PROGRAMME AND ABSTRACTS

8th Latin-American Symposium on Animal Cell Technology

Grand Mercure Rio de Janeiro Copacabana Hotel

Rio de Janeiro, Brazil

October 21-24, 2018

SPONSORS

SLATCC 2018 ORGANIZATION

http://slatcc2018.peq.coppe.ufrj.br

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ABOUT THE SLATCC CONFERENCE SERIES

2004 – First SLATCC meeting at UFRJ, Rio de Janeiro, Brazil
2006 – São Paulo, Brazil
2008 – Havana, Cuba
2010 – Montevideo, Uruguay
2012 – Santa Fe, Argentina
2014 – Valparaíso, Chile
2016 – Cocoyoc, Mexico
2018 – Rio de Janeiro, Brazil

PROGRAMME OVERVIEW

	21/10/2018	22/10/2018	23/10/2018	24/10/2018
9h		Poster session (odd posters)	Poster session (even posters)	Poster session (odd + even)
10h		Keynote lecture 2: Nathan Lewis, UCSD, USA	Keynote lecture 3: Laura Palomares, UNAM, Mexico	Keynote lecture 4: Martin Bonamino, INCA, Brazil
11h		Break	Break	Break
11h20		IT: Lucimara de la Torre, UNICAMP, Brazil	IT: Eduardo Mufarrege, UNL, Argentina	Session H: Viral and recombinant vaccines IT: Francesc Godia, UAB, Spain Alvim & Castilho, UFRJ, Brazil Pilatti et al., Unifesp, Brazil Gerdtzen et al., U. Chile, Chile Pose et al., UdeC, Chile
11h50		Session B: Genome editing and cell line development IT: Oliberto Sanchez, UdeC, Chile Alcorte et al., USP, Brazil Zapata et al., U.deC, Chile Latorre et al., PUC Valparaíso, Chile	Session E: Downstream process development IT: Aliuska Cardoso, CIM, Cuba Santos et al., UNESP, Brazil Carvalho et al., UFRJ, Brazil Esteves et al., FIOCRUZ, Brazil	
13h20		Tribute to Carlos A. Pereira Lunch	Lunch	Lunch Session I: Cell and nucleic acid therapies IT: Adriano Azzoni, USP, Brazil Silva et al., UFSCar, Brazil Sanchez et al., U. Chile, Chile Souza et al, UNICAMP, Brazil
14h40	Registration, check-in and setup of posters (Grand Mercure Copacabana Hotel, rooms Gávea and Leme)	Session C: Upstream process development IT: Alvio Figueredo, Fiocruz, Brazil Bettinardi et al., UFRJ, Brazil Gil et al., UFSCar, Brazil Silva et al., USP, Brazil Almeida et al., UFRJ, Brazil	Session F: Biosimilars IT: Kalinka Carrijo, ANVISA, Brazil IT: Guillermina Forno, Zelltek, Argentina Cruz & Castilho, UFRJ, Brazil Lima et al., UFRJ, Brazil	Presentation of poster prize winners
16h10		Break	Break	Closing ceremony
16h30	Opening ceremony	Session D: Product development and product quality IT: Claudia Berdugo-Davis, Catalent, USA Mufarrege et al., UNL, Argentina Amaral et al., USP, Brazil Attallah et al., UNL, Argentina Porras et al., UNAM, Mexico	Session G: Pharmaceutical Biotechnology in Brazil IT: José Fernando Perez, Recepta Biopharma, Brazil IT: Vanda Magalhães, Bionovis, Brazil IT: Renato Astray, I. Butantan, & Elena Caride, Fiocruz, Brazil	
17h	Keynote lecture 1: Alan Dickson, U. Manchester, UK			
18h	Session A: Biopharmaceutical trends IT: Manuel Carrondo, iBET, Portugal IT: Brandon DeKosky, KU, USA IT: Belinda Sanchez, CIM, Cuba Aguiar et al., USP, Brazil	Industrial workshop I IW: Alécio Pimenta Jr., GE, Brazil IW: Jack Palmer, Thermo Fisher, USA	Industrial workshop II IW: Chaya Kataru, Kerry, USA IW: Juliana G. Oliveira, Pall Biotech, Brazil IW: John Yoshi Shyu, Corning, Brazil	*IT: invited talk* *IW: industrial workshop*

Sunday, October 21

14:30 – 16:30 **Conference check-in and setup of all posters** (Gávea and Leme rooms)

16:30 – 17:00 **Opening ceremony**

17:00 – 18:00 **KL 1. Keynote lecture**

Cell factories: manufacturing next generation products from solid foundations
Alan Dickson, The University of Manchester, UK

18:00 – 19:55 **Session A: Biopharmaceutical trends**

Session chairs: Ricardo Medronho, Universidade Federal do Rio de Janeiro, Brazil
Manuel J. T. Carrondo, iBET – Instituto de Biologia Experimental e Tecnológica, Portugal

18:00 – 18:05 **Session sponsored by Janssen**

18:05 – 18:35 **IT 1. Invited talk**

Viral DSP development and cell therapy purification and concentration
Manuel J. T. Carrondo, Instituto de Biologia Experimental e Tecnológica, Portugal

18:35 – 19:05 **IT 2. Invited talk**

Rapid and facile discovery of monoclonal antibodies to fight infectious diseases
Brandon J. DeKosky, KU – The University of Kansas, USA

19:05 – 19:35 **IT 3. Invited talk**

Novel trends in cancer immunotherapy: CIM developments
Belinda Sánchez Ramírez, CIM - Centro de Inmunología Molecular, Cuba

19:35 – 19:55 **OP 1.** Recombinant human growth factors expressed in mammalian cells and exosomes from MSCs for skin regeneration
Bruna A. Aguiar, Paula Fratini, Mari Cleide Sogayar, Ana Claudia O. Carreira, USP - Universidade de São Paulo, Brazil

Monday, October 22

09:00 – 10:00 **Poster session** (authors of odd-numbered posters should stay with their posters)

10:00 – 11:00 **KL 2. Keynote lecture**

Engineering complex traits in the postgenomic era of CHO cell line development
Nathan Lewis, UCSD - University of California, San Diego, USA

11:00 – 11:20 **Break and networking time**

11:20 – 11:50 **IT 4. Invited talk**

Microfluidics - a promising tool for *in vitro* cell culture, biological models and drug research
Lucimara G. de la Torre, Unicamp – Universidade Estadual de Campinas, Brazil

11:50 – 13:10 **Session B: Genome editing and cell line development**

Session chairs: Mariela Bollati, Instituto Pasteur de Montevideo, Uruguay
Andrea Queiroz Maranhão, UnB – Universidade de Brasília, Brazil

11:50 – 11:55 **Session sponsored by Janssen**

11:55 – 12:25 **IT 5. Invited talk**

Fluorescence-assisted sequential insertion of transgenes: an approach to increase specific productivity in mammalian cells
Oliberto Sánchez, Rita P. Cerro, Felipe E. Bravo, Jannel Acosta, Natalie C. Parra, Jorge R. Toledo, UdeC - Universidad de Concepción, Chile

12:25 – 12:40 **OP 2.** Production and initial characterization of rCOL7A1 variants resistant to matrix metalloproteinases cleavage
Mariel Dourado Alcorte, Mari Cleide Sogayar, Marcos Angelo Almeida Demasi, USP - Universidade de São Paulo, Brazil

12:40 – 12:55 **OP 3.** iCAR-T cells: a new concept for the standardization and industrialization of the car t-cell therapy
Lionel Zapata, Mansilla R., Cifuentes P., Sanchez O., UdeC - Universidad de Concepción, Chile

12:55 – 13:10 **OP 4.** Combined effect of C-MYC and XBP1S on specific cell growth rate and protein productivity on CHO cells rh-EPO producer
Yesenia del C. Latorre, Mauro A. Torres, Shawal Spencer, Natascha Gödecke, Hansjörg Hauser, Dagmar Wirth, Julio A. Berrios and Claudia V. Altamirano, Pontificia Universidad Católica de Valparaíso, Chile

13:10 – 13:20 **Tribute to Carlos Augusto Pereira**

13:20 – 14:40 **Lunch**

14:40 – 16:10 **Session C: Upstream process development**

Session chairs: Claudia Altamirano, PUCV - Pontificia Universidad Católica de Valparaíso, Chile
Alvio Figueredo, Biomanguinhos/Fiocruz, Brazil

14:40 – 15:10 **IT 6. Invited talk**

Upstream process development
Alvio Figueredo, Biomanguinhos/Fiocruz, Brazil

15:10 – 15:25 **OP 5.** Hydrocyclones as cell retention devices for perfusion applications: investigation of variables relevant to hydrocyclone attachment to single-use bioreactor bags
Ioná W. Bettinardi, Andreas Castan, Ricardo A. Medronho, Leda R. Castilho, UFRJ – Universidade Federal do Rio de Janeiro, Brazil

15:25 – 15:40 **OP 6.** Taylor vortex flow bioreactor as an alternative for mesenchymal stromal cell expansion
Liseth V. G. Gil, Harminder Singh, Diogo P. Santos, Eric T. Katayama, Juliana S. Silva, Kamilla Swiech, Claudio T. Suazo, UFSCar – Universidade Federal de São Carlos, Brazil

15:40 – 15:55 **OP 7.** Evaluation of recombinant erythropoietin production in novel human cell lines
Luciano C. Silva, Virgínia Picanço-Castro, Kamilla Swiech, USP-Universidade de São Paulo, Brazil

15:55 – 16:10 **OP 8.** Process intensification in yellow fever virus production: a multiple harvest approach
Luiz Fernando C. Almeida, Felipe Tapia, Yvonne Genzel, Udo Reichl, Rodrigo C. V. Pinto, Leda R. Castilho, UFRJ – Universidade Federal do Rio de Janeiro, Brazil

16:10 – 16:30 **Break and networking time**

16:30 – 18:00 **Session D: Product development and product quality**

Session chairs: Octavio Ramírez, UNAM – Universidad Nacional Autónoma de México, Mexico
Ana Maria Moro, IB – Instituto Butantan, Brazil

16:30 – 17:00 **IT 7. Invited talk**

Process characterization strategy – a QbD driven control strategy
Claudia Berdugo-Davis, Catalent Biologics, United States of America

17:00 – 17:15 **OP 9.** Monocyte-macrophages and dendritic cells are more sensitive than pbmcs for the detection of immune response modulating impurities (IIRMIS) in therapeutic proteins
Eduardo F. Mufarrege, Sofía Giorgetti, Sonia Ricotti, Marina Etcheverrigaray and Daniela Verthelyi, UNL – Universidad Nacional del Litoral, Argentina

17:15 – 17:30 **OP 10.** 3D tumor spheroids: an in vitro bladder cancer model for drug screening
Robson L. F. Amaral, Mariza Abreu Miranda, Priscyla Daniely Marcato Gaspari, Ain-Hong Ma, Hongyong Zhang, Chong-Xian Pan, Kamilla Swiech, USP – Universidade de São Paulo, Brazil

17:30 – 17:45 **OP 11.** CHO-K1, HEK293 and NS0 cell lines: glycosylation impact on affinity, stability and neutralizing activity of a chimeric anti-rhIFN-α2b antibody
Carolina Attallah, María Fernanda Aguilar, Marina Etcheverrigaray, Marcos Oggero, UNL – Universidad Nacional del Litoral, Argentina

17:45 – 18:00 **OP 12.** Evaluation of mitochondrial redox potential and their effect in the n-glycosylation of Hemagglutinin recombinant, using the Baculovirus expression vector system (BEVS) at different dissolved oxygen tensions
Alberto Porras Sanjuanico, Martha A. Contreras, Ana Ruth Pastor, Vanessa Hernandez, Octavio Tonatiuh Ramírez, Laura A. Palomares, UNAM - Universidad Nacional Autónoma de México, Mexico

18:00 – 19:20 **Industrial Workshop I**

Session chairs: Viviane F. Botosso, Instituto Butantan, Brazil
Rimenys J. Carvalho, UFRJ, Brazil

18:00 – 18:40 **IW1.** Unlocking the potential for downstream efficiency
Alécio Pimenta Jr., GE Healthcare Life Sciences, Brazil

18:40 – 19:20 **IW2.** A case study: leveraging Gibco products to reduce regulatory hurdles and improve manufacturing process efficiencies for biosimilars
Jack Palmer, Thermo Fisher Scientific, USA

Tuesday, October 23

09:00 – 10:00 **Poster session** (authors of even-numbered posters should stay with their posters)

10:00 – 11:00 **KL 3. Keynote Lecture**

Challenges of bringing recombinant vaccines to the market: a case study for an Influenza vaccine
Laura Palomares, UNAM – Universidad Nacional Autónoma de Mexico, Mexico

11:00 – 11:20 **Break and networking time**

11:20 – 11:50 **IT 8. Invited talk**

Immunogenicity of therapeutic proteins
Eduardo F. Mufarrege, Universidad Nacional del Litoral, Argentina

11:50 – 13:20 **Session E: Downstream process development**

Session chairs: Jorge F. B. Pereira, UNESP – Universidade Estadual Paulista "Julio de Mesquita Filho", Brazil
Tânia P. P. Cunha, FIOCRUZ – Fundação Oswaldo Cruz, Brazill

11:50 – 12:20 **IT 9. Invited talk**

Industrial downstream optimization: a study case
Aliuska Cardoso Ramírez, Azalia Rodríguez Taño, Yanet Borrego Morales, Alejandro Portillo Vaquer, Centro de Inmunología Molecular, Cuba

12:20 – 12:40 **OP 13.** Kyte-doolittle hydrophilicity plot as a tool to predict the potential of aqueous biphasic systems to purify recombinant proteins from E. coli
Nathalia V. Santos, Danielle B. Pedrolli, Sandro R. Valentini, Jorge F. B. Pereira, UNESP – Universidade Estadual Paulista, Brazil

12:40– 13:00 **OP 14.** Development of an advanced purification step for recombinant factor IX
Rimenys J. Carvalho, Ana Caroline R. Vieira, Leda R. Castilho, UFRJ – Universidade Federal do Rio de Janeiro, Brazil

13:00 – 13:20 **OP 15.** Downstream process of LigANI protein, a vaccine candidate for human and animal lepstospirosis
Gabriela Santos Esteves, Mariana Miguez, Ana Carolina M Andrade-Góes, Marco A Medeiros, Biomanguinhos/Fiocruz, Brazil

13:20 – 14:40 **Lunch**

14:40 – 16:10 **Session F: Biosimilars**

Session chairs: Guillermina Forno, Zelltek S.A., Argentina
Vanda D. Magalhães, Bionovis, Brazil

14:40 – 15:10 **IT 10. Invited talk**

Biologics in Brazil: Overview and regulatory perspectives
Kalinka de Melo Carrijo, ANVISA – Agência Nacional de Vigilância Sanitária, Brazil

15:10 – 15:40 **IT 11. Invited talk**

Forced degradation studies as a tool for analytical comparability of biosimilars
Guillermina Forno, Zelltek S.A., Argentina

15:40 – 15:55 **OP 16.** Expression, purification and characterization of an anti-PCSK9 biosimilar mAb
Thayana A. Cruz & Leda R. Castilho, UFRJ – Universidade Federal do Rio de Janeiro, Brazil

15:55 –16:10 **OP 17.** Economic opportunity analysis of the development and manufacturing of an Evolocumab biosimilar in Brazil
Tulio M. Lima, Marcos B. Pinho, Leda R. Castilho, UFRJ – Universidade Federal do Rio de Janeiro, Brazil

16:10 – 16:30 **Break and networking time**

16:30 – 18:00 **Session G: Pharmaceutical Biotechnology in Brazil**

Session chairs: Aldo Tonso, USP – Universidade de São Paulo, Brazil
Francesc Godia, UAB – Universitat Autònoma de Barcelona, Spain

16:30 – 17:00 **IT 12. Invited talk**

Innovation in oncology
José Fernando Perez, Recepta Biopharma, Brazil

17:00 – 17:30 **IT 13. Invited talk**

Biosimilars in Brazil and the vision of Bionovis
Vanda D. Magalhães, Bionovis, Brazil

17:30 – 18:00 **IT 14. Invited talk**

Cell culture technology in two Brazilian public laboratories for the production of immunobiologicals
Renato Astray, Instituto Butantan, & Elena Caride, Biomanguinhos/Fiocruz, Brazil

18:00 – 19:40 **Industrial Workshop II**

Session chairs: Daniel A. Ribeiro, Biomanguinhos/Fiocruz, Brazil
Renata G. F. Alvim, UFRJ, Brazil

18:00 – 18:20 **IW3.** Importance of versatile media and feeds for biologics production in rCHO cells
Chaya Kataru, Kerry Biosciences, USA

18:20 – 19:00 **IW4.** New technologies of upstream bioreactor for industrial scale using adherent cells
Juliana Groba de Oliveira, Pall Biotech, Brazil

19:00 – 19:40 **IW5.** New directions in 3D cell culture – novel technologies and applications
John Yoshi Shyu, Christopher Suarez, Corning, USA

Wednesday, October 24

09:00 – 10:00 **Poster session** (authors of all posters should stay with their posters)

10:00 – 11:00 **KL 4. Keynote Lecture**

The cancer immunotherapy revolution
Martín H. Bonamino, INCA – Instituto Nacional do Câncer, Brazil

11:00 – 11:20 **Break and networking time**

11:20 – 12:50 **Session H: Viral and recombinant vaccines**

Session chairs: Renato M. Astray, IB – Instituto Butantan, Brazil
Luciane P. Gaspar, Biomanguinhos/Fiocruz, Brazil

11:20 – 11:25 **Session sponsored by Pall**

11:25 – 11:55 **IT 15. Invited talk**

Optimization of bioprocesses for virus-like particle production
Francesc Gòdia, UAB – Universitat Autònoma de Barcelona, Spain

11:55 – 12:10 **OP 18.** Upstream process development for the production of zika and yellow fever virus-like particles (VLPs)
Renata G. F. Alvim & Leda R. Castilho, UFRJ – Universidade Federal do Rio de Janeiro, Brazil

12:10 – 12:25 **OP 19.** Glycosylation pattern analysis of the rabies virus glycoprotein ectodomain produced in Drosophila melanogaster S2
Livia Pilatti, Flávia F. Barbosa, Renato M. Astray, Michael Butler, Elisabeth F.P. Augusto, Unifesp – Universidade Federal de São Paulo, Brazil

12:25 –12:40 **OP 20.** Optimization of PK15 cell culture for veterinary antigen production
Ziomara P. Gerdtzen, Felipe Véliz, Kurt Pohlhammer, Lorna León, Iván Valdés, Harold Oliva, Samuel Valdevenito, Universidad de Chile, Chile

12:40 – 12:55 **OP 21.** Expression and characterization of a vaccine candidate against bovine viral diarrhea virus
Alaín G. Pose, Raquel M. Seguí, Oliberto S. Ramos, Jorge R. Toledo, UdeC – Universidad de Concepción, Chile

12:55 – 14:10 **Lunch**

14:10 – 15:25 **Session I: Cell and nucleic acid therapies**

Session chairs: Adriano Azzoni, USP – Universidade de São Paulo, Brazil
Kamilla Swiech, USP – Universidade de São Paulo, Brazil

14:10 – 14:40 **IT 16. Invited talk**

Development of recombinant modular proteins for nucleic acid delivery
Adriano Azzoni, USP – Universidade de São Paulo, Brazil

14:40 – 14:55 **OP 22.** Strategies for mesenchymal stem/stromal cells expansion targeting the application of the wave-induced motion bioreactor
Juliana S. Silva, Liseth V. G. Gil, Eric T. Katayama, Fernanda P. Casciatori, Kamilla S. Antonietto, Dimas T. Covas, Claudio Alberto T. Suazo, UFSCar – Universidade Federal de São Carlos, Brazil

14:55 – 15:10 **OP 23.** Scale-up of human adipose-derived mesenchymal stem cells culture for diabetes cell therapy
Anamaria C. Sánchez, Lorna León, Esteban González E., Barbara A. Andrews, Ziomara P. Gerdtzen, Pablo Caviedes, Juan A. Asenjo, Universidad de Chile, Chile

15:10 – 15:25 **OP 24.** Periosteal tissue engineering: behavior of human dermal fibroblasts and Saos-2 osteoblasts on chitosan-based scaffolds
Renata F. B. Souza, Fernanda C. B. Souza, Cristiano Rodrigues, Diego Mantovani, Ketul C. Popat, Ângela M. Moraes, Unicamp, Brazil

15:25 – 15:40 **Presentation of the best poster and the best poster presented by a student**

15:45 – 16:00 **Closing ceremony**

LIST OF POSTERS

Session A: Biopharmaceutical trends

1. **N-glycoengineering of hIFN-α2b by fusion of ANITVNITV peptide: impact on physicochemical properties, *in vitro* bioactivity and pharmacokinetics**

   Agustina Gugliotta, Natalia Ceaglio, Ricardo Kratje, Marcos Oggero, Univ. Nacional del Litoral, Argentina

2. **An hGM-CSF-derived peptide as a novel glycoengineering tool to produce highly o-glycosylated therapeutic variants of HIFN- α2b**

   Francisco Iturraspe, Agustina Gugliotta, Verónica Ferrando, Ricardo Kratje, Marcos Oggero, Natalia Ceaglio, Universidad Nacional del Litoral, Argentina

3. **N2a cell line and neuronal primary cultures as platforms to evaluate neuroprotective and neuroplastic activities of human erythropoietin**

   Ma. de los Milagros Bürgi, Gabriela Aparicio, Ricardo Kratje, Camila Scorticati, Marcos Oggero, Universidad Nacional del Litoral, Argentina

4. **Development and standardization of tridimensional (3D) spheroids as an *in vitro* ovarian cancer model**

   Larissa B. Tofani, Robson L. F. Amaral, Juliana M. Marchetti, Kamilla Swiech, Univ. de São Paulo, Brazil

5. **Production and purification of the Brain Derived Neurotrophic Factor (BDNF) in bacteria *Escherichia coli***

   Pía A. Zamponi, Oliberto Sánchez, Romina A. Rojas, Universidad de Concepción, Chile

6. **Monoclonal antibodies against glycosylated recombinant human stem cell factor. a tool for quality control and bioprocess monitoring**

   Antonela Fuselli, Luisina Cappellino, Milagros Burgi, Ricardo B. Kratje, Claudio C. Prieto, Universidad Nacional del Litoral, Argentina

7. **Effect of synthetic phosphoethanolamine on the *in vitro* metabolism of bladder cancer cells**

   Vinicius Borroni Facanali, Eric Takashi Katayama, Diogo Peres dos Santos, Claudio Alberto Torres Suazo, Fernanda Perpétua Casciatori, Universidade Federal de São Carlos, Brazil

8. **Synthesis of cobalt ferrite nanoparticles doped with kiwi, sicilian lemon, okra and açai**

   Robson R. Bernardo, Luiz A. Oliveira, Brunno R. F. Verçoza , Braulio S. Archanjo, Giani C. Rodrigues, Alessandra C. Lima, Bruna C Coelho, Otoniel P Pereira, Univ. Federal do Rio de Janeiro, Brazil

9. **Obtaining L-Asparaginase by natural and synthetic pathways: search for a more effective biopharmaceutical against acute lymphoid leukemia**

   Tales A. Costa-Silva, Adalberto Pessoa-Jr, Gisele Monteiro, Universidade de São Paulo, Brazil

Session B: Genome editing and cell line development

10. **Expression of envelope protein domain III (EDIII) of Zika virus in *Drosophila* cells**

    Alane L. Xalega, Thaissa C. Bernardino, Renato M. Astray, Lennon Pereira, s P.S. Alves, Luis C. Ferreira, Viviane Botosso, Soraia A. C. Jorge, Instituto Butantan, Brazil

11. **Site-directed integration in CHO-DG44 cells: mapping genomic hotspots associated to high and stable expression levels of recombinant proteins**

    Luis RubenA. Quispe, Mari C. Sogayar, Marcos A. Demasi, Universidade de São Paulo, Brazil

12. **Evaluation of recombinant human acid alpha–glucosidase expression in HEK–293F cell line**

    Nathália P. S. Leite, Matheus H. Santos, Kamilla Swiech, Universidade de São Paulo, Brazil

13. **Characterization of cellular states of CHO-K1 cells adapted to serum free media and suspension growth through cell cycle and RNA-seq profiling**

    Ileana Tossolini, Fernando López-Díaz, Sebastián Antuña, Ricardo Kratje, Claudio Prieto, Universidad Nacional del Litoral, Argentina

14. **Development of biotechnological tools for differential diagnostic of infection by Mayaro or Chikungunya**

    Deny A Santos, Miriam A Silva, Letícia B Rocha, Roxanne MF Piazza, Lívia Pilatti, Flávia F Barbosa, Renato M Astray, Instituto Butantan, Brazil

15. **The portion of the ACTB promoter associated with a CpG island is capable of decreasing the silencing of recombinant genes in CHO cells**

    Matías Gutiérrez, Roberto Zúñiga, Norberto Collazo, Pablo Sotelo, Juan C. Aguillón and María C. Molina, Universidad de Chile, Chile

16. **Generation of stable reporter breast and lung cancer cell lines for NF-κB activation studies**

    Hellen Daghero, Romina Pagotto, Maribel G. Vallespí, Mariela Bollati-Fogolín, Inst. Pasteur Montevideo, Uruguay

17. **New amphiphilic amino acid derivatives for efficient DNA transfection *in vitro***

    Lucia C. Peña, Maria F. Argarana, Maria M. De Zan, Antonella Giorello, Sebastian Antuña, Claudio C. Prieto, Carolina M. I. Veaute, Diana M. Muller, Universidad Nacional del Litoral, Argentina

Session C: Upstream process development

18. **Recombinant acid alpha-glucosidase production in the human cell line HKB-11 cultured under serum free suspension conditions**

    Matheus H. Santos, Rafael T. Biaggio, Virginia Picanço-Castro, Kamilla Swiech, Univ. de São Paulo, Brazil

19. **Influence of mild hypothermia and glucose concentration on growth kinetics and production of MAbs in NS0 cells**

    Yandi Rigual, Claudia Altamirano, Julio Berríos, Pontificia Universidad Católica de Valparaíso, Chile

20. **Manufacturing of chimeric antigen receptor T cells for adoptive immunotherapy using Xuri bioreactor**

    Letícia Delfini Vaz, Amanda Mizukami, Pablo Diego Moço, Kelen Cristina Ribeiro M. Farias, Virgínia Picanço-Castro, Dimas Tadeu Covas, Kamilla Swiech, Universidade de São Paulo, Brazil

21. **Recombinant coagulation Factor VII expression in human cell lines**

    Tárik R Heluy, Rafael T Biaggio, Marcela CC de Freitas, Virgínia Picanço-Castro, Kamilla Swiech, Universidade de São Paulo, Brazil

22. **Effects of temperature shift on cell metabolism and production of zika virus-like particles (VLPS) in mammalian cells**

    Alexandre B. Murad, Pedro I. Barbosa, Antonio M. Roldão, Rodrigo C. Pinto, Manuel J. T. Carrondo, Leda R. Castilho, Universidade Federal do Rio Janeiro, Brazil

23. **Influence of shear stress by gas aspersion in Baculovirus production by Sf9 cells in bioreactor**
    Karina Klafke, Marlinda S Lobo, Aldo Tonso, Universidade de São Paulo, Brazil

24. **3D culture of the RT4 cell line in the Taylor vortices bioreactor**
    Eric T. Katayama, Diogo P. Santos, Lizeth V. G. Gil, Rebeca C. Emídio, Fernanda P. Casciatori, Kamilla Swiech, Claudio A. T. Suazo, Universidade Federal de São Carlos, Brazil

25. **The bottlenecks to develop an efficient scaling up process using Drosophila S2 cells system**
    Monize C. Decarli, Diogo P. Santos, Daniela M. Correia, Renato M. Astray, Fernanda P. Casciatori, Ângela M. Moraes, Claudio Alberto T. Suazo, Universidade Federal de São Carlos, Brazil

26. **Design of experiment approach for the evaluation of cell culture process robustness**
    María Belén Bosco, Ignacio Amadeo, Laura Mauro, Romina Zuqueli, Guillermina Forno, Zelltek, Argentina

27. **Evaluation of culture conditions for Chinese Hamster Ovary (CHO) cells scale-up**
    Andrés Bello-Hernández, Astrid Nausa-Galeano, Fabio Aristizabal, Rubén Godoy-Silva, Universidad Nacional de Colombia, Colombia

Session D: Product development and product quality

28. **Reversible microdevice for monitoring animal cell**
    Franciele Flores Vit, Naiara Godoi, Rony Nunes, Hernandes Faustino de Carvalho, Lucimara Gaziola de la Torre, Universidade Estadual de Campinas, Brazil

29. **Design and characterization of a novel dimeric TNFα inhibitor**
Viana Manrique-Suárez, Luis Macaya, Nelson Santiago Vispo, Oliberto Sánchez, Univ. de Concepción, Chile
30. **Design, production and characterization of a TNFα-blocking trimeric TNF receptor 2 (TNFR2) ectodomain**
Maria A Contreras, Luis A Macaya, Jorge R Toledo, Oliberto Sanchez, Univ. de Concepción, Chile
31. **Production of biologically active human TNFα in E. coli shuffle T7 express**
Maria A Contreras, Jorge R Toledo, Oliberto Sánchez, Universidad de Concepción, Chile
32. **Identification and characterization of novel TNFα-blocking peptides**
Maria A Contreras, Luis A Macaya, Jorge R Toledo, Oliberto Sanchez, Univ. de Concepción, Chile
33. **Effect of N-glycosylation on recombinant human interferon alpha immunogenicity**
María J. Leopold, Eduardo F. Mufarrege, Marina Etcheverrigaray, Univ. Nacional del Litoral, Argentina
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ABSTRACTS OF ORAL ACTIVITIES

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CELL FACTORIES: MANUFACTURING NEXT GENERATION PRODUCTS FROM SOLID FOUNDATIONS

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Key Words: expression, cell factories, novel formats, ATMPs

The past 25 years has seen tremendous advances in yields and range of biopharmaceutical products. In the early 1990's, yields of 0.1g/L were considered as good, the Chinese Hamster ovary system was overshadowed by lymphoid cell types, antibodies were products of the future and cytokines/hormones were emerging as therapeutics. Intellectual enquiry has revolutionised our understanding of complex systems (cells and products) and technological advances have enabled the potential to develop novel products (including Advanced Therapy Medicinal Products, ATMPs). In the current world of systems and synthetic biology, we stand at the cross-roads for the successful manufacture of new products (domain-engineered proteins/peptides, nucleic acids, cell-based therapeutics). The past has taught us the approaches that can bring imagination to reality but it has also warned that the pathways require innovation and a willingness to continue to engage with new technology. This presentation will review that future landscape.

VIRAL DSP DEVELOPMENT AND CELL THERAPY PURIFICATION AND CONCENTRATION

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Key Words: Viral DSP, mesenchymal stem cell DSP, continuous purification, integrated stem cell production

The last couple of years saw regulatory approval for cell and gene therapy biopharmaceuticals, targeting very critical unmet medical needs, in particular cancer and monogenetic, orphan indications.
These novel biopharmaceuticals are highly complex, bringing in needs for extraction and purification which are much more elaborate than for pharmaceutical proteins.
In this presentation, a brief overview of some new tools and processes for virus based therapy products will be presented, including design and testing of new membrane chromatographic tools as well as the use of continuous, highly reliable, processes.
For cell therapies, work carried out for clarification, washing and cell concentration will be presented. It will be shown that such complex processes can be run in a continuous, integrated mode required to ensure operation under complete sterility; this is essential given that the large size of the cell product precludes the use of any type of final sterile filtration commonly used in protein biopharmaceuticals.

RAPID AND FACILE DISCOVERY OF MONOCLONAL ANTIBODIES TO FIGHT INFECTIOUS DISEASES

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Key Words: Antibody therapeutics, single-cell technology, next-generation sequencing, vaccines

Antibodies are developed in response to natural infection and vaccination, and they form a key part of human immune responses. Antibodies are generated *in vivo* by B cells and comprise the most valuable class of protein drugs, providing highly specific therapies with minimal side effects. High-throughput sequencing of antibodies has emerged as a critical step for understanding of antibody responses following infection or vaccination, and for discovery of new antibody drugs. However, determining the complete antibody sequences for drug development remains a major challenge because antibodies are comprised of two different variable polypeptide chains, the heavy (VH) and light (VL) chains. Thus, single B cell analysis is required for antibody sequencing because of the dual-gene variability in B cell populations, and there are no available technologies that can adequately interrogate the $>10^6$ B cells in typical human blood samples.

To directly address this issue, we have developed a low-cost single-cell technology for sequencing complete antibody variable region repertoires from $>5x10^6$ B cells per experiment. Massive natively paired variable region heavy and light VH:VL repertoire analyses of human donors enabled rapid antibody discovery while providing novel insights regarding human anti-infective responses. We also leverage *in vitro* display technologies to determine antibody binding properties. Gene libraries of natively paired VH:VL amplicons are produced *en masse* and expressed in a yeast display platform optimized for antibody surface expression. The yeast library is then screened for binding to pathogen-derived proteins or peptides using flow cytometry, along with next-generation sequencing and computational analysis of the screening datasets. These projects have been applied for efficient isolation of HIV-1 broadly neutralizing antibodies (bNAbs) from the B cell repertoire of an HIV-1 slow progressor and high-affinity neutralizing antibodies targeting Ebola virus (EBOV) glycoprotein from a vaccinated donor. Additional studies related to the immune responses following Zika virus disease will also be discussed. Ongoing work continues to apply these comprehensive screening technologies for rapid antibody discovery against infectious disease targets and to improve the development of vaccines for enhanced protection against infectious disease.

NOVEL TRENDS IN CANCER IMMUNOTHERAPY: CIM DEVELOPMENTS.

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Key Words: Cancer, immunotherapy, antibody, vaccine, EGFR

The understanding of the mechanisms that mediate the relationship between the immune system and tumors has been the basis for the design of immunotherapies, whose success in the clinic is now a reality. There are several immunotherapies directed at targets in the tumor or at points of control of the immune response, which are already registered for the treatment of patients with various types of cancer. The Molecular Immunology Center, with more than 20 years of experience in the field of cancer immunotherapy, has monoclonal antibodies and vaccines for the treatment of patients. Among these are those directed to EGFR, an oncogene that in addition to contributing to tumor proliferation, favors the evasion of the tumor to the immune system. These antibodies and some vaccines are based on highly glycosylated proteins, which are produced in mammalian cells. Among these antibodies is nimotuzumab, registered for the treatment of patients with head-neck cancer, glioma, esophagus and pancreas. Among the vaccines are CimaVax-EGF, registered for the treatment of patients with non-small cell lung cancer, and HER1 and HER1 + HER2 vaccines, in clinical or preclinical trials respectively.

RECOMBINANT HUMAN GROWTH FACTORS EXPRESSED IN MAMMALIAN CELLS AND EXOSOMES FROM MSCs FOR SKIN REGENERATION

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Key Words: biopharmaceuticals, mammalian cell expression system, wound healing, Platelet-Derived Growth Factor, Vascular Endothelial Growth Factor.

The regenerative medicine aims treatments to accelerate the different phases of tissue repair: blood clotting and inflammation, cell proliferation, tissue remodeling. In scarring and deficient tissues, there is an impairment of the ideal levels of growth factors in the wound healing phases. By manipulating the growth factors (GF) composition, it is possible to accelerate or modify the process of regeneration and remodeling of damaged tissues. Currently, Mesenchymal Stem/Stromal Cells (MSCs) are considered to be promising sources for various types of Cell Therapies in the Regenerative Medicine field. These cells display a regenerative potential and may be used for repair and maintenance of different tissue types, however, the mechanisms by which this repair activity is exerted is not well known yet. In this way, the application of MSCs or their microvesicles/exosomes and growth factors could contribute to the correct repair in a timely manner. Two abundant GF at Platelet-rich plasma (PRP), platelet-derived growth factor BB (rhPDGF-BB) and vascular endothelial growth factor 165 (VEGF165) were expressed in a safe and reproductive heterologous system using mammalian cells HEK293, 293T or CHO cells, to obtain overproducing cell clones, to guarantee their quality and post-translational modifications, which are important for their production or stability. The recombinant GFs were purified using heparin affinity chromatography and tested using a specific in vitro biological activity assays for each growth factor. To test the in vivo biological activity, an animal model of dorsal wound healing was standardized in nude rowett rats using four wounds with surgical punch of 6 mm diameter. The results showed comparative efficacies in both treatments of wounds with 5ug rhPDGF-BB or rhVEGF165, with an accelerate cicatrization rate in comparison with the injury without treatment or treated with only the vehicle (saline solution). To improve the wound healing process, we propose the use of exosomes isolated from MSCs, treated with recombinant growth factors, to verify its therapeutic effect on the tissue regeneration. To this step we isolated exosomes from MSCs and characterized them by Western blot assay with CD81 antibody. Animals will be treated with exosomes isolated from MSCs treated with recombinant growth factors, described before, to evaluate its wound potential. We waiting to develop and offer an improved alternative and approach to wound healing treatment for patients and animals, with complex chronic ulcers and other damage in the skin, which are difficult to treat and associated with high treatment costs allowing. Ethical approval: *CEUA-FMVZ/USP nº 8941120916*.

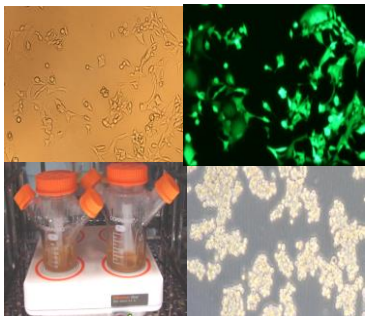

Figure 1- Mammalian Cells transfected with a vector containing both a peptide growth factor and the eGFP cDNAs and Scaling-up of Mammalian Cell Culture in Spinner

ENGINEERING COMPLEX TRAITS IN THE POSTGENOMIC ERA OF CHO CELL LINE DEVELOPMENT

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Key Words: CRISPR, Systems Biology, Genomics, CHO

Over the past 3 decades, Chines hamster ovary cells have become the predominant production hosts for biotherapeutic proteins, and now produce 6 of the top 10 grossing pharmaceuticals. However, the complexity of the protein-based drugs and the host cells pose major challenges that must be controlled to improve the safety, efficacy, and affordability of these pharmaceuticals. Seven years ago, with the publication of the first public genome sequence of CHO cells, it became possible to more rationally engineer CHO cells to address outstanding challenges in recombinant protein production. Here I will focus on recent work wherein we have utilized the novel genomic resources to develop large scale computational models of CHO cells, deploy novel genome editing tools, and engineer complex traits to improve CHO based bioprocessing.

MICROFLUIDICS - A PROMISING TOOL FOR *IN VITRO* CELL CULTURE, BIOLOGICAL MODELS AND DRUG RESEARCH

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Key Words: microfluidics, cell culture, concentration gradient, drug screening, organ on a chip.

Microfluidics is a multidisciplinary field that operates at the microscale with small amount of fluids. The hydrodynamic characteristics allow the control of chemicals, cells, lipids, nucleic acids in space and time. The microenvironment uses few amounts of sample and reagents that flows in microchannels in laminar regime, since no external force is applied. In this case, the micron scale minimize effects of mass and heat transfer, allowing integration of different techniques for data acquisition in different ways than macro scale. These characteristics open great opportunities for technological research. In the field related to the cultivation of cells, simulating the conditions found in the environments where mammalian cells grow is challenging. Multi-cell type communication and the presence of gradients of cytokines and/or other proteins, and biomechanical stimulation vary widely in time and space. Micro-fabricated systems are alternatives to better mimic real conditions. In this context, microfluidics expands the possibilities for cellular studies on dynamic conditions, enabling cellular studies that involve response mechanisms, chemotaxis and cytotoxicity, because it mimics cellular microenvironments. These microdevices can also be designed to evaluate reaction kinetics in real time, reducing analysis time. These systems have been applied for cellular investigations including evaluation of cellular behavior by gradient generator system, cultivating cells in microbioreactor system, generating droplets to encapsulate biological agents and even analyzing the dynamic of encapsulated cells through droplet-based systems. In addition, possibilities for using microfluidics increase when coupled to biophotonics and cell biology, to allow major advances in the biomedical and bio-engineering areas. In this way, concentration gradient generators can be explored to determine MIC (Minimum Inhibitory Concentration) and IC_{50} (half minimal 50% inhibitory concentration) in dynamic conditions, screening of drugs, cell growth in different conditions can be monitored simultaneously. The heterogeneity of cells during *in vitro* mammalian cells transfection can be tracked. Advanced and complex cultivation of different cells can be designed in microfluidic environment mimicking organs in devices named organ-on-a-chip. However, efforts are still required to design microdevices that operate in a simple way for daily use in research laboratories. In this situation, collaborative work of researchers from different fields is required. Therefore, microfluidics enables applications that would not be feasible at the macroscale demonstrating high potentiality as a tool for *in vitro* cell culture, biological models and drug research.

FLUORESCENCE-ASSISTED SEQUENTIAL INSERTION OF TRANSGENES: AN APPROACH TO INCREASE SPECIFIC PRODUCTIVITY IN MAMMALIAN CELLS.

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Key Words: List up to five key words.

Production of recombinant biopharmaceuticals in mammalian cells cultures predominantly use transformation approaches in which the gene of interest is randomly integrated into a host chromosome, and the desired protein is secreted into the culture medium to facilitated the downstream process. The specific productivity (Qp) of mammalian cell lines usually correlates directly with the transgene mRNA levels which, in turn, are dependent on the expression cassette used, on the transgene copy number integrated into the genome, and on the chromatin structure in the integration site.

Currently, the generation of cell lines for recombinant protein production is carried out through two main strategies; one based on dihydrofolate reductase (DHFR)-based methotrexate (MTX) selection, and the other on glutamine synthetase (GS)-based methionine sulfoximine (MSX) selection. For both systems, DHFR and GS, the selected high-producing clones usually harbor the transgene organized in tandem arrays of tens or hundreds of copies. Thus, it is not known what is the real contribution that makes each single copy of the transgene to the Qp of the selected clone. In addition, gene expression from these tandems is often unstable due to the loss of transgene copies resulting from recombination events, or due to epigenetic silencing.

In this work we assess the sequential insertion of transgenes as a new approach for developing cell clones producing high levels of a recombinant protein. We designed a Bicistronic expression cassette that allows the fluorescence-assisted selection of single copy high producing clones. After selection, the fluorescent marker can be removed by site-specific recombination, without affecting the transgene expression. Thus, the resulting non-fluorescent clone can be subjected to additional rounds of transgene insertion, increasing stepwise the transgene copy-number and simultaneously the cell line Qp. Clones generated using this approach show stable expression levels after more than 30 passages. As far as we know, this is the first report describing the sequential insertion of transgenes as an alternative to increase the specific productivity of cell lines.

PRODUCTION AND INITIAL CHARACTERIZATION OF rCOL7A1 VARIANTS RESISTANT TO MATRIX METALLOPROTEINASES CLEAVAGE.

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Key Words: Dystrophic Epidermolysis Bullosa (DEB), biopharmaceuticals, recombinant collagen VII, recombinant collagen VII variants.

Collagen VII (COL7A1) is a glycoprotein of 2,994 amino acids found mainly in the basement membrane, being the major component of the anchoring fibrils, responsible for attachment of the dermis to the epidermis. Mutations in the *COL7A1* gene are associated with the Dystrophic epidermolysis bullosa (DEB) skin disorder. The phenotype of this rare inherited disease is characterized by blistering and skin fragility caused by minor trauma or friction in affected tissues. Currently, DEB has no cure, and treatment is only palliative, aiming especially at managing the burden of blistering and skin infections. Some alternative treatment options are currently being explored including human recombinant collagen VII (rCOL7A1) replacement therapy. Studies employing knockout mice for the *Col7a1* gene demonstrated that the topical, intradermal injections and intravenous administration of rCOL7A1 enabled reconstitution of the anchoring fibrils, and a partial phenotypic reversal of DEB. Studies using a Col7a1 null mice indicated that the rCOL7A1 half-life is of approximately one month, suggesting that the administration frequency of rCOL7A1 would be once a month.. Although the endogenous turnover mechanism of the anchoring fibrils has not been fully elucidated, degradation of COL7A1 seems to occur through the action of matrix metalloproteinases 1 (MMP1) and 2 (MMP2), since inhibition of MMP1 and MMP2 facilitates anchoring fibrils formation. These data suggest that mutating COL7A1 at the MMP1 and MMP2 cleavage sites should increase the anchoring fibrils half-life at the dermis-epidermis junction. The main objective of this work is the structural and functional characterization of three rCOL7A1 variants (V1, V2 and V3) potentially resistant to MMP1 and MMP2 activity. Here, we describe the initial characterization of these rCOL7A1 variants, obtained through transient transfection of 293T cells with bicistronic expression vectors of the rCOL7A1 and its three variants. Additionally, stable expression of these proteins was obtained by transfection of the CHO-DG44 cell line. These preliminary results and further functional characterization of rCOL7A1 variants resistant to MMP1 and MMP2 induced proteolytic degradation may translate into an rCOL7A1 with extended half-life as a therapeutic option for DEB.

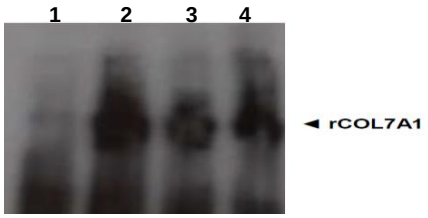

Figure 1 – Confirmation of transient expression and secretion by 293T cells of rCOL7A1 (lane 1) and its three variants V1, V2 and V3 (lanes 2, 3 and 4, respectively), by Western blot of samples of conditioned media from 293T cells transfected with pNU1-COL7A1 (line 1), pNU1-COL7A1V1 (line 2), pNU1-COL7A1V2 (line 3), pNU1-COL7A1V3 (line 4).

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iCAR-T cells: A new concept for the standardization and industrialization of the CAR T-cell therapy

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Key Words: Cart T cell therapy, iCAR-T cell, Lentiviral Transduction

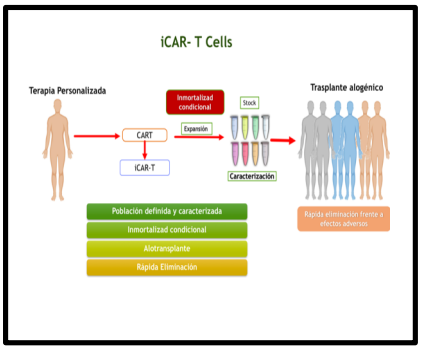

Figure 1 – Representation of iCAR-T cell system

During the last years, car t cell therapy has become a revolutionary tool in the fight against cancer. This immunotherapy takes advantage of the specificity and functionality of the adaptive immune response generated against tumor development. Using genetic engineering tools, T lymphocytes from cancer patients are transformed to express on their surface an "antigen receptor chimeric" (CAR). The CAR receptor allows the recognition of specific tumor antigens, redirecting the lymphocytes to eliminate the tumor cells[1]. CART therapy has shown high levels of efficacy in hematological cancer with a 90% total response in clinical trials[2]. Despite the remarkable results obtained, CART therapy shows limitations that threaten its overcrowding and reduce the possibility of covering the entire population that requires treatment.

This work proposes to face this problem through the development of "iCAR-T" cell line, a monoclonal cell line derived from a specific population of T lymphocytes, able to behave like an immortal culture in a controlled way, proposing a model that facilitates the production and massification of CART cell therapy. With this purpose, a controlled immortalization system (CIS) was designed, this expression system allows the specific managing of cell proliferation through the application of an external stimulus. The genetic sequence of the CIS was constructed from 3 plasmid vectors and cloned into the third generation lentiviral expression vector pLW. Then, $CD8^+$ memory T cells isolated from peripheral blood mononuclear cells (PBMCs) were transduced with a third generation chimeric receptor against CD38 antigen, which contained the activation domains of CD28, 4-1BB and CD3z, and were selected by cell sorting Finally, CART cells were transduced with the lentiviral vector that contained the CIS system. The control over the proliferation and function of iCAR-T cells modified with the CIS system was tested in-vitro by flow cytometry. As a result, it was demonstrated that the use of an inducer, which is innocuous for both cells and patients, is possible to promote the specific proliferation of iCAR-T cells. Using Western Blot and RT-PCR, was possible to observe a specific increase in the expression levels of molecules involved in the proliferation of T cells, without affecting the cellular phenotype. These results establish the basis for future studies of function and specific cytotoxicity in xenograft models.

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2. Yip A, Webster RM. The market for chimeric antigen receptor T cell therapies. *Nat Rev Drug Discov*. 2018. doi:10.1038/nrd.2017.266.

Combined effect of c-myc and xbp1s on specific cell growth rate and protein productivity on CHO cells rh-EPO producer.

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Key Words: CHO cells, erythropoietin, c-myc, xbp1s, protein productivity, growth rate.

The cells line more used in pharmaceutics industry for r-protein production is Chinese Hamster Ovary (CHO). This one is characterized for to do glycosylation with high similitude grade when is compared with human protein. However, there are associated limitations. On the one hand, the deficient use of glucose and, on the other the secretory capacity / protein processing in these cells. These problems affect cell viability and, as a consequence, r-protein's specific (qp) and/or volumetric productivity (Qp).

Recent studies suggested that overexpression of global regulation factor c-Myc, together with the optimization of culture conditions; allow improving the cell growth and glucose metabolism efficiency in processes based on CHO cell culture. Besides, studies suggested that overexpression of global regulation factor xbp1s has an effect on specific productivity. However the effect of overexpression of c-myc on the productivity (qp and Qp) of r-proteins in CHO cells has been poorly evaluated. Moreover the combined effect of c-myc with xbp1s over these parameters has not been evaluated.

rh-EPO producer CHO cells were randomly transfected with pCDH-puro-cMyc (addgene, #46970) and modified pCMV5-Flag-XBP1s (addgene, #63680, and hygromycin resistance) lipofectamine® was used, in accordance with the manufacturer's instructions. After that, the cells transfected pool was selected used puromicyn and hygromycin for the selection during two weeks. Later the clones were selected and incubated for 72h in 37ºC, 5%CO2 in a humidified incubator. The clones were cultured in Hyclone medium (SFM4CHO) serum free, with 20mM Glc and 4mM Gln in spinner flask at 60rpm. The culture control (CC) was CHO rh-EPO cells without transfect. The cultures were stopped when viability arrive to around 75%. For each clone the human recombinant protein was measured from supernatant, using ELISA kit (RayBio®, RB.ELH-EPO-1) in accordance with the manufacturer's instructions.

C-myc clone (CCL1) and C-myc/Xbp1s clone (XCCL1) were cultured by duplicate and characterized in terms of growth, metabolism and production. Both clones showed increases in its maximum growth rate and maximum cell concentration, 30% and 70%, respectively, compared to the CC. However, both, qp and Qp, showed different trends. This shows xbp1s has and additional effect on r-protein productivity. Qp and qp were significantly increased in comparison with CC and CCL1, as was expected.

Acknowledgment

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UPSTREAM PROCESS DEVELOPMENT

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Key Words: Upstream development, *QbD*, continuous processing, process intensification.

Cell culture is an essential part of processes for the production of therapeutic proteins and vaccines, as well as cell and gene therapies. Current trends in quality management guidelines of major regulatory agencies stress the need to develop biopharmaceutical production processes that have quality as an underlying concept. In harmony with this trend, several areas have gained significant attention lately, *e. g.:* the use of the quality-by-design (*QbD*) paradigm, the application of continuous processing, the search for highly productive processes and the use of modeling to understand and design cell culture processes (Gronemeyer *et. al.*, 2014).

The *QbD* paradigm, which uses product and process knowledge together with risk management to ensure product quality, is totally aligned with rational process development (Lee, 2015). It is popular in industry in the area of synthetic drugs, but the extreme complexity of biological processes has slowed down its full implementation in the biopharmaceutical area, with only a few remarkable exceptions, such as in the production of obinutuzumab and atezolizumab (Kelly, 2016). The central concept of QbD is the design of the operational space to establish the limits for key process variables (Figure 1).

Continuous processing has also received a great deal of attention lately and has gathered top scientists from academia and industry in the three editions of the “Integrated Continuous Biomanufacturing” conference celebrated so far. The main drivers for this renewed interest is that continuous processes lead to better product consistency in terms of critical quality attributes (CQA), increase the possibilities of process intensification leading to higher productivity, allow reduced plant footprint and make intensive use of disposable technologies (Castilho, 2014).

Mathematical models using the vast amount of data generated by *omics* techniques are required to improve our capacity to design cell culture processes. For large-scale production, the hydrodynamics of the system plays also a key role. The use of modeling is definitively increasing the capacity of production processes and will continue to do so in the future (Rathore, 2014)

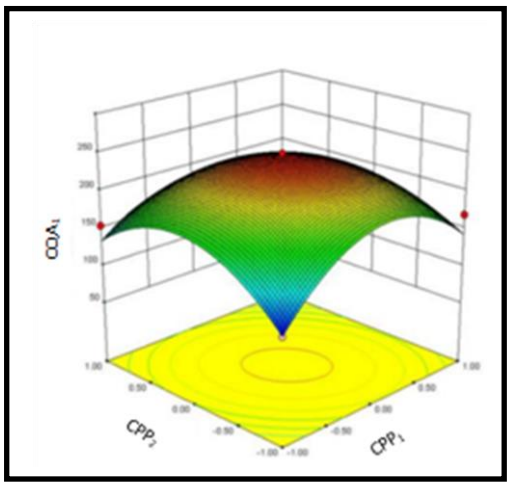

Figure 1 – Design of space, the central concept of QbD

The search for more productive processes, which has always been a goal for the industry, has recently started to be encouraged also by the regulatory agencies. This recognizes that unavailability of drugs due to shortage of product, caused by limited production capacity, is as serious as any other major quality problem (Woodcock, 2014). Among the strategies used to increase upstream process productivity we have: improvement of cell-specific productivity; design of nutritionally deep cell culture media; development of rational bioreactor culture strategies leading to higher cell density and/or integral of viable cell and therefore to higher titers; the use of productivity enhancers and mild hypothermia (Butler and Meneses-Acosta, 2012).

HYDROCYCLONES AS CELL RETENTION DEVICES FOR PERFUSION APPLICATIONS: INVESTIGATION OF VARIABLES RELEVANT TO HYDROCYCLONE ATTACHMENT TO SINGLE-USE BIOREACTOR BAGS

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Key Words: hydrocyclone, perfusion, cell separation, CHO cells, separation efficiency for resin beads

Hydrocyclones (HC) are very compact devices with no moving parts that enable particle separation under the action of a centrifugal field. Despite the small size, HCs have a large processing capacity and enable a long-term operation without clogging issues. The manufacturing of single-use HCs is a promising alternative for the integration of a cell retention device in single-use bioreactor bags developed for perfusion applications.
In the present work, rapid, batch tests performed at laboratory bench were carried out to investigate variables relevant to the attachment of the HC to a single-use bioreactor bag and its operation, such as: (i) particle concentration in the feed suspension; (ii) presence of a dip tube installed in the underflow port; and (iii) use of a peristaltic pump to control the overflow flow rate. The main response considered was their effect on HC separation efficiency. Slurries of Superose® 6 soft chromatography beads were used to mimic highly concentrated CHO cell suspensions, though no information regarding the impact on cell viability would be provided. The stainless-steel HC2015 designed for mammalian cell separation (Deckwer et. al., 2005) was selected for the batch tests, and also used as a benchmark for plastic prototypes produced by 3D-printing techniques.
The Superose® 6 chromatographic beads did not show to be an ideal representative model for CHO cells, since the average diameter (9.86 µm, measured using ViCell XR cell counter) was lower than typical CHO cells, decreasing the terminal settling velocity. The stainless-steel HC2015 operated at 2.3 bar provided a total separation efficiency (E_t) up to 96%, and a centrifugal separation efficiency (E') of 82% for a CHO cell suspension at 24E6 viable cells mL^{-1}. No significant reduction in the viability of cells recovered through the underflow was observed. The use of a peristaltic pump to control the overflow flow rate at values much lower than 720 L d^{-1} (the maximum overflow flow rate normally determined for the HC2015 operated in continuous mode) resulted in a reduction of the E' values and a consequent increase on cell concentration in the overflow. The reduction in the separation efficiency was probably due to a disturbance of the liquid flow pattern inside the hydrocyclone, since it was observed that the typical gas core coming out from the overflow was suppressed. The constriction of the underflow outlet by a dip tube with an internal diameter (I.D.) equal to the hose barb fitting (3/8") increased E_t values by means of increasing the flow ratio (R_f), however lower E' values (and, again, higher particle concentration in the overflow outlet) were obtained. It was determined that the connection of the HC underflow to an upper port of the bioreactor bag would require a short dip tube with a minimum I.D. of 7/8", in order to not interfere with the umbrella-type discharge in the underflow, and thus not affecting the separation efficiency. A 3D-printing hydrocyclone with equivalent geometry to the stainless-steel HC2015 was made and presented comparable separation efficiencies.

DECKWER, W. D., ANSPACH, F. B., MEDRONHO, R. A., LÜBBERSTEDT, M. (2005). "Method for separating viable cells from cell suspensions". Patent No. US 6,878,545 B2.

TAYLOR VORTEX FLOW BIOREACTOR AS AN ALTERNATIVE FOR MESENCHYMAL STROMAL CELL EXPANSION

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Key Words: Mesenchymal Stem Cells. Cell-microcarrier aggregates. Taylor Vortex Flow Bioreactor. Shear stress.

It is expected that the high demand of human mesenchymal stromal cells (hMSCs) for clinical applications can only be met through production stirred culture systems with bioreactors and microcarriers. The bioreactor more used in culture of anchorage dependent cells is a simplified version of the stirred tank known as spinner flask. However, it has shown heterogeneous distribution of hydrodynamic forces with maximum shear stress from 0.3 to 0.4 Pa and difficulty for controlling the cell-microcarrier aggregates size in cultures of MSCs. One of the bioreactors that has been gaining much interest for the culture of MSCs due to low shear stress, satisfactory mass transfer and easy scale-up is the Taylor Vortex Flow Bioreactor (TVFB). The TVFB consists of two concentric cylinders, an inner rotating cylinder and an outer stationary. The most relevant demands for a new bioreactor in the cultivation of MSCs are: to be able to create a low shear environment, to allow the control of the size of aggregates (microcarriers + MSCs) and to preserve the functional characteristics of the CEMs required for clinical applications.

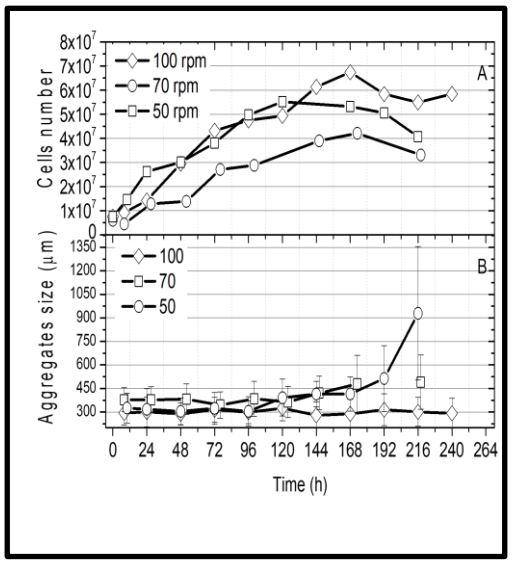

Figure 1 – (A) Growth profile; (B) Microcarrier-cell aggregate size.

Thus, in the present study was carried out the evaluation of expansion of hMSCs attached to macroporous Cultispher®S and MAG microcarriers in Alpha MEM culture medium supplemented with 2, 10 and 15 % (v/v) of fetal bovine serum in a CO_2 incubator at 37°C and pH of 7.1-7.4. The agitation speeds of the inner cylinder were 50, 70, and 100 rpm to controlled aggregates size during culture. In hMSCs cultured at 50 rpm the results were: maximum aggregate size of 929.47±425 µm, expansion factor (EF) of 7.35, maximum specific growth rate (μ_{max}) of 0,01 h^{-1} and maximum production of cells of $5.51x10^7$. The culture performed at 70 rpm showed a maximum aggregate size of 490.63±173 µm, EF of 7.02, μ_{max} and maximum production of cells of 0.017 h^{-1} and $4.21x10^7$, respectively. Finally, the results at 100 rpm were a maximum aggregate size of 324.49±80 µm, maximum productionof cells of $6.12x10^7$ cells with EF of 8.18 and μ_{max} of 0.029 h^{-1}(Figure 1).

EVALUATION OF RECOMBINANT ERYTHROPOIETIN PRODUCTION IN NOVEL HUMAN CELL LINES

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Key Words: Human cell lines; serum-free suspension culture, recombinant proteins, erythropoietin

Human cell lines have attracted great interest because they are capable of producing glycosylated proteins that are more similar to native human proteins, thereby reducing the potential for immune responses. Despite the great potential, some promising human cell lines have not been extensively exploited for recombinant protein production, especially under serum-free suspension conditions. This work aims to evaluate the production of recombinant erythropoietin (EPO) in the human cell lines Huh-7, HKB-11, SK-Hep-1, cultured under serum-free suspension conditions. Recombinant erythropoietin-producing cells were successfully generated by lentiviral transduction (MOI of 1) and then submitted to a sorting process resulting in homogenous cell populations with percentage of GFP positive cells higher than 90%. Firstly two serum-free media were evaluated to select the most suitable for cell growth and EPO production: CDM4CHO (Hyclone) and FreeStyle (Invitrogen). The best results were obtained using CDM4CHO for SK-Hep-1 and HKB-11 cells and FreeStyle for Huh-7 cells. The characterization of cell growth demonstrated that Huh-7, HKB-11, SK-Hep-1 cells presented maximum specific growth rates of 0,053; 0,051 and 0,027 h^{-1} respectively and maximum cell densities of $4{,}4x10^6$; $5{,}7x10^6$ and $2{,}3x10^6$ cell/mL, respectively, in the selected culture media. Higher levels of EPO expression were obtained for Huh-7 cells (maximum of 571 μg/mL) when compared to HKB-11 (maximum of 113 μg/mL) and SK-Hep-1 cells (maximum of 113 μg/mL). The EPO produced by the cells were purified and the analysis of the glycosylation profile is in progress. The growing demand for mammalian-derived recombinant therapeutics proteins is driving research to the development of optimized expression systems aiming at high yields and a high-quality product.

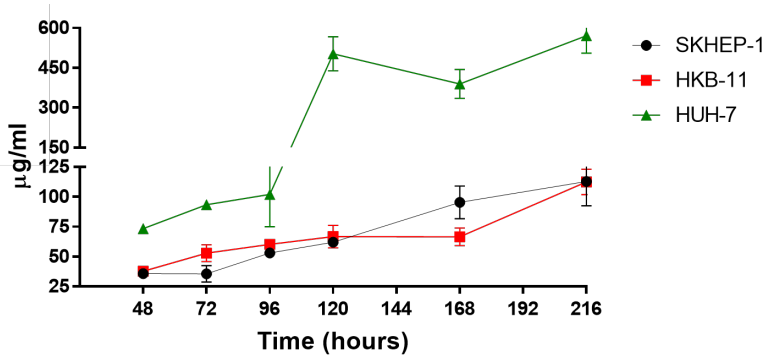

Figure 1. Kinetic of EPO production under serum-free suspension conditions (n=3).

PROCESS INTENSIFICATION IN YELLOW FEVER VIRUS PRODUCTION: A MULTIPLE HARVEST APPROACH

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Key Words: yellow fever virus production, serum-free Vero microcarrier cell culture, process intensification, multiple harvests, 17DD and 17D 204 RKI strains.

The vaccine against yellow fever virus (YFV) produced in embryonated eggs was described in 1937 by Theiler & Smith. Since then, egg-based yellow fever vaccine has been used for immunization of more than hundred million people, with a history of high efficacy and safety. The increase in demand for this vaccine, and the vaccine shortages experienced recently in Angola (2016) and Brazil (2017-2018), demonstrate clearly, the need to develop more advanced and scalable YF production technologies.

Vero is an adherent cell line which is accepted by the World Health Organization (WHO) for manufacturing of human vaccines and is susceptible for flavivirus infection. Industrial processes using this cell line to produce vaccines, e.g. against rabies and polio diseases, rely on microcarriers cultures, ideally under serum-free (SF) conditions.

The aim of this work was the development of a high yield cell culture process in SF media, and the establishment of an efficient infection strategy to produce yellow fever virus. For process intensification, different infection and harvesting strategies were evaluated for both 17DD and 17D-204 strains used in the currently approved live-attenuated YF vaccines.

Therefore, Vero cells (WHO cell bank, ECACC) were cultured on Cytodex 3 microcarriers (2 g L^{-1}) in 250-mL spinner flasks using single and multiple harvest modes (i.e. 1, 2, 3 and 5 harvests) in SF media (Opti MEM, Gibco, USA). Multiple harvests were done with three consecutive steps: microcarriers settlement, supernatant harvest, and addition of fresh media. In both harvest modes, the cell growth and virus production phases were evaluated at 37 and 34 °C, respectively.

The best conditions for cell propagation and viral infection resulted in a maximum cell density of 1.2×10^6 cells mL^{-1} and virus titers exceeded 10^7 PFU mL^{-1}. When multiple harvests (2, 3, and 5 harvests) were carried out, it was verified that the cells kept the ability to propagate YF virus, resulting in a significant increase in total virus particles obtained per batch cell culture.

Overall, the development of a multiple harvest-based mammalian cell platform for propagating the yellow fever virus using the Vero cell line under serum-free conditions is an important step towards the production of a new vaccine using a scalable and cost-effective technology.

Theiler M, Smith HH (1937), The use of yellow fever virus modified by in vitro cultivation for human immunization. J Exp Med. 65:787-800.

PROCESS CHARACTERIZATION STRATEGY – A QbD DRIVEN CONTROL STRATEGY

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Key Words: Process Characterization, Late Phase Development, QbD., Design Space.

Assuring product quality is a concern that can be addressed through the defined control strategy required to fulfill regulatory expectations. Late Phase Development and Process Characterization (PC) under the QbD approach can assist on the definition of the control strategy. The final purpose is to generate a consistent product quality based on improved process understanding and a definition of the Design Space. Different methodologies have been reported for Process Characterization which contain common elements such us risk assessment, scale-down model qualification, and statistical experimental approach. However, there is not a consensus on criteria for the selection of a unique or multiple experimental designs for the evaluation of CPP (Critical Process Parameters) at every stage. In addition, the lack of clarity in objectives and identification of CPP might lead to unnecessary large experimental designs with potentially confounding factors.

With the cumulative experience in the development of biopharmaceuticals it is possible to achieve a platform approach for the definition and execution of PC for the production of a monoclonal antibody. In this work we present a flexible path to evaluate molecule fit in platform approach for PC and alternative routes for different scenarios. The stepwise approach proposed represents and efficient experimental approach, which specifically target the appropriate number of experiments while achieving quality results. It is expected that by clearly identifying objectives and other inputs, an appropriate path can be outlined to assure success in conducting DOEs for process characterization. A DOE toolbox and analytical strategy supports our process characterization approach.

A case study will be presented to exemplify the step- wise platform approach for PC. DOE selection criteria will be discussed along with a sample of statistical analysis, modeling and statistical simulations toward the definition of a comprehensive control strategy.

MONOCYTE-MACROPHAGES AND DENDRITIC CELLS ARE MORE SENSITIVE THAN PBMCS FOR THE DETECTION OF IMMUNE RESPONSE MODULATING IMPURITIES (IIRMIS) IN THERAPEUTIC PROTEINS

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Key Words: Therapeutic Protein Immunogenicity, quality control, IIRMI, primary cultures.

Therapeutic proteins are agents produced through careful and complex multi-step manufacturing methods. However, there are numerous reports showing undesired immune responses in patients during therapy. Several factors may contribute to the product immunogenicity, such as route and frequency of administration, formulation and patient-related factors. In addition, some contaminants derived from different sources (host cell or manufacturing process) may escape the purification strategy and are found in the final product. These contaminants, previously defined as innate immune response modulating impurities (IIRMIs), have the ability to elicit an immune response even when present at trace levels. This response may lead to the development of neutralizing antibodies (NAbs) against the product and an exacerbated production of pro-inflammatory cytokines and chemokines. The use of PBMC has been proposed to assess the potential of impurities that activate the immune cells. Additionally, commercially available human and murine cell lines were also proposed to detect these entities. However, macrophages and dendritic cells, which are rich in pattern recognition receptors (PRR) and are known to initiate immune responses, are present at very low frequency or absent in peripheral blood (PB). To address this, we evaluated the use of monocyte-derived macrophages (mo-MØ) and immature dendritic cells (mo-iDC) as screening tools to detect IIRMIs. Using purified PRRAgonists (PRRAgs) as model IIRMI we show that both primary cultures are more sensitive than PBMC in detecting IIRMI. Interestingly, mo-MØ and mo-iDC showed different limits of detection (LLOD) for individual PRRAgs. In addition PRRAgs induced increased expression of a set of pro-inflammatory genes in mo-iDC and the profile of genes induced varied with the TLR agonist and concentration. Finally, we tested the capability of human PBMC, mo-MØ and mo-iDC for detecting impurities in a commercially available product for the treatment of autoimmune inflammatory diseases. While PBMC samples and a panel of HEK-BLUE cell lines transfected with individual TLRs were not activated by impurities present in the product, mo-MØ and mo-iDC were activated even when low amount of product where added to the cell culture.
In summary, primary monocyte-derived macrophages and dendritic cells are more sensitive than PBMC for the detection of impurities potentially present in protein therapeutic preparations.

3D TUMOR SPHEROIDS: AN *IN VITRO* BLADDER CANCER MODEL FOR DRUG-SCREENING

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Key Words: 3D culture, cancer, spheroids, ultra-low attachment plates, hanging drop

Reducing costs is still the major challenge during compound screening. More than 90% of candidates for a new anti-cancer drug that pass through preclinical phase do not reach the desired efficacy or safety required during clinical trials. The reason is that the *in vivo* (animals) and *in vitro* (2D culture) models usually used in preclinical studies are often inadequate to effectively model tumor biology. In this scenario, three-dimensional (3D) cultures emerged as a potential *in vitro* model for culturing tumor cells by enabling the recovery of conditions found in native tissues such as cell-cell and cell-extracellular matrix interaction that can influence the phenotype and function of individual cells. The present work aimed to develop a simple, reproducible and low-cost 3D bladder tumor model for *in vitro* drug screening. Initially, the generation of 3D spheroids with RT4 bladder cancer cell line was evaluated in 96-well Ultra-Low Attachment plates (Corning), and 96-well hanging drop plates (3D Biomatrix). Throughout culture several parameters were evaluated: morphology (microscopy images); shape parameters (ImageJ); growth profile (cell count using trypan blue); presence of apoptotic cells (Annexin V-FITC) and drug-sensitivity response to anti-cancer drug Doxorubicin (3D CellTiter-Glo®). The results showed that compacted spheroids were generated after 48 hours of culture, in both methods (Fig 1), with a satisfactory regular surface with no salient edges (solidity higher than 0.90) and ellipsoidal and spherical shapes (sphericity index close to 0.90). In both methods we were able to generate spheroids with a diameter between 300 to 500 µm, size that can be considered ideal to use in drug screening assays. The RT4 spheroids were more resistance to Doxorubicin treatment than 2D cultures with IC50 values of 1.00 µg/L and 0.37 µg/L, respectively. Spheroid formation in ULA plates was more robust, reliable and straightforward and was then the method chosen to continue the work using bladder tumor patient-derived cells. We used two previously established PDX-derived cells, BL0293 and BL0808, from advanced bladder cancer fragments. After tumor fragments digestion, cells were cultured in ULA plates with 5% of Matrigel. Both PDX-derived cells formed regular and round-shaped spheroids (roundness>0.8) with a diameter higher than 400 µm. The response of the spheroids to 10 µM of antineoplastic drugs, Cisplatin, Gemcitabine and the combination of both, were evaluated during 3 days. BL0293 spheroids were more resistant to Cisplatin and partial resistant to Gemcitabine while BL0808 spheroids were partial resistant to both. However, both were sensitive when treated with the combination Cisplatin + Gemcitabine. These results were similar to those observed in *in vivo* studies with BL0293 and BL0808. In conclusion, 3D cell culture models showed a higher drug resistance when compared to conventional 2D cultures and may predict the outcome of *in vivo* drug-screening assays. Therefore, it can be used as a low-cost strategy to perform high-throughput screening.

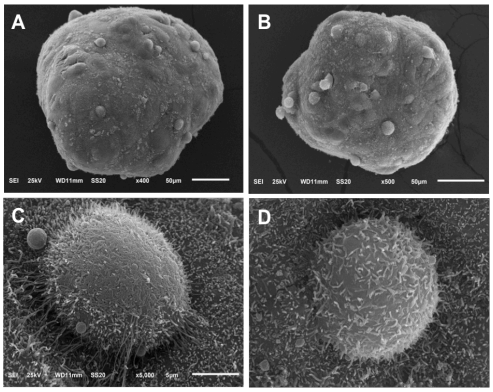

Figure 1. Scanning electronic microscopy of 72-hour RT4 spheroids from ULA (A and C) and HD (B and D). A and B: Scale bar = 50 µm; C and D: Scale bar = 5 µm.

CHO-K1, HEK293 AND NS0 CELL LINES: GLYCOSYLATION IMPACT ON AFFINITY, STABILITY AND NEUTRALIZING ACTIVITY OF A CHIMERIC ANTI-rhIFN-α2b ANTIBODY

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Key Words: antibody constant region, neutralizing activity, affinity constant, glycosylation

The monoclonal antibodies constitute a large subset of the marketed biotherapeutics, most of which are glycosylated, and thus produced in mammalian cells. Complete antibodies are bifunctional molecules, since the variable (V) regions are responsible of antigen binding and the constant (C) regions confer effector properties. However, this immunological dogma is in revision because several studies suggest that C regions of different class or subclasses of antibodies with identical V regions, influence the antigen binding activity (Casadevall, A. and Janda, A. 2012, Janda, A., Bowen, A., et al. 2016) Also, despite the glycosylation pattern strongly influences the antibody effector functions, this feature always was considered not to be important for binding antigen ability (Torres, M., Fernandez-Fuentes, N., et al. 2007). In this work, the impact of the different cell lines on the antigen-antibody affinity constant, the thermal and pH-dependent stability and antigen neutralizing ability of a chimeric anti-rhIFN-α2b murine single chain Fv fused to Fcγ1 (scFv-Fc) was studied. The proteins were produced by CHO-K1, HEK293 and NS0 cells. They showed the same stability degree both in association as no-dissociation condition, but differed when they were submitted to thermal analysis where CHO-K1 showed the lowest resistance to temperature. The also showed no significant differences in the affinity constant measured by competitive ELISA. The scFv-Fc neutralizing ability of IFN-a2b activity was measured by inhibition of GFP expression from Mx-GFP HeLa reporter. The inhibition capacity was higher using the molecule produced by CHO cells despite the three antibodies showed no difference in the antigen binding assay. In fact, the neutralizing activity of the same deglycosylated protein was considerably reduced. The present study invites us to critically discuss the choice of the cell line to produce biotherapeutic antibodies.

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EVALUATION OF MITOCHONDRIAL REDOX POTENTIAL AND THEIR EFFECT IN THE N-GLYCOSYLATION OF HEMAGGLUTININ RECOMBINANT, USING THE BACULOVIRUS EXPRESSION VECTOR SYSTEM (BEVS) AT DIFFERENT DISSOLVED OXYGEN TENSIONS.

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Key Words: Redox potential, roGFP2, rHA, DOT, N-glycosylation

Nowadays, there are several expression systems to produce therapeutic proteins, among them is the baculovirus expression vector system (BEVS). The viral infection with baculovirus induces the accumulation of reactive oxygen species (ROS) in the mitochondria, altering the redox potential of this organelle. Such alteration can cause general damage to the cell and consequently affects the quality and expression of recombinant proteins. The present work focused on the evaluation of the mitochondrial redox potential, at different dissolved oxygen tensions (DOT) and its effect on the production and N-glycosylation of recombinant hemagglutinin (rHA) of the H1N1 influenza virus. To evaluate the mitochondrial redox potential, we used the reduction-oxidation sensitive green fluorescent protein (roGFP2) which is a redox sensitive biosensor. We found that higher concentrations of rHA with less complex glycosylation were obtained with DOT of 20% and 5%, which resulted in a mitochondrial redox potential of -277.6 mV and -279.5 mV respectively. Inversely, lower concentration of rHA with more complex glycosylation were obtained with DOT of 60% and 0%, which resulted in a mitochondrial redox potential of -280.3 mV and -283.3 mV respectively. These data show that the mitochondrial redox potential could be an indicator of the complex glycosylation pattern of the expressed protein.

UNLOCKING THE POTENTIAL FOR DOWNSTREAM EFFICIENCY

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Key Words: continuous chromatography, in-line conditioning, column packing, protein A.

Improvements in productivity and efficiency are ranked as the single most important area on which the biomanufacturing industry should focus its efforts. These improvements are relevant for both existing and new facilities, and agility and flexibility in production are key elements. Novel technologies and innovative process strategies, such as intensified processing, enable more efficient biomanufacturing operations, even for existing facilities. Therefore, we will present the latest advances in the downstream bioprocess, such as continuous and connected chromatography, in-line conditioning of buffers, packing intensification process of industrial columns, binding capacity and alkaline stability advances in chromatographic resins based on protein A ligand.

A CASE STUDY: LEVERAGING GIBCO PRODUCTS TO REDUCE REGULATORY HURDLES AND IMPROVE MANUFACTURING PROCESS EFFICIENCIES FOR BIOSIMILARS.

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As the biosimilar industry becomes more competitive, there are increasing demands for speed to market and operational efficiencies. Industry leaders are faced with many challenges for their company when developing market competitive biologics. In the past, titer played a large role in if a molecule would be economically feasible. As the industry has evolved, titers have increased over 5 fold in the past 20 years and other challenges have evolved. The main challenges when developing a biosimilar is achieving the same product quality attributes as the innovator molecule, and demonstrating control of the manufacturing process. In addition to these, making the process more efficient in order to reduce labor and waste is key to keeping costs down. Efforts have been made to reduce these current challenges by providing GIBCO media and feed formats to reduce the complexity of formulation in the manufacturing process, as well as supplying feeds that target desired glycosylation profiles for a molecule.

CHALLENGES OF BRINGING RECOMBINANT VACCINES TO THE MARKET: A CASE STUDY FOR AN INFLUENZA VACCINE

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Key Words: Recombinant vaccines, influenza, licensure, insect cell-baculovirus expression system technology (BEST).

Influenza is a disease with human and veterinary importance. Its changing genome and the possibility of virus transmission between animal reservoirs and humans make its control highly relevant for global health. The main antigen in influenza vaccines is hemagglutinin (HA), a trimeric glycoprotein embedded in the envelope membrane of the virus that specifically recognizes sialic acids in the surface of target cells and determines tropism. Most vaccines in the market are inactivated subunit vaccines derived from chicken embryos or from animal cell culture. Manufacturing of such vaccines requires handling of live potentially pathogenic viruses, which must adapt to *in vitro* culture. It has been shown that such adaptation can result in variations different to circulating influenza viruses, reducing vaccine efficacy.

An alternative is the use of recombinant vaccines, which have proven to be more effective to protect against influenza than a traditional vaccine (Dunkle et al., 2017). Licensed recombinant influenza vaccines for human and veterinary applications contain recombinant HA (rHA), produced using the BEST. The BEST is especially suitable for the production of influenza vaccines because it is a plug and play technology that allows the fast exchange of the gene of interest to quickly produce the vaccine every season or under pandemic situations. The human version of the vaccines is Flublok®, which is licensed in the USA (Protein Sciences Corporation, a Sanofi Company) and Mexico (Laboratorios Liomont). The veterinary avian counterpart is produced by Boehringer Ingelheim Vetmedica. In this talk, the path for the development, scale up, and licensing of both vaccines in the USA and Mexico will be presented and discussed, with emphasis in the synergy of the academy and the industry in process and product development, characterization and approval. Current challenges will be discussed.

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IMMUNOGENICITY OF THERAPEUTIC PROTEINS

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Key Words: Therapeutic Protein, Immunogenicity, quality control, primary cultures.

Biologics are drugs widely used to treat several diseases. However, in some cases these products can trigger undesired immune responses that compromise the safety and efficacy of the therapy. Numerous factors may contribute to the overall product immunogenicity: dose, route and frequency of administration, contaminants, formulation, patient-related factors, B and T-cell epitopes.
Among the mentioned factors, this talk will deepen in describing current methods for evaluating T-cell mediated immune responses. These strategies usually include an *in silico* analysis using immune-informatic algorithms to predict potential T-cell epitopes within the protein sequence. Then, *in vitro* assays are used to validate the predictions. The most used experimental platforms are based on human and mouse cell primary cultures. Even though human Peripheral Blood Mononuclear Cells (PBMC) constitutes a suitable source of immune cells, other specific cells such as macrophages or dendritic cells may also be included in these protocols to provide a better alternative in terms of antigen presentation.
In addition, the use of animal models for *in vivo* studies will be discussed. Regarding this, H-2 KO transgenic mice expressing human MHC molecules (HLA) have shown more reliable immune responses than wild type mice constituting a suitable alternative for evaluating *in vivo* therapeutic protein immunogenicity.
Finally, this presentation will describe how impurities derived from the manufacturing process that escape the purification procedures enhance the product immunogenicity. These entities were defined in the literature as Innate Immune Response Modulating Impurities (IIRMIs) and may lead to the development of neutralizing antibodies (NAbs) against the product and an exacerbated production of pro-inflammatory cytokines and chemokines.
In conclusion, monitoring potential intrinsic product immunogenicity and contaminants that alone or in a synergistic manner may breakdown patient immune tolerance is essential during the product quality control process. This pre-clinical study provides valuable product safety information and may also accelerate the process of approval of therapeutic proteins.

INDUSTRIAL DOWNSTREAM OPTIMIZATION: A STUDY CASE.

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Key Words: Metal chelate affinity chromatography, ion exchange chromatography glycoproteins, hydrophobic interactions, isoforms

The changes made in the Erythropoietin (rh-EPO) process in search of continuous improvement caused changes in the performance of the purification process. That is why some changes were made to optimize the purification process in the intermediate purification stage. Immobilized metal-ion affinity chromatography (IMAC) was used with the purpose to remove impurities such as HCP, nucleic acids, endotoxins and viruses. During EPO purification in this chromatographic step, it was observed in the elution the presence of hydrophobic contaminants that affected significantly the purity and recovery. Considering that the ionic strength used in the equilibrium step could favoring unspecific adsorption of contaminants by hydrophobic interactions were performed different experiments decreasing the ionic strength in the equilibrium and adding a non-ionic detergent to avoid this type of interaction and to maintain protein integrity and prevent their aggregation. The rh-EPO was obtained with more than 98% of purity by RP-HPLC and a yield of 90%. The removal of contaminants was evaluated and the impact of the change in the polishing stage obtaining a molecule that fulfilled all the established quality specifications. As part of the optimization in the intermediate purification stage was studied the ionic interaction chromatography to isolate and purify the most acidic isoforms of rh-EPO. The cutting of isoforms taking place in the purification process of the Erythropoietin (rh-EPO) allows obtained consistently the presence of six major acid isoforms and 2 minorities in the range of pH between 3.5-5.5. These isoforms allows a high biological activity (> 80 000 IU/mL) and a sialic acid content higher 10 mol sialic acid/mol of protein. In search of a molecule with of isoforms pattern enriched in the acidic isoforms with more than 100 000 UI/ml of biological activity, we decided to study new operating conditions in this chromatographic step that allowed achieve this goal. From the studies undertaken was possible to obtain a profile of the most acidic isoforms of rh-EPO, similar to the biological reference product. The molecule obtained was compared with the biological reference product, observing that it is possible to obtain a biosimilar molecule.

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KYTE-DOOLITTLE HYDROPHILICITY PLOT AS A TOOL TO PREDICT THE POTENTIAL OF AQUEOUS BIPHASIC SYSTEMS TO PURIFY RECOMBINANT PROTEINS FROM *E. COLI*

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Key Words: aqueous biphasic systems, protein purification, fluorescent proteins, Kyte-Doolittle hydrophilicity plot.

The market of biological products is one of the fastest growing industrial segments. However, the high costs of production and purification of biomolecules still prevent this sector of reaching its full potential. Much of the difficulties of purifying recombinant bioproducts is the lack of tools to predict the capacity of the purification techniques. Hence, the goal of this study was to develop a method to predict the potential of a purification technique (specifically, Aqueous Biphasic Systems - ABS) to purify different recombinant proteins from *Escherichia coli (E. coli)*, using the Mean Relative Hydrophobicity in Kyte-Doolite scale (MRH). Polymer/salt ABS with different relative hydrophobicities were selected to evaluate the impact of the MRH in the partition of recombinant proteins. For this study, three recombinant Fluorescent Proteins (FP) were selected (Enhanced Green Fluorescent Protein, GFP, produced by *E. coli* BL21(DE3); Fezziwig Yellow Fluorescent Protein, YFP, and Red Fluorescent Protein, RFP, expressed by *E. coli* TOP10). 3D fluorescence spectroscopy was used to evaluate the purification (high FP/Total protein (TP) ratios (GFP/TP, YFP/TP and RFP/TP) correspond to high purity levels). Previously, Ishihama *et al.* calculated the MRH for the most abundant intracellular proteins from *E. coli* (-0.25, where lower values indicating higher hydrophilicity). In this work, the MRH for GFP (-0.467), YFP (-0.293) and RFP (-0.743) was determined, and the subsequent extraction from cell lysate using ABS carried out. It was observed that the FP with highest difference between MRH and the most abundant proteins in *E. coli* were the most purified (high FP/TP relation) using the ABS under study. In addition, it was also observed that polymer/salt ABS with high water ratio between coexisting phases are the most effective for the purification of GFP. Although more studies are still required for the application of this tool, our results suggest that Kyte-Doolittle hydrophilicity plot can be a useful parameter to predict the partition behavior of recombinant proteins using ABS. Acknowledgments: FAPESP, CAPES and CNPq.

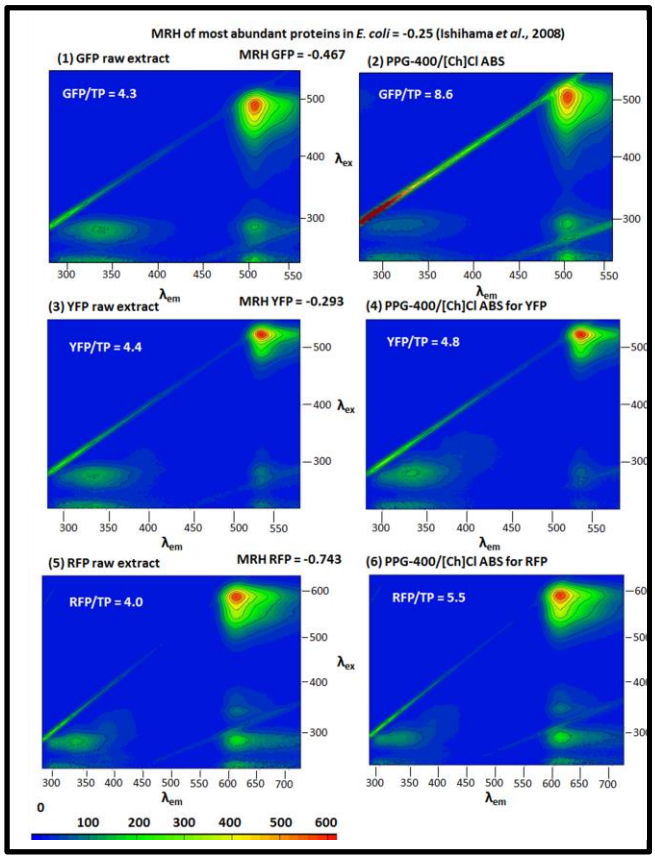

Figure 1 – MRH of the FP (GFP, YFP and RPF), and 3D fluorescence spectra with respective FP/TP ratio before and after purification using ABS.

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DEVELOPMENT OF AN ADVANCED PURIFICATION STEP FOR RECOMBINANT FACTOR IX

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Key Words: Protein purification, recombinant factor IX, liquid chromatography, multimodal chromatography, cation exchange chromatography.

Factor IX (FIX) is one of the blood factor bloods that take part of the blood coagulation system. The deficiency of this protein causes hemophilia B, and the usual treatment is a replacement therapy administering plasma-derived or recombinant FIX concentrates. Plasma-derived FIX (pdFIX) can pose a potential risk of transmission of pathogens, and its production depends on the availability of plasma from human donors as raw material. Therefore, in several countries of the world the trend is the preferential use of recombinant FIX (rFIX). In order to establish efficient and affordable rFIX manufacturing technologies, both high-titer cell culture processes and efficient purification processes must be developed. Regarding product purification, liquid chromatography techniques are most commonly employed, and several sequential steps are usually required to remove impurities, such as host-cell proteins (HCP) and DNA, down to the levels established by the regulatory authorities.

In the present work, we investigated a third purification step for recombinant FIX (rFIX), using different liquid chromatography strategies and techniques, based on a cation exchange membrane adsorber (Sartobind S75, Sartorius, Germany) and on a multimodal resin (Capto^{TM} Adhere, GE, Sweden). The first step (anion-exchange chromatography - AEX) and the second purification step (hydrophobic interaction chromatography - HIC) were carried out as previously developed in our group [1,2].

The studies with the CEX membrane adsorber were carried out in flow-through mode, for adsorption of the impurities. Dilutions (2, 4 and 8-fold) of the eluate of the second purification step (HIC) to yield different conductivities, and the pH was kept at 7.2. As expected, low or no adsorption of impurities was obtained for the highest conductivity (2-fold dilution). Thus, 4 and 8-fold dilution were run again at pH 7.2 and 6.2. The best recovery (87.7%) was obtained at pH 6.2 with 8-fold dilution, resulting in a 1.4-fold purification factor, both based on rFIX biological activity. The second strategy evaluated used the multimodal resin, which had shown in previous experiments a performance better than other resins, such as Capto MMC and phenylboronate. According to adsorption studies, a lower concentration of $(NH_4)_2SO_4$ (135 mM) provided higher recovery. Based on that, elution studies were carried out using this concentration during the conditioning and wash. Using 15% of elution buffer (1.5 M NaCl + 40% ethylene glycol) for desorbing low-binding molecules and 50% of it for rFIX elution, a 2.3-fold purification factor and a complete recovery were achieved.

Based on these results, further optimization of the whole 3-step process, using Capto Adhere as the advanced purification step, are under way, aiming to meet the regulatory requirements for critical contaminants.

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DOWNSTREAM PROCESS OF LIGANI PROTEIN, A VACCINE CANDIDATE FOR HUMAN AND ANIMAL LEPSTOSPIROSIS

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Key words: recombinant, leptospirosis, downstream, Ligs, protein purification

Leptospirosis is one of the most common zoonosis in the world according to Brazilian Ministry of Health, the number of leptospirosis cases have been increasing over the years. In Brazil, 40,407 cases have been reported in the last 10 years (until July of this year), and 3,453 have died. Our group has been working with Leptospiral immunoglobulin-like proteins as diagnostic and subunit vaccine candidate and in 2007, we demonstrated the potential of the non-identical LigA region (LigANI) as a vaccine candidate, as it was able to protect 67-100% of the animals against lethal challenge (Silva et al., 2007). Thus, the aim of this work was to establish the methodologies to obtain the recombinant LigANI protein with a high degree of purity and homogeneity. Considering that these informations are essential for the development of a product with biotherapeutic application (Jozala et al., 2016), initially we performed the scale up from 80 mL bioreactor to 2 liters of culture obtaining 1.06 g of LigANI per liter. Then, the biomass was disrupted in a homogeneizer, the lysate was centrifuged and the supernatant was recovered, filtered and purified using three chromatographic steps: immobilized metal affinity chromatography (IMAC), gel filtration (GF) and ion exchange (IEX). The total yield of the process was 219.52 mg of LigANI per liter of culture with a purity of 99.70 ± 0.24%. Stability data showed that the protein remained stable when stored at -80°C and lyophilized for 120 days. In summary, we demonstrated the technical feasibility of LigANI protein as a candidate in the development of a vaccine against human and veterinary Leptospirosis.

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BIOLOGICS IN BRAZIL: OVERVIEW AND REGULATORY PERSPECTIVES

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Key Words: Anvisa, biological and biosimilar products.

In 2010 Brazilian Health Regulatory Agency - Anvisa published a new regulation for biological and biosimilar products – RDC 55/2010. According this regulation there are two classes of biotherapeutic products: new biological products and biological products. New biological products are defined as innovative biological drugs, and biological products are the copy biological drugs or non-innovative biological products. The approval of new biological products follows the classical regulatory pathway and is based on a full registration dossier including a complete physical-chemical and biological characterization and non-clinical and clinical data. Otherwise, biological products can be licensed by the comparative regulatory pathway (biosimilar approach) or by the stand-alone approach. The comparative regulatory pathway requires a head-to-head comparison at the levels of quality, safety and efficacy to demonstrate that the product and the comparator biological (reference biological product) have similar profiles, proving that there are no significant differences between them. The comparative analysis comprises quality, non-clinical and reduced clinical data, and may allow extrapolation to other indications. A product licensed by the comparative pathway is called biosimilar. In the stand-alone approach the applicant needs to present complete data regarding quality issues but these data do not have to be comparative. For the license by the stand-alone pathway, the comparative Phase III study with the innovator biological product is mandatory. Extrapolation of indications is not accepted in the stand-alone approach. Brazilian regulation for biological and biosimilar products is aligned with WHO guidelines. Regardless of the regulatory pathway chosen to license a biological product in Brazil, RDC 55/2010 requires proof of quality, safety and efficacy of all products. At the end of 2017, Anvisa has launched some regulations to speed up the analyses and to make it easier for the biological product to reach the Brazilian population. Since 2015, Anvisa is a Regulatory Member of ICH, and its highest priority for the coming years is to improve the harmonization of the Regulation with The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

RELEVANCE OF STABILITY STUDIES ON DEMONSTRATING BIOSIMILARITY

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Key Words: forced degradation studies, biosimilars, comparability exercise.

Forced degradation studies are useful to predict biopharmaceuticals stability and provide information about analytical methods ability to detect degradation products. In addition, they play a key role in establishing biosimilarity. Typical stress tests include degradation factors such as heat, hydrolytic, oxidative, and agitation.

The aim of this work was to compare a set of analytical methods for detecting instability of erythropoietin and etanercept during stress conditions and to use the most sensitive ones to compare degradation profile of AmegaBiotech products with those of reference products. Harsh storage conditions such as low and high pH, temperature, oxidation, freeze-thaw cycles and agitation were evaluated. Samples were analyzed in order to evaluate purity, impurities, post-translational modifications, higher order structure and biological activity.

The results demonstrated that a number of analytical techniques were able to detect and characterize various alterations in structure, changes in physical stability, binding to a specific target, and biological activity of the studied proteins. Then, biosimilar and reference products were subjected to stress conditions and differences in the relative abundance of degradation products were studied. Overall, the changes that occurred under stress conditions and the degree at which they occurred were similar between AmegaBiotech and reference products.

Stress stability studies are expected to be included in a comparability exercise. It has been have demonstrated that AmegaBiotech products have degradation profiles highly similar to the ones showed by the reference products.

EXPRESSION, PURIFICATION AND CHARACTERIZATION OF AN ANTI-PCSK9 BIOSIMILAR MONOCLONAL ANTIBODY

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Key Words: anti-PCSK9 humanized monoclonal antibody, expression in CHO cells, biosimilars, IRES-mediated tricistronic vectors, critical quality attributes

Monoclonal antibodies (mAbs) represent the most important class of biopharmaceuticals. These complex recombinant proteins are indicated for the treatment of high-incidence diseases, such as several cancers and chronic disorders, and account for about half of the annual sales of the biopharmaceutical market. The growing demand for mAbs and the development of biosimilars are driving a trend to achieve more efficient, lower-cost production processes and to globalize biopharmaceuticals manufacturing.
Since 2015, a new class of mAbs was approved by FDA and EMA. These mAbs target the enzyme PCSK9, involved in the regulation of low-density lipoprotein (LDL) cholesterol homeostasis, and significantly reduce the circulating LDL cholesterol levels. Therefore, anti-PCSK9 mAbs are an efficient treatment for a quite large amount of patients who present adverse effects to the traditional statins (small lipid-lowering synthetic drugs) or who present the genetic disorder familial hypercholesterolemia.
In this study, we selected signal peptides, selection markers and IRES elements from the literature to construct different IRES-mediated tricistronic vectors to express an anti-PCSK9 biosimilar mAb. A total of 4 vectors were constructed and validated by traditional molecular biology approaches presenting the following cassette structure: CMV-IE promoter, signal peptide (SP1 or SP2), light chain gene (LC), EMCV IRES, signal peptide (SP3), heavy chain gene (HC), attenuated IRES, selection marker gene (neo or hygro). Several restriction enzyme sites were included to allow the manipulation of each gene individually, in order to have a flexible expression platform for a wide range of mAbs. CHO-K1 cells (ATCC, USA) previously adapted to suspension culture in TC-LECC medium (Xell AG, Germany) were transfected (or co-transfected with plasmids encoding two different selection markers) with Lipofectamine 3000 (Gibco, USA), using either circular or linearized plasmids.
Supernatants harvested 48h post-transfection showed a similar expression level for all constructs. After 2 months under selection pressure with the respective antibiotics, the stable cell pool transfected with single circular SP1-LC-SP3-HC-NEO plasmid was chosen for further studies. The growth, metabolism and mAb production profile of this stable pool was investigated. mAb production showed to be growth-associated. Batch cultures in shake flasks attained 13.9E6 cells/mL on day 7 and showed an specific growth rate of 1.2 d^{-1} during the exponential growth phase. The mAb purified from cell culture supernatant using protein A affinity chromatography showed high purity and homogeneity by SDS-PAGE and Western blot. Further critical quality attributes were evaluated such as: (1) affinity of the purified mAb to PCSK9; (2) mAb homogeneity, aggregates, monomers and fragments content; (3) mAb N-glycan profile; (4) primary sequence. The results confirmed the expected structure, highlighting the success of the proposed platform for stable mAb expression. It can be a useful tool for fast production of different mAbs and can provide a high-quality product for characterization, formulation and pre-clinical studies. FACS experiments to enrich cell pools and to obtain clonal high-producer cell lines, as well as comparability assays using the reference product Repatha™ (evolocumab, Amgen, USA) are currently under way.

ECONOMIC OPPORTUNITY ANALYSIS OF THE DEVELOPMENT AND MANUFACTURING OF AN EVOLOCUMAB BIOSIMILAR IN BRAZIL

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Key Words: Technical and economic evaluation, anti-PCSK9 monoclonal antibody (mAb), evolocumab, biosimilars.

In this work, we present a technical/economic evaluation of the production of a biosimilar of evolocumab, aiming analyzing the feasibility of the construction of an industrial plant with the capacity for producing the drug in sufficient quantity to meet the Brazilian demand in 2030, considering that the patent of the innovator product expires in 2029.

Evolocumab is a recombinant human monoclonal antibody, which has therapeutic indications for patients presenting high-cholesterol levels that suffer from adverse effects of statins, as well as for homo- and heterozygous familial hypercholesterolemia patients, which is a genetic disorder causing high cholesterol levels, which can lead to cardiovascular accidents and very early death.

Based on the incidence and prevalence of the therapeutic indications of this mAb in the Brazilian population, on government purchases of medicines for hypercholesterolemia and on the Brazilian population growth rate, the annual demand was calculated to be 60.5 kg/year by 2030. A plant was then designed to produce the biosimilar, considering the estimated demand. The manufacturing process was defined according to platform technologies reported in literature for mAbs combined with in-house experience regarding the upstream and downstream processes, as well as considering the biopharmaceutical manufacturing trends anticipated for the coming years.

After the process was defined it was possible to establish mass balances and raw material demands for the manufacturing process, which allowed the calculation of the costs of raw materials. The costs of the main equipment needed for the plant was obtained from literature (and updated using established cost indices) or by direct quotation with companies. In this way, a total capital investment, which included also the anticipated costs for biosimilar clinical development, was determined to be approximately USD 84 million. Unit production costs were calculated to be USD 0.223 per mg of biosimilar evolocumab.

The cash flow for a plant lifetime of 10 years was calculated, showing that even considering a sales price lower than the lowest price found in the market the project showed an internal rate of return (IRR) of 14 % per year and a net present value of $7.3 million at a 12.3 % per year minimum acceptable rate of return.

Variations to the base case originally considered were also evaluated, providing sensitivity analyses regarding important variables and assumptions. The feasibility of the project was confirmed even considering adverse scenarios.

INNOVATION IN ONCOLOGY

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Key Words: Innovation, Oncology, Immunotherapy, Monoclonal Antibodies

Recepta is a Brazilian biotech company dedicated to research and (clinical and non clinical) development of new biological drugs for the treatment of cancer. Currently Recepta´s focus is on the clinical development of new immunomodulating antibodies. Ongoing clinical trials with aPD1 and aCTLA4 are showing very promising results. Recepta was the first Brazilian company to ever internationally licensing the IP of drug for the treatment of cancer. The NaPi2b monoclonal antibody now in clinical development as an ADC XMT1536 by Mersana Therapeutics. Recepta´s pipeline include peptides with immunomodulatory properties.

BIOSIMILARS IN BRAZIL AND THE VISION OF BIONOVIS

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Brazil is one of the largest single purchasers of highly complex biologics in the world. Despite being an important consumer, there were no local manufacturing of biopharmaceutical API in the country until very recently, when companies such as Eurofarma and Libbs have started their own efforts to produce filgrastim and rituximab. Bionovis, Orygen and Biomm are other examples of which are helping to include Brazil in the world map of biologics. The importance of biosimilars in the world and in Brazil emerge as a clear opportunity that can help to increase access to this very expensive medicines and, in the particular Brazilian case, also an opportunity to build up in the country state-of-the-art production facilities, train highly qualified human resources and master the entire technology to manufacture locally some of the most advanced therapeutic proteins, including diverse monoclonal antibodies. The governmental PDP – Partnerships for Productive Development – politics put together state owned institutions and private Brazilian and multinational companies in a joint effort to transfer, absorb and implement this technology in Brazil. Bionovis is fully engaged in this effort together with Bio-Manguinhos/FIOCRUZ, to receive technologies to manufacture not only biosimilars, but also originator products, from partners like Janssen, Merck, Samsung Bioepis and Fresenius Kabi. Developing PDP programs is part of Bionovis' strategy, which includes developing its own biosimilars and innovative biopharmaceuticals, having Brazil as a hub to fulfil not only Latin American demands but also to export to Europe, USA and Asia.

CELL CULTURE TECHNOLOGY IN TWO BRAZILIAN PUBLIC LABORATORIES FOR THE PRODUCTION OF IMMUNOBIOLOGICALS

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Key Words: Cell culture; Vaccine; Biologicals

Instituto Butantan and Bio-Manguinhos / Fiocruz are important public Brazilian laboratories dedicated to research, development and production of Immunobiologicals. The main and historical products of these laboratories comprise vaccines and hyperimmune serum. However, both have recently entered in product development partnerships with private industry or have started new projects targeting the biological drug market. This lecture will focus on giving a general view of the initiatives of these laboratories comprising the current utilization of animal cells in the institutions. The speakers will share non-confidential information on the ongoing research and development projects giving examples of products or services obtained through cell culture technology and comment on the new perspectives. The provided information will permit to know the status of cell culture technology used or considered to be used and the relative importance of this subject in the two laboratories.

IMPORTANCE OF VERSATILE MEDIA AND FEEDS FOR BIOLOGICS PRODUCTION IN rCHO CELLS

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Key Words: CHO cells, Chemically defined media and feeds, Complex feeds and supplements, hydrolysates, Biologics.

Chinese Hamster Ovary (CHO) cells are the preferred host for recombinant protein production. Biopharmaceutical companies routinely utilize supplements and feed systems for maximizing the yield of recombinant proteins in bioprocesses involving CHO cells. Although high productivity is desirable for monoclonal antibodies (mAbs) production, utilizing supplements and feeds might affect the quality of recombinant proteins. Our work compares the productivity and quality of recombinant proteins produced in CHO-cell based fed-batch processes using our chemically defined (CD) media - AmpliCHO CD medium and MAM-PF®77 CD medium, CD feed - AmpliCHO CD feed and a variety of complex supplements and feed systems - Sheff-CHO supplements and Sheff-Pulse feeds.

The CD media, feeds and supplements were tested in three CHO cell lines, each expressing a different monoclonal antibody. Each cell line was sequentially adapted to the CD medium being evaluated. Multiple fed-batch experiments were performed in shake-flasks with combinations of different media, supplements and feeds. At regular intervals, samples were assessed for viable cell density, cell viability, nutrient metabolism and IgG titers for the different conditions. Glycosylation patterns were also evaluated for the samples obtained at the end of the fed-batch process. The medium/feed combinations which demonstrated high protein productivity and high cell viability at the end of the culture process were further tested in bioreactors to evaluate scalability of the medium/feed combinations in the different cell lines.

The use of Kerry CD medium with either CD feed or complex feed supplements resulted in comparable cell growth and viability at the end of the fed-batch process in addition to similar IgG titers across CHO cell lines utilizing both dihydrofolate reductase (DG44) and glutamine synthetase (CHO-GS) selection. In terms of the product quality, the various CD medium, supplement and feed combinations tested resulted in desirable glycosylation patterns for the respective IgG molecules. These results demonstrate the versatility of AmpliCHO CD medium and MAM-PF®77 CD medium, AmpliCHO CD feed and CHO U-feed mix, Sheff-CHO supplements and Sheff-Pulse feeds.

NEW TECHNOLOGIES OF UPSTREAM BIOREACTOR FOR INDUSTRIAL SCALE USING ADHERENT CELLS

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Key Words: Bioreactor; Adherent Cells; Upstream; Viral Vaccine; Gene Therapy

Today, the vast majority of human and veterinary vaccines are produced using anchorage dependent cell lines (ADCL): primary cells such as chicken embryo fibroblasts (CEF), human diploid cells such as MRC-5 or continuous cell lines such as Vero and MDCK1. Traditionally ADCL have been cultivated in non-controlled, labor-intensive and space-demanding multi-tray stacks (MT) or roller bottles (RB). Microcarrier technologies are one alternative allowing drastic reduction of footprint and labor requirements together with potential increase in productivity using a controlled environment in bioreactor. However, the expertise required to transfer a process from 2D supports to microcarriers and the time necessary to develop a process from scratch can be a strong barrier for some companies. An alternative to 2D-supports and microcarriers is the fixed-bed (FB) technology. Although the principles of fixed-bed have been known and used for years in cell culture, its adoption has been quite limited due to intrinsic limitations: difficulties of fixed-bed packing and bioreactor preparation, potential cells or nutrient gradients in the fixed-bed, aeration limitation and difficulties during scale-up of a process.

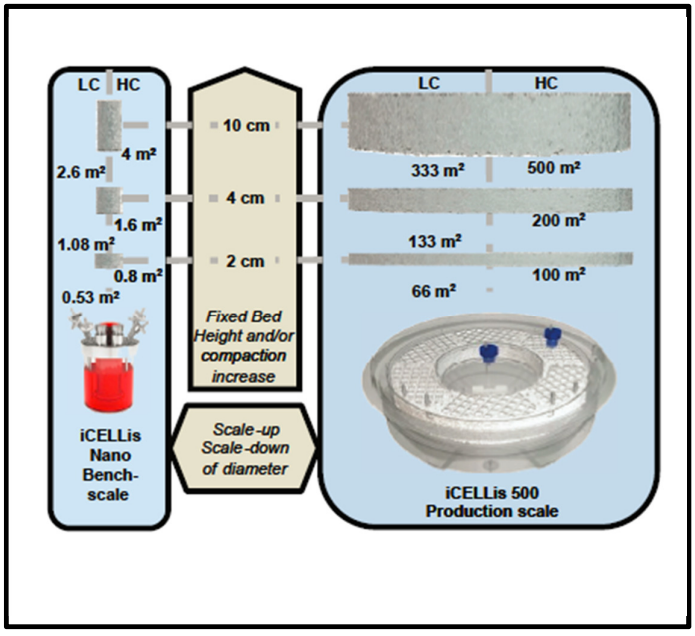

Figure 1 – iCELLis bioreactor bench (Nano) and manufacturing (500) scales and their modular size fixed bed. LC = Low compaction; HC = High compaction.

The iCELLis technology is an efficient and cost-effective technology that is proving his worth in biotechnology field, as reflected by increasing number of users and published data. The iCELLis® platform, a scalable line of single-use bioreactors, is specially designed for adherent cell culture applications (e.g. recombinant protein and viral vaccine production). Fixed-bed bioreactors allow mammalian cells cultivation at high cell density with low shear stress. Central to the iCELLis® bioreactor is the use of a compact fixed-bed pre-packed with medical grade proprietary microcarriers (polyester microfibers) providing a large growth surface area in a compact bioreactor volume.

Reference: Pall Biotech; Application Note - Bringing Fast Process Development to Adherent Cell Culture Processes: Demonstration of Linear Scalability from 1.6 to 200 m2 in Single-Use Fixed Bed Bioreactor

NEW DIRECTIONS IN 3D CELL CULTURE – NOVEL TECHNOLOGIES AND APPLICATIONS

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Corning, Inc.

Key Words: Three-dimensional cell culture, novel applications, 3D growth.

Three-dimensional (3D) cell culture systems have gained increasing interest in recent years due to their advantages in providing more *physiologically relevant information and more predictive data* compared to convention two-dimensional (2D) cell culture. For specialized cell types such as primary cells and stem cells, a two-dimensional (2D) growth substrate may not be sufficient to support complex cellular behaviors such as cell polarity, morphology, spheroid formation, signal transduction, and tissue-specific gene expression. This seminar and discussion will highlight novel applications that rely on the use of 3D growth substrates and specialized culture systems and the benefits they can provide from basic research to advanced drug screens.

THE CANCER IMMUNOTHERAPY REVOLUTION

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Key Words: Cancer, Immunotherapy, Chimeric Antigen Receptors (CARs),

The recent years have witnessed a revolution in cancer treatment by the introduction of the new therapeutic modalities based on monoclonal antibodies and gene-modified cells designed to promote immune responses to tumors.
The use of Immune Checkpoint Blockade (ICB) antibodies has led to impressive results in some tumors. This new therapeutic modality has now been extensively incorporated in the anti-tumor arsenal although we are just starting to understand the overall mechanisms underlying the immune regulation promoted by these new drugs. Furthermore, the capacity to predict the patients responding to this new therapeutic modality is still in its early stages.
Along with the ICBs, a second immunotherapeutic approach has established new paradigms for the cell-based immunotherapies. This revolution is based on the transgenic expression of artificial receptors in T lymphocytes, such as the Chimeric Antigen Receptors (CARs). This strategy has been recently approved for commercialization in the US and Europe with CARs specific for the B cell antigen CD19.
Several CAR designs and target molecules are being tested by groups around the world. Innovative T cell expansion protocols are being developed and gene delivery tools being used to transfer transgenes. At INCA we have been working on non-viral gene delivery systems and on alternative protocols to simplify the laboratory-based strategies for the generation of CAR expressing T cells.

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Optimization of bioprocesses for VIrus Like Particle production

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Key Words: HIV-1 VLPs, bioreactor perfusion, additives, shRNA, characterization, HEK293.

Gag polyprotein from HIV-1 can generate Virus-Like Particles (VLPs) when recombinantly expressed in animal cell platforms. HIV-1 VLP production in HEK293 cells can be improved using different strategies for increasing product titers. One of them is the so-called Extended Gene Expression (EGE), based on repeated medium exchanges and retransfections of the cell culture to prolong the production phase. Another approach to improve transient transfection results is media supplementation with gene expression enhancers such as valproic acid and caffeine, despite their detrimental effect on cell viability. Valproic acid is a histone deacetylase inhibitor while caffeine has a phosphodiesterase inhibition effect. This work has three main objectives. First, the combination of the EGE protocol with valproic acid and caffeine supplementation to maximize VLP production; second, the replacement of these chemical additives by shRNA for obtaining the same inhibition action and third the bioreactor scale-up of the process.

The combination of the EGE protocol with caffeine and valproic acid supplementation resulted in a 1.5-fold improvement in HIV-1 VLP production compared with the EGE protocol alone, which is an 18-fold improvement over a conventional batch cultivation. shRNAs encoded in the expression vector were tested to substitute valproic acid and caffeine. This novel strategy enhanced VLP production by 2.5-fold without any detrimental effect on cell viability, which results in obtaining higher quality VLPs. Finally, the combination of shRNA with EGE resulted in more than 14-fold improvement compared with the batch standard protocol traditionally used. This protocol enables the production of high-quality HIV-1 VLPs avoiding toxic effects of the additives but maintaining high product titers. When EGE process was performed at bioreactor scale, cell viability was comparable through all processes in the two systems tested (bioreactor and shake flasks as control); however, the bioreactor allowed for much higher cell densities and specific growth rates. Transfection efficiency was also comparable and successfully achieved in both systems. GagGFP fluorescence quantification revealed similar VLP titres in both shake flasks and bioreactor. A product quality assessment was also carried out to evaluate the two systems. Presence of VLPs in all samples was confirmed by transmission electron microscopy. Nanoparticle tracking analysis showed that the ratio of VLPs/total particles (VLPs and cell vesicles) was higher in the shake flask than in the bioreactor, possibly due to higher cell densities achieved in the bioreactor. Similarly, host cell DNA and host cell protein analyses revealed higher impurity concentrations for the bioreactor compared with shake flasks. Finally, observations on the budding process of VLP using super resolution confocal microscopy will also be presented.

UPSTREAM PROCESS DEVELOPMENT FOR THE PRODUCTION OF ZIKA AND YELLOW FEVER VIRUS-LIKE PARTICLES (VLPs)

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Key Words: virus-like particles (VLPs), yellow fever virus, zika virus, upstream process development, perfusion processes

The emergence of recent outbreaks caused by zika and yellow fever (YF) viruses have shown the importance of developing effective strategies to prevent the transmission of these pathogens and to diagnose them. Zika virus has spread to more than 60 countries since 2015, causing Guillain-Barré syndrome in adults and serious congenital malformations in fetuses from infected mothers. Even though several vaccine candidates are under development, there are no approved vaccines in the market so far, and zika-specific serological diagnostics are still a challenge.

In the case of yellow fever, the infection can lead to acute viral haemorrhagic disease, with very high mortality rates. The existing egg-based live-attenuated commercial vaccines have shown to be very efficacious, but their production process is limited in terms of scalability and ability to respond to outbreaks. Furthermore, due to the live-attenuated virus, it can cause rare, but fatal adverse effects. After recent major YF outbreaks in Africa (2016) and Brazil (2017-2018), the need for a new non-egg derived YFV vaccine has become paramount, especially considering the risk of YFV spreading to highly populated areas with no vaccination coverage, where the mosquito vector is present, such as Asia.

Therefore, in this work virus-like particles (VLPs) have been studied as a possible platform for the development of such zika and yellow fever vaccines, since VLPs mimick the organization and conformation of native viruses and are considered safe, because they don't contain the viral genome.

With the aim of developing a cost-effective technology to produce ZIKV and YFV vaccines, we focused on generating stable cell lines that constitutively express zika and YF VLPs. Different gene constructs, containing different signal peptides and genes coding for wild-type, mutated or chimeric variants of the structural proteins prM (pre-membrane) and E (envelope), were designed. The most promising transiently transfected HEK293-3F6 cell cultures were put under selection pressure to generate stable cell pools and then used to investigate pseudoperfusion processes in spin-tubes and perfusion processes in stirred-tank bioreactors, using a chemically-defined, animal-component free medium (HEK TF, Xell AG, Germany). Besides evaluating the use of high-cell-density perfusion processes as a platform to produce the VLPs and investigating different cell retention devices, chemical additives have been studied as another strategy to enhance productivity.

Results have confirmed that higher product concentrations are obtained in pseudoperfusion and perfusion processes in comparison to batch mode, which was made possible by the stability of the cell pools for over 16 weeks after transfection, even in the absence of selection markers.

The use of FACS to sort for high producer cells allowed obtaining enriched cell pools producing significantly higher amounts of VLPs and confirmed the hypothesis that secreted VLPs can be transiently detected on the cell membrane surface.

Qualitative analysis of the secreted VLPs were done by transmission electron microscopy (TEM) and dynamic light scattering (DLS) after a two-step chromatography purification process, showing that the produced VLPs resemble the respective native viruses in both shape and size.

GLYCOSYLATION PATTERN ANALYSIS OF THE RABIES VIRUS GLYCOPROTEIN ECTODOMAIN PRODUCED IN *DROSOPHILA MELANOGASTER* S2

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Key Words: Glycosylation, Rabies Virus Glycoprotein, *Drosophila melanogaster* S2.

Rabies is a viral zoonoses which produces a fatal form of encephalomyelitis, affecting both humans and domestic and wild animals, with death occurring within the first seven days of the disease. The rabies virus envelope is composed of only one transmembrane glycoprotein (RVGP – rabies virus glycoprotein) that is responsible for the infection in host cells. The main vaccines against rabies are based on inactivated virus obtained from cell culture, but its production includes steps with high risk due to the handle of high amount of infectious material, which demands a high level of biosafety. Thus, is important to develop more efficient and cheaper vaccines that minimize these problems, and also, develop immunoglobulin and antigens for composition of diagnostic kits, or for use in controlling vaccine production steps. The use of the rRVGP (recombinant RVGP) produced in animal cells has become an attractive alternative for this purpose.

In the production of recombinant proteins, the choice of the host cell line and the culture conditions, such as nutrients and mode of operation, are particularly important factors for the glycoforms profile of the final protein, which directly impacts the expected function of the protein. Thus, the study of the glycosylation pattern is essential to evaluate the quality of a recombinant protein.

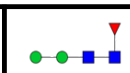
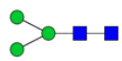
Structure		Percentage (%)		
		1.	2.	3.
F(6)M2		10.15	13.90	8.02
M3		8.61	10.94	8.10
F(6)M3		78.96	72.45	78.57
F(6)A1		2.28	-	3.09
Mn5		-	2.71	2.20
■ N-acetyl-glucosamine; ▲ Fucose; ● Mannose.				

Figure 1 – Identification by UPLC-FLR-MS of glycoprotein structure of rRVGPEcto expressed in different culture conditions.

Drosophila melanogaster Schneider 2 cells expressing rRVGP ectodomain (rEctoRVGP) were established in previous work. In this study, these cells were used, aiming the purification and evaluation of the cellular response in terms of glycosylation patterns.

Cells were grown in 100mL schott flasks with 20 mL of SF900III (Gibco) (100rpm, 28ºC), and the protein rEctoRVGP was expressed under different conditions (amount of inducer and time of expression): 1. 0,7mM of CuSO4, collected 72h after induction; 2. 0,7mM of CuSO4, collected 96h after induction; 3. 1,0mM of CuSO4, collected 72h after induction).

The ectodomain was purified by Immobilized Metal Affinity Chromatography, and its glycosylation pattern was analyzed by UPLC-FLR-MS (Figure 1). For the three conditions studied, P (6) M3, between 70 and 80% of the glycolic structures, followed by F (6) M2, between 8 and 13%, M3, between 8 and 10 %. The structures F (6) A1 was found in the conditions 1 and 3, and the Mn5, in the conditions 2 and 3, however, in amounts of less than 5%.

OPTIMIZATION OF PK15 CELL CULTURE FOR VETERINARY ANTIGEN PRODUCTION

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Key Words: Veterinary vaccines, suspension culture, metabolic model, scaling, PK15.

Porcine circovirus associated disease (PCVD) severely affects pigs, and it is responsible for important economic losses in the industry. A vaccine based on a viral antigen has been developed to prevent this disease, and it is produced industrially in the pig's kidney cell line PK15. However, due to the adherent nature of these cells, process scalability is seriously limited.
The aim of this work is to characterize the culture of PK15 adherent cells and to determine a strategy for optimization and scale-up of the vaccine production process.
The key parameters that characterize the growth of PK15 adherent cells were obtained experimentally and metabolites of the central carbon metabolism were measured. Based on this information, a culture strategy was designed for the suspension culture of PK15 cells by means of a progressive adaptation process.
Cells were grown in suspension, maintaining a viability above 90%, a lactate to glucose ratio, cell density and antigen production equivalents to 1.1, 0.7 and 0.5 times of those achieved in the original adherent culture, respectively. Metabolic flux analysis was performed to characterize the efficiency in the use of nutrients and to perform a rational design of culture medium using a stoichiometric model.
A fed-batch culture strategy allowed us to increase the cell density achieved to 1.4 times with respect to the original adherent culture, showing a decrease of a 57% in the lactate to glucose ratio. Changes in the infection protocol allowed to increase the viral antigen production by one order of magnitude. Exploratory studies with perfusion culture indicate that there is room for further improvement in the quantity of biomass produced.
Results show that it is possible to grow PK15 cells in suspension for process scalability without compromising productivity. These results are the first step towards the optimization of a PCVD vaccine production process that can be transferred to the production setting.

EXPRESSION AND CHARACTERIZATION OF A VACCINE CANDIDATE AGAINST BOVINE VIRAL DIARRHEA VIRUS

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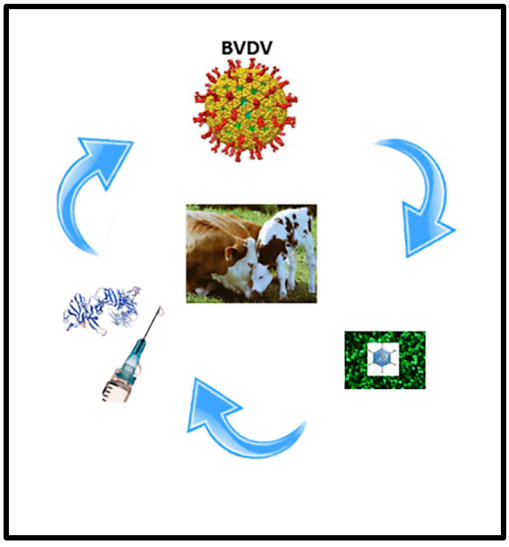
Key Words: BVDV, adenoviral vector, expression system, HPLC, cell culture.

Bovine viral diarrhea virus (BVDV) causes important economic losses in cattle worldwide. In this study, a vaccine candidate against this infectious agent was expressed in mammalian cell culture for further characterization.

Gene expression was carried out by transducing several mammalian cell lines from different species with a recombinant adenoviral vector (rAdV) coding the molecule of interest. This molecule was composed by two distinct sequences of the E2 protein from BVDV, which is the most immunogenic component of this virus, attached to a histidine tag followed by the Fc segment of human immunoglobulin (BVDE2Fc). Chimeric protein was purified by immobilized metal affinity chromatography (IMAC) and the polysaccharides profile was assessed by HPLC using an Amide-80 column.

After verifying the authenticity of the chimeric protein BVDE2Fc, coded in a transcriptional unit containing the gene of interest under the control of early/immediate CMV promoter, a rAdV was assembled using the same construction. Several mammalian cell lines were transduced with the recombinant adenoviral vector in order to secrete BVDE2Fc protein.



Graphic abstract: Vaccine candidate against BVDV.

As densitometric analysis did not show differences in BVDE2Fc secretion from distinct cell lines, the MDBK cell line was selected as the final expression system because of its bovine origin. The supernatant of MDBK cell transduced with the rAdV was submitted to a purification process, which allowed more than 90 % purity of the BVDE2Fc protein. A N-deglycosylation reaction showed a distinct molecular weight during the band pattern analysis of BVDE2Fc protein, indicating the polysaccharide transfer to potential N-glycosylation sites. This result was corroborated by HPLC, where a polysaccharide profile with some degree of sialylation was observed.

This research constitutes one of the initial stages aimed to the obtaining of an effective vaccine candidate against BVDV, which could improve the livestock industry by diminishing or controlling the detrimental effects of this viral disease in cattle.

DEVELOPMENT OF RECOMBINANT MODULAR PROTEINS FOR NUCLEIC ACID DELIVERY

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Key Words: modular proteins, nuclei acid delivery, intracellular trafficking.

The low efficiency of nucleic acids delivery to mammalian cells is a recurring problem in gene therapy studies using non-viral vectors. This is mainly caused by the difficulty in directing and transporting the therapeutic molecules to the target cells due to the presence of numerous physical, enzymatic and diffusional barriers. Over the past few years, our research group has developed multifunctional recombinant proteins specially designed for gene delivery, seeking to mimic the ability of viruses to infect cells exploiting signaling, extra- and intra-cellular responses. Here we report the development and characterization of new multifunctional recombinant proteins capable of self-organizing into nanoparticles and efficiently transport nucleic acids (plasmid DNA or small interfering RNA) into mammalian cells. The pDNA-Protein and siRNA-Protein nanoparticles were found capable of efficiently protect, and facilitate cell uptake and intracellular traffic of transgenes. The kinetics of nanoparticle formation, stability and physical-chemical parameters, such as hydrodynamic diameter and zeta potential were evaluated and correlated with the efficiency of gene delivery for different cell lines. The pDNA-Protein nanoparticles were also capable to exploit the intracellular trafficking *via* interaction with the Dynein molecular motors, in a similar mechanism found in the intracellular trafficking of viral particles. These results demonstrate the potential of the rational design of recombinant modular proteins as efficient non-viral vectors for future gene-based therapies.

STRATEGIES FOR MESENCHYMAL STEM/STROMAL CELLS EXPANSION TARGETING THE APPLICATION OF THE WAVE-INDUCED MOTION BIOREACTOR

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Key Words: Umbilical-Cord Matrix Derived Mesenchymal Stem/Stromal Cells (UCM-MSC); Wave-Induced Motion Bioreactor; Cellbag; Single Use Bioreactor; Disposable Device.

In the last 15 years, Mesenchymal Stem/Stromal Cells (MSCs) have been highly targeted for use in the development and improvement of therapeutic techniques in different clinical areas. MSCs are present in low concentrations in the tissues, making necessary *in vitro* expansion to enable research and therapeutic applications. The use of bioreactor technology for MSCs expansion has been a key factor in boosting *in vitro* productivity with bioprocesses in accordance with the Good Manufacturing Practices (GMP). The Single Use (SU) technology, represented here by the Wave-Induced Motion Bioreactor (WIMB), offers the possibility of medium to large cell expansion scale with culture variables control, reduced operating costs, high biosafety level and low shear environment. However, there are precedents that point to problems that cause low expansion factors (EFs) and cell death in adherent cells culture in the WIMB due to the formation of aggregates deposits (microcarriers and cells) on the cellbag plastic. The objective of this study was to evaluate strategies for the effective expansion of Umbilical-Cord Matrix derived Mesenchymal Stem/Stromal Cells (UCM-MSCs) in a 2/10 WAVE Bioreactor^{TM} of 600 mL avoiding the aggregates deposits formation. For such purpose, the use of low cell density inoculum (1.65×10^4 cells/mL) was tested and a homemade device with two plastic plates was used to compress and verticalize the longitudinal ends of the cellbag. UCM-MSCs were expanded using the microcarrier Cultispher-S® and α-MEM medium (+10% v/v fetal bovine serum) at 37°C and pH 7,1-7,4. The modified culture system was evaluated in triplicate (n = 3) with the cell adhesion phase performed in a Spinner flask of 200 mL and the expansion phase in the WAVE Bioreactor. For comparison purposes, duplicate expansion (n = 2) of UCM-MSCs was performed in Spinner flask under culture conditions similar to those used in the WAVE. In the Spinner was observed: EF of 54.7 ± 1.8 in 7 days, maximum specific growth rate (μ_{max}) of 0.037 h^{-1}, $Y_{lac/glc}$ of 1.50 ± 0.25 $mol_{lactate}/mol_{glucose}$ and maximum mean diameter of aggregates of 320 ± 30 µm. In the WAVE Bioreactor the results were: EF of 8.4 ± 1.3 in 10 days, μ_{max} of 0.022 ± 0.002 h^{-1}, $Y_{lac/glc}$ of 1.43 ± 0.18 $mol_{lactate}/mol_{glucose}$ and maximum mean diameter of aggregates of 250 ± 10 µm. The harvested cells from both culture systems showed adequate expression for specific MSCs markers and maintenance of the differentiation capacity in adipocytes, osteocytes and chondrocytes. In general, the operational strategies evaluated were effective in enabling the expansion of UCM-MSCs in the WAVE Bioreactor, whose potential use in the expansion of MSCs can be further improved to become an interesting alternative.

SCALE-UP OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS CULTURE FOR DIABETES CELL THERAPY

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Key Words: Cell therapy, Human adipose-derived stem cells, Suspension culture, Scale-up, Differentiation

Type I Diabetes is a disease that has shown an increasing incidence in the last few years. The current treatments involve exogenous insulin administration, strict diet control, and exercise. However, adequate glycemic control is still difficult to attain, as they do not provide a system to regulate blood glucose levels. For this reason, the development of new therapeutic alternatives is an urgent medical issue. Over the last few years, cell therapy using adipose-derived stem cells (hASC) has emerged as a promising treatment alternative. Adipose/fat tissue provides a readily available source of mesenchymal stem cells. Yet, their expansion is still limited and not easily scalable.

The general aim of this work is to scale-up the expansion of hASCs for their further differentiation into insulin and glucagon expressing cells (IPC and GPC), to cluster both types to form islet equivalent units for transplant in patients. This could represent a cellular-based therapy for type I diabetes. The hASC cells were characterized in 2D culture, and the effect of glucose and calcium concentration on cell growth and metabolism was determined. Then, cells were adapted to suspension culture and compared to adherent culture, in terms of proliferation, viability and metabolites production. These results are the first approach for the improvement of biomass production in suspension culture of these cells. Protocols have been previously designed for the differentiation of hASC into IPCs and GPCs, nevertheless, these protocols have yet to be adapted for large-scale suspension culture, to achieve our major ulterior goal of producing large amounts of islet equivalent units.

PERIOSTEAL TISSUE ENGINERING: BEHAVIOUR OF HUMAN DERMAL FIBROBLASTS AND SAOS-2 OSTEOBLASTS ON CHITOSAN-BASED SCAFFOLDS

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Key Words: tissue engineering, periosteum, xanthan, chitosan, phosphorylation

Many diseases may affect the bone tissue, resulting in structural defects and leading to impairment of its functions. In the case of large bone defects, conventional therapies are usually inefficient and more specialized treatment modalities are required to enhance tissue repair. Tissue engineered scaffolds able to mimic the periosteum, a membrane present in the bone tissue which is fundamental in the healing process, are particularly interesting because they can significantly improve bone regeneration in severely injured tissues[1,2]. Natural polymers, such as polysaccharides, are vastly used in tissue engineering applications since they are biocompatible, biodegradable and able to mimic the extracellular matrix. Xanthan gum is a polysaccharide that may interact with chitosan resulting in complexes with improved properties in comparison to matrices produced with each polysaccharide alone[3,4]. Chemical modifications, such as the phosphorylation of chitosan, can be performed to enhance the osteoconductivity of the resulting scaffold[5]. In this work, phosphorylated chitosan (Chp) was combined with xanthan (X), in the presence or not of the porogenic agent Kolliphor® P188 (K) and the silicone rubber Silpuran® 2130, used to improve mechanical properties, to obtain porous and mechanically reinforced matrices for the application as scaffolds in periosteal tissue engineering. Indirect toxicity towards fibroblasts (HDF) and osteoblasts (Saos-2), cells chosen as representatives of the periosteum tissue, was analyzed. Evaluation of cell cultures under phase-contrast optical microscope revealed no significant change in their morphology as well as no difference in cell monolayer confluence after exposure to the degradation products of scaffold samples. Besides, the number of live and dead cells evaluated after 24 hours of culture in the presence of samples extracts was similar to control (untreated cells). Moreover, an evaluation of mineralization promoted by differentiated adipose derived stem cells (ADSC) after culture on the scaffolds in the presence of osteogenic media was performed. High deposition of minerals, such as calcium phosphate and oxalate crystals, and formation of extracellular matrix were observed. These results, in addition to physicochemical and mechanical evaluation of the materials, indicate that the formulations studied are good candidates for the application proposed.

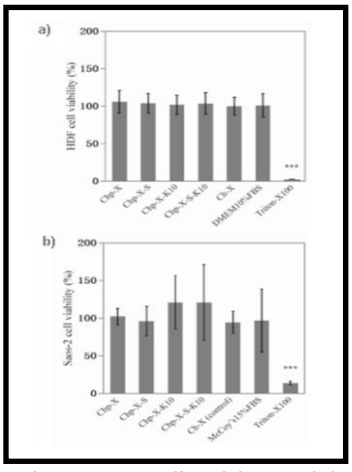

Figure 1 – Fibroblasts (a) and osteoblasts (b) viability after exposure to scaffolds extracts.

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ABSTRACTS OF POSTERS

N-glycoengineering of hIFN-α2b by fusion of ANITVNITV peptide: impact on physicochemical properties, in vitro bioactivity and pharmacokinetics

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Key Words: rhIFN-α2b, glycoengineering, pharmacokinetics, antiproliferative activity

IFN-α is a multifunctional cytokine since it presents antiviral, antiproliferative and inmunomodulatory activities. Therefore, it has been used as a therapeutic agent for the treatment of viral and tumor diseases. However, due to its rapid plasma clearance, rhIFN-α has to be administered in high and repeated doses in order to reach the desired effect, resulting in the appearance of some undesired symptoms. In order to overcome this kind of problems that arise not only for IFN but for other therapeutic proteins, different engineering strategies have been developed and successfully applied. One of the commonly used strategies involves the chemical and genetic fusion to a peptide, polypeptide or a synthetic polymer. In this sense, the 9-mer peptide ANITVNITV, which contains 2 potential N-glycosylation sites, has been described as a useful tool for improving FSH pharmacokinetics (Perlmann et al., 2003). Other strategy involves the mutation of the protein sequence in order to introduce potential N-glycosylation sites. rhIFN-α2b has been previously modified by our group by replacing aminoacids Pro4, Arg23, Lys70 and Asp77 by Asn. This hyperglycosylated variant -called IFN4N- showed a 25-fold increased half-life and a 20-fold reduced clearance compared to the non-glycosylated IFN-α2b (Ceaglio et al., 2008).
In this work, two hIFN-α2b variants were designed and constructed by fusing ANITVNITV peptide to the N-terminus of IFNwt and IFN4N. Thus, IFNwtNter and IFN4NNter were produced in CHO-K1 cells and purified by immunoaffinity chromatography in order to study the effect of this modification on the physicochemical, biological and pharmacokinetic properties of the molecule.
The physicochemical characterization of IFNwtNter and IFN4NNter was performed by SDS-PAGE and isoelectric focusing (IEF) followed by Coomasie blue staining. The comparison between modified and unmodified proteins revealed that ANITVNITV-derived glycans contributed both to the apparent molecular weight increment and to the isoelectric point reduction of both analogs. In accordance with this, the sialic acid content was higher for the peptide fusion proteins compared to the corresponding unmodified ones. As it was expected, the pharmacokinetic analysis of IFNwtNter and IFN4NNter revealed a notable improvement of parameters such as T_{max}, AUC and CL_{app}. The *in vitro* biological activity analysis revealed a reduction of the antiviral specific biological activity (SBA) of IFNwtNter and IFN4NNter compared to IFNwt and IFN4N, respectively. Despite the fact that a decreased *in vitro* antiproliferative SBA was expected for both proteins, it was only observed for IFNwtNter. Particularly, the peptide addition to IFN4N caused a 5-fold increment of its *in vitro* antiproliferative SBA. To conclude, the fusion of the ANITVNITV peptide constitutes a useful strategy to improve the physicochemical and pharmacokinetic properties of both IFN variants. Moreover, this modification increased the growth-inhibitory function of IFN4N, resulting in a promising agent for the treatment of tumor diseases.

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An hGM-CSF-derived peptide as a novel glycoengineering tool to produce highly O-glycosylated therapeutic variants of hIFN-α2b

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Key Words: hIFN-α2b; glycoengineering; O-glycosylation; fusion protein

One of the major concerns regarding administration of protein biotherapeutics for disease treatment lies in their low stability and short plasma half-life. Glycosylation is the most important co/postranslational event carried out by eukaryotic cells. Its manipulation through glycoengineering represents an effective tool in order to improve plasma half-life, as well as solubility, bioactivity, secretion and antigenicity of therapeutic proteins. For these reasons, we have developed a novel glycoengineering approach to generate proteins bearing O-glycans through their fusion to a 15-mer peptide tag named GMOPm. That sequence comprises the first 7 amino acids of the N-terminal region of the human granulocyte-macrophage colony stimulating factor (hGM-CSF) together with 8 more residues that have been added with the aim of generating 6 potential O-glycosylation sites. The goal of this work was to study the ability of GMOPm to improve the biological properties of a widely used human biotherapeutic, human interferon-α2b (hIFN-α2b).

Five chimeras were constructed by adding GMOPm to the N- and/or C-terminal ends of hIFN-α2b in different proportions, to obtain variants with 7 to 29 potential O-glycosylation sites as predicted in silico: GMOPm-IFN; (GMOPm)2-IFN; (GMOPm)3-IFN; (GMOPm)2-IFN-GMOPm and (GMOPm)3-IFN-GMOPm. CHO-K1 recombinant cell lines were cultured for IFN variants production, achieving concentrations between 0.7 and 8 $\mu g.ml^{-1}$ in culture harvests. The chimeras were purified from culture supernatant by affinity chromatography, using a monoclonal antibody anti-hIFN-α2b, with yields ranging from 40 to 100%. SDS-PAGE and IEF analysis of purified proteins demonstrated that the more the number of GMOPm tags added, the higher the molecular mass and the lower the isoelectric point of the fusion protein. Both analysis deeply suggest that O-glycans have been attached at least to some of the potential O-glycosylation sites. Interestingly, all IFN variants retained *in vitro* antiviral activity, although it decreased concomitantly with the number of fused tags. However, it is widely known that *in vitro* activity generally does not reflect *in vivo* efficacy of a biotherapeutic. Indeed, improvement of pharmacockinetic properties is considered so vital to a protein´s *in vivo* activity that many times it is achieved at the expense of *in vitro* activity. Thus, the increment in glycosylation achieved by GMOPm addition may result in an improved pharmacokinetic profile and, consequently, an improved *in vivo* bioactivity. This will be analyzed in a near future.

In summary, in this work we have produced purified and *in vitro* characterized five GMOPm/IFN chimeras which exhibited promising properties for the *in vivo* evaluation of their pharmacokinetic and biological activity.

N2A CELL LINE AND NEURONAL PRIMARY CULTURES AS PLATFORMS TO EVALUATE NEUROPROTECTIVE AND NEUROPLASTIC ACTIVITIES OF HUMAN ERYTHROPOIETIN

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Key Words: erythropoietin, neuroprotection, neuroplasticity, N2a cell line, rat hippocampal neurons.

Neurodegenerative diseases are pathologies that affect the nervous system and cause cognitive disorders, behavior disorders and physiological changes. They are characterized by their chronicity and progressive evolution. According to the World Health Organization (WHO), neurodegenerative diseases affect millions of people around the world, mainly due to the increase in life expectancy and the concomitant increase in the world's elderly population. Nowadays, there is not treatment with perceptible advantages for these pathologies. Because of that, researchers are involved in the development of neuroprotective and/or neurotropic agents. In this aspect, hEPO (human erythropoietin) has an important role considering its antiapoptotic, cytoprotective, angiogenic and antioxidant properties.

In this work, the *in vitro* neuroprotective and neuroplastic activity of rhEPO (recombinant human erythropoietin) were determined employing murine neuroblastoma cell line (N2a) and primary cultures from hippocampal neurons. In both cases, the capacity of the cytokine to reverse the staurosporine (STP)-induced apoptosis was studied. Different conditions were assayed, demonstrating the cytoprotective role of rhEPO to protect STP-treated N2a cell lines and primary cultures at 5 and 11 DIV (days *in vitro*). rhEPO was capable to significantly reverse the STP-induced apoptosis in a dose-response effect in both cells types (between $p<0.01$ and $p<0.001$).

The neuroplastic role of rhEPO in N2a cells and in hippocampal neurons was also tested. First, N2a cells were employed to quantify the neuritogenesis after incubation with 10, 50 and 300 ng/ml of rhEPO for 3 hs in starving condition. The neuritogenesis was estimated as the number of primary neurites, the average of the neurite length and the longest neurite per cell. N2a cells treated with 50 and 300 ng/ml of rhEPO showed a significant increase in the neurite length ($p<0.05$ and $p<0.001$) and number ($p<0.05$ and $p<0.01$) compared with the control cells. Then, to complete neuroplasticity evaluation, filopodia formation and synapses were studied in cultured hippocampal neurons. Neurons were incubated with 10, 50 and 300 ng/ml of rhEPO. Those neurons treated with 50 and 300 ng/ml of rhEPO showed a significant increase in filopodia density along 20 µm of dendrite length ($p<0.05$ and $p<0.001$) and the number of synapses ($p<0.01$ and $p<0.01$) compared with control cells.

In summary, both N2a cells and hippocampal neurons are useful and complementary platforms to evaluate the neuroprotective and neuroplastic role of rhEPO and derivatives of it. These derivates of rhEPO are specifically being designed to display neuroprotection and neuroplasticity during the evolution and recovery of neurologic illnesses.

DEVELOPMENT AND STANDARDIZATION OF TRIDIMENSIONAL (3D) SPHEROIDS AS AN *IN VITRO* OVARIAN CANCER MODEL

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Key Words: 3D cell culture, spheroids, ultra-low-attachment plates, SKOV-3 cells, drug screening assays.

Compared to 2D culture, tridimensional (3D) in vitro cancer models can better mimic native tissues, since the tumor microenvironment established in 3D often plays a significant role cellular responses to drugs. This work shows the establishment and characterization of 3D spheroids using human ovarian cancer cells (SKOV-3) as a platform for drug screening assays. For this, SKOV-3 cell were initially cultured in RPMI medium supplemented with 10% (v/v) fetal bovine serum in ultra-low attachment (ULA) plates (Corning) using different initial cell concentrations: $5.0x10^3$; $12.5x10^3$; $25.0x10^3$ cells/mL. Under these conditions it was not observed the formation of compact spheroids, even after initial plate centrifugation. To improve spheroids formation, the addition of methylcellulose (MC) (0.25% and 0.5% v/v) was then evaluated. The addition of MC promoted the formation of compact SKOV-3 spheroids with diameters ranging from 138 to 751 µm throughout 120 h of culture. In this period, it was not observed cell growth. A higher number of the microvilli were observed in the spheroids, when compared with 2D culture (Figure 1). The cells under 3D conditions presented a higher level of the apoptosis when compared with 2D condition, probably due to nutrients/metabolites diffusion issues. After treatment by 48 h with the anti-cancer drug Paclitaxel, the spheroids also showed higher resistance, when compared with 2D (Figure 2). The results obtained demonstrate that these 3D models better mimic the *in vivo* tumor microenvironment.

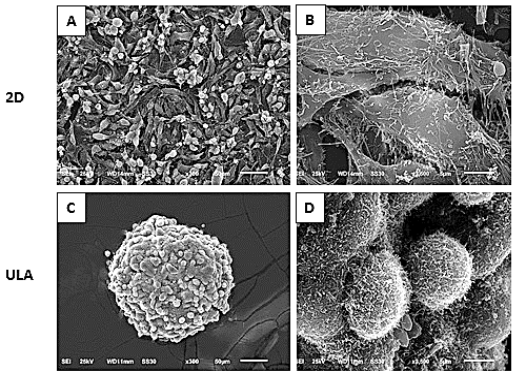

Figure 1 - *Screening electronic microscopic images of SKOV-3 cells in 2D (**A, B**) and ULA **(C, D)**. 3500x objective, scale bar = 5 µm.*

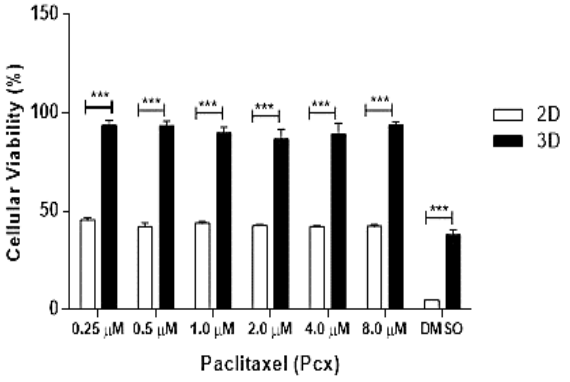

Figure 2 - *Cellular Viability of the spheroids SKOV-3 in ULA plates and 2D after 48 h of treatment with Paclitaxel drug. Statistical analysis was performed by Two-way ANOVA test followed by Bonferoni. *** (p<0.001) compared to control group* (n=4).

Production and purification of the Brain Derived Neurotrophic Factor (BDNF) in bacteria *Escherichia coli*

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Key Words: BDNF recombinant, Neurotrophic factor, *Escherichia coli, Biopharmaceutical.*

The Neurotrophic Factor Derived from the Brain (BDNF) is a molecule of great interest for its participation in processes of synaptic plasticity and neurogenesis. Its deficiency influences the development of neurodegenerative diseases, turning it into a target with significant therapeutic potential. The objective of the present work is to obtain human recombinant BDNF, of high purity to obtaining a biopharmaceutical based on this neurotrophin. After testing different expression conditions in *E. coli* bacteria, SHuffle and BL21 strains, the protein was obtained as an inclusion body. For this, plasmid pET-22b(+) was used, in which the human BDNF gene was inserted in the NdeI and XhoI cloning sites, including a Histidine tag. After transforming the bacteria with the vector, cultures were prepared with Luria-Bertani medium and induction of BDNF was performed using isopropyl-β-D-1-thiogalactopyranoside (IPTG) 0.4 mM for 4 hours at 37°C. Cell disruption was carried out by sonication, and the visualization of soluble and insoluble proteins, was carried out by SDS-PAGE 15% and Western-Blot, using an anti-6xHis antibody. The results show a reinforced band of 14 kDa, corresponding to the expected weight of BDNF (14.676 kDa). Because the protein was mostly expressed insoluble in strain BL21, the solubilization conditions were established using Urea 8M plus B-mercaptoethanol. The purification was carried out using metal ion affinity chromatography (IMAC), obtaining 95% purity. Finally, we proceeded to establish the renaturation conditions of the BDNF obtained, to get it in its native form. Currently, in vitro tests are being carried out to evaluate cell proliferation and viability in the PC12 cell line.

MONOCLONAL ANTIBODIES AGAINST GLYCOSYLATED RECOMBINANT HUMAN STEM CELL FACTOR. A TOOL FOR QUALITY CONTROL AND BIOPROCESS MONITORING.

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Key Words: recombinant human Stem Cell Factor, monoclonal antibodies, glycoprotein.

Stem Cell Factor (SCF) is an early-acting cytokine capable of promoting proliferation, differentiation, migration and survival which depends on the cell type. It plays a crucial role in hematopoiesis, gametogenesis, melanogenesis, intestinal motility, and in normal development function and recovery of nervous and cardiovascular systems. Potential therapeutic applications of SCF comprise anemia treatment, mobilization of hematopoietic stem/progenitor cells to peripheral blood for harvest and transplantation and increasing gene transduction efficiency for gene therapy. Furthermore, human glycoproteins produced by mammalian cell cultures may present some advantages in comparison to other expression systems, such as authentic native glycosylation, higher *in vitro* or *in vivo* molecular stability, among others. Thus, it is of major interest the production of glycosylated recombinant human SCF as well as the proper monitoring in each step of the process.

In order to study product quality during the different stages of the glycoprotein production process a set of murine monoclonal antibodies (mAbs) against glycosylated recombinant human SCF (rhSCF) was produced and characterized.

The cell line producing rhSCF was generated through lentiviral transgenesis of HEK293 cells. Antibiotic selection was performed in order to increase productivity. A HisX6 tag was used to purify rhSCF from culture supernatant by Immobilized Metal-ion Affinity Chromatography. BALB/c mice were inoculated with purified rhSCF following an immunization plan comprising four different strategies. After fusion protocol, hybridoma cell lines expressing specific immunoglobulins were cloned. Three clones with the highest productivities were amplified *in vivo* to produce murine ascites. mAbs were purified from ascites by protein A chromatography and characterized by their isotype, specificity and title.

Two mAbs were able to detect the glycoprotein in Western blot and indirect ELISA assays, showing adequate limits of detection and titles; and two mAbs were good candidates for the capturing step in sandwich ELISA, which permitted to develop an assay for rhSCF quantification in different samples. A panel of three anti-rhSCF mAbs was developed and used to identify and quantify the protein during different stages of the production process. According to these results it is possible to use the monoclonal antibodies panel as a tool to further optimize rhSCF production process. This panel might also be useful for affinity chromatography and different determinations in regenerative medicine, developmental biology and immunology.

EFFECT OF SYNTHETIC PHOSPHOETHANOLAMINE ON THE *IN VITRO* METABOLISM OF BLADDER CANCER CELLS

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Key Words: Animal cells culture; RT4 tumoral cells; Cell metabolism; Anticancer drugs.

Cancer is one of the most worrying diseases nowadays, reaching more and more people around the world. The importance of research on the disease is therefore indisputable, especially since the treatments available until now are not always efficient, cause resistance and can generate toxicity, in addition to the severity of the side effects caused in the patient's body. One of the hypotheses that has been raised is that synthetic phosphoethanolamine (PEA), a primary amine linked to cellular metabolism, is a potential candidate for anticancer drug with minimal side effects. On the above, the objective of the current work was to evaluate the effect of the presence of synthetic PEA on the *in vitro* metabolism of bladder cancer cells, with desired inhibitory effect.

The experimental work consisted in culturing the bladder cancer RT4 cells in T-type culture flasks 25 cm^2, using culture media McCoy's 5A with L-glutamine (Sigma-Aldrich, EUA) supplemented with 10 % v/v of bovine fetal serum (Hyclone, EUA) and sodium bicarbonate ($NaHCO_3$, Cultilab, Brazil), in the absence (control) and in the presence of three concentrations of synthetic PEA: 10, 30 and 100 mM. For each run, a flask 'control' (C) was incubated at 37 ºC and 5 % CO_2 in parallel with a flask 'test' (named T10, T30 and T100, according to respective PEA concentrations), in triplicate. The effect of the tested substance was evaluated by counting of cells in Neubauer chamber and analysis of glucose and lactate final concentrations.

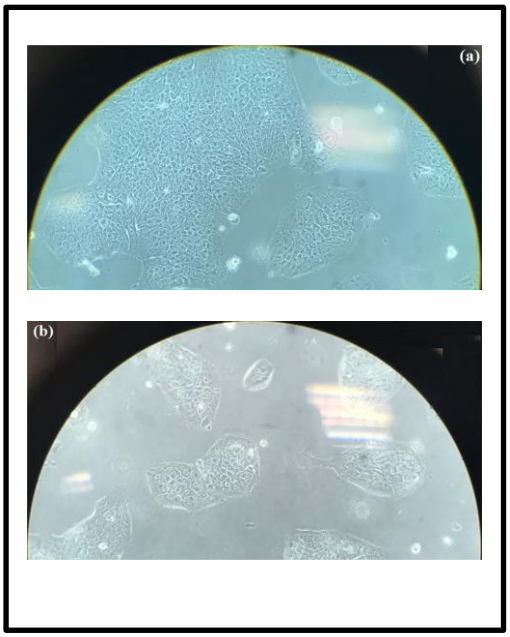

Figure 1 – RT4 cells morphology after 5 days (20x oversized) (a) control; (b)100 mM PEA.

First run was carried with C and T100. Average expansion factor (f) was 9.7 ± 2.5 for C and 4.9 ± 0.1 for T100. Final glucose and lactate concentrations were, respectively, 14.9 mM ± 0.01 and 1.4 mM ± 0.1 for C and 15.8 mM ± 0.3 and 1.5 mM ± 0.2 for T100. Therefore, a significant inhibitory effect of PEA on RT4 metabolism was observed (significance level 10 %). Second run was carried with C and T10. Average f was 2.4 ± 0.1 for C and 2.5 ± 0.3 for T10. Final glucose and lactate concentrations were, respectively, 15.5 mM ± 0.1 and 1.2 mM ± 0.04 for C and 15.3 mM ± 0.05 and 1.2 mM ± 0.05 for T10. Finally, third run was carried with C and T30. Average f was 2.8 ± 0.2 for C and 1.7 ± 0.03 for T30. Final concentrations of glucose and lactate were, respectively, 13.0 mM ± 1.4 and 1.5 mM ± 0.1 for C and 12.8 mM ± 1.4 and 1.5 mM ± 0.1 for T30. Hence, PEA concentrations up to 30 mM did not play significant effect on RT4 cells *in vitro* metabolism. Further tests are required for a most trustable conclusion, but PEA concentrations from 100 mM showed to have potential to inhibit RT4 bladder cancer cells to grow and aggregate, as shown in Figure 1, where larger aggregates of cells were formed in the flask control in comparison to flask with 100 mM PEA.

SYNTHESIS OF COBALT FERRITE NANOPARTICLES DOPED WITH KIWI, SICILIAN LEMON, OKRA AND AÇAI

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Key Words: Nanoparticles, Cobalt Ferrite andX-Ray diffraction (XRD)

In the last decades, the nanoparticles have been the object of intense studies, since intrinsic characteristics such as their size and morphology, allow a range of physical and chemical properties, making possible the manipulation of functionalities and applications mainly in the biomedical area, whose research has evidenced several advantages in pharmacodynamics and pharmacokinetics proving ability in the drug delivery system, drug-delivery for example. In order to produce nanoparticles with respect to the principles of green chemistry, and with that to reduce the generation of harmful substances, we use as raw material four easily found fruits: kiwi (*Actinidia deliciosa*), Sicilian lemon (*Citrus limonia*), okra (*Abelmoschus esculentus* L. Moench) and the açaí (*Euterpe oleracea*), which effected stability to the synthesis due to the presence of polysaccharides and biopolymers through its various components such as carbohydrates, proteins, anti-oxidant agent such as vitamin C and a series of cationic minerals. In this work we are looking for the synthesis of Cobalt Ferrite with interest in its magnetic properties, adding this functionality to our nanoparticles (NPs) with the successful objective of addressing the drug to them incorporated by inducing a magnetic field and provoking a called hyperthermia, a method that has been effectively applied in the field of oncology in the fight against tumor. In parallel, important steps are underway, such as the cytotoxicity test of NPs, and the introduction of them in a culture medium of the *Trypanosoma cruzi* protozoa to evaluate the capacity of maintenance of the activity and their viability within the microorganism, through the study of its growth curve. The Cobalt Ferrite NPs were synthesized by the sol-gel method with the solvent MiliQ water, where they were dissolved in Iron Nitrate and Cobalt separately for each fruit, submitted to heating to form Cobalt Ferrite ($COFe_2O_4$). The samples were centrifuged for washing and subjected to oven drying at 100 ° C and subsequent calcination at 600 ° C for one hour. The samples were analyzed by X-ray diffraction (XRD) by the Full Prof program and Rietveld method, as shown in figures 1, 2, 3 and 4, which showed better results for Kiwi fruit and Sicilian lemon due to the characteristic peaks of the crystalline structure. However, both syntheses show that the particles aggregate in front of the curves enlargement, and such conclusions lead us to revalidate the steps of the synthesis of the particles in order to actually reach the desired dimension by glimpsing our perspective of application.

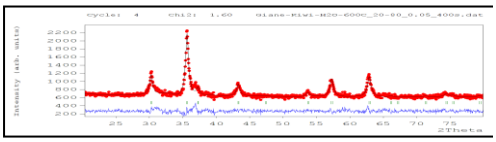

Figure 1: DRX Cobalt ferrite with Kiwi (Actinidia deliciosa)

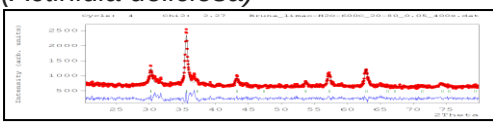

Figure 2: DRX Cobalt ferrite with Sicilian lemon (Citrus limonia)

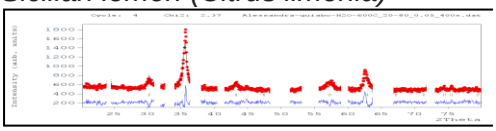

Figure 3: DRX Cobalt ferrite with okra (Abelmoschus esculentus L. Moench)

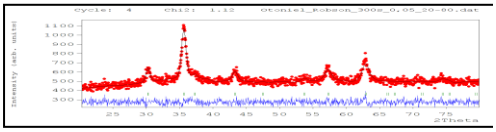

Figure 4: DRX Cobalt ferrite with Acai (Euterpe oleracea)

OBTAINING L-ASPARAGINASE BY NATURAL AND SYNTHETIC PATHWAYS: SEARCH FOR A MORE EFFECTIVE BIOPHARMACEUTICAL AGAINST ACUTE LYMPHOID LEUKEMIA

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Key Words: L-asparaginase; Anticancer enzyme; Submerged Fermentation; Fungal cell culture; site-directed mutagenesis.

L-Asparaginase (L-ASNase E.C.3.5.1.1) is a biopharmaceutical used for treatment of acute lymphocytic leukemia (ALL). Several side effects were registered by using of bacterial L-ASNase during ALL treatment, including thrombosis, pancreatitis, hyperglycemia, hepatotoxicity and resistance. Others relevant problems associated with L-asparaginase treatment are hypersensitivity reactions, low thermal stability, human proteases degradation and rapid clearance. Innumerable techniques have been used to overcome these downsides such as bioprospecting eukaryotic sources or modification of commercial bacterial L-asparaginases by site-directed mutagenesis. In order to find eukaryotic sources of L-ASNase, 50 filamentous fungi were used in this study, which were isolated from the microbiome of the jellyfish *Olindias sambaquiensis* – Figure 1A. Fifteen fungi samples isolated from jellyfish tentacles (brown structures in jelly fish responsible to toxin production) showed L-asparaginase production by submerged fermentation containing. The highest activity was shown by strain OS03 (Figure 1B) with 4.98 U/g, and lowest shown by sample OS50 (Figure 1C) grown in seawater media containing proline (10% w/v), showing 0.90 U/g. Results of this screening are promising because filamentous fungi L-asparaginase can shows less adverse effects due to post-translational properties (which generate the active enzyme) that occur in these microorganisms and absent in beings prokaryotic (Sarquis et al., 2004). Regarding protein engineering of commercial bacterial L-asparaginases by site-directed mutagenesis we intend using bioinformatics analysis to obtain new structure of L-asparaginase with improved properties: eliminated glutaminase activity, resistance to human proteases and decreased immunogenicity. For example, L-asparagine in the position 24 in *Escherichia coli* (EcAII) type II ASNase has been identified as primary cleavage site for two lysosomal proteases, asparaginase endopeptidase and cathepsin B (Maggi et al., 2017). Designing an N24 mutation based on this assumption should lead to relevant changes in L-asparaginase protease-resistance.

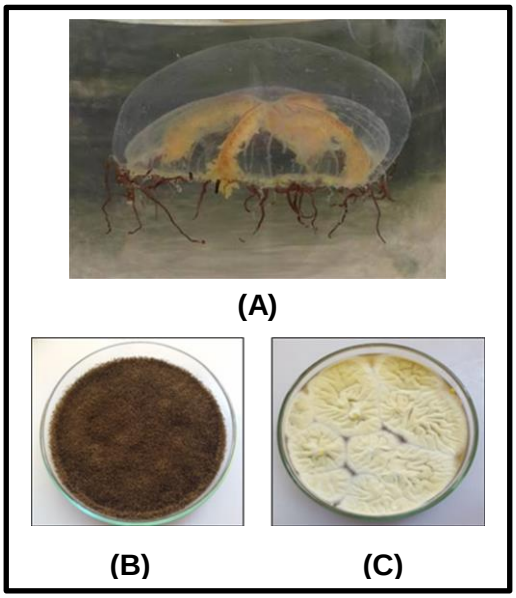

Figure 1. ***(A)*** *Side view of live Olindias Sambaquiensis in seawater.* ***(B)*** *and* ***(C)*** *filamentous fungi isolated from jelly fish tentacles.*

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EXPRESSION OF ENVELOPE PROTEIN DOMAIN III (EDIII) OF ZIKA VIRUS IN DROSOPHILA CELLS.

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Key Words: ZIKV, EDIII, S2, Immunofluorescence, diagnosis.

The Zika virus (ZIKV) is a single-strand positive RNA that belongs to the genus *Flavivirus* in the family Flaviviridae. The ZIKV Envelope (zE) glycoprotein is composed of three ectodomains (DI, DII, and DIII) and is involved in various aspects of the viral cycle, mediating binding and membrane fusion. The availability of tests for the laboratory diagnosis of Zika infection is still very restricted, so there is a need to develop an affordable and more accurate test, reducing the risk of cross-reaction. The Neutralizing antibodies reacting with the zEDIII are generally specific for each virus and don't cross-neutralize other viruses. In this way, we pretend to express analyze the EDIII domain of ZIKV in drosophila S2 cells to develop a rapid immunofluorescence diagnostic test able to detect the infection of ZIKV in serum of human patients.

The fragment encoding EDIII-ZIKV was cloned in the pAcV5-HisA-Hygro drosophila vector, and the product was transformed and screened into DH5α. The vector obtained, pAcEIII-ZIKV-Hygro, were transfected into S2 cells. Schneider 2 cells (S2 cells), are derived from the late embryonic stage of the *Drosophila melanogaster* (20-24 hours old), which have been used as hosts for the expression of heterologous gene products. They are relatively cheap cells to maintain and produce heterologous proteins translated accurately and correctly processed. In our studies, we constructed the plasmid carrying the EDIII domain of ZIKV. This plasmid was transfected into S2 cells, and after the selection we established the S2 Ac EDIII-ZIKV cell line. This recombinant cell line is being used to analyze the expression of zEDIII domain by dot blotting.

Financial Support: FAPESP, CAPES, Fundação Butantan.

SITE-DIRECTED INTEGRATION IN CHO-DG44 CELLS: MAPPING GENOMIC HOTSPOTS ASSOCIATED TO HIGH AND STABLE EXPRESSION LEVELS OF RECOMBINANT PROTEINS.

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Site-directed insertion, Biopharmaceutical production, CHO cells, , lentiviral vector

In the last decades, observed number of improvements have been introduced at several stages of the biopharmaceuticals manufacturing process, resulting in a significant increase in the production capacity of the biopharmaceutical sector. This is based, in part, on the development of improved expression vectors and cell lines, as well as optimization of culture media and bioreactor operating strategies. Among the main limiting factors related to the establishment of a production platform based on mammalian cells are the extended periods of time and high costs associated with the process of obtaining highly overproducing cell clones. These limitations are intimately related to the methodologies commonly employed for the generation of stable cellular transfectants, which involve random genomic integration events of expression vectors, and use of gene amplification protocols, which are associated to, respectively, insertional events into chromosomal sites with unstable transcriptional activity and drastic genetic rearrangements. These processes result in a rather heterogeneous stably transfected population of cells with respect to protein expression stability and levels, drastically reducing the probability of isolating a highly producing cell clone. An alternative that has been explored in recent years is based on site-directed insertion of the expression cassette into regions of the host cell genome previously associated with stable and high-level gene expression. The biopharmaceutical industry has shown a growing interest in this type of alternative for the generation of overproducing clones in CHO cells, one of the most widely utilized expression platforms for the production of biopharmaceuticals. Therefore, there is a great demand to accumulate knowledge about genomic regions which support a stable and high level expression of therapeutic proteins producing cell lines, such as CHO cells. Accordingly, the present project aims at mapping some of these genomic sites present in the CHO-DG44 genome, which could be employed for the site-directed insertion of expression vectors of interest through the CRISPR-Cas9 methodology. Our proposal involves the use of an expression bicistronic lentiviral vector as a tool for screening genomic sites present in CHO-DG44 cells associated with high and stable expression of the protein of interest. Preliminary results include the construction and functional characterization of the pLV-spEYFP-Dhfr expression vector, used to generate cell populations stably producing a secreted variant of the EYFP (spEYFP) protein. This was accomplished either by transfection or stable transduction of CHO-DG44 cells, followed by selection of events of stable genomic integration of the expression vector by biochemical complementation based on the exogenous expression of Dihydrofolate reductase, and cell clones isolation through limiting cell cloning. The next steps include determining the integrated spEYFP coding sequence copy number per cell among the CHO-DG44 cell clones obtained, as well as characterizing the genomic integration sites associated with single genomic insertional events of the expression vector.

Financial support: CAPES, CNPq, FAPESP, INCT-Regenera, FINEP, MCTI, MS-DECIT.

EVALUATION OF RECOMBINANT HUMAN ACID ALPHA–GLUCOSIDASE EXPRESSION IN HEK–293F CELL LINE

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Key Words: Pompe disease, rhGAA, enzyme replacement therapy, cell line development, human cell lines

Pompe disease is a disorder classified as an inborn error of metabolism (IEM) that has a genetic origin and implies in an alteration of glycogen metabolic pathway. The pathology is also referred as glycogen storage disease type II (GSDII) and is related to a deficiency of the enzyme acid alpha–glucosidase (GAA) that hydrolyzes lysosomal glycogen, leading to its accumulation in the cells. The consequences depends on age, the associated mutation and genetic polymorphisms, but it is often observed progressive cardiomyopathy, hepatomegaly, weakness, hypotonia and cardiorespiratory failure[1,2]. Nowadays, the treatment is based on enzyme replacement therapy with a recombinant GAA synthetized in CHO cells. Despite the effectiveness of this treatment, undesired immunological responses have been observed, probably caused by different patterns of protein glycosylation. Glycoproteins that are produced on non–human cells can present different groups of carbohydrates that are not present on human body and therefore lead to an activation of the immune system, decreasing the efficacy of the treatment[3]. This study is focused on the expression of the GAA enzyme in the human HEK293F cell line cultured under serum-free suspension condition. Firstly, a transient transfection protocol was employed to evaluate GAA expression using polyethylenimine (PEI) (DNA:PEI ratio of 2:1) at different cell concentrations (1, 2 and $3x10^6$ cells/mL). Under this conditions it was not possible to obtain good transfection levels. Only 16% of GFP positive cells were obtained at the concentration of $3x10^6$ cells/mL. Experiments are in progress to improve transfection levels (at least ≥50%). In addition, the expression of GAA will also be evaluated after the generation of a stable cell line through lentiviral transduction.

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CHARACTERIZATION OF CELLULAR STATES OF CHO-K1 CELLS ADAPTED TO SERUM FREE MEDIA AND SUSPENSION GROWTH THROUGH CELL CYCLE AND RNA-SEQ PROFILING

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Key Words: Bioprocess, Cell cycle, CHO cell culture, Flow cytometry, RNA-Seq.

Chinese Hamster Ovary (CHO) derived cell lines are the preferred host system for the production of therapeutic proteins. Knowing how CHO cells behave during bioprocessing has until now relied heavily on empirical studies with a limited knowledge of the intracellular dynamics. The recent availability of CHO genome reference and several -omics data has led to new areas of research for better understanding of CHO cells metabolic behavior.
The aim of this work was to explore the regulation of adapted to serum-free suspension CHO-K1 host cell line bioprocesses, especially under a temperature gradient from 37°C to 31°C. We analyzed cell cycle behavior through flow cytometry of Propidium Iodide stained cells and high throughput transcriptome dynamics by RNA sequencing.
During 20 days, three replicates of CHO-K1 cells were cultured in 1 L bioreactors. Equal operating conditions were used and the temperature was shifted gradually from 37°C to 31°C. For cell cycle study, a daily sample was taken and DNA content was determined by propidium iodide staining followed by flow cytometry analysis and cell cycle modeling. Besides, RNA samples were collected on day 1, and during the exponential (days 7 and 8) and the stationary (days 14 and 15) growth phases for strand-specific RNA-Sequencing analysis. Differential gene expression (DE) between culture phases, Gene Ontology enrichment and pathways analysis were performed.
We found a cell culture state characterized by G0/G1 synchronization, mainly during the late exponential growth phase and towards the last days of the stationary phase. Particularly during stationary phase nearly 80% of the cells analyzed were in G0/G1. Clustering analysis reveals that days 7 and 8 clustered together with day 1, and days 14 and 15 comprised another group, indicating that the most important gene expression changes occurred throughout the stationary phase, markedly predominating the gene up-regulation. We successfully identified key genes and pathways connected with the particular culture states such as response to low temperature, modulation of the cell cycle, regulation of DNA replication and repair, apoptosis, among others. The DE analysis showed that at 31°C 443 genes were up-regulated and 182 genes were down-regulated.
Our RNA-seq data analysis enabled the identification of target genes for mechanism-based cell line engineering and bioprocess modification, an essential step to translate gene expression data from CHO-K1 host cells into bioprocess-related knowledge. Further efforts aim at increasing desirable phenotypes of CHO cells and promoting efficient production of high quality therapeutic proteins can highly benefit from this type of studies.

DEVELOPMENT OF BIOTECHNOLOGICAL TOOLS FOR DIFERENTIAL DIAGNOSTIC OF INFECTION BY MAYARO OR CHIKUNGUNYA

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There are several pathogenic *Alphaviruses* for humans causing severe encephalitis or polyarthralgia. They are transmitted by hematophagous arthropods as mosquitoes and ticks. *Chikungunya* (CHIKV) and *Mayaro* (MAYV) viruses can be transmitted by *Aedes sp* mosquito or *Haemagogous sp* mosquito respectively endemic in tropical and subtropical countries. In Brazil MAYV outbreaks were registered between 1950 and 2012, mainly in middle-west and north regions with some suspect cases in 2014 in the south-est region. After its introduction in Brazil in 2014, CHIKV presented fast circulation and infected people were diagnosed throughout the country. Currently, MAYV and CHIKV infections can be clinically misdiagnosed as caused by *Dengue* (DENV) or even *Zika* (ZIKV) viruses, with consequences for the decisions taken about patient treatment. The development of biotechnological tools for correct diagnosis of these four arboviruses co-circulating in Brazil is very demanded. The main objective of this work is to obtain monoclonal antibodies to differential epitopes on the surface proteins of CHIKV and MAYV. The genetic sequences corresponding to each differential epitope were synthesized as double strand DNA fragments (G-blocks) and cloned in the appropriated vectors. The activity of these vectors were further confirmed by *in vitro* evaluation of gene expression. Mice (Balb/C, SPF) were immunized with four doses at intervals of seven days each. After the second booster, blood samples were taken by the orbital plexus and antibody titer was measured by ELISA. Three days before the fusion procedure mice with the highest antibody titers received an additional booster dose. Mice were euthanized for the removal of popliteal lymph nodes. Lymphocytes were fused with myeloma cells and the resulting hybridomas were screened in HAT and HT medium on a feeder layer of peritoneal cells taken from mice and added to the cell culture dish. Several hybridomas were evaluated for antibody secretion, resulting in the isolation, expansion, subtyping and subcloning of some cells expressing anti-MAYV or anti-CHIKV antibodies.

THE PORTION OF THE ACTB PROMOTER ASSOCIATED WITH A CPG ISLAND IS CAPABLE OF DECREASING THE SILENCING OF RECOMBINANT GENES IN CHO CELLS.

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Key Words: Chinese Hamster Ovary cells, promoter engineering, cell line development, gene silencing, recombinant antibody expression.

Some CpG Islands can avoid the genetic silencing of housekeeping genes. These have been used to decrease the silencing of recombinant genes in cells of animal origin. The β actin gene (ACTB) of eukaryotes possesses a CpG island that covers much of its promoter. We set out to study the utility of the promoter region of the ACTB gene of *Cricetulus griseous* that is associated with its CpG island in the reduction of the silencing of recombinant genes in CHO cells. The promoter portion associated with this CpG island, (RegCG), which contains the proximal promoter, exon I, and the intron I of the ACTB gene, was cloned into expression vectors. In addition, chimeric promoters that combine RegCG with the CMV promoter and another that also contains a tandem of 5 glucocorticoid response elements (GRE) between RegCG and CMV were generated. These constructions were analyzed in a reporter system of transcription in transient expression tests and stable lines. We verified that RegCG behaves as a promoter which is more resistant to silencing than the CMV promoter. We found that, although RegCG transcriptional activity is mainly commanded by the CAAT and CArG boxes of the proximal promoter, the transcription starts mainly an intronic transcription start site (TSS) before the proximal TSS. The chimeric promoters were shown to be more resistant to silencing than the CMV and RegCG promoters separately. They were also able to maintain the expression of recombinant antibodies in stable clones for 40 days at an average level 4 times higher than the clones generated with the CMV promoter. Finally, clones generated with the chimeric promoter containing GRE, showed a volumetric production of 132 mg/L, in batch mode. Taken together, this data show that this promoter can be used for the generation of clones for industrial applications.

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GENERATION OF STABLE REPORTER BREAST AND LUNG CANCER CELL LINES FOR NF-KB ACTIVATION STUDIES.

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Key Words: NF-κB, Reporter cell lines.

Nuclear Factor kappa B (NF-κB) is a conserved transcription factor involved in the expression of genes that are critical to inflammation and cell survival. Exposure to particular signals results in phosphorylation of NF-κB inhibitor proteins, which in turn allows NF-κB dimers to translocate to the nucleus and induce gene expression. Pathologic consequences of NF-κB impaired activation are vast, mainly because of the pleiotropic roles that NF-κB-induced genes have on inflammation, cell proliferation and apoptosis. Thus, experimental models assessing NF-κB activation have direct screening applications for drug discovery. In this scenario, pathway-specific reporter cell systems become valuable tools to identify and elucidate the mechanism of action of novel compounds. Here, we describe the generation, characterization, and validation of human cancer epithelial reporter cell lines for functional studies of NF-κB activation by different pro- and anti-inflammatory mediators. NCI-H460 (lung) and T-47D (breast) cells were transfected with a pNF-κB-hrGFP plasmid which contains the GFP gene under the control of NF-κB binding elements. The pro-inflammatory cytokine TNF-α was able to activate the reporter systems in a concentration-response manner, which corresponds to the activation of the NF-κB signaling pathway. Finally, the reporter cell lines were validated using dexamethasone, a common anti-inflammatory drug, a synthetic inhibitor of NF-κB (BAY 11-7082) and a new anti-cancer peptide (CIGB-552). The precise mechanism of action of CIGB-552 remains partially unknown, but it is believed that part of its anti-tumor activity may be driven by NF- κB inhibition. We have established robust H460-NF-κB-hrGFP and T-47D-NF-κB-hrGFP reporter cell lines which represent a useful cancer model for primary screening and identification of compounds with anti-inflammatory activity.

NEW AMPHIPHILIC AMINO ACID DERIVATIVES FOR EFFICIENT DNA TRANSFECTION *IN VITRO*

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Key Words: Gemini, Transfection, Amphiphile.

Gene therapy is a promising approach, with a potential to improve human health. A successful gene therapy depends on efficient, safe and stable gene delivery systems. Chemically mediated non-viral vectors, such as cationic lipids, exhibit low immunogenicity, compared to viral vectors [1]. Amphiphilic gemini, a specific group of cationic lipids, has shown efficient transfection activity [2].These are dimeric amphiphiles with two hydrophilic heads and two hydrophobic groups per molecule, separated by a covalently bound spacer chain at the head groups. These agents have a versatile chemical structure, can be easily produced on a laboratory scale, can compact DNA to nano-sized lipoplexes and show relatively low toxicity. Several classes of natural amino acid-based gemini have been synthesized and characterized for the purpose of gene delivery. The current study presents the development of amphiphilic carriers aimed to achieve high DNA in vitro transfection efficiency. We designed and synthesized four new amphiphilic amino acid derivatives (cysteine-based) of low molecular weight, formed by the same pentapeptide (GEM: WWCOO) N-acylated, with different hydrophobic chains containing from 12 to 18 carbons, named GEM-C_n, which dimerize by oxidation in the presence of pLenti-CMV-GFP Puro plasmid (P) in the respective gemini. We determined critical micelle concentration (CMC), cytotoxicity and hemolytic activity for the derivatives obtained. We assessed complex formation between plasmid DNA and gene delivery vector and then we determined particle size, ζ-potential and transfection efficiency in CHO-K1 and HEK293T/17 cells, for the complex. We found that the carriers had low critical micelle concentrations and formed nanoparticles with plasmid DNA. The nucleic acid nanoparticles with all the carriers showed low hemolytic activity and cytotoxicity. All the compounds tested were active for both cell lines tested, significantly higher against CHO-K1 cells, except GEM-C_{12}. In particular, GEM-C_{18} was able to transfect 30% - 40% more CHO-K1 cells. In comparison, GEM-C_{18} transfected 67% CHO-K1 cells, obtaining a value similar to the one obtained for PEI (67%) and greater than the one obtained for Lipofectamine 2000 (55%). Further studies on a greater number of cell lines are required to establish specificity of action, to correlate the physicochemical and structural properties for GEM-C_n/pLenti-CMV-GFP Puro complexes with in vitro transfection of CHO-K1 cells, and to contribute to a better understanding of the gene delivery process.

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RECOMBINANT ACID ALPHA-GLUCOSIDASE PRODUCTION IN THE HUMAN CELL LINE HKB-11 CULTURED UNDER SERUM FREE SUSPESION CONDITIONS

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Key Words: Recombinant acid alpha-glucosidase; serum-free suspension culture; human cell line

The current treatment of Pompe disease, a lysosomal storage disorder, is performed by enzyme replacement therapy using a human recombinant acid alpha-glucosidase (rhGAA) produced in Chinese Hamster Ovary (CHO) cells. Despite the satisfactory results, cases of serious immunological reactions have been reported probably due to the differences between the recombinant protein and the native one. There is a growing enthusiasm for the use of human cell lines as a platform for production of recombinant glycoproteins because of their ability to produce such proteins as close as possible to those naturally found in the human body, minimizing possible immunogenic reactions. In this work, we evaluate two strategies to produce rhGAA in the human cell line HKB-11: transient transfection and stable lentiviral transduction. In the transient transfection approach, two conditions of cell concentration and DNA concentration was evaluated, $1x10^6$ cell/mL (2 and 5µg DNA/10^6 cell) and $2x10^6$ cell/mL (1 and 2µg DNA/10^6 cell) with a DNA:PEI ratio of 1:2 (Polyethylenimine, PEIpro®). The cells were cultured and transfected under serum free suspension conditions in mini bioreactor tubes in FreeStyle serum-free medium (Invitrogen). The percentage of positive GFP cells was monitored up to 120 hours post transfection. Percentage above 50% of GFP positive cells was obtained by this method and these values remained high up to 120 hours post transfection (Figure 1). The rhGAA activity remained in the range 19-31 nmol/min/mL during the experiment. For the lentiviral transduction, a multiplicity of infection of 1 (MOI 1) was employed. By this approach it was possible generate a stable recombinant cell line with 90% of GFP positive cells after the sorting (FACS). Preliminary results indicated that these cells were able to produce 11 nMol/min/mL in FreeStyle medium and 9 nMol/min/mL in CDM4CHO medium at 96 hours of culture. The characterization of the kinetics of rhGAA production as well as the kinetics of cell growth and metabolism are in progress.

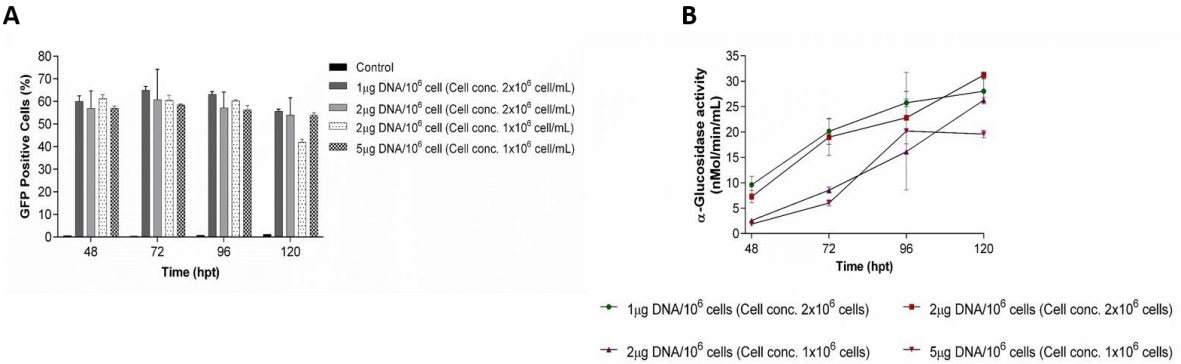

Figure 1. Genetic modification of cells under serum-free suspension conditions and recombinant acid alpha-glucosidase activity. A) GFP positive cells by transient transfection using PEIpro (n=2). B) Recombinant acid alpha-glucosidase activity.(n=2).

INFLUENCE OF MILD HYPOTHERMIA AND GLUCOSE CONCENTRATION ON GROWTH KINETICS AND PRODUCTION OF MAbs IN NS0 CELLS

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Key Words: NS0 cells, mild hypothermia, glucose concentration, Monoclonal antibodies.

Mammalian cells have glycosylation patterns similar to human cells, allowing to obtain biopharmaceuticals for human therapy. However, they have low levels of productivity leading to the search for strategies of optimization for their increase. One of these strategies is the cell culture under mild hypothermia (MH) conditions.
MH has shown positive results, for example, an increase in the longevity of the cell culture and the specific productivity of the product. As there is no exact consensus to explain this phenomenon and the vast majority of studies are in CHO cells, it is new to know precisely what happens in other producer cells of interest such as NS0.
The number of studies on MH in NS0 cells producing Monoclonal Antibodies (MAbs) is poor. The most complete publication on this topic is that of Swiderek and Al-Rubeai in 2007. However, these authors only studied a temperature that is considered as MH so in this sense their study is limited. In addition, in none of these works the combined impact of temperature and glucose concentration (GC) on the growth and production of MAbs in NS0, was study. In contrast, in our study two temperatures were studied within the range considered as MH and two GC values.
To analyze the influence of MH on the relationship between the kinetics of cell growth and the production of the MAb of interest, batch cultures were performed at 37, 33 and 31 °C and two GC (15 and 30 mM) in Spinner Flask with a working volume of 150 mL. Each condition studied was replicated. The methodology of MH used was the shift of temperature. The results obtained showed a significant decrease in the specific growth rate maximum, the maximum cell concentration of the culture and the specific glucose consumption rate with the decrease in temperature and the increase in glucose concentration. On the other hand, the decrease in temperature caused a decrease in the specific production rates of toxic metabolites such as ammonium ($q_{ammonium}$) and lactate ($q_{lactate}$), being more marked at 33 °C than at 31 °C. Considering that only at 33 °C and 15 mM of glucose the cells began to consume lactate and that the greatest reduction in $q_{ammonium}$ occurred (3.7 times, taking as reference 37 °C and 15 mM of glucose), it is possible to suggest that to this condition the metabolism is more efficient. Also, it was observed that the yields of lactate-glucose and ammonium-glucose were practically reduced by half with respect to the reference condition. Due to these facts, in this condition there was a significant increase in the longevity of the culture and specific productivity of the MAb. The results regarding the effect of moderate hypothermia coincide with that reported by several authors for CHO cells.
On the other hand, conditions with 30 mM of glucose showed growth inhibition, which was evidenced by a significant increase in $q_{lactate}$ and $q_{ammonium}$, negatively affecting q_{MAb}. Considering all the above elements, it is possible to say that the work contributes in the Upstream processes development for the production of MAbs in this cell line.

Acknowledgment:
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MANUFACTURING OF CHIMERIC ANTIGEN RECEPTOR T CELLS FOR ADOPTIVE IMMUNOTHERAPY USING XURI BIOREACTOR

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Key Words: Immunotherapy; CAR-T cells; Lymphocyte; Cell therapy; Bioreactor

Cell-based immunotherapy has attracted clinical and pharmaceutical interest over the last few years. In particular, adoptive cellular therapy using chimeric antigen receptor (CAR)-T cells target to CD19 has demonstrated clinical efficacy in several hematologic cancers. The highly demanding cell doses used in clinical trials require a scalable, efficient and GMP-compliant manufacturing process. This work aims to establish a bioprocess to expand immunocompetent T cells transduced with CD19CAR. T-cells were collected by apheresis from healthy donors and isolated by density gradient centrifugation (Ficoll-Hypaque™, Thermo Fisher Scientific). Thereafter, cells were enriched by positive immunomagnetic CD3 selection (StemCell Technologies) and activated with CD3/CD28 beads (Thermo Fisher Scientific) in a proportion of 1:3 (beads/cells). On the next day, the cells were transduced with lentiviral vectors (MOI 5) and cultivated using RPMI culture medium supplemented with 10% of AB human serum, 100 IU/ml of IL-2 (GE Healthcare Life Sciences) in static T-flasks. Activation beads were removed at day 7 and at day 9 the cells were transferred to 1L Xuri bag (working volume of 100 mL) and maintained in static conditions. At day 11, the bag was transferred to the Xuri platform (GE Healthcare Life Sciences) with a working volume of 390 mL under controlled conditions: 10 rpm, 6º and 37ºC. Cell proliferation was monitored daily and percentage of CAR expression was quantified in TCD3, TCD4 and TCD8 populations by flow cytometry. The amount of cells expanded (994 x 10^6 cells) after 14 days are sufficient for 6 infusions (80 kg) assuming 2x10^6 cells/kg. We next evaluated the cytolytic potential of 19CAR-T cells produced, co-culturing with B cell lines Sup-B15 ($CD19^+$) and LAMA-84 ($CD19^-$). A ratio of 10 effector cells for 1 target cell was used. The results showed that our 19CAR-T cells are functionally active and detect only B cells that are CD19+. Further experiments will be performed to improve the expansion bioprocess for therapeutic applications.

RECOMBINANT COAGULATION FACTOR VII EXPRESSION IN HUMAN CELL LINES.

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Key Words: Recombinant coagulation factor VII, serum-free suspension culture, vitamin K, lentiviral transduction, recombinant proteins.

The blood coagulation factor VII (FVII) belongs to the group of vitamin K dependent glycoproteins that is mainly produced in the liver. When blood is exposed to the tissue factor (TF), the activated FVII binds TF and initiate the coagulation cascade. Currently this factor has been used for hemophiliac patients who have developed inhibitory antibodies against conventional treatments (recombinant factors VIII and FIX). The commercial products are all recombinant proteins expressed in mammalian cell lines such as CHO and BHK. Although they have similar structures compared to the native proteins found in humans, they can exhibit non-human post-translational modifications pattern that can cause immunogenic reactions. In this work we evaluate the expression of recombinant FVII in promising novel human cells lines, SK-HEP-1, HUH-7, HKB-11 and HEK 293, cultured under serum-free suspension conditions. SK-HEP-1, HKB-11 and HEK 293 were cultured in CDM4CHO (HyClone) medium and HUH-7 in CD 293 AGT (Gibco), supplemented with 1% Glutamax (Gibco), all cells were maintained in 5% CO_2, 37°C environment and 150 rpm. The cells were generated by lentiviral transduction (MOI=1) and then submitted to a sorting process. Initially two transduction cycles were performe, being the culture then supplemented with vitamin K. The results obtained indicate a high vitamin K cytotoxicity. To overcome this problem, a study was performed to evaluate the cell response to different vitamin K concentrations (0; 0.2; 0.4; 0.6; 0.8; 1.0 µg/mL) in 6-well plates. The results showed that HUH-7 cells were more sensitive to the vitamin K, surviving only in absence of the vitamin and in a concentration of 0.2 µg/mL, while the other cells did not have differences in the curves of growth and viability in any concentration used. Therefore, it has been defined that the cell lines SK-HEP-1, HKB-11, HEK 293, will be adapted with 1 µg/mL, while HUH-7 cells will be adapted only with 0.2 µg/mL of the vitamin K. The next step of this work will evaluate the rFVII expression analysis in the vitamin-K adapted cells.

EFFECTS OF TEMPERATURE SHIFT ON CELL METABOLISM AND PRODUCTION OF ZIKA VIRUS-LIKE PARTICLES (VLPs) IN MAMMALIAN CELLS

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Key Words: mammalian cell culture, Zika virus-like particles (VLPs), temperature, metabolism

Zika virus (ZIKV) is a positive-sense, single-stranded RNA genome virus that belongs to the *Flaviviridae* family. ZIKV infection usually causes no symptoms or mild symptoms, such as mild fever, rash, joint pain and red eyes. In the current decade, however, ZIKV infection has been shown to be associated to neural complications in adults (such as Guillán Barré syndrome) and congenital abnormalities in fetuses from infected mothers. In 2016, Zika virus was considered a “Public Health Emergency of International Concern” by the World Health Organization (WHO) due to its quick spread to dozens of countries and to the complications caused by the disease (WHO, 2016). The best way to protect the population against ZIKV infection is by developing a safe and efficacious vaccine. Different studies involving different technologies have been carried out in the last few years to find an adequate vaccine candidate (Poland et al., 2018). Virus-like particles (VLPs) represent one of these technologies, presenting as advantages the safety and ability to generate good immune responses (Espinosa et al., 2018).

This work had as a main objective the evaluation of the effects of temperature shift in mammalian cell cultures regarding cell metabolism and VLP production. Recombinant CHO-K1 and HEK293-3F6 expressing ZIKV VLPs were cultured in shake flasks at 37°C, 33°C and 30°C. Half of the cultures were grown at the subphysiological temperatures from the start, whereas the other half was kept at 37°C until the third day of cultivation, when when a temperature downshift to 33°C or 30°C was carried out. Cell concentration was measured daily using an automated cell counter (ViCELL, Beckman Couter); and glucose and lactate were measured daily using a biochemistry analyzer (YSI 2700). Cells cultured from the start at lower temperatures reached low cell densities and had high longevity (13-14 days of culture), with no depletion of glucose along the whole period. On the other hand, cells that underwent a growth phase at 37°C and had the temperature downshifted thereafter, experienced glucose depletion on the 4th day, but kept growing for approximately 8 days, regardless of the temperature maintained after the growth phase (37°C, 33°C or 30°C). Amino acid profiles and VLP formation along time will be presented to discuss the importance of temperature effects on VLP production and of amino acids as secondary carbon sources.

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INFLUENCE OF SHEAR STRESS BY GAS ASPERSION IN BACULOVIRUS PRODUCTION BY SF9 CELLS IN BIOREACTOR

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Key Words: Biopesticide, Baculovirus, Bioreactor, STR, Bubble free.

Corn crop has extremely economic importance for representing a major part of human and animal diet, being a high value commodity product. Hence, the difficulties in plantation could cause a great damage, pests, for example, could account for 30% in reduction of production. The application of chemical solutions brings not only a relevant expend but also an important environmental and human health impact. Along with increasing demands in agriculture yield, biopesticides emerge as a pertinent option. Biopesticides are biologically formulated products derived from living organisms or their products. It is used as biological regulator of a species that harms the crop or which has undergone uncontrolled population increase, seeking ecological balance. Brazil is an example of one of the most successful cases of biopesticides field use in the Biological Control Program of the Velvetbean Caterpillar. This was achieved by the production *in vivo* of a baculovirus, a target specific virus, which is a natural controller of Arthropoda phylum. Although effective, it is a labor-intensive method that results in a high-cost, low-competitive product. In contrast, *in vitro* production using cell culture in bioreactor brings the possibility of greater process control, with higher quality and high volume in a smaller space. The aim of this project is to produce *in vitro* SfMNPV baculovirus with Sf-9 insect cells. To increase cell concentration at the moment of infection, different medium supplementation were tested, with cysteine and glutamine, although without positive results. Another approach to enhance cell number and improve virus infection was to minimize shear stress caused by gas aspersion inside the bioreactor.

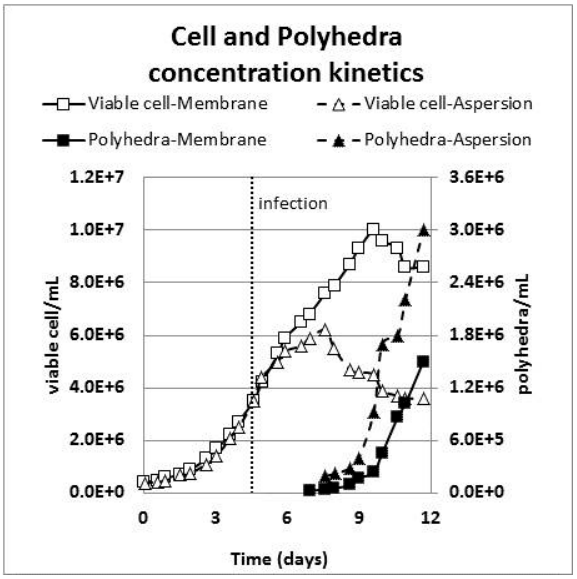

Figure 1: Comparison in cell growth and virus infection between R10(aspersion) and R11 (membrane aeration).

To do so a comparison was made between the SfMNPV baculovirus infection in the regular STR bioreactor with aspersion aeration (BioFlo 110 – indicated as R10) and in a bubble free bioreactor (BioStat B – Sartorius – indicated as R11), equipped of a silicon tubing, which is permeable to gas (membrane aeration). The results indicate that the stress shear may affect more the pos infection phase, since the maximum specific growth rate (μ_{max}) was similar (0.59 and 0.53 day^{-1}), but the maximum cell concentration reached was significant higher in membrane aeration (R11 - $10.0x10^6$ cells/mL) in comparison with aspersion aeration (R10 - $6.2x10^6$ cells/mL). Although both runs reached 100% of cells with occlusion derived virus (polyhedron) in their inside visually, R10 produced $5.0x10^6$ polyhedra/mL and R11 $3.0x10^6$ polyhedra/mL. In conclusion, shear stress by gas aspersion causes great impact on post-infection cell growth.

3D CULTURE OF THE RT4 CELL LINE IN THE TAYLOR VORTICES BIOREACTOR

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Key Words: 3D culture, spheroids, bioreactor, Taylor vortices flow, *in vitro* cancer models

Nowadays, cancer has been responsible for a high number of morbidity and mortality in a global scale. From the anticancer drugs that have been developed, 95% of them fails during clinical trials despite the indication of activity presented in the pre-clinical *in vitro* tests. This occurs because these drugs are tested in a 2D models (monolayer), and tumors in the organisms grow in a 3D microenvironment with a morphology similar to spheroids (ACHILLI; MEYER; MORGAN, 2015). Because of this, 3D culture methodologies have been studied and developed for tumor cell lines. There are already a variety of methodologies to produce spheroids *in vitro*, but to produce high quantities of spheroids a dynamic culture using a bioreactor is recommended. The problem of using bioreactors is that cells are exposed to high shear stress. Trying to avoid this problem, other types of bioreactors have been developed and it is suggested on of the being the Taylor vortices bioreactor (TVB). The advantages of the TVB are that they generate low shear stress compared with the conventional spinner bioreactor (NEMRI et al., 2013). Other advantages to the use of bioreactors are the possibility to increase, low handling to the operator and the possibility of long time cultures. For the reasons mentioned, the TVB (Figure 1A) was uses to test the production of spheroids from the RT4 cell line, a bladder tumor type of cell using the McCoy medium. The agitation methodology adopted was intermittent during the first 6 hours (5 minutes agitating and 55 minutes stationary) and continuous afterwards. The speed rotation was 110 rpm, the work volume of 100 mL, the initial cell concentration was $2{,}5 \cdot 10^5$ cell·mL^{-1}, the pH was maintained between 7,0 to 7,4, the temperature 37 ºC and the CO_2 concentration was between 10 to 20%. In these conditions, it was possible to obtained productivity around 100 spheroids·mL^{-1}·day^{-1}, 85 ± 70 µm of medium diameter and sphericity around 0,80 ± 0,10. These results indicate that a high number of spheroids can be obtained utilizing the Taylor vortices bioreactor, and that with further study in this bioreactor, it may be possible to obtain spheroids with higher diameter and higher quantities.

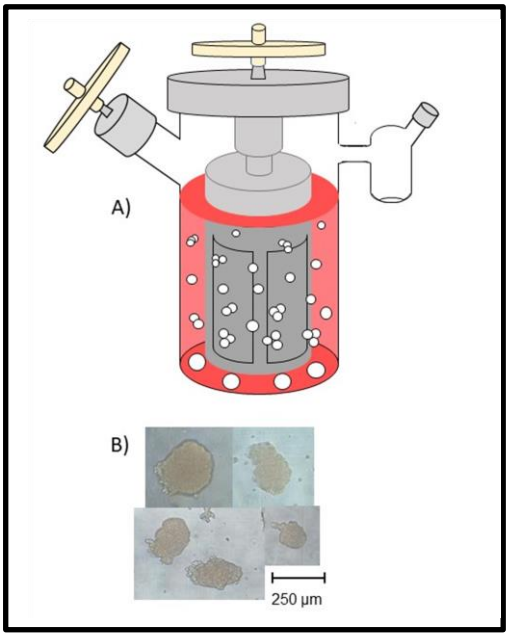

Figure 1 – A) Model of the Taylor vortices bioreactor utilized to produce the RT4 spheroids. B) Image of the spheroids obtained in the bioreactor.

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THE BOTTLENECKS TO DEVELOP AN EFFICIENT SCALING UP PROCESS USING *DROSOPHILA* S2 CELLS SYSTEM

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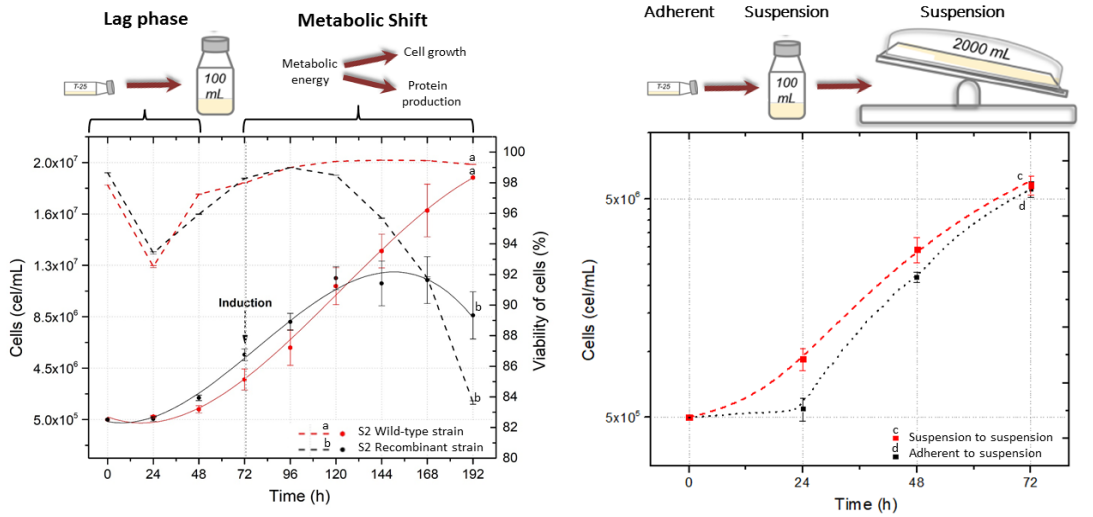
Key Words: *Drosophila* S2 cells; S2 cells system; scaling-up process; recombinant protein production; high-level production.

The *Drosophila* S2 cell system has been successfully used as a robust platform to produce a series of complex recombinant proteins. Recently, it has been demonstrated that these cells are able to support the production of vaccines, such as dengue virus, HIV-1 virus and rabies[1,2]. Thus, to achieve high-level production using the S2 expression platform, it is imperative to develop efficient large-scale processes for S2 cells culture[3]. In this context, the objective of this work was to identify the main bottlenecks in the scaling up the culture process of S2 cells carrying inducible promoters, aiming to design a high-performance bioprocess for heterologous proteins production. For this purpose, a robust recombinant lineage engineered by our group for the expression of rabies virus glycoprotein (rRVGP) using a copper-inducible promoter was cultivated in T-flaks, Schott flasks and in a 2 L Cellbag in a WAVE Bioreactor™ 2/10. Cell culture conditions were the same in all cases: inoculum of 5×10^5 cells/mL, Sf900-III culture medium, culture induction with 0.7 mM of CuSO4 at 72 h, 28 °C in a pH range convenient both for cell growth and rRVGP production (6.2–7.0) and no nutrient exhaustion. The first bottleneck identified was attributed to a metabolic shift in cells after cell induction aiming to prioritize the production of foreign proteins at the expense of cell growth (Fig. 1). If the induction time was not carefully defined, limitations on cell growth and acceleration of the transition from exponential to stationary phase, even at low concentration of cells in the bioreactor. The second bottleneck was observed when changing the culture from semi-adherent monolayer to suspension. This change resulted in an unproductive time in the process, with a large lag phase, given that cell viability decreased significantly in both strains. Therefore, these two bottlenecks can significantly affect the development of bioprocesses involving S2 cells. A complete understanding of both situations can be very helpful in overcoming threats to the successful scaling up of the bioprocess.

Fig. 1: Bottlenecks in the *Drosophila* S2 cells system during heterologous protein production.



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DESIGN OF EXPERIMENT APPROACH FOR THE EVALUATION OF CELL CULTURE PROCESS ROBUSTNESS

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Key Words: cell culture robustness, productivity, product quality, design of experiment

Robustness is a desirable feature of any cell culture manufacturing process. It can be defined as the ability of a process to tolerate variability of materials and changes of the process and equipment without negative impact on quality.

The objective of this study was to investigate the effect of perfusion rate and glucose concentration in the cell culture medium for the production process of a highly glycosylated protein expressed in a recombinant CHO cell line. Cell growth, productivity, downstream processing performance and quality of active pharmaceutical ingredient batches were measured for each experimental condition defined using a factorial 2^2 experimental design. Controlled bioreactor cultures were established in a 5 l bioreactor in perfusion mode. Daily samples were taken, and after each run a three-step downstream process was carried out for obtaining the pharmaceutical grade recombinant protein.

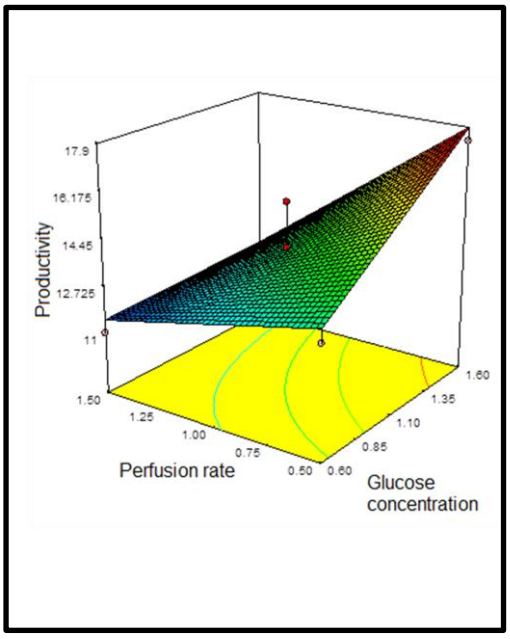

Figure 2

Perfusion rate and glucose in cell culture medium did not affect product quality in all the design of space, since protein glycosylation, potency, host cell proteins and oligomers content complied with the specifications. However, maximum cell density, growth rate and productivity were significantly affected by changes in those operative parameters (Figure 1).

These studies suggest that the design of experiments applied to cell culture processes has the potential to demonstrate robustness and significantly increase productivity through process optimization, with assurance of product quality consistency.

EVALUATION OF CULTURE CONDITIONS FOR CHINESE HAMSTER OVARY (CHO) CELLS SCALE-UP

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Key Words: Scale-up, CHO cells, monoclonal antibodies.

Monoclonal antibodies (mAbs) are the basis of several treatments for high-impact diseases including inflammatory conditions such as asthma and rheumatoid arthritis, lupus, osteoporosis and several types of cancer. Different mammalian cell lines are industrially used to produce monoclonal antibodies; among them, Chinese Hamster Ovary (CHO) cells are the most common because of their efficient post-translational processing of complex proteins, the high similarity between the glycosylation patterns of native recombinant proteins and CHO-derivatives and their robust behavior in bioreactor. In this work, we evaluated the behavior of six different CHO clones expressing one of two different monoclonal antibodies (three each). Temperature, osmolality, pH, agitation speed, and media composition were evaluated in three experimental stages at increasing working volumes, to explore the process design space for antibody cell culture.

In the first stage, we used 6-well plates with a working volume of 3 mL. The six clones were cultured in CD FortiCHO™ medium for 5 days; a Box Bhenken response surface design was used to evaluate shaking speed, osmolality and initial pH at 37°C and 8% CO_2. Response variables included final cell concentration, antibody titer, mAb specific productivity, and lactic acid specific productivity. Optimal conditions were clone-dependent. Subsequently, two additional levels of temperature were evaluated for every clone at the optimal conditions. To check the effect of the medium composition, a series of 7 days long, batch cultures were performed using two different commercially available Chemical Defined Culture Media (CD FortiCHO™ and Dynamis™). Clone-dependent behavior was observed in the two media. Same behavior occurred with specific growth rates; however, the yields of biomass and lactic acid based on glucose were significantly higher for FortiCHO™.

The second stage was performed at 40 mL working volume in baffled Erlenmeyers using Dynamis™ medium. Batch cultures resulted in about 25 to 35% reduction of the maximum cell concentration and an increase in the specific glucose consumption rate respect to 3 mL cultures for every clone. A discrete, supplementary addition, every two days from day 4 up to day 12, of a EfficientFeed C+ AGT™ feed stream, amounting from 5 to 12.5% of the original culture volume, was then evaluated; five out of six clones improved their maximum cell concentration when the addition amounted 8,75% of the initial volume, reaching in one case up to 3.5×10^7 cells·mL^{-1}; however, better specific mAb production rates (11 to 24 pg·cell^{-1}·h^{-1}) were obtained when addition amounted 12.5% of the initial volume, the third stage was performed at 200 mL working volume in spinner flask for only one of the clones. 90, 120 and 150 rpm were evaluated. A maximum cell concentration of 0.84×10^6 cells·mL^{-1} was observed at day three; afterwards, both cell concentration and viability went down, reaching 40% viability after 8 days of culture; since glucose concentration was never below 3,5 g·L^{-1}, but lactate went up to 2.1 g·L^{-1}, we suspect oxygen limitation might be causing this behavior. At present we are evaluating k_La as a scaling-up strategy for mAb production.

REVERSIBLE MICRODEVICE FOR MONITORING ANIMAL CELL

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Microfluidics is the science that works with the manipulation of small amounts of fluids in channels with micrometric dimensions. Its purpose is to remodel conventional procedures, through systems that present more compact and versatile forms. Microfluidic devices allow the construction of different geometries with different materials and different techniques. Therefore, a wide range of microfluidic platforms have been applied in different areas such as: chemical engineering, pharmaceutical, modern medicine and biotechnology. Microfluidic devices are ideal tools for creating and controlling concentration gradients, thus reducing waiting time to choose an ideal concentration. These microenvironments can be artificially created to investigate cell behavior. The use of concentration gradients allows the evaluation of different cultivation conditions, with simultaneous information acquisition, reducing stages of the process, and consequently it minimizes the repetition time of experiments, reagent expenses and possible operation errors. The technique commonly employed for the manufacture of microfluidic devices is soft lithography, which involves many steps and some of them quite complex .This technique creates devices that are not reversible, which ends up being a great disadvantage of these devices, besides sterilization for the use of animal cells .Due to this, most irreversible devices become disposable, which results in a time of manufacture and high cost. In this context, the present work aims to construct reversible devices for the cultivation of animal cells, using the CO_2 laser ablation technique and as a commercial polydimethylsiloxane material (PDMS laminated). The devices were evaluated operationally (flow test and diffusive gradient generation) and biologically (cell behavior analysis). The results show that reversible devices are a promising alternative for the study of animal cells in microfluidic.

Key Words: microfluidics, concentration gradient, cell, animal cells.

Design and characterization of a novel dimeric TNFα inhibitor

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Key Words: TNFα inhibitor, dimerization domain, VEGF.

Neutralization of TNFα and/or blocking its binding to receptors has become a major strategy in the therapy of inflammatory diseases, such as rheumatoid arthritis, Crohn's disease, psoriatic arthritis, and psoriasis. Among the antagonists actually approved by FDA are engineered monoclonal antibodies and fusion proteins containing the extracellular domain of TNFα receptors (TNFR) linked to the Fc portion of human immunoglobulin (etanercept) [1]. The dimeric nature of these biologic inhibitors increases its affinity by 50 to 1000 folds in comparison to soluble monomeric forms [2]. We report a novel dimeric TNFα inhibitor, a fusion protein composed by of TNFR2 extracellular domain and VEGF dimerization domain. The last one includes point mutations that decrease its binding to its receptor and its biological activity without affecting its dimerizing properties. *In silico* systematic mutations on VEGF amino acid sequences were performed using BeAtMuSiC server, in order to identify key residues in VEGF which mediates binding to its receptors, KDR and FLTR-1. VEGF-receptor complex interface residues were identified by a difference superior to 5% in solvent accessibility between the complex and the free structure[3]. Two new mutations that decreased VEGF binding to both receptors were identified. Y21P (ΔG = 5.89Kcal/mol; 4.02 kcal/mol) and Y25G (ΔG = 5.24Kcal/mol; 5.29 kcal/mol), were included in the recombinant molecule. The fusion protein (TNFR2-VEGF*) coding sequence was cloned in the pEntry vector and expressed in stably transfected Chinese hamster ovary (CHO) cells.

Purified TNFR2-VEGF* didn´t show *in vitro* significant angiogenic activity quantified by endothelial cell tube formation on gelled basement membrane extract assay[4]. This suggests no biological activity of the mutated VEGF dimerization domain. Our fusion protein binds TNFα with the same affinity of etanercept (anti-TNFα fusion protein approved by FDA), without significant statistical differences in their Kd values, determined by thermophoresis assay. Furthermore, TNFR2-VEGF* exhibited similar biological activity to etanercept in inhibiting TNFα induced cytotoxicity on L929 cells *in vitro*.

These results make TNFR2-VEGF* a novel anti-TNF-α drug candidate, being the VEGF* dimerization domain a new strategy for the production of dimeric recombinant biomolecules.

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DESIGN, PRODUCTION AND CHARACTERIZATION OF A TNFα-BLOCKING TRIMERIC TNF RECEPTOR 2 (TNFR2) ECTODOMAIN

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Key Words: Tumor necrosis factor alpha, TNFα antagonist, protein designs.

Tumor necrosis factor alpha (TNFα) is a pro-inflammatory cytokine with a central role in pathogenesis of autoimmune diseases such as rheumatoid arthritis, psoriasis and *Crohn's* disease among others. Therefore, neutralization of TNFα has become a very successful therapeutic strategy for the treatment of these pathologies.
The main goal of this work was to develop a new TNFα neutralizing molecule. Taking into account that the ectodomain of the TNF receptor 2 (TNFR2) has been used to block TNFα and TNFR2 is a trimmer membrane protein in the nature, we designed a chimeric protein that comprises the ectodomain of TNFR2 attached to the trimerization domain of human collagen XV.
The chimeric protein (3cTNFR2) was stably produced in HEK-293 cells, and it was successfully purified by IMAC from cell culture medium. 3cTNFR2 monomers were detected as a 50-kDa protein in SDS-PAGE; dimers and trimmers of the protein were detected by non-reducing SDS-PAGE. There was performed a microscale thermophoresis assay which determined that 3cTNFR2 interacts with TNFα with a Kd of 18.51±7.22 nM. Finally, in an *in vitro* assay using L929 cells, 3cTNFR2 neutralized the cytotoxic activity of TNFα.
This work is the first report about the development of a trimmer TNFR2 to block TNFα. Although further studies are required to demonstrate the anti-inflammatory effect of 3cTNFR2, to date our results suggest that 3cTNFR2 has the potential to be used as TNFα neutralizing agent.

PRODUCTION OF BIOLOGICALLY ACTIVE HUMAN TNFα IN E. COLI SHUFFLE T7 EXPRESS

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Key Words: Human TNFα, Protein expression, Microscale thermophoresis.

TNFα is an inflammatory cytokine with many actions that are central to the pathogenesis of autoimmune diseases, and the neutralization of TNFα is a therapeutic strategy for the treatment of these pathologies. However, the discovery of new TNFα antagonist molecules is still a challenge, and the first step in this challenge is the production of sufficient amount of biologically active TNFα.
Our main goal in this work was to produce and purify human TNFα from *E. coli*. For that, soluble human TNFα gene was cloned in pET-22b(+) between NdeI and XhoI sites in frame with a 6xHis tag; the resulting plasmid was transformed into *E. coli* T7 SHuffle. TNFα expression was induced with IPTG 0.1 mM for 6 hours at 30°C. The bacterial rupture was performed using a French press, and then TNFα was successfully purified by IMAC from soluble fraction. TNFα monomers were detected as a 17-kDa protein in SDS-PAGE and western blot against 6xHis tag.
In order to check the quality of the TNFα produced, a comparison was made with a commercial TNFα. In first place, the binding with Adalimumab was characterized by microscale thermophoresis; homemade TNFα and commercial TNFα interact with Adalimumab with a Kd of 6.79 ± 3.07 nM and 13.36 ± 4.52 nM respectively. In second place, the biological activity of TNFα was demonstrated in an in vitro assay on L929 cells, there both molecules induced cell cytotoxicity but homemade TNFα was more potent than commercial TNFα in the same conditions.
These results demonstrate that the production of biologically active TNFα in *E. coli* was successful and it may be used to search new TNFα-antagonist molecules.

IDENTIFICATION AND CHARACTERIZATION OF NOVEL TNFα-BLOCKING PEPTIDES

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Key Words: TNFα antagonist, peptide phage display.

TNFα is an inflammatory cytokine with a central role in pathogenesis of autoimmune diseases, such as rheumatoid arthritis, psoriasis and *Crohn's* disease among others. Hence the neutralization of TNFα is a viable strategy for the treatment of these pathologies. However, the discovery of new TNFα antagonist molecules remains to be a challenge.

The main objective of this work was to identify new TNFα antagonist peptides. For that, a peptide-expressing phage display library was constructed by Kunkel mutagenesis with a resulting diversity of $5.8x10^9$. The screening of the phage library was performed against immobilized human TNFα, and after rounds of selection, individual clones of phages were isolated. The phage clones were sequenced and two different peptides were identified.

In order to check that the identified peptides bound TNFα, these were obtained by chemical synthesis with a biotin tag to the peptide detection. The binding assay was performed by ELISA and the K_d of the interaction TNFα-peptides was determined by microscale thermophoresis, it result in 48.12 ±12.6 μM and 64.57 ± 14.26 μM for each peptide. Finally, the neutralization of TNFα was studied in an *in vitro* cytotoxicity assay using L929 cells; here both peptides were capable of neutralizing TNFα cytotoxicity.

Furthermore, molecular docking studies have been realized to predict the binding sites of the peptides to TNFα. Currently, we are working in the implementation of modifications to the peptides to increase the affinity to TNFα and to increase the half-life of the peptides for its utilization as therapeutic molecules.

EFFECT OF N-GLYCOSYLATION ON RECOMBINANT HUMAN INTERFERON ALPHA IMMUNOGENICITY

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Key Words: Biobetters, Therapeutics proteins, Immunogenicity, Quality control, N-glycosilation.

Recombinant human Interferon alpha 2b (rhIFN-α2b) is a therapeutic protein used for treatment of a variety of human viral diseases and cancers. Repeated dosing of IFN over several months induces neutralizing antibodies (NAb) against the therapeutic in up to 80% of patients. Moreover, type I IFNs can both induce and unmask sub-clinical autoimmune diseases. This undesired immune response may impose a limitation for its clinical use. In addition, its short circulating half-life constitutes another limiting factor for IFN-α therapy. To circumvent this inconvenient, highly glycosylated IFN variants were developed and designated as: IFN-2NM47/95, IFN-3NM47, IFN-3NM47/95 and IFN3NM47 Nter. These new molecules showed different glycosylation patterns and improved pharmacokinetic properties compared with the original molecule. However, no immunogenicity analysis was carried out to evaluate product safety. For this, the aim of this study was to investigate the immunogenicity of these new IFN variants through a comparative ex vivo study that also included the unmodified IFN version (IFN-WT).

IFN variants were produced in CHO-K1 cell supernatants and purified by immunoaffinity chromatography. For ex vivo assays, blood samples were collected from 12 healthy donors and peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Hypaque. HLA-DR1 allotypes were determined by Luminex technology. Monocytes were isolated by plastic adherence, differentiated into immature dendritic cells (iDCs) and incubated with each IFN variant. Upon maturation with recombinant TNF-α, antigen-pulsed DCs were incubated with autologous T-cells. After 48-72 h, supernatants were collected and assayed for IFN-gamma and IL-4 production by sandwich ELISA. A stimulation index (SI) criteria was defined as the ratio of cytokine concentrations from protein challenged PBMCs and unchallenged PMBCs (culture media). A response was considered positive when $SI \geq 2$.

T-cell proliferation assays showed that all tested proteins exclusively induced IFN-gamma production, but in different levels in a protein and donor-depending manner. In particular, HLA-DRB1*08, HLA-DRB1*09, HLA-DRB1*13 and HLA-DRB1*16 alleles were directly involved in IFN-derived peptide presentation. Also, a comparative analysis revealed that 3NM47/95 was the variant which exhibited the higher immunogenicity with 42% of positive responses. In contrast, IFN-3NM47-Nter was less immunogenic than the other IFN variants (25% of responders). In addition, a similar proportion of responders (33%) was observed for IFN-2NM47/95, IFN-3NM47 and IFN-WT. It is important to highlight that IFN-3NM47-Nter is the most glycosylated IFN version. Therefore, this suggests that higher glycan contents played a role in antigen recognition, processing and/or presentation.

Considering the reduced immunogenicity for IFN-3NM47-Nter observed here and its superior pharmacokinetic properties, altogether these results highlight IFN-3NM47-Nter as a promising candidate for clinical use in antiviral therapy.

CYTOTOXICITY AND CELLULAR PERMEABILITY EVALUATION OF POLYMERIC NANOPARTICLES CONTAINING RIVASTIGMINE USING CACO-2 CELLS

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Key Words: cytotoxicity, permeability, drug carrier, nanoparticles, Caco-2.

The use of nanoparticles offers a promising perspective to encapsulate and transport drugs through blood-brain barrier (BBB) improving their bioavailability for brain diseases. Nano-bio interface and the behavior of nanoparticles in biological environment are important biophysicochemical characterization. To date, no standard screening method has been established for nanoparticles toxicity assay due to the variability of materials and cell types. This work proposed to evaluate (i) the cytotoxicity and (ii) the cellular permeability of the drug rivastigmine encapsulated in poly(methyl methacrylate) (PMMA) and poly(methyl methacrylate co acrylic acid) (PMMA-co-AA) nanoparticles using Caco-2 cell monolayers. The nanoparticles were synthesized with 7.5 %w/w acrylic acid using miniemulsion polymerization methodology. The encapsulation of 0.3 %w/w of the drug was carried out in situ. Peptide TAT was conjugated to P(MMA-co-AA) nanoparticles using EDC, aiming to increase their penetration through the BBB. Nanoparticles of 75 nm with 96% efficiency of rivastigmine encapsulation and 70% bioconjugation efficiency were obtained as an aqueous suspension. Cytotoxicity assay used confluent Caco-2 cells treated with encapsulated rivastigmine (0.11 – 250 µg/mL) for 72 h at 37 °C. Untreated, drug-treated and non-encapsulated nanoparticles were used. Cell viability was determined by quantitation of ATP (Celltitter Glo kit) and the luminescence was measured using FlexStation 3. CC_{50} value was determined by linear regression. Cellular permeability test used confluent Caco-2 cells (2 x 10^5 cells/well) seeded in Transwell® for 3 days followed by incubation with 100 µg/mL P(MMA-co-AA) nanoparticles for 4 hours. Non-encapsulated nanoparticles, EDTA treated and untreated cells were used as controls. The permeability was calculated by fluorescence reading in SpectraMax 190 Microplate Reader. The results showed that although the free drug presented no cytotoxicity effect (CC_{50}> 250 µg/mL), CC_{50} value for nanoparticles with or without drug was approximately 52 µg/mL, showing more toxicity to Caco-2 cells (Table 1). The permeability of EDTA treated cells was statistically similar (± 25%) to the nanoparticles formulations (Table 2). We conclude that (i) cytotoxic response could be associated with the nanoparticle material or with the dispersive medium components, (ii) the immobilization of TAT did not show a significant influence to the nanoparticles transport and (iii) Caco-2 cells demonstrated satisfactory sensibility and reproducibility for the evaluation of nanoparticle biocompatibility and permeability studies.

Table 1 - Cytotoxicity in Caco-2 cells.

CC_{50} (µg/mL rivastigmine) ± standard deviation	
Drug	> 250
PMMA	52.31 ± 7.46
P(MMA-co-AA)	34.52 ± 10.80
PMMA + Drug	52.86 ± 9.28
P(MMA-co-AA) + Drug	51.26 ± 16.76

Table 2 - Permeability in Caco-2 cells.

Cellular Permeability (%) ± standard deviation	
EDTA Treated Cells	24.40 ± 0.92
P(MMA-co-AA)	27.10 ± 2.13
P(MMA-co-AA) + TAT	23.64 ± 0.27
P(MMA-co-AA) + Drug	24.05 ± 0.03
P(MMA-co-AA) + Drug + TAT	23.60 ± 1.32

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CHARACTERIZATION OF A IGG TYPE MONOCLONAL ANTIBODY BY MASS SPECTROMETRY: TECHNIQUE EXPLORATION FOR BIOPHARMACEUTICAL PRODUCT ANALYSIS

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Key Words: Biopharmaceuticals, mass spectrometry, monoclonal antibody, glycosylation, peptide mapping

Biopharmaceuticals have large structural complexity that influences their quality as health products. Regulatory agencies demand for its registration extensive structural characterization, with use of analytical methods in the state of the art. Among them, mass spectrometry stands out for its vast range of applications, reaching diverse levels of structure information, being widely used for this purpose. This present work aims the characterization of a commercial monoclonal antibody with mass spectrometry under different approaches. The sample was obtained by purification of anti-IL-8 antibodies enriched supernatant of CHO DP-12 cell line culture. The antibody was precipitated by solvent addition and submitted to: (1) molecular weight analysis of intact structure by MALDI-TOF; (2) tryptic digestion in solution and SDS-PAGE gel and LC-MS analysis; (3) antibody N-deglycosylation by PNGase F, purification of its free glycans and MALDI-MS analysis by three different setups. As results, an experimental average molecular weight of 150594 Da was obtained, with an error smaller than 0.1% in comparison with its theoretical mass. LC-MS analyses showed a global sequence coverage of 70.9%, with higher performance observed in solution digestion and nanoLC analyses, with the identification of the glycosylation consensus site. The glycosylation profile by different analytical approaches showed complex fucosylated glycan as the majority species. Structural data obtained of this work was endorsed by literature, corroborating the mass spectrometry utility for characterization of biopharmaceutical molecules.

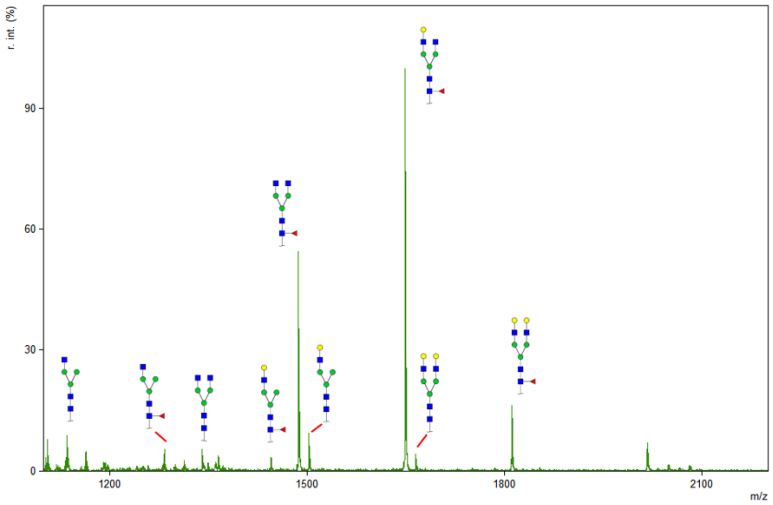

Figure 1 – Mass spectrum of PNGase F free glycans from monoclonal antibody

DEVELOPMENT OF STRAIGHT-THROUGH INTEGRATION OF CLARIFICATION AND CAPTURE OF MONOCLONAL ANTIBODIES

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Key Words: Monoclonal antibody purification, flocculation, anion-exchange chromatography, single-pass tangential flow filtration.

The monoclonal antibody (mAb) market has been presenting a significant growth rate in the last two decades, which increased the interest of biopharmaceutical companies in this product class. Many improvements have been achieved in the upstream processing of mAbs, leading to significant increases in bioreactor titers. However, the production costs are still high, specially due to downstream processing costs, which can represent a major part of the of overall production costs.

Traditional mAb platform processes include a very selective, but high-cost Protein A (PrA) affinity chromatography as the first purification step (capture). Several approaches have been recently explored in order to replace PrA chromatography. In this work, we propose a new, low-cost strategy for integrating clarification and capture step for mAbs using flocculation followed by a straight-through process with single-pass tangential flow filtration (TFF) and suspension anion-exchange (AEX) chromatography.

First, the recombinant anti-IL8 mAb were produced by CHO-DP12 cells (ATCC, USA) in shake flasks at 180 rpm and 37°C using TC-LECC medium (Xell, Germany). After harvest, cells were flocculated using 5 pg per total cells at pH 6.5, allowing 15 min for settling of cells. Subsequently, the resulting supernatant and a Q-Sepharose resin (GE, Sweden) were pumped in equal amounts to a vessel, where a residence time for AEX adsorption of 15 min was applied, with the aim of allowing contaminants to adsorb to the resin. The resulting supernatant/AEX resin suspension was pumped out of the vessel into a 0.22-µm hollow fiber system (GE, Westborough, MA, USA). The mAb was recovered in the permeate, whereas the AEX resin remained in the retentate and could undergo elution, regeneration and sanitization for reusing. Two process variations were evaluated, which were combined resulted in 4 different process strategies: (i) the ratio of clarified supernatant to AEX resin; (ii) the use of an inclined lamella settler (Biotechnology Solutions, USA) after the flocculation step to ensure a cleaner supernatant and to allow reducing the cell settling time.

The integrated clarification-capture process showed to be simple and fast. Steady-state conditions were obtained during adsorption and filtration for all conditions studied. The average recovery of mAb during the steady-state was 48.5% ± 2%, which means a loss of approximately 3% of mAb product, since it was 2-fold diluted by the 1:1 mix with the resin suspension. However, considering the overall process, from start to final permeate recovery, global yields between 61% and 85% were obtained. These results are mainly related to the void volume of inclined lamella settler. The best global recovery was obtained when the settler was not included in the process.

Regarding impurities removal, in all 4 process strategies evaluated more than 85% of DNA was removed, and approximately 50% of HCP removal could be achieved. Taking into account that two different supernatant/AEX resin ratios tested, a lower supernant/resin ratio (41) provided a higher DNA clearance (34 fold), compared to less than half of this clearance when sample/resin ratio was doubled to 82. New process strategies will be evaluated aiming to avoid the large settler void volume and simultaneously adopt a lower supernant/resin ratio.

EXPRESSION, PURIFICATION, AND CHARACTERIZATION OF RECOMBINANT hIFN-α2b USING THE *E. Coli* EXPRESSION SYSTEM.

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Key Words: Interferon-α2b, *Escherichia coli,* soluble, antiviral activity, API.

Human alpha interferon (hIFN-α) is the largest family of type I interferons that contains 13 subtypes. These have an important role in the defense against viral infection thanks to its antiviral, antiproliferative and immunomodulatory functions, it also has anticancer and antifibrinolytic activity. The subtype hIFN-α2b, due to its structural characteristics and its high affinity to specific cellular receptors, is a therapeutic agent approved by the Food and Drug Administration (FDA), which has been used for the treatment of viral and oncological diseases.

The objective of this research was to develop an efficient production process for the pilot level of recombinant hIFN-α2b as a future active pharmaceutical ingredient (API). By establishing the optimum culture conditions for a 5 L fed-batch fermentation using *Escherichia coli* as a recombinant system to overexpress the soluble hIFN-α2b, 92% expression of this protein in the soluble fraction was obtained. A laboratory-scale purification procedure based on tangential flow ultrafiltration, mixed mode and cation exchange chromatography was standardized, obtaining a purified protein concentration equal to 335 mg starting from the fermentation of 5 L until the end of the process. SEC-HPLC analysis was performed, also a qualitative and quantitative analysis with RP-HPLC to analyze the purity of the molecule, resulting in a purity greater than 90%. For the determination of yields and quantification of hIFN-α2b in each stage, a sandwich ELISA assay was used. The concentration of hIFN-α was determined based on a calibration curve constructed from a commercial hIFN-α standard, obtaining a recovery at the end of the process of 15%. The antiviral activity of the recombinant hIFN-α2b was checked by *in vitro* assay in the Hep-2 cell line with the analysis of the cytopathic effect with Mengovirus; resulting in a specific activity similar to the commercial standard.

From these data, it is concluded that the recombinant hIFN-α2b obtained by this process is biologically active with a similar antiviral activity as the commercial products. In this way, you can obtain a pharmaceutical ingredient that will allow the development of different formulations with innovative therapeutic strategies.

PRODUCTION AND PURIFICATION OF FULLY BIOACTIVE RECOMBINANT HUMAN EPIDERMAL GROWTH FACTOR (hEGF) IN PICHIA PASTORIS.

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Key Words: hEGF, P. pastoris, ultrafiltration, chromatography, proliferation.

The human Epidermal Growth Factor (hEGF) is a cytokine that stimulates migration and proliferation of epithelial cells and fibroblasts. Due to the hEGF mitogenic potential, is used as a healing agent in the treatment of acute and chronic wounds in skin, gastrointestinal tract and renal tissue. Therefore, hEGF plays an important therapeutic role, however currently it can be produced as a recombinant protein using costly processes. Because of this, a great interest has arisen for developing cheaper and more efficient methods to produce hEGF. The objective of this work was standardize a process for hEGF production and its purification from *P. pastoris*, using a 3-steps purification system based on ultrafiltration and ion exchange chromatography. The hEGF gene was cloned into yeast expression vector under control of AOX1 promoter. Then, the cassette of expression was transformed and integrated into *P. pastoris* genome by homologous recombination. Positive clones with successful insertion of hEGF gene were identified by dot-blot. A positive clone was selected and it was standardized a 5 liters fed-batch fermentation process for hEGF expression. After 75 hours of induction with methanol, hEGF expression reached a concentration of 100 mg/L into the culture supernatant. Later, it was standardized a purification process from clarified culture supernatant. The downstream process includes a tangential flow ultrafiltration step in a 10 kDa cassette, ion exchange chromatography step and mixed mode chromatography step. The standarized process, allowed the production of high yield (70%) recombinant hEGF with high purity (≥90%). The purity of recombinant hEGF was confirmed by Tricine/SDS-PAGE, RP-HPLC, SEC-HPLC, and yield was determined by a commercial ELISA/sandwich assay. On the other hand, it was observed that the cell proliferation activity of the purified recombinant hEGF was higher than the commercial hEGF with a EC_{50} of 6,9 ng/ml in BALB/3T3 A31 fibroblast. Furthermore, purified hEGF was able to improve cell migration in an *in vitro* wound healing assay performed in mouse fibroblast. Overall, the results demonstrate that the optimized upstream and downstream process enable the production of high purity of recombinant hEGF biologically active.

CHARACTERIZATION OF YFV AND ZIKV VIRUS-LIKE PARTICLES (VLPs) PURIFIED BY A TWO-STEP CHROMATOGRAPHY PROCESS

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Key Words: virus-like particles (VLPs), yellow fever virus, zika virus, chromatographic purification, characterization.

Recent outbreaks of yellow fever virus (YFV) and zika virus (ZIKV) in several countries have demonstrated that these viruses represent a serious threat to global public health. The high mortality rate of YFV infection, and the association of ZIKV with Guillain-Barré syndrome and with congenital malformations in fetuses from infected mothers make outbreaks of these viruses in endemic and non-endemic areas potentially catastrophic. The need for a mass immunization alternative to allow a rapid response in case of new outbreaks or to keep a permanent high vaccination coverage in endemic areas is urgent.

Although the main transmission route for zika virus is by infected mosquitoes, it is known that this virus can remain infective in body fluids for long periods and can be transmitted via the sexual route, which can lead to the transmission of the disease even to non-endemic areas. To this date, there is no approved vaccine or drug to prevent or treat ZIKV infections. Yellow fever live-attenuated vaccines are available since the 1930's. They are safe and provide life-long immunity against infection, but since they are made from the attenuated virus, it can cause rare, but serious (fatal) adverse effects. Moreover, the vaccine production platform is based on virus propagation in specific pathogen-free eggs, making production scale-up difficult and impairing a rapid response in case of outbreaks.

Virus-like particles (VLPs) are tridimensional structures spontaneously formed from recombinant viral structural proteins, which mimic the native virus conformation. They lack the viral genome and present the antigen in a repetitive way, being a promising alternative to other vaccine platforms (e.g. to live-attenuated virus).

Virus and VLPs are usually purified by sucrose gradient ultracentrifugation, which is difficult to scale-up. In this work we investigated a chromatographic purification process for YFV and ZIKV VLPs directly from HEK293-derived cell culture supernatant, adapting the previous process established by Pato (2015) for whole YF virus produced in Vero cells.

Prior to chromatography the supernatant was clarified by either filtration or centrifugation. The purification was done by two steps of chromatography. A bind-and-elute anion exchange chromatography was first performed using a Q membrane adsorber (Sartorius), in order mainly to concentrate the VLPs from the supernatant. Then a flow-through multimodal chromatography step using the CaptoCore 700 resin (GE) was performed, aiming at the removal of host-cell proteins (HCP) and monomeric proteins.

To evaluate the purity profile and correct formation of the VLPs in each step of the process, the purified VLPs were characterized by several techniques such as ELISA, SDS-PAGE, Western blot, transmission electron microscopy (TEM) and dynamic light scattering (DLS). The purified VLPs presented the expected structure, with a size distribution similar to the respective whole viruses and to VLPs of flaviviruses reported in the literature.

We are currently working on a straight-through process (STP) integrating the chromatography steps. The process developed is robust and can be easily scaled-up to a commercial production. It also serves as a basis for the purification of other flavivirus VLPs that have been recently expressed at LECC/COPPE/UFRJ (DENV 1-4, SLEV, WNV, etc.).

EXTRACTION OF RECOMBINANT PROTEIN PspA4Pro FROM *E. COLI* CELLS USING [$P_{4,4,4,14}$]Cl AQUEOUS SOLUTION

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Key Words: Extraction; Purification; Recombinant protein; Surface Active Ionic Liquids.

Streptococcus pneumoniae is a major pathogen to causing pneumonia and meningitis. Several pneumococcal proteins have been identified as potential vaccine candidates, such as pneumococcal surface protein A (PspA), in particular PspA4Pro that was shown high cross-reactivity among strains of the pathogen. Currently, *Escherichia coli* is used as a protein-expression system for PspA4Pro immunogen production because of easy operation and low cost [1, 2]. However, since its production is intracellular, the search for simpler and more reliable methods to extract and purify the PspA4Pro is still fundamental for a proper downstream processing design. Some amphiphilic compounds have been suggested as effective cell membranes disrupting agents, particularly, because these chemical compounds can interact with the lipid bilayers, permeabilizing it or even causing its entire disintegration. Recently, surface active ionic liquids (SAILs) like tributyl-1-tetradecylphosphonium chloride ([$P_{4,4,4,14}$]Cl) were used in the recovery of recombinant proteins from *E. coli* cells [3]. Therefore, the aim of this work was to evaluate the ability of [$P_{4,4,4,14}$]Cl to extract and even purify the recombinant PspA4pro from *E. coli* cells. For the protein extraction, 1.0g of pellet from *E. coli* BL21 DE3 pET37b+ cells was homogenized with 9.0g of an aqueous solution of [$P_{4,4,4,14}$]Cl (250mM) during 1.0 h 25ºC and 50.0 rpm in an orbital shaker. Afterwards, the extract was centrifuged in 5000 x*g* for 30 min at 4ºC. The supernatant was collected and preserved at –20°C. A positive control for the protein extraction was carried out using an ultrasound (US) standard method. After the extraction samples of both extraction methods were collected, incubated in 2:1 (v/v) Laemmli's buffer with β-mercaptoehtanol, and then applied into a discontinuous (5% and 12% (w/w)) SDS-PAGE electrophoresis [4]. The gels were revealed by Coomassie Brilliant Blue dye and the pictures obtained by GelDoc® XR+ BioRad. As shown in Figure 1, the use of [$P_{4,4,4,14}$]Cl aqueous solution was not only able to extract the PspA4Pro protein but also to purify it from other proteins that are inside the *E. coli* cells. These results are quite interesting, since it demonstrated that the addition of low concentrated SAILs aqueous solutions can be an effective method to recover PspA4Pro, allowing its pre-purification and an easy integration with further purification steps, such as liquid-liquid extraction, liquid chromatography or precipitation.

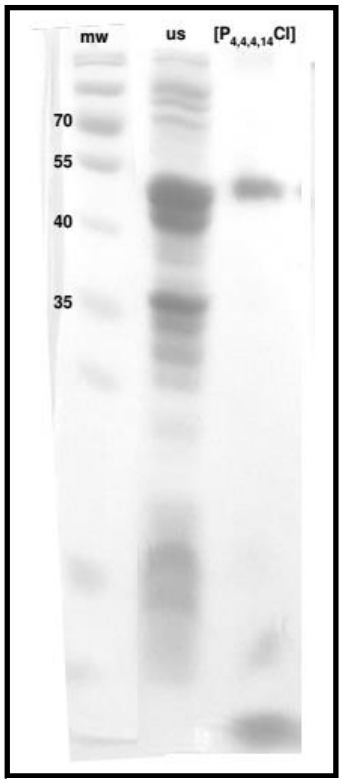

Figure 1 – SDS-PAGE of the ultrasound and [$P_{4,4,4,14}$]Cl aqueous solutions extracts

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RECOMBINANT ANTIGENS OF *MYCOBACTERIUM. BOVIS* RECOGNIZED BY BOVINE SERA WITH KNOWN BOVINE TUBERCULOSIS STATUS USING AN ELISA ASSAY

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Key Words: Bovine Tuberculosis, *Mycobacterium bovis*, recombinant microorganisms, M. bovis antigens, MPB70 and MPB83, protein expression and purification, IMAC, RP-HPLC, ELISA, Comercial ELISA IDEXX.

Bovine Tuberculosis is a zoonotic disease with worldwide distribution caused by *Mycobacterium bovis* (*M. bovis*), which affects cattle from all ages. This pathology annually generates huge economic losses around 3.2 million dollars to the meat and dairy industry. Currently, field bovine tuberculosis diagnosis is performed through the tuberculin skin test that has shown several disadvantages, becoming more difficult its control and future eradication. The aim of this research was to express, in recombinant microorganisms, and purify *M. bovis* antigens MPB83 and MPB70, and to optimize an ELISA assay using an anti-bovine IgG conjugated antibody, which allows the recognition of these antigens by bovine tuberculosis positive animal sera. Both proteins were expressed in *E.coli* BL21 strain and purified by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (RP-HPLC). The antigens were obtained with a level of purity higher than 90% after RP-HPLC. In order to optimize an ELISA format, plates were incubated with 1ug/well of MPB70 and MPB83. Consequently, different serial dilutions from 1:20000 to 1:160000 were tested for the conjugated antibody and two dilutions 1:50 and 1:100 for the bovine sera were used to run the ELISA. To develop the reaction obtained from the recognition of the antibodies contained in each sera sample against the antigens, OPD substrate was added. The results from the ELISA showed that the optimal dilution for the conjugated antibody was 1:10000 and for the bovine sera was 1:50. Once the ELISA was optimized, new plates with both antigens were coated to determine whether specific antibodies from the sera samples are able to recognize these proteins. These results were compared with results previously obtained in a Commercial ELISA IDEXX for the same samples. According to this, from a total of 18 sera screened there were 17 that shared the same bovine tuberculosis status in both assays, however, only 1 sample had different result for each test. The level of concordance between both ELISAs was high with a Kappa value of 0.886. In conclusion, in this study it was demonstrated that MPB70 and MPB83 were recognized by serum antibodies using the optimized ELISA system.

PRODUCTION AND CHARACTERIZATION OF ZIKA VIRUS-LIKE PARTICLES (ZIKV-VLPs) PRODUCED IN INSECT CELLS

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Key Words: Zika virus, virus-like particles, recombinant baculovirus

In recent years, arboviruses have become a major public health problem in the tropical and subtropical regions of the world. The Zika virus (ZIKV) is an arboviral disease prevalent in the Americas, Africa and Asia and has increased its area of endemicity and it is considered a major public health problem in our country. The diagnosis of the infection is made through molecular techniques and serological tests, but these may be non-specific. Due to this and to the advances of the infections caused by the ZIKV it is of extreme importance the development of tools that allow the adequate combat to the ZIKV. Thus, we intend to present a new vaccine proposal against the infection caused by ZIKV. Virus-like particles (VLPs) vaccines appear as an enormous potential for use as extremely effective antiviral vaccines, since they mimic the viral particle, inducing immune response and, as they don't have the genetic material of the virus they won't replicate making them safe as viral particles. We intend to establish a methodology for production and characterization of VLPs containing the structural proteins C, prM and E of ZIKV produced in insect cells and using gene expression system derived from baculovirus. In order to obtain the recombinant baculovirus (BV-ZIKV), vectors containing the sequences of the proteins of interest were constructed to obtain the recombinant bacmid. This bacmid was transfected into *Spodoptera frugiperda* (Sf-9) insect cells. After that, stocks of BV-ZIKV were obtained for infection. Infection kinetics were performed to determine the multiplicity of viral infection (MOI) of the inoculum to be used to infect the cells and the best time for BV-ZIKV production. Sf-9 cells infected with BV-ZIKV were collected at the times 96h, 120h and 144h. The results showed a correct generation of recombinant baculovirus. The Dot Blotting assay showed that the best MOI for infection was 5 and that the best BV-ZIKV collection time was 144 hours post-infection. The purification step to separate the ZIKV-VLPs from the BV-ZIKV will be done. The ZIKV proteins expression in this system will be analyzed by SDS-PAGE and Western Blotting. The ZIKV-VLPs will be analyzed by electron microscopy.

Financial Support: FAPESP, CAPES and Butantan Foundation

SIZING AND ECONOMIC OPPORTUNITY ANALYSIS OF A PLANT FOR THE PRODUCTION OF A NEW, INACTIVATED YELLOW FEVER VACCINE

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Key Words: purified inactivated yellow fever vaccine, serum-free Vero bioreactor cell culture, demand estimation and plant sizing, economic opportunity analysis

Outbreaks of yellow fever (YF) have recently occurred, alarmingly, in countries in Africa and Latin America. The first ever YF cases registered in Asia were reported in 2016, being imported from the African outbreak. Shortage of the current egg-derived, live-attenuated commercial vaccine in these recent outbreaks have shown that there is an urgent need for a new vaccine based on a non-replicating antigen, in order to reduce the risk of the rare, but serious adverse effects associated to the current vaccine, as well as to allow its administration also to immunosuppressed people, children under 9 months of age, people allergic to egg proteins and lactating women. The new vaccine should additionally be produced by a scalable platform, in order to avoid vaccine shortage and enable adequate response in case of outbreaks.
Thus, the present work focuses on an economic evaluation of a new YF vaccine candidate recently developed by a joint COPPE-UFRJ/BioManguinhos-FIOCRUZ project, which involves the production of the YF 17DD virus by cultivating Vero cells on microcarriers in bioreactors under animal-derived component free conditions, followed by two steps of chromatographic purification and then inactivated with beta-propiolactone. This purified, inactivated vaccine (PIV) candidate was tested in mice and shown to confer protection against challenge of the animals with the virus.
For this economic opportunity analysis, at first a market analysis was carried out and an annual demand of 60 million doses of vaccine was predicted. Based on this demand, the production capacity of the plant was established, and the main upstream and downstream processing equipment was sized and quoted.
Adopting the method based on the main pieces of equipment of the plant, a capital investment of USD 9.1 million was calculated as needed to build a production plant for the Active Pharmaceutical Ingredient (API). Mass balances for the raw materials led to a unitary production cost of USD 0.31/dosis. The calculation of net present value, internal rate of return, and payback showed that the proposed project is economically feasible. Sensitivity analyses were also performed on critical parameters, such as selling price per product dose, minimum attractiveness rate and costs of clinical studies, indicating that the new PIV YF vaccine platform is technically and economically feasible.

RECOMBINANT EXPRESSION OF RABIES VIRUS-LIKE PARTICLES (VLPS)

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Key words: *Lyssavirus rabies*; virus-like particles; Baculovirus; rabies virus glycoprotein and rabies virus matrix protein.

Introduction: Rabies is a zoonotic disease responsible for more than 60.000 human deaths annually. The rabies affects different species of mammals. The main method to prevent the rabies is the vaccination. The virus encodes 5 proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (RVM), glycoprotein (RVGP) and polymerase (L). The (RVGP) is the only protein exposed on the virus surface and it is able to induce neutralizing antibodies that make it interesting to be expressed in many systems. It is assumed that the matrix protein (RVM) constitutes major structural components of the rabies virus, and it has an important role in virus assembly and budding. Different vaccines proposals have been studied in order to establish an immune response mediated by neutralizing antibodies more effective and improve the level of cellular immune response, among them, the use of virus-like particles (VLPs). It's our interesting that recombinant baculovirus system be able to producing VLPs containing the RVM and the RVGP. Therefore, the production of rabies VLPs would be system safer and cheaper vaccine candidates. **Objective**: The focus of this paper is to produce recombinant baculovirus bearing RVM and RVGP proteins of rabies virus and to produce VLPs that are able to inducing immune response. **Methods**: In this work, we constructed 2 recombinant baculovius, one containing the matrix protein gene (BVM) and the second containing the rabies glycoprotein (BVG). The batches are titration using Sf9 Easy Titer cells. We coinfected Sf9 cells with BVM and BVG, we used MOI 0,01 for each of them. After 96 h post infection the supernatant and cells were collected, clarified and concentrated by sucrose gradient (50%, 30% and 10%). The samples were analyzed: western blotting and negative contrast electron microscopic. **Results and Discussion**: Sf9 cells coinfected showed typical cytopathic effects such as ceasing of cell growth and decreasing cell viability. The sucrose gradient allowed us to separate the VLPs from the BVs. We can detect the VLPs in ≈ 32% of sucrose gradient and the BVs detect in the pellet. The coinfection showed that proteins, RVM and RVGP were detected in the supernatant cell culture. **Conclusion**: In our studies, rabies VLPs including G and M protein were constructed and the BVM and the BVG are able to express the proteins in their conformational structure. It's our expectancy that this assay produce VLP with RVGP in its native form and its be able to produce neutralizing antibodies.

Supported by: FAPESP, CNPq and CAPES

Obtaining virus-like particles (VLPs) from Mayaro virus using baculovirus

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Key Words: Mayaro virus; Baculovirus; Virus like particles (VLPs); Alphavirus

The Mayaro virus (MAYV) and Chikungunya virus (CHIKV) are Alphavirus that cause diseases characterized by arthralgia, fever, headache, vomiting and diarrhea. In Brazil, recently thousands of chikungunya cases have been reported, being one of the main problems of public health of the country. The Mayaro, being a similar virus, has great potential to cause serious epidemics in the near future. Therefore, studies are necessary to develop preventive and diagnostic methods for these diseases. This work aims to produce VLP (Virus Like Particles) of Mayaro virus, using baculovirus expression system (BEVS). Construction of a recombinant baculovirus containing the corresponding genes for structural proteins of the MAYV, through the Bac-to-Bac system (Invitrogen). The recombinant baculovirus obtained is then used to infect Sf-9 and Hi-5 cells and the production of VLP was analyzed by Western blot and Immunofluorescence indirect (IFI). The Western blot results indicated the expression of structural proteins, where it is possible to visualize the E2 and C proteins in their expected size (50 and 25 KDa, respectively). Indirect immunofluorescence evidenced the presence of Alphavirus proteins on the surface of baculovirus infected cells. Preliminary results evidenced the expression of the alphavirus structural proteins. The next experiments aim at the detection and characterization of the generated VLPs.

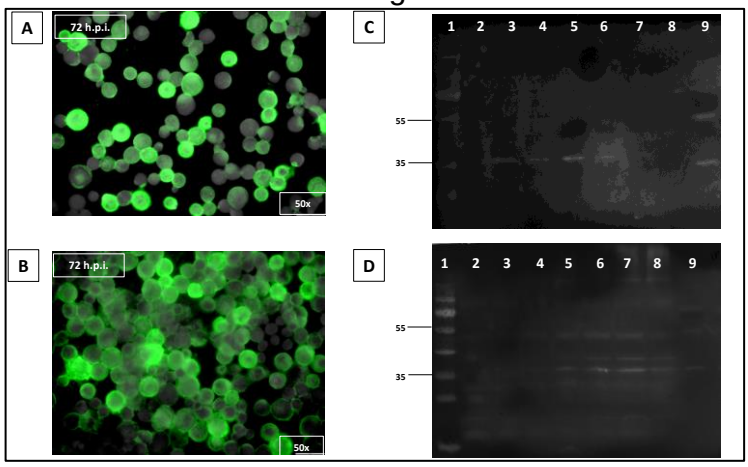

Figure 1 – *Immunofluorescence indirect assay: (**A**) Sf-9 and (**B**) Hi-5 infected with recombinant baculovirus; and Western blot assay: (**C**) Sf-9 and (**D**) Hi-5, infected with recombinant baculovirus and analysis in different times (24, 48, 72, 96, 120 e 144 h.p.i.); Using α-MAYV policlonal; 1 – Marker Pageruler Plus (Thermo Scientific); 2 – Negative control; 3 – 24 h p.i.; 4 – 48 h p.i.; 5 – 72 h p.i.; 6 – 96 h p.i.; 7 – 120 h p.i.; 8 – 144 h p.i.; 9 – Mayaro wild.*

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DOWNSTREAM PROCESS FOR PRODUCTION OF ZIKA INACTIVATED VACCINE

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key words: zika virus, inactivated vaccine, downstream process

The emerging of Zika Virus in Northeast of Brazil in early´s 2015 stimulate several studies about virus pathology, development of diagnostic tools and strategies for vaccine development around the world. To prevent the infections mainly in pregnant women, Butantan Institute, that is one of the main producers of immunobiologicals in Brazil, is working to develop a scalable process for production of purified and inactivated ZIKV vaccine prototype. The aim of this work was to evaluate the best methodology of downstream process in order to aggregate high yield of viruses and the best viral antigen purity. The virus's production was performed using VERO cells in serum free media and the first steps of downstream were clarification by filtration at 0.22/0.45 µm followed by concentration and diafiltration by tangencial flow filtration and by an ionic exchange chromatography step. These 3 steps promoted good virus recover and the residual DNA reached acceptable levels (< 100 pg/dose). As the Host Cell Protein (HCP) level was still high, we studied two designs of the production processes including methodologies for reducing the HCP: density gradient centrifugation and multimodal chromatography methodology. Both showed very promising results regarding the purity (< 100 ng/mg) and virus recovery (> 1+E07 pfu/ml), but the multimodal chromatography has the advantage of avoid the step for removal the excess of sucrose. The downstream technology was established and is ready to enter the next phases of vaccine development. This project has been funded in whole or in part with Federal funds from the U.S. Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority, under Grant No. IDSEP130015 and Butantan Foundantion.

	Protein (µg/mL)	DNA (ng/mL)	HostCell (ng/mL)	PFU/ml
Process 1				
TFF	404	216	14563	2,2E+10
Ionic exchange	109	8,9	10316	4,46E+9
Multimodal Cromatigraphy	54,8	<0,2	46,50	1,01E+7
Process 2				
TFF	388	216	14563	2,2E+10
Ionic exchange	205	<0,2	10316	2,3E+9
Ultracentrifuge	37,2	<0,2	20	8,5E+9

Table 1: Comparative performance of 2 downstream process of zika vaccine prototype. Results of total protein and DNA measure, determined by fluorimetry, and PFU determined by the plaque forming unit technique after a complete production process (1) TFF →ionic Exchange chromatography→ multimodal chromatography; (2) TFF →ionic Exchange chromatography→ densit gradiente centrifugation).

EXPRESSION OF ALPHAVIRUS STRUCTURAL PROTEINS IN THE DROSOPHILA SCHNEIDER 2 CELL SYSTEM

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key words: Alphavirus, Mayaro, Chikungunya, VLP, S2 cells.

In the group of emerging and reemerging infectious diseases, mosquito-borne arboviruses are considered major public health challenges. The transmission of Chikungunya (CHIKV, Togaviridae, *Alphavirus*) in America continent was first reported in Central America and Caribbean region. The CHIKV epidemics reached Brazil through zoonotic transmission by *Aedes spp* mosquitoes, particularly *Ae. Aegypti* and *Ae. albopictus*, two invasive and cosmopolitan species. Another *Alphavirus*, the Mayaro virus (MAYV) present mainly in the Amazon region has been showing evidence of a real urbanization, increasing concerns about its transmission by urban mosquitoes. Therefore, it is clear that studies on strategies for the control and prevention of MAYV and CHIKV infection are needed. Currently no vaccines or antiviral therapy specific for CHIKV and MAYV infection are available. This work describes for the first time an efficient expression, purification and analysis of virus-like particles (VLP) of CHIKV and MAYV Alphaviruses in S2 (*Drosophila melanogaster*) insect cells. The genes coding for the structural proteins of the MAYV and CHIKV viruses were cloned into vectors for recombinant expression in S2 cells. This platform proved to be suitable for the processing of CHIKV and MAYV glycoproteins and to produce VLP, since we detected the presence of particles in the culture supernatant by Western blotting and analyse particle structure by Transmission Electron Microscopy (TEM) and Light Scatterring (Zeta Sizer). Furthermore, it was possible to select cells for greater production of VLP and to establish a method for particle purification using ultracentrifugation and dialysis.

TEST IN BACTERIOPHAGE M13 AS AN ANTIGEN CARRIER

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Key Words: Phage Display, M13, Mycobacterium Tuberculosis.

The bacteriophage M13 is a virus that only infects bacteria, and It is composed of a circular single-stranded DNA molecule encased in a thin flexible tube. This structure has been exploited in the Phage Display technique, which consists in the study of the protein-protein interaction, principally. Phage Display has been used for various types of investigations, among which is the studies as antigen carrier based on this principle, due to the ease in production and purification of the phage. In this study, the generation of an antigen attached phage was proposed, using a *Mycobacterium Tuberculosis antigen*.

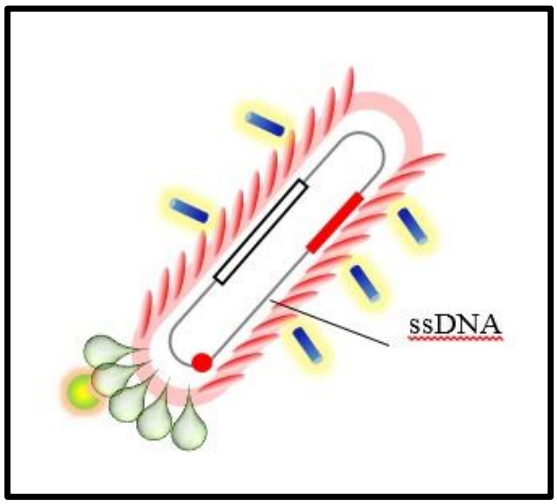

Figure 1 - Structure of M13 Phague

Acr protein was chosen to be attached to the pIII protein of the phage. Acr-M13 phage was purificated through PEG/NaCL method, and the phage titer was about 10^{10} pfu/µL. The presence of Acr in M13 phage was studied through ELISA, which proved that Acr-PIII joined correctly the phage.

Finally, it was proposed to scaling to immunogenicity assay in mouse to prove immune response and scheme a possible vaccine.

DEVELOPMENT OF A RECOMBINANT VACCINE CANDIDATE AGAINST HANTAVIRUS

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Key Words: vaccine, hantavirus

Abstract

Andes virus is the main causative agent of Hantavirus cardiopulmonary syndrome (HCPS) in South America. There are currently no vaccines or treatments against Andes virus. However, there are several evidences suggesting that antibodies against Andes virus envelope glycoproteins may be enough to confer full protection against HCPS. The main goal of the present work was to develop a vaccine candidate against hantavirus, based on the surface glycoproteins Gn and Gc. With this purpose, the sequence encoding for the extracellular domains of both antigens was introduced into the methylotrophic yeast Pichia Pastoris. After induction with methanol, the recombinant antigens accumulated intracellularly as insoluble aggregates. After cell disruption, the recombinant antigens were solubilized and purified by metal-ion affinity chromatography. The immunogenicity of both antigens was determined in immunization assays in both mice and Syrian hamsters. In both species it was possible to detect the presence of specific antibodies against Gn and Gc. Part of these antibodies showed neutralizing activity. The results obtained to date suggest that the Gn and Gc antigens from Andes virus, produced in P. pastoris, have the potential to become the first commercial vaccine against HCPS.

ANTIVIRAL POTENTIAL AGAINST HUMAN ROTAVIRUS TYPE A OF CRUDE EXTRACT FROM PERUVIAN SEA CUCUMBER

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Key Words: Antiviral activity, human rotavirus type A, sea cucumber, *Patallus mollis*, marine organism.

The acute diarrhea syndrome caused by rotavirus is one of the most important viral etiological agents of childhood diarrhea worldwide.Its incidence has decreased thanks to vaccination programs; however, outbreaks of this disease continue to occur, which is why the search for new effective antiviral remedies is crucially required. Sea cucumber is one of the most used marine organisms as a traditional food in Asia due to its delicate flavor and texture, it has been used in traditional oriental medicine and has been attributed a series of biological activities. The aim of this study was to test the in vitro anti-viral activity of the crude tegument extract of *Pattalus mollis*, endemic sea cucumber from the coasts of Peru, against human rotavirus type A (RVA). An aqueous crude extract was prepared with the dry tegument of *P. mollis* and its cytoxicity in the VERO cell line was evaluated, being the CC_{50} for the crude extract of 27042.10 µg/mL. To determine the antiviral activity against RVA, the crude extract was applied by pre and post-treatment before and after the viral inoculation and in conjunction with it. A good antiviral activity was observed by pretreatment, with a percentage of inhibition (PI) greater than 99.9%, an IC_{50} of 96.39 µg/mL and a selectivity index (IS) of 280.55; unlike post treatment that showed no antiviral activity. In the treatment as a whole, a PI greater than 99.9% was observed, an IC_{50} of 64.29 µg/mL and an IS of 420.63 (Figure 1). The outcomes offer the scientific basis for the use of extracts of *Pattalus mollis* against RVA. They are worth to focus for finding effective active compounds in the next step and detecting the mechanism of action.

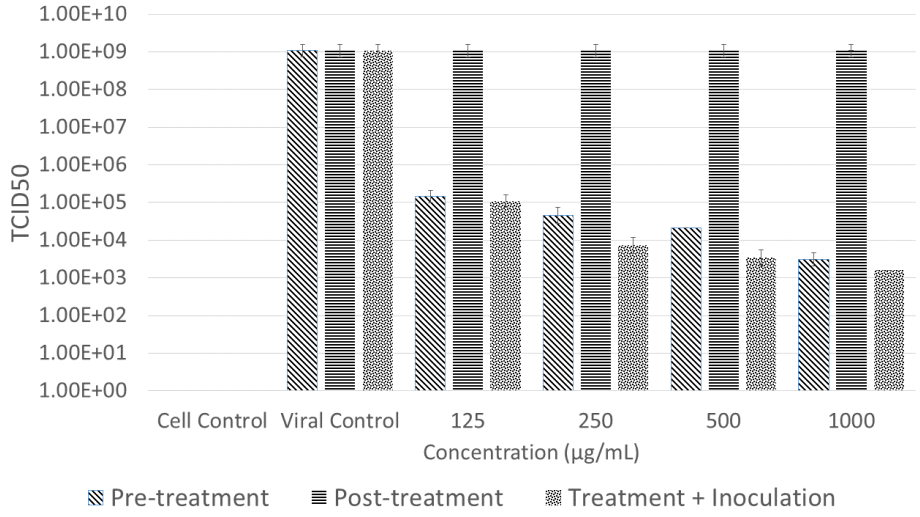

Figure 1 - Effect of the crude extract of the Pattalus mollis on the infectious dose in culture of VERO cells (TCID50) by human rotavirus type A (RVA).

ECTODOMAIN OF RABIES LYSSAVIRUS GLYCOPROTEIN EXPRESSED IN S2 CELLS

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Key words: Rabies glycoprotein, Ectodomain, Rabies lyssavirus, S2 cells.

Rabies is a viral neglected disease considered fatal, that affects mammals and with only 14 cases of survival reported in humans. Despite the existence of preventive vaccines, rabies still represents a serious public health problem, with a major impact in vulnerable populations around the world, causing about 60,000 human deaths per year. The membrane glycoprotein of the rabies lyssavirus (RVGP), specifically the ectodomain portion, has been intensively studied for the development of recombinant proteins due to its ability to interact with neutralizing antibodies and induce immune responses. Producing a membrane-independent RVGP using an insect cells expression system (*Drosophila melanogaster* Schneider 2) can contribute to the advancement expected in the vaccine production process. This project aims to construct a plasmidial vector containing only the ectodomain portion and detect the soluble RVGP expressed by recombinant *Drosophila*. The gene encoding ectodomain was synthesized by GenScript® and subcloned into pMtBipV5HisC using *Eco*RI and *Not*I enzymes. Products of pMtBip-RVGPecto constructions were screened after DH5α tranformation. Then plasmid constructed was transfected into S2WT cells using Lipofectamine® 2000 to obtain the stable lineage S2MTBip_RVGP-ecto, subsequently reselected by limiting dilution. Schott flasks were inoculated and the cultures were induced at different concentrations of $CuSO_4$ (0,5mM, 0,7mM or 1mM) and sampled at different times (24, 48 and 72 hours). Protein detection was done by qualitative Dot and Western blotting analysis using mouse anti-rabies virus Glycoprotein Antibody (LS-C75309) non-conformational monoclonal antibody. Transformation of pMtBip-RVGPecto into DH5α generates two colonies. After mini preparation, those samples had a banding pattern analyzed by enzyme digestion and agarose gel, confirming the presence of ectodomain insert. The recombinant cell population (S2MTBip_RVGP-ecto) was capable to express rabies virus glycoprotein in soluble form under different induction and sampling conditions, although, part of RVGP is still detected in the cells. Better detections condictions were observed at times for cultures induced with 0.7 mM (24, 48 and 72 hours), 0,5mM and 1mM (24 hours). Western Blotting results show the presence of a band corresponding to a molecular mass of approximately 50 kDa in culture media, that correspond with expected value since the recombinant protein does not present complete RVGP. The limiting dilution re-selected a subpopulation (S2MTBip_RVGP-ectoG5) resulting in a 1.5x increase in the expression of RVGP-ecto in relation to the population S2MTBip_RVGP-ecto, suggesting the possibility of optimization of the production.

EXPLOITING DYNEIN MOLECULAR MOTOR TO ENHANCE GENE DELIVERY EFFICIENCY TO MAMMALIAN CELLS

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Key Words: modular proteins, gene delivery, intracellular trafficking.

The high efficiency of viral vectors for gene delivery relies essentially on the ability to exploit the molecular interactions through which cells respond to extra- and intracellular stimuli. One example is the fact that viruses are able to interact and hijack the cell´s molecular motors in order to enhance intracellular trafficking. In an effort to mimic viral transduction capabilities by non-viral vectors, we designed modular recombinant proteins containing an LC8 Dynein molecular motor interacting domain and a DNA binding domain based in arginine clusters. We found that these modular proteins were able to interact with plasmid DNA (pVAX1-Luc) and to form stable nanoparticles in different buffer solutions and pH conditions. By increasing the number of arginine residues in the DNA binding domain, from 6 to 12, a 10-fold increase in transfection efficiency was found, in terms of Luciferase reporter enzyme expression. Additionally, by using the specific AAA+ ATPase motor cytoplasmic Dynein inhibitor, Ciliobrevin D, we found strong evidence of the direct role of Dynein retrograde transport in transfection efficiency. These results demonstrate the potential of the rational design of recombinant modular proteins as efficient non-viral vectors for gene-based therapies.

SCALE-UP OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS CULTURE FOR DIABETES CELL THERAPY

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Key Words: Cell therapy, Human adipose-derived stem cells, Suspension culture, Scale-up, Differentiation

Type I Diabetes is a disease that has shown an increasing incidence in the last few years. The current treatments involve exogenous insulin administration, strict diet control, and exercise. However, adequate glycemic control is still difficult to attain, as they do not provide a system to regulate blood glucose levels. For this reason, the development of new therapeutic alternatives is an urgent medical issue. Over the last few years, cell therapy using adipose-derived stem cells (hASC) has emerged as a promising treatment alternative. Adipose/fat tissue provides a readily available source of mesenchymal stem cells. Yet, their expansion is still limited and not easily scalable.

The general aim of this work is to scale-up the expansion of hASCs for their further differentiation into insulin and glucagon expressing cells (IPC and GPC), to cluster both types to form islet equivalent units for transplant in patients. This could represent a cellular-based therapy for type I diabetes. The hASC cells were characterized in 2D culture, and the effect of glucose and calcium concentration on cell growth and metabolism was determined. Then, cells were adapted to suspension culture and compared to adherent culture, in terms of proliferation, viability and metabolites production. These results are the first approach for the improvement of biomass production in suspension culture of these cells. Protocols have been previously designed for the differentiation of hASC into IPCs and GPCs, nevertheless, these protocols have yet to be adapted for large-scale suspension culture, to achieve our major ulterior goal of producing large amounts of islet equivalent units.

MANUFACTURING OF CHIMERIC ANTIGEN RECEPTOR T CELLS FOR ADOPTIVE IMMUNOTHERAPY USING XURI BIOREACTOR

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Key Words: Immunotherapy; CAR-T cells; Lymphocyte; Cell therapy; Bioreactor

Cell-based immunotherapy has attracted clinical and pharmaceutical interest over the last few years. In particular, adoptive cellular therapy using chimeric antigen receptor (CAR)-T cells target to CD19 has demonstrated clinical efficacy in several hematologic cancers. The highly demanding cell doses used in clinical trials require a scalable, efficient and GMP-compliant manufacturing process. This work aims to establish a bioprocess to expand immunocompetent T cells transduced with CD19CAR. T-cells were collected by apheresis from healthy donors and isolated by density gradient centrifugation (Ficoll-Hypaque™, Thermo Fisher Scientific). Thereafter, cells were enriched by positive immunomagnetic CD3 selection (StemCell Technologies) and activated with CD3/CD28 beads (Thermo Fisher Scientific) in a proportion of 1:3 (beads/cells). On the next day, the cells were transduced with lentiviral vectors (MOI 5) and cultivated using RPMI culture medium supplemented with 10% of AB human serum, 100 IU/ml of IL-2 (GE Healthcare Life Sciences) in static T-flasks. Activation beads were removed at day 7 and at day 9 the cells were transferred to 1L Xuri bag (working volume of 100 mL) and maintained in static conditions. At day 11, the bag was transferred to the Xuri platform (GE Healthcare Life Sciences) with a working volume of 390 mL under controlled conditions: 10 rpm, 6º and 37ºC. Cell proliferation was monitored daily and percentage of CAR expression was quantified in TCD3, TCD4 and TCD8 populations by flow cytometry. The amount of cells expanded (994 x 10^6 cells) after 14 days are sufficient for 6 infusions (80 kg) assuming 2x10^6 cells/kg. We next evaluated the cytolytic potential of 19CAR-T cells produced, co-culturing with B cell lines Sup-B15 ($CD19^+$) and LAMA-84 ($CD19^-$). A ratio of 10 effector cells for 1 target cell was used. The results showed that our 19CAR-T cells are functionally active and detect only B cells that are CD19+. Further experiments will be performed to improve the expansion bioprocess for therapeutic applications.

EX VIVO GENERATION OF ERYTHROID CELLS

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Key Words: Hematopoietic Stem/Progenitor Cells, Erythroid cells, growth factors, hEPO

Erythroid cells in different maturation stages have relevant applications, both as therapeutic agents themselves and as potential cell vehicles for drug delivery. They could also be used for drug screening or for the detection of antibodies against erythroid antigens in patient samples in order to identify transfusion matches or allo-immunized individuals. Besides, the difficulty to find safe cell supplies in cases of rare phenotypes or poly-immunized patients has prompted efforts to produce erythroid cells *ex vivo* from hematopoietic stem/progenitor cells (HSPCs). However, the available protocols are still not cost effective, which prevents its application in transfusional practices and limits other uses. Thus, this work aimed to apply strategies to reduce expensive growth factors utilization in cultures. Two hypothesis were established: experimental design development would allow obtaining an optimal formulation of the HSPCs expansion medium. Additionally, HSPCs lentiviral transgenesis with the erythropoietin (hEPO) gene would make cells secrete hEPO into the culture medium and, consequently, it would reduce its utilization as supplement.

In order to optimize the medium formulation, a *Plackett-Burman* design was applied in order to study the significance of 11 factors' effects over HSPCs expansion. It was evaluated by cell counting and cytometric analysis of the CD34 HSPCs marker. Three factors were significant: bovine seric albumin (BSA), granulocyte and macrophages colony-stimulating factor (GM-CSF) and hydrocortisone (H). Then, a *Box-Behnken* design was used to optimize this factors' levels, maintaining the others in constant levels. After analyzing the same responses, the optimal concentrations of BSA, GM-CSF and H were 2% (W/V), 20 ng/ml and $4x10^{-5}$ M, respectively. Optimized cultures-derived cells were induced to differentiate in a reported medium: 571-fold cell expansion and progressive erythroid differentiation were observed. Cell differentiation was assessed by cytometry immunophenotypic analyses, staining morphological studies, hemoglobin detection and colony forming units assays. They showed that culture conditions allowed high erythroid selectivity.

The second approach implied the generation of modified HSPCs with hEPO gene. Cultures of transduced cells without commercial EPO [eHSPCs-EPOc (-)] were compared with non-modified cell cultures with EPOc [nmHSPCs-EPOc (+)]. Both cell lines reached similar maturation states and showed erythroid commitment. This was proven by a reduction in CD34 and CD45 expression and an increase in $CD71^+$ and $CD235a^+$ cells. In addition, morphological changes and a shift in dyes affinity, characteristics of erythroid maturation, were evidenced. The differentiation in eHSPCs-EPOc (-) cultures was shown to be induced by secreted hEPO: control nmHSPCs-EPOc (-) cultures did not show erythroid cells; also, hEPO secretion was proven by ELISA and isoelectric focusing, which showed similarity between EPOc and secreted hEPO isoforms. Thus, eHSPCs were self-induced to differentiate *in vitro* without EPOc, into populations highly enriched in erythroid cells, which allows reducing the amount of EPOc added into cultures and the associated cost.

Both strategies are interesting to be applied in order to improve the processes intended for *ex vivo* production of erythroid cells, which are in development and optimization stages.

CHARACTERIZATION ASPECTS OF MESENCHYMAL STEM CELL SPHEROIDS

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Key Words: mesenchymal stem cell spheroids; spheroids; 3D cultures; viability assays.

3D cell organization in vitro in the form of spheroids can closely mimic the natural microenvironment of organs and tissues, consisting of an attractive approach for the development of tissue engineering and cell therapies[2]. The objective of this work was to evaluate the effectiveness of techniques traditionally used in the analysis of conventional 2D monolayers of mesenchymal stem cell (MSC) cultures to characterize MSC spheroids.
For this purpose, MSC spheroids were produced by culturing the cells during 7 days in micro-molded nonadhesive hydrogels in DMEM medium, 37 °C and 5% CO_2. Images were obtained with phase contrast microscopy and analyzed using the Image J software (n=28). Parameters such as Feret diameter, sphericity index and solidity were measured. To compare spheroids dissociation with trypsin-EDTA solution and Trypple Express Dissociation Reagent, spheroids were dissociated in different concentrations and times of exposure (n=9) and were analyzed for viable cell concentration and viability using hemacytometer by the trypan blue 0.04% exclusion method. To compare the performance of different cell viability assays in both 2D and 3D cell cultures, the same amount of cells found in 7-day spheroids were cultured as monolayers in 96-well plates[1]. Resazurin solution (Sigma-Aldrich) and Cell Titer-Glo 3D (Promega) kit were used to determine viable cell counts.
Spheroids with appropriate spherical shape were obtained (mean sphericity index of 0.92±0.02) and regular surface (mean solidity of 0.98±0.01). Most of the spheroids (75%) presented diameters between 300 and 400 μm. Using trypsin-EDTA solution 1X, a period of 15 minutes was necessary for complete the dissociation of the spheroids, what caused a decreased in cell viability (53±2).Using trypsin-EDTA solution 3X, 10 minutes were necessary for complete dissociation, providing adequate cell viability (86±3). Using trypsin-EDTA solution 5X, 7 minutes were necessary for complete dissociation, but severe damages were observed in cell membranes, resulting in about 100% of cell death and significant presence of cell debris. By means of the Trypple Express Dissociation Reagent, both 1x and 3X solutions required exposure periods above 20 minutes to effectively dissociate spheroids. This excessive exposure time caused alterations in cell morphology, damage in cell membranes and decreased cell viability (17±1 and 31±2 respectively). Both resazurin and Cell Titer assay showed, respectively, fluorescence and luminescence signals higher in 2D than in 3D cultures. However, luminescence results obtained with the Cell Titer assay for 3D cultures showed to be closer to that of 2D cultures than the values observed with resazurin fluorescence, indicating that the former test may be more sensitive to detect viable cells in MSC spheroids than resazurin. These results show that there is still a demand for more robust, reliable and sensitive reagents and techniques applicable to the characterization of 3D cultures, especially in compacted spheroids.

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ANALYSIS OF DIFFERENT MORPHOLOGICAL, PHYSICO-CHEMICAL AND STRUCTURAL PARAMETERS OF THE ISOLATED PLATELET-RICH PLASMA GEL AND ASSOCIATION WITH HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Key Words: Tissue engineering; Medicine regenerative; Mesenchymal stem cells; Platelet Rich-Plasma; Adipose tissue.

In recent year, the Platelet Rich-Plasma (PRP) has been stood out as a potential scaffold in cultivation of mesenchymal stem cells. Studies have pointed out to the effectiveness of this interaction in the regenerative process, through growth factors and inflammatory and modulatory mediators released by the platelet concentrate, as well as by the immunomodulatory and plasticity properties of the mesenchymal stem cells. The association of human adipose-derived mesenchymal stem cells (ADSC) with PRP is able to optimize the tissue repair process, stimulating angiogenesis and recruitment of cells to the lesion site. The physico-chemical aspects of the PRP-gel, as well as the interaction and behavior of the ADSC when associated with the gel are still not well defined, and knowledge improvement becomes fundamental for future clinical applications in the treatment of tissue lesions. Therefore, the aim of this study was to evaluate different morphological, physico-chemical and structural parameters of the isolated PRP-gel or in association with ADSC (PRP/ADSC). The PRP gels were submitted to scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy technique (FTIR). The ADSC viability during and after association with the gel-PRP was also evaluated. The results showed that after association, the feature properties of PRP and the ADSC viability were maintained. (Figure 1). There were no structural changes in the gel-PRP isolated and PRP/ADSC, maintainig the physical-chemical profiles of the samples. Additionally to these results, it was observed that the PRP-gel may provide a favorable environment for cells, allowing its proliferation. In summary, it is possible to conclude that PRP-gel can be used as a potential scaffold maintaining cell viability and physical-chemical aspects when associated with ADSC.

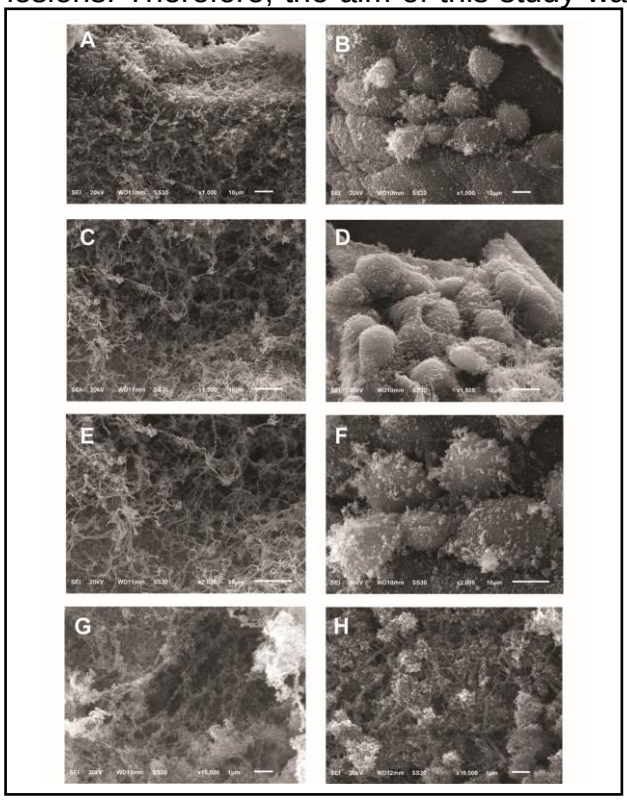

Figure 1 – Scanning electron microscopy of PRP gels isolated (A, C, E, G) and with ADSC (B, D, F, H).

PRODUCTION OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES AND STEM CELLS LABELING

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Key Words: superparamagnetic iron oxide nanoparticles, cell tracking, stem cells, therapy, in vitro imaging.

Superparamagnetic iron oxide nanoparticles (SPIONs) have been used for diagnoses in biomedical applications, due to their unique properties and their biocompatibility for humans. The possibility of efficiently labeling cells with these magnetic nanoparticles has stimulated the use of magnetic resonance imaging as an excellent tool for high-resolution visualization of the fate of cells after transplantation and for evaluation of therapeutic strategies. Application of mesenchymal stem cells (MSC) tracking approaches is necessary to determine tissue distribution and lifetime of stem cells following their injection and help understand stem cells mechanisms of tissue repair[1,2].
In this work, magnetite nanoparticles ware obtained by the co-precipitation method from Fe^{II} and Fe^{III} in an alkaline medium under mechanical stirring at 60°C[3]. The magnetic nanoparticles ware washed with distilled water and employed in stem cells labeling. Magnetic cell labeling and intracellular iron quantification ware executed following the protocol procedure was described in the literature[1].

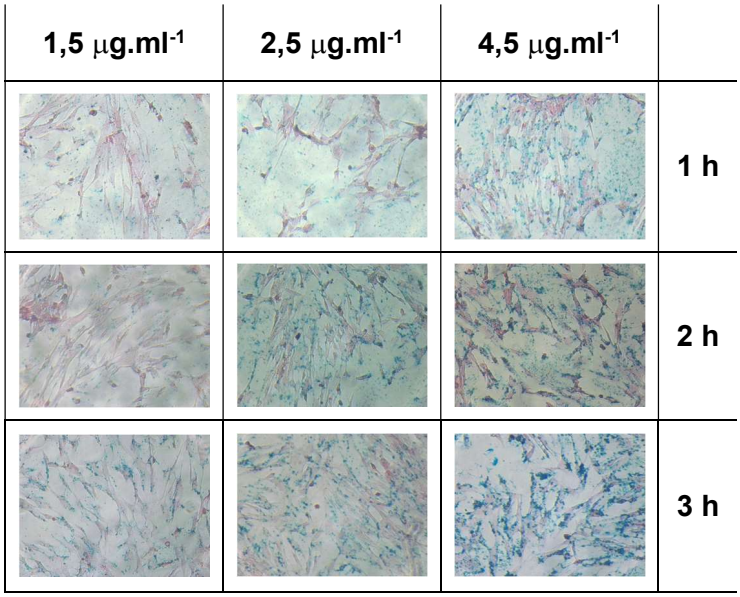

Figure 1 – Dose and incubation time dependent MSC labeling with SPIONs.

The obtained nanoparticles showed good stability in aqueous solution, mean diameter of 12.0 nm, saturation magnetization of 76.0 emu.g^{-1} (at 300 K) and demonstrated to be promising for internalization in mesenchymal stem cells (Figure 1). It is important to highlight that both dose and incubation time are significant variables, and contribute positively, in the labeling process. Based on the obtained results, it is suggested that the labeling be carried out at greater times, to guarantee the internalization of the particles and not only adhesion to the cell membrane, and in larger concentrations, as long as the cellular viability is not affected, in order to increase the labeling efficiency .

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PERFORMANCE OF SMOOTH MUSCLE CELLS SEEDED IN CHITOSAN-PECTIN TUBULAR SCAFFOLDS

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Key Words: chitosan, pectin, collagen, tubular scaffold, tissue engineering.

Scaffolds are artificial extracellular matrices, capable of supporting cell growth and three-dimensional tissue formation. Along with cells and growth factors, scaffolds are one of the three key components for tissue engineering[1]. However, cell seeding in these scaffolds is often difficult due to the limited ability of cells to penetrate the three-dimensional structure. Cell encapsulation within a matrix can be used to facilitate cell seeding to porous scaffolds[2], allowing to obtain materials with uniform properties by promoting homogeneous distribution of cells. Collagen (Col) gel is an outstanding biomaterial used for cell encapsulation since it is the main component of most tissues and plays an important role in maintaining the biological and structural function of the extracellular matrix[3]. Thus, Col gel was used to encapsulate human smooth muscle cells (SMC, $0.5x10^6$ cells/mL_{gel}) and facilitate their penetration in chitosan-pectin (Ch-P) porous scaffolds. Chitosan and pectin are polysaccharides widely used in the composition of biomaterials due to their biocompatibility and biodegradability[4]. In this work, both polymers were combined aiming to obtain scaffolds to be used in tissue engineering applications. The scaffolds were designed to have tubular geometry, being therefore valuable for the development of engineered tissues that show annular cylindrical geometry *in vivo*, such as intestines, veins and arteries. Histological staining on transversal sections of samples showed that SMC seeding to the tubular structures was successfully achieved by using Col gel for cell encapsulation, as most of the scaffold pores were filled with the gel and cells were uniformly distributed throughout the structure after 7 days of culture. Moreover, the biomaterial presented dimensions (inner diameter of 5 mm and wall thickness of 1.5 mm) and stability appropriate for its use as scaffold in tissue engineering, in particular for vascular tissues, in which there is a need for biocompatible constructs with diameters lower than 6 mm for replacing small caliber blood vessels (Figure 1). Next steps of the work include static maturation of the cellularized constructs to assess cell viability and their mechanical behavior.

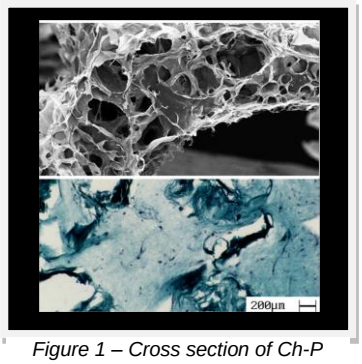

Figure 1 – Cross section of Ch-P tubular scaffold assessed by scanning electron microscopy (a) and Masson's Trichrome staining of the transversal section of the scaffolds (b).

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IMPLEMENTATION OF XENO-FREE BIOPROCESS IN STIRRED-TANK BIOREACTOR FOR EXPANSION OF UMBILICAL CORD DERIVED-MESENCHYMAL STROMAL CELLS CONDITIONED WITH HYPOXIA

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Key Words: mesenchymal stromal cells, cell therapy, immunomodulation, hypoxia.

Multipotent Mesenchymal Stromal Cells (MSC) have been widely explored as promising candidate cell-based therapy of a variety of immuno-mediated, inflammatory and degenerative diseases, due to their immunomodulatory and regenerative properties. However, some limitations, such as cell senescence by excessive *in vitro* expansion, reduction or inconsistency of the therapeutic potential and low survival of transplanted cells, require immediate search for new approaches GMP-compliant expansion to produce robust and functional MSCs for therapies. Here, we have established a scalable bioprocess to expand MSC from human umbilical cord (hUC-MSC) conditioned with hypoxia under xenoantigen-free conditions. hUC-MSCs were isolated, expanded in stirred-tank bioreactor coupled to human collagen coated microcarriers using AB human serum and conditioned with hypoxia (dissolved oxygen concentration maintained to 5%). Subsequently, were characterized by immunophenotyping and differentiation and immunossupressive potentials. To access *in vitro* immunossupressive function, peripheral blood mononuclear cells were stained with CFSE (3uM) and stimulated with Dynabeads^{TM} activator (1:1). hUC-MSC expanded on 4-hour adhesion phase and intermittent shaking, showed significant cell expansion and presented excellent rate of cellular recovery. The established protocol enabled the production of 1,36 (±0,01) × 10^5 cells/mL after five days of culture, corresponding to a fold expansion of 6,11 (±0,63) based on the percentage of adherent cell to microcarriers (56,25 ±6,25%), similar normal DO (20%) conditions. Regarding immunophenotypic analysis, decreased CD105 expression post-expansion was observed. Multipotential capacity to differentiaed into adipogenic and osteogenic lineages was retained and showed elevated immunossupressive potential, able to inhibit lymphocyte proliferation in the different ratios. These results represent an important step toward the implementation of a GMP-compliant large-scale production system for hUCMSCs in hypoxic with hypoxia. New experiments are needed to evaluate the effect of priming with hypoxia to immunomodulatory and regenerative *in vivo* potential.