60th Anniversary of Artificial Cells

In conjunction with

XVI ISBS Int. Symposium Blood Substitutes & Oxygen Therapeutics
V ISNS Nanomedicine Conference

13-15 November 2017, Montreal, Quebec, Canada

www.medicine.mcgill.ca/artcell/meetings/pdf

CANADA 150

vive375

Sponsored by

McGill

Faculty of Medicine | Faculté de médecine

and

ISABB International Society for artificial Cells, Blood Substitutes and Biotechnology

Above photo: McIntyre Medical Sciences Building , Faculty of Medicine, McGill University
(Meeting venue at Holiday Inn, 999 St. Urbain, Montreal, QC, Canada)

60th Anniversary of Artificial Cell. This is to celebrate the 60th anniversary of the invention of Artificial Cells and the contribution of researchers at this centre and numerous groups of researchers around the world.
The first artificial cells were prepared in 1957 at McGill University (Chang, 1957 B.Sc www.medicine.mcgill.ca/artcell/514.pdf ,followed by Chang 1964 Science, Chang & Poznanski, 1968 Nature Chang, 1971 Nature, Chang 1972 Monograph on Artificial Cells http://www.medicine.mcgill.ca/artcell/1972bookCovercr.pdf . He emphasizes that this is not to reproduce biological cells since nature and cell culture can do a much better job. His aim is to use this basic principle to innovate and go outside the box – especially there can be unlimited variations in the content, dimensions and membranes. Since then, extensive efforts by him and researchers in his centre and numerous groups around the world have evolved this principle of artificial cells into Micro-nano systems, Nanobiotechnology, Nanomedicine, Blood Substitutes, Synthetic Biology, Biosorbents, Bioencapsulation, Biotherapeutics, Drug Delivery Systems, cell/stem cell therapy, microbe, enzyme therapy, cancer therapy, nano-robotics etc.
Nature Rev Drug Disc: http://www.medicine.mcgill.ca/artcell/2005NatureRev.pdf
2007 Monograph http://www.medicine.mcgill.ca/artcell/2007%20ebook%20artcell%20web.pdf

This is held in conjunction with the XVI ISBS and V Nanomedicine Conference since all have related interests of artificial cells and of the international society (network) of **International Society of Artificial Cells, Blood Substitutes & Biotechnology (ISABB)**, www.medicine.mcgill.ca/artcell/isabi.pdf

Official Journal of Society: Artificial Cells, Nanomedicine & Biotechnology, an international journal,(Editor in chief: Chang) **Reuter Impact factor: 5.605** (2016) and **World Ranking: 4th/**among 77 Biomedical Engineering journals (2017). Taylor & Francis Publisher. Presenters can submit their papers for publication after peer review. http://www.tandfonline.com/action/showMostReadArticles?journalCode=ianb20

The biannual ISBS *has voted to hold the XVI ISBS in Montreal, Quebec, Canada. Most recent ISBS was 2011 XIII at Harvard, 2013 XIV at Blood Transfusion Institute of China and 2015 XV at Lund Sweden. We welcome experienced pioneers, established researchers, new researchers, students, clinicians, developers, regulators and blood bankers and others. Areas for this meeting* O_2 *carriers,* O_2 *therapeutics,* CO_2 *carriers, antioxidants, vasoactivity, stem cells, cord blood, recombinant source, platelet substitutes, safety and regulatory, transfusion medicine and other related topics.*

V ISNS Nanomedicine Conference *has voted to hold this in Montreal since artificial cell is the forerunner of nanomedicine www.medicine.mcgill.ca/artcell/nanobk_ch1.pdf Areas for this meeting micro-nano systems, applications in therapeutics, drug delivery,* Synthetic Biology *diagnostics and other areas with emphasis on present & future perspectives.*

Local organizer (centre and centre alumni):
TMS Chang, McGill, chair and honorary president (McGill 57',61'.65'),
L Chang (McGill Diploma 58')
F.D'Agnillo, FDA/NIH (McGill 97'),
P. Keipert, Consultant & President, Keipert Corp, San Diego, CA, USA *(McGill 86'),*
S. Prakash, McGill (McGill 96'),
BL Yu, Harvard (McGill 02')
Artificial Cells & Organs Research Centre and Centre alumni, Departments of Physiology, Medicine & Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, QC, Canada

International Organizing committee
Budak G, Bülow L, Chang L, Chang TMS, Chen C, Ergan F, D'Agnillo F, Estep T, Greenburg AG, Jahr S, Hoesli C, Keipert P, Kinsella M, Neufeld R, Piskin E, Poncelet D, Prakash S, Sakai H, Su ZG, Yang CM, Yu BL, Zhou H.

International Scientific Advisory Committee
Alayash A, Abuchowski A. *Bäumler, H* ,Bian Y, Biro G, Budak G, Bucci E, Bülow L, Burhop K, Chandra R, Chang TMS, Chen C, Chen GC, Cooper C, D'Agnillo F, Dixit V,
Estep T, Feola M, Gould S, Greenburg AG, Gu KF, Han JQ, Hsia C, Huang YB, Intaglietta M, Jahr JS, Juncker D, Keipert P, Kim HW, Kluger R, Kobayashi K, Krafft MP, Liu Q, Liu JX, Lotan M, Ma L, Meßmer K, Mozzarelli A, Maysinger D, Neufeld RJ, Palmer A, Piskin E, Poncelet D, Poznansky M, Prakash S, Privalle C, Pugach I, Rausch C, Riess JG, Sakai H, Shi Z, Simoni J, Selivanov E, Su ZG, Tsai AG, Wang, ZY,Wei G, Wong B, Wong JT, Xiu RJ, Yang CM, Yu BL, Zafiris G , Zal F, Zapol W, Zhao L, Zhou H, Zhu YJW

Center and center alumni Advisory Committee
Blais, MC, Barre P, Bian YZ , Budd N, Cattaneo MV, Chan G, Chow KM, D'Agnillo F, Daka JN, Zolotarova E, Ergan F, Fustier C, Georges E, Grunwald J, Gu JS, Gu KF, Gu LM, Guo.C, Hoesli C, Jiang WH, Keipert P, Kinsella M, Esquisabel A, Lau A, Lee C, Liu ZC, Lyold- george I, Ma S, Mobed-Miremadi M, Neufeld RJ, Nicolau D, Nelseiski P, Ning J, Nishiya T, Piskin AK, Piskin E, Poznansky M, Prakash S, Rong ZX ,Shi Z, Shum-Tim D, Sorrini P, Stefanescu A ,Tabata Y, Tso J, Varma R, Wang Y, Wang Z, Wei G,Wong R, Yu BL, Yu, WP. Zhao YQ, Zhu JW,

MEETING VENUE: Holiday Inn, 999 Saint Urbain, Montreal, PQ, Canada

Nov 13th	Nov 13th	Nov 13th	Nov 13th		Nov 14,15	Nov 14th	Nov 14th	Nov 14th	Nov 15th	Nov 15th
	Ballroom	Habiscu	Jasmine			Ballroom	Habiscu	Jasmine	Ballroom	Habiscu
8:30 am	registration									
9:15 am	seating				8:30 am	Clinical	CANCER	GEN/CVS	Protein Eng	Nanocap
9:30 am	Addresses/ Opening lect									
11:00am	Coffee				10:30 am	coffee	coffee		coffee	coffee
11:30am	KEYNOTE & Plenary				11 am	Moving forward	1,world 2.PFC	DIABETES/ microbes	Microcirculat	Extraction purification
1 – 2:30	Lunch your own				1 – 2:30	Lunch your own	Lunch your own	Lunch your own	Lunch your own	Lunch your own
2:30-5:30	Preclinical	Xfusion Med	Drug del/ Reg Med		2:30-5:30	Oxidative	Hemoper		Molecular/ chemical	Nanomed/ Diagnosis

7:00 pm Monday symposium banquet

Admission complementary

If have name-tag.

GUIDELINE OF TIME FOR PRESENTATION

We have such a large number of excellent speakers that even with 2 to 3 simulaneous sessions we are still press for time. Having more simultaneous sessions than this will result in participants missing too many important presentations. Thus, I hope that you would bear with me and collaborate as follows for the benefit all participants.

Address: 5-10 mins
Plenary lecture: as indicated
Session keynote: 25 mins
Sessions lecture: 20 mins

Session lecturers spesk for the full 20mins, then continue with the next speaker with interruption for questions

Questions and Discussions: In specialized symposia like ours, participants prefer to have questic and discussions at the end of each session after all the ideas and proposals have been presented most sessions, if the session chairs are strict with the time limit, there should be time for discusssio

Informal interactions: Unlike large congresses and conventions, we purposely limit the numbe audience. As you know, participation is by application only. This is partly for safety reason and alsc allow ample time for informal interaction and discussion during coffee breaks and lunch breaks. In afternoon sessions, even though the sessions officialy end at 5:30pm the rooms are available unt pm.

Breaks: Breaks are held between the two 2 hour morning sessions. We purposely have a long lu break of 90 mins for participants to rest and relax and sightseeing. This way we should be ready return for the 3 hour afternoon sessions with no breaks.

MONDAY, NOVEMBER 13TH 2017

Registration 8:30 – 9:15 Ballroom Foyer

9:15 Assemble in ballroom:

9:30 OPENING ADDRESSES Ballroom

Fortier, S (Canada) Principal of McGill University
Welcome address for McGill University

Eidelman D (Canada) Vice-Principal and Dean of Medicine, McGill University (Canada)
Welcome address for Faculty of Medicine

White, J (Canada)
Professor & Chair, Department of Physiology, McGill University
Welcome for Department of Physiology where artificial cells was invented

Poznansky M (Canada)
First PhD (Physiology) graduate of Prof Chang
Emeritus Professor and Emeritus Director, Robart Institute, University of Western Ontario, Canada
Welcome address for Centre Alumni

Quirion, Remi (Canada) Chief Scientist of Quebec, in charge of all three Quebec Research Councils
Welcome address

XIONG, Sheng (China) consule and Head, Office of Education, Science & Technology and Culture,Chinese consulate Montreal
Welcome address to Overseas Chinese and Chinese delegates

60th ANNIVERSARY LECTURE
Chang TMS (Canada)
Honorary President, of ISBS and of ISNS,Director, Artificial Cells & Organs Research Centre, Departments of Physiology, Medicine & Biomedical Engineering, Faculty of Medicine, McGill University, Canada
A story of the roles of individuals and researchers around the world in the Invention and Evolution of Artificial Cells to Nanomedicine, Nanobiotherapeutic, blood substitutes, Bioencapsulation, Hemoperfusion, Regenerative Medicine etc

***11:00-11:30 Coffee* Ballroom Foyer**

11:30-12 PLENARY KEYNOTE LECTURE: (30 mins)
Peixun Zhang, Na Han, Yuhui Kou, Xiaofeng Yin, Baoguo Jiang (China)
Peking University People's Hospital, Beijing, China
"Peripheral nerve intersectional repair by bi-directional induction and systematic remodeling: biodegradable conduit tubulization from basic research to clinical application
(Chang, Editor-in-chief's comment: "Professor Jiang, dean of medicine of Peking University and his team published a 2017 review in our journal describing how they moved from the laboratory to the treatment of 30 patients with limb paralyses caused by stroke or trauma. They use a biodegradable growth factor releasing conduit to connect a branch of the proximal C7 on the normal side to the opposite distal C7T1 trunk of the paralyzed side. This results in neural connection and the restoration of the function of the paralyzed limbs - a major breakthrough for patients with stroke or trauma. Professor Jiang receives the inaugurate annual best 2017 paper award in Artificial Cells, Nanomedicine & Biotechnology, an international journal - Reuter world ranking of journal 4th among 77 Biomedical Engineering journals)

12:00 - 12:50 pm PLENARY LECTURES

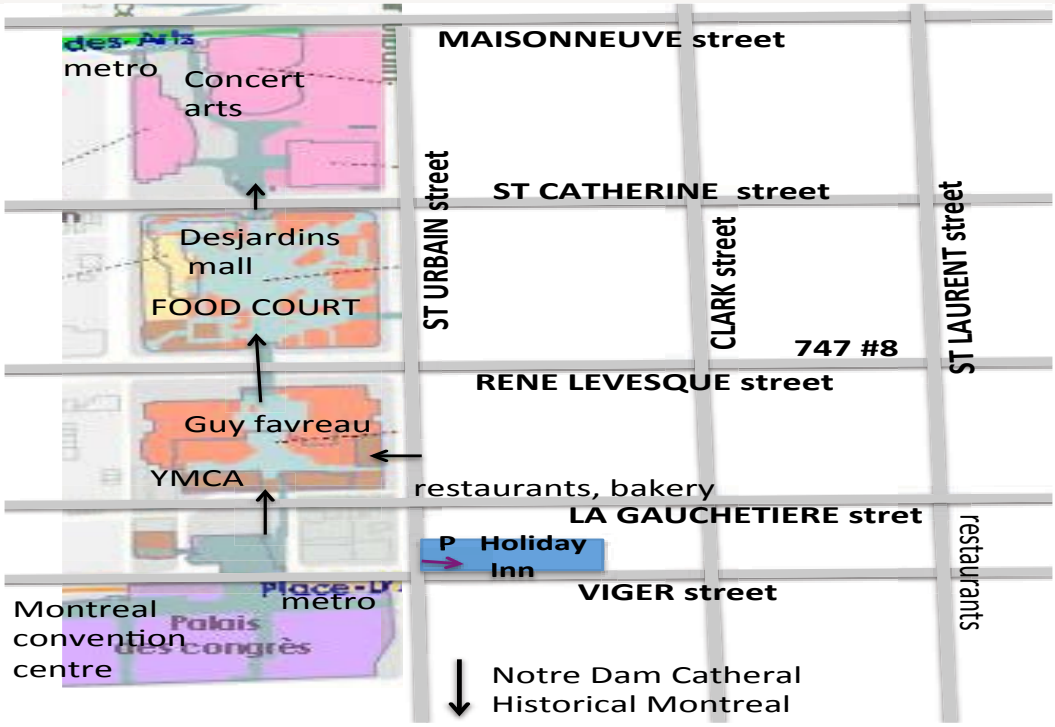
Jahr JS (U.S.A.) (25 mins)
Professor Emeritus of Anesthesiology David Geffen School of Medicine at UCLA, California, U.S.A.
Hemoglobin glutamer-250 (bovine) in South Africa: consensus usage guidelines .from clinician experts who have treated patients

Prakash, S (Canada) (25 mins)
Professor, Artificial Cells and Organs Research Centre & Department of Biomedical Engineering, McGill University,
Artificial Cells Biomedical technologies for human health with emphasis on microbiome and cardiac sten

1:00 to 2:30 LUNCH

On your own to the many nearby restaurants, sightseeing or exercise before returning to the nonstop 3 hour afternoon session !

MAP OF AREA AROUND THE HOLIDAY INN



Restaurants:
This is Montreal's tourist area and there are numerous restaurants, food courts, bakeries,, take outs nearby within 2-5 mintues walk (map above). There are many government and large business buildings in the area and restaurants are particularly competitive in reasonable and quick lunch service. A number of high-end French resturants are in the Historical Montreal area within longer walking distances. The hotel also has its own restaurant.

Symposium banquet Monday 7pm (Complementary, but needs Name Badge)
Kam Fung Restaurant (1071 Saint Urbain Street). If you exit from Holiday Inn Saint Urbain exit, please turn right and within 3 minutes you should be there after passing La Gauthetiere. Please see above map. After entrance, go straight ahead to end, then turn left and take escalator to 2nd floor). (This is also Google's top ranking for Dim Sum lunch but there maybe long line up for lunch.)

Underground city

Montreal is known for its underground city. The above map shows only a very small section of this. You can try this by going into the Saint Urbain street entrance (arrow in map above), into the Guy-favreau building. After entering follow the long hall on the first turn to the right. Go all the way to the end of the long hall. Then go down the escalator. Then go through the underground tunnel passing the post office on your left, then go up the escalator. Right in front is a fountain (picture above right), and behind it, is the large international food court. If you keep going passing the food court, you will come to the Place des Arts where there are some art exhibits and many concert halls. Going up further is the Place des Arts metro station**.** (just across the street from Holiday Inn is the Montreal Convention Centre with the Place d'arm metro subway station)

.

2:30 pm-5:30 pm **PRECLINICAL (except for keynote 20 mins each) Ballroom**
Co-Chairs: Chen C (China) & D'Agnillo F (U.S.A.)

D'Agnillo F (U.S.A.) Keynote lecture 25 mins
Senior Investigator, Laboratory of Biochemistry and Vascular Biology, Division of Hematology, ,Center for Biologics Evaluation and Research (CBER), Food and Drug Administration, Bethesda, MD, USA
(Alumni of Artificial Cells & Organs Research Centre)
FDA recommendations on the chemistry and manufacturing controls (CMCs) aspects of HBOC development.

Kassa, T*[1], Fantao Meng*[1], Michael Brad Strader[1], Sirsendu Jana[1], Darón I. Freedberg[2], Felice D'Agnillo[1], and Abdu I. Alayash[1] (USA)
[1]Laboratory of Biochemistry and Vascular Biology, and [2] Laboratory of Bacterial Polysaccharides, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring,
Biochemical and Biophysical Characterization of Hemoglobin-Based Oxygen Carriers (HBOCs): Not All HBOCs Are Created Equal

Ning Jing, MD,PhD (Canada)
Clinical evaluator, Clinical Evaluation Division, Hematology & Oncology Centre for Evaluation of Radiopharmaceuticals and Biotherapeutics/Biologics and Genetic Therapies Directorate, Health Product and Food Branch, Health Canada, Government of Canada (Alumni, Artificial Cells and Organs Research Centre)
The key consideration of the clinical assessment for biosimilars

Chen C (China)
President, Chinese Society of Blood Substitutes,
Vice President Northwest University, Xian, China
Preclinical investigation of Polymerized Porcine Hemoglobin (pPolyHb)

Chen G, Yaojin Li, Hong Wang, Jiaxin Liu*, Chengmin Yang*(China)
Assistant Professor, Blood Transfusion Institute of Chinese Academy of Medical Sciences
(Alumni of Artificial Cells & Organs Research Centre)
Novel Red Blood Cell Substitute: the Principle, Design and Its Effectiveness for Hemorrhagic Shock

Yaojin Li, Peipei Sang, Weinan Li, Shen Li, Gang Chen, Wentao Zhou, Hong Wang, Jiaxin Liu*, & Chengmin Yang* (China)
Institute of Blood Transfusion, Chinese Academy of Medical Sciences, Chengdu, P.R. China
Polymerized human placenta hemoglobin dissolved in hydroxyethyl starch solution as a novel oxygen-carrying plasma expander

Song, Bjorn K. (USA)
William H. Nugent[1], Ramon F. Cestero[2], Kevin Ward[3], Ronald Jubin[4], Abe Abuchowski[4], Bjorn K. Song[1]
1Song Biotechnologies, Baltimore, MD
2. University of Texas Health San Antonio, San Antonio, TX
3. University of Michigan Medical School, Ann Arbor, MI
4. Prolong Pharmaceuticals, South Plainfield, NJ
Efficacy of SANGUINATE™ versus Standard of Care in Three Rat Models of Hemorrhagic Shock

Guo C and TMS Chang (Canada)
Artificial Cells & Organs Research Centre, McGill University, Canada
Immunological study of poly-[Hb-CAT SOD CA]: a nanobiotherapeutic

***2:30 pm-5:30 pm TRANSFUSION MEDICINE* (except for keynote 20 mins each) Habiscus**
Co-chairs : Robillard, Pierre (Canada) & Binglan Yu (U.S.A.)

Yang CM (China)*:* Keynote lecture 25 mins
Professor Emeritus and Director Emeritus, Institute of Transfusion medicine, CAMS/PUMC.
Former Director, Chinese Red Cross National Blood Center.
Recent development of Transfusion Medicine in China

Robillard, Pierre (Canada)
Medical Director, Hema-Quebec, Montreal, Quebec, Canada
Hemovigilance from an international perspective

Christopher P. Stowell, MD, PhD (USA)
Director, Blood Transfusion Service, Department of Pathology, Massachusetts General Hospital
Associate Professor of Pathology, Associate Director, Harvard Transfusion Medicine Fellowship Program
Harvard Medical School, U.S.A
The Clinical Impact of Red Blood Cell Storage: What Have the RCTs Told Us?

Pelletier, P (Canada)
Director of Transfusion Medicine Service, McGill University Hospital Centre designated transfusion center
Faculty of Medicine, McGill University, Montreal, Quebec, Canada
Ethnic differences in red blood cell antigens and how they affect transfusion.

Ponka P (Canada)
Lady Davis Institute and Department of Physiology, Associate member of Artificial Cells & Organs Research Centre, McGill University
Physiology and Pathophysiology of Iron Homeostasis: Implications for Therapy of Iron Overload

Yu BL (U.S.A.)
Assistant Professor, Mass General Hospital, Harvard Medical School (Alumni of Artificial Cells & Organs Research Centre)
Inhalation of nitric oxide in blood transfusion

Blais, MC (Canada)
Professor, Montreal University (Alumni of Artificial Cells & Organs Research Centre)
Research on blood groups in animal

Scott, M (Canada)
Senior Scientist - Clinical Professor, Canadian Blood Services and University of British Columbia
Immunocamouflaged Cells: Applications in Transfusion and Transplantation Medicine"

Kwan D (Canada)
Assistant Professor-Dept. of Biology, Centre for Applied Synthetic Biology, Concordia University
Engineering blood group antigen-cleaving enzymes by directed evolution to modify red blood cells and remove antigenicity

2:30 pm-5:30 pm
DRUG DELIVERY/ REGENERATIVE MEDICINE (except for keynote 20 mins each) Jasmine
Co-Chairs: Chen GQ (China) & E Piskin (Turkey)

Chen GQ (China) Keynote lecture 25 mins
Professor of Microbiology and Biomaterials, Department of Biological Sciences and Biotechnology, School of Life Sciences, Tsinghua University Beijing 100084 China
Drug Targeting Systems Based on PHA Granule Binding Protein PhaP

Shum-Tim (Canada)
Professor of Surgery, Associate member of Artificial Cells & Organs Research Centre, Faculty of Medicine, McGill University
Authors: [1]D. Shum-Tim, MD., [2]A. Paul. Ph.D., H. [1]Al-Kindi, MD., [3]S. Prakash, Ph.D.
[1]Departments of Surgery, and Surgical Research, McGill University Health Center, McGill University, Faculty of Medicine, Montreal, Quebec, Canada. [2]Departments of Chemical and Petroleum Engineering, University of Kansas, Lawrence Kan, [3]Biomedical Technology and Cell Therapy Research Laboratory, Department of Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.
Novel Application of Micro-Nanoparticles in the Treatment of Heart Diseases

Maysinger D (Canada)
Professor, Department of Pharmacology and Therapeutic, McGill University
Anti-inflammatory dendrimers

Piskin E (Turkey)
President, Biomaterial and Bioprocessing Congresses,
Hacettepe University and Biyomedtek/NanoBMT, Cyberpark-Bilkent University/ Tekmer-Başkent University, Ankara, Turkey (Alumni of Artificial Cells & Organs Research Centre)
Engineering of Bone and Cartilage Tissues

Peixun Zhang, Yuhui Kou, Na Han, Xiaofeng Yin, Baoguo Jiang (China)
Peking University People's Hospital, Beijing, China.100044
Peripheral nerve system repair with the bi-directional induction and system remodeling from central system and target organs

Jiuxu Deng#, Ming Li#, Jian Weng, Yuhui Kou, Peixun Zhang, Na Han, Bo Chen, Xiaofeng Yin*, Baoguo Jiang*
Department of Orthopedics and Trauma, Peking University People's Hospital, Beijing, China.
#Equal contributors and co-first authors.
Comparison of different number autologous sural nerve grafts repair common peroneal nerve defects

Ming Li[#], Jiuxu Deng[#], Jian Weng, Fei Yu, Yuhui Kou, Na Han, Xiaofeng Yin, Peixun Zhang[*] & Baoguo Jiang*(China)
Department of Orthopedics and Trauma, Peking University People's Hospital, Beijing, China
Autologous sural nerve repair long common peroneal nerve defect by biodegradable conduit small gap tubulization

Se

7 pm SYMPOSIUM BANQUET (Complementary, but needs Name Badge)
Kam Fung Restaurant (1071 Saint Urbain Street pleae see map above). If you exit from Holiday Inn Saint Urbain exit, turn right and within 3 minutes you should be there after passing La Gauthetiere. After entrance, go straight ahead to end, then turn left and take escalator to 2nd floor).

TUESDAY, NOVEMBER 14TH 2017

8:30-10:30 am CLINICAL TRIAL RESULTS (A) : **(except for keynote 20 mins each) Ballroom**
Co-Chairs: 1. Greenburg AG (U.S.A.) & Mackenzie, C (U.S.A)

Greenburg AG (U.S.A.) Keynote lecture 25 mins
Past president, ISBS Int Sym Blood Substitutes, Emeritus Professor of Surgery, Brown University (U.S.A)
Discussion of clinical trial result of Hemoglobin based oxygen carriers

Mackenzie, Colin MD (U.S.A)
Emeritus Professor, University of Maryland School of Medicine ,Baltimore ,MD 21201 USA
Lessons Learned from 22 clinical trials of HBOC-201

Abuchowski, A (U.S.A.)
CEO, Prolong Pharmaceuticals
SANGUINATE®: A Clinical Update

Keipert P (U.S.A.)
Consultant & President, Keipert Corp, San Diego, CA, USA (Alumni of Artificial Cells & Organs Research Centre)
Challenges Facing HBOC Development in Trauma - What have we learned to minimize the risk going forward

Estep T (U.S.A.)
Chart Biotech Consulting, LLC
Moving HBOCs Forward - Testing Hypotheses in the Clinic

Biro, G (Canada)
Ottawa University, Ottawa, Ont, Canada
Concurrent disease states that may modify the response to intravascular HBOC

8:30 am-10:30 pm CANCER (20 mins each) Habsicus
CoChairs: Ramesh Chandra (India) & Palmer, AF (U.S.A)

Donald A. Belcher[1], Julia Ju[2], Jin Hyen Baek[3], Ayla Yalamanoglu[3], Paul W. Buehler[3], Daniele M. Gilkes[2,4], Andre F. Palmer (USA)
1.William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH. 43224, USA
2.Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, Baltimore, MD 21218, USA 3.Division of Blood Components and Devices, Laboratory of Biochemistry and Vascular Biology, FDA/CBER, Silver Spring, MD 20993, USA 4.Department of Oncology and Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA
The Quaternary State of Polymerized Human Hemoglobin Regulates Oxygenation of Breast Cancer Solid Tumors: A Theoretical and Experimental Study.

Zhou, Dongfang, Xing Wei, Yubin Huang*
Assistant Professor, State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, ChangChun 130022, People's Republic of China
A Facile Way to Prepare Functionalized Dextran Nanogels for Conjugation of Hemoglobin

Kinsella M (Canada)

Bioengineering Department, McGill University (Associate member of Artificial Cells & Organs Research Centre)
Engineering Nanomaterials to Diagnose and Track Cancer from the Cellular to the Tissue Leve
Vartika Tomar[1], Satya Prakash[2] and Ramesh Chandra[1]
Laboratory of Drug Discovery and Metabolism [1]Department of Chemistry, University of Delhi, Delhi-110007, India
[2]Department of Biomedical Engineering, McGill University, Montreal, Canada
Metabolism of Anticancer Agents Noscapine and Analogs

Wang,Y[1,2] and TMS Chang[1]
[1] PhD Research done at Artificial Cells and Organs Research Centre, McGill University, Montreal, Canada
[2] Now Research staff, 3rd Hospital of Peking University Medical School (Alumni of Artificial Cells & Organs Research Centre)
Biodegradable Nanocapsules Containing A Nanobiotechnological Complex for the Suppression of A Melanoma Cell Line B16F10
www.medicine.mcgill.ca/artcell/2016Wang&Chang.pdf

Prabha, Shashi 1, **Bahar Ahmed 2, and Dr. Mohd Aqil*1 (India) (to be confirmed)**
1,Dept. of Pharmaceutics, Jamia Hamdard, New Delhi-, India.2.Dept. of Pharmaceutical Chemistry, Jamia Hamdard, New Delhi-India.
Preparation and in-vitro characterization of 9-bromonoscapine for preparation of cancer drug delivery nano formulations for use in breast and other cancers

8:30 am-10:30 am GENERAL AND CVS (except for keynote 20 mins each) Jasmine
Cochair: Best, R (U.S.A.) & Poncelet D (France)

Chandra R (India) Keynote lecture 25 mins
Professor and Director, Laboratory of Drug Discovery and Metabolism
Department of Chemistry, University of Delhi, Delhi-110007, India
From Laboratory to the bedside

Best, Robert (U.S.A.) ;
Professor of Biomedical ScieProfessornces, associate Dean for Faculty Affairs, University of South Carolina School of Medicine
Technological versus Traditional Approaches to Medicine in an Age of Rapid Change and Declining Resources

Mobed-Miremadi, M (U.S.A)
Santa Clara University, CA, U.S.A. (Alumni of Artificial Cells & Organs Research Centre)
The Legacy of Artificial Cells in Biomedical Engineering Education

Poncelet D (France)
President, International Bioencapsulation Group, Professor, ONIRIS, UMRS, CNRS, GEPEA, France
(Alumni Artificial Cells and Organs Research Centre)
Microencapsulation : a, human story

Lomis, Nikita [1,2], Francis Gaudreault[3], Meenakshi Malhotra[4], Susan Westfall[1], Dominique Shum-Tim[5] and Satya Prakash (Canada)
[1,*]Biomedical Technology and Cell Therapy Research Laboratory, Department of Biomedical Engineering, [2,*]Division of Experimental Medicine,; [3]Human Health Therapeutics, National Research Council Canada, [4]Department of Radiology, Stanford University School of Medicine, Stanford, CA, , USA; [5]Division of Cardiac Surgery and Surgical Research, Royal Victoria Hospital,
Development of a novel nanoparticle based therapy for cardiovascular diseases

Westfall S, Lomis N, Iqbal U, Prakash, S
Biomedical Technology and Cell Therapy Research Laboratory, Dept. of Biomedical Engineering,

Artificial Cells and Organs Research Centre, McGill University,
Ferulic acid is a cross-talk mediator between L. fermentum NCIMB 5221 and the host metabolic, anti-oxidant and immune systems

***11:00-11:30 Coffee* Ballroom Foyer**

11:00-1:00 MOVING FORWARD Ballroom
Cochairs: Biro,G (Canada) Keipert, P (U.S.A)

Chang TMS (Canada)
Honorary President, of ISBS and of ISNS,
Director, Artificial Cells & Organs Research Centre, Faculty of Medicine, McGill University, Canada
Individual Roles of (1) Oxygen carriers, (2) Oxygen carries with antioxidant and (3) Oxygen carries with antioxidant and CO2 transport. www.medicine.mcgill.ca/artcell/translation.pdf

Hsia C (U.S.A.)
Carleton Jen-Chang Hsia Ph.D. Chairman and CEO, NanoBlood LLC, Sioux Falls, SD., 57107
SanFlow as a Universal Golden Hour Drug for the Treatment of Hemorrhagic and Ischemic Stroke

Simoni, J (U.S.A)
Professor, Texas University, Texas.
Requirements for HBOC to be highly effective in the treatment of myocardial ischemia

William Richard Light[1], Ashok Malavalli[1], Kim Vandegriff[1], Joseph Tucker[1], Roberto Lopez[2], Paulo Fontes[2]
[1] VirTech Bio, Inc., Natick, MA, USA [2] University of Pittsburgh, Pittsburgh, PA, USA
Development of a new hemoglobin-based oxygen carrier solution (VIR-XV1) for liver allograft preservation in combination with machine perfusion

Zal F (France) Keynote
President, HEMARINA S.A. | Aéropole centre | Biotechnopôle |
Use of HEMO2life - an Innovative Oxygen Carrier in Organ Transplantation.

Polard,Valérie & Pierre Alix* (France)
Responsable Préclinique/ Preclinical Development Manager
Aéropole centre – Biotechnopôle, 29600 MORLAIX, FRANCE
Evaluation of a specific oxygen carrier (M101®) added to pig liver cold storage solutions to improve post-transplant graft function.

Kim Hae Won (U.S.A.)
insights into acellular HBOC-mediated hypertension and potential pharmacologic remedies

Rausch C (U.S.A.) (to be confirmed) *The development and the difficulties as well as the opportunities of blood substitutes*

11:00am to 1:00 pm (1) AROUND THE WORLD (2) PFC Habiscus
(20 mins each)
Co-Chairs: Liu JX (China.) & Speiss,B (U.S.A)

Bülow L (Sweden)
Past president, 2015 ISBS Int Sym Blood Substitutes,
Professor and Chair, Dept of Pure and Applied Biochemistry, Lund University, Sweden
Present status of research on blood substitutes in Europe

Liu JX (China)
Secretariat, Chinese Society of Blood Substitutes,
Professor and Interim Director, Blood Transfusion Institute of Chinese Academy of Medical Sciences

Present status of research on blood substitutes in China

Hiromi Sakai[1], Koichi Kobayashi[2]
[1]Department of Chemistry, Nara Medical University, Kashihara,; [2]Keio University, Tokyo, Japan;
Present Status of Blood Substitute Research in Japan

Spiess, Bruce D (U.S.A)
Professor and Associate Chair (Research) Department of Anesthesiology University of Florida College of Medicine. Gainesville, FL
Perfluorocarbon Emulsions as Respiratory Gas Diffusion Enhancers- A Path towards Medical Breakthroughs.

Latson, Gary W. M.D.(U.S.A)
Director Neurosurgical Anesthesiology, Scott and White Memorial Hospital, Baylor Scott and White Healthcare
Adjunct Associate Professor, Anesthesiology, Texas A&M University
Perftoran (Vidaphor), an intravenous perfluorocarbon emulsion from Russia: Introduction to Western Medicine.

Latson, Gary W. M.D.(U.S.A)
Director Neurosurgical Anesthesiology, Scott and White Memorial Hospital, Baylor Scott and White Healthcare
Adjunct Associate Professor, Anesthesiology, Texas A&M University
Intravenous Perfluorocarbon Emulsions as a treatment for vascular gas embolism and decompression sickness.

Ferenz, Katja (Germany)
Universitätsklinikum Essen (AöR), Institut für Physiologische Chemie, Hufelandstraße 55
Functionality of albumin-derived perfluorocarbon-based artificial oxygen carriers in the Langendorff-heart"

11:00 am -1:00 pm METABOLIC : DIABETES, MICROBES **(20 mins each) Jasmine**
CoChairs: Shi. Z (U.S.A) & R.J. Neufeld (Canada)

R.J. Neufeld[1],(Keynote lecture 25 mins) C. Pinto Reis[1,2], B. Sarmento[1,3], C. Woitiski[1,2], F. Veiga[2], A. Ribeiro[2], D. Ferreira[3], C. Damgé[4])
[1]Queen's University, Kingston, Ontario, Canada[2]University of Coimbra, Portugal[3]University of Porto, Portugal [4]Université Louis Pasteur, France
BIOMATERIAL CHOICES IN DESIGN OF COMPLEX NANOPARTICULATE CARRIERS FOR ORAL DELIVERY OF INSULIN

Hoesli C (Canada)
Department of Chemical Engineering Université McGill University, Associate member of Artificial Cells & Organs Research Centre
"Pancreatic beta cell bioencapsulation by emulsification and internal gelation"

Zou, Hequn (China)
Vice-president, Chinese Society of Apheresis
Director, Institute of Nephrology and Urology, Southern Medical University
. Nanomedicine in the early diagnosis of diabetes

Shi Z (U.S.A.),
Vice President, Clinical Development, REMD Biotherapeutics Corp, California. (Alumni of Artificial Cells & Organs Research Centre)
Zhiqing Shi[1], Jeremy Pettus[2], Dominic Reeds[3], Tricia Santos[2], Schafer Boeder[2], Michelle Levin[2], Edda Cava[3], Dung Thai[1], Hai Yan[1], Edgar Bautista[1], John McMillan[1], Robert Henry[2], Samuel Klein[3]
(1: REMD Biotherapeutics, Inc. USA; 2*: University of California San Diego, USA; 3. Washington University School of Medicine, USA)*

A Fully Human Glucagon Receptor (GCGR) Antibody Reduces Daily Insulin Requirements and Improves Glycemic Control in People with Type 1 Diabetes

Scott, M (Canada)
Senior Scientist - Clinical Professor, Canadian Blood Services and University of British Columbia
Modulating the Immune System via Bioreactor Produced miRNA-Based Therapeutics

1:00 to 2:30 LUNCH
On your own to the many nearby restaurants and sightseeing before returning to the nonstop 3 hour afternoon session !

2:30pm-5:30pm OXIDATIVE/HEME MEDIATED TOXIXITY (except for keynote 20 mins each) Ballroom
Co-Chairs: Alayash A (U.S.A.) & Simoni, J (U.S.A)

Alayash A (U.S.A.) Keynote lecture 25 mins
Laboratory of Biochemistry and Vascular Biology, Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20993, USA
Mechanisms of Toxicity and Modulation of Hemoglobin-based Oxygen Carriers (HBOCs)

Stefano Bruno (Italy)
Authors**:** Esra'a Ali Mohammad Alomari[1], Stefano Bruno[1]*, Luca Ronda[2], Gianluca Paredi[1], Riccardo Piano[2], Stefano Bettati[2], Davide Olivari[3], Francesca Fumagalli[3], Deborah Novelli[3], Giuseppe Ristagno[3], Roberto Latini[3], Chris Cooper[4], Brandon Reeder[4], Andrea Mozzarelli[1]
[1]DEPARTMENT OF FOOD AND DRUG, UNIVERSITY OF PARMA, PARMA, ITALY; [2]DEPARTMENT OF MEDICINE AND SURGERY, UNIVERSITY OF PARMA, PARMA , ITALY; [3]ISTITUTO DI RICERCHE FARMACOLOGICHE 'MARIO NEGRI', MILAN, ITALY; [4]SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF ESSEX, COLCHESTER, UNITED KINGDOM
High- and low-affinity PEGylated hemoglobin-based oxygen carriers: differential oxidative stress in a Guinea pigs transfusion model

D'Agnillo F (U.S.A.)
Senior Investigator, Laboratory of Biochemistry and Vascular Biology, Division of Hematology, ,Center for Biologics Evaluation and Research (CBER), Food and Drug Administration, Bethesda, MD, USA
(Alumni of Artificial Cells & Organs Research Centre)
Reversible renal glomerulr dysfunction in guinea pigs infused with polymerized cell-free hemoglobin

Michael Brad Strader, PhD[1], Tigist Kassa, PhD[1], Sirsendu Jana, PhD[1], Fantao Meng, PhD[1], Wayne Hicks, PhD[1], John S. Olson[2], and Abdu I. Alayash, PhD[1]
[1]DBCD/OBRR/CBER/FDA,
[2]BioScience Department, Rice University, Houston, TX
Characterization of oxidative toxicity in mutant Hemoglobins and Hemoglobin Based Oxygen Carrier (HBOCs) candidates using high resolution accurate mass (HRAM) mass spectrometry

Nicholas L. Robbins,
1RESTOR™ Program, 59th Medical Wing, JBSA Lackland AFB, TX, University of Texas Health Sciences Center at San Antonio, San Antonio, TX,
Discussant

Yang, Bo[1], Li Wang[1], Chao Chen[1,2], Hongli Zhu[1,2] (China) **(abstract only)**
1.College of Life Science, Northwest University, Xi'an 710069, P. R. China
2.National Engineering Research Center for Miniaturized Detection Systems, Northwest University, Xi'an 710069, P. R. China
pPolyHb protects myocardial H9C2 cell against ischemia-reperfusion injury by regulation of Pink1-Parkin mitochondrial autophagy pathway

Li, Weinan , Wanjing Li, Wentao Zhou, Yaojin Li, Shen Li, Hong Wang, Jiaxin Liu*(China) (abstract only)

Institute of Blood Transfusion, Chinese Academy of Medical Science, Chengdu, P.R. China
Preliminary Study of Oxidative Stress Caused by Polymerized Hemoglobin and Intervention in Rats

2:30-5:30 pm
HEMOPRERFUSION, PLASMAPHARESIS, ADBSORBENT
www.medicine.mcgill.ca/artcell/hpbk_ch1.pdf **Habiscus**
(except for keynote 20 mins each)
Cochairs: Zou, Hequn (China) & Barre, P (Canada)

Barre, P (Canada)
Associate Professor-McGill University, Medical Director-Chronic Kidney Disease Clinic, Montreal General Hospital- Division of Nephrology, L4.521 , Associate member, Artificial Cells & Organs Research Centre
Hemoperfusion at McGill University Health Centre

Wang , Shenqi (China) Keynote lecture 25 mins
Marie Curie Fellow(MC-IIF) Ph.D
Professor,School of Life Science & Technology,Huazhong University of Science and Technology, Wuhan,P.R.China
The Challenge of Adsorbent for Hemoperfusion in China

Zou, Hequn (China) , Keynote lecture 25 mins
Vice-president, Chinese Society of Apheresis,Director, Institute of Nephrology and Urology, Southern Medical University
Adsorbent baed plasmapheresis for autoimmune/inflammation diseases

Lulu Han, Jingyu Li, and Lingyun Jia* (China)
School of Life science and Biotechnology, Dalian University of Technology, Dalian 116023, P. R. China
Removal of indoxyl sulfate by water-soluble poly-cyclodextrins in dialysis

Jun Ren*, Lingyun Jia (China) renjun@dlut.edu.cn
School of Life Science and Biotechnology Dalian University of Technology, Dalian, China 116024
Preparation of hydrophobic charge induction adsorbent for selective removal of antibody from human plasma

Lingyun Jia*, Jun Ren, Xiaobo Bao (China)
Liaoning Key Laboratory of Molecular Recognition and Imaging, School of Life science and Biotechnology, Dalian University of Technology, Dalian, China 116024
Removal of Beta-2-microglobulin from Human serum using Single Domain Antibody as Ligand

Yu, Huibin and Professor Shenqi Wang (China),
School of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China.
Preparation of Zn^{2+} loaded chitosan beads based adsorbent for the removal of human testosterone in plasma

Chen, Jian 1, Guanghui Cheng1, Yamin Chai1 and Lailiang Ou*1 (China)
1Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China.
Preparation of nano-CaCO3/polystyrene nanocomposite beads for efficient bilirubin removal

Li, Xing [1], Sheng Wang[1], Lailiang Ou[2], Yaoting Yu[2], Shenqi Wang[11] (China) (to be confirmed)
[1]Huazhong University of Science and Technology, Wuhan, China.[2]Nankai University, Tianjin, China
A Novel Polystyrene Beads Adsorbents Containing Mesopores and Linear Decapeptide Segments as Ligands for the Removal of β2-Microglobulin from Human Plasma

WEDNESDAY, NOVEMBER 15TH 2017

8:30-10:30 am PROTEIN ENGINEERING **(except for keynote 20 mins each)** ***Ballroom***
Co-Chairs: Bülow L (Sweden) & Reeder, Brandon (U.K.)

Bülow L (Sweden) Keynote lecture 25 mins
Past president, 2015 ISBS Int Sym Blood Substitutes,
Professor and Chair, Dept of Pure and Applied Biochemistry, Lund University, Sweden
Protein Engineering for Hemoglobin Based Oxygen Carriers

Reeder, Brandon (U.K.) Keynote lecture 25 mins
Brandon J. Reeder(1)*, Michelle Simons(1), Nélida Leiva Eriksson(2), Leif Bulow(2), Andrea Mozzarelli(3), Luca Ronda(4), Andras Eke(5), Natalie Syrett(1), Victoria Allen-Baume(1), Chris Cooper(1)
(1) School of Biological Sciences, University of Essex, Colchester, Essex, CO4 3SQ, UK.
(2) Pure and Applied Biochemistry, Lund University, Sweden
(3) Department of Food and Drug, University of Parma, Italy
(4) Department of Medicine and Surgery, University of Parma, Italy
(5) Department of Physiology, Semmelweis University, Budapest, Hungary
A novel recombinant hemoglobin-based blood substitute with combined enhanced ferryl and ferric reductase activit

Sun, Jian [1], Bin Cao[2], Qinggui Meng[2] (China)
1Department of Molecular and Cellular Pharmacology, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, P R China; 2Shandong Wan'an pharmaceutical co., LTD., Dongying, Shandong 25700, China
Recombinant hemoglobin as oxygen carrier by gene engineering: a review

Nélida Leiva Eriksson [(1)] and Leif Bulow [(1)]
[(1)] Department of Pure and Applied Biochemistry, Lund University, Box 188, 221 00 Lund, Sweden
A green alternative for the development of HBOCs

Karin Kettisen* & Leif Bülow (Sweden)
Pure and Applied Biochemistry, Lund University
Impact of cysteine residues in recombinant fetal hemoglobin

Carlsson, Magnus [1], Selvaraju Kanagarajan[1], Sandeep Chakane[2], Karin Kettisen[2], Khuanpiroon Ratanasopa[2,3], Leif Bülow[2], Li-hua Zhu[1] (Sweden)
[1]Department of Plant Breeding, Swedish University of Agricultural Sciences, Alnarp, Sweden [2]Department of Pure and Applied Biochemistry, Lund University, Lund, Sweden [3]Örebro Life Science Centre, School of Science & Technology, Örebro University, Örebro, Sweden
Human fetal hemoglobin expression, purification and characterization in Nicotiana benthamiana

Shen, Yuesheng[1], Geng Niu[1], Yuwei Bai[1], Chao Chen[1,2], Hongli Zhu[1,] (to be confirmed)
1.College of Life Science, Northwest University, Xi'an, P. R. China
2.National Engineering Research Center for Miniaturized Detection Systems, Northwest University, Xi'an, P. R. China
Preliminary study on pharmacokinetics of Polymerized Porcine Hemoglobin (pPolyHb)

Yuhao Lu, Meng Du, Ziyuan Wang* (China) (to be confirmed)
School of life Science, Xuzhou Normal Universit, Xuzhou, P.R. China
Enhancement of recombinant hemoglobin production in P. pastoris containing the HRG-4 heme transpot system

8:30-10:30 am NANOCAPSULES AND NANOPARTICLES (except for keynote 20 mins each) Habiscus
CoChairs: Sakai H (Japan) & *H. Bäumler* (Germany)

Sakai H (Japan) Keynote lecture 25 mins
Professor Nara Medical University, Nara, Japan
Translational Research of Hb-vesicles (Artificial Red Cells) for a Transfusion Alternative and O_2/CO Therapeutics

***H. Bäumler* (Germany)**
Institute of Transfusion Medicine, Charité-Universitätsmedizin Berlin, Germany
Hemoglobin-Based Oxygen Carriers HbMP-700 can deliver more than oxygen

Tomoko Kure, Hiromi Sakai (Japan)
Department of Chemistry, Nara Medical University, Kashihara 634-8521, Japan
Transmembrane Difference in Colloid Osmotic Pressure Affects the Lipid Membrane Fluidity of Liposomes Encapsulating a Concentrated Hb Solution

Doctor, Allan (U.S,A,)
Professor of Pediatrics and Biochemistry, Washington University School of Medicine
Pediatric Critical Care Medicine, Saint Louis Children's Hospital ,St. Louis, Missouri
ErythroMer (EM), a Nanoscale Bio-Synthetic Artificial Red Cell: proof of concept and in vivo efficacy results

Konetski, D., Zhang, D., Gong, T., Baranek, A., Worrell, B., Bowman, C. (U.S.A.)
University of Colorado, Boulder
Production of Artificial Cell Membranes Bearing New Characteristics or Behaviors Using "Click" Chemistries

Tajparast, F and Mladen I. Glavinović (Canada)
Departments of Civil Engineering & Applied Mechanics & Physiology, McGill University, Montreal, PQ, Canada
Forces acting on objects in nanopores with irregularities

Zhang, ZB (United Kingdom)
Past President, President of Symposium on biocompatible capsules (UK)
Professor and Deputy Director of the China Institute, University of Birmingham,Birmingham
Understanding the mechanical properties of cells, microspheres and microcapsules

Huang, Y (Wang, Yupeng ,Yubin Huang*) (China)
Professor, State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, ChangChun 130022, People's Republic of China
Hemosome formed by protein−polymer conjugate assembly as oxygen carrier

***11:00-11:30 Coffee* Ballroom Foyer**

11:00am-1:00pm MICROCIRCULATION (except for keynote 20 mins each) Ballroom
Cochairs: Intaglietta, M (U.S.A.) and. Friedman, Joel M (U.S.A.)

Intaglietta, M (U.S.A.) Keynote lecture 25 mins
Professor, University of California at San Diego
Authors: Amy G. Tsai, Pedro Cabrales, Joel M. Friedman, Daniel M. Tartakovsky, & Marcos Intaglietta
POST-TRANSFUSION INCREASE OF HEMATOCRIT PER SE DOES NOT IMPROVE CIRCULATORY OXYGEN DELIVERY DUE TO INCREASED BLOOD VISCOSITY

Pedro Cabrales (U.S.A)
Dept. of Bioengineering, University of California, La Jolla, CA
Polyethylene Glycol Camouflaged Earthworm Hemoglobin

Torres Filho, Ivo MD, PhD (U.S.A.)
Research Physiologist, US Army Institute of Surgical Research, JBSA Fort Sam Houston, TX
In vivo Enhancement of Flow and Oxygen Transport in Microvessels: The Nanomedicine Approach During Ischemia

Seetharama A. Acharya, Savita Bhutoria, Dongxia Li, M. Prabhakaran, Amy G. Tsai, Marcos Intaglietta, and Craig Branch (U.S.A)
PATTERN OF PEGYLATION OF HB IMPACTS THE EFFICACY TISSUE OXYGENATION BY PEG HB: EAF P3K6 HB IS AN ANTI-ANEMIA THERAPEUTIC OPTIMIZED FOR OXYGEN TRANSFER CATALYTIC ACTIVITY.

Amy G. Tsai ,Pedro Cabrales, Joel M. Friedman and Marcos Intaglietta (U.S.A)
ROLE OF CYTOKINES IN PROMOTING OXYGEN DELIVERY AFTER BLOOD TRANSFUSION. Amy G. Tsai,

Joel M. Friedman (U.S.A)
ENHANCING SAFETY AND THERAPEUTIC EFFICACY OF BOTH HBOCS AND RBC BASED TRANSFUSIONS THROUGH THE SYSTEMIC NANOPARTICLE-BASED DELIVERY OF NO BIOACTIVITY

11:00am-1:00pm *PROCESSES INCLUDING EXTRACTION AND PURIFICATION* (20 mins each) Habiscus
Cochairs: Gu KF (U.S.A.) Cattaneo M (U.S.A.)

Elmer, J (U.S.A.)
Department Chemical Engineering, Villanova, PA, U.S.A.
Prolonging the Shelf Life of Lumbricus terrestris Erythrocruorin for Use as a Novel Blood Substitute

Ka Zhang[(1)]*, Tongchang Zhou[(1)], Lei Ye[(1)], Leif Bülow[(1)], (Sweden)
[(1)] Division of Pure and Applied Biochemistry, Lund University, Lund, Sweden
Purification of recombinant human hemoglobin from crude cellular extracts using molecularly imprinted polymers

Guo C , M Gynn & TMS Chang (Canada)
[1] Ph.D. Research done at Artificial Cells and Organs Research Centre, McGill University, Montreal, Canada
Extraction of Superoxide Dismutase, Catalase and Carbonic Anhydrase from red blood cell hemolysate for the preparation of Poly-[Hb-CAT SOD CA]: a nanobiotherapeutic with enhanced rbc functions:

Gu KF (U.S.A.) Alumni of Artificial Cells & Organs Research Centre
Novel Feeding Strategy Development for Enzyme/Protein Production

Cattaneo M (U.S.A.)
President, BioVolutions Laboratories Inc., Cambridge, Massachussetts, (Alumni of Artificial Cells & Organs Research Centre)
Continuous Manufacturing of Monoclonal Antibodies

1:00 to 2:30 LUNCH
On your own to the many nearby restaurants and sightseeing before returning to the nonstop 3 hour afternoon session !

2:30pm-5:30pm
NOVEL MOLECULAR & CHEMICAL APPROACHES (20 mins each) Ballroom
CoChairs: Kluger R (Canada) and Acharya SA (U.S.A.)

Kluger R (Canada)
Professor of Chemistry, U of Toronto
Authors : Ronald Kluger and Aizhou Wang
HBOCs from Hb-Hb Coupling Deliver Oxygen and Avoid Nitric Oxide

Acharya SA (U.S.A.)
Redesign of EAF PEG Hb to function as a targeted oxygen transfer catalyst under anemia to improve tissue oxygenation of the hypoxic areas: Application in Sickle Cell Anemia.

Palmer A (U.S.A.)
Professor and Chair, Dept of Chemical Engineering and Biomolecular Engineering, Ohio State University.
Engineering polymerized hemoglobin size regulates side-effects

Li Ma[1] and Carleton Jen-Chang Hsia[2] U.S.A.)
Georgia Department of Physics, Georgia Southern University,Statesboro, GA[1] and NanoBlood LLC, Sioux Falls, SD[2].,
SanFlow with Crystalloid as Blood Substitute

Komatsu , Teruyuki (Japan)
Department of App1ied Chemistry, Faculty of Science and 1-13-27 Kasuga Bunkyo-ku, Tokyo 112-8551, Japan
Hemoglobin-Albumin Cluster "HemoAct™" as an Artificial O_2-Carrier

Takashi Matsuhira, Keizo Yamamoto, Hiromi Sakai (Japan)
Department of Chemistry, Nara Medical University, Kashihara 634-8521, Japan
Reactivity of Cys b93 of native and b-crosslinked Hbs

Craig A Branch[1,2], Min-Hui Cui[1], Sangeetha Thangaswamy, PhD[3], and Seetharama Acharya[2.3] (U,S.A.)
1Gruss Magnetic Resonance Imaging Center/Dept of Radiology, 2 Department of Physiology and Biophysics, 3 Albert
Einstein College of Medicine, Bronx, NY; 3 Division of Hematology, Dept. Medicine, Albert Einstein College of Medicine, Bronx, NY
Semisynthetic Plasma Expanders, EAF PEG Alb and EAF PEG Hb, differentially affect oxygen deficit in animal models of sickle cell disease

Gang Chen, Tingting Wu, Can Huang, Hanfeng Zheng, Yaojin Li,
Hong Wang, Jiaxin Liu*, Chengmin Yang* (China) **(Abstract book only)**
Institute of Blood Transfusion, Chinese Academy of Medical Science,
Chengdu City, Sichuan Province, P. R. China
The reduction of human cord blood methemoglobin by vitamin C

ANNOUNCEMENT for 2019 ISBS
Professor Yang and Professor Sakai

2:30-5:30 pm NANOMEDICINE/ DIAGNOSTIC (except for keynote 20 mins each) Habiscus
Co-Chairs: Budak, G (Turkey) & Daka JN (Canada)

Budak, G (Turkey) Keynote lecture 25 mins

President,ISNS International Society for Nanomedical Sciences
Associate Professor, Director, Academy of Nanomedicine and Advance Technology, Ankara, Turkey
Prextrolin®- The Next Generation Nuclear Stain Biomarker for Cellular Analysis

Daka JN (Canada)
Government Research Scientist, Radiation Protection Bureau, Health Canada, Ottawa, CANADA
(Alumni of Artificial Cells & Organs Research Centre)
A Simple Plate Reader Method for Determination of Taurine in Human Urine Samples as a Potential Radiation Biomarker in Extreme Radiological/Nuclear Exposure Situations.

Piskin AK (Turkey)
Professor, Hacettepe University, Ankara (Turkey) (Alumni of Artificial Cells & Organs Research Centre)
Authors: Ayse Kevser Ozden*, Seda Atay**, Monirah Bakhshpour**, Fatma Yilmaz** , Handan Yavuz**, Adil Denizli**
*Hacettepe University, Faculty of Medicine, Medical Biochemistry Department
**Hacettepe University, Faculty of Science, Biochemistry Department, Ankara, Turkey
Quartz Crystal Microbalance (QCM) based biosensors for detecting breast cancer cells via their membrane receptors

Moghtader, Farzaneh [1,2], Orhan Erdem Haberal[2,3], Aysel Tomak[4], Hadi M. Zareie[5], Erhan Piskin[1,2]
[1]Hacettepe University, Nanotechnology and Nanomedicine Division
and Chemical Engineering Department, Beytepe, Ankara, Turkey
[2]NanoBMT, Beysukent/Cyberpark-Bilkent – KOSGEB/Tekmer-Başkent, Ankara, Turkey
[3]Başkent University, Biomedical Engineering Department, Baglıca, Ankara, Turkey
[4]İzmir Institute of Technology, Department of Material Science and Engineering, 35430, Urla, Izmir, Turkey
[5]University of Technology, School of Physics and Advanced Materials,
Microstructural Analysis Unit, Sydney, Ultimo NSW 2007, Australia
Bacterial Detection by SERS Using Nanoparticles and Bacteriophages

Chen, Jie, Wenyan Han, Jian Chen,† Weichao Wang, Wenhui Zong, Guanghui Cheng,Yaoting Yu, Lailiang Ou* (China)
*Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China
Computer-Aided Design of Small-Molecular Peptide Ligands of Adsorbent Targeting Tumor-Necrosis Factor-α (TNF-α)

Ursula Stochaj[1]* (Canada)
Authors:Dana Abou Samhadaneh[1], Khalid A. Alqarni[1], Ossama Moujaber[1], Dusica Maysinger[2], Ursula Stochaj[1]*
[1]Department of Physiology, McGill University, Montreal, Canada
[2]Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada,
GOLD NANOPARTICLES IMPAIR NUCLEAR FUNCTION AND PROTEOSTASIS IN CANCER CELLS

Dipanjan Pan* [1, 5, 6, 7, 8] (U.S.A)
Authors: Mao Ye,†, 1 Santosh Misra,†, 1 Arun K. De,†, 2 Fatemeh Ostadhossein,1 Kuldeep Singh,4 Laurie Rund,2 Lawrence Schook2,5 and Dipanjan Pan* [1, 5, 6, 7, 8]
1 Department of Bioengineering, University of Illinois at Urbana-Champaign, USA. 2 Department of Animal Sciences, University of Illinois, Champaign-Urbana, Illinois, USA. 3Agricultural Animal Care and Use Program, University of Illinois at Urbana-Champaign, Illinois, USA. 4Veterinary Diagnostic Laboratory, University of Illinois, Champaign-Urbana, Illinois, USA. 5Beckman Institute of Advanced Science and Technology, University of Illinois at Urbana Champaign, Illinois, USA. 6Mills Breast Cancer Institute, Carle Foundation Hospital, 502 N. Busey, Urbana, Illinois, USA. 7Department of Materials Science and Engineering, University of Illinois-Urbana Champaign, Illinois, USA. 8Carle-Illinois College of Medicine, Urbana, Illinois, USA. †Contributed equally.
*Corresponding author:
Nano-enabled Orphan Nuclear Receptor Activation Regulates Metabolism, Transport and

Programmed Cell Death Pathways in Soft Tissue Sarcoma of Xenograft Mice and Transgenic Oncopigs

Qi,Yanxin (China)
Yanxin Qi,Yupeng Wang,Yubin Huang*
State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, ChangChun 130022, People's Republic of China
Protein-Resistant Biodegradable Amphiphili Graft Copolymer Vesicles as Protein Carriers

Juncker D (Canada)
Professor of Biomedical Engineering, Micro and Nanobioengineering Laboratory McGill University
Authors: Grant Ongo, Sa Xiao, Susan Westfall, Andy Ng, Satya Prakash & David Juncker
Cell microarrays tissue constructs, and artificial gastrointestinal tract in a box.

Sang, Peipei, Yaojin Li, Gang Chen, Shen Li, Wentao Zhou, Hong Wang, Chengmin Yang* Jiaxin Liu ***(abstract only)***
Institute of Blood Transfusion, Chinese Academy of Medical sciences & Peking Union Medical College, Chengdu, P.R. China.
Effects of polymerized human placenta hemoglobin combined with hydroxyethyl starch on tissue organs in hemorrhagic shock rats

Zhao, Mengye[1], Chengbin Yan[1], Ying Xiao[1], Chao Chen[1,2], Hongli Zhu[1,2] **(to be confirmed)**
1.College of Life Science, Northwest University, Xi'an 710069, P. R. China
2.National Engineering Research Center for Miniaturized Detection Systems, Northwest University, Xi'an 710069, P. R. China
The effect of Polymerized Porcine Hemoglobin (pPolyHb) on hemodynamic stability and oxygen delivery in a rat model of perioperative blood transfusion

ABSTRACTS

1. OPENING PLENARY LECTURES pages 22-24

2. BLOOD SUBSTITUTES AND XVII ISBS RELATED AREA 25-62

3. V ISNS NANOMEDICINE CONFERENCE AND RELATED AREAS, 63-85

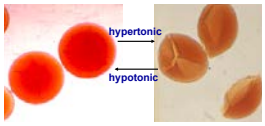
(Aphabetical : last name of presenter)

60th ANNIVERSARY LECTURE

Professor Chang TMS thomas.chang@mcgill.ca Honorary President, of ISBS and of ISNS, Director, Artificial Cells & Organs Research Centre, Depts of Physiology, Medicine, Biom Eng, Faculty of Medicine, McGill University,QC, Canada

A story of the roles of individuals and researchers around the world in the Invention and Evolution of Artificial Cells to Nanomedicine, Nanobiotherapeutic, blood substitutes, Bioencapsulation, Hemoperfusion, Regenerative Medicine etc

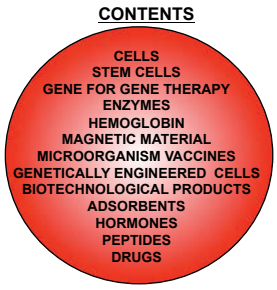
Stimulated by Physiology professor Sir Arnold Burgen's teaching on cell physiology, Chang invented and prepared the first artificial cell in his McGill dormitory room during his honours Physiology B.Sc. at McGill (Chang 1957) http://www.medicine.mcgill.ca/artcell/514.pdf . He continued this research while in medical school.becuase his wife, Lancy, has been most selflessly supported this. After this there was no one to look after his Ph.D. research. Luckily, Professor MacIntosh chair of Physiology agreed to take up this role, but insisted that Chang should be the sole author of the first paper (Chang **Science** 1964, Chang, Mason, McIntosh, **JCP** 1966). Then as assistant professor Chang & Poznanski **Nature** 1968; associate professor and MRC career investigator Chang **Nature**1971; then full professor and MRC career investigator, Chang, **Artificial Cell Monograph** 1972 that is for all to veiw on http://www.medicine.mcgill.ca/artcell/1972bookcovercr.pdf.



He emphasizes that he is not trying to reproduce biological cells since nature and cell culture can do a much better job. His aim is to use this basic principle to innovate and go outside the box – especially since there can be unlimited variations in the content, dimensions and membranes (figure on lower left).

He and researchers in this centre and numerous researchers around the world have since developed the idea of artificial cell into a very large area in the macro, micro, nano or soluble nanodimension complexes. This leads to many uses (Table). More details are summarized in http://www.medicine.mcgill.ca/artcell/2005NatureRev.pdf

ARTIFICIAL CELLS : variations



DIMENSIONS
Macro
Micro:
Nano:
Soluble complex

MEMBRANES
Polymeric
Biodegradable
Lipid
Xlinked protein
Conjugated
Carriers
Antigen, antibody

A few examples are:

(1) **Hemoperfusion:** After Professor Kolff, inventor of artificial kidney, listened to Chang's ASAIO lecture on artificial cell surface modified adsorbents for hemoperfusion, Kolff and Prof Bate's strong support resulted in extensive MRC research grants that allowed Chang to develop and carry out clinical trials. Chang's *clinical design showing its superiority over artificial kidney* resulted in quick FDA approval. Then, Dean Sam Freedman recognized this group as the Artificial Cells and Organs Research Centre. Hemoperfusion has since been used routinely in patients for acute poisoning and detoxification around the world. Extensions have resulted in treatment of liver failure, kidney failure and immunosorbent hemoperfusion for immunological diseases like Lupus etc. A 2017detailed review is available for all to viewn on: http://www.medicine.mcgill.ca/artcell/hpbk_ch1.pdf

ARTIFICIAL CELLS : USES

Micro and nano based miniature devices
Drug delivery:
Blood Substitutes and oxygen therapeutics:
Enzyme and gene therapy:
Cell & Stem Cell Therapy:
Biotechnology & Nanobiotechnology
Nanomedicine
Regenerative medicine
Agriculture, Industry, Aquatic culture
Nanocomputers and nanorobatics
Nanosensors etc

(2) **Blood substitutes:** There was no serious interest in his original basic research on glutaraldehyde crosslinked nanobiotechnology polyhemoglobin (Chang 1971 BBRC) www.medicine.mcgill.ca/artcell/1971ChangBBRC.pdf . It was only HIV contaminated blood in 1987 that led to belated catch up development of this and others without the much needed basic research and knowledge http://www.medicine.mcgill.ca/artcell/2014kimgrb.pdf Two U.S. groups have developed this basic idea, independently and tested clinically in the U.S. One of these and is now approved for routine use in South Africa to avoid the use of H.I.V. contaminated donor blood but it is 15 years too late since many patients around the world were infected with HIV, before screening test becomes available. However, short memory of the HIV disaster, resulted again in minimal basic research support for the many important needs like severe blood loss, other infections, etc. Chang's group is concentrating on a novel nanobiotherapeutic with enhancement of all 3 red blood cell functions with the *aim to show superior over blood in clinical trial*, having shown this in a 90 min sustained severe hemorrhagic shock rat model http://www.medicine.mcgll.ca/artcell/translation.pdf

(3) Artificial cell has led to the whole area of **Nanomedicine:** http://www.medicine.mcgill.ca/artcell/nanobk_ch1.pdf

(4) **Bioencapsulation of cells/stem cells/genetic engineered cells** followed his original research on cell encapsulation. Numerous researchers around the world are looking into its use for liver failure, kidney failure, genetic diseases etc http://www.medicine.mcgill.ca/artcell/2005NatureRev.pdf .

(5) Artificial cells has evolved into Micro-nano systems, Nanobiotechnology, Nanomedicine, Blood Substitutes, Synthetic Biology, Biosorbents, Bioencapsulation, Biotherapeutics, Drug Delivery Systems, cell/stem cell therapy, microbe, enzyme therapy, cancer therapy, nano-robotics etc as summarized in his 2007 monograph that is available on http://www.medicine.mcgill.ca/artcell/2007%20ebook%20artcell%20web.pdf Rapid increase in activities has resulted in the Artificial Cell, Nanomedicine and Biotechnology, an international journal, (Chang, editor in chief) Francis & Taylor Publisher, U.K. having to increase its annual pages by 4 times. Its Reuter world ranking is now 4th in Biomedical Engineering journals (77). World interest is also shown in 2011 when researchers in this area voted him out of 700 McGill nominee as the "Greatest McGillian in McGill's 190 years history". http://www.medicine.mcgill.ca/artcell/voting%20result.pdf . Other details in this area can be found on his public service website: http://www.medicine.mcgill.ca/artcell

PLENARY KEYNOTE LECTURE:

Peixun Zhang, Na Han, Yuhui Kou, Xiaofeng Yin, Baoguo Jiang
zhangpeixun@bjmu.edu.cn, jiangbaoguo@vip.sina.com
Peking University People's Hospital, Beijing, China

Peripheral nerve intersectional repair by bi-directional induction and systematic remodelling: biodegradable conduit tubulization from basic research to clinical application

Artificial Cells, Nanomedicine & Biotechnology, an international journal: 45; issue 8, 1463-1463, 2017 | DOI: 10.1080/21691401.2017.1390843, 2017

Qouted from above papter:

Abstract: In terms of the clinical effect of peripheral nerve injury repair, the biological degradable conduit 2 mm small gap tubulization is far better than the traditional epineurial or perineurium neurorrhaphy. The assumption of the bi-directional induction between the central system and the terminal effector during peripheral nerve regeneration is purposed and proved in clinical by our group. The surgical approach of transferring a portion of or the whole contralateral C7 nerve to repair a part of or the whole ipsilateral brachial plexus injury is clinically promoted, in which the most important idea and practice is to use the cone conduit designed by the group to repair thick nerves with fine nerves. Some of the patients suffering from cerebral palsy or cerebral haemorrhage and those who got cerebral infarction yet have not reached recovery after 3–6 months could regain some functions of the ipsilateral upper limb and improve the life quality by transfer of a portion of or the whole contralateral C7 nerve and connection by cone conduit.

"Currently, China has over 70 million patients with limb paralysis resulting from stroke or cerebral palsy, and the patient number increases by 20 million each year. If patients with the limb paralysis, resulting from stroke or cerebral palsy, do not recover after systematic rehabilitation exercise, there is little more doctors can do to help. Peripheral nerve transposition is a new strategy for repairing the injured nerves (brachial plexus injury) and treating the patient with limb paralysis resulting from the stroke or cerebral palsy. Unilateral brachial plexus avulsion can repair upper limb function by transposing a portio n of or the whole contralateral C7 nerve. Similarly, unilateral paralysis resulting from the stroke (cerebral hemorrhage or cerebral infarction) or cerebral palsy (perinatal hypoxia) can be treated using transposition of a portion of or the whole contralateral C7 nerve, which would repair the whole brachial plexus at the dysfunctional upper limb side, with potentially partial recovery of the functions of shoulder, elbow, wrist and hands."

"Based on the application of biodegradable conduit 2mm small gap tubulization for peripheral nerve mutilation and the deeply comprehension of bi-directional induction and systematic remodeling between central system and target organ, this new technique has brought new recovery hope for the patients with limb paralysis (Zhang P et al. 2013). This surgical approach has been successfully applied to 30 patients in clinic and has a greater extent of improvement in the life quality of patients. This continuous research had accomplished translation from basic research to clinical application."

Quoted from editorial: **A novel approach to restore function of stroke or trauma related paralyzed limbs**

Thomas Ming Swi Chang Editor in chief, 45: issue 8; 1463-1463, 2017 | DOI: 10.1080/21691401.2017.1390843, 2017

"Professor Jiang, dean of medicine of Peking University and his team published a 2017 review in our journal describing how they moved from the laboratory to the treatment of 30 patients with limb paralyses caused by stroke or trauma. They use a biodegradable growth factor releasing conduit to connect a branch of the proximal C7 on the normal side to the opposite distal C7T1 trunk of the paralyzed side. This results in neural connection and the restoration of the function of the paralyzed limbs - a major breakthrough for patients with stroke or trauma. **Professor Jiang receives the inaugurate annual best 2017 paper award in Artificial Cells, Nanomedicine & Biotechnology, an international journal** - Reuter world ranking of journal 4th among 77 Biomedical Engineering journals

PLENARY LECTURES

Jahr JS (j.s.jahr@ucla.edu

Hemoglobin glutamer-250 (bovine) in South Africa: consensus usage guidelines from clinician experts who have treated patients

Jahr S, Professor Emeritus of Anesthesiology David Geffen School of Medicine at UCLA

Hemopure (hemoglobin glutamer-250 [bovine]; HBOC- 201) is a hemoglobin (Hb)-based oxygen carrier registered with the Medicines Control Council of South Africa. It is indicated for the treatment of adult patients who are acutely anemic, for the purpose of maintaining tissue oxygen delivery thus eliminating, delaying, or reducing the need for allogeneic red blood cells (RBCs). HBOC-201 is also a volume expander, and circulatory volume must be carefully monitored for signs of fluid overload. HBOC-201 is not as effective as RBCs for restoring Hb content and concentration, but in cases of severe anemia where allogeneic blood is not an option or is unavailable, it may offer an immediate alternative for improving oxygen transport. This presentation provides clinical recommendations on the safe and effective use of HBOC-201 based on the postmarketing experience in South Africa as well as a better understanding of HBOC-201 properties reflected in recent publications.

Mer, et al. Transfusion 2016; 56(10):2631-2636. DOI 10.1111/trf.13726
Levien presentation at Fort Detrick, Maryland, 2017.

Prakash, S satya.prakash@mcgill.ca

Artificial Cells Biomedical technologies for human health with emphasis on microbiome and cardiac stents

Satya Prakash, Professor, Artificial Cells and Organs Research Center, Biomedical Engineering, Physiology, Experimental Medicine and Surgery, Faculty of Medicine, McGill University

Artificial cell research is a frontier in biomedical research driven by its clinical applications. Various innovative artificial cell based therapies have recently been proposed in several areas. Artificial cell transplants, biohybrid kidney/organs, artificial cell stem cell /gene therapy, artificial cell control release/blood substitutes, artificial cell targeted drug delivery are few areas that are currently being researched with great pace around world. In these areas, we have made significant contributions. In this meeting, contributions made in probiotics and microbiome and in designing next generation and stent will be discussed.

The human microbiome, signifies the full range of microorganisms (the microbiota) that live on and in humans. It represents a diverse collection of microorganisms. Bacterial population alone is estimated at as high as 200 trillion individual organisms. We have shown that oral feeding of live artificial cell bacterial cells can be used amongst others in managing kidney diseases (1), Heart Diseases (2), Vitamin D (3), Nonalcoholic fatty liver disease (NAFLD) (4), Colon Health (5,6), Cholesterol (6), Gastrointestinal Health (8) Neurological Disorders and Obesity (9), Metabolic Syndrome, Diabetes and other important health conditions. Details of these studies and how they can be used as next generation of therapeutics will be discussed.

Coronary artery disease like atherosclerosis caused by hardening of arteries is a leading cause of death in the developed countries. Most of these patients undergo angioplasty and stenting for impoved blood flow. But stenting has serious complications such as restenosis. Several approaches have been used to improve stent design and performances such as the covered stent to improve biocompatibility, use of intracoronary radiation therapy and drug eluting stent. But these are followed by potential side effects, drug washouts and effects on nontarget cells. Thus, there is an immediate need for a better and more biocompatibe coated vascular stent which will be thrombosis and restenosis-free and we have designed a new stent. Our developed a novel stent technology which can successfully deliver therapeutic genes and promote endothelial recovery. For this, for the first time, we used (i) biologically safe recombinant baculovirus, artificial cell microparticles and stretchable hydrogel to design a new (10) (ii) commercially viable, (iii) bioactive stent platform (11) (iv) to promote re-reendothelialization (12). McGill patented and successfully transfer this technology to industry and subsequently, based on these researches in 2014 a company called “MangoGen Pharma” was spin-off (www.mangogen.com) which in 2015 raised US $ 37.24 million USD from GP health care group. Recently (2017), Australian (# 2014252680), US (#15335408) patents has been approved while pending in EU, China, India, Canada and other geographies. Details of these patented technology in stent and other applications will be discussed.

References:

1. Prakash S and Chang, T.M.S. (1996). Nature Medicine, 2 (8):883-7.
2. Prakash S., et al and Bhathena, J (2005). Expert Opinion on Biological Therapy. Oct;5(10):1281-301.
3. Jones, M.L. et al and Prakash, S. (2013). Journal of Clinical Endocrinology Metabolism 98(7):2944-51.
4. Bhathena, J. et al and Prakash, S (2013). PLoS One. 2013; 8(3): 10.137.
5. Kahouli et al and Prakash, S (2017). Applied Microbiology Biotechnology. 101(5):1999-2019.
6. Urbanska, A. et al and Prakash, S. (2015). Cell Biochemistry and Biophysics. 72(3):757-69.
7. Tomaro-Duchesneau et al and Prakash S. (2015). Beneficial Microbes. 5; 6(6):861-9.
8. Jones, M. L et al and Prakash (2012). British, Journal of Nutrition. 107(10):1505-13.
9. Westfall S. et al and Prakash, S (2017). Cell Molecular Life Sciences Jun 22.1007.
10. Paul A, Elias CB, Shum-Tim D, Prakash S (2013). Nature Scientific Report. 3:2366
11. Paul A, Shum-Tim D, Prakash S. (2013). Methods Molecular Biology.1036:137-49.
12. Paul A, Hasan A, Rodes L, Sangaralingam M, Prakash S.(2014). Advance Drug Delivery Review. 71:115-30

2. BLOOD SUBSTITUTES AND XVII ISBS RELATED AREA

Abuchowski, A
abe@prolongpharma.com
AAbuchowski@prolongpharma.com

SANGUINATE®: A Clinical Update

Abraham Abuchowski, PhD
Prolong Pharmaceuticals, South Plainfield, NJ

SANGUINATE® is a dual carbon monoxide and oxygen delivery agent under development by Prolong Pharmaceuticals. As such, it is being developed as a therapeutic agent to correct hypoxia caused by a variety of anemic and ischemic disorders. The three components that comprise SANGUINATE® provide multiple therapeutic effects such as anti-inflammatory and anti-vasoconstrictive properties from carbon monoxide, improved rheology from polyethylene glycol, and oxygen delivery specifically to hypoxic tissues from PEGylated bovine hemoglobin.

A series of preclinical and clinical studies have been conducted that demonstrate safety and provide evidence of efficacy. Animal models of hemorrhagic shock and focal cerebral ischemia displayed the ability of SANGUINATE® to down-regulate inflammatory markers, deliver oxygen, and improve survival. A range of diseases and clinical studies that have been undertaken to date to support the safety of this investigational product as well as determine its efficacy.

Over 200 subjects have been exposed to either single or multiple doses of SANGUINATE®. Patients received as many as 16 units (8L) in 18 days and four units (2L) in 24 hours. SANGUINATE® was well tolerated and no meaningful adverse effects were identified that were attributed to SANGUINATE®. Signs of efficacy include improved neurological status, reduction of inflammatory markers, improved cerebral blood flow, and reduction/elimination in vasopressor intervention.

Other studies are ongoing and expected to be completed in 2017 (see Table below). Design of pivotal studies are under discussion in the areas of Septic Shock and Vaso-Occlusive Crisis in Sickle Cell Disease.

Indication	Phase	Dosing	Status	Location
Healthy Subjects	1	Single	Completed	Australia
Stable Sickle Cell Disease (SCD) Patients	1b	Single	Completed	Latin America
Delayed (kidney) Graft Function (DGF)	2	Multiple	Completed	USA
Delayed Cerebral Ischemia (DCI)	2	Multiple	Completed	USA
Life-Threatening Anemia	1/2	Multiple	Enrollment complete	USA
SCD- Leg Ulcers	2	Multiple	Enrollment complete	Latin America
SCD – Vaso-Occlusive Crisis (VOC) hospitalized	2	Multiple	Enrollment complete	Latin America
Humoral Sensitization	2	Single	Enrollment complete	USA
SCD - VOC ambulatory	2	Single	Ongoing	USA
Beta-Thalassemia	2	Multiple	Ongoing	Thailand
Beta-Thalassemia with Pulmonary Hypertension	2	Multiple	Ongoing	Thailand

Acharya SA seetharama.acharya@einstein.yu.edu

Pattern of PEGylation of Hb Impacts the Efficacy Tissue Oxygenation by PEG Hb: EAF P3K6 Hb is an anti-anemia Therapeutic Optimized for Oxygen Transfer Catalytic Activity

Seetharama A. Acharya[1], Savita Bhutoria[1], Dongxia Li[1], M. Prabhakaran[1], Amy G. Tsai[2], Marcos Intaglietta[2], and Craig Branch[3]
Division of Hematology[1], Department of Medicine of Physiology and Biophysics and Guss Magnetic Resonance and Imaging Center[3], Albert Einstein College of Medicine, Bronx NY and Deprtment of Bioengineering, University of California San Diego, La Jolla, CA[2]

Background: Surface decoration of hemoglobin (Hb) with PEG chains attenuates vasoconstriction of acellular Hb due to: (i) Increased oxygen (O_2) affinity of PEG Hb; (ii) PEGylation induced colloidal plasma expander like properties; (iii) Increase of nitrite reductase activity of Hb; and, iv) A combination of these effects. Disadvantages of PEG-Hb are increased O_2 affinity and PEGylation induced Hb tetramer dissociation. Extension Arm Facilitated hexaPEGylation of Hb with 6 copies of PEG-5K chains (EAF P5K6 Hb) is the only PEGylation protocol that does not induce a weakening of inter dimeric interactions, formulated at 4 g % has the viscosity of colloidal plasma expanders. However, MP4 of Sangart, a prototype of EAF P5K6 Hb, did not deliver much O_2 in extreme hemodilution studies, while P5K2 Hb yields very good tissue oxygenation. Increasing EAF PEGylation of Hb from 2 to 6 to 10 copies marginally reduces O_2 affinity of Hb PEG. But as the level of PEGylation increases the O_2 affinity of PEG Hb in the presence of strong allosteric effectors like IHP and L-35 is reduced. As PEGylation increases, there is preferential stabilization of the oxy conformation of PEG-Hb, and accessibility to the deoxy conformational state is reduced. The structure of larger PEG shells lowers O_2 escape and efficacy of tissue oxygenation. Thus we propose reducing the size of the PEG-shell by surface decoration with 6 copies of PEG 3K (EAF P3K6 Hb) using this PEG Hb at 6 g % to have the same viscosity as a solution of 4 g % EAF P5K6 Hb. Tissue oxygenation and structure of this EAF P3K6 Hb was compared with that of EAF P5K6 Hb.
Results: Surface decoration of Hb by EAF hexaPEGylation with PEG 3K chains reduces the molecular radius and therefore molecular volume of PEG-shell by more than 50 %, and increases the packing density of the PEG shell by about 50 %. The molecular shape of EAF P5K6 Hb is ellipsoidal while that of P3K6 Hb is globular. The O_2 affinity of EAF P3K6 is comparable to that of EAF P5K6 Hb. In extreme hemodilution studies in hamster, the efficacy of tissue oxygenation by EAF P3K6 Hb is better than that of EAFP5K6 Hb and is comparable to that of P5K2 Hb and P10K2 Hb. In PxK2 pattern, the increasing the molecular mass(X) of PEG chain from 5K to 10K has little influence on tissue oxygenation, while in EAF PxK6 pattern reducing the molecular size PEG from 5K to 3K improves efficacy.
Discussion and Conclusions: We demonstrate that pattern of PEGylation of Hb impacts the efficacy of oxygenation of hypoxic tissues. The amount of EAF P3K6 Hb and other PEG Hbs in plasma in these anemic hamster accounts for 20 to 25 % of total Hb (Hb in RBC and in plasma). We suggest that the high O_2 affinity of PEG Hb facilitates better extraction of O_2 from oxyHb in RBC to plasma and diffusion to tissues, i.e., PEG Hb in plasma acts as a catalyst for O_2 transfer to tissues while the oxy Hb in RBC serves as the reservoir for O_2, i.e., PEG Hb is as an anti-anemia therapeutic when the hematocrit is close to the transfusion trigger and O_2 delivery by RBC is not adequate. Consistent with this a 10 % top load of EAF P3K6 Hb protects trasnsgenic sickle cell mouse NY1DD from hypoxia-reoxygenation induced vaso-occlusion and from vaso-occlusion present in transgenic BERK mice, a hemolytic model of sickle cell disease.

Alayash A (U.S.A.) alayash@cber.fda.gov

Mechanisms of Toxicity and Modulation of Hemoglobin-based Oxygen Carriers (HBOCs)

Abdu I. Alayash, PhD, DSc
Laboratory of Biochemistry and Vascular Biology, Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20993, USA

Several adverse events have been associated with the infusion of hemoglobin-based oxygen carriers (HBOCs) in humans including transient hypertension, gastrointestinal, pancreatic and liver enzyme elevation, cardiac and renal injury. Although several mechanisms have been suggested, the basis of HBOC toxicity is not fully understood. Scavenging of vascular endothelial nitric oxide (NO) by Hb and its heme-mediated oxidative side reactions were thought to be the major causes of toxicity. Several attempts were made by industry and some in the research community including short term fixes to control hemodynamic imbalances specifically vasoconstriction and hypertension with little or no long-term tangible improvements in organ toxicities. Based on more recent preclinical studies, oxidative pathways driven by the heme prosthetic group appear to play a more prominent role in the overall toxicity of free Hb or HBOCs. Additionally, a better understanding of the complex redox chemistry of HBOCs is emerging, together with emergence of specific

countermeasures designed to slow down and/or prevent HBOCs oxidative pathways may ultimately provide a platform for delivering safe and effective hemoglobin-based oxygen therapeutics.

***Bäumler* H** hans.baumler@charite.de

Hemoglobin-Based Oxygen Carriers HbMP-700 can deliver more than oxygen

H. Bäumler[(1)], A. Stephen[(1)], R. Georgieva[(1)], L. Zhao[(2)], N. Suwannasom[(1,3)], Y. Xiong[(1)]*
[(1)]Institute of Transfusion Medicine, Charité-Universitätsmedizin Berlin, 10117 Berlin, Germany
[(2)]Institute of Transfusion Medicine, Academy of Military Medical Sciences, Beijing, China
[(3)]Faculty of Medical Sciences, University of Phayao, 59000 Phayao, Thailand

The synthesis of hemoglobin particles (HbMP-700) is based on a CCD-technique, C: co-precipitation of hemoglobin (Hb) with $MnCO_3$ immediately followed by addition of human serum albumin (HSA), C: cross-linking of Hb and D: dissolution of the $MnCO_3$ template resulting in polymerized submicron HbMP-700 with an average size of around 710 ± 60 nm. These HbMP-700 possess a high oxygen affinity (*p*50 of 6 mmHg) compared to 26.5 mmHg of Hb in solution and does not scavenge NO, which are important properties of the new generation of HBOCs. [1-3]
The HbMP-700 can be loaded with hydrophobic or hydrophilic nanoparticles (NPs) or with superparamagnetic NPs (e.g. SPIONs) during the precipitation. Since HbMP-700 are not recognized by phagocytizing cells they can fulfill several functions – they delivery oxygen to the tissue with low pO_2, can release slowly the immobilized NPs or enzymes and can be detected by MRI if SPIONs are incorporated. Surface modifications with antibodies or peptides allows the HbMP-700 to target endothelia cells, circulating tumor cells, monocytes, granulocytes and others. The HbMP-700 are endocytosed/phagocytized, accumulated in the targeted tissue and release their content into the cells. Due to the combination of oxygen and drug delivery with contrast enhancing particles the HbMP-700 could serve as THERANOSTICS.
[1] Bäumler, H. et al. Artificial Organs 38(8), 708–714, 2014
[2] Xiong, Y. et al. ACS Nano 7(9), 7454–7461, 2013
[3] Bäumler, H. & Georgieva R. Biomacromolecules 11, 1480–1487, 2010

Key-words: Artificial oxygen carriers, Hemoglobin, Nanoparticles, Oxygen affinity, Theranostics

Belcher DA belcher.305@buckeyemail.osu.edu

The Quaternary State of Polymerized Human Hemoglobin Regulates Oxygenation of Breast Cancer Solid Tumors: A Theoretical and Experimental Study.

Donald A. Belcher[1], Julia Ju[2], Jin Hyen Baek[3], Ayla Yalamanoglu[3], Paul W. Buehler[3], Daniele M. Gilkes[2,4], Andre F. Palmer[1]
1. William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH. 43224, USA
2. Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, Baltimore, MD 21218, USA
3. Division of Blood Components and Devices, Laboratory of Biochemistry and Vascular Biology, FDA/CBER, Silver Spring, MD 20993, USA
4. Department of Oncology and Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA

A major constraint in chemotherapeutic treatment of triple negative breast cancer is inadequate oxygenation. Hypoxic conditions in the tumor microenvironment induce quiescence in cancer cells, which reduces the effectiveness of cancer-therapies. Polymerized human hemoglobin (PolyhHb) can be transfused to increase solid tumor oxygenation and improve the efficacy of anti-cancer therapeutics. In this study, we analyzed the biophysical properties of synthesized PolyhHbs with low and high oxygen (O_2) affinity. By locking PolyhHb in the relaxed (R)-state, O_2 offloading at low O_2 tensions (<20 mmHg) may be increased, while O_2 offloading at high O_2 tensions (>20 mm Hg) is facilitated with tense (T)-state PolyhHb. Therefore, R-state PolyhHb may deliver significantly more O_2 to hypoxic tissues. To yield low and high affinity PolyhHbs respectively, we first polymerized T and R state hemoglobin with glutaraldehyde. Clarification, purification, and concentration of the PolyhHb was performed via tangential flow filtration. The diameter, cooperativity, O_2 tension at 50% saturation (P_{50}), and rapid offloading kinetics of the synthesized PolyhHbs was analyzed. The resulting biophysical parameters were used to populate an O_2 transport model into the tumor tissue. In general, we found that increasing the volume of transfused PolyhHb decreased the apparent viscosity of blood in the arteriole. In addition, we found that PolyhHb transfusion decreased the wall shear stress at large arteriole diameters (>20 μm), but increased wall shear stress for small arteriole diameters (<10 μm). Transfusion of both T- and R-state PolyhHb may lead to elevated O_2 delivery at low $pO_{2,in}$. In addition, transfusion of R-state PolyhHb may be more effective than T-state PolyhHb for O_2 delivery at similar transfusion volumes. Decreases in the apparent viscosity resulting from PolyhHb transfusion may result in significant changes in flow distributions throughout the tumor microcirculatory network. The difference in wall shear stress implies

that PolyhHb may have a more significant effect in capillary beds through mechano-transduction. In an experimental animal model, periodic top-load transfusion of PolyhHb into mice with MDA-MB-231 seeded breast cancer tumors confirmed the oxygenation potential of both PolyhHbs. Tissue section analysis demonstrated primary PolyhHb clearance occurred in the liver and spleen.

Biro, G (Canada) Biro.george@gmail.com

CONCURRENT DISEASE STATES THAT MAY MODIFY THE RESPONSE TO INTRAVASCULAR HBOC.

George P. Biro, MD, PhD,
Emeritus Professor. University of Ottawa. Canada

HBOC within the vascular system is known to modify NO mediated vasomotor control in experimental animals and is manifested as hypertension in human subjects. The principal mechanism involves the high-affinity binding of NO to hemoglobin (HGb) released from endothelial thereby reducing its bioavailability. This inhibits relaxation of vascular smooth muscle. In addition, evidence for other vasoconstrictor mechanisms affecting arterial blood pressure (BP) has also been found (angiotensin, endothelin, prostanoids) to operate in the presence of HBOC. Normal endothelial function is dramatically modified in a variety of diseases, collectively identified as endothelial dysfunction (ED). Its principal manifestation is failure to induce vasodilation by cholinergic agonists, acetylcholine, etc. The disease states exhibiting ED are generally also involve chronic inflammatory states and oxidative stress, such as vascular and metabolic diseases, arthritides, as well aging *per se*. In those patient populations in which such conditions are prevalent it is important to be aware that the responses to HBOC's are different from those in healthy patients. In clinical trials involving HBOC's it is essential that the presence of such concurrent diseases, as response-modifying factors, be identified and accounted for in the statistical analysis. Attention focused on the rise in BP neglects the significantly far more important effects of *altered vasoregulation* (vasoconstriction) in critical organ vascular beds. Comparisons between treated groups should not neglect possible differences with respect to the presence of disease states with ED. The risks and benefits of three treatment modalities need to be assessed: (1.) red cell (rbc) transfusion: the expected benefit of improved O_2 supply against the known frequency of direct and indirect transfusion- associated adverse outcomes; (2.) HBOC "transfusion": the realistically expected benefit of improved O_2 supply against the HBOC-associated adverse outcomes occurring in normal and concurrent disease states with ED ; and (3.) No treatment: tolerance of reduced O_2 supply against the absence of transfusion- and HBOC-related adverse outcomes observed in (1.) and (2) above.*[The author has no conflict with any producer or product in this field]*

Blais MC MC.Blais@umontreal.ca
(Alumni of Artificial Cells & Organs Research Centre)

Canine Transfusion Therapy: Can the Veterinary Literature Improves Canine Models?

Marie-Claude Blais, DMV, DACVIM, Université de Montréal, QC, Canada

In order to support anemic and bleeding canine patients requiring sophisticated veterinary care, the demand for blood transfusions has increase dramatically. This presentation will review canine blood banking basics and highlight important immune-hematologic particularities of dogs.

CANINE BLOOD TYPES & ALLOANTIBODIES

Dogs have > 12 blood group systems mostly known as dog erythrocyte antigens (DEA). Clinically, the most important canine blood group system is DEA 1, both because of its well-documented immunogenicity and of its overall prevalence (50%). A DEA 1+ blood transfusion in a DEA 1- dog will invariably elicits a strong alloantibody production, a lead to documented hemolytic transfusion reaction if subsequent DEA 1+ blood is transfused. Standardized DEA 1 blood typing is readily available using monoclonal antibodies based on agglutination reactions (DMS/RapidVet-H) or on chromatographic techniques (Alvedia Quick Test).

First-time transfusions to dogs are considered safe without prior cross-matching, as dogs do not possess clinically-significant naturally-occurring antibodies. A cross-match is essential on any dogs that has received a transfusion >4 days previously or have an unknown transfusion history. Gel column techniques (DiaMed or Ortho Clinical and DMS gel tube assay) are simple, sensitive, and standardized methods to crossmatch dogs. The blood group DEA 4 and Dal have been associated with hemolytic transfusion reactions may pose particular challenges given their very high frequency. The rare Dal- phenotype has been identified in few research Beagles.

CANINE ESTIMATED BLOOD VOLUME

The circulating blood volume (CBV) of dogs is estimated at 85 ml/kg, but may significantly vary from individuals/sex/reproductive status/age/breed. In response to sympathetic stimulation (e.g., exercise, hypoxia, or hemorrhage), the canine spleen rapidly contracts to release extra-RBC into the circulation, increasing circulating hematocrit, blood volume, and O_2-carrying capacity during exercise 1.3- to 1.5-fold above resting levels. In a splenectomysed canine hypovolemia model, knowing both the HBOC volume

infused and its concentration in plasma (HemoCue photometer) allows for determining the CBV quickly and with reasonable accuracy.

CANINE BLOOD COLLECTION, STORAGE AND ADMINISTRATION

Since dogs can safely donate from 15 to 20 ml/kg, a standard close-system collection set (450 ml collection bag) can safely be used in >25 kg dogs, allowing splitting the unit aseptically into components (e.g. packed red blood cells, FFP, etc). Blood is usually drawn aseptically from the jugular vein – sedation is rarely required. Canine pRBC can only be stored for 35 days (CPDA-1 with Optisol or Nutricel) to 37 days (Adsol). Delivery of canine RBCs via mechanical delivery systems (volumetric and syringe pumps) has been associated with a marked decrease in short-term probability of survival compared to RBCs delivered by gravity flow. Dogs are considered fairly resistant to hemoglobinuria (usually no renal damage), although acute, life-threatening or fatale, non-immune hemolytic transfusion reactions have been reported. Symmetric dimethylarginine (SDMA) has now proven to be a useful biomarker for early detection of renal dysfunction in dogs.

CONCLUSION

Dogs age five-to eight-fold faster than do humans, share environments with their owners, are usually kept until old age and receive a high level of health care. The increased appreciation of the unique and comparative value of the dog as a model for diverse human disease could accelerate research, leading to new treatments and improved health care for both humans and our best friends.

Bruno, S stefano.bruno@unipr.it

High- and low-affinity PEGylated hemoglobin-based oxygen carriers: differential oxidative stress in a Guinea pigs transfusion model

Esra'a Ali Mohammad Alomari[1], Stefano Bruno[1]*, Luca Ronda[2], Gianluca Paredi[1], Riccardo Piano[2], Stefano Bettati[2], Davide Olivari[3], Francesca Fumagalli[3], Deborah Novelli[3], Giuseppe Ristagno[3], Roberto Latini[3], Chris Cooper[4], Brandon Reeder[4], Andrea Mozzarelli[1]

[1]DEPARTMENT OF FOOD AND DRUG, UNIVERSITY OF PARMA, PARMA, ITALY; [2]DEPARTMENT OF MEDICINE AND SURGERY, UNIVERSITY OF PARMA, PARMA , ITALY; [3]ISTITUTO DI RICERCHE FARMACOLOGICHE 'MARIO NEGRI', MILAN, ITALY; [4]SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF ESSEX, COLCHESTER, UNITED KINGDOM

Hemoglobin (Hb)-based oxygen carriers (HBOCs) are investigational replacements for blood transfusions based on chemically or genetically modified Hbs. HBOCs are known to cause oxidative damage to cells and tissues, but the possible linkage between these detrimental effects and the oxygen binding properties has never been thoroughly investigated for products that are otherwise identical and within the same experimental framework. To fill this gap, we produced and tested human Hb conjugated to polyethylene glycol (PEG) either in the oxygenated state (PEG-Hb^{oxy}, high affinity and low cooperativity) or in the deoxygenated state (PEG-Hb^{deoxy}, low affinity and unmodified cooperativity). Groups of 7-9 Guinea pigs underwent either i) isovolumetric autotransfusion, or ii) isovolumetric transfusion with PEG-Hb^{oxy}, or iii) isovolumetric transfusion with PEG-Hb^{deoxy}. The surviving animals were sacrificed after 7 days and their organs harvested. Plasma samples were analysed for biochemical markers of inflammation, tissue damage and organ dysfunction. Protein heart extracts were analysed for oxidative damage, measuring: i) protein carbonyl content, ii) protein S-nitrosylation; iii) protein S-glutathionylation; iv) protein adducts with 4-hydroxynonenal; v) protein adducts with malondialdehyde. Overall, significant differences were observed among the groups, with both HBOCs producing higher oxidative stress in comparison to autotransfusion. Particularly, protein S-nytrosylation and 4-hydroxynonenal adducts, as well as plasma levels of 8-oxo-2'-deoxyguanosine, proved to be useful markers of oxidative stress in this model and could be used for the evaluation of new HBOCs.

Bülow L leif.bulow@tbiokem.lth.se
Past president, 2015 ISBS Int Sym Blood Substitutes,

Present Status of Research on Blood Substitutes in Europe

Leif Bülow, Pure and Applied Biochemistry, Lund University, SWEDEN

Two main different approaches have been pursued to create blood substitutes. The first uses perfluorocarbons that can physically dissolve high concentrations of oxygen. The alternative methodology involves the use of the red cell blood hemoglobin as the starting material. These latter are often referred to as Hemoglobin Based Oxygen Carriers (HBOC) and include modifications allowing for enhanced stability and efficacy outside the red blood cell environment. First generations HBOC products failed due to enhanced toxicity outside the protective environment of the red cell. For instance, hemoglobin can scavenge the vasodilator nitric oxide and it was assumed that the toxicity of these products was therefore due to this vasoconstrictive property.

Basic hemoglobin research is very strong in Europe and also has a long tradition in many laboratories. The activities involve hemoglobins from a range of biological origins. These efforts have provided a genera

understanding of possible intrinsic chemical reactions of hemoglobins involving among others NO but also other compounds. This background knowledge on hemoglobin toxicity has in many aspects been instrumental also for developing novel and safe HBOC products. The ambition of this presentation is not to give a complete overview of all European activities in the field, but rather highlight some important examples that can be key to convert academic research into useful clinical products.

Bülow L leif.bulow@tbiokem.lth.se
Past president, 2015 ISBS Int Sym Blood Substitutes,

Protein Engineering for Hemoglobin Based Oxygen Carriers – An Attractive Alternative to Chemical Approaches

Leif Bülow, Pure and Applied Biochemistry, Lund University, SWEDEN

In the past decades, significant research efforts have been directed towards the production and application of functional hemoglobin-based oxygen carriers (HBOC), which have potential to be transfused in place of the RBCs. A cell-free Hb, lacking the antioxidant network of RBCs, may participate in several deteriorating reactions causing physiological stress and organ damage. Heme mediated toxicity, radical reactions and reactions involving nitric oxide (NO) are often regarded as major complications associated with cell-free Hb. When applying protein engineering strategies in the design of HBOCs many of these issues can be ameliorated. In this presentation, I will discuss the use of site-directed mutagenesis and gene fusion to optimize the behavior of blood substitutes. Special focus will be given to fetal hemoglobin (HbF) which has several favorable properties in comparison with the adult protein.

Chemical cross-linking and chemical modifications, e.g. pegylation, are often regarded as essential parts in the manufacturing process of HBOCs, but these steps may alter the structural properties and final yield of the product. We have used the XTEN technology to modify Hb genetically, obviating chemical modification steps. XTEN is a recombinant polymer composed of negatively charged amino acid residues. This XTEN polymer was genetically linked to fusion fetal hemoglobin (fHbF), and used as an alternative to addition of PEG groups.

Cabrales, P pcabrales@ucsd.edu

Polyethylene Glycol Camouflaged Earthworm Hemoglobin

Pedro Cabrales
Dept. of Bioengineering, University of California, La Jolla, CA

Nearly 21 million components of blood and whole blood and transfused annually in the United States, while on average only 13.6 million units of blood are donated. As the demand for Red Blood Cells (RBCs) continues to increase due to the aging population, this deficit will be more significant. Despite decades of research to develop hemoglobin (Hb) based oxygen (O_2) carriers (HBOCs) as RBC substitutes, there are no products approved for clinical use. Lumbricus terrestris erythrocruorin (LtEc) is the large acellular O_2 carrying protein complex of the earthworm Lumbricus terrestris. LtEc is a stable protein complex, resistant to autoxidation, and capable of transporting O_2 to tissue when transfused into mammals. These characteristics render LtEc a promising candidate for the development of the next generation HBOCs. LtEc has a short half-life in circulation, limiting its application as a bridge over days, until blood became available. Conjugation with polyethylene glycol (PEG-LtEc) can extend LtEc circulation time. This study explores PEG-LtEc pharmacokinetics and pharmacodynamics. To study PEG-LtEc pharmacokinetics, hamsters instrumented with the dorsal window chamber were subjected to a 40% exchange transfusion with 10 g/dL PEG-LtEc or LtEc and followed for 48 hr. The vascular response of PEG-LtEc were studied in the hamsters dorsal window chamber following multiple infusions of 10 g/dL PEG-LtEc or LtEc solution to increase plasma LtEc concentration to 0.5, 1.0, and 1.5 g/dL, while monitoring the animals' systemic and microcirculatory parameters. Results confirm that PEGylation of LtEc increases its circulation time, extending half-life to 70 hr, 4 times longer than that of non-PEGylated LtEc. However, PEGylation increased the rate of LtEc oxidation in vivo. Vascular analysis verified that PEG-LtEc did not cause microvascular constriction or systemic hypertension. The molecular size of PEG-LtEc did not change the colloid osmotic pressure or blood volume expansion capacity compared to LtEc, due to LtEc's already large molecular size. Taken together, these results further encourage the development of PEG-LtEc as an O_2 carrying therapeutic.
Support: NIH grants from the Heart Lung and Blood Institute, P01-HL110900, R56-HL123015, and R01-HL126945. Authors declare no competing financial interests.

Carlsson, Magnus magnus.l.carlsson@slu.se

Human fetal hemoglobin expression, purification and characterization in *Nicotiana benthamiana*

Magnus Carlsson[1], Selvaraju Kanagarajan[1], Sandeep Chakane[2], Karin Kettisen[2], Khuanpiroon Ratanasopa[2,3], Leif Bülow[2], Li-hua Zhu[1]
[1]Department of Plant Breeding, Swedish University of Agricultural Sciences, Alnarp, Sweden [2]Department of Pure and Applied Biochemistry, Lund University, Lund, Sweden [3]Örebro Life Science Centre, School of Science & Technology, Örebro University, Örebro, Sweden

In the hemoglobin (Hb)-based oxygen carrier (HBOC), Hb suffers from the major disadvantages of short circulating half-life and tetramer dissociation into dimers (αβ). Also, main problem in producing Hb in bacterial systems is formation of inclusion bodies. Fetal hemoglobin (HbF) is considered to be more stable than human adult Hb (HbA). Hence, current study was carried out through fusing α and γ globin chains of HbF (fHbF) together with a specific linker and transiently expressing the protein in *N. benthamiana*. The fHbF protein with the size of approximately 32 kDa was then purified using cation exchange chromatography, followed by anion exchange chromatography. SDS-PAGE analysis and western blot analyses were also carried out to confirm the fHbF protein. Molecular size of the fHbF was also determined using size exclusion chromatography and MALDI-TOF. Authenticity of fHbF was further confirmed by liquid chromatography tandem-mass spectrometry. The absorbance spectra of fHbF in binding with ligands (oxygen and carbon monoxide) indicated that fHbF had proper quaternary structure, folded properly and contained a functional heme moiety with full heme cofactor incorporation as assessed by UV/Vis. The absorption spectra of the oxy, deoxy and carboxy forms of fHbF showed a typical spectrum which are identical to those of human HbF. Together, these results provided evidence that *N. benthamiana* can be used as an expression system to produce HbF and fHbF have a potential to be considered as a possible starting material for Hb-based oxygen carrier.

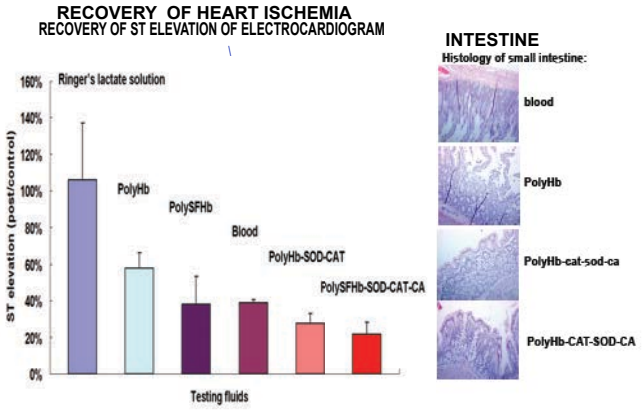
Chang TMS artcell.med@mcgill.ca

Individual Roles of (1) Oxygen carriers, (2) Oxygen carries with antioxidant and (3) Oxygen carries with antioxidant and CO2 transport.

Honorary President, of ISBS and of ISNS,
Professor and Director, Artificial Cells & Organs Research Centre,
Faculty of Medicine, McGill University, Canada

If would be folly to make a general statement regarding which of the following is the best approach: (1) Oxygen carriers, (2) Oxygen carries with antioxidant or (3) Oxygen carries with antioxidant and CO2 transport. Indeed there are many situations where none of these is needed and only volume replacement is required. Furthermore, why use a more complicated system when the clinical condition only requires a simple oxygen carrier. For those conditions with potential for ischemia-reperfusion or oxygen radical problems, it would be folly not to use oxygen carries with antioxidant properties. The use of oxygen carries with antioxidant and CO2 transport is most likely only needed in conditions where there is severe blood loss resulting in insufficient red blood cells to function for the carbonic anhydrase C02 transport.

For this situation, we have designed a soluble nanobiotherapeutic, Poly-[hemoglobin-superoxide dismutase-catalase-carbonic anhydrase]. This can have the same function as rbc or it can also have enhancement of all 3 red blood cell (rbc) functions: transports oxygen, removes oxygen radicals and transports carbon dioxide. In a 90 min 2/3 blood volume loss hemorrhagic shock rat model, the one with enhancement of rbc function is more effective than blood in the recovery of intracellular pCO2, cardiac ischemia, plasma lactate, troponin, histology of the heart, intestine and kidney.



The lyophilized form can be stored for 1 year at 4C (compared to 42 days for rbc) and 40 days at room temperature (compared to 1 day for rbc). The lyophilized form can be heat pasteurized and retain enzyme activity. We have also developed a method to extract the needed enzymes from rbc at low costs. Our preliminary result shows that bovine enzymes are nanoencapsulated within the excess hemoglobin and therefore not exposed to immunological reaction when tested by 4 weekly intravenous injections in rats. Translation to clinical use is time consuming and past experience shows that we cannot wait until it is again too late. Translation also includes the analysis of how results in animal studies can be applied to clinical use in patients. www.medicine.mcgill.ca/artcell/translation.pdf

One of the reason in designing this with enhanced rbc function is my personal bias based on the clinical trial with hemoperfusion that it will be more conclusive to design clinical trial to shown superiority to rbc rather than to test for equivalency.

Chen C (China)
President, Chinese Society of Blood Substitutes,
Vice President Northwest University, Xian, China
Preclinical investigation of Polymerized Porcine Hemoglobin (pPolyHb)
cchen898@yahoo.com

Chen, Gang, biomedicinechen@163.com
(Alumni of Artificial Cells & Organs Research Centre)

Novel Red Blood Cell Substitute: the Principle, Design and Its Effectiveness for Hemorrhagic Shock
Gang Chen, Yaojin Li, Hong Wang, Jiaxin Liu*, Chengmin Yang*
The Blood Substitutes Research Group in Institute of Blood Transfusion,Chinese Academy of Medical Science & Peking Union Medical CollegeChengdu, P. R. China

Broadly speaking, the concept of blood substitutes should contain several aspects including red blood cell substitutes, platelet substitutes, plasma substitutes and so on. The plasma substitutes, usually were named plasma expander, has been achieved the industrialization in world. Now, it is red blood cell substitute product that current research focuses on and clinical trial urgently need. However, there haven't been any systematic researches or reports about the idea and concept on the design of products of blood red cell substitutes. During the past years, according to the updating of clinical transfusion principle and the reality of China, several principles about red blood cell substitute product designing were proposed in our research group.

1. Functions and clinical indications: the red blood cell substitutes were designed for oxygen supply for the ischemic tissues to prevent or alleviate the pathological changes or injuries caused b hypoxia, and also maintain the circulation capacity in vessel. Thus, the red blood cell substitutes could be used in the first aid for patients with traumatic large blood loss and also in the rescue therapies for acute anemia patients.

2. Designing principles: according to the principle of non-equal volume reperfusion in clinical transfusion and dose response relationship in the occurrence of side effects in hemoglobin-based oxygen carriers (HBOCs), the content of polyhemoglobin in products could be lower down in some extent to meet the oxygen need for hypoxia tissues as prerequisite. In addition, a certain amount of colloidal macromolecules should be contained in the preparation to ensure the required colloid osmotic pressure to maintain the plasma expanding function. Furthermore, the antioxidant and buffer should also be contained for the stability during the long-time preservation.

3. Effectiveness for the hemorrhagic shock: after building the experimental rats model of 60% blood loss, the designed red blood cell substitute products were equal volume re-infused in the time of 30min after bleeding and postoperative observation for 120min. The indexes for hemodynamics, homology, enzymes and tissue oxygen levels were tested and the results showed effective recoveries. The survival of the experimental rats after re-infusion was nearly 100% within 24 hours and above 80% within 72 hours, which were 50% higher than the control groups (HES130/0.4), respectively. Moreover, the results from the 4±2℃ long-time preservation investigation showed that the majority of the quality indexes were kept in the required ranges during the one-year storage time, indicating that the designed red blood cell substitute product could be stored for more than one year under the condition of 4±2℃. However, the results also showed that, if the storage temperatures were higher, the methemolgobin content in products would be much increased and be higher than required value.

The aim of our research is to obtain the ideal red blood cell substitutes and the safety, effectiveness, economy and other realities are needed to be considered during this process before the industrialization.

Key words: Red blood cell substitutes; Principles; Design; Hemorrhagic shock;

The reduction of human cord blood methemoglobin by vitamin C (abstract only)
Gang Chen, Tingting Wu, Can Huang, Hanfeng Zheng, Yaojin Li, Hong Wang, Jiaxin Liu, Chengmin Yang**
Institute of Blood Transfusion, Chinese Academy of Medical Science,Chengdu City, Sichuan Province, P. R. China

Background and Objective: Hemoglobin-based oxygen carriers (HBOCs) have proven to be of great value to the field of red blood cell substitutes and oxygen therapeutics. HBOCs avoid many problems associated with traditional blood transfusion, such as the requirement for blood type matching, short storage duration, transportation limitations, viral infections and so on. However, the oxidation toxicity has been considered to be a critical obstacle in the development of HBOCs. Thus, a means to control the methemoglobin (MetHb) content in HBOC solutions has become an important strategy to increase the safety and effectiveness of

HBOCs. In previous research by our group, vitamin C (Vc) was shown to provide remarkable antioxidant protection to HBOCs derived from human cord blood and lowered the MetHb content of HBOCs. In line with this, the objective of the current study is to investigate the reducing effect of Vc on human cord blood-derived MetHb.
Methods: Several factors influencing the reduction of MetHb by Vc were investigated, including pH value and temperature. Subsequently, the molecular structures and functions of hemoglobin were characterized before and after the redox process through the testing of oxygen affinity (P50 values), ultraviolet-visible spectroscopy, (2)
circular dichroism spectroscopy (CD) and differential scanning calorimetry (DSC).
Results: Human cord blood-derived MetHb was reduced effectively by Vc, and this effect was further promoted at an increased pH value and decreased temperature. The P50 values were found to be similar before and after the redox process. However, the disorder of the secondary structure of hemoglobin was elevated after the reduction reaction. In addition, the precipitation temperature point of reduced hemoglobin was shown to be lower through differential scanning calorimetry, indicating the decreased stability of human cord blood hemoglobin after being reduced from MetHb.
Conclusion: This research identified that Vc has remarkable reducing effects on human cord blood-derived MetHb. The side effects following Vc reduction need to be further researched in future work.
Key words: Hemoglobin-Based Oxygen Carriers (HBOCs); Methemoglobin (MetHb); Vitamin C (Vc); Reduction;

D'Agnillo F felice.dagnillo@fda.hhs.gov
(Alumni of Artificial Cells & Organs Research Centre)

Reversible Renal Glomerular Dysfunction in Guinea Pigs Infused with Polymerized Cell-Free Hemoglobin

Xiaoyuan Zhang[1], Matthew C. Williams[1], Otgonchimeg Rentsendorj[1], and **Felice D'Agnillo**[1]

[1]Laboratory of Biochemistry and Vascular Biology, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20993, USA

Renal exposure to hemoglobin-based oxygen carriers (HBOCs) may alter the functional or structural integrity of the glomerular filtration barrier. Using a guinea pig exchange transfusion model, we examined the effects of bovine polymerized hemoglobin (HbG) on glomerular permeability and filtration barrier components including podocytes, endothelial cells, and the glomerular basement membrane. HbG induced marked proteinuria at 4 and 12 hours post-infusion that resolved by 72 hours. This was characterized by the urinary excretion of high molecular weight proteins (≥66 kDa) including albumin, immunoglobulin, and transferrin suggesting a loss of glomerular barrier function. HbG-induced proteinuria also correlated temporally with the reduced expression of podocyte markers (podocin, nephrin, and Wilms' Tumor protein) and endothelial cells (ETS-related gene and claudin-5). Glomerular glycocalyx assessment revealed marked but reversible alterations in the glycosaminoglycan-rich coating of glomerular structures. Intraglomerular 4-HNE deposition, a marker of oxidative stress, was also detectable. Taken together, these findings suggest that HbG induces reversible glomerular barrier dysfunction that may have broad implications for understanding the overall renal response to HBOCs.

D'Agnillo F felice.dagnillo@fda.hhs.gov
(Alumni of Artificial Cells & Organs Research Centre)

Hemoglobin Based Oxygen Carriers: Regulatory Perspectives on Chemistry, Manufacturing, and Controls.

Laboratory of Biochemistry and Vascular Biology, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20993, USA

Hemoglobin-based oxygen carriers (HBOCs) are in development as oxygen and volume replacement therapies for clinical indications including emergency resuscitation and elective surgery. These products may serve an important role particularly when needed red blood cells are not available, contraindicated, or refused based on religious grounds. HBOCs have been manufactured using different hemoglobin sources (e.g. human, bovine, recombinant technology) and a variety of protein modification strategies including intra- and intermolecular crosslinking, conjugation, and polymerization. HBOCs also differ with respect to purity and added excipients. Defining the chemistry, manufacturing, and controls (CMCs) of a HBOC is a critical component of product development that requires broadly applicable tests as well as product-specific assessments given that not all HBOCs are structurally or biochemically equivalent. As a general principal, the overall CMC profile and specifications of a HBOC should be sufficient to assure the proper identity, quality, purity, and potency of the product. The extent of CMC information required during product development will vary with the phase of the investigation, the proposed formulation, and details of the studies. This talk will provide a broad overview of regulatory concepts, methods, and criteria that are relevant to the CMC profiling of these products.

Doctor, Allan (U.S,A,) doctor_A@kids.wustl.edu
Professor of Pediatrics and Biochemistry, Washington University School of Medicine
Pediatric Critical Care Medicine, Saint Louis Children's Hospital ,St. Louis, Missouri
ErythroMer (EM), a Nanoscale Bio-Synthetic Artificial Red Cell: proof of concept and in vivo efficacy results

Elmer, J Jacob.elmer@villanova.edu

Prolonging the Shelf Life of *Lumbricus terrestris* Erythrocruorin for Use as a Novel Blood Substitute

Elmer, J
Department Chemical Engineering**,** Villanova, PA, U.S.A.

Limitations associated with the storage of red blood cells have motivated the development of novel blood substitutes that are able to withstand long-term storage at elevated temperatures. The hemoglobin of the earthworm *Lumbricus terrestris* (LtEc) has been shown to effectively deliver oxygen in mice and hamsters, but its stability during storage at high temperatures for long times has not yet been investigated. Several factors were investigated in this study to optimize the thermal and oxidative stability of LtEc during storage, including pH, antioxidant supplements, and deoxygenation. A strategy for the reduction of fully oxidized LtEc with antioxidants was also developed. In addition, the structural stability of LtEc was also enhanced in two ways: (1) cross-linking it with glutaraldehyde and (2) conjugating it to a polyacrylic acid (PAA) mesh. Overall, the optimal storage buffer appears to be Ringer's Modified Lactate solution at pH 7.0, since LtEc exhibits the highest melting temperature (T_m = 56°C) and lowest oxidation rate (k_{ox} = 0.04 x 10^{-3} hr^{-1} at 20°C) in this buffer. Cross-linking LtEc with glutaraldehyde significantly increased its thermal stability (T_m = 67°C), but had no effect on its oxidation rate. Alternatively, LtEc-PAA conjugates are stable enough to be autoclaved (in the presence of carbon monoxide) without denaturing or aggregating, but they are prone to hemichrome formation. Deoxygenation of LtEc was also observed to virtually eliminate oxidation. As an alternative strategy, we also show that oxidized LtEc can be completely reduced (98.3% ± 1.3%) with 0.33 mg/mL ascorbic acid (AA). Most importantly, the oxygen transport properties of LtEc were unaffected by storage at high temperatures or oxidation followed by reduction with AA. In contrast, both the glutaraldehyde cross-linked LtEc and the LtEc-PAA conjugate exhibited significantly higher oxygen affinity than native LtEc. Overall, these results show that LtEc can be stored at high temperatures (37°C) for at least 7 days without any significant loss of function or structure.

Eriksson N L nelida_leiva.eriksson@tbiokem.lth.se

A green alternative for the development of HBOCs

Nélida Leiva Eriksson [(1)] and Leif Bulow [(1)]
[(1)] Department of Pure and Applied Biochemistry, Lund University, Box 188, 221 00 Lund, Sweden
*Corresponding author: nelida_leiva.eriksson@tbiokem.lth.se

It is more than three decades since the development of blood substitutes based on human Hemoglobin (Hb) started. Despite the advances made on the understanding of its redox chemistry no product has been given therapeutic licensure (except in South Africa and Russia) due to its adverse effects. Another problem with the use of human Hb is related to its production which is mainly recombinant. Since human Hb has the intrinsic capacity to induce oxidative reactions, the down-stream processing becomes complicated and expensive.
Here we propose the development of HBOCs based on plant Hbs. Plant Hbs have the typical myoglobin-fold (3-on-3 structure). The main difference between plant Hbs and human Hbs is the coordination of Fe^{+2} in the heme group. Plant Hbs are hexacoordinated. It means that the heme group is attached to the globular protein by covalent bonds to the proximal and distal Histidines (His). Interestingly, such a hexacoordination does not hinder these proteins to bind exogenous ligands. Thus, our candidate plant Hb binds oxygen at a rate similar to other oxygen transporters (57×10^6 M^{-1} s^{-1}). Such hexacoordination offers several advantages over human Hb, such as high stability, low autoxidation and low heme-loss. As a result, the possibility of oxidative damage is lower with plant Hbs. For instance, our candidate plant Hb degrades 40% less DNA than human Hb under the same conditions. Another advantage of plant Hbs over human Hb is its production. The heterologous expression of plant Hbs in *E. coli* is 30-40% higher compared to human Hb. In addition, no stabilizing agents are needed when carrying out the down-stream processing making its purification less complicated and, therefore, cheaper.
Given the advantages above described, the possibility of having plant Hbs as starting material for the development of a new generation of HBOCs with high stability and at a low cost is promising.

Keywords: Plant Hemoglobin, HBOC, hexacoordination,

Estep T chartbiotech@aol.com

Moving HBOCs Forward – Testing Hypotheses in the Clinic

T.N. Estep
Chart Biotech Consulting, Erie, Colorado

The development of hemoglobin based oxygen carriers (HBOCs) has been stymied by concerns that this class of products increases the risk for mortality, myocardial infarction and other serious adverse events, despite the demonstration of clinical efficacy with respect to blood sparing in several clinical trials, and the lifesaving support of compassionate use patients at otherwise lethal hematocrits. While hemoglobin catalyzed destruction of nitric oxide and generation of reactive oxygen species have been proffered as potential mechanisms of HBOC toxicity, specific hypotheses relating these chemistries to HBOC SAEs observed in clinical trials have been lacking, as has human data which would either support or refute these potential toxicity mechanisms. It is suggested that formulating more specific hypotheses and systematically testing them against both preclinical and clinical results could elucidate whether HBOCs are or are not risk factors for SAEs of concern. In addition, such analyses could lead to better patient selection criteria, improved product dosing schedules, possible strategies for side effect mediation, enhanced correlation of preclinical and clinical results, and a more informed direction for future product development. It is also likely that many HBOC clinical trials have suffered from suboptimal dosing regimens that have contributed to undesired side effects because of the unique characteristics of this class of product compared to existing therapies. By way of example, an analysis of one particular toxicity mechanism and one aspect of hemoglobin dosing will be presented using both preclinical and clinical data.

Ferenz, Katja (Germany) Katja.Ferenz@uk-essen.de

Functionality of albumin-derived perfluorocarbon-based artificial oxygen carriers in the Langendorff-heart

Katja Bettina Ferenz*, Anna Wrobeln[*], Klaus-Dieter Schlüter[#] and Michael Kirsch[*]
*University of-Duisburg-Essen, University Hospital Essen, Institute of Physiological Chemistry, Essen, Germany
[#]Institute of Physiology, Justus Liebig University, Giessen Germany

Despite long lasting efforts, at present a harmless, effective artificial oxygen carrier is missing for clinical use both in Europe and USA. To bypass this bottleneck albumin-derived perfluorocarbon-based nanocapsules (nanocapsules) were designed as a novel artificial oxygen carrier[1]. Most importantly, nanocapsules do not contain any chemical emulsifier. Nanocapsules are synthesized in different size ranges (Ø 100-1500 nm) by using ultrasonics[1]. Physical assessment of size (DLS, REM/LSM), oxygen transport capacity or the charging of erythrocytes is performed.

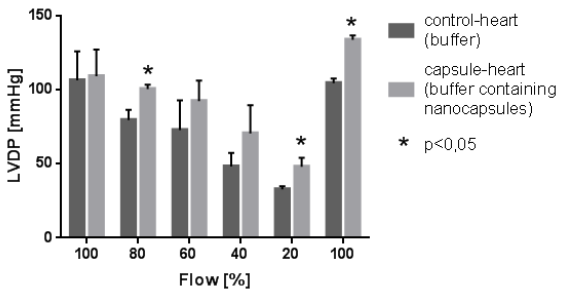


Fig. 1 Left Ventricular Developed Pressure (LVDP) during flow reduction down to 20% and reflow up to 100%. Dark grey show the untreated control hearts and light grey the treated hearts.

The aim of this study[2] was to examine the functionality of the albumin-derived perfluorocarbon-based nanocapsules, more clearly, to check if they supply enough oxygen to remain and isolated organ functional.
The functionality of the nanocapsules was analyzed in a flow-controlled Langendorff perfusion apparatus. Rat hearts were retrograde perfused with a modified Krebs Henseleit (KH)-buffer in the absence or presence of albumin-derived perfluorocarbon-based nanocapsules as artificial oxygen carriers. In different protocols (such as stepwise flow-reduction) the abilities to deliver oxygen and remove carbon dioxide were tested. The left ventricular developed pressure and the rate pressure product were determined along with other parameters.
In all protocols used hearts perfused with modified KH-buffer in the presence of nanocapsules showed increased left ventricular developed pressure and rate pressure product compared to control hearts (perfused in the absence of nanocapsules).
Due to their oxygen and carbon dioxide transport capacity albumin-derived perfluorocarbon-filled nanocapsules preserve the beat of rat hearts much better than modified KH-buffer does[2]. Because of the positive results the next step will be to proof the functionality in a more complex system, e.g. the rat normovolemic hemodilution model.

References:

Wrobeln A., Laudien J., Groß-Heitfeld C., Linders J., Mayer C., Wilde B., Knoll T., Naglav D., Kirsch M., **Ferenz K. B**. Albumin-derived perfluorocarbon-based artificial oxygen carriers: A physico-chemical characterization and first *in vivo* evaluation of biocompatibility. European Journal of Pharmaceutics and Biopharmaceutics. 2017; 115: 52-64.
Wrobeln A., Schlüter K. D., Linders J., Zähres M., Mayer C., Kirsch M, **Ferenz K. B.** Functionality of albumin-derived perfluorocarbon-based artificial oxygen carriers in the Langendorff-heart. Artificial cells, Nanomedicine and Biotechnology. 2017; 45 (4): 723-

Friedman J M. joel.friedman@einstein.yu.edu

Enhancing safety and therapeutic efficacy of both HBOCS and RBC based transfusions through the systemic nanoparticle-based delivery of NO bioactivity.

Joel M. Friedman MD, Ph.D, Department of Physiology and Biophysics,
Albert Einstein College of Medicine, Bronx, NY 10461

Transfusion of either red blood cells or HBOCs seeks to restore adequate tissue perfusion to tissues under conditions of acute and chronic blood loss. This requirement translates typically translates into restoration of diminished functional capillary density (FCD) to a level that supports tissue perfusion. The strategy of merely increasing oxygen carrying capacity of the blood is not always sufficient or even needed to ameliorate poor tissue perfusion. In case of HBOCs and hemolyzed RBC's, nitric oxide scavenging, oxidative stress and proinflammatory events can offset potential benefits of enhanced oxygen transport and raise safety issues. Additionally, the enhanced viscosity associated with current high dosing for RBC based transfusion can also offset increased oxygen content of the blood. An approach to enhance the efficacy and safety of HBOC and RBC based transfusions is being pursued based on the use of IV infused biodegradable nanoparticles capable of sustained delivery of NO bioactivity through several strategies. Collaborative studies with the UCSD group have shown that these nanoparticles are both vasoactive, anti-inflammatory and increase FCD. They are effective at countering the toxicity of HBOCs and the inflammatory cascade associated with both hemorrhagic shock and systemically induced LPS. Additionally these nanoparticles can prevent reperfusion and reoxygenation injuries. The presentation will cover the preclinical results to date and an overview of the technology.

Guo C

Extraction of Superoxide Dismutase, Catalase and Carbonic Anhydrase from stroma-free red blood cell hemolysate for the preparation of the nanobiotechnological complex of PolyHemoglobin-Superoxide Dismutase-Catalase-Carbonic Anhydrase

C. Guo, M.Gynn and TMS Chang*
Artificial Cells and Organs Research Centre
Departments of Physiology, Medicine and Biomedical Engineering
Faculty of Medicine, McGill University, Montreal, Quebec, Canada. H3G 1Y6
*Corresponding author: Professor TMS Chang, artcell.med@mcgill.ca

We report a novel method to simultaneously extract superoxide dismutase (SOD), catalase (CAT) and carbonic anhydrase (CA) from the same sample of red blood cells (RBC). This avoids the need to use expensive commercial enzymes thus allowing this to be cost effective for large-scale production of a nanobiotechnological polyHb-SOD-CAT-CA with enhancement of all 3 red blood cell functions. The best concentration of phosphate buffer for ethanol-chloroform treatment results in good recovery of CAT, SOD and CA after extraction.Different concentrations of the enzymes can be used to enhance the activity of polyHb-SOD-CAT-CA to 2, 4 or 6 times that of RBC.

Guo C

Immunological study of bovine poly-[Hb-CAT SOD CA]: a nanobiotherapeutic

Guo C and TMS Chang artcell.med@mcgill.ca
Departments of Physiology, Medicine, Biomedical Engieering
Artificial Cells & Organs Research Centre, McGill University, Canada

In this research, the long-term safety and immune responses of rats are investigated under four-week immunization (via IV injection) and 30% blood volume infusion with bovine sourced blood substitutes containing bovine hemoglobin (Hb) and higher content of red blood cell enzymes: catalase (CAT), superoxide dismutase (SOD) and carbonic anhydrase (CA). Body weight growth, the heart rates and plasma biochemistry were recorded or tested to evaluate the safety of injection with bovine HBOCs to rats. Mean arterial pressures (MAP) before and after IV injection every week have been compared to detect anaphylactic shock. After immunization, the immunized rats were infused with large volume of the same samples used for immunization. The MAP, total IgE, histamine and tryptase levels were compared before and after infusion to see if there are any anaphylactic shock and allergic reactions. Total IgG and IgM were tested after immunization and infusion. More detailed research was conducted to test the antibodies produced against the HBOCs by Ouchterlony double diffusion, to quantify the antibodies produced to against the components (bovine Hb, CAT, SOD and CA) in HBOCs, and to determine the complement fragment 3a (C3a) activation after immunization and infusion respectively. The results show no safety problem to immunize the rats and all the rats survived one week after infusion with large volume of samples. No adverse immune responses were detected. This study suggests the possibility to use bovine HBOCs with enhancement of CAT, SOD and CA for clinical transfusion

Greenburg AG agerson4@yahoo.com

Discussion of clinical trial result of Hemoglobin based oxygen carriers

Past president, ISBS Int Sym Blood Substitutes,
Emeritus Professor of Surgery, Brown University (U.S.A)

Huang, Y ybhuang@ciac.ac.cn

Hemosome formed by protein−polymer conjugate assembly as oxygen carrier

Yupeng Wang, Yubin Huang*
State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, ChangChun 130022, People's Republic of China

Nanocarriers based on nature's biomaterials such as peptides and proteins have shown great advantages in the field of nanomedicine such as inherent biocompatibility, biodegradability and immunogenicity. However,

the complicated preparation and additional denaturation of protein may limit their further uses. Herein, a novel protein - nanoparticle drug delivery system was prepared based on the self-aggregated property of proteins in the state of isoelectric point with mild reaction conditions, simple preparation method and good biocompatibility. In this study, albumin and hemoglobin was chosen as model protein for the preparation of empty and drug-loaded nanoparticles, and did not denaturation or inactivation in the process of preparation. The protein particles are stable in physiological buffers and could release their therapeutic payload quickly into cancer cells after a time dependent cellular uptake owing to the PH sensitive of Schiff base crosslinked bond. Furthermore, as a function of protein, the ability of transport oxygen of hemoglobin can enhanced antitumor activity of photosensitizer compared with that of free photosensitizer in vitro. This new approach for protein particle preparation are applicable to variety proteins with not denaturation or inactivation had great potential as a drug delivery for cancer therapy.

Hsia C cjchsia@yahoo.com

SanFlow as a Universal Golden Hour Drug for the Treatment of Hemorrhagic and Ischemic Stroke

Carleton Jen-Chang Hsia Ph.D. Chairman and CEO, NanoBlood LLC, Sioux Falls, SD., 57107

A recent review suggested that acute stroke treatment has entered into a golden age, envisioning development of novel treatment paradigms. These would combine neuroprotection with intravenous thrombolysis within the initial 3.0-4.5 hours after stroke onset as well as the use of intra-arterial device-based therapy. The authors anticipate the use of neuroprotectants will increase the number of patients who can be treated despite long transport times and
consequences of ischemia and reperfusion injuries (1). To expand therapeutic window and increase the treatable patient population, we have developed a conjunctive neuroprotective agent for both hemorrhagic and ischemic stroke that can be safely used in pre-hospital setting without the need for time consuming neuroimaging. In this report we describe the discovery of such an agent. We propose polynitroxylated pegylated hemoglobin (PNPH aka SanFlow) is such a universal conjunctive neuroprotective stroke drug. Polynitrxylation confers superoxide
dismutase mimetic activity to SanFlow to control cell free hemoglobin toxicity and restores focal endogenous nitric oxide deficiency to enhance cerebral blood flow as a golden hour drug for both hemorrhagic and ischemic stroke. These safety and neuroprotective activities of SanFlow have been demonstrated in a hemorrhagic traumatic brain injury (TBI) model in vivo as well as in four in vitro neuroprotective models (2). We have also published preclinical efficacy studies in a stroke model following transient occlusion of the middle cerebral artery and demonstrated that
the cerebral blood flow in the ischemic border region can be sustained for more than 2 hours by SanFlow (3). Recent ischemic stroke trials with stent retrievers have demonstrated a benefit on neurologic outcome by endovascular thrombectomy (4); however many of these patients do not regain full neurologic recovery and the probability of poor outcome increases progressively with prolonged ischemia and delayed reperfusion. These clinical findings have sparked renewed interest in developing adjunctive neuroprotective agent like SanFlow that: 1) can better maintain oxygen delivery during ischemia through the collateral circulation before reperfusion is
established; 2) provide neuroprotection after reperfusion is established and; 3) is safe to use for both ischemic and hemorrhagic stroke patients. The combined neuroprotective data for SanFlow in hemorrhagic TBI and ischemic stroke models demonstrated that it may represent a viable adjunct neuroprotective therapy for thrombolytic and endovascular treatment by stabilizing collateral blood flow and by mitigating reperfusion injuries without the need for inhospital neuroimaging to effectively treat more acute stroke patients.

1) Catanese L. et.al. Acute Ischemic Stroke Therapy Overview, Circulation Research. 2017;120:541-558.
2) Shellington DK et.al. Polynitroxylated Pegylated Hemoglobin: A Novel Neuroprotective Hemoglobin for Acute Volume-Limited Fluid Resuscitation after Combined Traumatic Brain Injury and Hypotension in Mice. Crit Care Med. 2011 Mar; 39(3):494-505.
3) Cao S. et.al. Transfusion of Polynitroxylated Pegylated Hemoglobin Stabilized the Pial Artery Dialation and Reduces Infract Volume in a Middle Cerebral Artery Occlusion Model of Stroke. J. of American Heart Association, 2017 (in Press).
4) Saver JL et. al. Time to Treatment With Endovascular Thrombectomy and Outcomes From Ischemic Stroke: A Meta-analysis JAMA. 2016;316(12):1279-1289.

Intaglietta, M mintagli@ucsd.edu

POST-TRANSFUSION INCREASE OF HEMATOCRIT *PER SE* DOES NOT IMPROVE CIRCULATORY OXYGEN DELIVERY DUE TO INCREASED BLOOD VISCOSITY

Amy G. Tsai [1], Pedro Cabrales [1], Joel M. Friedman [2], Daniel M. Tartakovsky [3], and Marcos Intaglietta [1]

[1]Dept. of Bioengineering, University of California, San Diego, La Jolla, CA, [2]Dept. of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY, and [3]Dept. of Energy Resources Engineering, Stanford University, Stanford, CA.

The effects of blood transfusion in treating anemia were analyzed by calculating DO_2 (blood oxygen delivery) = OCC (oxygen carrying capacity) x CO (cardiac output) where CO is inversely proportional to blood viscosity, which is directly proportional to hematocrit (Hct). In this model the circulation is assumed to be a rigid arterial hydraulic system that accommodates blood volume changes in the venous circulation. We analyze the effects of transfusing 0.5-3.0 units of 65% Hct packed red blood cells (pRBCs) calculating microcirculatory DO_2 after accounting for the increased blood viscosity relative to anemia conditions, assuming that blood pressure does not change. We account for O_2 diffusion out of the circulation prior to blood arriving to the nutritional circulation, a function of blood flow velocity. We show that up to 3 units transfusions of pRBCs increase DO_2 only when treating anemia where Hct (or hemoglobin) is below 60% of normal, provides no benefit when Hct is 50% of normal, and progressively reduces DO_2 beyond this threshold. Our physics based analysis that accounts for the effect of increasing blood viscosity on blood flow due to increasing Hct, shows a small increase in DO_2 in severe anemia and transfusing 3 units of pRBCs, and no increase in DO_2 for conventional 1-2 units transfusions used to treat acute anemia with < 70% DO_2 deficit. However, clinical observations that transfusions can provide a beneficial effect suggest the existence of mechanisms that compensate the absence of DO_2 improvement solely due to increased Hct.

Supported in part by NIH 5P01 HL110900 and USAMRAA award W81XWH1120012.

Kassa, Tigist Tigist.Kassa@fda.hhs.gov

Biochemical and Biophysical Characterization of Hemoglobin-Based Oxygen Carriers (HBOCs): Not All HBOCs Are Created Equally

Tigist Kassa*[1], Fantao Meng*[1], Michael Brad Strader[1], Sirsendu Jana[1], Darón I. Freedberg[2], Felice D'Agnillo[1], and Abdu I. Alayash[1]
[1]Laboratory of Biochemistry and Vascular Biology, and [2] Laboratory of Bacterial Polysaccharides, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20993, USA

Hemoglobin-based oxygen carriers (HBOCs) have been under active investigation for the last three decades but the development of these products has been hampered by safety concerns and adverse events including transient hypertension, gastrointestinal, pancreatic and liver enzyme elevation, cardiac and renal injury in humans. Chemical and/or genetic modifications intended to stabilize HBOCs in tetrameric or polymeric forms introduce conformational constraints that can impact hemoglobin allosteric responses and oxidative side reactions. Here, we present for the first time a comprehensive biochemical and biophysical investigation of some human, bovine and genetically engineered HBOCs and their analogues. We evaluated oxygen equilibrium and ligand binding kinetics under different experimental conditions. We studied their autooxidation kinetics, redox reactions, heme orientations, protein unfolding and heme release. We also determined the effects of HBOCs on cellular respiration and redox states. These experiments provide a better understanding of the relationship between the structure-function and oxidative toxicity of these proteins. We demonstrate here that these products display a diversity of physicochemical properties including molecular size/crosslinking oxygen/redox characteristics and even oxidative inactivation by protein/heme clearing mechanisms. These diverse properties can therefore be manipulated independently, leaving open the possibility of engineering a safe and effective HBOC with ideal characteristics.
*Equal contribution

Kettisen, K karin.kettisen@tbiokem.lth.se

Impact of cysteine residues in recombinant fetal hemoglobin

Karin Kettisen* & Leif Bülow
Pure and Applied Biochemistry, Lund University

The development of HBOCs is pursued to alleviate the increasing demands of blood in clinical healthcare. As an oxygen-carrying component of such a therapeutic, the compatibility of oxygen-distributing properties of hemoglobin (Hb) is critical. The adult Hb (HbA), which is available in relatively large quantities from outdated donated blood, or alternatively bovine Hb, have been tested mostly to formulate blood substitute candidates. However, inherent adverse effects of extracellular Hb such as structural integrity breakdown, heme loss, nitric oxide scavenging and oxidative reactions leading to functionality loss and radical formation, are issues that need to be addressed [1]. The approach of using recombinant technology to probe and develop designed Hb molecules is a powerful tool to manage the drawbacks of native Hb.

We are researching fetal Hb (HbF) as an alternative starting material to develop an oxygen therapeutic. HbF is more stable than HbA in terms of tetramer strength and exposure to alkaline conditions. Moreover, recombinant HbF production gives higher yield than HbA in *E. coli* production systems [2]. HbF also has three-fold less DNA cleavage activity compared to HbA [3].

Cysteine carries a redox active side-chain that is linked to the oxidation pathways of the Hb molecule. The well-known oxidative hotspot at position 93 in the β-chain of HbA, close to the heme pocket, is also conserved in HbF on the γ-chain [4]. To elucidate the impact of cysteine residues in HbF, site-directed mutagenesis was used to create mutants; αA19C, γC93A, and a mutant with both substitutions, αA19C + γC93A. Through analysis of oxidation reactions it was shown that these amino acid substitutions could alter the oxidation pattern of HbF. γ93C appears to play an important role, similar to β93C in HbA, to the functions of the protein in terms of oxidative stability. The mutations also proved that electron transfer from position 93 in the γ-chain to position 19 on the surface of the α-chain is possible. This ability of α19C to act as a compensatory hotspot influences oxidative reactions such as autoxidation and ferryl Hb formation during exposure to H_2O_2. The residue α19C is positioned on the surface of the protein, and is able to interact with the surroundings. An agarose gel based assay thus showed that the DNA cleavage activity of HbF was elevated upon addition of the redox active cysteine residue by forming a surface-exposed radical site [3].

[1] A.I. Alayash, Blood substitutes: why haven't we been more successful?, Trends in Biotechnology 32(4) (2014) 177-185.

[2] K. Ratanasopa, Cedervall, T., and Bülow L., Possibilities of Using Fetal Hemoglobin as a Platform for Producing Hemoglobin-Based Oxygen Carriers (HBOCs), in: L.T.S. Elwell C.E., Harrison D.K. (Ed.) *Oxygen Transport to Tissue XXXVII*, Advances in Experimental Medicine and Biology, Springer, New York, NY, 2016, pp. 445-453.

[3] S. Chakane, Matos, T., Kettisen, K., and Bülow, L., Fetal hemoglobin is much less prone to DNA cleavage compared to the adult protein, Redox Biology 12 (2017) 114-120.

[4] K. Ratanasopa, Strader, M. B., Alayash, A. I. and Bülow L., Dissection of the radical reactions linked to fetal hemoglobin reveals enhanced pseudoperoxidase activity, Frontiers in Physiology 6 (2015) 39.

Kim Hae Won Hae_Won_Kim@Brown.edu

insights into acellular HBOC-mediated hypertension and potential pharmacologic remedies

Hae Won Kim
Department of Molecular Pharmacology, Physiology and Biotechnology
Brown University, Providence, RI 02912, USA

One key impediment to regulatory approval of current acellular HBOCs appears to be an undesirable BP elevation (hypertension) observed following IV administration in clinical studies. FDA indicated that all HBOC products it reviewed were vasoactive at the doses proposed for clinical use. Because concomitant increases in the cardiac output does not occur, the hypertensive effect of HBOCs is primarily due to vasoconstriction which can cause subnormal tissue blood flow to key organs. Therefore, HBOC administration could lead to critical organ dysfunction and failure especially when present concurrent cardiovascular pathologies (e.g., atherosclerosis, hypertension, diabetes, systemic inflammatory response syndrome). However, the precise mechanism(s) of HBOC-mediated hypertension observed in recent HBOC clinical trials has not been fully elucidated. While moderate hypertensive response may even be desirable in certain clinical conditions (e.g., hemorrhagic shock), an acute elevation in systemic and organ/regional blood pressures (e.g., pulmonary, cerebral) could cause serious vascular/organ damages in vulnerable patients. It would be especially detrimental when HBOC-mediated vasoconstriction would exacerbate the low blood flow conditions due to existing/concurrent vascular pathologies (atheroma/thrombosis), tissue edema or other impeding conditions. The etiology of HBOC-mediated vasoactivity appears to be complex and may involve multiple pathways. Currently proposed mechanisms include HBOC scavenging of endothelial NO, activation of endothelin (ET) release, Hb redox reaction mediated vascular damage, vascular autoregulatory mechanisms, adrenergic receptors and other vasoactive agents. Regardless of mechanisms, it appears that the HBOC-mediated hypertension could be modulated by pharmacologic interventions including nitrovasodilators, Ca^{++} channel blockers, ACE inhibitors and ET_A/ET_B receptor antagonists. However, choice of drugs and finding optimal dosing would pose significant challenges because many target patients (trauma/hemorrhage) would be hemodynamically unstable and some drugs may detrimentally interact with HBOCs reducing their O2 carrying/delivery function and intravascular stability. This presentation will review currently proposed acellular HBOC-mediated hypertension/vasoactivity mechanisms and discuss potential pharmacologic modulation.

Keipert *K* Keipertcorpconsulting@gmail.com
(alumni, Artificial Cells and Organs Research Centre)

Challenges facing HBOC development in Trauma: What have we learned to minimize the risk going forward? Peter E. Keipert, PhD*§
**President, KEIPERT CORP. Life Sciences Consulting, San Diego, CA, USA §Formerly, V.P., Clinical Development, SANGART Inc. (San Diego, CA*

Development of hemoglobin-based oxygen carriers (HBOCs) in trauma represents an ideal setting to employ HBOCs as adjunct "oxygen therapeutic" agents to temporarily provide an oxygenation bridge until medical or surgical interventions (including RBC transfusion, if required) can be initiated. This became the focus for most first-generation HBOCs that were being tested for resuscitation of hypotensive shock in acute trauma. These early studies relied primarily on systolic hypotension (SBP< 90 mmHg), as the main inclusion criterion. These trials all failed due to safety issues (cardiac events, mortality), and certain protocol design limitations. Despite the best available resuscitation efforts in trauma, organ dysfunction or failure due to hypoperfusion in critical tissues remains a risk, and can be detected by the presence of lactic acidosis. As a result, SANGART's trauma program with MP4OX added a physiological biomarker as a key inclusion criterion (i.e., lactate > 5 mmol/L) to prospectively identify those patients who had sufficient hemorrhage to incur a tissue oxygen debt. This was key to the successful conduct of a Phase 2b study in 329 trauma patients, which demonstrated that a greater proportion of MP4OX patients were alive and discharged at Day 28 (57% *vs.* 50%; p=0.18), and that the mortality was slightly lower than in Controls (11.6% *vs.* 13.9%; p=0.73) with no between-group differences in adverse event rates. Multiple secondary endpoints also showed positive trends in the MP4OX group, including fewer days on ventilator, in ICU and in the hospital, and faster times to complete resolution of organ failure. These positive results convinced FDA that SANGART's Phase 2c MP4OX dose-comparison study in trauma could include US clinical sites. Despite this regulatory path forward, SANGART failed to secure new funding and had to terminate development and cease operations in Dec 2013.

Kluger R rkluger@chem.utoronto.ca

HBOCs from Hb-Hb Coupling Deliver Oxygen and Avoid Nitric Oxide

Ronald Kluger and Aizhou Wang
Department of Chemistry, University of Toronto, Toronto, Ontario M5S 3H6

The results of clinical trials of HBOCs as analyzed by Natanson implicated a problematic source of vasoactive species. The logical conclusion from various reports is that the materials contained hemoglobin tetramers or dimers that extravasate through the pores of endothelia where they bind NO. The size of the pores is typically slightly larger than diameter of HbA. Thus, we developed chemical methods to connect HbA to HbA in a permanent way that makes the material too large to extravasate while maintaining oxygen delivery. The methods to produce the bis-tetramers are based on specific interactions between species that are not found elsewhere on proteins, The methods involve Cu(I) catalyzed azide-alkyne cycloaddition, strain-promoted cycloaddition, and coupling of Hb-biotins to avidin. The resulting physical and physiological properties of various products reveal that the designed materials avoid the problems of earlier HBOCs while retaining their useful characteristics

Komatsu , Teruyuki (Japan) komatsu@kc.chuo-u.ac.jp

Hemoglobin-Albumin Cluster "HemoAct™" as an Artificial O_2-Carrier

Teruyuki Komatsu
Department of App1ied Chemistry, Faculty of Science and 1-13-27 Kasuga Bunkyo-ku, Tokyo 112-8551, Japan

Hemoglobin (Hb)-based O_2-carriers of several kinds have been developed and evaluated as red blood cell (RBC) substitutes. However, because of several concerns, none has been assigned yet for practical use. Recently, we have synthesized covalent core-shell structured protein cluster comprising Hb in the center and human serum albumins (HSA) at the periphery, Hb-HSA_3 cluster (HemoAct™), as a novel O_2-carrier designed for RBC substitute [1-3]. The protein cluster was prepared by covalent linkage between surface Lys amino groups of Hb and Cys-34 residue of HSA using heterobifunctional cross-linker, *α*-succinimidyl-*ω*-maleimido. The major product was isolated using anion exchange chromatography. The 3D reconstruction of Hb-HSA_3 based on transmission electron microscopy images revealed a complete triangular structure [4]. The HemoAct™ showed higher O_2-affinity (P_{50} = 9 Torr) than the native Hb. Intravenous administration of HemoAct™ into anesthetized rats did not elicit an unfavorable increase in systemic blood pressure by vasoconstriction. The half-life of ^{125}I-labeled HemoAct™ in the blood circulation was markedly longer than that of HSA. These results suggest that the HemoAct™ solution can be of great medical importance not only for alternative material of RBC transfusion, but also for O_2-providing therapeutic reagent in various clinical situations. Moreover, we have recently prepared recombinant canine serum albumin (rCSA) by yeast expression technique, and synthesized Hb-$rCSA_3$ cluster as a RBC substitute for dogs [5].

References

[1] D. Tomita, T. Komatsu, et al., *Biomacromolecules* 2013, *14*, 1816.
[2] H. Hosaka, T. Komatsu, et al., *PloS One* 2014, *9*, e110541.
[3] R. Haruki, T. Komatsu, et al., *Sci. Rep.* **2015**, *5*, 12778.
[4] R. Shinohara, T. Komatsu, et al., *J. Phys. Chem. Lett.* **2017**, *8*, 819.
[5] K. Yamada, T. Komatsu, et al., *Sci. Rep.* **2016**, *6*, 36782.

Kure T kure@naramed-u.ac.jp

Transmembrane Difference in Colloid Osmotic Pressure Affects the Lipid Membrane Fluidity of Liposomes Encapsulating a Concentrated Hb Solution

Tomoko Kure, Hiromi Sakai
Department of Chemistry, Nara Medical University, Kashihara 634-8521, Japan

Hb-vesicle (Hb-V) is an artificial oxygen carrier encapsulating a highly concentrated hemoglobin solution (40 g/dL) in a liposome. The *in vivo* safety and efficacy of Hb-V suspension as a transfusion alternative and structural stability during storage have been studied extensively. Because the intra-liposomal Hb aqueous solution can possess colloid osmotic pressure (COP, 200–300 Torr), which is much higher than that of blood plasma (20–25 Torr), a question arises of whether the lipid membrane senses the transmembrane difference in COP. We examined the membrane microviscosity using a fluorescence polarization technique (Langmuir 2017;33:1533-40). To avoid the interference of red Hb on fluorescence measurement, we used human serum albumin (HSA) as a substitute of Hb. Both HSA and Hb solutions show high COP depending on the concentration. Encapsulation of HSA solution (40 g/dL) in the liposome decreased membrane microviscosity at a lower temperature (949±8 cP → 607±10 cP at 25 °C). The result indicates that the transmembrane osmotic stress induced by HSA encapsulation expands the liposome maximally with increasing spherical surface area, and the membrane fluidity is increased extremely. Even for such a condition, the lowest membrane microviscosity, 377±10 cP at 60 °C, is much higher than that of DPPC liposome (40±2 cP at 60 °C). Accordingly, Hb-V as well as HSA-V maintains a spherical structure and mechanical stability under transmembrane stress caused by high COP, as described in earlier reports of the literature.

Kwan D david.h.kwan@gmail.com

Engineering blood group antigen-cleaving enzymes by directed evolution to modify red blood cells and remove antigenicity

Kwan D Assistant Professor-Dept. of Biology, Centre for Applied Synthetic Biology, Concordia University

Blood transfusion is a critically important medical procedure used to treat blood-loss due to trauma or during surgery, and also in the treatment of chronic blood disorders. However, due to the presence of blood antigens, careful blood-typing is necessary to avoid adverse and sometimes fatal reactions that may result from a mismatched blood transfusion. The ability to generate antigen-null red blood cells would be a break-through development, allowing for transfusion without the need to find a positive match. The major A and B carbohydrate antigens are clinically the most important blood antigens for blood transfusion. We are currently investigating enzymatic methods of removing these antigens from the surface of red blood cells. The principal antigenic determinants of A and B blood groups are the terminal trisaccharide components; GalNAc-α-1,3-(Fuc-α-1,2)Gal in the A-antigen, and Gal-α-1,3-(Fuc-α-1,2)Gal in the B-antigen. The enzymatic removal of these A and B antigens from cell surfaces using glycosidases has long been proposed as a method for allowing transfusion from an otherwise mismatched donor, however, a major practical limitation has been the lack of suitably efficient glycosidases. We have developed a high-throughput method to screen for glycosidases active upon blood group antigens using chemoenzymatically synthesized fluorogenic substrates that closely mimic the natural blood group antigens. We have utilized this methodology both in the screening of mutant libraries for the directed evolution of a blood group antigen-cleaving glycosidase, and have also demonstrated its potential for the functional screening of metagenomic libraries in order to identify as yet undiscovered blood group antigen-cleaving enzymes.

Latson, Gary gary.latson@bswhealth.org

Perftoran (Vidaphor) – Introduction to Western Medicine

Gary W. Latson, M.D.
Director, Neurosurgical Anesthesiology, Baylor Scott and White Healthcare, Temple Texas.
Adjunct Associate Professor, Anesthesiology, Texas A&M University Health Science Center College of Medicine.

Perftoran, which has been re-branded as Vidaphor for marketing in North America, is an Intravenous emulsion of the perfluorocarbons perfluorodecalin and perfluoromethylcyclohexyl piperidine in a surfactant (Proxanol 268) and electrolyte mixture. It was developed in Russia as an oxygen-carrying plasma additive for hemorrhagic anemia and a variety of ischemic conditions and was approved for clinical use in Russia in 1996 and used by the Russian Armed Forces and in civilian medical care. It has been administered to over 35,000

patients with significant evidence of benefit and relatively mild and manageable adverse effects. It may have significant potential for use in hemorrhagic shock if human red blood cells are not available, and for several other applications including treatment of vascular gas embolism, cerebral or spinal trauma, and regional ischemia. A newly formed United States Corporation (FluorO2 Therapeutics, Inc.) intends to manufacture the product in the United States under GMP standards and make it available for clinical use in Mexico (where it was previously approved). They also intend to seek approval in other Latin American countries and conduct research to support eventual approval in the US for human and veterinary use. This presentation will briefly review some of the key information about this product and provide references for additional information.
Disclosure: The author has been a consultant to FluorO2 Therapeutics Inc. The information in this article is summarized from previously published articles, patent applications, or book chapters authored in part by persons with past or present relationships with FluorO2 Therapeutics, Inc

Latson, Gary gary.latson@bswhealth.org

Intravenous Perfluorocarbon Emulsions as a treatment for vascular gas embolism and decompression sickness.

Director Neurosurgical Anesthesiology, Scott and White Memorial Hospital, Baylor Scott and White Healthcare
Adjunct Associate Professor, Anesthesiology, Texas A&M University

Intravenous perfluorocarbon emulsions (IVPFC) are plasma additives containing various liquid perfluorocarbons in emulsions with surfactants or phospholipids which have been developed for several applications including enhanced oxygen transport for severe anemia ("artificial blood substitutes"). They have a high affinity for many gases and have the capability of dissolving, transporting, and facilitating diffusion of oxygen, carbon dioxide, nitrogen, and other gases. Another significant potential application is for the treatment of gas bubbles in the bloodstream, as can occur in severe decompression sickness, pulmonary barotrauma with arterial gas embolism, or iatrogenic vascular gas embolism. Studies with several IVPFC products over nearly four decades in multiple animal models have consistently demonstrated that IVPFC can speed resolution of gas bubbles, enhance oxygen delivery to affected tissues, modify the immune response, and improve outcome. The US Navy has sponsored significant research in this area with promising results. This presentation will provide an overview of the physiologic basis for these effects and briefly review the most important studies which provide insight into potential future applications.

Preliminary Study of Oxidative Stress Caused by Polymerized Hemoglobin and Intervention in Rats

Weinan Li, Wanjing Li, Wentao Zhou, Yaojin Li, Shen Li, Hong Wang, Chengmin Yang*, Jiaxin Liu*
Institute of Blood Transfusion, Chinese Academy of Medical Science, Chengdu, P.R. China
*Corresponding author: chengminyang2602@126.com; jxliu8122@vip.sina.com

Background: As the continuing lack of blood supply, the emergence of new virus transmitted from blood transfusion, the requirement of cross matching in blood transfusion, and other issues, blood substitutes were quickly developed. Hemoglobin-based oxygen carriers are a kind of blood substitutes, having the expansion ability and being able to carry and to release oxygen. It is easy to pass through the distal end of the damage blood vessels, alleviating the body hypoxia timely, and has a good effect in the treatment of acute anemia. However, the side effects of HBOCs, including oxidative stress, vasoactivity and gastrointestinal reaction restrict the development and commercialization. We have been engaged in the study of polymerized human placental hemoglobin (PolyPHb) for a long time, and previous study has demonstrated the efficacy of PolyPHb in rat shock model and blood exchange model. Therefore, based on the previous study, this study deeply investigates the oxidative stress induced by PolyPHb in rats and the intervention effect of vitamin C.
Objective: Investigate the effect of low concentration of PolyPHb on oxidative stress in rats, and the intervention effect of vitamin C on oxidative stress caused by PolyPHb in rats.
Methods: Establish the rat 70% blood exchange model, and divide the rats into NaCl group, (2%, 4%, 6%) PolyPHb group, 2%PolyPHb + (0.025%, 0.05%, 0.1%) V_C group. Observe rat mean arterial pressure, heart rate and other physiological indexes before and after the blood replacement. After finishing the blood replacement, take arterial blood, measure the arterial blood gas. Then, separate plasma to detect Reduced Glutathione (GSH) concentration, Superoxide Dismutase (SOD) activity, Total antioxidant capacity (T-AOC), Methemoglobin (MetHb) concentration, Malondialdehyde (MDA) concentration, 8-hydroxy-2 deoxyguanosine (8-OHDG) and vitamin C concentration to evaluate the level of oxidative stress in rats.
Results: After 70% blood replacement, compared with NaCl group, SOD activity, GSH concentration, T-AOC, MetHb concentration, and MDA concentration in different concentrations of PolyPHb group were significantly increased (P<0.01), but the 8-OHDG concentration had no obvious change (P>0.05). After adding different concentrations V_C into 2% PolyPHb, low concentration V_C's SOD activity, GSH

concentration, T-AOC, MetHb concentration, and MDA concentration had no obvious change (P>0.05), while middle and high concentration V_C's MDA concentration were significantly decreased (P<0.01).
Conclusions: Infusion of low concentration PolyPHb can effectively alleviate oxidative stress in rats. In addition, PolyPHb can induce lipid peroxidation in rats, without DNA damage. High concentration V_C can enhance the body antioxidant capacity and reduce lipid peroxidation in rats.
Key words: PolyPHb, oxidative stress, lipid peroxidation, DNA damage, Vitamin C

Li YJ

Polymerized human placenta hemoglobin dissolved in hydroxyethyl starch solution as a novel oxygen-carrying plasma expander
Yaojin Li, Peipei Sang, Weinan Li, Shen Li, Gang Chen, Wentao Zhou, Hong Wang, Jiaxin Liu*, & Chengmin Yang*
Institute of Blood Transfusion, Chinese Academy of Medical Sciences, Chengdu, P.R. China
*Corresponding author: jxliu8122@vip.sina.com and chengminyang2602@126.com

Background and Objective Artificial colloids such as hetastarch or dextrans are used as plasma expanders to restore blood volume in clinical practice, but void of oxygen carrying ability. Functionality and application of colloidal fluids may be increased by adding hemoglobin-based oxygen carriers (HBOCs) to provide oxygen-carrying capacity. This research was designed to investigate the efficacy and safety of a novel oxygen-carrying plasma expander (polymerized human placenta hemoglobin dissolved in hydroxyethyl starch) in a series of animal experiments. **Materials and Methods** Mesenteric microvascular observation in a rat model was performed to assess the effects of oxygen-carrying plasma expander on hemodynamics and microcirculation function. Tissue oxygen pressure, blood perfusion, inflammatory response and enzymatic changes of heart, liver and kidney were measured to evaluate the feasibility for treating severe hemorrhagic shock with 60% blood loss. The oxidative stress induced by one component of polymerized hemoglobin and antioxidant effect of vitamin C were also studied in a rat model of exchange transfusion. **Results** Infusion with oxygen-carrying plasma expander recovered the perfusion of mesenteric microcirculation and the diameters of vessel, and reduced the number of adherent leukocytes to vessel wall comparing to Voluven group. Resuscitation with oxygen-carrying plasma expander could maintain higher tissue oxygenation and perfusion than control group. Oxygen-carrying plasma expander group significantly decreased TNF-α、IL-6 、MPO activities, but increased the level of CK, LDH and ALT after 24 hours of resuscitation when comparing to hydroxyethyl starch alone group. Low concentration of polymerized hemoglobin in oxygen-carrying plasma expander induced oxidative stress and lipid peroxidation in rat, which can be relieved by appropriate doses of vitamin C. **Conclusion** The novel oxygen-carrying plasma expander shows dual functions of oxygen carrying and volume expansion, which has great potential applications in ex hospital first aid, although there are still some problems need to be solved.
Key-words: plasma expander; hemoglobin-based oxygen carriers (HBOCs); hemorrhagic shock; microcirculation;

Light, R wrlight@mac.com

Development of a new hemoglobin-based oxygen carrier solution (VIR-XV1) for liver allograft preservation in combination with machine perfusion
William Richard Light[1], Ashok Malavalli[1], Kim Vandegriff[1], Joseph Tucker[1], Roberto Lopez[2], Paulo Fontes[2]
[1] VirTech Bio, Inc., Natick, MA, USA
[2] University of Pittsburgh, Pittsburgh, PA, USA
Liver transplantation is the ultimate therapy for end-stage liver disease and acute liver failure. Machine Perfusion (MP) is being developed as the new standard for liver preservation, with red blood cells (RBCs) often used as the oxygen carrier. RBCs *ex vivo* present numerous complications, so we originally examined the use of a bovine-derived glutaraldehyde polymerized hemoglobin-based oxygen carrier (BD HBOC) solution as the perfusate in a swine model with liver allografts preserved with MP (Liver Assist® device) for 12 hours at 21°C[1]. Following that success, VirTech Bio designed the first HBOC for *ex vivo* applications. VIR-XV1 is human derived, glutaraldehyde polymerized, with a higher molecular weight ((MW >500 kD, p50 = 36 mmHg) and is engineered for cost-effective production through scalable filtration technology to be Contract Manufacturing Organization 'friendly'. We present here in the context of our historical BD-HBOC control, (n=3, tHb=3.5g/dL)) a new 12-hour study with VIR-XV1, study group (n=3, tHb=3 g/dL). The perfusate was sampled for ABGs and biochemical analysis every 15 minutes during the first hour and every 3 hours for the duration of the experiments. Liver biopsies were obtained before and after organ procurement and every 3 hours during MP. Partial tissue oxygenation (ptO2) was continuously measured with an intrahepatic oximetry probe. Fresh liver samples were obtained every 3 hours for mitochondrial function, ROS and nitrate/nitrite measurements. Liver allografts cleared lactate very effectively while sustaining a

stable pH. In spite of the low tHb, pO_2 were >500 mmHg and ptO_2>200mmHg on both groups. The pCO_2 were much lower (<20 mmHg) in the study group. Mitochondrial function (RCR, Maximal Respiratory Rate) was properly sustained on both groups, and levels of ROS (H_2O_2) remained low (<6000 pmol/min/mg). Histological and EM analysis showed sustained integrity of the hepatic tissue for the first 9 hours. The VIR-XV1 group remained histological and EM intact throughout the entire duration of the experiments while the original BD HBOC group showed progressive endothelial cell damage, early detachment and progressive amount of debris accumulation in the sinusoids from 9 to 12 hours. Both HBOCs provided effective liver oxygenation and CO_2 removal over the 12-hour period. VIR-XV1 promoted superior hepatic integrity after 9 hours. While nitrate and nitrite were only measured in the VIR-XV1 group, they compared favorably (10 to 100 times lower, <0.04 and < 16 μM) to previous *in vivo* studies performed with BD HBOC. This has obvious *in vivo* implications for VIR-XV1 which are currently being explored as well. In conclusion, VIR-XV1 has the potential to increase the current number of viable liver transplants, shorten the waiting list time, and dramatically improve patient outcomes.

1) Fontes P, Lopez R, van der Plaat, A, Vodovotz Y, Minervini M, Scott V, Soltys K, Shiva S, Paranjpe S, et al. Am J Transplant 2015; 15: 381-394.

Liu JX jxliu8122@vip.sina.com

Study on Blood Substitutes and Oxygen Carries in China

Jiaxin Liu, Hong Wang, Chenmin Yang
Institute of Blood Transfusion, CAMS,Chengdu, China

Nowadays, Chinese government attaches great importance to Blood Substitutes and Oxygen Carries research. Last decade, significant progress had happened.

1. Supported by National key projects: From 1996 to this day, this project obtain about 0.1 billion China Yuan funding which come from state, province and city level. And get fifty million China Yuan funding which come from Enterprise financing. From end of 2016, bring this project into line with CAMS Innovation Fund for Medical Sciences. This fund supported by the Finance Ministry and the National health and Family Planning Commission. Every year Chinese government will give about 0.4 billion to CAMS Innovation Fund, and this project is a part of CAMS Innovation Fund.

2. Research and development unit: CAMS&PUMC Institute of Blood Transfusion; CAMS&PUMC Institute of Biomedical Engineering; Academy of Military Medical Sciences Institute of Blood Transfusion in field battle; CAS Institute of Process Engineering. CAS Changchun Institute of Applied Chemistry; Third Military Medical University Institute of Battle Surgery; Chinese Northwestern University; Jiangsu Normal University; University of Shanghai for Science and Technology; and Tianjin Union Biotechnology Co ., Ltd; Shanxi lifegen Co ., Ltd; Union colleague of science and technology (Tianjin) Co ., Ltd; etc.

3. All kind of research and progress

3.1 In 1960s, Third Military Medical University cooperated with CAS Shanghai Institute of Organic Chemistry. Project lead by Professor Huisun Chen. And found perfluorocarbon emulsion (PFCE) particles which named “white blood”. Then, PFCE get a Phase 2 clinical trial at Shanghai zhongshan hospital and other hospital. Pre-clinical program got good effect and no obvious side effects were found. However, this project is interrupted by PFCE’s long time of metabolic and other side effects. In recent years, Beijing SL Pharmaceutical Co., Ltd cooperate with Alliance Pharma Co., Ltd, is planning to development of PFCE.

3.2 From 1992, IBT ,CAMS, Professor Chenmin Yang and Jiaxin Liu started Hemoglobin-based oxygen carriers (HBOCs) research. This project used human umbilical cord blood as raw materials. In the past 20 years, this project basically finished poly-hemoglobin manufacturing process and put forward a new idea about blood transfusion. The new idea is a new formula which designed by poly-hemoglobin and colloid Plasma substitute mixture. This project also set up a large amount of blood loss shock and microcirculation of rat model. Our team cooperated with Hong Kong New β Innovation Co ., Ltd, finished HBOCs physical and chemical quality standard and testing methodology. And key files is checked by National Institutes for Food and Drug Control.

3.3 CAS Institute of Process Engineering cooperated with Beijing Kaizheng Biotech Co ., Ltd. Project lead by Professor Zhiguo Su. This project used bovine blood as raw materials. This kind of HBOCs got a Phase 1 clinical trial. Later, this project make significant progress on manufacturing process and basic research.

3.4 Chinese Northwestern University cooperated with Shanxi lifegen Co ., Ltd. Project lead by Professor Chao Chen. This project used swine blood as raw materials. This project basically finished pilot process and pre-clinical pharmacology, toxicology, and immunogenicity etc. Now, this project will declare Phase 1 clinical trial.

3.5 CAS Changchun Institute of Applied Chemistry, Project lead by Professor Yubin Huang. This project focus on Micro capsule material and cellular type Red blood cell substitutes. In the last decade, this project make many pioneer advances in idea and method.
3.6 Jiangsu Normal University, Project lead by Professor Ziyu Wang. This project focus on genetically engineered Hemoglobin. For HBOCs side effects, like as vascular activity and free radical injury, this team increase the products size and alter charge of surface. Later, this team will be change Hb gene mutational site, make albumin jointed with Hb, make antioxidant enzyme jointed with Hb.
3.7 Tianjin Union Biotechnology Co ., Ltd cooperated with Tianjin Medical University Cancer Hospital, Tianjin Ophthalmic Hospital, West China Hospital of Sichuan University, Tianjin Institute of Medical and Pharmaceutical Science. Project lead by Professor Chenmin Yang. In the last decade, this project used Nano-HBOC treated with acute myocardial infarction, Retinal infusion, Myocardial function protection, Cerebral ischemia injury protection. This project make many pioneer advances in idea and method.
3.8 Hong Kong New β Innovation Co ., Ltd, Project lead by Dr. Bingmiu Wong. This project focus on Hb-nitric oxide (NO) mixture, and added appropriate antioxidant. Results show that Hb-NO has effective in treatment, and no obvious side effects were found, like as vascular activity and free radical injury. In 2016, the United Kingdom approved Hb-NO got a Phase 1 clinical trial. And Hong Kong also approved Hb-NO used for increasing the effect of oncotherapy.
3.9 CAMS&PUMC Institute of Biomedical Engineering, Project lead by Professor Tianjun Liu. This project focus on Synthetic hematin chloride.

SanFlow with Crystalloid as Blood Substitute

Li Ma, Ph.D. xrglima@yahoo.com[1] Carleton Jen-Chang Hsia
Georgia Depart of Physics, Georgia Southern University,Statesboro, GA[1] and NanoBlood LLC, Sioux Falls, SD[2].,

SanFlow, aka polynitroxylated pegylated hemoglobin (PNPH), is being developed for critical care and transfusion medicine to meet the challenge posted by to (1). NanoBlood will present preclinical efficacy data showing the superiority of SanFlow (4g/dl hemoglobin) compared to ~ 14g/dl of whole blood. SanFlow is a paradigm change, which manages volume expansion with crystalloid, making it a safer and more practical therapy than fresh whole blood in civilian and military critical care and transfusion medicine. Instead of replacing the lost oxygen carrying capacity with large quantities of HBOC, we found that restoration of blood flow with residual 50% of oxygen carrying capacity of the anemic blood at ~7 g/dl red blood cell hemoglobin is the optimum resuscitation strategy. With crystalloid for volume replacement, the use of SanFlow to restore the focal or global endogenous vascular nitric oxide level which serve to maintain hemodynamic stability of the cardiovascular system. We have demonstrated that the requisite normal physiological oxygen consumption and CO2 release needs are fully satisfied. Thus SanFlow with supplemental crystalloid for volume replacement was shown to be better substitute for whole blood transfusion. This paradigm change avoided the high dose of cell free hemoglobin toxicity of previous generations of HBOC that failed from meta-analysis of their clinical trials due to myocardial infraction and death (2). In fact, we have proposed the use of SanFlow over 96 hours in combat casualty care to replace the fresh blood transfusion in the battlefield. Likewise, in remote areas or developing countries where transfusion of fresh blood is not readily available, SanFlow, without need for refrigeration and blood typing, may be more desirable and practical. In our recent publications, the function of SanFlow with crystalloid simply make the anemic blood flow better to serve the bodies physiological functional needs (3-4). The following is a translation of the SanFlow data in these three papers in Layman's terms: We have exceeded the NIH/DOD/FDA challenge by demonstrating that extremely small volume i.e. ~ 1/10th of shed blood volume of SanFlow, in conjunction with crystalloid, was shown to be capable to restore mean arterial pressure (MAP) to a more stable and higher level than fresh whole shed blood in a mouse model of traumatic brain injury (TBI) with hemorrhagic shock (HS). SanFlow was shown to maintain cerebral perfusion pressure (CPP) of the hemorrhagic brain to provided critical oxygen delivery to serve neurological functional needs without resulting in increased intracranial pressure (ICP) and without cerebral edema of the mouse brain. These therapeutic benefits were achieved in an anemic state (~7g/dl hemoglobin) with normal physiological parameters (3-4). These and other preclinical efficacy data were presented to the FDA and led to the FDA's ruling that SanFlow is a small volume therapeutic drug. Proposal of SanFlow for combat casualty care have received a combined satisfaction score of 90%. With unlimited supply of bovine hemoglobin as raw material, we propose that SanFlow can be developed to meet global critical care and blood transfusion needs in both developing and developed countries worthy of the support of the WHO.

[1] NIH/DOD/FDA Workshop Report "Oxygen Therapeutics", July 25, 2011: http:// www.nhlbi.nih.gov/research/reports/2011-therapeutics
[2] Natanson C. et.al. Cell free hemoglobin-based blood substitutes and risk of myocardial infraction and death: a meta-analysis. JAMA. 2008 Sep 17; 300 (11): 1300.
[3] Brockman EC et.al. Polynitroxylated Pegylated Hemoglobin-A Novel, Small Volume Therapeutic for Traumatic Brain Injury Resuscitation: Comparison to Whole Blood and Dose Response Evaluation. J Neurotrauma. 2017 Apr 1; 34(7):1337-1350.

Lu, Y

Enhancement of recombinant hemoglobin production in P. pastoris containing the HRG-4 heme transpot system

Yuhao Lu, Meng Du, Ziyuan Wang* School of life Science, Xuzhou Normal Universit, Xuzhou, P.R. China
*Corresponding author: zwang22299@aliyun.com

Rcombinant human hemoglobin is a good raw material for oxygen carrier substitute. *P. pastoris* is being developed as a widely used host organism for cost-effective and efficient eukaryotic protein expression system. But the insufficient availability of heme prosthetic groups is a major limitation in the production of recombinant globin in P. pastoris. *C. elegans* is natural heme auxotroph and must acquire the heme cofactor from the environment to incorporate into hemoglobin. Recent studies identified that HRG-4 protein of *C. elegans* at the apical plasma membrane is responsible for importing heme across plasma membrane.

Objectives：To construct a transgenic platform of *P. pastoris* for raising the heme level and improving recombinant human hemoglobin production.

Rerults： HRG-4 expression plasmid was designed、linearized and transformed into GS115 *P. pastoris* cells by electroporation. Eight stable transformants of *P. pastoris* were selected. The HRG-4 gene expression was confirmed by RT-PCR analysis. Then the second plasmid containing hemoglobin α and β gene was transformed into yeast cells. The second plasmid was designed to insert into the rDNA locus and multicopy integrants could be selected. Finally, the coexpressing of human hemoglobin and HRG-4 heme transport genes in heme-containing medium was studied. An increase of recombinant hemoglobin in 1.3 times was detected.

Conclusion: Our preliminary results indicate that coexpression of HRG-4 has enhancement effect on hemoglobin production in *P. pastoris* cells.

References:

1 Rajagopal A, Rao AU, Amigo J, Tian M, Upadhyay SK, Hall C, Uhm S, Mathew MK, Fleming MD, Paw BH, Krause M, Hamza I. Haem homeostasis is regulated by the conserved and concerted functions of HRG-1 proteins. Nature. 453(7198):1127-31, 2008.
2 Yuan X, Protchenko O, Philpott CC, Hamza I. Topologically conserved residues direct heme transport in HRG-1-related proteins. J Biol Chem. 287(7):4914-24, 2012.
3 Marx H, Mecklenbräuker A, Gasser B, Sauer M, Mattanovich D. Directed gene copy number amplification in *Pichia pastoris* by vector integration into the ribosomal DNA locus. FEMS Yeast Res. 9(8):1260-70, 2009.

Key words: HRG-4, heme, recombinant expression, *P. pastoris*

Mackenzie, Colin MD (U.S.A) cmack003@gmail.com
Emeritus Professor, University of Maryland School of Medicine ,Baltimore ,MD 21201 USA

Lessons Learned from 22 clinical trials of HBOC-201

Matsuhira T mattu@naramed-u.ac.jp

Reactivity of Cys b93 of native and b-crosslinked Hbs

Takashi Matsuhira, Keizo Yamamoto, Hiromi Sakai
Department of Chemistry, Nara Medical University, Kashihara 634-8521, Japan
mattu@naramed-u.ac.jp

There have been many reports of PEG modification of Hb for the usage of artificial oxygen carriers. Maleimide-PEG (mal-PEG) is a well known reagent for PEG modification of Hb, while maleimide conjugates to two active thiols ascribed to Cys b93 on the surface of b subunits.
In this work, several kinds of mal-PEG were conjugated to native and b-crosslinked Hb (bXLHb). The reaction between maleimide and Cys b93 was monitored by electrophoresis and size exclusion chromatography. When using branched PEG with multiple maleimide terminals, multimers of Hbs and bXLHbs were obtained but the structures of multimers were different, suggesting the difference in reactivity of Cys b93 to maleimide

Jing Ning jing.ning@hc-sc.gc.ca
(Alumni of Artificial Cells & Organs Research Centre)

Biosimilars and the Key Considerations for the Clinical Assessment

Jing Ning, MD. PhD
Senior Clinical Evaluator, Clinical Evaluation Division Biologics and Genetic Therapies Directorate (BGTD)
Health Canada

A biosimilar biologic drug (biosimilar) is a biologic drug that obtains market authorization subsequent to a version previously authorized in Canada, and with demonstrated similarity to a reference biologic drug.

The highlights of the new guidance document for biosimilars published in 2016 by Health Canada will be presented.
Foundation of the biosimilar development program and demonstration of similarity between the reference biologic product and the biosimilar will be summarized. The presentation will focus on the key considerations of the clinical assessment of biosimilars.

Palmer A palmer.351@osu.edu

Engineering polymerized hemoglobin size regulates side-effects
Andre Francis Palmer
William G. Lowrie Department of Chemical & Biomolecular Engineering
The Ohio State University, Columbus, Ohio
Universal O_2-carrying solutions that can replace the O_2 storage and transport functions of red blood cells will greatly improve clinical outcomes for trauma patients when blood is not available such as the site of accidents or on the battlefield. My talk will address a simple approach for designing hemoglobin-based O_2 carriers (HBOCs). Our design strategy is based on the observation that transfusion of cell-free hemoglobin results in vasoconstriction, systemic hypertension and oxidative tissue injury. The root cause of these side-effects stem from the ability of hemoglobin to extravasate into the tissue space through pores in the blood vessel wall, and scavenge nitric oxide from the surrounding vasculature, as well as catalyze production of reactive oxygen species. Therefore, our design strategy will focus on increasing the molecular diameter of HBOCs so that they are unable to traverse across the wall of blood vessels into the tissue space. In this talk, I will discuss the design, biophysical properties and *in vivo* response of polymerized hemoglobins or varying size. This work is significant in that it will lead to the development of polymerized hemoglobins that are safe and efficacious for use in transfusion medicine.

Pelletier, P patricia.pelletier@mcgill.ca

Ethnic differences in red blood cell antigens and how they affect transfusion
Pelletier, P
Director of Transfusion Medicine Service, McGill University Hospital Centre designated transfusion center
Faculty of Medicine, McGill University, Montreal, Quebec, Canada

The prevalence of certain red blood cell antigens can vary greatly among ethnic groups. While antigenic differences between donor and recipient always carry the risk of antibody formation, this is accentuated when recipients come from a different ethnic group than the majority of blood donors. This situation can pose a challenge when antibodies are made against highly prevalent antigens or against a combination of multiple antigens, requiring the search for “rare blood”. These issues are especially complex in patients with sickle cell anemia, a disease that combines antigenic differences, high rates of antibody formation and significant requirements for blood transfusion.

Polard,Valérie valerie.polard@hemarina.com

Evaluation of a specific oxygen carrier (M101®) added to pig liver cold storage solutions to improve post-transplant graft function.
Polard,Valérie & Pierre Alix* (France) valerie.polard@hemarina.com
Responsable Préclinique/ Preclinical Development Manager
Aéropole centre – Biotechnopôle, 29600 MORLAIX, FRANCE

Background: Cold storage (CS), using an appropriate solution remains the gold standard in liver graft preservation. However continuous hypothermic machine perfusion (CHMP) has been shown to be more efficient than CS since it provides oxygen to the graft cells, allowing ATP synthesis and subsequent protection of cell membranes. CHMP requires sophisticated tools, expensive ancillary products and stringent monitoring that limit its clinical use. Addition of a high-affinity oxygen carrier, in CS solutions, may improve the preservation quality at least equal to that of CHMP.
The aim of this study was to evaluate the performance of a CS liver graft solution supplemented with a specific oxygen carrier, issued from a marine worm (M101®).
Methods: We used an orthotopic pig liver transplantation (OLT) model and compared four groups of five animals transplanted with a graft stored for 6 hours by CS, CS + M101 (1g/L) , CHMP and CHMP + M101 (1g/L).
The CS solution was the UW-CS® (Bridge to Life company) and the CHMP solution was the UW-MP® (Bridge to Life company).

Post-transplant liver functions and histological analysis were compared until the 7th postoperative day.
Results: CS + M101 conservated group decreased reperfusion injury compared to CS conservated group with a lower peak of LDH and AST released after OLT (1140 vs. 2150 U/L, P=0,159; 752 vs. 1675, P=0,03). There was no significant difference between CS + M101 and CHMP or CHMP + M101.
Moreover, the histological analysis showed less inflammatory cells activation in CS + M101 group compared to CS group.
Conclusion: Actually, M101 seems to improved the hepatic graft conservation with lower reperfusion graft injury.
Otherwise, results of compared enzymatic activity of the mitochondrial respiratory chain complexes after 12 hours of liver conservation by the sames methods are waiting.

Ponka P prem.ponka@mcgill.ca
(Associate member, Artificial Cells & Organs Research Centre)

Physiology and Pathophysiology of Iron Homeostasis: Implications for Therapy of Iron Overload
Prem Ponka**,** *Lady Davis Institute and Department of Physiology, McGill University, Montréal, QC, Canada*
Iron is a vitally important element in virtually all organisms, because of its unsurpassed versatility as a biological catalyst. It serves as metal cofactor for many proteins and enzymes, either non-heme or hemoproteins. In the latter, iron is inserted like a gem in the center of protoporphyrin IX. Under normal conditions both intracellular iron trafficking and heme levels are impeccably regulated, preventing the accumulation of noxious free iron and highly toxic intermediates of heme biosynthesis. In mammals, there is an iron cycle that entails the movement of iron from plasma transferrin (Tf) to hemoglobin (Hb) in developing red blood cells, and the release of iron back to plasma Tf from macrophages that recycle the Hb iron of senescent erythrocytes. Under normal circumstances, Hb-processing macrophages simultaneously convey iron to plasma at the same rate as the metal is delivered from Tf to developing erythroid cells. The crucial role in this loop is played by heme oxygenase 1 (HO1) that catabolizes heme to biliverdin upon the release of Fe^{2+} and carbon monoxide. Importantly, we have recently reported that HO1 is expressed also in erythroid cells. Despite numerous mechanisms that maintain iron homeostasis, organisms can face the threat of iron overload. Patients with iron overload accumulate excessive amounts of iron in various organs including the liver, pancreas, and heart, and, consequently, they suffer from conditions that include cirrhosis, diabetes, and heart dysfunction.

Different types of hereditary hemochromatoses are caused by mutations in genes that control iron metabolism (HFE, hemojuvelin, hepcidin, Tf receptor 2, ferroportin, and hepcidin). The simple and effective treatment for genetically-based iron overload consists of performing periodic phlebotomies that remove excessive iron. Secondary iron overload commonly develops in thalassemia, the most common inherited single gene disorder in the world. Unpaired globin chains that accumulate in thalassemic erythroblasts are bound to heme that likely induces HO1. We hypothesized that in thalassemic erythroblasts HO1-mediated release of iron from heme is the major culprit responsible for cellular damage. To investigate the contribution of HO1 to the pathology associated with thalassemia, wild-type and thalassemic (th3/+) mice were injected with tin-protoporphyrin IX (SnPP, HO1 inhibitor). We showed that β-thalassemic mice injected with SnPP have an increase in hemoglobin levels as well as red blood cell counts and display a decrease in liver iron content. Our results suggest that new therapies that suppress heme catabolism may be beneficial in ameliorating the anemia and ineffective erythropoiesis in thalassemias.

Rausch C (U.S.A. and Hong Kong China)
Newai Corp, Hong Kong
The development and the difficulties as well as the opportunities of blood substitutes
carl.rausch@gmail.com

Robillard, Pierre (Canada) . Pierre.robillard@hema-quebec.qc.ca
Medical Director, Hema-Quebec, Montreal, Quebec, Canada
Hemovigilance from an international perspective

Nicholas L. Robbins, robbinsN@uthscsa.edu
1RESTOR™ Program, 59th Medical Wing, JBSA Lackland AFB, TX, , University of Texas Health Sciences Center at San Antonio, San Antonio, TX, 4Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC

Brandon J. Reeder reedb@essex.ac.uk

A novel recombinant hemoglobin-based blood substitute with combined enhanced ferryl and ferric reductase activity

Brandon J. Reeder(1)*, Michelle Simons(1), Nélida Leiva Eriksson(2), Leif Bulow(2), Andrea Mozzarelli(3), Luca Ronda(4), Andras Eke(5), Natalie Syrett(1), Victoria Allen-Baume(1), Chris Cooper(1)
(1) School of Biological Sciences, University of Essex, Colchester, Essex, CO4 3SQ, UK.
(2) Pure and Applied Biochemistry, Lund University, Sweden
(3) Department of Food and Drug, University of Parma, Italy
(4) Department of Medicine and Surgery, University of Parma, Italy
(5) Department of Physiology, Semmelweis University, Budapest, Hungary
*Corresponding author: reedb@essex.ac.uk

There is a clinical need to generate a synthetic blood substitute/oxygen therapeutic that is long lasting and sterile. Hemoglobin (Hb) is the natural oxygen carrier in the body, therefore the use of cell-free synthetic Hb is a good starting material for an oxygen therapeutic. However, these Hemoglobin Based Oxygen Carriers (HBOCs) display an inherent capacity to induce oxidative reactions, causing cell and tissue damage. The ability of Hb to function as redox enzymes outside their normal environment has been known for many decades, although the implications and extent of this redox chemistry is not fully understood. Nonetheless, the capacity of cell-free Hb to damage organs such as the kidney is well known.
We have developed mutations designed to decrease the intrinsic damaging oxidative reactivity of Hb, specifically enhancing the ability of endogenous plasma reductants such as ascorbate and urate to remove cytotoxic high oxidation state of Hb (ferryl). These have used redox activate surface tyrosine residues as initial electron acceptors, linking the electron transfer from exogenous reductants to the heme iron[1,2]. A subset of these mutations also showed enhanced plasma ascorbate ferric reductase activity, enabling them to remain longer in the oxygen carrying ferrous form. For adult Hb, our lead compound is a Thr84Tyr mutation in the beta chain and for fetal Hb a Lys96Tyr mutation in the gamma chain. These mutations have exhibited a decreased capacity to induce damaging oxidation reactions to cells and cell membranes, whilst maintaining stable oxygen binding, a normal rate of autoxidation and good stability of the heme moiety. The PEGylated form (using Euro-PEG[3]) of this Thr84Tyr mutation in adult Hb exhibits an enhanced *in vivo* lifetime in a mouse model over that of the PEGylated wild-type adult Hb.
Based on the data collected so far, the introduction of the through-protein electron transfer pathway is a promising step in a new generation of stable HBOCs with decreased redox activities. We have also added mutations to decrease nitric oxide (NO) scavenging capacity of Hb without associated damaging heme release. The combined effects of the tyrosine and NO mutations is currently being studied to determine the effects on renal toxicity.
[1] Reeder et al, J. Biol. Chem,
[2] Silkstone et al, Biochem J. (2016) 473, 3371-3383
[3] Portoro et al, Biochim. Biophysc. Acta, (2008)1784, 1402-1409,
Keywords: Hemoglobin, HBOC, tyrosine, nitric oxide, oxidative stress, electron transfer pathways.

Scott, M mdscott@mail.ubc.ca

Stealth Erythrocytes: Treatment and Prevention of RBC Alloimmunization by Polymer-Grafting
Senior Scientist - Clinical Professor, Canadian Blood Services and University of British Columbia

While ABO/Rh(D) red blood cell (RBC) matched transfusions are generally considered as safe, a significant risk of alloimmunization to non-A/B blood group antigens exists; especially in chronically transfused patients. Indeed, alloimmunization to non-A/B antigens can be so severe that RBC transfusion can no longer be safely administered without the risk of a potentially deadly immune hemolytic reaction. Currently, no satisfactory solutions exist to either prevent blood group alloimmunization or to cost-effectively treat patients with severe alloimmunization. To address this problem, we have pioneered the “immunocamouflage” of donor RBC. The immunocamouflaged (Stealth) RBC is manufactured by the covalent grafting of polymers (e.g., methoxypolyethylene glycol) to RBC membrane proteins. As a result of the grafted polymer, non-A/B blood group antigens are biophysically and immunologically masked. The polymer-modified RBC are morphologically normal and, in mice, exhibit normal *in vivo* survival at immunoprotective grafting concentration. As demonstrated by the clinically validated monocyte monolayer assay, recognition of mismatched human RBC by patient-derived alloantibodies was significantly attenuated. Of particular interest, immune recognition of the Rh(D) antigen was readily blocked; a finding that could potentially improve blood inventory and transfusion safety in emergency situations.

Sakai H hirosakai@naramed-u.ac.jp

Present Status of Blood Substitute Research in Japan

Hiromi Sakai[1], Koichi Kobayashi[2]
[1]Department of Chemistry, Nara Medical University, Kashihara, Japan; [2]Keio University, Tokyo, Japan;

In the early 1950s, Minoshima was the first in Japan to attempt preparation of 'artificial blood' with a cobalt-histidine complex as an oxygen carrier model. Following the pioneering studies conducted by Chang, several HBOCs, acellular and cellular, were developed in Japan. Pyridoxalated Hb-polyoxyethylene conjugate (PHP) was developed in the 1980s by Ajinomoto Co. Inc. Fluosol-DA emulsion was the first clinically approved oxygen-carrying infusion in 1993. Since then, pioneering R&D of 'artificial blood' has continued actively in Japan in industry and academia. In the 1990s, difficulties related to transfusion-related infection, a low degree of self-sufficiency in blood products, and anticipated shortages of blood products in aging societies have spurred the Japanese government to support artificial blood product R&D for substitution of the functions of red blood cells, platelets, plasma proteins, etc. The Society of Blood Substitutes, Japan (SBSJ) was established in 1993 (past presidents, Tsuchida, Kobayashi; present president, Takeoka). Since then, SBSJ symposia have been held annually. Now SBSJ has nearly 100 members and 4 supporting corporate members. Along with recombinant technologies and studies of ES-derived and iPS-derived blood cells, a wide scope of research

Sakai H hirosakai@naramed-u.ac.jp

Translational Research of Hb-vesicles (Artificial Red Cells) for a Transfusion Alternative and O_2/CO Therapeutics

Hiromi Sakai
Department of Chemistry, Nara Medical University, Kashihara 634-8521, Japan

A fluid of Hb-vesicles, Hb-V, which comprises a concentrated particle dispersion ([Hb] = 10 g/dL, occupied particle volume = 40%, particle size = 250 nm), is being developed as a transfusion alternative with physicochemical characteristics comparable to those of blood [1–3]. Encapsulation of a concentrated Hb solution within phospholipid vesicles can mitigate the toxic side effects of cell-free Hb. Through establishment of the production method and confirmation of its safety [4,5] and efficacy [1,6,7] as a transfusion alternative, we are preparing for the next stages of translational research. From 2015, this project has been supported by the Japan Agency for Medical Research and Development (AMED); GLP preclinical safety studies are underway. Recent studies have also clarified Hb-V as effective as an organ perfusate [8], a CO carrier for inflammatory disease [9,10], and a photosensitizing agent for the port-wine stain model [11].

References: [1] J Funct Biomater 2017;8:E14; [2] Langmuir 2017;33:1533-40; [3] ACS Chem Biol 2017, in press; [4] Immunopharmacol Immunotoxicol 2017;39:140-147; [5] PLoS One 2016;11: e0165557; [6] BMC Anesthesiol 2017;17:44; [7] PLoS One 2017;12: e0178724; [8] Transplantation 2015;99:687-691; [9] Int J Nanomed 2016;11:5611-20; [10] J Control Release 2016;234:49-58; [11] Plast Reconstr Surg 2017;139:707e-16e.

Effects of polymerized human placenta hemoglobin combined with hydroxyethyl starch on tissue organs in a rat model of hemorrhagic shock

Peipei Sang, Yaojin Li, Gang Chen, Shen Li, Wentao Zhou, Hong Wang, Jiaxin Liu*
Institute of Blood Transfusion, Chinese Academy of Medical sciences & Peking Union Medical College, Chengdu, P.R. China.* Corresponding author: jxliu8122@vip.sina.com

Background: Our previous study showed that polymerized human placenta hemoglobin (PolyPHb) combined with hydroxyethyl starch (HES) increased the survival of rats in a severe hemorrhagic shock model. Aim of the current study was to further investigate the organ effects of PolyPHb combined with HES.
Methods: Male SD rats were subjected to severe hemorrhagic (60% loss of the total blood volume) and randomly divided into the following three groups: 1) PolyPHb/HES (hemoglobin content 2 g/dl) group, 2) RBC/HES (hemoglobin content 2 g/dl, RBC was derived from rats blood) group, 3) HES group. The volume of resuscitative fluid was identical to the removal blood volume (60% of blood volume). The concentration of TNF-α, IL-6 and MPO activity in intestinal tissue was measured at 2 h after resuscitation. And the level of CK, LDH, AST, ALT, BUN and Cr in plasma were measured at baseline, shock, 2 h and 24 h after resuscitation. At the end of study, the rats were sacrificed and the liver, kidney and heart tissues were quickly incised to fix using 10% formaldehyde for the measurement of histopathologic changes.
Results: The level of TNF-α, IL-6 and MPO activity in intestinal tissue were decreased by PolyPHb/HES group compared with HES group. The level of CK and LDH in PolyPHb/HES group were higher than HES and RBC/HES group at 2 h and 24 h after resuscitation. The level of AST and ALT has no significant changes in three groups at 2 h after resuscitation but increased significantly at 24 h after resuscitation, and

the level of ALT in PolyPHb/HES group was higher compared with the other two groups. The level of BUN at 2 h after resuscitation, and the level of Cr at 2 h and 24 h in PolyPHb/HES group were higher compared with RBC/HES and HES group. Pathological analysis showed that there is no changes in heart, kidney and liver tissues in RBC/HES group. But changes of kidney and liver tissues were observed in PolyPHb/HES group and HES group. And heart pathophysiology lesion was also observed in PolyPHb/HES group.

Conclusion: PolyPHb/HES could attenuate inflammation response of intestinal tissue but has heart, kidney and liver side effects.

Shen, Yuesheng[1], Geng Niu[1], Yuwei Bai[1], Chao Chen[1,2], Hongli Zhu[1,2] zhuyjw@hotmail.com

1.College of Life Science, Northwest University, Xi'an, P. R. China
2.National Engineering Research Center for Miniaturized Detection Systems, Northwest University, Xi'an, P. R. China

Preliminary study on pharmacokinetics of Polymerized Porcine Hemoglobin (pPolyHb)

Simoni, J jan.simoni@ttuhsc.edu

Requirements for HBOC to be highly effective in the treatment of myocardial ischemia

Jan Simoni
Texas HemoBioTherapeutics & BioInnovation Center and Texas Tech University Health Sciences Center, Lubbock, Texas 79424, USA

Myocardial ischemia occurs when the blood flow to the myocardium is rapidly obstructed by a blockade of a coronary artery that leads to ST-segment elevation myocardial infarction (STEMI). Although restoration of blood flow and myocardial reoxygenation is the standard of care for STEMI, it is frequently associated with ischemia-reperfusion injury (I/R). Also percutaneous intervention procedures (PCI) by obstructing the blood flow during balloon inflation may aggravate hypoxia of the distal portion of coronary artery. Hypoxic ischemic myocardial injury and oxidative stress in PCI subjects are caused by imbalance between the production of reactive oxygen species and detoxification of reactive intermediates, which leads to the inflammatory responses and dysfunction of the endothelium.

The concept of perfusion of the coronary artery during PCI is not new. Initially, in order to reduce myocardial ischemia, it was proposed to perfuse arterial blood through the central lumen of the angioplasty catheter. This technique, however, showed to be disadvantageous due to the high viscosity of blood and hemolysis. The use of crystalloids was unable to prevent ischemia because they do not carry oxygen.

Since the late 80's, the blood substitute field has recognized the need for applying oxygen-carrying solutions in prolonging balloon occlusion time and the restoration of heart function after elective coronary revascularization. To date several attempts with fluorocarbons and HBOCs took place. Although, Fluosol (Green Cross Corp., Osaka, Japan) showed to be effective in reducing myocardial ischemia during a prolonged balloon occlusion time in 1994 was removed from the market. The reported problems were related to the premature release of oxygen and side effects, including thrombocytopenia and flu-like symptoms. The infusion of HBOC-201 (glutaraldehyde-polymerized bovine hemoglobin; Biopure/OPK Biotech, Cambridge, MA, USA) has been shown to reduce the infarct size and restore myocardial tissue oxygen tension in experimental myocardial ischemia in dogs. The mechanism by which HBOC-201 increased myocardial oxygenation and ameliorated myocardial ischemia was related to its ability to reach and oxygenate post-stenotic or poorly perfused tissue, where RBCs were not able to pass. A multicenter phase II study in the setting of elective angioplasty and stent procedures proved that intracoronary infusion of oxygenated HBOC-201 is capable of preserving left ventricular function and protect against myocardial ischemia in patients experiencing complete coronary occlusion, likely through maintenance of myocardial oxygenation. However, HBOC-201 used in other clinical trials did not perform so well. When injected in a larger volume, serious adverse events occurred in some tested subjects, including death, hypertension, myocardial infarction, cerebral vascular accident, cerebrovascular ischemia, transient ischemic attack and acute renal failure. These clinical observations established a new standard. It is now clear that HBOC useful for effective treatment of myocardial ischemia should: (i) non toxic, (ii) provide controlled oxygen delivery during reperfusion to prevent I/R, (iii) induce coronary vasodilation, (iv) alleviate oxidative reactions, (v) reduce endothelial inflammatory responses by attenuating the expression of adhesion molecules, and also (vi) decrease platelet aggregability and release of serotonin (5-HT) in response to platelet aggregation agonists, particularly collagen.

Some HBOCs already met these new standards, particularly: Hb conjugated with antioxidant enzymes superoxide dismutase and catalase (U.S. Patent No. 5,606,025 A), and Hb pharmacologically modified with vasodilators, anti-inflammatory and antioxidant agents: o-ATP, o-adenosine and reduced glutathione (U.S. Patent No. 7,759,306 B2).

Song, Bjorn K. bjorn@songbiotechnologies.com

Efficacy of SANGUINATE™ versus Standard of Care in Three Rat Models of Hemorrhagic Shock

William H. Nugent[1], Ramon F. Cestero[2], Kevin Ward[3], Ronald Jubin[4], Abe Abuchowski[4], Bjorn K. Song[1]
1. Song Biotechnologies, Baltimore, MD
2. University of Texas Health San Antonio, San Antonio, TX
3. University of Michigan Medical School, Ann Arbor, MI
4. Prolong Pharmaceuticals, South Plainfield, NJ

Hemorrhage is a leading cause of death in young adults. Once hemorrhage is controlled, two progressive factors determine resuscitative outcomes: magnitude of hemorrhagic shock and time to treatment. Hemoglobin-based oxygen carriers are well suited to bridge circulatory function when whole blood or its constituents are not available, but characterization across the spectrum of hemorrhagic shock scenarios in a preclinical setting is a necessary precursor to clinical trials. SANGUINATE™ is a novel PEGylated bovine carboxyhemoglobin-dual gas transfer agent that has shown early promise in improving clinical outcomes for situations of hemolytic anemia and is now evaluated against standard resuscitated adjuncts for impact on tissue oxygenation and survival in three preclinical, lethal models of hemorrhagic shock.

In three separate experiments, Sprague Dawley rats were subjected to a controlled blood withdrawal (45% blood volume) to induce varying degrees of hemorrhagic shock by altering the withdrawal rates (1.0 - 3.5 ml/kg/min). This resulted in three different models of hemorrhagic shock - acute, delayed, and prolonged - where untreated animals would die approximately 10, 20, and 90 minutes after hemorrhage, respectively. Hemorrhaged animals were resuscitated (20% blood volume) with either SANGUINATE™ or a volume control, Hextend™, immediately prior to death when mean arterial pressure had fallen to < 22 mmHg.

All severities of hemorrhagic shock produced a non-detectable (0 - 2 mmHg) level of peripheral tissue oxygenation ($P_{ISF}O_2$) in the spinotrapezius muscles of all animal groups. Fluid resuscitation was initiated at 6 ± 3.7, 15 ± 3.6, and 81 ± 6.2 minutes after hemorrhage for acute, delayed, and prolonged models, respectively. All SANGUINATE™ resuscitated animals across groups showed significant improvement in $P_{ISF}O_2$, while all Hextend™ resuscitated animals remained at non-detectable levels. SANGUINATE™ also extended survival times vs. Hextend™ for 'delayed' (N = 2) and showed significant improvement vs. Hextend™ in 'acute' (136 ± 49 vs 83 ± 24 minutes; $p < 0.001$), and prolonged (216 ± 67 vs 76 ± 25 minutes; $p < 0.01$) groups.

SANGUINATE™ increases circulatory oxygen delivery sufficiently to show an increase in peripheral tissue oxygenation across a range of pathological severities of hemorrhagic shock. This enhanced oxygen delivery correlates to increased survival times, warranting further study of SANGUINATE™ as a trauma resuscitant.

Bruce D. Spiess BSpiess@Anest.UFL.EDU

Perfluorocarbon Emulsions as Respiratory Gas Diffusion Enhancers- A Path towards Medical Breakthroughs.

Bruce D. Spiess, MD, FAHA
Professor and Associate Chair (Research) Department of Anesthesiology University of Florida College of Medicine, 1600 Archer Road Box 100254, Gainesville, FL 32610-0254

Perfluorocarbon (PFCs) compounds have been a hereto fore under realized pharmaceutical class of intravenous emulsions and respiratory adjuvants researched extensively since the late 1970's. This lecture will focus upon how PFCs differ from HBOCs and how they have been successful in providing O_2 delivery to ischemic tissues. PFCs as non-polar oils enjoy a high level of respiratory gas solubility. Solubility is much different than molecular binding of O_2. As such, PFCs represent a distinctly different class of pharmaceutical artificial oxygen (and other gas) transporters than are hemoglobin based oxygen carriers (HBOCs). These two classes of agents have contrasting mechanisms for respiratory gases transport, therefore each have different advantages and side effects. Both PFCs and HBOCs have suffered from a misguided historical research effort to outperform human banked blood. The PFCs should be viewed as pharmaceuticals possessing unique gas solubility and diffusion characteristics such that they may relieve ischemia of tissues with low/flow- no flow states therefore they can enhance tissue salvage while other definitive treatments are being sought. This lecture will focus on how diffusion of gas is what is tremendously important with PFCs and how that one factor should be exploited for medical usages. The enhanced solubility has led to not only O_2 delivery but also to N_2 removal form blood. PFCs have been approved by the United States FDA in the early 1990's for treatment/prevention of coronary ischemia. They have shown great utility in animal models for tissue ischemia, traumatic brain injury (a microcirculation problem) and now for enhancement of radiation therapy in solid tumors. These applications are notably not in hemorrhagic shock or in situations wherein the red cell mass is decreased. It is the enhanced diffusion of respiratory gases when microcirculation dysfunction occurs that perhaps is the best application of PFCs. Work form our laboratories in the past has shown that indeed PFCs if instilled prior to vascular occlusion may continue to supply O2 to areas of no capillary flow. That is due to the fact that O2 is highly diffusible and if a column of PFC exists in a vascular

bed then forward convective flow may not be required. Think about he implications for medicine- an intravenous agent that can enhance O2 delivery with little or no microvascular blood flow. Pharmaceutical development has stumbled in the past because of a hemoglobin centric thinking wherein PFCs and HBOCs were pursued as a "blood substitute". Perhaps, PFCs as short term enhanced tissue oxygen (and other gas enhancements) delivery vehicles should have varied and potentially game changing medical potentials.

Christopher P. Stowell, MD, PhD (USA) CSTOWELL@mgh.harvard.edu
Director, Blood Transfusion Service, Department of Pathology, Massachusetts General Hospital
Associate Professor of Pathology, Associate Director, Harvard Transfusion Medicine Fellowship Program
Harvard Medical School, U.S.A
The Clinical Impact of Red Blood Cell Storage: What Have the RCTs Told Us?

Michael Brad Strader Michael.Strader@fda.hhs.gov

Characterization of oxidative toxicity in mutant Hemoglobins and Hemoglobin Based Oxygen Carrier (HBOCs) candidates using high resolution accurate mass (HRAM) mass spectrometry
Michael Brad Strader, PhD[1],\ Tigist Kassa, PhD[1], Sirsendu Jana, PhD[1], Fantao Meng, PhD[1], Wayne Hicks, PhD[1], John S. Olson[2], and Abdu I. Alayash, PhD[1]
[1]DBCD/OBRR/CBER/FDA, [2]BioScience Department, Rice University, Houston, TX

Worldwide demand has driven the development of hemoglobin (Hb)-based oxygen carriers (HBOCs) as potential acellular oxygen therapeutics. HBOCs have the potential to provide an oxygen bridge to patients and minimize current problems associated with supply and storage of donated blood. However, to date, safety and efficacy issues have hampered the approval of viable HBOCs in the United States. These previous efforts have underscored the need for a better molecular understanding of toxicity in order to design safe and oxidatively stable HBOCs. Our lab has employed high resolution accurate mass (HRAM) mass spectrometry (MS) to characterize oxidative toxicity in studies focused toward designing viable HBOCs. When integrated with other analytical techniques, HRAM data has been invaluable in providing mechanistic insight into the extent of oxidative modification by quantifying oxidation in amino acids near the reactive heme or at specific "oxidative hotspots". In addition to providing a deeper understanding of Hb oxidative toxicity, HRAM MS studies have also been employed toward developing suitable HBOCs using a "two-prong" strategy that involves: 1) understanding the mechanism of Hb toxicity by evaluating mutant Hbs identified in patients with hemoglobinopathies and 2) utilizing this information toward designing against (or for) these reactions in acellular oxygen therapeutics that will result in oxidatively stable protein. Here we present an overview of the two-prong strategy; information from mutant Hb studies successfully led to engineering one protein that is oxidatively more stable and is currently being evaluated as a candidate acellular oxygen therapeutic.

Sun, Jian *Sunj@tju.edu.ca*

Preparation of recombinant hemoglobin as oxygen carrier by gene engineering
Jian Sun[1], Bing Cao[2], Qinggui Meng[2]
[1]*Department of Molecular and Cellular Pharmacology, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, P R China;* [2]*Shandong Wan'an pharmaceutical co., LTD., Dongying, Shandong 25700, China*

Blood transfusion is a life-saving treatment, which quickly provides the tissues with oxygen. However, blood transfusion have disadvantages, including blood type match, infectious agent contamination and blood storage and resource limitation. Blood substitution is an alternative solution. Transfusion with purified human or animal hemoglobin (Hb) as oxygen carrier (HBOC) have been trialed for many years, but not succeeded so far, due to severe side effects: renal toxicity, hypertension, etc. Recombinant hemoglobin as oxygen carrier by gene engineering is a promising strategy, because they have several advantages: no resource limitation; no pathogen; reduction of side effects by point mutations. Recently the extracellular hemoglobin consisting of eight different globin chains from marine lugworm has been found to be better HBOCs. One of the eight globin chains B2 globin chain has reported to be produce as a recombinant hemoglobin successfully in *E. Coli.* by Zal's group. In this study, we have tried to express different globin chains in *E. Coli.* to analyzed their capacity of oxygen carrier further.

Mladen I. Glavinović MLADEN.glavinovic@mcgill.ca

FORCES ACTING ON OBJECTS IN NANOPORES WITH IRREGULARITIES

Mohammad Tajparast[1] and Mladen I. Glavinović[2]
Departments Civil Engineering and Applied Mechanics[1] and Physiology[2], McGill University, Montreal, PQ, Canada

Nanopore applications had an explosive growth over last several decades owing to the development of advanced fabrication techniques. The ability to control nanopore transport properties, but also its dimensions allows an estimation of the size of different types of objects passing through (proteins, DNA, viruses and nanoparticles) by measuring the change in ion current. However, in nanopores with irregularities the nano-size (and meso-size) objects can also be distinguished by their shape from the changes of the currents recorded, but it is still not clear what forces determine the movement (translational and rotational) of objects through such nanopores and requires simulations. The transport of K^+, glutamate$^-$, Na^+ and Cl− was simulated within a nanopore using the Poisson–Nernst–Planck equation, and was coupled to the transport of water using the Navier–Stokes equations.

We determined three forces acting on the object in the nanopore: a) Coulomb force (due to fixed charges on the surface of the object when present in an electric field), b) dielectric force (caused by the permittivity mismatch between the material of the object and the solution, when placed in a non-uniform electric field and determined from the Maxwell stress tensor), and c) hydrodynamic pressure. Charged object was positioned opposite a positively or negatively charged protrusion, and in between these protrusions. Both the object and protrusions altered significantly the radial and axial water velocity. The hydrodynamic pressure on the object wall, which is significant even in the absence of external pressure, diminishes with radial distance, but the overall hydrodynamic pressure force on the object is very low. The Coulomb forces on individual surfaces are as expected much greater than corresponding dielectric forces. However, the overall contribution of dielectric forces, which is more significant than expected, contributes significantly to the object rotation.

Torres Filho, Ivo ivo.p.torresfilho.civ@mail.mil

In vivo Enhancement of Flow and Oxygen Transport in Microvessels: The Nanomedicine Approach During Ischemia

Ivo P. Torres Filho, M.D., Ph.D.
Research Physiologist, US Army Institute of Surgical Research, JBSA Fort Sam Houston, TX

The in vivo study of microvascular oxygen transport requires accurate and technically difficult measurements of several mass transfer parameters. Although recommended, blood flow and oxygenation are typically not measured in many studies where treatments for ischemia are tested. Since all physiologically relevant events in oxygen transport take place at microvascular level, we have implemented and used the phosphorescence quenching technique coupled with non-invasive intravital videomicroscopy for quantitative evaluation of these events in vivo. Rodent experimental models and various approaches have been employed to induce ischemia including hemorrhage, micro and macro-embolism, as well as microvessel occlusion. Measurements indicated that microvascular blood flow as well as intravascular and tissue oxygen partial pressure (PO2) decreased following these procedures. The nanomedicine approach to minimize or reverse the effects of ischemia was tested by adding oxygen carriers such as different perfluorocarbons and by measuring well-defined end-points such as blood flow and tissue PO2 that will have significant effect on tissue survival and outcome. In several cases, enhancement of flow and oxygen transport could be demonstrated. Similar results could be found in vitro, when perfluorocarbons were mixed with blood (from healthy donors and sickle cell disease patients), and an enhanced oxygen transport could be demonstrated by the perfluorocarbon emulsion.

Supported by US Army Medical Research & Materiel Command.

Amy G. Tsai agtsai@ucsd.edu

ROLE OF CYTOKINES IN PROMOTING OXYGEN DELIVERY AFTER BLOOD TRANSFUSION.

Amy G. Tsai [1], Pedro Cabrales [1], Joel M. Friedman [2] and Marcos Intaglietta [1]
[1]Dept. of Bioengineering, University of California, San Diego, La Jolla, CA and [2]Dept. of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY.

The cardiovascular effects due to treating anemia by transfusing packed RBC were investigated in the window chamber hamster model rendered anemic (60% Hct deficit) post 24 hr exchange transfusion of human serum albumin. It was found that transfusion of either 0.5, 1.0 or 2.0 units of pRBCs increased blood flow by an average of 50% over that in the anemic state, independently of the number of units transfused, restoring microcirculatory DO_2 to near normal values. These effects coincided with the post transfusion (2 hr) significant elevation of the serum inflammatory cytokine levels of IL-1b, IL-6, IL-10 and TNF-α, and were eliminated by pre-treating transfusion with the anti-inflammatory dexamethasone. These results resolve in

part the paradox posed by finding that solely on physical principles blood transfusion does not increase DO_2. These results also show an alternative to using RBCs, hemoglobin solutions and fluorocarbon suspension for increasing DO_2, based on solely inducing vasodilatation. This was investigated in the hamster anemia model using nitric oxide (NO) generating high viscosity plasma expanders and NO carrying/delivering biodegradable nano particles. This approach produced similar and better results than using pRBCs transfusions for treating anemia in the hamster model.

Supported in part by NIH 5P01 HL110900 and USAMRAA award W81XWH1120012.

Yang CM yun5323@163.com

Recent Development of Transfusion Medicine in China

Chengmin Yang, Jiaxin Liu, Hong Wang
Institute of Blood Transfusion, CAMS,Chengdu, China

The research of transfusion medicine and the clinical blood transfusion have made great progress in China in the past 20 years.

1. Blood Source

In 1997, the Chinese government promulgated the “Blood Donation Law of the People’s Republic of China”, which was implemented on October 1, 1998. Since then, China has implemented voluntary blood donation in the general public, and incorporated the blood management into the legal system.

In recent years, the blood capacity taken in China has been increasing year by year, with an average annual growth of about 10%. The total blood capacity taken in 2016 is more than 4,000 tons, but still cannot meet the needs of clinical transfusion therapy, the gap of which amounts to almost ¼ of the total need. To 2016, the Chinese population blood donation rate is 10/1000 person. In addition, the Red Cross Society of China has set up the "Chinese Bone Marrow Bank", which includes the information of over 2,000,000 voluntary donors, all of whom are detected by DNA, and out of which new allele of HLA is found in 1429 cases.

2. Blood Safety in China

(1) Transfusion infectious risk:

the nucleic acid tests have been conducted in China, which shorten the window period of HIV from 22 days to 12 days, and decrease HCV from 70 days to 14 days. But there are still cases of transfusion inflicted infections in china.

(2) Non-infectious risk:

the common adverse transfusion reactions such as fever, allergies, and etc., have been significantly decreased from 3% in 2005 to below 1% in 2015. Severe adverse transfusion reactions, such as acute lung injury, hemolytic reaction, and graft versus host disease, have also been overtly reduced. The main reason for the occurrence of adverse transfusion reactions, still, is due to the incompatibility of donors’ blood types.

(3) Bacterial contamination, which takes place primarily in platelet transfusion, occur currently at a rate of about 1/4000.

(4) In Shenzhen, Dongguan, and other places, the method of “Electronic Cross Blood Matching” is now on trial, which improves the accuracy of blood matching, and generate results in just a few seconds.

3. Clinical Blood Transfusion

(1) The clinical transfusion in China is now on the course of transition from the "Opening Strategy" to the "Restrictive Strategy". And the "Personalized Science-Oriented Transfusion" is currently being vigorously carried out.

(2) The blood transfusion indication has been decreased from below 100 g/L to 70 g/L with respect to the level of Hb. Also, studies have been promoted gradually focusing on "pre - transfusion evaluation and post transfusion efficacy evaluation", laying the foundation of the implementation of the accurate transfusion.

(3) Around 90% of the patients in need of clinical blood transfusion are given the blood components transfusion. And only the patients with acute massive hemorrhage are salvaged with whole blood transfusion.

(4) The exploration of the combination of traditional Chinese medicines and western medicine in the clinical blood transfusion is now on its track. A new system is to be setup, which comprises of the combination of western and traditional Chinese medicine, through the integral therapy of tradition Chinese medicine in the treatment of diseases of red blood cell, platelet, and white (granular) cell, and that of its western counterpart in emergency cases.

(5) Post – Trauma Blood Transfusion:

Relevant Chinese authorities attache great importance to problems of transfusion in accidents inflicted traumas, with research institutions dedicated to tackle with these problems. There has been urgent need for the development and application of blood substitutes, due to the blemishes of natural blood in first – aids, which limits its efficiency in rescuing lives facing the scarcity of time.

4. Basic Research and Professional Transfusion Education

(1) The research departments of blood transfusion in China has attached great importance to the combination of popularization and refinement, not only of the research of principles of blood transfusion, blood preservation, transfusion risk prevention, and other basic scientific research, but also, of overcoming

bottlenecks in blood safety and clinical practice of blood transfusion, for instance, the recruitment and management of blood donors, gene detection and matching technology, blood collection, blood transfusion , and the prevention of adverse reactions.

(2) Transfusion medicine education:

6 medical institutions in China currently provide undergraduate courses in transfusion medicine. In 2013, Beijing Union Medical College established its Department of Transfusion Medicine. Another 10 universities and research institutes qualify for doctoral, master's degrees of transfusion medicine. Also on the go are the vocational training of science and technology personnel in China, with different courses available each year.

(3) The establishment of the subject of transfusion medicine and the publication of relevant monographs and periodicals:

Approved by the state in 2016, the subject of transfusion medicine has been qualified as the second - degree subject in medical schools, which includes third – degree subjects such as Basic Transfusion Medicine, Clinical Blood Transfusion, Blood Donor Recruitment, Transfusion Technology, and others. Also established are the Chinese Blood Transfusion Association, the Clinical Blood Transfusion Branch of the Chinese Medical Association, the Blood Transfusion Medicine Branch of the Chinese Medical Doctor Association and etc.

(4) Over the past 10 years, China has published more than 50 Monographs in transfusion medicine which exceeding 500, 000 words. The “Chinese Transfusion Science”, edited by Chengmin Yang, Jin Liu and Tongmao Zhao, is to be published by the end of 2017. Currently, 3 types of professional journals of transfusion medicine are available in China, for instance, the "Chinese Journal of blood transfusion".

5. Blood Transfusion Organizations

Presently in China, there are one national research institute of blood transfusion and five provincial institutes. Blood centers have been set up in each province and municipality, together with 452 central blood stations in urban areas, and more than 1,000 blood transfusion departments in Grade A Class-Three Hospitals.

6. Academic Communication

(1) In China, national conference of blood transfusion, organized by the China Association of Blood Transfusion and the Clinical Blood Transfusion Branch of the Chinese Medical Association is held every 1-2 years, with participants exceeding 1,000. In addition, academic forums of different specialties in transfusion medicine are held each year in different provinces.

(2) As a member of the International Society of Blood Transfusion, China actively participates in various academic meetings. The national and local blood transfusion organizations in China have been involved in the cooperation of research, education, and academic exchanges with more than 20 international blood transfusion organizations.

Yang, Bo[1], Li Wang[1], Chao Chen[1,2], Hongli Zhu[1,2] (China) zhuyjw@hotmail.com
1.College of Life Science, Northwest University, Xi’an 710069, P. R. China
2.National Engineering Research Center for Miniaturized Detection Systems, Northwest University, Xi’an 710069, P. R. China

pPolyHb protects myocardial H9C2 cell against ischemia-reperfusion injury by regulation of Pink1-Parkin mitochondrial autophagy pathway

Yu BL BYU1@mgh.harvard.edu
(Alumni of Artificial Cells & Organs Research Centre)

Therapeutic Inhalation of Nitric Oxide in HBOC Transfusion

Binglan Yu[1], PhD, Warren M. Zapol[1], MD, Donald B. Bloch[1,2], MD
[1]Anesthesia Center for Critical Care Research, Department of Anesthesia, Critical Care and Pain Medicine, [2]Division of Rheumatology, Allergy and Clinical Immunology, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA 02114

Inhaled nitric oxide (NO) is a selective pulmonary vasodilator that has been used to treat pulmonary hypertension and to increase systemic oxygenation in babies and adults since 1990. Plasma cell-free oxyhemoglobin scavenges endothelium-derived NO, which is responsible for vasoconstriction produced by transfusing hemoglobin-based oxygen carriers (HBOCs). In awake mice, we demonstrated that infusion of HBOC-201 (Hemopure, OPK Biotech) induces systemic hypertension, which can be prevented by breathing 80 parts per million (ppm) NO for 15 or 60 minutes or with 200 ppm NO for 7 minutes. In awake lambs, pretreatment with inhaled 80 ppm NO for 1 hr prevents systemic and pulmonary hypertension induced by subsequent infusion of HBOC-201, without causing intracellular methemoglobinemia. Our data have also shown that mice with endothelial dysfunction (reduced NO bioavailability, as may be caused by diabetes or 4-6 weeks of a high-fat diet), but not healthy wild-type mice, experience vasoconstriction during transfusion of PolyHeme (Northfiled Laboratories, Inc.), and this can be prevented by inhaled NO. In awake lambs,

infusion of PolyHeme induces pulmonary hypertension, that can be prevented by breathing 80 ppm NO for 1 hr before infusion, followed by a low dose of 5 ppm NO during transfusion. In conclusion, the administration of inhaled NO is a promising approach to the safe transfusion of HBOCs without producing vasoconstriction.

Zal F franck.zal@hemarina.com ; nelly.rolland@hemarina.com

Use of HEMO2life - an Innovative Oxygen Carrier in Organ Transplantation.

Zal, F
President, HEMARINA S.A. | Aéropole centre | Biotechnopôle |

Organ transplantation is the elective treatment in case of end-stage organ failure, but despite the advancement in surgical procedures its success is still limited by the ischemia-reperfusion injury (IRI).
Early function of transplanted organ is a predictor of poor short- and long-term outcomes after g transplantation. In kidney transplant delayed graft function (DGF) is the most important predictor for graft survival. In lung transplant, Primary graft dysfunction (PGD) is a common complication of lung transplantation characterized by acute pulmonary edema associated with bilateral pulmonary infiltrates and hypoxemia. Development of both DGF and PDG are linked to the degree of IRI.
HEMO2life® is a novel natural oxygen carrier extracted from *Arenicola Marina* and prepared as a commercially available solution to be used as additive to organ preservation solutions. Thanks to its high oxygen carrying capabilities coupled with unique anti-oxidant properties, HEMO2life® can act on both cold ischemic and reperfusion phases, limiting the impact of IRI with benefits in term of survival, metabolic activity and cellular integrity and as consequence improving organ functioning once transplanted.
We report two studies in IRI animal models (pigs) for kidney and lung transplantation with HEMO2life® used as an additive to standard organ preservation solutions. In both studies, following the addition of HEMO2life® functional parameters and IRI biological markers improved when compared to control groups.
In kidney transplantation study, serum creatinine during first two weeks post-transplant improved showing an early function recovery. Long term follow-up (3 months) confirmed the trend observed in the first two weeks.
In lung transplantation study, 5 hours after transplant key hemodynamic and functional parameters improved in HEMO2life® treated group: reduction of graft vascular resistance ($p<0.05$) and increase in graft oxygenation ratio ($p<0.05$). Several ischemia-reperfusion related biomarkers showed positive trends in the HEMO2life® group: expression of HMG B1 (allarmine) in serum tended to be lower (2.1+/- 0.8 vs 4.6+/-1.5) compared with Perfadex® group demonstrating protective effects against sudden reperfusion injuries.
Taken in conjunction, these two studies demonstrate for the first time the therapeutic potential of HEMO2life® in dealing with the effects of IRI. Clinical trials are ongoing with promising results so far

The effect of Polymerized Porcine Hemoglobin (pPolyHb) on hemodynamic stability and oxygen delivery in a rat model of perioperative blood transfusion

Zhao, Mengye[1], Chengbin Yan[1], Ying Xiao[1], Chao Chen[1,2], Hongli Zhu[1,2] zhuyjw@hotmail.com
1.College of Life Science, Northwest University, Xi'an 710069, P. R. China
2.National Engineering Research Center for Miniaturized Detection Systems, Northwest University, Xi'an 710069, P. R. China

Ka Zhang ka.zhang@tbiokem.lth.se

Purification of recombinant human hemoglobin from crude cellular extracts using molecularly imprinted polymers

Ka Zhang [(1)], Tongchang Zhou [(1)], Lei Ye [(1)], Leif Bülow [(1)],*
[(1)] Division of Pure and Applied Biochemistry, Lund University, Box 124, 221 00, Lund, Sweden

Large amounts of recombinant Hb can be produced from especially transgenic *Escherichia coli* host strains. One of the key challenges is to devise an efficient and cost-effective protein purification strategy to ensure highly purified Hb preparations for the desired application. Molecularly imprinted polymers (MIPs), which can be described as artificial plastic receptors with the ability to specifically recognize template molecules, offer a valuable alternative source for binding and capture of Hb [1-2]. The aim of this study has been to examine the potentials of MIPs as a chromatographic resin to enable a specific isolation of Hb directly from crude and complex biological extracts. The results from this work showed that human HbA, HbF and a fusion HbF could all be isolated in one step by an Hb-MIP column. The binding was strictly pH dependent and elution of the adsorbed protein could be achieved by increasing pH from 6 to 8. This chromatographic modality also allowed identification of changes related to amino acid substitutions on the Hb surface. For instance, when a lysine residue was introduced, the HbA αY42K mutant eluted later than wildtype HbA. Aspartic acid residues mitigated the interaction between the protein and imprinted polymers, therefore αA12D/ αA19D HbF mutants eluted ahead of wildtype HbF. Hb-MIP particles could also be used to identify that *E.coli* glyceraldehyde-3-

phosphate dehydrogenase (GAPDH) could interact specifically with Hb, especially HbF. This host cell protein could thus be co-eluted from the Hb-MIP column under controlled conditions [3].

[1] Shen, X, Zhou T, Ye L (2012) Molecular imprinting of protein in Pickering emulsion. *Chemical Communications* 48: 8198-8200.
[2] Zhou T, Zhang K, Kamra T, Bulow L, Lei Y (2015) Preparation of protein imprinted polymer beads by Pickering emulsion polymerization. *Journal of Materials Chemistry B* 7:1254-1260.
[3] Zhang K, Zhou T, Ye L, Bulow L, Characterization of protein-protein interactions in recombinant hemoglobin producing Escherichia coli cells using molecularly imprinted polymers. Advances in Experimental Medicine and Biology (In press, 2017).

3. OTHER AREAS INCLUDING NANOMEDICINE,

Barre, P (Canada) Paul.Barre@Muhc.Mcgill.Ca
(Associate member, Artificial Cells & Organs Research Centre)
Hemoperfusion at McGill University Health Centre
Associate Professor-McGill University, Medical Director-Chronic Kidney Disease Clinic, Montreal General Hospital- Division of Nephrology, L4.521 ,
Hemoperfusion was perfected at the MUHC in the 1970's by Dr. T.M.S. Chang and his associates. Clinical experience was carried out in several Montreal hospitals for end-stage kidney disease, complications of uremia, liver failure, intoxications, aluminum overload.
Hemoperfusion was carried out with several forms of activated charcoal. The nature of the activated charcoal provided very high clearances of many uremic toxins over a short period of time with excellent patient outcomes.
This presentation will provide background on these clinical successes over a 15-year period.

Best, Robert, rbest@ghs.org ; rbest@mailbox.sc.edu ;
Technological versus Traditional Approaches to Medicine in an Age of Rapid Change and Declining Resources
Robert G Best, PhD, University of South Carolina School of Medicine Greenville
Nanomedicine provides technological tools for disease diagnosis and intervention that have already proven to be powerful, with many of the most powerful applications yet to emerge. As these technologies progress through developmental stages toward practical clinical applications, traditional approaches toward treatment and diagnosis continue to prevail in the practice of medicine worldwide. At the same time, resources for health care, medicine, and biomedical research have become increasingly strained, with rapid changes evident in the mechanisms of reimbursement and resource allocation. Such economic pressure requires increasingly thoughtful consideration of the way that technologies can be made to bear on the diseases and conditions that we anticipate in the years ahead. We will consider an approach that takes stock of the complex array of factors related to resource allocation and the consideration of ethical perspectives at the earliest point in research and development to maximize the benefits from nanomedical technologies and innovations as an element of proper stewardship of limited resources in health care.

Budak, G drgurerbudak@yahoo.com

Prextrolin® **"The Next Generation Nuclear Stain Biomarker for Cellular Analysis"**
Dr. Gürer G. Budak (MD, PhD, EMBA)
Associate Professor, Director, Nanobiomed Inc- NanoMedicine & Advanced Technologies Research Center, Member, European Technology Platform on Nanomedicine, President, International Society for Nanomedical Science, web: www.nanobiomed.com.tr Ankara - TURKEY

In modern medicine, cellular analysis technologies and histo-pathological examinations are accepted as the "***gold standard***" for final diagnosis and decision appropriate treatment for diseases, especially for cancer. *Hematoxylin* is the only cell stain biomarker widely used for all diagnostic techniques and no alternatives have been developed for over 100 years. Despite an intense demand on *Hematoxylin* across the world; its production does not meet the needs steadily because of the source limitation. The raw material of *Hematoxylin* is *Logwood Trees* that grown in tropical climatic zone and a member of rain forests.
Based on an awareness of such needs and reason, we launched a research to develop a sustainable and cost-effective diagnostic colorimetric biomarker for alternative to *Hematoxylin*. As a result of basic R&D for

about 10 years, we discovered a new, eco-friendly, and highly efficient stain biomarker which named; ***Prextrolin***®.

Prextrolin® is a disruptive innovation, which has a capacity to ongoing paradigm shift in scientific, medical, social, environmental and economic dimensions.

It is highly active and selective conjugated micro molecule binds phosphates of DNA irreversibly and structural proteins of the cell reversibly. This selective binding capacity highlights the tissue and cell details, provides mapping and probing for cytological parameters for diagnosis. ***Prextrolin***® is not only a new product itself, but chemical synthesis technology has also very important innovative steps. New chemically synthesis techniques & re-design process was applied to end-product production. The chemical structure of the ***Prextrolin***® is registered by ***American Chemical Society*** as a new molecule.

In addition to conventional histopathology and cytology applications, ***Prextrolin***® is also possible to use in advanced and/or automated diagnostic protocols (nucleic acid labeling, flow cytometry, spectrophotometry, polymerase chain reaction (PCR), cell-tissue microarray technology, cell signaling pathway/signal transduction, immunohistochemistry, in situ hybridization, colorimetric biochemical tests etc). These techniques make it possible to visualize the distribution and localization of specific cellular components within cells and in proper tissue context.

Prextrolin® will also help all clinicians for screening tests and routine diagnostics in such as; *fine needle biopsies for cancers diagnosis, *intra-operative evaluation with Frozen Section sampling, *post-operative evaluation for final diagnostic conclusions, *differentiation between cancer and noncancerous tissue, *identifying prognostic grading-staging, *assessment of cancerous subtypes and *defining the origin of the metastatic cancer cells.

Cattaneo M mvcattaneo@gmail.com
(Alumni, Artificial Cells & Organs Research Centre)

Continuous Manufacturing of Monoclonal Antibodies

Maurizio Cattaneo, PhD,
President and CEO, bioVolutions Laboratories Inc. U.S.A.

Continuous manufacturing (CM) offers significant advantages over batch processes in terms of shorter processing time, high productivities, higher quality, reduced equipment footprint and reduced operating and capital cost. Despite these advantages, the industry is usually hesitant to adopt this approach due to low risk tolerance and the need to design new facilities that incorporate improvements in technology. However, cost pressures in the biopharmaceutical industry, the expanding interest in monoclonal antibody therapy and a favourable regulatory environment are the main drivers of this innovative technology. A flexible and cost effective Continuous Manufacturing platform for the production of therapeutic mAbs is presented. By following a QbD approach, the identification of critical process parameters (CPPs) and critical quality attributes (CQAs) helps to define a design space to meet both yield as well as product quality criteria. Key improvements of our process over current knowledge will be outlined using a recent DOE study for manufacturing of therapeutic mAbs using a Continuous Manufacturing platform.

Chandra. R acbrdu@hotmail.com

Metabolism of Anticancer Agents Noscapine and Analogs

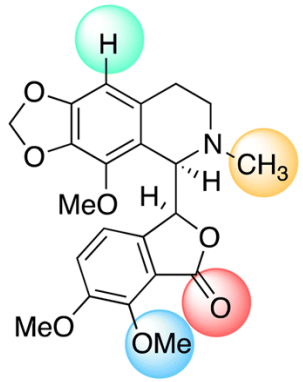
Vartika Tomar, Satya Prakash and **Ramesh Chandra**
Laboratory of Drug Discovery and Metabolism
Department of Chemistry, University of Delhi, Delhi-110007, India
Department of Biomedical Engineering, McGill University, Montreal, Canada

Many nitrogen-moiety containing alkaloids derived from plant origins are bioactive and play a significant role in human health and emerging medicine. Noscapine is an isoqiunoline alkaloid found in opium latex. Unlike most other alkaloids obtained from opium latex, noscapine is not sedative and has been used as antitussive drug in various countries. Recently, it has been introduced as an anti-mitotic agent. This drug can be used orally .When the resistance to other anti-cancer drugs such as paclitaxel menifests, noscapine might be effective. Therefore, noscapine and its analogs have great potential as novel anticancer agents. From the urine of rats, rabbits and humans treated with noscapine, two novel metabolites were isolated and identified as 7-hydroxy-6-methoxyphthalide (MA-1) and 6-hydroxy-7-methoxyphthalide (MA-2), mainly by mass spectrometry.

Our laboratory has reported that two brominated derivatives of noscapine, 5-bromonoscapine (5-Br-nosc) and reduced 5-bromonoscapine (Rd 5-Br-nosc), have higher tubulin binding activity than noscapine and affect tubulin polymerization differently from noscapine. In addition, they are able to arrest cell cycle progression at mitosis at concentrations much lower than noscapine. Interestingly, whereas noscapine-arrested cells have nearly normal bipolar spindles, cells arrested by 5-Br-nosc and Rd 5-Br-nosc form

multipolar spindles. Nevertheless, noscapine and the two derivatives all affect the attachment of chromosomes to spindle microtubules and they impair the tension across paired kinetochores to similar degrees. 5-Br-nosc and Rd 5-Br-nosc are also more active than noscapine in inhibiting the proliferation of various human cancer cells, including those that are resistant to paclitaxel and epothilone. Our lab investigation thus indicate a great potential for the use of 5-Br-nosc and Rd 5-Br-nosc both as biological tools for studying microtubule-mediated processes and as chemotherapeutic agents for the treatment of human cancers.

We have synthesized various Noscapine derivatives to describe recent breakthroughs that have led to an elucidation of the noscapine biosynthetic pathway, and to discuss the pharmacological properties that have prompted intensive evaluation of the potential pharmaceutical applications of noscapine and several semi-synthetic derivatives. We have speculated the future potential for the production of Noscapine using metabolic engineering and synthetic biology in plants and microbes.



Noscapine, a phthalideisoquinoline alkaloid derived from Papaver somniferum, has been used as a cough suppressant since the mid 1950s, illustrating a good safety profile. Noscapine has since been discovered to arrest cells at mitosis, albeit with moderately weak activity. Immunofluorescence staining of microtubules after 24 h of noscapine [illegible] of chromosomes to complete congression to the equatorial plane for proper mitotic separation. A number of noscapine analogues possessing various modifications have been described within the literature and have shown significantly improved antiprolific profiles for a large variety of cancer cell lines. Several semisynthetic antimitotic alkaloids are emerging as possible candidates as novel anticancer therapies.

Chandra R acbrdu@hotmail.com

From the laboratory to the bedside

Chandra R
Laboratory of Drug Discovery and Metabolism
Department of Chemistry, University of Delhi, Delhi-110007, India

Chen GQ chengq@mail.tsinghua.edu.cn

Drug Targeting Systems Based on PHA Granule Binding Protein PhaP

George Guo-Qiang CHEN
School of Life Sciences, Center for Synthetic and Systems Biology (CSSB)
Tsinghua University, Beijing 100084 China
Tel: +86-10-62783844, Fax: +86-10-62794217, e-mail: chengq@mail.tsinghua.edu.cn

Polyhydroxyalkanoates (PHA) is a family of intracellular biopolyesters produced by many bacteria. PHA granule binding protein PhaP is able to bind to hydrophobic polymers. A receptor mediated drug specific delivery system was developed in this study based on PhaP. The system consists of PHA nanoparticles, PhaP and ligands fused to PhaP. The PHA nanoparticles were used to package mostly hydrophobic drugs, PhaP fused with ligands produced by over-expression of their corresponding genes in *Pichia pastoris,* or *E. coli* was able to attach to hydrophobic PHA nanoparticle. At the end, the ligands were able to pull the PhaP-PHA nanoprticles to the targeted cells with receptors recognized by the ligands. It was found in this study that the receptor mediated drug specific delivery system Ligand-PhaP-PHA nanoparticles was taken up by macrophages, hepatocellular carcinoma cell BEL7402 *in vitro* and hepatocellular carcinoma cells *in vivo,* respectively, when the ligands were mannosylated human a1-acid glycoprotein (hAGP) and human epidermal growth factor (hEGF), respectively, which were able to bind to receptors of macrophages or hepatocellular carcinoma cells. The system was clearly visible in the targeted cells and organs under fluorescence microscopy when rhodamine B isothiocyanate (RBITC) was used as a delivery model drug due to the specific targeting effect created by specific ligand and receptor binding. The delivery system of hEGF-PhaP-nanoparticles carrying RBITC was found to be endocytosed by the tumor cells in an xenograft tumorous model mouse. Thus, the Ligand-PhaP-PHA specific drug delivery system was proven effectively both *in vitro* and *in vivo*.

References

1. Zhao Hongyu, Wang Hui, Liu Xi, Yao Zhengyu, Xu Manyu, Wei Daixu, Wang Jiawei, Wang Xinquan and Chen GQ. Structural Insights on PHA Binding Protein PhaP from *Aeromonas hydrophila*. Scientific Report 6 (2016) 39424-39424

2. Chen GQ. A Polyhydroxyalkanoates Based Bio- and Materials Industry. Chem Soc Rev 38 (2009) 2434–2446
3. Yao YC, Zhan XY, Zou XH, Wang ZH, Xiong YC, Zhang J, Chen J and Chen GQ. A Specific Drug Targeting System Based on Polyhydroxyalkanoate Granule Binding Protein PhaP Fused with Targeted Cell Ligands. Biomaterials 29 (2008) 4823–4830
4. Wang ZH, Wu HN, Chen J, Zhang J and Chen GQ. A novel self-cleaving phasin tag for purification of recombinant proteins based on hydrophobic nanoparticles. Lab on a Chip **8 (2008) 1957-62**

Preparation of nano-$CaCO_3$/polystyrene nanocomposite beads for efficient bilirubin removal

Jian Chen[1], Guanghui Cheng[1], Yamin Chai[1] and Lailiang Ou[*1]*
[1]Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China.
Corresponding author's email: **ouyll@nankai.edu.cn**

As a pathogenic toxin, bilirubin is generally removed from blood by hemoperfusion based on adsorbents for the remission of liver disease or to bridge patients to transplantation. However, bilirubin adsorbents with satisfactory adsorption performance, excellent blood compatibility and high mechanical strength, are still in strong demand. In this research, a novel nano-CaCO3/polystyrene nanocomposite adsorbent (NPS-8) was synthesized for efficient bilirubin removal from blood plasma. Comparison with the polystyrene adsorbent (PS-8), which was without nano-CaCO3 incorporation, revealed that NPS-8 had superior bilirubin adsorption capacity and mechanical strength. The resulting nano-CaCO3 reinforced PS-8 (NPS-8) was tested by transmission electron microscopy (TEM), scanning electron microscopy (SEM), mechanical strength test, and bilirubin adsorption assays. Adsorption results indicated that NPS-8 displayed better adsorption capacity for bilirubin (91%) than that of PS-8 (75.88%). The mechanical strength of NPS-8 was significantly higher than that of PS-8. In addition, PS-8 and NPS-8 both had good blood compatibility properties (a negligible hemolytic activity and platelet adhesion). Therefore, NPS-8 has a high potential to be used as an efficient bilirubin adsorbent for blood purification in clinical practice. At the same time, the success of organic-inorganic nanocomposite adsorbents might provide a new insight for the improvement of adsorbents in hemoperfusion.
Keywords: nano-CaCO3/polystyrene composite beads, adsorbent, bilirubin, adsorption, blood purification.
This abstract is funded by Ministry of Science and Technology of the People's Republic of China, grant: 2017YFC1104400, and the Natural Science Foundation of Tianjin, grant: 14ZCZDSY00011, 14JCTPJC00 487 and 13JCQNJC14200.

Computer-Aided Design of Small-Molecular Peptide Ligands of Adsorbent Targeting Tumor-Necrosis Factor-α (TNF-α)

Jie Chen, Wenyan Han, Jian Chen,† Weichao Wang, Wenhui Zong, Guanghui Cheng,Yaoting Yu, Lailiang Ou[*] [*]Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China

Computer-aided molecular design is an effective tool in almost all areas of health science research. Traditional ligands targeting the proinflammatory cytokine TNF-α focus on antibodies and receptors, which always associate with risks and limitations. In this study, two active peptide ligands (T1 and T2) were designed based on the weak interactions between TNF-α and its receptor TNFR-1 by computer simulation technology. The Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) calculated binding free energies between the peptide ligands and TNF-α were −24.71 and −15.56 kcal/mol, respectively. The small molecular peptide ligands were fixed on PVA microspheres to assess the affinity between TNF-α and the ligands designed. Affinity adsorption tests showed that the PVA microspheres modulated with T1 and T2 ligands both exhibited a stronger affinity for TNF-α than PVA microspheres (79.20±1.32 pg/mg and 74.27±1.10 pg/mg vs. *39.03±1.25 pg/mg). Therefore, our results indicated that computer-aided molecular design could be a valuable method to create a novel immunoadsorbent to efficiently capture TNF-α in blood.*

Keywords : *Computer Aided Design; Peptide; Ligands; TNF-α*

Daka JN joseph.n.daka@hc-sc.gc.ca
(Alumni of Artificial Cells & Organs Research Centre)

A Simple Plate Reader Method for Determination of Taurine in Human Urine Samples as a Potential Radiation Biomarker in Extreme Radiological/Nuclear Exposure Situations.

Joseph N. Daka, PhD.
Government Research Scientist

Radiation Protection Bureau, Health Canada, 775 Brookfield Road, Ottawa, ON K1A 1C1, CANADA.

Presently, with the developed techniques of the Artificial Cells & Organs Research Centre (McGill University) various artificial cells containing one or more biologically active materials , including radionuclides or radioisotopes, for various applications in internal medicine, can be made. Diagnostics and radiotherapy in cancer treatment are one of the examples. While direct exposure of radiological/nuclear substances to interior tissues may cause harmful effects to humans, published results of Chang et al have confirmed that after these same toxic materials have been encapsulated or made into artificial cells, some can provide effective alternative tools for medical testing and treatment. In recent years, he Radiation Protection Bureau at Health Canada (Ottawa) has been conducting experiments with urine and blood samples to determine whether internal exposure to ionising radiation does produce, in humans, some low-molecular-weight biochemical compounds called radiation biomarkers. So far, most of the existing knowledge of radiation metabolomics has come from studies with animal models.. Generally, humans may experience extreme exposure to ionising radiation during radiological/nuclear accidents or irradiation cancer treatments. Since taurine was identified as a reliable biomarker of ionizing radiation, a simple method for its rapid measurement in urine or blood has been developed. The approach is based on using: 1) EZ:Faast adsorbents kits from Phenomenex; 2) a derivatization technique with dinitrofluorobenzene; and 3) a measurement on a 96-well UV/Vis microplate reader. The final results have shown for example, that during an event of a radiological/nuclear emergency, taurine measurement in biofluids can be used as a biomarker for rapid screening (or triage) of victims to identify those needing immediate medical attention.

Comparison of different number autologous sural nerve grafts repair common peroneal nerve defects

Jiuxu Deng#, Ming Li#, Jian Weng, Yuhui Kou, Peixun Zhang, Na Han, Bo Chen, Xiaofeng Yin*, Baoguo Jiang*
Department of Orthopedics and Trauma, Peking University People's Hospital, Beijing, China.
#Equal contributors and co-first authors.
*Correspondence to: Xiaofeng Yin, M.D., xiaofengyin@bjmu.edu.cn . Baoguo Jiang, M.D., jiangbaoguo@vip.sina.com .

Objective: To investigate and compare the effect of different number autologous cable-style nerve grafts repair peripheral nerve defects. Methods: Sprague-Dawley (SD) rats of common peroneal nerve defect were established and different number autologous sural nerve grafts were applied. Three months after operation, nerve functional and morphology recovery conditions were observed. Results: The results showed that regenerated nerve of cable-style nerve graft with one, two and three sural nerves graft was 1815±30, 1838±23 and 1842±27 respectively (P>0.05). The common peroneal nerve function index (PFI) was 39.00±2.37, 36.67±1.75 and 36.33±1.37 (P>0.05). The motor nerve conduction velocity (MNCV), recovery of myodynamia and wet weight ratios of tibialis anterior muscle of one single sural nerve graft group were also no statistical differences compared with two sural nerves and three sural nerves group. Conclusions: One single small sural nerve graft could satisfy repair huge common peroneal nerve defect. This indicates one single small nerve graft may obtain good therapeutic effect for huge peripheral nerve defect.
Keywords: peripheral nerve defect, autologous nerve graft, functional recovery, nerve regeneration.

Gu KF kgshanghai@yahoo.com
(Alumni of Artificial Cells & Organs Research Centre)

Novel Feeding Strategy Development for Enzyme/Protein Production

Kangfu Gu

A DO/pH cycling feeding strategy was designed and developed, which is applied in defined media fermentation. Also a RQ feeding strategy was designed and developed, which is used in complex media fermentation. These two qualitative control feeding strategies not only make fermentation process development and scale up easy, simple, robust and convenient but also achieve high cell density and high enzyme/protein expression.

Han, Lulu

Removal of indoxyl sulfate by water-soluble poly-cyclodextrins in dialysis

Lulu Han, Jingyu Li, and Lingyun Jia* lyj81@dlut.edu.cn
School of Life science and Biotechnology, Dalian University of Technology, Dalian 116023, P. R. China

Chronic kidney disease (CKD) is caused by the loss of renal function in various levels. This loss will lead to the accumulation of uremic toxins in the blood which could make a great damage to the body. Indoxyl sulfate (IS), a kind of uremic toxins, has been linked with the promotion of CKD progression. However, 90 % of IS in

the blood binds to human serum albumin (HSA), so the bound IS can't pass through the dialysis member. As a result, conventional hemodialysis (HD) cannot effectively clear IS. Cyclodextrin, a kind of natural cyclic oligosaccharides with a hydrophobic cavity and a hydrophilic outer surface, has been widely used as a hydrophobic compound adsorbent. In this work, we demonstrated a new strategy to clean the IS from blood by adding low concentration of poly-cyclodextrins to the dialysate. The results showed that maximum adsorption capacity of poly-cyclodextrins to IS was 79 mg/g. Therefore, this method can effectively remove the IS in blood, and will open a new avenue to clear the hydrophobic blood toxins during the dialysis therapy.
Key Words：Cyclodextrin; adsorbent agent; indoxyl sulfate; dialysis; removal

Hoesli C corinne.hoesli@mcgill.ca
Department of Chemical Engineering Université McGill University, Associate member of Artificial Cells & Organs Research Centre

Umar Haris Iqbal umar.iqbal@mail.mcgill.ca

Lactobacillus fermentum NCIMB 5221 as a Potential Therapy for the Metabolic Syndrome
Umar Haris Iqbal [1], Susan Westfall [1], Leila Farahdel [1], Nikita Lomis [1], and Satya Prakash [1]
(1) Biomedical Technology and Cell Therapy Research Laboratory, Dept. of Biomedical Engineering, Dept. of Experimental Medicine, Faculty of Medicine, McGill University, 3775 University Street, Montreal, Quebec, H3A2B4, Canada. *Tel: 1-514-398-3676, Fax: 1-514-398-7461. Email:satya.prakash@mcgill.ca
The metabolic syndrome currently affects approximately one in five Canadians with its world-wide prevalence increasing over time. This syndrome is characterized by increased levels of triglycerides and glucose levels, abdominal obesity, and reduced HDL cholesterol affecting the energy homeostasis of an individual. It has also been associated with insulin resistance and a proinflammatory state. A person diagnosed with this condition is twice as likely to develop cardiovascular disease and is five times as likely to develop type 2 diabetes mellitus. Current therapies starting with life style changes and advancing to medication and surgery have shown to be either not effective or associated with adverse effects. Current research has found a relationship between the gut microbiome and metabolic pathways and have suggested that bacteria in the gut can play a significant role in one's health. The current study uses a high-fat diet animal model to investigate the use of Lactobacillus fermentum NCIMB 5221 as a possible therapy for the metabolic syndrome. In this study, the animals were given a daily dosage of probiotic treatment over a period of six weeks. At the end of the probiotic treatment phase, animal weight was reduced by approximately 12% over a period of 6 weeks. Furthermore, serum total cholesterol, LDL cholesterol, and triglycerides values also had reduction. Interestingly, when probiotic treatment was stopped, reduction in weight began for about two weeks, after which it plateaued. With this preliminary study, it can be concluded that L. fermentum NCIMB 5221 has the potential to be a therapy for the metabolic syndrome however further studies need to be done to get a clearer picture as to how it works.

Lingyun Jia lyjia@dlut.edu.cn

Removal of Beta-2-microglobulin from Human serum using Single Domain Antibody as Ligand

Lingyun Jia*, Jun Ren, Xiaobo Bao
Liaoning Key Laboratory of Molecular Recognition and Imaging, School of Life science and Biotechnology, Dalian University of Technology, Dalian, China 116024

Removal of beta-2-microglobulin (B2M) from the blood circulation of the patients with kidney failure is an important therapeutic modality for the clinical treatment of dialysis related amyloidosis (*DRA*). Here we describe the development of a new type of highly selective adsorbent using single domain antibody as ligand. The antibody was obtained by screening immune libraries, and the process of oriented immobilization

of ligands through epoxy-sulfydryl coupling has been systemically studied by considering several key reaction conditions. The resultant adsorbent showed an equilibrium dissociation constant of 1.5×10^{7} M. Oriented immobilization of single domain antibody has proven effective in increasing the B2M binding capacity in human serum. Using serum samples (initial B2M concentration of 32 mg/L) from kidney dialysis patients, over 80% of B2M was removed after 15 min of adsorption when the gel/serum ratio was 1:20, without detectable non-specific adsorption of other proteins. This work provides a potential effective strategy for preparing highly specific adsorbents for blood purification by using single domain antibody.

Juncker D david.juncker@mcgill.ca

Cell microarrays tissue constructs, and artificial gastrointestinal tract in a box.
Juncker D

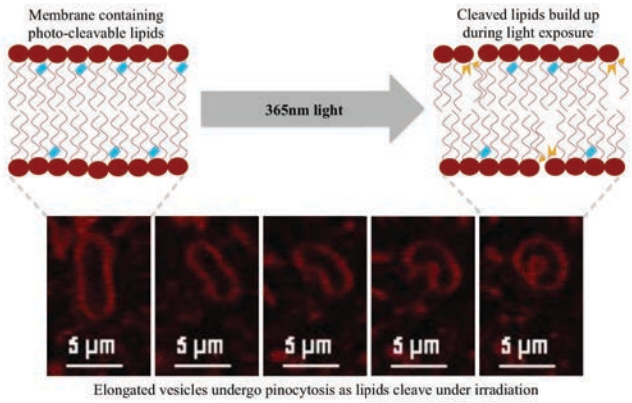
Professor of Biomedical Engineering, Micro and Nanobioengineering Laboratory McGill University
Authors: Grant Ongo, Sa Xiao, Susan Westfall, Andy Ng, Satya Prakash & **David Juncker**
The development of designer organs and tissues for in vivo and in vitro use greatly benefits from microscale technologies. Here, I will review efforts from our lab towards making 1D, 2D and 3D tissue constructs and bioreactors. The use of threads, and different methods of weaving, knitting and knotting for creating cell-laden constructs will be shown. Next, the creation of artificial tissues using direct writing will be discussed, and the use of integrated de-clogging mechanism for robust operation shown. The use of alginate as permanent or sacrificial templating material in combination with various hydrogels and cells will be introduced. 3D printed monolithic pin heads will be presented as a new low-cost platform for printing and patterning nanoliters of different viscous hydrogel solutions containing cells; it constitutes the first example of low-volume spotting of highly viscous hydrogels to the best of our knowledge. Cell microarrays of cancer cells with different metastatic predisposition surrounded by stromal cells will be shown. The application of these co-culture microarrays for studying cancer-stroma interactions and cellular communities arising at this interface will be discussed. To conclude this presentation, the development of a miniaturized Gastrointestinal tract in-a-box (GITBOX) will be presented. The GITBOX is an automated in vitro gut system mimicking the stomach, upper and lower intestines along with their physiological conditions, and enables culture of complex microbiota communities from a fecal inoculum in simulating the human gut microbiome. The features and characteristics of the GITBOX, as well as the response of the microbiota to challenges by antibiotics and probiotics will be presented.

Kinsella M joseph.kinsella@mcgill.ca

Engineering Nanomaterials to Diagnose and Track Cancer from the Cellular to the Tissue Level ser
Kinsella M
Bioengineering Department, McGill University (Associate member of Artificial Cells & Organs Research Centre)
Cancer diagnoses, and increasingly treatment, are progressively being aided by the development of new medical and molecular imaging technologies. Central to the utility of these procedures has been the concurrent development of contrast agents that enable clinicians to clearly delineate tumor peripheries, determine pathologies, and develop personalized intervention strategies. Nanotechnology has provided innovative materials that have been proven to provide elevated contrast in a number of different imaging modalities. Specifically, tumor tissue-specific nanoparticles have shown great potential as contrast agents for the direct *in vivo* detection of a number of cancers. Our research focuses on developing". The composite material exploits the dipolar coupling of superparamagnetic nanoparticles trapped within a secondary inorganic matrix, and has been shown to enhavationnce the transverse relaxivity contrast in a 3 T MRI more than 1.6 fold when compared to similar, unencapsulated Fe_3O_4 nanoparticles. An alternative contrast agent that enhances the visualization of breast cancer in X-ray microComputed Tomography was achieved by synthesizing 10 nm Bi_2S_3 nanoparticles modified to display a tumor targeting peptide (LyP-1, CGNKRTRGC). In these studies the accumulation of the nanoparticle contrast agent within the tumor was increased by 260% compared to nanoparticles that did not contain the homing peptide.

Konetski, D **Danielle.konetski@colorado.edu**

Production of Artificial Cell Membranes Bearing New Characteristics or Behaviors Using "Click" Chemistries
Konetski, D., Zhang, D., Gong, T., Baranek, A., Worrell, B., Bowman, C.
University of Colorado, Boulder
Towards the development of an artificial cell, it is preferable to generate liposomes containing synthetic lipids capable of imparting valuable new characteristics and behaviors to the membrane system. Here, we present a more facile method for the incorporation of distinct moieties onto the phospholipid structure using "click" chemistries. To demonstrate one utility of this system, phospholipids bearing a photocleavable moiety have been synthesized. When these lipids are incorporated into a liposome and irradiated, non-spherical vesicles undergo pinocytosis, enabling uptake of resources from the external environment. This has the potential to greatly expand the functional lifetime of artificial cells leading to greater product generation and getting closer to a "living" cell.



Elongated vesicles undergo pinocytosis as lipids cleave under irradiation

Autologous sural nerve repair long common peroneal nerve defect by biodegradable conduit small gap tubulization

Ming Li[#], Jiuxu Deng[#], Jian Weng, Fei Yu, Yuhui Kou, Na Han, Xiaofeng Yin, Peixun Zhang[*] & Baoguo Jiang[*]
Department of Orthopedics and Trauma, Peking University People's Hospital, Beijing, China
*Correspondence to: Peixun Zhang, M.D., zhangpeixun@bjmu.edu.cn.Baoguo Jiang, M.D., jiangbaoguo@vip.sina.com.

To investigate and compare the effect of biodegradable conduit small gap tubulization on varying anastomotic stoma combined with single autologous sural nerve on the repair of long common peroneal nerve defect. Rat models of common peroneal nerve defect were established and biodegradable conduit small gap tubulization on different anastomotic stoma combined with single autologous sural nerve were performed. After postoperative 12 weeks, nerve morphological variation and functional recovery were observed. The results showed that regenerated nerve in group of biodegradable conduit small gap tubulization on both proximal and distal anastomotic stoma (1817±31) was more than group of proximal anastomotic stoma (1645±30, P<0.05), group of distal anastomotic stoma (1719±32, P<0.05), and group of epineurium suture (1542±52, P<0.05), but less than normal group (2047±66, P<0.05). The maximum tetanic contraction force and wet weight ratios of tibialis anterior muscles in group of biodegradable conduit small gap tubulization on both proximal and distal anastomotic stoma were also better than other experimental groups (P<0.05).The common peroneal nerve function index in small gap tubulization groups were superior than group of epineurium suture (P<0.05) and inferior to normal group (P<0.05), but there was no statistically significant differences between intergroup (P>0.05). The motor nerve conduction velocity in experimental groups were lower than normal group (P<0.05), but there was no statistically significant differences between intergroup (P>0.05). This study suggested that when use autologous sural nerve repair long common peroneal nerve injury, performing biodegradable conduit small gap tubulization on both proximal and distal anastomotic stoma may be a better choice for nerve regeneration and functional recovery.
Keywords: biodegradable conduit small gap tubulization, long common peroneal nerve injury, autologous sural nerve, nerve regeneration, functional recovery.

Li, Xing

A Novel Polystyrene Beads Adsorbents Containing Mesopores and Linear Decapeptide Segments as Ligands for the Removal of β2-Microglobulin from Human Plasma

Xing Li[1], Sheng Wang[1], Lailiang Ou[2], Yaoting Yu[2], Shenqi Wang[12]shenqiwang131@hust.edu.cn
[1]*School of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China.*
[2] *he Key Laboratory of Bioactive Materials, Ministry of Education, Nankai University, Tianjin 300071, China*

As a consequence of renal failure, the concentration of β2-MG increases, ultimately giving rise to deposition of the protein into amyloid fibrils and development of the disorder, dialysis-related amyloidosis (DRA). It can effectively alleviate the syndrome of amyloidosis for the patients suffering from long term dialysis by removing β2-MG from the blood. In this paper, a couple of linear decapeptide segments KD and KE were designed to adsorb the β2-MG specifically by Autodock Software. Due to β2-MG molecule is about 8nm in diameter, we developed chloromethylated crosslinked polystyrene microspheres (CMCPS) containing uniform mesopores(2-50nm) as carriers and linear decapeptide segments as ligands for removing β2-MG. Via amino groups of linear decapeptide segments with chloromethyl groups on CMCPS in N, N-dimethyl formamide (DMF) solution by nucleophilic substitution reactions, we synthesized novel polystyrene beads adsorbents(CMCPS-KD and CMCPS-KE). The obtained adsorbents can get rid of β2-MG from human plasma during the hemodialysis and harmless to the human body as well. Latex immunoturbidimetry assay was used to detect the concentration variations of the β2-MG before and after adsorption, meanwhile, the adsorption mechanism was discussed. The hydrophobic force and electrostatic force played a predominant role in the binding of polystyrene beads adsorbents with β2-MG. Subsequently, the prepared adsorbents were characterized by Fourier transform infrared spectroscopy (FT-IR), elemental analysis (EA) and scanning electron microscope (SEM) test. Eventually, the results indicated that the new adsorbents

CMCPS-KD and CMCPS-KE have high adsorption capacity for β2-MG reaching 90% and 88% respectively (wet polystyrene beads adsorbents). By the way, albumin and parathyroid hormone (PTH) were also removed during the process, and the adsorption capacity of CMCPS-KD for albumin lower than 15%.

Keywords: β2-microglobulin; dialysis-related amyloidosis; polystyrene beads adsorbents(CMCPS-KD and CMCPS-KE)

Table 1.Using Latex immunoturbidimetry assay detects the concentration variations of the β2-MG before and after adsorption

	blank	standard protein	plasma	MCPS-KD[a]	MCPS-KE[b]	CMCPS
Variation of absorbance (ΔA)	0.0015	0.0276	0.0268	0.0050	0.0054	-0.0001
	-0.0023	0.0311	0.0261	-0.0014	0.0047	0.0066
	0.0003	0.0448	0.0233	0.0021	-0.0028	0.0036
			0.024	0.0026	0.0013	-0.0065
Mean value of absorbance	0.0009	0.0345	0.0251	0.0032	0.0038	0.0051
Concentration of β2-MG (mg/L)	0	11.5000	8.2827	0.7872	0.9926	1.4375
Adsorption capacity				90.50%	88.02%	82.64%

The grey data are eliminated due to unreasonable of negative values.

[a] The CMCPS-KD refers to polystyrene loaded linear decapeptide segment KD.

[b] The CMCPS-KE refers to polystyrene loaded linear decapeptide segment KE. The adsorption capacity of CMCPS-KE for PTH can reach 30%(the figure isn't shown in the Table 1.).

Table 2. Using bromocresol green method detects the concentration variations of the albumin before and after adsorption

	blank	plasma	MCPS-KD	MCPS-KE	CMCPS
Optical density (OD)	0.0791	~~0.1969~~	~~0.2049~~	0.2123	~~0.3432~~
	0.0838	0.2337	0.2196	0.2189	0.2242
	0.0836	0.2546	0.2240	0.2130	0.2474
		~~0.2556~~	0.2253	~~0.2062~~	0.2034
		0.2514	~~0.2433~~	~~0.2337~~	~~0.1935~~
Mean value of optical density	0.0822	0.2466	0.2230	0.2147	0.2250
Concentration of albumin (g/L)	0	52.1418	44.6552	42.0434	45.3003
Adsorption capacity			14.36%	19.37%	13.12%

The Maximums and minimums are eliminated during the process of calculating the mean values of optical density

Lomis, Nikita nikita.lomis@mail.mcgill.ca

Development of a novel nanoparticle based therapy for cardiovascular diseases

Nikita Lomis[1,2], Francis Gaudreault[3], Meenakshi Malhotra[4], Susan Westfall[1], Dominique Shum-Tim[5] and Satya Prakash[1,*]

[1,*]Biomedical Technology and Cell Therapy Research Laboratory, Department of Biomedical Engineering, 3775 University Street, Montreal, QC, H3A 2B4, Canada; [2,*]Division of Experimental Medicine, 1110 Pins Avenue, Montreal, QC, H3A 1A3, Canada; [3]Human Health Therapeutics, National Research Council Canada, 6100 Royalmount Avenue, Montreal, QC, H4P 2R2, Canada; [4]Department of Radiology, Stanford University School of Medicine, Stanford, CA, 94305, USA; [5]Division of Cardiac Surgery and Surgical Research, Royal Victoria Hospital, 1001 Boulevard Décarie, Montréal, QC, H4A 3J1, Canada.

Cardiovascular diseases (CVDs) are the leading cause of mortality across the globe. Congestive heart failure (CHF), a type of CVD, is caused by buildup of plaque in the coronary artery which obstructs the flow of blood and oxygen to the heart. This causes stiffness and irreversible necrosis of the heart tissue. The common treatments for CHF involve insertion of coronary stents along with administration of inotropic or diuretic drugs. Milrinone (MRN) is an inotrope drug, which is known to improve myocardial contractility and function, however, its efficacy is limited. Therefore, in this study we report the development and optimization of novel MRN carrying nanoparticles for use in CHF. The nanoparticles were prepared from the blood plasma protein, human serum albumin (HSA) owing to properties such as target specificity, biocompatibility, biodegradability and non-immunogenicity. The MRN-HSA-NPs were optimized by following the ethanol desolvation technique. Key parameters such as HSA and MRN concentration, pH of preparative solution, ethanol volume, glutaraldehyde content and polymerization time, were optimized. Results showed that the MRN-HSA-NPs size was 154.2±5.8 nm and zeta potential was 29.5±2.9 mV. The drug encapsulation efficiency was 41.1±1.7 %. Molecular docking studies were performed to determine the nature of binding between MRN and HSA. The Wilma and SIE softwares predicted that MRN binds in the hydrophobic cavity (Sudlow's Site 1) present on sub-domain IIA of the HSA molecule with a binding affinity of -8.6 kcal/mol. Circular dichroism

studies further confirmed that when MRN is mixed with HSA in a HSA/MRN molar ratio of 1:5, a change in the secondary structure of HSA was observed. These studies indicate that MRN-HSA-NPs may potentially be used for targeted drug delivery to the heart.

Maysinger D dusica.maysinger@mcgill.ca

Anti-inflammatory dendrimers

Dusica Maysinger
Professor, Department of Pharmacology and Therapeutics, Faculty of Medicine, McGill University, 3655 Promenade Sir William Osler, Montreal, Canada.

Inflammation and particularly neuroinflammation is associated with many pathologies in aging and neurodegenerative disorders. Cellular processes and activation of signal transduction pathways in inflammation are initiated and driven by numerous stimuli including bacterial endotoxins, metabolic disorders (metaflammation), mechanical injury and others. Neuroglia are key modulators of neuroinflammation; they can promote inflammatory processes, or participate in the resolution of inflammation.
Current non-steroidal anti-inflammatory agents are not effective unless applied early in the disease. Nanostructured dendrimers (highly organized branched polymeric structures) with sulfate terminal groups exhibit intrinsic anti-inflammatory activity and affect synaptic plasticity by normalizing hyperactive neuroglia. Mechanisms implicated in degenerative changes in the hippocampus and cortex involve autocrine and paracrine regulation of lipocalin 2 (cytokine mainly synthesized by astrocytes). Anti-inflammatory dendritic nanostructures can re-establish homeostasis in the brain structures by reducing deleterious effects of lipocalin-2 and by attenuating hyperactivity of neuroglia.

Mishra, N neerajdops@rediffmail.com

Surface modified microparticulate carriers of Embelin for their beneficial Pharmacological potential in ulcerative colitis

Neeraj Mishra[1*], Nidhi[1], Saurabh Sharma[2]
[1]Department of Pharmaceutics; [2]Department of Pharmacology, I.S.F. College of Pharmacy, Ghal Kalan, Ferozpur, G.T road, Moga- 146001, Punjab, India.

Present study was aimed to developed enteric coated microsphere of Embelin and their pharmacological potential was investigated in acetic acid induced ulcerative colitis. The optimized formulation of embelin loaded microspheres has shown significant sustained release of embelin. Further this formulation significantly reduced the ulcer activity score, oxidative stress and attenuates the inflammatory changes. Thus it may be concluded that embelin loaded enteric coated microparticles has shown delayed release capacity than plain Embelin and exerts colon ulcer protective effect in rats.
Key words: Multiparticulate carrier, pH dependent, Colon, Time dependent, Eudragit S 100

Mobed-Miremadi, M **mmobedmiremadi@scu.edu**
(Alumni, Artificial Cells & Organs Research Centre)

LEGACY OF ARTIFICIAL CELLS IN BIOMEDICAL ENGINEERING EDUCATION

Mobed-Miremadi, M
Santa Clara University, CA, U.S.A. (Alumni of Artificial Cells & Organs Research Centre)

Over the past ten years, the advances in bio-fabrication methods namely 3D printing and soft lithography as well as simulation tools have revolutionized the speed and flexibility of design of biomimetic membranes. Consequently, modeling the diffusive and mechanical behavior of immuno-isolation membranes of different morphology, size and composition has reached the rapid-prototyping stage in biomedical engineering classrooms. The use of simulation tools used in artificial cell characterization in small and large scale bioreactors will be presented.

Farzaneh Moghtader *farzaneh_moghtader@yahoo.com*

Bacterial Detection by SERS Using Nanoparticles and Bacteriophages

Farzaneh Moghtader[1,2], Orhan Erdem Haberal[2,3], Aysel Tomak[4], Hadi M. Zareie[5], Erhan Piskin[1,2]
[1]Hacettepe University, Nanotechnology and Nanomedicine Division
and Chemical Engineering Department, Beytepe, Ankara, Turkey
[2]NanoBMT, Beysukent/Cyberpark-Bilkent – KOSGEB/Tekmer-Başkent, Ankara, Turkey
[3]Başkent University, Biomedical Engineering Department, Baglıca, Ankara, Turkey
[4]İzmir Institute of Technology, Department of Material Science and Engineering, 35430, Urla, Izmir, Turkey

[5]University of Technology, School of Physics and Advanced Materials,
Microstructural Analysis Unit, Sydney, Ultimo NSW 2007, Australia

The main aim of this study is to develop bacterial detection strategies using bacteriophages with nanoparticles by "Surface Enchanced Raman Spectroscopy" (**SERS**). *E.coli*, *S.aureus and S.infantis* were selected as the targets and their specific phages were used as the bioprobe. Phages were propagated/purified in large enough quatities by rather traditional techniques using the target bacteria and stored at +4°C. Bacterial cultures were prepared freshly in each work day from the stokes. Gold nanorods were synthesized by using CTAB as the reducing agent and stabilizer. Both a commercial Raman System and also a home-made Raman probe/reader (designed and constructed by us) were used alternatively to obtain the SERS data. TEM/SEM/AFM images demonstrated their morphologies. The average particles size and distribution and charges were obtained by a Nanosizer. LSPR and UV visible spectra were used to demonstrate their optical properties (adsorption spectra). It was possible to obtain quite sharp (intense) peaks (even at one bacterial cell level) - fingerprints of the target bacteria without using any bacteriophages. We were able to detect the target bacteria using phages in a real time contacting system very effectively in quite simple tests.

Acknowledgement: This study is partially supported by EU-FP7-IAPP Nanobacterphage-*SERS*, TÜBİTAK 1003 Project-No: 113O864, NanoBMT-KOSGEB-Tekmer-Başkent Project. E. Piskin was supported as the honorary member of Turkish Academy of Sciences (TUBA).

Neufeld RJ neufeld@queensu.ca
(Alumni of Artificial Cells & Organs Research Centre)

BIOMATERIAL CHOICES IN DESIGN OF COMPLEX NANOPARTICULATE CARRIERS FOR ORAL DELIVERY OF INSULIN

R.J. Neufeld[1], C. Pinto Reis[1,2], B. Sarmento[1,3], C. Woitiski[1,2], F. Veiga[2], A. Ribeiro[2], D. Ferreira[3], C. Damgé[4]
[1]Queen's University, Kingston, Ontario, Canada [2]University of Coimbra, Portugal [3]University of Porto, Portugal
[4]Université Louis Pasteur, France

Nanoparticulate multilayer structures were developed for oral delivery of insulin and potentially other peptide/protein-based biopharmaceuticals. Formulation methods involved one or a combination of: nanoemulsion dispersion/triggered *in situ* polymer gelation; ionotropic colloidal pregel/polylectrolyte complex coating; and nanospray drying. The most effective polymeric formulation involves an insulin-loaded alginate-dextran nanoparticulate core, complexed with a chitosan-PEG shell and finally with an albumin coat.
In vitro bioactivity was assessed by stimulation of rat L6 myoblasts with insulin released from nanoparticulate complex, assayed through detection of phosphoylated AKT, a downstream product of the insulin signaling cascade. Insulin released from nanoparticulate complex was shown fully bioactive.
Oral dosage of nanoparticulate insulin to diabetic rats reduced glycemia over a 24h period, in a dose dependent manner with the maximum effect after 14h. Increased plasma insulin levels improved glycemic response to an oral glucose overload. Pharmacological activity was 42% and relative bioavailability was 30% for the orally dosed formulation, relative to subcutaneous insulin administration. Labelled insulin/nanoparticles adhered strongly to villus apical enterocytes and Peyer's patches, and were internalized via the intestinal mucosa. The antidiabetic effect of oral dosed insulin was thus preserved in nanoparticulate form, explained by the protective effect of albumin against gut proteases, by the pH-responsive impermeable structure of alginate polymer in gastric pH, by the mucoadhesive properties of chitosan and PEG, and by the role of chitosan in opening tight junctions between cells, improving insulin and nanoparticulate absorption.
Toxicological assessment after 15 days oral administration to diabetic rats included haematological, biochemical and urine-based assays, and organ and tissue histology. Absence of toxicological effects was shown over the short term, with some observed effects attributed to diabetes physiopathology, diabetes inducement or diabetes evolution status. Lack of toxicity associated with nanoparticulate administration was also demonstrated by absence of mortality over the short term.
Rationale behind the selection of the various polymers used in the nanoparticulate complex will be discussed, as it may apply to other therapeutic applications.

Erhan Pişkin piskin@hacettepe.edu.tr
(Alumni of Artificial Cells & Organs Research Centre)

Engineering of Bone and Cartilage Tissues

Erhan Pişkin
Hacettepe University and Biyomedtek/NanoBMT, Cyberpark-Bilkent University/

Tekmer-Başkent University, Ankara, Turkey

Tissue engineering is one of the recent theraputic approaches for both soft and hard tissue repair. Healthy cells taken from the host own tissues or from other sources are used together with scaffolds. Target specific (eg., osteoblasts, chodrocytes, etc.) or preferentially stem cells are isolated, differentiated (in the case of stem cells), or even genetically modified (for instance to express growth factors, eg., BMPs) and are used in two diffetrent approaches. In the first approach they are just loaded into the scaffolds (as seeds) and apply as biohybrid implants. Alternatively, cells are propagated within the pores of scaffolds within bioreactors (*in vitro*) to form tissue-like structures and then they are implanted for tissue replacement. Scaffolds have large and interconnected pores which allows 3D-cell ingrowth are used. They have to be degradable *in vivo*, means that they should degrade such a rate that the new forming tissues to replace them properly. Of course both they and their degradation products must be biocompatible. They are produced several techniques, such as moulding/salt extraction, electrospinning, cryogelation, etc. They are made of several natural polymers (e.g., collagen and its denaturated form gelatin) and synthetic polymers (e.g., lactides, glycolide and ε-caprolactone). Several bioactive agents (e.g., growth factors, etc.) may be also incorporated (usually as controlled release formulations) to trigger the regeneration rate and proper new tissue formation. After careful *in vitro* biocompatibility test, tissue engineering scaffolds (loaded with cells) or biohybrid implants are applied *in vivo* in proper animal models. Critical size defects (means that the defects do not recover by themselves) are created in animals. In the maxillofacial applications, cranium, cleft palate, zgyoma, mandibula, etc. models have been used for bone tissue engineering. Ear defects are created to study cartilage repair. Several macro-, histological, molecular techniques are used to investigate tissue regeneration. This talk briefly reviews the topics mentioned above by using the experience of the author's group in this field.

Dipanjan Pan dipanjan@illinois.edu

Nano-enabled Orphan Nuclear Receptor Activation Regulates Metabolism, Transport and Programmed Cell Death Pathways in Soft Tissue Sarcoma of Xenograft Mice and Transgenic Oncopigs

Mao Ye,†, 1 Santosh Misra,†, 1 Arun K. De,†, 2 Fatemeh Ostadhossein,1 Kuldeep Singh,4 Laurie Rund,2 Lawrence Schook2,5 and Dipanjan Pan* 1, 5, 6, 7, 8
1 Department of Bioengineering, University of Illinois at Urbana-Champaign, USA. 2 Department of Animal Sciences, University of Illinois, Champaign-Urbana, Illinois, USA. 3Agricultural Animal Care and Use Program, University of Illinois at Urbana-Champaign, Illinois, USA. 4Veterinary Diagnostic Laboratory, University of Illinois, Champaign-Urbana, Illinois, USA. 5Beckman Institute of Advanced Science and Technology, University of Illinois at Urbana Champaign, Illinois, USA. 6Mills Breast Cancer Institute, Carle Foundation Hospital, 502 N. Busey, Urbana, Illinois, USA. 7Department of Materials Science and Engineering, University of Illinois-Urbana Champaign, Illinois, USA. 8Carle-Illinois College of Medicine, Urbana, Illinois, USA. †Contributed equally. *Corresponding author:

Background: Sarcomas are a rare and heterogeneous cancer variant of mesenchymal origin. Their genetic heterogeneity coupled with uncertain histogenesis make them difficult to treat and is associated with poor prognosis. The lack of therapeutics providing high efficacy and better patient outcome warrants pursuit of novel therapies. The orphan nuclear receptor (NR) agonists can play role in programmed cell death and a safer transport and metabolism across cytoplasmic compartment could be additional advantage for future human use.
Methods: Towards this aim, we provide an 'in-silico-to-in-vivo' approach to identify and synthesized a novel RXR agonist and developed it as a nanotherapy treatment for 'soft" tissue sarcoma which can provide potent and controlled delivery of newly discovered retinoid x-receptor (RXR)-selective agonist. Novel RXR-selective agonists were identified through structure-based drug discovery and computational modeling to transcriptionally activate the orphan nuclear receptor target. The delivery of the potent agent has been accomplished by a micellar polymeric nanoparticle (20 nm). In order to realize the translational supremacy of the work, the agents were studied simultaneously in a rodent and a transgenic swine model (Oncopigs) of soft tissue sarcoma. The Oncopig provides an inducible and reproducible tumor model in a large animal, which is comparable to humans in both size and physiology and is being used for development, validation, safety and efficacy assessment of the RXR agent intended for human translation. Studies with rodent and Oncopig models elucidate mechanistic insights into the modulations of RXR, _PPAR transcriptional activation and downstream target genes along with genes involved in phase-I, phase-II and phase-III metabolism and transport regulation in treated and control tumor tissues.
Results: The structurally novel scaffold-based RXR selective agonist is different from either bexarotene or alitretinoin (9-cis-retinoic acid)-based structures, basic frame for most of reported RXR ligands. Structure-based drug discovery involving computational modeling was used to identify a novel RXR agonist ligand and synthesized as a bis(indolyl)methane derivative. Our results demonstrated significant efficacy in vitro. This agent was delivered using a polymeric micellar nanoparticles derived from an amphiphilic polymer (polystyrene-b-polyacrylic acid) for evaluation of efficacy and safety in transformed sarcoma cells, 63-3 Cre

and 141-10 Cre of pig origin and in rodent xenograft models. Responses at gene and protein levels established the treatment approach as a highly effective RXR agonist across cell, rodent and oncopig models. Interestingly, RXR-8 was not only able to modulate metabolic and transporter genes related to orphan nuclear receptors but also played a major role in modulating programmed cell death in sarcomas, developed in oncopigs. Results suggest that this novel agent induced expression of RXR related gene modulations in tumor as well as in liver of treated animals including rodents and onco-pigs as well. As RXR forms a heterodimer with other orphan nuclear receptors to induce expression of drug metabolism genes, the expression of other several nuclear receptors also increased. A significant upregulation of Phase-I drug metabolism genes (CYP isoforms) also was reported with a few exceptions supporting the no-retention possibility of newly synthesized drug molecule RXR-8 and negate the possibility of retention related toxicities in subjects. In general, the nano-RXR was more effective than the RXR drug alone in tumor regression, gene expression and protein modulation.
Conclusion: The agents developed in this work can significantly contribute to translational research. We anticipate that outcome of this work would have a necessary advancement on significant translational impact to achieve the survival of patients with soft tissue sarcoma.

Ayse Kevser Ozden (Piskin AK) kpiskin@hacettepe.edu.tr
(Alumni of Artificial Cells & Organs Research Centre)

Quartz Crystal Microbalance (QCM) based biosensors for detecting breast cancer cells via their membrane receptors

*Ayse Kevser Ozden *, Seda Atay**, Monirah Bakhshpour**, Fatma Yilmaz** , Handan Yavuz**, Adil Denizli***
**Hacettepe University, Faculty of Medicine, Medical Biochemistry Department*
***Hacettepe University, Faculty of Science, Biochemistry Department. Ankara, Turkey*

Breast cancer is the most common cancer among women and the second most common cancer overall. Treatment of metastatic breast cancer is still a big challenge despite developing strategies. Early diagnosis and detection of metastasis saves many lives. Detection and characterization of tumor cells are crucial in cancer treatment. Therefore, it is of prime importance to detect these cells in tumors as well as in the circulation. Furthermore, discrimination of breast cancer cells with high metastatic potential from those of low potential may shed light on treatment regimes. A quartz crystal microbalance (QCM) biosensor was developed to detect breast cancer cells by functionalizing the gold sensor surface with ligands that are specific for membrane receptors, namely, transferrin and mammalian target of rapamycin (mTOR). The MDA-MB 231 breast cancer were used as target cells. First, poly(2-hydroxyethyl methacrylate) (PHEMA) nanoparticles were prepared by mini-emulsion polymerization of hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA). Nanoparticles were characterized with a zeta-sizer and then their suspension is dropped on the surface of the QCM and the dried QCM surface was modified further by activation with carbodiimide and transferrin or mTOR antibody attachment. The QCM biosensor was analyzed by using atomic force microscopy (AFM), ellipsometry, Fourier transform infrared spectrophotometry (FTIR) and contact angle measurements. The cells were applied to the derivatized QCM biosensor to investigate the affinity and binding kinetics. The nanoparticles and lagands were found to form a monolayer on the QCM surface. Binding kinetics were best fitted to the Langmuir–Freundlich adsorption model. The QCM signal was correlated well with the number of receptors on cells. It is concluded that the QCM biosensor functioning via receptor interactions may be used to detect breast cancer cells or discriminate the highly metastatic type of breast cancer cells. with high metastatic potential.

Denis Poncelet denis.poncelet@bioencapsulation.net

Microencapsulation : a human story

Denis Poncelet
Oniris, GEPEA UMR CNRS 6144, Nantes, France; # denis.poncelet@oniris-nantes.fr

Life “apparition” is generally linked to the development of the biochemistry. However, to get a living organism, the biochemistry needs to be structured as biological cells, i.e. encapsulated. Microencapsulation provides material immobilization and isolation, its protection, control of the transfer in and out of the cell, liquid-to-solid structuration and especially power to create sophisticated functions. As an example, production of ATP would not be possible without membrane and no energy would be available to the system to counteract the entropy.
The challenge for the scientists and engineers working in encapsulation is then to mimic the biological cells. Chemical components are separated to avoid unwanted reaction, vitamins are protected from oxygen, profile

of drug release is tuned-up, essential oil is converted to powder before incorporation in feed, monomers are encapsulated to develop self-healing material.

HISTORY It exists a large panel of microencapsulation methods, combining equipment or dispersion method (Spray, dripping, extrusion, fluid bed, emulsion, …) and a process of encapsulation (physical, chemical, …). Each author defines the starting point of microencapsulation depending on the domain and the methods. Table 1 provides some key dates of microencapsulation development.

APPLICATIONS Microcapsules are generally not the end product but assists in developing innovative products or processes. It is then not surprising that man in the street ignores the microencapsulation.

However, microcapsules are incorporated in many of our daily products. Most of the big companies (P&G, Unilever, Nestlé, 3M, Dupont, …) have developed products based on microcapsules. We have identified several thousand companies working on or with microcapsules. The world market count several billions of euros and is supposed to increase by 9% per year.

Far to be exhaustive, we could cite the following applications of microcapsules : drug delivery, aroma addition to food powders, artificial organs, long action of cosmetic products, agrochemical formulation, self-blocking screws, cosmeto-textiles, enzyme-loaded laundry powders, cell immobilization, liquid crystal, carbon-less paper, incorporation of additives in paint or feed …

Qi, Yanxin yxqi@ciac.ac.cn

Protein-Resistant Biodegradable Amphiphili Graft Copolymer Vesicles as Protein Carriers

Yanxin Qi,Yupeng Wang,Yubin Huang*
State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, ChangChun 130022, People's Republic of China

The protein adsorption and self-assembly behavior of biocompatible graft copolymer, poly(lactide-co-diazidomethyl trimethylene carbonate)-g-poly(ethylene glycol) [P(LA-co-DAC)-g-PEG],were systematically studied. The graft copolymers showed enhanced resistance to non-specific protein adsorption compared with their block copolymer counterparts, indicative of the increased effect of PEG density beyond PEG length. Diverse nanostructures including vesicles can be assembled from the amphiphilic graft copolymers with well-defined nano-sizes. Hemoglobin (Hb), as a model protein, can be entrapped in the formed vesicles and keep the gas-binding capacity. The reduce release rate of Hb from graft copolymer vesicles indicated the relatively stable membrane packing compared with block copolymer counterpart.

Keywords: graft copolymer; polymersome; protein adsorption; protein carriers

Jun Ren renjun@dlut.edu.cn

Preparation of hydrophobic charge induction adsorbent for selective removal of antibody from human plasma

Jun Ren*, Lingyun Jia
School of Life Science and Biotechnology Dalian University of Technology, Dalian, China 116024

Immunoadsorption is an important type of blood purification therapy, and has proven effective in treating myasthenia gravis (MG) and a range of other autoimmune diseases. Protein based affinity ligands, such as recombinant Protein A, antibody and autoantigens are usually employed for immunoadsorbents (Ren et al., 2011). They have high specificity for target molecules, but also suffer from the drawbacks mainly associated with their protein nature, such as high cost and low stability. Thus, synthetic compounds have been explored as alternatives for producing specific adsorbents for the treatment of autoimmune diseases. Benzotriazole-5-carboxy has been found as a potential hydrophobic charge induction ligand, which could allow rapid separation of target antibodies from human plasma when immobilized on agarose beads. The adsorbent has a dynamic binding capacity of 57.7 mg/mL gel for human IgG, while only bind 4.3 mg/mL of BSA. With the sample of human serum, Benzotriazole-based adsorbent could yield an antibody component with the purity more than 90%, serving as a potential adsorbent for antibody removal therapy.

Acknowledgment
This work was supported by the National Key Technologies R&D Program（2016YFC1103002）and Dalian Innovation supporting program (2016RQ023).

Reference

Ren J., Bai Y., et al. 2011, Amelioration of Experimental Autoimmune Myasthenia Gravis Rats by Blood Purification Treatment using 4-Mercaptoethylpyridine Based Adsorbent, J. Biomed. Mater. Res. A, 98A:589-595.

Scott, M mdscott@mail.ubc.ca

Modulating the Immune System via Bioreactor Produced miRNA-Based Therapeutics

Scott, M
Senior Scientist - Clinical Professor, Canadian Blood Services and University of British Columbia

The interface of polymer-engineered cells with the immune system is most often viewed as a 'local' event – *i.e.,* encapsulation of donor cells prevents immune injury at the site of implantation. Surprisingly however, grafted polymers also have the potential to induce potent systemic immunomodulatory effects. We have previously demonstrated that the covalent grafting of methoxy poly(ethylene oxide) to allogeneic leukocytes induced a persistent pro-tolerogenic state both *in vivo* and *in vitro*. The polymer-dependent tolerance was characterized by the upregulation of regulatory T cells (Tregs) and downregulation of effector T cell (Teff) subpopulations. Surprisingly, cell-free conditioned media or plasma obtained from these cells were similarly capable of mediating the immune modulation both *in vitro* and *in vivo*. Initially, it was hypothesized that soluble cytokines mediated this effect; however subsequent studies demonstrated that the immunomodulatory activity resided in the cytokine poor fraction and that microRNA (miRNA) were the essential effector elements. Using a bioreactor manufacturing system, two complex miRNA therapeutics (TA1 and IA1) of miRNA have been manufactured which induce (*in vitro* and *in vivo*) either a potent tolerogeneic (TA1) or inflammatory (IA1) immune response. The immunomodulatory and clinical efficacy of the TA1 therapeutic was examined in the Non-Obese Diabetic (NOD) mouse model of autoimmune Type I diabetes. In the NOD mouse, TA1 effectively reset the immune system from a proinflammatory to a tolerogenic state as evidenced by increasing Tregs and decreasing Teff cell populations. Consequent to the increase in the Treg:Teff ratio, TA1 prevented or delaying the onset of autoimmune diabetes. In contrast, the *in vitro* treatment of naïve human lymphocytes with the proinflammatory IA1 miRNA-therapeutic decreased the Treg:Teff ratio and dramatically enhanced their killing of human tumor cells. These findings provide significant new insights into the potential utility of miRNA-based therapeutics as an approach to treating immune dysfunctions.

Shashi, Prabha prabhashashi6@gmail.com

Preparation and in-vitro characterization of 9-bromo noscapine for preparation of cancer drug delivery nano formulations for use in breast and other cancers

Shashi Prabha 1, Bahar Ahmed 2, and Dr. Mohd Aqil*1 prabhashashi6@gmail.com
1. Dept. of Pharmaceutics, Jamia Hamdard, New Delhi-110062, India.
2. Dept. of Pharmaceutical Chemistry, Jamia Hamdard, New Delhi-110062, India.

Breast cancer is the most common invasive cancer in females worldwide. It accounts for 16% of all female cancers and 22.9% of invasive cancers in women. Various diagnostics and treatment methods are available but they are not adequate as they are associated with several limitations. Therefore, there is a need to develop new formulation.

Noscapine and its derivatives represent a group of microtubule-targeting agents that possess great potential as novel anticancer agents. Nanoparticles and vesicles hold promise for improving cancer treatment for e.g. they can guide drugs directly to tumors increasing effectiveness and reducing side effects. However, significant challenges need to be overcome before these promising formulation make it to the clinic. The most challenging task is to design and prepare formulations that effective and safe. In various studies Noscopine analogues have shown promising results. However, their synthesis are very challenging. The present study proposes synthesis and characterization of noscapine bromo analogue for designing nano formulation for management cancer. Noscapine bromo was prepared using 1.0 g of noscapine (2.42 mmol) using 48% hydrobromic acid solution (-2 mL) in a flask and freshly prepared bromine water (-12.5 mL) was added drop wise to the reaction mixture and neutralized to pH 10.0 using ammonia solution. The obtained precipitate was crystallized 70% using ethanol to yield 9-bromonoscapine. NMR analysis was performed to melting point (mp), 169 °C to 170 °C; IR, 2945 (m), 2800 (m), 1759 (s), 1612 (m), 1500 (s), 1443 (s), 1263 (s), 1091 (s), 933 (w) cm–1.); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96 (d, J= 8.309 Hz, 1H), 6.26 (d, J=8.309 Hz, 1H), 6.023 (s, 2H), 5.392 (d, J=4.721Hz, 1H), 4.270 (d, J=4.721, 1H), 4.077 (s, 3H), 3.999 (s, 3H), 3.872 (s, 3H), 2.831-2.746 (m, 1H), 2.670-2.579 (m, 1H), 2.516(s, 3H), 2.496-2.422 (m, 1H), 2.024-1.913 (m, 1H).^{13}C NMR (75 MHz, $CDCl_3$) δ 167.95, 152.24, 147.67, 146.47, 141.17, 139.90, 134.10, 130.26, 119.57, 118.90, 118.25, 117.45, 101.01, 95.50, 81.23, 62.24, 60.86, 59.37, 56.72, 48.34, 45.13, 25.85. MS (ESI) *m/z* 492 $[M+H]^+$; HR-MS (ESI) Calcd for $C_{22}H_{22}NO_7Br$ $[M+H]^+$:492.0657, found: 492.0636. This method resulted in a yield of 82% bromonocapine. Detials of the preparation and characterization process will be discussed.

* Corresponding authors

Erhan Pişkin
(Alumni of Artificial Cells & Organs Research Centre)

Engineering of Bone and Cartilage Tissues

Erhan Pişkin
Hacettepe University and Biyomedtek/NanoBMT, Cyberpark-Bilkent University/
Tekmer-Başkent University, Ankara, Turkey

Tissue engineering is one of the recent theraputic approaches for both soft and hard tissue repair. Healthy cells taken from the host own tissues or from other sources are used together with scaffolds. Target specific (eg., osteoblasts, chodrocytes, etc.) or preferentially stem cells are isolated, differentiated (in the case of stem cells), or even genetically modified (for instance to express growth factors, eg., BMPs) and are used in two diffetrent approaches. In the first approach they are just loaded into the scaffolds (as seeds) and apply as biohybrid implants. Alternatively, cells are propagated within the pores of scaffolds within bioreactors (*in vitro*) to form tissue-like structures and then they are implanted for tissue replacement. Scaffolds have large and interconnected pores which allows 3D-cell ingrowth are used. They have to be degradable *in vivo*, means that they should degrade such a rate that the new forming tissues to replace them properly. Of course both they and their degradation products must be biocompatible. They are produced several techniques, such as moulding/salt extraction, electrospinning, cryogelation, etc. They are made of several natural polymers (e.g., collagen and its denaturated form gelatin) and synthetic polymers (e.g., lactides, glycolide and ε-caprolactone). Several bioactive agents (e.g., growth factors, etc.) may be also incorporated (usually as controlled release formulations) to trigger the regeneration rate and proper new tissue formation. After careful *in vitro* biocompatibility test, tissue engineering scaffolds (loaded with cells) or biohybrid implants are applied *in vivo* in proper animal models. Critical size defects (means that the defects do not recover by themselves) are created in animals. In the maxillofacial applications, cranium, cleft palate, zgyoma, mandibula, etc. models have been used for bone tissue engineering. Ear defects are created to study cartilage repair. Several macro-, histological, molecular techniques are used to investigate tissue regeneration. This talk briefly reviews the topics mentioned above by using the experience of the author's group in this field.

Shi, ZQ
(Alumni of Artificial Cells & Organs Research Centre).
Vice President, Clinical Development, REMD Biotherapeutics Corp, California.

A Fully Human Glucagon Receptor (GCGR) Antibody Reduces Daily Insulin Requirements and Improves Glycemic Control in People with Type 1 Diabetes

Zhiqing Shi[1], Jeremy Pettus[2], Dominic Reeds[3], Tricia Santos[2], Schafer Boeder[2], Michelle Levin[2], Edda Cava[3], Dung Thai[1], Hai Yan[1], Edgar Bautista[1], John McMillan[1], Robert Henry[2], Samuel Klein[3] (1: REMD Biotherapeutics, Inc. USA; 2: University of California San Diego, USA; 3. Washington University School of Medicine, USA)

Studies conducted in rodent models of T1D have shown that glucagon receptor blockade normalizes plasma glucose concentration without the need for exogenous insulin. We conducted a randomized, double-blind, placebo (PBO) controlled trial in 21 subjects with T1D (8 men,13 women) to evaluate the effect of a single 70 mg SC injection of REMD-477, a human monoclonal antibody against the GCGR, on daily insulin requirements and glycemic control. After obtaining baseline insulin use and data from Continuous Glucose Monitoring (CGM), subjects were admitted to the Clinical Research Unit for 5 days. Insulin was provided by continuous IV infusion to maintain postabsorptive and postprandial plasma glucose between 90-120 mg/dl and <180 mg/dl, respectively. Standard meals were provided to ensure the same daily energy and macronutrient contents were consumed during the inpatient study. Drug/PBO was given on the second day of admission. The primary endpoint was the comparison between groups in the change in daily insulin requirements on day 4 from day 1. An interim analysis of the first 17 subjects found REMD-477 treatment reduced daily insulin use by 32% (4.2%, 60%) vs PBO on Day 4 (p=0.027). Average daily glucose assessed by CGM was 19 mg/dL (6.2, 31; p=0.006), and 26 mg/dL (8.2, 45; p=0.008) lower in the REMD-477 group than in the PBO group at Weeks 2 and 3 after treatment, respectively. Glucose time-in-range (70-180 mg/dL) for REMD-477 was 9.5% (2.7%, 16%; p=0.009) and 13% (1.9%, 25%; 0=0.026) greater in the REMD-477 group than in the PBO group during Weeks 2 and 3 after treatment, respectively. REMD-477 therapy was well tolerated and no episodes of severe hypoglycemia were noted. These data demonstrate that a single SC injection of REMD-477 reduces daily insulin requirements while simultaneously improving glucose control, as measured by average glucose concentration and glucose time-in-range, without increasing hypoglycemia in subjects with T1D

Shum-Tim (Canada)
(Associate member of Artificial Cells & Organs Research Centre)

Novel Application of Micro-Nanoparticles in the Treatment of Heart Diseases

[1]Professor D. Shum-Tim, MD., [2]A. Paul. Ph.D., H. [1]Al-Kindi, MD., [3]S. Prakash, Ph.D.
[1]Departments of Surgery, and Surgical Research, McGill University Health Center, McGill University, Faculty of Medicine, Montreal, Quebec, Canada. [2]Departments of Chemical and Petroleum Engineering, University of Kansas, Lawrence Kan, [3]Biomedical Technology and Cell Therapy Research Laboratory, Department of Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

Introduction: The technology of Micro-nanoparticles (MNPs) has advanced tremendously over the past decades that increasing innovative applications have been used in the diagnostic and therapeutic fields in medicine. Recently, our laboratory has applied this technology in drug and/or gene delivery for the treatment of ischemic heart diseases in experimental models. The MNPs may increase drug bioavailability, decrease drug toxicity, and side effects, while enhancing drug/gene deliveries by active or passive mechanisms. In this abstract, we will describe two applications of this technology for gene delivery in a coronary stent model of vascular injury and drug delivery in the treatment of ischemic heart failure model.

Methods: 1) Polymeric nanoparticles were used to encapsulate recombinant baculovirus containing genomic DNA (VGF1/Fluorescent green), known to promote vascular endothelial recovery after injury. A conventional coronary stent was then coated with MNPs loaded with recombinant baculovirus, which was deployed in the pneumatically injured femoral artery. The patency rate and immunochemical analysis of the stented arteries were compared between conventional bare metal stents vs. bioactive stents 8 weeks after deployment. 2) Polymeric MNPs were loaded with Milrinone using a double emulsion-solvent evaporation technique. Acute myocardial infarction (MI) was created by coronary ligation in a rodent model. One week after MI, conventional free-form of Milrinone vs. Milrinone prepared in microparticles was given intravenously, and vital signs and transthoracic echocardiography were monitored at pre-determined times for 24 hours. In-vitro release kinetics and characterization of the MNPs were evaluated at physiological conditions in both studies.

Results: 1) Fluorescent green was observed within hours of deployment in the vicinity of the stent and complete re-endothelialization of the denuded endothelial layer were observed in the bioactive stent. In addition, superior stent patency rate compared with bare metal stent was observed in the injured arteries 8 weeks post-operatively. 2) In rodents injected with Milrinone encapsulated with MNPs, the left ventricular ejection fraction at 90 min, and 3, 6, and 12 hours was significantly greater than in group given Milrinone at a conventional intravenous form. The plasma level of Milrinone was significantly higher, while the intercellular adhesion molecule and cytokine-induced neutrophil chemoattractant-1 levels were significantly lower in the encapsulated Milrinone group compared with the conventional group.

Conclusion: Our experimental studies have shown the feasibility of using micro-nanotechnologies to deliver genetic substrates to specific site of interest to exert its therapeutic effects. This target-specific delivery may enhance therapeutic efficacy, while minimizing toxic side effects. Micro-nanotechnology can also be used to deliver inotropic drug that may increase the drug circulation time with minimal hemodynamic side effect, and pass across biological barriers to allow subsequent internalization and distribution within tissue. We propose a new strategy for future gene/drug delivery in patients with ischemic heart disease and end-stage heart failure.

Stochaj, Ursula ursula.stochaj@mcgill.ca

GOLD NANOPARTICLES IMPAIR NUCLEAR FUNCTION AND PROTEOSTASIS IN CANCER CELLS

Dana Abou Samhadaneh[1], Khalid A. Alqarni[1], Ossama Moujaber[1], Dusica Maysinger[2], **Ursula Stochaj[1]***
[1]Department of Physiology, McGill University, Montreal, Canada
[2]Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada,
*Corresponding author:

Gold nanoparticles are promising tools for cancer research and therapy. The molecular mechanisms through which gold nanoparticles promote cancer cell killing are only beginning to emerge. The size and morphology of gold nanoparticles plays a role for the damage they inflict on cells. However, an in-depth understanding of these processes is still missing. To this end, we have assessed the impact of gold nanoparticles of different sizes and morphologies on human cancer cells. We have focused on the cell nucleus and proteostasis, because they provide read-outs for essential biological activities that control cell survival. We show that depending on size and morphology, gold nanoparticles alter the nuclear organization and function. Moreover, our studies reveal that gold nanourchins are particularly efficient for the impairment of protein homeostasis. Our work emphasizes the importance of proper gold nanoparticle design to achieve the efficient killing of cancer cells.

Keywords

Gold nanoparticles, nucleus, proteostasis, stress

Biodegradable Nanocapsules Containing A Nanobiotechnological Complex for the Suppression of A Melanoma Cell Line B16F10

Wang,Y[1,2] and TMS Chang[1]
[1] PhD Research done at Artificial Cells and Organs Research Centre, McGill University, Montreal, Canada
[2] Now Research staff, 3rd Hospital of Peking University Medical School (Alumni of Artificial Cells & Organs Research Centre)

www.medicine.mcgill.ca/artcell/2016Wang&Chang.pdf

The incidence of melanoma, a fatal skin cancer, has increased rapidly in the last few years. The American Cancer Society's estimates for melanoma in the United States for 2016 are 76,380 new cases of melanomas and about 10,130 people are expected to die of melanoma in 2016[1]. Once metastasized only palliative therapies are available. Immunotherapy, photodynamic therapy, chemotherapy and radiation therapies with their adverse side effects cannot improve the survival rates in advance melanoma.[1-3] Thus, there is an urgent need for new therapeutic strategy.

Melanoma depends on tyrosine (Tyr) for growth.[4] Recent interest in nanomedicine has resulted in the extension of artificial cells[5-7] to prepare nano-dimension nanocapsules, nanoparticles and soluble nanobiotechnological complexes.[8,9] For example, polyhemoglobin-tyrosinase can be prepared by the crosslinking of hemoglobin (Hb) and tyrosinase into a soluble nanobiotechnological complex for testing in melanoma mice.[10] However, this can only delay but not suppress the growth of melanoma in mice model.[10,11] In this paper, we combine two nanobiotechnological technologies into a single therapeutic agent. A soluble nanobiotechnological complex is first formed by crosslinking haemoglobin and tyrosinase into a soluble polyhemoglobin-tyrosinase complex. This is then nanoencapsulated into biodegradable polylactiide (PLA) nanocapsules to form nanocapsules containing polyhemoglobin-tyrosinase. We tested this in a highly malignant melanoma cell line B16F10 on inhibition of tumor growth, migration and colonization. We also analyzed possible mechanisms of action like ROS generation, apoptosis induction and effect on cell cycle.

Ferulic acid produced by the probiotic *Lactobacillus fermentum* NCIMB 5221 reduces developmental time through a dTOR-mediated mechanism

Westfall S1, Lomis N1, Iqbal U1, Prakash, S1 suswes00@gmail.com
1Biomedical Technology and Cell Therapy Research Laboratory, Dept. of Biomedical Engineering, McGill University, 3775 University Street, Montreal, Quebec Canada. +1-514-398-3676, satya.prakash@mcgill.ca

The gut microbiota is related to several energy regulating diseases including diabetes, obesity, metabolic syndrome and neurodegeneration. However, the mechanisms of communication between the gut microbiota and the host's physiology that maintain energy homeostasis remain elusive. The gut microbiota is a dynamic bidirectional system that communicates with the host either through direct interactions between resident microorganisms and the intestinal lining or through the secretion of bacterial metabolites that penetrate the epithelium and influence host physiology. Probiotics are health-promoting bacteria that when consumed, alter the balance of resident microorganisms conferring health benefits. To assess the role of the gut microbiota on energy homeostasis in the context of development, *Drosophila melanogaster* larvae were orally administered the probiotic *Lactobacillus fermentum* NCIMB 5221 or its metabolic product, ferulic acid: a potent anti-inflammatory and anti-oxidant hydroxycinnamic acid. In *Drosophila* larvae, both treatments advanced the nutritionally-dependent stages of development (P1-P3) in a dose-dependent manner while not affecting the hormonally-controlled puparition. These treatments correspondingly accelerated the developmental-phase dependent 20-hydroxyecdysone and insulin receptor gene expression surges and altered the phasic expression of downstream insulin signalling factors including Akt, TOR and FOXO indicating a deep level of nutritionally-dependent regulatory control. Co-feeding flies ferulic acid with the TOR inhibitor rapamycin eliminated the physiological and molecular developmental advances indicating that microbial ferulic acid affects energy utilization in a dTOR-dependent manner. TOR conservation from flies to humans indicates a therapeutic potential for the gut microbiota, specifically *L. fermentum* NCIMB 5221, in several human energy regulatory diseases such as obesity, diabetes and cancer.

Describing the novel prebiotic activity of Triphala extract and its impact on its anti-oxidant, immune and metabolic processes

Susan Westfall, Nikita Lomis, Umar Iqbal, Imen Kahouli, Satya Prakash
(1) Biomedical Technology and Cell Therapy Research Laboratory, Dept. of Biomedical Engineering, Dept. of Experimental Medicine, Faculty of Medicine, McGill University, 3775 University Street, Montreal, Quebec,

H3A2B4, Canada. *Tel: 1-514-398-3676, Fax: 1-514-398-7461. Email: satya.prakash@mcgill.ca suswes00@gmail.com

The gut microbiota is composed of a plethora of diverse bacterial species which is critical for energy utilization, gut motility and the digestive process. When balance of the bacterial species is lost (dysbiosis), a variety of diseases manifest including energy regulating diseases (diabetes, obesity, the metabolic syndrome), inflammatory diseases (irritable bowel syndrome, Crohn's disease, etc.) and imbalances in endocrine hormones, notably the HPA axis. Prebiotics are fermentable food ingredients that promote growth of beneficial gut microbiota and can shift dysbiosis towards a healthy bacterial population. Recently, the definition of prebiotics has expanded to include plant-derived polyphenols and phytochemicals that can act like prebiotics in the intestinal milieu. The present study for the first time characterizes the prebiotic activity of a novel Triphala water extract and determines its antioxidant, anti-inflammatory and metabolic effects. Triphala, a poly-herbal compound composed of three unique fruits, stimulated the growth of beneficial Lactobacillus and Bifidobacteria species in isolated cultures to the same or greater extent as the glucose control. Importantly, the Triphala water extract was more active than the prebiotic controls (inulin and fructooligosaccharides) at stimulating growth in the isolated culture. Using an automated *in vitro* model of the human gastrointestinal tract consisting of 5 bioreactors (the stomach, small intestine and the ascending, transverse and descending colon) in a temperature- and pH-controlled environment, it was shown that the Triphala water extract elevated levels of beneficial bacteria (*Lactobacillus* spp., *Bifidobacteria* spp., *Prevotellaceae*, *Ruminococcus*, etc.), reduced levels of pathogenic species (*Enterococcus* spp., *E. coli*, *Stapphylococcus*, etc.) and elevated the gut microbial metabolic products, namely the short-chain fatty acids . Based on these observations, the activity of the Triphala water extract was determined in an *in vivo* *Drosophila melanogaster* model. Through a variety of biochemical and enzymatic assays, Triphala water extract was shown to have potent anti-oxidant and anti-inflammatory activities while imparting beneficial energy-regulating changes on various markers of metabolism in diet-challenges diabetes and obesity models including total and circulating glucose, total triglycerides and genetic markers of metabolic stress (insulin receptor, dAkt-TOR-FOXO pathway, etc.). Many of these beneficial variations could be linked with the prebiotic activity of Triphala in the *Drosophila* gut. Overall, Triphala was determined to have very potent prebiotic activity which was associated with several physiological states (oxidative, inflammatory and energy homeostasis) that could ultimately be used therapeutically against many gut microbiota associated diseases including diabetes, obesity, arthritis, IBD, IBS, colon cancer and more.

Elucidating microbiome-host communication: Ferulic acid is a cross-talk mediator between *L. fermentum* NCIMB 5221 and the host metabolic, anti-oxidant and immune systems

Susan Westfall1, Nikita Lomis1, Umar Iqbal1, Satya Prakash1* suswes00@gmail.com
1Biomedical Technology and Cell Therapy Research Laboratory, Dept. of Biomedical Engineering, McGill University, Montreal, Canada

Probiotics are known to alleviate microbiome dysbiosis and corresponding diseases such as diabetes, obesity and neurodegeneration; however, the mechanisms of communication between the microbiome and host remain elusive. The present study investigates how the probiotic *Lactobacillus fermentum* NCIMB 5221 (Lf5221) impacts fundamental mechanisms of metabolic, anti-oxidant and immune pathways through the action of its secreted metabolite ferulic acid (FA). To test metabolic effects, *Drosophila melanogaster* were fed a high-fat or high-sugar diet. Co-treatment with FA or Lf5221, but not heat-inactivated Lf5221, significantly reduced diet-induced disturbances in body weight, glucose and triglyceride levels. Mechanistically, there were dramatic effects on insulin receptor, *dilp* and the upstream dAkt/dTOR/dFoxo pathway expression. Co-treatment with rapamycin, a TOR inhibitor, abolished the effects of Lf5221 and FA indicating that dTOR may be a key mechanistic link. Oxidative stress and immune deregulation also intertwine the microbiome with disease. Survivability of flies challenged with either hydrogen peroxide stress or *S. aureus* infection was enhanced when pre-treated with FA or Lf5221. This survivability was reflected in positive changes in immunological gene expression, namely Duox/Nox, IMD and the antimicrobial peptides and increased expression of the cytokine Spatzle, whose activity was assessed with antimicrobial assays. Survival to oxidative stress was reflected in the elevated activity of anti-oxidant enzymes and reduction in oxidants and lipid peroxidation. Together these mechanisms of metabolic, anti-oxidant and immune regulation could contribute to the host protective effects of the probiotic Lf5221 through the action of FA.

Yu WP (Canada) weipingy@lipont.com
President and CEO, Lipont Pharmaceuticals (Alumni of Artificial Cells & Organs Research Centre)
Liposome drug delivery: challenges and opportunities

Preparation of Zn^{2+} loaded chitosan beads based adsorbent for the removal of human testosterone in plasma

Huibin Yu[a], Shenqi Wang[a,*]
School of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China.
Corresponding author: Shenqi Wang, E-mail: shenqiwang131@hust.edu.cn, Fax: +86-27-87792205; Tel.: +86-27-87792216. yuhuibin2014@hust.edu.cn
More and more evidences have demonstrated that reducing the testosterone (T) level from human plasma can effectively alleviate the syndrome of patients suffering from prostate cancer. Due to most of testosterone is bound to sex hormone-binding globulin (SHBG) and there are two binding sites for Zn^{2+} on the surface of SHBG, we prepared Zn^{2+} loaded chitosan beads as adsorbent for the removal of testosterone from human plasma. The factors affecting their adsorption ability for testosterone were investigated. The adsorption percentage for testosterone reached about 35% at 90 min when 1,6-hexanediamine was used as spacers. The adsorbent was stored at 4℃ for 1 month and the adsorption percentage did not significantly decrease ($P>0.05$). The adsorption for testosterone was mainly ascribed to the adsorption for T-bound SHBG that could compete Zn^{2+} binding sites on beads with albumin. The adsorption percentage for total proteins in plasma was less than 5%. Therefore, it is a potential adsorbent for the removal of human testosterone in plasma.
Key Words: testosterone, chitosan bead, zinc ion, 1,6-hexanediamine, adsorbent

Zhang, Zhibing Z.Zhang@bham.ac.uk ;

Understanding the mechanical properties of cells, microspheres and microcapsules

Past President, President of Symposium on biocompatible capsules (UK)
School of Chemical Engineering, University of Birmingham, Birmingham B15 2TT, UK

Many biological and non-biological materials in microscopic particles such as cells, microspheres and microcapsules are used to produce functional products for a wide range of industrial sectors including pharmaceutical and medical, chemical, agrochemical, food and feed, personal and household care. Understanding the mechanical properties of microparticles is essential for predicting their behaviour in manufacturing and processing, and for maximising their performance in end-use applications. However, it had not been possible to determine the mechanical properties of single microparticles until a novel micromanipulation technique was developed at the University of Birmingham, UK in 1990. The technique is capable of determining the mechanical properties of both biological and non-biological particles as small as 400 nm in diameter, and can be used for obtaining force-displacement data of single microparticles at high deformations, including those corresponding to rupture. The technique was enhanced by mathematical modelling and finite element analysis that allow intrinsic material properties to be determined, for example, the particle (or particle wall) elastic modulus, viscoelastic and plastic properties, and stress/strain at rupture. For biological materials, applications of this technique include understanding mechanical damage to animal cells in suspension cultures, yeast and bacterial disruption in downstream processing equipment, biomechanics of chondrocytes and chondrons for tissue engineering, and adhesion and cohesion of biofilms and food fouling deposits. For non-biological materials, applications include understanding and controlling particle breakage in processing equipment, and the formulation of microcapsules with optimum mechanical strength to achieve controlled release and targeted delivery of functional active ingredients. The knowledge generated has helped a number of companies commercialise particulate functional products, and the details will be presented.

Peripheral nerve system repair with the bi-directional induction and system remodeling from central system and target organs

Peixun Zhang, Yuhui Kou, Na Han, Xiaofeng Yin, Baoguo Jiang
Peking University People's Hospital, Beijing, China.100044
., zhangpeixun@bjmu.edu.cn.Baoguo Jiang, M.D., jiangbaoguo@vip.sina.com.

Peripheral target organs reversely induce the structural and functional remodeling of various levels of the proximal peripheral nervous system, spinal cord and upper central nerves according to their own functional needs.The various peripheral nerve transfer operations derived from the surgical repair mentioned above, such as the use of a proportion of or the whole C7 to contra-laterally repair the C5-8 branchial plexus injuries, allowed the patient's normal C7 to contralaterally control elbow flexion, elbow extension and wrist extension after a period of rehabilitation exercise. These results indicated that changes in the peripheral target organs on the injured side induced the positioning and differentiation at various levels up to the central nerves that originally controlled the normal C7 effectors, causing the establishment of new connections between the peripheral nerves and the injured target organs and the consequent effective re-control of the peripheral target organs. Those clinical cases and animal models all showed that the nerve cross transfer after the surgical repair allows the controlling nerves to function as the controlled effector. This suggests there is structural and functional remodeling of the corresponding proximal nerves.

Dongfang Zhou, east@ciac.ac.cn

A Facile Way to Prepare Functionalized Dextran Nanogels for Conjugation of Hemoglobin

Dongfang Zhou, Xing Wei, Yubin Huang*
State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, ChangChun 130022, People's Republic of China
Nanogels with several special advantages have been widely applied in protein delivery. However, biocompatible and biodegradable nanogels used for hemoglobin (Hb) delivery are far less explored. Herein, we developed a facile method to prepare functionalized dextran nanogels for conjugation of Hb. *In situ* cross-linked and aldehyde group functionalized nanogels (FNGs) were prepared from dextran-g-succinic anhydride-g-dopamine conjugate (Dex-SA-DA) assembly by simple pH adjustion and oxidization in water. Hb was further conjugated into the swelling FNGs by Schiff base reaction under mild condition. The obtained hemoglobin-loaded nanogels (HbNGs) exhibited high stability, oxygen affinity and good hemo-compatibility, suggesting the potential for oxygen carriers. We expected that the designed functionalized nanogels with high stability and loading capacity could bring a new opportunity for protein delivery.

Key words: Oxygen carriers, Hemglobin, Self-assebly, *In situ* chemical cross-linking, Functionlized nanogels.

Zou, Hequn Zou (China) , hequnzou@hotmail.com
Vice-president, Chinese Society of Apheresis
Director, Institute of Nephrology and Urology,
Southern Medical University, Guangzhou, China

Adsorbent Based Plasmapheresis for Autoimmune/Inflammation Diseases

Hequn Zou, Xinyu Liu, Xiaohong Wang, Bin Li, Yongqiang Li, Shao Xiaofei, Jiamin Li.
Institute of Nephrology and Urology, The Third Affiliated Hospital of Southern Medical University, Guangzhou 510630, China
Our blood purification center actively applied plasma adsorption (PA) therapy in treating different kinds of rheumatics and auto-immune/inflammation diseases, and further deeply understood the great foreground in clinical application of plasmapheresis techniques.
Methods: More than 200 cases of rheumatics and auto-immune/inflammation diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis, antibody-mediated transplant kidney rejection, multiple sclerosis, ankylosing spondylitis, vasculitis and amyotrophic lateral sclerosis were treated with plasma adsorption (PA) therapy. Plasauto-IQ plasmapheresis machine (Japan Asahi company), Plasmaflo OP-08W plasma separator and IMMUSORBA PH-350 or TR-350 absorbers were respectively used for PA treatment. The quantity of blood flood was 80-120ml/min. The treatment plasma quantity for each time was 1.5 times of plasma volume of each patient.
Results: In patients with SLE, including pregnant cases, SLEDAI score, serum auto-antibodies, IgG, C3 and 24-hour urine protein were significantly reduced after PA treatment. Morning stiffness time was shortened and the sign of weakness was improved in 80% (8/10) cases of rheumatoid arthritis. Joint swelling and pain were alleviated in 70% (7/10) cases. Among the 10 cases of rheumatoid arthritis, 7 cases had positive rheumatoid factor (RF) that turned to be negative after plasmapheresis. Joint pain was improved in all cases (100%) of ankylosing spondylitis. Based on Arnett Standard of function improvement, the effective rate of PA therapy was 86%. After plasma absorption, all cases of amyotrophic lateral sclerosis got muscle strength improvement. The case receiving mechanical assisted ventilation could intermittently wean from mechanical ventilation. For patients with multiple sclerosis and vasculitis, significant efficacy was shown in improving not only symptoms but also organ function.
Conclusion: Our results suggested significant advantages of PA therapy in treating rheumatics and auto-immune/inflammation diseases and its mechanisms might be related to rapid and effective clearance of in vivo pathogenic substances in patient blood. When combined with drug treatment, it can rapidly control the disease, increase the efficacy and reduce complications of immunosuppressant.
Keywords: Adsorbent, plasmapheresis, rheumatics, autoimmune/inflammation diseases.

Zou, Hequn Zou (China) , hequnzou@hotmail.com
Vice-president, Chinese Society of Apheresis
Director, Institute of Nephrology and Urology,
Southern Medical University, Guangzhou, China

Nanomedicine in the Early Diagnosis of Diabetes

Dongfeng Gu, Luca Musante, Yongqiang Li, Xinyu Liu, Shuiwang Hu, Xiaomeng Xu, Hanfei Lin, Harry Holthofer, Hequn Zou
Institute of Nephrology and Urology, The Third Affiliated Hospital of Southern Medical University, Guangzhou 510630, China; Centre for BioAnalytical Sciences (CBAS), Dublin City University, Dublin 9, Ireland; Department of Pathophysiology and Key Laboratory of Functional Proteomics of Guangdong Province, Southern Medical University, Guangzhou 510515, China
Purpose: To search for potential urine biomarkers for early diagnosis of prediabetes and early diabetic nephropathy based on proteomic analysis of urine exosomes enriched with a novel namomembrane separation technique developed by my team.
Methods: Community screening involving 2142 residents aged 18 years or older was performed in the three communities randomly selected from a Southern city of China. Demographic data, living style, physical activity, personal and family history were obtained. Physical examination and blood and urine biochemical tests were performed. Urine exosomes were enriched, by means of a novel nanomembrane concentrator method developed by my team, from residents screened and diagnosed respectively as prediabetes, diabetes mellitus with normal urine protein output, diabetic nephropathy with microalbuminuria, diabetic nephropathy with macroproteinuria, and normal controls. Transmission electron microscope (TEM) nanoparticle tracking analysis (NTA) were used to observe the morphology and intensity of exosomes. Differential gel electrophoresis (DIGE) and ABI 4800 Proteomic Analyzer MALDI-TOF-MS were used respectively to isolate and identify protein from exosomes.
Results: MASP2 and CALB1 were significantly increased while Protein S100 A8 and Protein S100 A9 were significantly decreased in the urine exosomes from the community residents respectively diagnosed as prediabetes and early stage of diabetic nephropathy even without microalbuminuria.
Conclusion: It is suggested by our study with the novel nanomembrane concentration technique that prediabetes and early stage of diabetic nephropathy is characterized by a distinct change in urine exosomal protein signature. It is also suggested that the novel nanomembrane separation technique and NTA are useful assays to find potential biomarkers from urine exosomes and furthermore the accurate pathways involved in prediabetea and early diabetic kidney injury.
Keywords: nanomembrane, NTA, exosome, proteomics, prediabetes, diabetic nephropathy.