratorio de Inmunidad Innata, Programa de Inmunología, Facultad de Medicina, Universidad de Chile, Santiago, Chile

[2] Ragon institute of MGH, MIT and Harvard, Massachusetts, USA

[3] Laboratorio de Biofármacos Recombinantes, Universidad de Concepción, Concepción, Chile

Contact to: gzvm.hardaz@gmail.com

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) characterized by an exacerbated intestinal immune response, alternating between active and remission states. IL-33 (IL-1 family member), associates with the UC pathophysiology, binding to ST2 receptor, inducing the secretion of proinflammatory cytokines, in addition to helping tissue repair. The ST2 protein increases in serum and colon of UC patients and is considered a possible activity biomarker. A potential tool in ST2 recognition is the use of ScFv (Single Chain Variable Fragment), therefore, in this study, a ScFvα-ST2 was expressed in bacteria, recovered from inclusion bodies and analyzed for detection and ST2 affinity. ScFv (selected from the phage library) was cloned into a His/HA-tag vector for bacterial expression (BL21-DE3-Ori), analyzed for purity by FPLC and renaturation by dichroism. Additionally, an expression vector for ST2 production in bacteria (BL21 DE3-Ori) was used to test ScFvα-ST2 by immunoprecipitation using αHA requirements, affinity was assessed by ELISA. Finally, native ST2 protein from HMC1 (human mast cell-1) cells was obtained for detection of ScFvα-ST2 by flow cytometry. ScFvα-ST2 showed a purity of 97% and the dichroism reading spectrum corresponded to secondary structures. The immunoprecipitation of ST2, using ScFvα-ST2, resulted in bands with a molecular weight corresponding to the ST2 molecule, with affinity within the parameters established. ScFvα-ST2 recognized ST2 protein in HMC1 cells, as shown by flow cytometry (88.9% relative to control). ScFvα-ST2 recognizes the ST2 protein and can be used in functional experiments and to monitor as a potential biomarker of IBD.

Session 4: Vaccines I: COVID19

S4-45

Development of a quantitative sandwich ELISA as a tool for RBD bioprocess optimization

Acuña Intrieri, M.E.[1]; Deriane, M.A.[1]; Rey Serantes, D.A.[2]; Czibener, C.[2]; Goldbaum, F.A.[1]; Comerci, D.J.[2]; Cerutti, M.L.[1]

[1] Centro de Rediseño e Ingeniería de Proteínas (CRIP – IIB – UNSAM), Argentina

[2] Instituto de Investigaciones Biotecnológicas (IIBIO – UNSAM – CONICET), Argentina

Contact to: macunaintrieri@iib.unsam.edu.ar

COVID-19 is an ongoing pandemic caused by the respiratory coronavirus SARS-CoV-2. Up to now, this virus has infected more than 550 million of people and caused more than 6.9 million deaths globally. Among the viral proteins, the RBD domain of the surface Spike glycoprotein constitutes the target of most SARS-CoV-2 neutralizing antibodies, positioning it as a key antigen for the development of effective therapeutic treatments and diagnostic tests. Moreover, newer RBD-based vaccine candidates are emerging as stand-alone or booster vaccines alternatives for low- and middle-income countries due to its higher production rates than the full Spike trimer. Taking into consideration the increasing demand to improve recombinant RBD production, we here present the development and validation of a quantitative sandwich ELISA for bioprocess sample monitoring. The assay is based on a set of matched-pair highly specific in-house developed anti-RBD monoclonal antibodies (mAbs) directed against native RBD epitopes. ELISA validation parameters such as sensitivity, repeatability, reproducibility, limit of detection and limit of quantification were determined. Sample matrix effects were also assessed by analysing recovery yields in supernatant samples from HEK293 cultures grown in chemically defined or serum supplemented media. Performance comparison against routinary SDS-PAGE procedures corroborated its higher specificity and sensitivity for RBD detection and quantification in bioprocess samples. Interestingly, Spike samples were not detected in this ELISA format, pointing out that one or both native anti-RBD epitopes remains occluded in the trimeric antigen. Experiments aimed to address the ELISA versatility against highly divergent RBD variants are underway.

S4-46

Development of clonally-derived HEK293 cell lines expressing trimeric spike protein of SARS-COV-2 variants for the development of a multivalent COVID-19 vaccine candidate

Marsili, F. F.[1,2]; Alvim, R. G. F.[1]; Silva, T. G.[3]; Castilho, L. R.[1,2]
[1] Cell Culture Engineering Laboratory, PEQ/COPPE, Federal University of Rio de Janeiro (UFRJ), Brazil
[2] Biochemistry Graduate Program, IQ – UFRJ, Brazil
[3] Cenabio – UFRJ, Brazil
Contact to: ffm@peq.coppe.ufrj.br

The development of COVID-19 vaccines in 2020 and the mass vaccination campaigns since then have allowed to significantly decrease the incidence of severe COVID-19 cases, hospitalizations and deaths. However, the rapid and recurrent emergence and spread of new highly transmissible SARS-COV-2 variants currently imposes new challenges. In this context, the development of multivalent and updatable vaccines targeting emerging variants could represent an important tool for public health. Most approved COVID-19 vaccines so far, across vaccine platforms (mRNA, subunit, viral vector), focus on the trimeric spike glycoprotein of SARS-COV-2 as antigen, due to its ability to elicit neutralizing antibodies.

In this work, parental HEK293 cells were stably transfected with plasmids encoding the sequence of the spike (S) protein of SARS-COV-2 variants D614G, gamma and delta, using cationic lipids for transfection and G418 sulfate as selective pressure. The resulting stable cell pools were immunolabeled using an anti-S monoclonal antibody and a fluorophore. Cell pools were then submitted to single-cell deposit in 96-well plates using a FACS Aria. A commercial semi-solid medium developed to support single-cell cloning was used to enable the expansion of sorted cell clones. The cells confirmed to be derived from a single cell clone were then frozen and simultaneously expanded to higher plate formats, being then finally transferred to shake flasks for suspension cultivation. After that, the most promising clones were selected and further evaluated in fed-batch cell culture experiments using chemically defined culture medium and nutrient solution. The workflow described herein allowed obtaining clonally-derived recombinant stable cell lines with a production yield that potentially enables their future use in industrial processes for the production of the active ingredient of multivalent COVID-19 vaccines.

Design, optimization and validation of a RBD indirect ELISA for detecting antibodies against SARS-CoV-2

Rodríguez, M.C.[1]; Ceaglio, N.[1]; Fuselli, A.[1]; Villarraza, J.[1]; Garay, E.[1]; Antuña, S.[3]; Gastaldi, V.[1, 3]; Fontana, D.[1, 3, 4], Gugliotta, A.[1]; Prieto, C.[2, 3,4]

[1] Universidad Nacional del Litoral (UNL), CONICET, Facultad de Bioquímica y Ciencias Biológicas (FBCB), Centro Biotecnológico del Litoral (CBL), Santa Fe, Argentina

[2] Laboratorio de Desarrollo Biotecnológico (LDB) – UNL – FBCB – CBL, Argentina

[3] Biotecnofe S.A., PTLC, Santa Fe, Argentina

[4] Cellargen Biotech – SRL – FBCB – LDB, Argentina

Contact to: rodriguezmaceleste@gmail.com

Testing strategies are essential tools to mitigate the effect of COVID-19, help to diagnose the disease and provide data for surveillance and epidemiologic studies. Virus neutralization tests (VNTs) are the gold standard of serology assays. However, they exhibit several drawbacks, including the requirement of containment facilities and the difficulty of standardization. In this context, serological binding tests based on antigen-antibody interaction represent a more versatile alternative.

In this study, we report the production and purification process of the receptor binding domain (RBD) of SARS-CoV-2 in HEK293 cells, which allowed the design, validation and optimization of an indirect ELISA (iELISA) for the detection of human anti-RBD antibodies. To find the optimal conditions of this iELISA, an optimization procedure through design of experiments (DoE) and response surface methodology (RSM) approach was performed, applying a full factorial design at three levels. Thereafter, an optimal condition was found: 136.36 ng of RBD as coating antigen and 1/3225 of commercial HRP-conjugated anti-human IgG for detection (Santa Cruz Biotechnology).

Then, the assay was validated, exhibiting a sensitivity of 94.24 (86.01-98.42%; 95% CI) and a specificity of 95.96% (89.98-98.89%; 95% CI). Besides, the degree of agreement between quality results assessed using Kappa´s value was 0.92. Hence, this iELISA represents a high-throughput technique, simple to perform, reliable and feasible to be scaled-up to satisfy the current demands of the region. Since RBD is proposed as the coating antigen, the intended use of this iELISA is not only the detection of previous exposure to the virus, but also the possibly of detecting protective immunity.

Development, characterization and validation of a sensitive and low-cost method to detect antibodies against SARS-CoV-2

Soldano, N.[1]; Raimondi, A.[1]; Vogt, L.[1]; Shaurli, D.[1]; Bosco, B.[1]; Zuqueli, R.[1]; Amadeo, I.[1]; Forno, G[1].

[1] Amega Biotech, RN 168 - Paraje El Pozo, Parque Tecnológico Litoral Centro (3000) Santa Fe. Argentina.
Contacto to: nsoldano@amegabiotech.com

The Receptor Binding Domain (RBD) of the Spike protein of Sars-CoV2 was used as an antigen in the development of a sensitive and low-cost indirect method to detect antibodies against SARS-CoV-2 in human serum. The portion of DNA encoding the RBD was fused to the sequence corresponding to a signal peptide and to a HisTag sequence at the C-terminus to facilitate subsequent purification. Afterwards, it was cloned into a mammalian cell expression vector. CHO-S cells were transfected with this construction, obtaining a cell line that stably expresses RDB. This CHO-RBD cell line was adapted to grow at high cell densities in serum-free medium. Productivity tests were carried out using shaking cultures in Batch and Fed-batch mode. Subsequently, production tests were carried out in 5-liter bioreactors in perfusion mode, reaching cell densities nearby $26x10^6$ cells/ml. The obtained supernatant was purified using affinity chromatography. The purified RBD quality was analyzed and the protein was used as an antigen to develop an indirect ELISA for the detection of anti-SARS-CoV-2 antibodies. Serum from volunteer donors recovered from Covid-19 were used for method validation. The method was proven to be specific, precise, sensitive and inexpensive. In addition, a sanitary registration was obtained in Argentina under the name of immunoenzymatic assay for the qualitative detection of IgG specific for the RBD antigen of the spike protein of the SARS-CoV-2 virus and the test is currently being commercialized.

S4-49

Analysis of the concentration step of SARS-CoV-2 produced in Vero cells in serum-free medium for antigen production

Perini de Arújo, A.P.[1]; Rodrigues Ueoka, A.[1]; Ferreira Barbosa, F.[1]; Mancini Astray, R.[1]
[1] Laboratório Multipropósito, Instituto Butantan, Brazil.
Contact to: renato.astray@butantan.gov.br

The tangential flow filtration (TFF) is a widely used method for virus concentration step in the biotechnology industry. The performance of TFF is dependent on the amount of contaminants in the harvested and clarified material. The SARS-CoV-2 virus has been produced in Vero cells in serum-free medium, concentrated by TFF and purified by sucrose-gradient ultracentrifugation prior to horse immunization for antiserum production. In this work the TFF performance was evaluated during several ranges of concentration times to define the most appropriate concentration level which was able to conserve antigen concentration and characteristics. The level of DNA and proteins were also measured for the analysis of the partial purification of this step. Results showed that the ideal TMP during TFF process was between 10-12 psi, using the Pellicon 2 300kDa – C screen of 0,1 m^2. When the product was concentrated over than 10X, the material showed aggregation by TEM analysis. The concentration level of 5X resulted in good virus recovery (82,5 %) without many aggregates, good host cell protein removal (less 67,1 %) which is the most critical contaminant for antiserum production. Finally, only a small amount of residual DNA was removed (less 33,1 %) in the process. Benzonase treatment was already assayed in different protocols, showing promisor results for DNA removal.

Production and purification of SARS-CoV-2 soluble antigens, potential tools for the development of diagnostic tests and vaccine candidates

Villarraza, J.[1]; Fuselli, A.[1]; Gugliotta, A.[1]; Garay, E.[1]; Antuña, S.[3]; Gastaldi, V.[1,3]; Tardivo, B.[3]; Rodríguez, M. C.[1]; Alvarez, D.[5]; Castro, E.[5]; Cassataro, J.[5]; Fontana, D.[1]; Ceaglio, N.[1]; Prieto, C.[2,3,4]

[1] UNL, CONICET, FBCB, CBL, Santa Fe, Argentina
[2] Laboratorio de Desarrollo Biotecnológico (LDB) – UNL – FBCB – CBL, Argentina
[3] Biotecnofe S.A., PTLC, Santa Fe, Argentina
[4] Cellargen Biotech SRL – FBCB – LDB, Argentina
[5] Instituto de Investigaciones Biotecnológicas "Dr. Rodolfo A. Ugalde" UNSAM-CONICET, San Martín Prov Buenos Aires, Argentina
Contact to: jvillarraza@fbcb.unl.edu.ar

SARS-CoV-2, the etiologic agent of COVID-19 pandemic disease, presents 4 structural proteins. Spike (S) proved to be the main responsible for neutralizing antibodies production. Thus, its sequence is highly considered for the design of chimeric proteins that might be used in diagnostic tests and as vaccine candidates. In this work, two chimeric proteins were designed based on the SARS-CoV-2 Spike protein. One of them is the Spike ectodomain which contains a foldon trimerization tag (Spike-ED) and the other is the receptor binding domain (RBD). Both proteins contain a signal peptide for their secretion to the culture supernatant and a His-tag for chromatographic purification. The sequences were cloned into animal cells expression vectors and used for cell line generation by sHEK293T transfection and antibiotic selection. The best producer cell lines were adapted to suspension growth using a chemically defined serum-free medium and cultured in 1 L stirred tank bioreactor operated in perfusion mode for 10 days. Protein expression was confirmed and monitored by SDS-PAGE/western blot. Harvests of Spike-ED and RBD were clarified and purified by IMAC. As a result, a purity greater than 98% was achieved for both proteins. SARS-CoV-2 antigens were used for immunogenicity analysis, using two different adjuvants. Higher anti-RBD titers were induced by Spike-ED. Further analysis confirmed that Spike-ED elicits neutralizing antibodies against SARS-CoV-2. To conclude, the proteins designed in the present work, RBD and Spike-ED, can be used for the design of serological tests. Also, vaccine candidates to fight against COVID-19 may be formulated using Spike-ED.

A novel Rabies/SARS-CoV-2 fusion glycoprotein for VLPs expression: a potential vaccine candidate for COVID19

Garay, E.[1]; Fontana, D.[1]; Villarraza, J.[1,], Fuselli, A.[1*]; Gugliotta, A.[1]; Antuña, S.[3]; Gastaldi, V.[1,3]; Tardivo, B.[3], Rodríguez, M.C.[1]; Ceaglio, N.[1]; Prieto, C.[2,3,4]

[1] Universidad Nacional del Litoral (UNL), CONICET, Facultad de Bioquímica y Ciencias Biológicas (FBCB), Centro Biotecnológico del Litoral (CBL), Santa Fe, Argentina

[2] Laboratorio de Desarrollo Biotecnológico (LDB) – UNL – FBCB – CBL, Argentina

[3] Biotecnofe S.A., PTLC, Santa Fe, Argentina

[4] Cellargen Biotech – SRL – FBCB – LDB, Argentina

* Authors contributed equally

Contact to: egaray@fbcb.unl.edu.ar

Virus-like particles (VLPs) are highly immunogenic nanoparticles that have been widely used for vaccine applications. For COVID19, VLPs have been engineered to obtain vaccine candidates through expression of SARS-CoV-2 S, M, E and N proteins. The production of these native VLPs is challenging, as multiple proteins must be expressed to assure VLP budding. As an alternative, we designed a novel rabies glycoprotein, fused to the SARS-CoV-2 Spike ectodomain stabilized in the pre-fusion conformation, with the goal to express and characterize chimeric VLPs (cVLPs) as a COVID19 vaccine candidate. The fusion protein was expressed in HEK293 cells, and its plasma membrane localization was confirmed by immunofluorescence. Using anti-RBD mAbs and convalescent plasma we specifically detect the exposition of key SARS-CoV-2 antigenic epitopes in its structure. Moreover, the correct interaction between our fusion protein with ACE2 receptor was confirmed in HEK293-ACE2 cells transduction assay, using pseudotyped GFP expressing lentiviruses. Further, cVLPs were expressed, purified and its morphology was assessed by transmission electron microscopy (TEM) and immuno-TEM. Finally, the immunogenicity of cVLPs was evaluated in mice vaccinated with two doses of VLPs, 3 weeks apart. Specific antibodies response was detected using either purified RBD or the trimeric spike protein in ELISA assays. High antibody titers were observed, even 3 months after priming. Furthermore, after a booster the animals showed a significative increment of the antibody titers, higher than the obtained 15 days after second dose. In conclusion, we were able to obtain a novel cVLP that presents SARS-CoV-2 native-like morphology and induces a specific humoral immune response in mice, becoming a promising candidate for vaccine applications.

Session 5: Vaccines II

S5-52

Biological recombinant adjuvant for viral diseases in aquaculture

Ortega Rivas, L.A.[1]

[1] Laboratorio de Biotecnología y Biofármacos, Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción. Concepción, Chile

Contact to: leonardoortegabq@gmail.com

Salmon industry is one of the main economic activities in Chile. Some pathogens have a huge impact on exportations generating multiple deaths in farm fields. Nowadays vaccination is the main prophylactic measure. In Chile, there are inactivated and subunit vaccines approved for IPNv (infectious pancreatic necrosis virus). However, these vaccines have not completely eradicated the disease. The use of elements that boost the potency to vaccination can contribute significantly to increase the efficacy of these vaccines. One of the elements that can be co-administered for these purposes are interferons, proteins with antiviral and immunomodulatory activities in mammals and fish. Many studies have shown cytokines as effective adjuvants for vaccines in mammals but little is known in salmonids. In this study, we established a protocol to express and purify a biologically active molecule IFN-a1 from *Salmo salar* using DNA recombinant technology and IMAC. Also, we demonstrated that IFN was able to protect against the cytopathic effect after the infection of IPNv in CHSE-214 cells. Besides, the biological activity was tested through its effects on the induction of interferon-stimulated genes in head kidney leukocytes. Finally, we tested the ability to boost immune response when IFN was coformulated with subunit antigens using VP2 as a model. Results demonstrate that co-administration of VP2 antigens plus IFN-a1 evoked an increase of the expression of the immune related gene expression significantly higher than the control groups. These results represent a promising adjuvant strategy for enhancing the protection of future viral subunit vaccines.

S5-53

Early and solid protection afforded by the thiverval vaccine provides novel vaccination alternatives against classical swine fever virus

Lamothe-Reyes, Y.[1,2]; Bohórquez, J. A.[1]; Wang, M.[1]; Alberch, M.[1]; Pérez-Simó, M.[1], Rosell, R.[1,3]; Ganges, L.[1]

[1] OIE Reference Laboratory for Classical Swine Fever, Institut de Recerca i Tecnologia Agroalimentàries, Centre de Recerca en Sanitat Animal IRTA-CReSA, 08193 Barcelona, Spain

[2] Recombinant Biopharmaceuticals Laboratory, Pharmacology Department, School of Biological Sciences, Universidad de Concepción, 4030000 Concepción, Chile

[3] Departament d'Agricultura, Ramadería, Pesca i Alimentació (DARP), 08007 Generalitat de Catalunya, Spain

Contact to: llilianne.ganges@irta.cat

Classical swine fever virus (CSFV) remains a challenge for the porcine industry. Inefficient vaccination programs in some endemic areas may have contributed to the emergence of low and moderate virulence CSFV variants. This work aimed to expand and update the information about the safety and efficacy of the CSFV Thiverval-strain vaccine. Two groups of pigs were vaccinated, and a contact and control groups were also included. Animals were challenged with a highly virulent CSFV strain at 21- or 5-days post vaccination (dpv). The vaccine induced rapid and strong IFN-α response, mainly in the 5-day immunized group, and no vaccine virus transmission was detected. Vaccinated pigs showed humoral response against CSFV E2 and E^{rns} glycoproteins, with neutralizing activity, starting at 14 days post vaccination (dpv). Strong clinical protection was afforded in all the vaccinated pigs as early as 5 dpv. The vaccine controlled viral replication after challenge, showing efficient virological protection in the 21-day immunized pigs despite being housed with animals excreting high CSFV titers. These results demonstrate the high efficacy of the Thiverval strain against CSFV replication. Its early protection capacity makes it a useful alternative for emergency vaccination and a consistent tool for CSFV control worldwide.

A novel display platform for vaccine applications based on chimeric VLPs produced in cell suspension mode and serum free medium

Garay, E.[1]; Fontana, D.[1, 2]; Leschiutta, L.[1], Kratje, R.[2]; Prieto, C.[1]

[1] Laboratorio de Desarrollo Biotecnológico, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. Santa Fe, Argentina

[2] Laboratorio de Cultivos Celulares, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. Santa Fe, Argentina

Contact to: egaray@fbcb.unl.edu.ar

Virus like particles (VLPs) are novel vaccine candidates that have a structure similar to a virus but are biosafe due the fact that they lack the viral genome. In our laboratory we previously developed a rabies VLP vaccine which is highly immunogenic, being able to trigger neutralizing antibodies and protect mice from infection with live virus. Furthermore, it can be continuously produced using stable HEK293 cells in perfusion mode using serum free medium. In this work we engineered these VLPs to expose an heterologous antigen in its surface, to establish a new heterologous antigen display platform for vaccine applications. To engineer the rabies VLPs, we analyzed the structure of rabies glycoprotein (RVG) and performed a bioinformatics study to discover sites that are suitable for the insertion of foreign epitopes without altering thestructure and trimerization of the glycoprotein. Two insertion sites were discovered on RVG, and a model epitope was inserted on each one individually. The selected foreign epitope was the main neutralizing epitope of Foot-and-Mouth Disease Virus, named G-H loop. The fusion proteins were able to expose the heterologous sequence both on the plasma membrane as wellas in the surface of VLPs, and its antigenic conformation was adequate. Moreover, VLPs were able to induce specificantibodies against G-H loop on mice, as well as RVG neutralizing antibodies. This sets the base for a new heterologous display platform for vaccine application which does not require inactivation procedures and has the potential to be scaled up using serum free medium.

Optimization of the production of GaHV-1 in LMH using T-flask and scaling to Cellcube-Bioreactor system

Quispe, J.[1]; Flores-Santos, J.C.[1]; Caballero, M.[1]; Mendoza-Espinoza, A.[1]
[1] Desarrollo e investigación. Laboratorio de Virología Molecular. QUIMTIA S.A., Lima, (CP15073), Peru
Contact to: juana.quispe@quimtia.com

Gallid herpes virus type 1 (GaHV-1) is the causative agent of an important respiratory disease of domestic fowl termed infectious laryngotracheitis (ILT). This virus is able to replicate in LMH, who is attachment-dependent cell, however, for a larger-scale production is necessary to increase the surface area. This work aims to optimize the production of GaHV-1 in T-flask and, then, scale to a Cell cube-Bioreactor system. For this, LMH cells were infected with an GaHV-1 strain that was isolated from Peru and a multiplicity of infection (MOI) of 1, 0.1 and 0.01 was evaluated. Then, cells were scaled up from T-Flask 225 cm^2 to Cellcube 21250 cm^2 and Cellstack 6360 cm^2 to Cellcube 85000 cm^2 in DMEM medium with 10% of fetal bovine serum. In Cellcube-bioreactor system, cells were cultured to 60% to 80% confluence and infected with an MOI of 0.1, and the virus replication was tracked via medium analysis at 48, 72, 96 and 120 hours post infection (hpi) to determine the time of harvest. Results show that virus infectivity titre of $1x10^9$ $TCID_{50}$/mL was achieved when infected the LMH cells at 60-80% confluence using an MOI of 0.1 and harvest at 96 hpi in a Cellcube-Bioreactor system. This LMH culture platform in a Cellcube-Bioreactor system can be used to evaluate the production of another avian virus like a FAdV, NDV and reovirus and formulate an inactivated vaccine.

In-process controls for the evaluation of harvest point of Zika virus in a fixed-bed bioreactor

Barbosa, F. F.[1]; Perini de Araújo, A. P.[1]; Fongaro Botosso, V.[2]; Mancini Astray, R.[1]
[1] Laboratório Multipropósito, Instituto Butantan, Brazil
[2] Laboratório de Virología, Instituto Butantan, Brazil
Contact to: renato.astray@butantan.gov.br

The analysis of metabolites is a common method for the evaluation of the production phase of a biotechnology process. Other methods can be used when working with a lytic virus as the product of the bioprocess. The activity of Zika virus is responsible for intense cell lysis in a short period of time, making the measurement of the accumulation of cell components in the supernatant an alternative to define the end of the production phase. In this work, Vero cells (CCL-81) were cultivated in a fixed-bed bioreactor and infected with Zika virus. Accumulation of DNA, host cell protein and cell in the supernatant were monitored. Results showed slow accumulation of contaminants in the supernatant during the early kinetics. A high increase in the number of cells in the supernatant was the first signal that the virus production phase was ending, with virus productivity per cell decreasing afterwards. DNA and host cell protein experienced significant increase in the last 24 h of the production cycle before the virus titer started to drop. The results showed that the production of Zika virus can the extended until 40 % of the total cells are suspended in the supernatant, after this point, the amount of contaminants reached high levels and virus titer declined.

The iCELLis® Nano Bioreactor provides a reliable method to produce rabies vaccines with high titer and high potency

Groba, J.[1]; Lundeen, T.[2]
[1] Pall Corporation, SP, BR
[2] Pall Corporation, MA, USA
Contact to: leticia_parizotto@pall.com

In Brazil, rabies is still a concern of public health. For a long time, the public vaccination campaign of domestic animals has been successful. However, in the last few years, vaccine availability and quality issues led to disruptions in this campaign. Currently, vaccine production from adherent cell cultures using roller bottles or 2D flasks is limited by small volumes, little process control, and high manual manipulation. To overcome these challenges, a bioreactor process is required to assure the safety, quality, and scalability of vaccine production. This work aimed to transfer a current rabies virus production using Vero cells in roller bottles (RBs) to the iCELLis Nano bioreactor. The iCELLis Nano bioreactor is a single-use, fixed-bed bioreactor that provides a controlled environment for adherent cells, is Good Manufacturing Practice (GMP) compliant, and has a reduced operational cost and capital investment compared to traditional methods.Keeping the same media volume per surface area ratio (0.29 mL/cm^2) and Multiplicity of Infection (MOI) (0.016), the process was successfully scaledup and resulted in three-fold higher viral production in the bioreactor. Furthermore, the quality attributes of the viral units produced in the iCELLis Nano bioreactor were assessed and presented adequate immunogenicity characteristics and a higher potency level than in the RBs. This higher potency allows the dilution of the product, resulting in more doses per batch and higher productivity. Thus, the iCELLis Nano bioreactor is a suitable bioreactor for scaling up rabies virus production.

S5-58

Baculovirus AcMNPV genome modification with the GPC fragment of the Andes virus to generate a pseudotyped baculovirus as a new biotechnology tool against HCPS

Jerez, B.[1]; Camacho, F.[1]; Sánchez, O.[1].

[1] Recombinant Biopharmaceutical Laboratory, Department of Pharmacology, Faculty of Biological Sciences, University of Concepción, Chile.

Contact to: bejerez@udec.cl

Hantaviruses infect various animals, including rodents that spread the virus via aerosols of their secretions and excretions. Through zoonoses, these viruses infect humans and cause diseases such as the Hantavirus Cardiopulmonary Syndrome (HCPS), which is prominent in the American continent. In Chile and Argentina, the etiological agent of HCPS is called the Andes Virus, which has a mortality rate of over 40% and is the only type of hantavirus that presents person-to-person transmission. To date, no preventive or therapeutic drug against SCPH has been approved by the FDA or its equivalents. In this work, the GPC fragment of the Andes Virus was inserted into the genome of the baculovirus Autographa californica multiple polyhedrovirus (AcMNPV), using molecular technology of baculovirus expression vector systems, MultiBac. This fragment is responsible for producing the envelope glycoproteins Gn and Gc, which are highly immunogenic. The insert was integrated into the genome under the promoters of polyhedrin for insect cells, and cytomegalovirus for mammalian cells. This modification allowed the expression of both glycoproteins in two remarkable scenarios: On the surface of mammalian cells infected with the modified baculovirus, and on the surface of the modified virus, generating a pseudotyped baculovirus in the latter case. This pseudotyped virus could be of great applicability for both the diagnosis and prevention of HCPS since it could be used for a serum-neutralization diagnostic test or as a vaccine.

Development of a biotechnological platform for vaccine production based on VLP using porcine circoviruses recombinant protein

Gastaldi, V. [1,3]; Garay, E.[1]; Fontana, D.[1]; Prieto, C.[2,4]
[1] UNL, CONICET, FBCB, CBL, Santa Fe, Argentina
[2] UNL, FBCB, CBL,LDB, Santa Fe, Argentina
[3] Biotecnofe S.A., PTLC, Santa Fe, Argentina
[4] Cellargen Biotech SRL, FBCB, LDB, Santa Fe, Argentina
Contact to: vic_gastaldi@hotmail.com

Surgical castration and vaccination against Porcine Circovirus (PCV2) are common practices in pig farming. The first has the goal of reducing aggressiveness, sexual behaviorand changes in the smell/taste of meat, and the second, protecting them from PCV2, a virus that produce an important disease with an extensive economic impact. CAP protein of PCV2 is highly immunogenic, strongly reacts with the pig serum infected with this virus, and has been reported that is able to self-assemble forming Virus Like Particles (VLPs). Therefore, CAP is a good candidate for the design of recombinant vaccines. While surgical castration is a required practice, it is a traumatic and irreversible situation for animals, being the immunocastration a viable alternative that has been growing in recent years. This procedure consists on inducing neutralizing antibodies against GnRHI in order to block its action in the hypothalamus-pituitary-gonadal axis. In this context, we proposed to generate a new platform based on chimeric CAP VLPs containing GnRHI peptide in its surface, in order to immunize swine against PCV-2 and immunocastrate animals by a single treatment (dual vaccine). For this, we designed, cloned and expressed different chimeric construction expressing the CAP protein from porcine circovirus, fused to different repetitions of the GnRHI peptide, in *Escherichia coli*. Chimeric proteins were correctly expressed on soluble fractions of bacterial lysate, with a correct molecular weight. Chimeric constructs were detected by anti-PCV-2 and anti-GnRHI antibodies on a bi-specific sandwich ELISA, proving that the inserted peptide has a correct antigenic conformation. Furthermore, were also detected by western blot using both antibodies. Transmission electron microscopy analysis of soluble fractions revealed the presence of round particles with a size of 20 nm, similar to that of native PCV-2 virions, indicating that fusion proteins could be forming chimeric VLPs. These results set the base for a novel dual base vaccine candidate based on novel chimeric VLPs.

Analysis of the performance of working cell bank in the production of Candid #1, a live attenuated Junin Virus Vaccine

Mogetta, M. H.[1]; Maiza, A.[1]; Arias, A.[1]; Abalo, M.[1]; Riera, L.[1]; Saavedra, C.[1]
[1] Instituto Nacional de Enfermedades Virales Humanas "Dr. Julio I. Maiztegui" INEVH-ANLIS, Argentina
Contact to: mhmogetta@anlis.gob.ar

The diploid cell line FRhL-2 was involved in obtaining attenuated Junin viral strain, Candid #1, during the vaccine development and is used to replicate the virus in the production of the vaccine. To ensure an adequate supply of equivalent, well-characterized cells exist for production, a cell bank was created. The original FRhL-2 cell batch was obtained from The Salk Institute (passage 11) and was used to generate the Master Cell Bank (MCB, passage 21), that is used to generate the Working Cell Bank (WCB, passage 25). To analyze the performance of the WCB, 30 batches of Candid #1 produced between 2002 and 2018, were analyzed, and the following attributes were evaluated: cell morphology, viability, cell recovery, % of confluence, and the ability to replicate the virus (measured as viral potency). The cell viability was 88.11% ± 7.53, the cell recovery was 87.49% ± 9.94, no morphology changes were observed and a 70% of cell confluence on the third day of thawing. These parameters characterized the consistency of the vaccine production process that could be demonstrated in the replicative capacity obtained, in all cases, within the established specifications (10^5 PFU/mL). The results show the sustained quality of the cell substrate for 16 years.

Session 6: Development of cell-based technologies and therapies

Cytotoxic analysis of pulverized endodontic files – clinical correlation with the occurrence of endodontic file fracture

Soimu, G.[1]; Carvalho da Silva, G. C.[2]; Oliveira Freitas Lione, V.[2]; Voight, D.[1]; Heggendorn, F. L.[1]

[1] Posgraduate Program in Dentistry, University of Grande Rio (UNIGRANRIO), Rio de Janeiro, Brazil

[2] Laboratory of Pharmaceutical Bioassays, School of Pharmacy, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

Contact to: giulianasoimu@hotmail.com

Fractured endodontic files present a major problem. These difficulties in preparation may predispose to persistent root canal infection and risk for treatment success. To remove the metallic fragment, several techniques are used, but none is shown to be completely safe. On the other hand, the phenomenon of corrosion in endodontic instruments leads to degradation and metallic dissolution of the instrument, in parallel with the biofilm formation. The aim of this study was to determine the *in vitro* cytotoxic effect of pulverized endodontic files on two cell lines: L929 and VERO, after 24 hours of exposure. 40 previously used endodontic files, Kerr type, were pulverized and divided into four groups (5 µg L., 10 µg L., 25 µg L. e 50 µg L.). Groups were organized in an increasing concentration of pulverized steel to determine the cytotoxicity index (IC_{50}) of the metal particles in contact with the cell lines, by means of MTT assays. The MTT assay revealed an inversely proportional relationship between cell viability and the concentration of pulverized endodontic files, for both the Vero cell line and the L929 cell line. However, when exposed to 50 µg., was observed a cytotoxic effect on L929 cells. The concentration of pulverized endodontic files suggested a reduction in cell viability. In a clinical correlation, metal particles could cause undesirable cytotoxic effects on the periapical tissues, resulting in local inflammatory processes.

Gene and cell therapies in Chile: a regulatory challenge

Hechavarría Núñez, Y.[1]; Salas Duguet, V. [1]; Crisóstomo Landeros J.[1]; Carmona Sepúlveda P.[1]
[1] Instituto de Salud Pública de Chile, Santiago de Chile, Chile
Contact to: mailto:yhechavarria@ispch.cl

Gene and cell therapy products represent a therapeutic alternative for chronic and seriously debilitating or life-threatening diseases like arthritis, cancer, and amyotrophic lateral sclerosis. In Chile, researchers from various institutions are interested in developing these products; nevertheless, there is a lack of specific regulatory guidelines. Thus, to identify the current status of gene and cell therapy products development in Chile, guidelines on biological products were analyzed to detect regulatory gaps. A three-way approach was used to compare guidelines issued by the Health Institute of Chile (ISP), FDA, and EMA. Finally, through research using keywords and revising web pages, institutions involved, the products in development, and their intended use were identified. Consequently, the use of cell therapy products was mainly evidenced, while gene therapy products are included in the pipeline for the near future. Besides, partial implementation of Good Manufacturing Practices (GMP) guidelines was noticed during diagnostic visits performed in 2016 by ISP to some manufacturing sites. Although some products are already introduced in clinical practice, there was no evidence of clinical trial or marketing authorizations. Recently, the ISP issued a guideline for clinical trial submissions of biological products, including gene and cell therapy products. Furthermore, the ISP called for a voluntary listing of establishments aiming to identify the products (in use or investigational), their intended use, the manufacturers, and the responsibilities of each manufacturer. Still, the development of specific GMP guidelines and marketing approval is needed. Moreover, active dissemination is required for a successful voluntary establishment listing process.

Conditioned medium derived from murine BM-MSCs cultured as spheroids exhibit *in vitro* immunomodulatory capacity

Fuentes, P.[*1]; Torres, M. J.[*1]; Vergara, M.[1]; Altamirano, C.[1,2]
[1] Escuela de Ingeniería Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[2] CREAS, Centro Regional de Estudios en Alimentos Saludables, Valparaíso, Chile
[*] Equally contributing author
Contact to: palm.fuentesrobert@gmail.com

In recent years, conditioned medium (CM) obtained from the culture of mesenchymal stromal/stem cells (MSCs) has been shown to effectively modulate the immune response *in vitro* and in animal models of lung, renal, cardiac, or hepatic injury as well as in models of burn injury of different organs. Conditions that are typically accompanied by a strong inflammatory response. Dynamic culture conditions, such as fluid flow, shear stress and three-dimensional aggregates cultivation (3D-MSCs), has been demonstrated substantially impact cellular behavior and secretome profile. For this reason, in this work we evaluate the immunomodulatory capacity *in vitro* of CM derived from 3D-MSCs cultures. Murine BM-MSCs were cultured as spheroids at two densities: $2.5x10^6$ and $5x10^6$ cells/mL (D1, D2) in shake flasks with serum-free medium and incubated at 70 rpm, 37°C, 21% O_2 and 5% CO_2 for 96 h. Samples were taken every 24 h to evaluate: morphological parameters of spheroids, re-adherence capacity of MSCs, key metabolites (glucose, lactate, ammonia), viability (secreted LDH), and immunosuppressive potential of the CM by a PBMC proliferation assay. Our results showed that dynamic culture of 3D-MSCs is feasible using basal medium without any type of animal/human supplementation, which represents a clear advantage for commercial production of CM. In both cultures, glucose was depleted at 96 h, while lactate increased and at 48 h began to decrease. In addition, we observed that the CM obtained from spheroids induce a significant decrease in lymphocyte proliferation, displaying an immunosuppression percentage of 79 and 76% (D1 and D2, respectively).

Mammal intestinal organoids for studying zoonotic pathogens

Cancela, S.[1,2]; Pagotto, R.[1]; Francia, M.E.[3,4]; Crispo, M.[2,5]; Yim, L.[6]; Bentancor, L.[6]; Bollati-Fogolín, M.[1,2]

[1] Cell Biology Unit, Institut Pasteur de Montevideo, Uruguay

[2] Molecular, Cellular and Animal Technology Program (ProTeMCA), Institut Pasteur de Montevideo, Uruguay

[3] Laboratory of Apicomplexan Biology, Institut Pasteur de Montevideo, Uruguay

[4] Department of Parasitology and Mycology, Institute of Hygiene, Faculty of Medicine, University of Republic, Montevideo, Uruguay

[5] Laboratory Animal Biotechnology Unit, Institut Pasteur de Montevideo, Uruguay

[6] Department of Biotechnological Development, Institute of Hygiene, Faculty of Medicine, University of Republic, Montevideo, Uruguay

Contact to: scancela@pasteur.edu.uy

Intestinal organoids are self-organized three dimensional (3D) structures composed of a layer of polarized intestinal epithelial cells surrounding a hollow lumen. They recapitulate *in vitro* the intestinal multicelular composition, architecture and physiology. The aim of this work was to set up organoid models for studying zoonotic pathogens such as *Salmonella* and *Toxoplasma gondii*. *T. gondii's* sexual cycle is restricted to felid's intestines, which are characterized by an excess of linoleic acid given by the lack of delta-6-desaturase activity. "Felinized" murine intestinal organoids were generated for triggering *T. gondii*'s sexual differentiation *in vitro*. For this purpose, murine intestinal organoids from C57BL/6 mice were established from crypt isolated intestinal stem cells (2D or 3D) and incubated in the presence of 20 µM delta-6-desaturase inhibitor and 200 µM linoleic acid. Under these conditions no cytotoxicity of felinizing compounds was observed until 5 days of incubation. To optimize *T. gondii's* infection, intestinal organoids were incubated with tachyzoites (at three distinct multiplicities of infection, MOIs) and evaluated by immunofluorescence assays (IFAs) at three time points post-infection. In order to set up a *Salmonella* infection model, intestinal organoids from farm animals (cow and sheep) were established and characterized by light microscopy and RT-PCR of specific markers. Forward steps will involve bovine intestinal organoids exposure to *Salmonella enterica* reporter strains at different MOIs, and bacteria invasion/proliferation evaluation at two time points after infection by extra and intracellular bacteria quantification and IFAs. Our results highlight the versatile uses of intestinal organoids as a powerful in *vitro* tool for modeling zoonotic diseases, contributing to the principle of reducing the use of experimental animal models.

Preliminary evaluation of cell-based carriers of novel conjugated polymer nanoparticles with antitumoral action

Beaugé, L.[1]; Palacios, R. E. [1,4]; Ibarra, L. E. [2,3]

[1] Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA), Universidad Nacional de Río Cuarto (UNRC) y Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Río Cuarto, 5800, Córdoba, Argentina

[2] Instituto de Biotecnología Ambiental y Salud (INBIAS), Universidad Nacional de Río Cuarto (UNRC) y Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), UNRC, Río Cuarto, 5800, Córdoba, Argentina

[3] Departamento de Biología Molecular, Facultad de Ciencias Exactas Fisicoquímicas y Naturales, UNRC, Río Cuarto, 5800, Córdoba, Argentina

[4] Departamento de Química, Facultad de Ciencias Exactas Fisicoquímicas y Naturales, UNRC, Río Cuarto, 5800, Córdoba, Argentina

Contact to: lbeauge@exa.unrc.edu.ar

In recent years, the use of cells to transport drugs has gained importance as a way to increase the arrival and accumulation at necessary sites, such as solid tumors. Conjugated polymer nanoparticles (CPNs) are an example of cargos to be transported by cells, due to the intrinsic ability to emit fluorescence and generate cytotoxic damage after application of external stimuli such as light. We have previously demonstrated the ability of immune cells to transport a kind of CPNs-F8BT to brain tumors. At the present, we are looking to develop new CPNs and evaluate them as cargo for cell-based delivery. Two types of CPNs were synthetized using CPs: PCPDTBT ((Poly[2,6-(4,4-bis-(2-ethylhexyl)-4H-cyclopenta[2,1-b; 3,4-b']dithiophene)-alt-4,7(2, 1, 3-benzothiadiazole)]) and PFOTBT (Poly[2,7-(9,9-di-octyl-fluorene)-alt-4,7-bis(thiophen-2-yl)benzo-2,1,3-thyadiazole). The method used was nanoprecipitation and the resulting CPNs were characterized by UV-Visible absorption, fluorescence emission spectroscopy and dynamic light scattering (DLS). The biological evaluation of cell incorporation and biocompatibility was done with monocytes from human cell lines THP-1 and U937. The incorporation of CPNs into cells could be successfully determined using flow cytometry and Odyssey® CLx Infrared Imaging System. Both CPNs showed excellent fluorescent brightness for bioimaging, were not toxic at the concentrations evaluated and were taken up successfully by monocytes. CPNs synthesized with the chosen CPs would be ideal candidates for cell labelling and trafficking evaluation in preclinical animal models.

Session 7: Innovations and challenges

Precipitation using polyethylene glycol and zinc chloride as a purification step for monoclonal antibodies capture

Moreno Pássaro, A. C.[1]; Orban de Souza, M. P.[1]; Rocha Antunes Pereira Bresolin, I.[1]; Peroni, L. A.[2]; Lazzarotto Bresolin, I. T.[1]

[1] Universidade Federal de São Paulo, Departamento de Engenharia Química. Rua São Nicolau, 210, 09913-03, Diadema/SP, Brazil

[2] Rheabiotech D. P. C. Produtos de Biotecnologia Ltda. Rua Ângelo Vicentim, 107, 13084-060, Barão Geraldo, Campinas - SP, Brazil

Contact to: bresolin@unifesp.br

Antibodies have become fundamental in several areas of study, such as clinical and diagnostics, due to their ability to recognize molecules present in a complex mixture. Among many applications, their use in rapid serological tests for diseases, such as COVID-19, has become increasingly popular worldwide. For the antibody purification step, precipitation stands out, which is based on reducing the solubility of a solute in a solvent by adding a precipitating agent. Thus, this paper aimed to study the use of precipitation as a technique to purify anti-human IgG1 monoclonal antibodies (mAbs) produced by hybridomas cultivated in RPMI-1640 medium. Supplementation with fetal bovine serum and amino acids was also performed whenever necessary. The experiments were carried out using polyethylene glycol of different molecular weights in different concentrations (4000 and 6000 g mol^{-1}, varying from 1 to 16%) and $ZnCl_2$ (0 to 16 mmol L^{-1}) as precipitating agents for these antibodies, determining their solubility curves and the most promising conditions for batch precipitation, as well as optimizing the resuspension step and accomplishing a viscosity analysis for the resuspended phase. The amount of antibodies obtained during the experiments were evaluated through protein A chromatography and the impurities were quantified through size exclusion chromatography. For batch precipitation, the combination of PEG 6000 16% and $ZnCl_2$ 3 mmol L^{-1} achieved a yield of 67% and a purity of 31% for the mAb. In conclusion, this technique presents great potential as a recovery step in mAb purification and an alternative to chromatography technology.

Application of QbD concepts to the development of a recombinant veterinary product liquid formulation

Rodríguez, M. C,[1]; Villarraza, J.[1]; Antuña, S.[2]; Tardivo, M. B.[2]; Fontana, D.[1, 2] ; Ceaglio, N.[1] ; Prieto, C. [2,3]

[1] UNL, CONICET, FBCB, Centro Biotecnológico del Litoral (CBL), Ciudad Universitaria, Ruta Nacional 168- Km 472,4-C.C. 242- (S3000ZAA), Santa Fe, Argentina

[2] Biotecnofe S.A. PTLC, Ruta 168 Pje El Pozo, (3000), Santa Fe, Argentina

[3] UNL, FBCB, Centro Biotecnológico del Litoral (CBL), Ciudad Universitaria, Ruta Nacional 168- Km 472,4-C.C. 242- (S3000ZAA), Santa Fe, Argentina

Contact to: rodriguezmaceleste@gmail.com

Protein formulation and drug characterization are one of the most difficult and time-consuming tasks because the complexity of biotherapheutic proteins. Hence, maintaining a protein drug in its active state typically requires preventing changes in its physical and chemical properties for maintaining its shelf-life.

Quality by Design (QbD) is a systematic approach that emphasizes product and process understanding and process control. Design of Experiments (DoE) is one of the most important QbD tool, allowing the possibility to modify the formulation attributes within a defined design space.

The critical quality attribute (CQA) considered in this analysis was reCG stability, evaluated as the intact reCG (%) amount obtained by a previously validated RP-HPLC method. The design space was defined throughout a DoE approach. First, we applied a Placket-Burman screening design to determine the effect of several factors on reCG stability. Thereafter, the well know response surface methodology (RSM) was applied to optimize the reCG liquid formulation, under accelerated (25°C, 60% RH) and stress (40°C, 75% RH) conditions. A robust design space was obtained, which should facilitate the scaling-up stage and allow possible modifications of the obtained liquid formulation within the design space. Finally, one of the best condition was validated. After 180 days under accelerated conditions 96 ± 10% of reCG remained intact, whereas at real conditions (4°C) the reCG persisted unaltered, demonstrating the high versatility and efficiency of QbD tools as DoE during biotherapeutics´ formulation stage.

Development of a platform based on the cooperative adsorption of monoclonal antibodies on gold nanoparticles

Campos, I.[1, 2, 4]; Riveros, A.[2]; Altamirano, C.[3]; Kogan, M.[2]; Hermoso, M.[4]; Molina, M. C.[1]

[1] Laboratorio de Anticuerpos Recombinantes e Inmunoterapia, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile, Chile

[2] Laboratorio de Nanomedicina y Nanoteranóstica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Chile

[3] Facultad de Ingeniería, Escuela de Ingeniería bioquímica, Pontificia Universidad Católica de Valparaíso, Chile

[4] Laboratorio de Inmunidad Innata, Programa Disciplinario de Inmunología, ICBM, Facultad de Medicina, Universidad de Chile, Chile

Contact to: ivo.campos@ug.uchile.cl

Many emerging nanobiotechnologies rely on the proper function of proteins, such as monoclonal antibodies (AcMo), immobilized on gold nanoparticles to enhance their stability and targeting compared to their soluble form, synthesizing constructs of high specificity, biocompatible and predictable pharmacokinetics. This project aims to develop a linker-free protocol, called: Cooperative Adsorption, controlling the conjugation of AcMo (Rituximab, Infliximab, anti-MICA, or anti-ST2) in ordered domains onto the surface of the nanoparticle. Gold nanospheres were prepared by a seeded-growth technique and then monofunctionalized with an AcMo. Potential Z, hydrodynamic diameter, and Uv-vis spectrophotometry confirmed the development of a protein corona on the gold nanoparticles. The monofunctionalized nanospheres conserved the ability to recognize the antigen based on gold detection in antigen-sensitized plates. The amount of AcMo conjugated on the nanospheres was quantified by an ELISA assay. The synthesized constructs were stable at different times (0, 12, 24, 48, 72, and 168 hours) in water, phosphate buffer, and citrate solution. This novel protocol represents a new tool to enhance the use of gold nanoparticles as a biocompatible platform, aiming for a bi-functionalized nanoparticle, with two glycoproteins adsorbed into their surface, that can detect two antigens and modulate the cellular interaction on a nanometer scale.

Acknowledgments: This work was supported by Research Grants from the Chilean National Agency for Research and Development (ANID): 21221729 from scholarship program DOCTORADO NACIONAL, 1221031 from National Fund for Scientific and Technological Development, FONDECYT; ACT210068 from Research: Science and Technology, ANILLO; and ID20I10106 from Fund for Promotion of Scientific and Technological Development, FONDEF.

Engineering PCV2's VLP as a new and adaptable biotechnological platform for vaccine development

Olivero, N.[1]; Frigini, E.[2,5]; Ramos, N.[3]; Carrión, F.[1]; Arbiza, L.[3]; Pritsch, O.[1,4]; Pantano, S.[5]; Ortega, C.[6]

[1] Laboratorio de Inmunovirología, Institut Pasteur de Montevideo, Uruguay
[2] Instituto de Matemáticas Aplicada San Luis (IMASL), CONICET, Universidad Nacional de San Luis, Argentina
[3] Sección Virología, Facultad de Ciencias, Universidad de la República, Uruguay
[4] Departamento de Inmunobiología, Facultad de Medicina, Uruguay
[5] Laboratorio de Simulaciones Biomoleculares, Institut Pasteur de Montevideo, Uruguay
[6] Unidad de Proteinas Recombinantes, Institut Pasteur de Montevideo, Uruguay
Contact to: ckortega@pasteur.edu.uy

Porcine circovirus type 2 (PCV2) is an important pathogen in swine herds, causing severe economic losses to the pig industry worldwide. Despite the availability of vaccines, the virus infects persistently more than 12% of the pigs globally. Since neutralizing antibodies recognize structural epitopes on the virions' surface, traditional immunization strategies using linear epitopes show low efficacy. Moreover, new PCVs types 3 and 4 have been recently reported. PCV4 is, so far, restricted to China, but its capability of infecting other livestock species raises serious concern. Hence, it is urgently necessary to potentiate our armamentarium with new tools for diagnosis and prevention.
We have implemented a high-yield protocol to produce PCV2's virus-like particles (VLPs) based on Expi cells technology. In parallel, we used cutting-edge computational methods developed by us to simulate and predict point mutations stabilizing the assembly of VLPs without altering their external surface.
We can create super-stabilized VLPs to use them as stiff molecular scaffolds to introduce arbitrary modifications, mimicking simply and efficiently the outer surface of emergent variants. Hence, this technology could provide an innovative platform for vaccine development.

Humanized mouse model to evaluate an immunotherapeutic nanomedicine

Mitarotonda, R.[1,2]; Arévalo, A. P.[3]; Pagotto, R.[4]; Bollati-Fogolín, M.[4]; Crispo, M.[3]

[1] Laboratorio de Nanosistemas de Aplicación Biotecnológica, Universidad Nacional de Hurlingham, Villa Tesei, Buenos Aires, Argentina

[2] Farmacología y Toxicología. Departamento de Ciencias Básicas. Universidad Nacional de Luján, Luján, Buenos Aires, Argentina

[3] Laboratory Animals Biotechnology Unit, Institut Pasteur de Montevideo, Uruguay

[4] Cell Biology Unit, Institut Pasteur de Montevideo, Uruguay

Contact to: romina.mitarotonda@unahur.edu.ar

Nanomedicines (NM) are studied as alternative treatments for anticancer agents. However, the evaluation of NM by conventional methods is not consistent with clinical translation. Here we report the development of a humanized mouse model to test an immunotherapeutic NM. For that, 3-week-old NOD-SCID IL2Rg^{null} female mice were transplanted with human PBMCs. Then, 15 days later, BT-474 cells labelled were subcutaneously injected for in vivo imaging studies. Experimental groups were defined as wild type (WT, n=5), humanized mouse (HM, n=6), humanized mouse with BT-474 cells (HBT, n=6) and wild type with BT-474 cells (WBT, n=5). Finally, animals were treated with the NM via i.v. and their efficacy were studied (data not shown). We evaluated the implantation and development of the human immune system in these mice. In the humanized animals, significant differences were observed in the amount of WBC (> 50% cells/L) and lymphocytes. Also, the concentration of monocytes increased from 0,03.10^9 cells/L in WT to 0,2.10^9 cells/L in HM. Moreover, the concentration of neutrophils increased (4.6%) in animals that have undergone humanization and/or xenograft. We also evaluated cell implantation by studying bone marrow from animals in which we observed the presence of a new cell population. Finally, there were no significant differences between groups in the concentration of mouse eosinophils, basophils, red blood cells and platelets. In addition, NM increased the concentration of proinflammatory cytokines. Our data showed that the humanized mouse model, unlike the immunocompetent models with xenografts, is relevant for analyzing NM due to their immunological implications.

Production of IgY avian antibodies against the structural protein E^{rns} of the Bovine viral diarrhea virus

Gutierrez, N.[1,3]; Hidalgo, A.[1]; González, E. [1]; Villavicencio, C. [1]; Manríquez, V.[2]; Camacho, F.[2,3]; Toledo, J.[1]

[1] Biotechnology and Biopharmaceutical Laboratory, Pathophysiology Department, Faculty of Biological Sciences, University of Concepción, Chile

[2] Recombinant Biopharmaceutical Laboratory, Pharmacology Department, Faculty of Biological Sciences, University of Concepción, Chile

[3] Combinatory and Bioprocess Laboratory, Pharmacology Department, Faculty of Biological Sciences, University of Concepción, Chile

Contact to: nicogutierrez@udec.cl

Bovine viral diarrhea virus (BVDV) is a *Pestivirus* belonging to the family *Flaviviridae* which affects cattle populations causing clinical signs that range from subclinical immunosuppression to severe reproductive and respiratory problems considering it as a sanitary problem for the cattle industry worldwide. BVDV is classified into 2 subtypes, Cytopathic and Non- Cytopathic (NCP). If BVDV infects a pregnant animal and is the NCP subtype, the virus can infect the fetus and birth a persistently infected (PI) animal, which is considered the principal reservoir of the virus in nature. E^{rns} protein is a structural protein of the BVDV and one of the responsible for avoiding the immune system to generate PI animal and one of the most immunogenic proteins of the virus. This protein has been utilized in immunoassays for the detection of PI animals and currently, there are still looking for new alternatives for the detection of the PI animals.

In this work, we describe the production of avian IgY anti- E^{rns} antibodies. Recombinant E^{rns} (rE^{rns}) protein of the BVDV was obtained from the culture media of suspension cultures of the SF-21 insect cell line and purified by immobilized metal affinity chromatography (IMAC). We demonstrated that IgY extracted from the egg yolk of immunized hens detect the rE^{rns} in ELISA. These results show that IgY could be a new alternative for BVDV detection.

CO_2 conversion into Polyhydroxybutyrate (PHB) through a bioelectrochemical system by *Cupriavidus necator*

Travali Barriga, M.[1]; Martínez Ruano, J. A. [1]; González Romo, E.[1]

[1] School of Biochemical Engineering, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

Contact to: jimmy.martinez.r01@mail.pucv.cl

Cupriavidus necatur is a gram-negative facultative aerobic bacteria able to growth both heterotrophic or autotropic producing polyhydroxybutyrate (PHB). PHB is a biopolymeric compound with high biocompatibility comparable to those of some petroleum-derived plastics. This compound can be produced by *C. necatur* using carbon dioxide (CO_2) as a substrate, which is attractive for industrial gas fermentation using bioelectrochemical systems. Despite these facts, the microbial growth and PHB production in these systems using is not well known. The objective of this study was to evaluate the growth kinetic and PHB production during the culture of *C. necatur* in a bioelectrochemical system using CO_2 and gas mixtures. Experiments were performed using an H-type reactor where the chambers were separated by a cation exchange membrane. The cathodic and anodic chambers contained minimal culture medium, which were filled with 1 L and 0,3 L of the medium, respectively. The bioelectrochemical system was operated with a fixed potential of -1000 mV (*versus* Ag/AgCl) and NiMo coated copper electrode was used as a working electrode. A gas mixture composed mainly of CO_2 was recirculated during the cultures. Samples were periodically taken for the measurement of pH, biomass and PHB. As a result, it was found that the specific growth rate (μ) and PHB production in *C. necatur* cultures is higher when the reactors were operated only with CO_2 compared to a gas mixture composed of O_2 and CO_2 (5% and 95%), $\mu=0.0107\ h^{-1}$ and $\mu=0.0064\ h^{-1}$, respectively. While the PHB production increased by 90%.

CONTACT LIST

Participant	Affiliation	Country	e-mail
Acuña Intrieri, María Eugenia	Centro de Rediseño e Ingeniería de Proteínas, UNSAM, CONICET	Argentina	macunaintrieri@iib.unsam.edu.ar
Aguilar, Juan J.	Instituto de Virología FCM-UNC	Argentina	virolog@cmefcm.uncor.edu
Alfonso, Victoria	INTA	Argentina	alfonso.victoria@inta.gob.ar
Altamirano, Claudia	Pontificia Universidad Católica de Valparaíso	Chile	claudia.altamirano@pucv.cl
Álvarez, Alejandro	Lobov	Argentina	lobov@lobov.com.ar
Álvarez, Irene	INTEA SA	Argentina	alvarez.irene@inta.gob.ar
Amadeo, Ignacio	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	iamadeo@fbcb.unl.edu.ar
Amalfi, Sabrina	INTA	Argentina	amalfi.sabrina@inta.gob.ar
Amaranto, Marilla	DQBRC-CIQUIBIC, Universidad Nacional de Córdoba	Argentina	marilla.amaranto@unc.edu.ar
Amor, Juan Francisco	Merck Life Science	Argentina	juan-francisco.amor@merckgroup.com
Andrade Dias, Georgia	Cytiva	Brazil	georgia.andrade@cytiva.com
Antuña, Sebastián	Biotecnofé SA	Argentina	antuna@biotecnofe.com.ar
Baravalle, María Eugenia	ICIVET-Litoral UNL-CONICET	Argentina	ebaravalle@live.com.ar
Baztarrica, Josefina	Biogénesis Bagó	Argentina	josefina.baztarrica@biogenesisbago.com
Beaugé, Lucía	Universidad Nacional Río Cuarto	Argentina	lbeauge@exa.unrc.edu.ar
Becerra, Luciana	INTEA SA	Argentina	becerra.maria@inta.gob.ar
Benedetto, Leonardo	Dirección de Evaluación y Control de Biológicos y Radiofármacos - INAME	Argentina	leonardo.benedetto@anmat.gob.ar
Bollati Fogolín, Mariela	Instituto Pasteur de Montevideo	Uruguay	mbollati@pasteur.edu.uy
Borba Cruz, Paula	Instituto Fiocruz	Brazil	paula.cruz@bio.fiocruz.br
Brito, Catarina	iBET/ITQB NOVA	Portugal	anabrito@ibet.pt
Burgardt, Noelia I.	ANLAP	Argentina	burgardtnoelia@gmail.com
Bürgi, María de los Milagros	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	mburgi@fbcb.unl.edu.ar
Campos, Ivo	Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile	Chile	ivo.campos@ug.uchile.cl
Cancela, Saira	Instituto Pasteur de Montevideo	Uruguay	scancela@pasteur.edu.uy
Carmona, Fernando	Cytiva	Argentina	fernando.carmona@cytiva.com
Cassataro, Juliana	Universidad Nacional de San Martín-CONICET	Argentina	jucassataro@iib.unsam.edu.ar
Caviedes, Pablo	Facultad de Medicina, Universidad de Chile	Chile	pcaviedes@ing.uchile.cl
Ceaglio, Natalia	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	nceaglio@fbcb.unl.edu.ar
Cerutti, María Laura	Center for Protein Engineering and Redesign IIB-UNSAM	Argentina	mcerutti@iib.unsam.edu.ar
Cervetti, Joaquín	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	joaquin.cervetti@gmail.com

Compaired, Diego	Instituto de Virología, CICVyA-INTA Castelar	Argentina	dcompaired@yahoo.com.ar
Corona, Daniel	Laboratorio de Hemoderivados	Argentina	daniel.corona@unc.edu.ar
Cragnaz, Lucía	Sartorius	Argentina	latam.marcom@Sartorius.com
Crisóstomo Landeros, José	Instituto de Salud Pública de Chile	Chile	jcrisostomo@ispch.cl
Curcio, Mariana	Thermofisher	Brazil	mariana.curcio@thermofisher.com
De María, Valeria	Universidad de Chile	Chile	valeria.demaria@ing.uchile.cl
Delgado López, Nidia	Instituto Nacional de Canerología	Colombia	ndelgado@cancer.gov.co
Delorenzi, Juan	Vigenius Biotech	Argentina	juan.delorenzi@vigenius.com.ar
Depetris, Matías	BioSynaptica SA	Argentina	mdepetris@biosynaptica.com
Días, Eduardo	Merck Life Science	Brazil	eduardo.dias@merckgroup.com
dos Reis Castilho, Leda	Universidade Federal do Rio de Janeiro	Brazil	leda@peq.coppe.ufrj.br
Estruch, Belén	Instituto de Investigaciones Biotecnológicas, UNSAM - CONICET	Argentina	beestruch@gmail.com
Etcheverrigaray, Marina	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	marina@fbcb.unl.edu.ar
Fabro, Sebastián	Merck Life Science	Argentina	sebastian.fabro@merckgroup.com
Fassolari, Matías	Merck Life Science	Argentina	matias.fassolari@merckgroup.com
Fernández Linderman, Macarena	Pontificia Universidad Católica de Valparaíso	Chile	m.fernandez.linderman@gmail.com
Filgueira Risso, Lucas	mAbxcience	Argentina	lucas.filgueirarisso@mabxience.com
Flores Mendeville, Gerardo	Universidad de Chile		gerardo.flores@ug.uchile.cl
Fontana, Diego	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	dfontana@fbcb.unl.edu.ar
Forno, Guillermina	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	gforno@fbcb.unl.edu.ar
Franco, Lautaro	INTEA SA	Argentina	franco.lautaro@inta.gob.ar
Fuentes Robert, Paloma	Pontificia Universidad Católica de Valparaíso	Chile	palm.fuentesrobert@gmail.com
Gagliardi, Delfina	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	gagliardi.delfina@gmail.com
Garay Novillo, Javier N.	DQBRC-CIQUIBIC, Universidad Nacional de Córdoba	Argentina	jgaraynovillo@unc.edu.ar
Garay, Ernesto	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	egaray@fbcb.unl.edu.ar
Garcia, Gustavo F.	Embiotec	Argentina	ventas@embiotec.com.ar
García, Juan Manuel	Embiotec	Argentina	info@embiotec.com.ar
Garrido, María José	Facultad de Medicina - Universidad de Chile	Chile	maria.garridog@utem.cl
Gasser, Fátima	ICIVET-Litoral UNL-CONICET	Argentina	fatima.gasser@icivet.unl.edu.ar
Gastaldi, Victoria	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	vic_gastaldi@hotmail.com
Gatica, Oscar	Pontificia Universidad Católica de Valparaíso	Chile	oscarnanuepara@hotmail.com
Gerdtzen, Ziomara	Universidad de Chile	Chile	zgerdtze@ing.uchile.cl
Gil, Pedro	Instituto de Virología Dr. José María Vanella, FCM, UNC	Argentina	pedroignaciog@gmail.com
Godia Casablancas, Francesc	Universidad Autónoma de Barcelona	Spain	francesc.godia@uab.cat

Godino, Agustina	DQBRC-CIQUIBIC, Universidad Nacional de Córdoba	Argentina	agustina.godino@unc.edu.ar
Godoy, Andrea	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	agodoy@fbcb.unl.edu.ar
González, Mauricio	Instituto de Ciencias Biomédicas, Universidad de Chile	Chile	mauricio.gonzalezo@utem.cl
Grabois, Marcelo	Universidad Nacional del Litoral/ ITERA SRL	Argentina	mgrabois@unl.edu.ar
Groba, Juliana	Pall do Brazil	Brazil	juliana_groba@pall.com
Gugliotta, Agustina	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	agugliotta@fbcb.unl.edu.ar
Gutiérrez Mella, Nicolás A.	Universidad de Concepción	Chile	nicogutierrez@udec.cl
Hechavarría Núñez, Yanet	Instituto de Salud Pública de Chile	Chile	yhechavarria@ispch.cl
Iturraspe, Francisco	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	iturraspefrancisco@gmail.com
Jerez Monsalves, Belén	Universidad de Concepción	Chile	belen.jerezmonsalves@gmail.com
Julín, Hernán	Dirección de Evaluación y Control de Biológicos y Radiofármacos - INAME	Argentina	hernan.julin@anmat.gob.ar
Junod, Gabriela	Pall Argentina	Argentina	gabriela_junod@pall.com
Koliqi, Ilir	Tosoh	Canada	Ilir.Koliqi@tosoh.com
Konigheim, Brenda S.	Instituto de Virología FCM-UNC	Argentina	brenda.konigheim@unc.edu.ar
Kratje, Ricardo	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	rkratje@fbcb.unl.edu.ar
Lagos Muñoz, Paola A.	Laboratorio de Biofármacos Recombinantes, Universidad de Concepción	Chile	paolalagos@udec.cl
Lamothe Reyes, Yaneysis	Universidad de Concepción	Chile	lyaneysis@gmail.com
Leopold, María Jesús	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	jesu.leopold@hotmail.com
López, María Gabriela	INTA	Argentina	lopez.mariag@inta.gob.ar
Lorca, Gustavo	Thermofisher	Chile	gustavo.lorca@thermofisher.com
Machado Weber, Sebastian	Roche	Brazil	sebastian.weber.sw3@roche.com
Manca, Mabel	MP Biomedicals LLC	Argentina	mabel.manca@mpbio.com
Mancini Astray, Renato	Instituto Butantam	Brazil	renato.astray@butantan.gov.br
Marsili, Federico	Universidade Federal do Rio de Janeiro	Brazil	ffm@peq.coppe.ufrj.br
Martínez Ruano, Jimmy A.	Pontificia Universidad Católica de Valparaíso	Chile	jiamartinezru@unal.edu.co
Martinez, Franco	Sartorius	Argentina	franco.martinez@Sartorius.com
Martínez, Hugo D.	Laboratorio de Hemoderivados	Argentina	daniel.martinez@unc.edu.ar
Martínez-Marignac, Verónica	CICYTTP- CONICET, Prov.ER y UADER	Argentina	veromm99@gmail.com
Martini, Georgina	Instituto de Investigaciones Biotecnológicas, UNSAM - CONICET	Argentina	martini@iprobyq-conicet.gob.ar
Masin, Marianela	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	mmasin@fbcb.unl.edu.ar

Medina, Daiana	Laboratorio de Investigación en Proteínas - IQUIBA-NEA CONICET	Argentina	daim824@gmail.com
Mejías, María Pilar	Biogénesis Bagó	Argentina	pilar.mejias@biogenesisbago.com
Mencacci, Nicolás	Biogenets LLC	Argentina	nm@biogenets.com
Mitarotonda, Romina	Universidad Nacional de Hurlingham	Argentina	romina.mitarotonda@unahur.edu.ar
Mogetta, María Herminia	ANLIS - Instituto Malbrán	Argentina	mhmogetta@anlis.gob.ar
Molina, María Carmen	Facultad de Medicina - Universidad de Chile	Chile	mcmolinas@uchile.cl
Molinas-Burgo, Temistocles	Faculty of Physical and Mathematical Sciences (FCFM), University of Chile.	Chile	temomb@gmail.com
Morante, Luisina			
Moreno Pássaro, Ana Carolina	Federal University of São Paulo (UNIFESP)	Brazil	ana.passaro28@unifesp.br
Mori, Francisco	BIOSYNAPTICA SA	Argentina	franciscomorifmm@gmail.com
Mufarrege, Eduardo	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	mufarrege@fbcb.unl.edu.ar
Mussio, Pablo	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	pablo.esteban.mussio@gmail.com
Notcovich, Cintia	STAMM	Argentina	suppliers@stamm.bio
Novoa Belman, Mario	Infors	México	m.novoa@infors-ht.com
Núñez Soto, Constanza Belén	U. de Chile. PUCV - USM	Argentina	cony.ldea@gmail.com
O´Donell, John Kevin	Tosoh	USA	Kevin.ODonnell@tosoh.com
Oggero Eberthardt, Marcos	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	moggero@fbcb.unl.edu.ar
Ojeda Zachara, Emilia W.	Dirección de Evaluación y Control de Biológicos y Radiofármacos - INAME	Argentina	emiliaojedaz@gmail.com
Olivero, Natalia	Instituto Pasteur de Montevideo	Uruguay	natalia.olivero@gmail.com
Olszevicki, Nicole	Pall Argentina	Argentina	nicole_olszevicki@pall.com
Ortega Rivas, Leonardo	Universidad de Concepción	Chile	leoortega@udec.cl
Ortega, Claudia	Instituto Pasteur de Montevideo	Uruguay	ckortega@pasteur.edu.uy
Peña, Lucía	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	luciacarolinape@gmail.com
Pereira Alves, Maria Isabel	Dirección de Evaluación y Control de Biológicos y Radiofármacos - INAME	Argentina	isabel.pereira@anmat.gob.ar
Pérez, Rodrigo M.	Secretaría de Ciencia y Tecnología - Formosa	Argentina	rodrigoprm@gmail.com
Plastine, María del Pilar	INTA	Argentina	plastine.maria@inta.gob.ar
Prato, Cecilia Arahí	Instituto de Investigaciones Biotecnológicas, UNSAM - CONICET	Argentina	arahi.prato@gmail.com
Prieto, Claudio	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	cprieto@fbcb.unl.edu.ar
Queiroz Maranhão, Andrea	University of Brazilia	Brazil	andrea.q.maranhao@gmail.com
Quesada, Johan	Pontificia Universidad Católica de Valparaíso	Chile	johan__120293@hotmail.com
Quispe Conislla, Juana L.	Universidad Nacional Mayor de San Marcos	Perú	juana.quispe@quimtia.com
Ramírez, Octavio	IBT-UNAM	México	tonatiuh.ramirez@ibt.unam.mx
Reynoso, Diego	Cytiva	Argentina	diego.reynoso@cytiva.com

Ricotti, Sonia	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	sricotti@fbcb.unl.edu.ar
Rivarosa, Florencia	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	rivarosaflorencia@gmail.com
Rodriguez Siza, José A.	Pontificia Universidad Católica de Valparaíso	Chile	jose.rodriguez.s01@mail.pucv.cl
Rodríguez, María Laura	Vigenius Biotech	Argentina	laura@vigenius.com.ar
Roldán, Patricia	Pall Argentina	Argentina	patricia_roldan@pall.com
Salvatore, Esteban	Sartorius	Argentina	Latam.Marcom@Sartorius.com
Sánchez Daza, Anamaría C.	Faculty of Physical and Mathematical Sciences (FCFM), University of Chile.	Chile	anitaasd@gmail.com
Sánchez Ramos, Oliberto	Universidad de Concepción	Chile	osanchez@udec.cl
Siqueira, Rafael	Pall	Brazil	rafael_siqueira@pall.com
Soares, Caio	Merck Life Science	Brazil	caio.soares@merckgroup.com
Soimu, Giuliana	UNIGRANRIO	Brazil	giulianasoimu@hotmail.com
Soldano, Nicolás	Zelltek SA	Argentina	nsoldano@amegabiotech.com
Sposito, Felipe	Merck Life Science	Brazil	felipe.sposito@merckgroup.com
Suhr, Eric	Secretaría de Ciencia y Tecnología - Formosa	Argentina	ericsuhr@hotmail.com
Taboga, Oscar	INTA	Argentina	taboga.oscaralberto@inta.gob.ar
Tardivo, María Belén	Biotecnofé SA	Argentina	btardivo@biotecnofe.com.ar
Tello Aguayo, Samantha A.	Facultad de Medicina - Universidad de Chile	Chile	samantha.tello.a@gmail.com
Tencer, Juan Pablo	Roche	Argentina	juan.tencer@roche.com
Toledo Alonso, Jorge R.	Universidad de Chile	Chile	jotoledo@udec.cl
Toledo Stuardo, Karen G.	Instituto de Ciencias Biomédicas, Universidad de Chile	Chile	karen.toledo.stuardo@gmail.com
Tonso, Aldo	Universidade de São Paulo	Brazil	atonso@usp.br
Tribulatti, María Virginia	Instituto de Investigaciones Biotecnológicas, UNSAM - CONICET	Argentina	vtribulatti@iib.unsam.edu.ar
Vallone, Andrea	Lobov	Argentina	avallone@lobov.com.ar
Vásquez Moreno, Gonzalo E.	Facultad de Medicina - Universidad de Chile	Chile	gzvm.hardaz@gmail.com
Veas Inostroza, Rhonda	Pontificia Universidad Católica de Valparaíso	Chile	rhondaveas@gmail.com
Villarraza, Javier	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	jvillarraza@fbcb.unl.edu.ar
Villavicencio Acurio, Carla J.	Universidad de Concepción, Chile	Chile	cvillavicen2019@udec.cl
Wandel-Petersen, Valentina	Centro Biotecnológico del Litoral-FBCB-UNL	Argentina	wandelp.valentina@gmail.com
Weremko, Eva	Lobov	Argentina	eweremko@lobov.com.ar
Zucchelli, Alejandro	Thermofisher	Argentina	alejandro.zucchelli@thermofisher.com

IX SLATCC

2022

UNL • FACULTAD
DE BIOQUÍMICA Y
CIENCIAS BIOLÓGICAS