THE 3RD AMDI POSTGRADUATE COLLOQUIUM 2017

25-26 JULY 2017

SEMINAR ROOM, ANIMAL RESEARCH CENTRE, IPPT

Themes of the particular interest include, but not limited to:

- Applied Sciences and Technologies
- Biological Sciences
- Biotechnology
- Medical and Health Sciences
- Bioinformatics
- Biomedical Engineering
- Chemical Sciences
- Physical Sciences

http://www.amdi.usm.my/index.php/colloquium17

CONTENTS

WELCOMING MESSAGE

Assalamualaikum WBT and Salam Sejahtera to all.

Welcome to the 3rd AMDI Postgraduate Colloquium 2017 organised by the Lifestyle Science Cluster of the Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia. This colloquium is now in its third edition in the running with its inception in 2014 and the second edition in 2015.

AMDI is known as a postgraduate research institute specializing in specific medical and dental sciences. It plays a vital role in producing competent medical and dental specialists who are, among others, capable of conducting clinical trials in the northern region of Malaysia, at our established Clinical Trial Centre. This colloquium allows critical reflection on actual research efforts and venturing into pioneer research.

I would like to congratulate Lifestyle Science Cluster and the Organising Committee for taking the responsibility to organize this postgraduate colloquium. It is my humble wish that the colloquium will promote knowledge exchange, research and educational links for all the postgraduate students at the Advanced Medical and Dental Institute.

I also would like to acknowledge the significant participation of our guest speakers from other academic institutions in sharing their knowledge and the commonalities of their research findings and exchange ideas with fellows and academic members of the institute.

Last but not the least, I wish you all an intellectually stimulating time at this colloquium.

Thank you.

Dr. Norehan Mokhtar

Director
Advanced Medical and Dental Institute
Universiti Sains Malaysia

Patron
The 3rd AMDI Postgraduate Colloquium 2017

WELCOMING MESSAGE

Assalamualaikum WBT and Salam Sejahtera to all.

Greetings to our distinguished guest speakers and all the participants. I take this pleasure to warmly welcome you to the 3rd AMDI Postgraduate Colloquium 2017, which is organized by the Lifestyle Science Cluster of the Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia.

I would like to congratulate the Lifestyle Science Cluster for having the initiative to continue this event. It gives me great pleasure to know that the academic staffs of this institute have taken the lead in organizing such an intellectual event on top of their teaching and research activities.

This edition of the colloquium will see young researchers from our institute showcasing their research abilities that they have fostered during their time here at AMDI and sharing their exciting findings. The program for this colloquium was designed to bridge the gap between the different field of sciences, making it possible for non-experts in each area to gain insight into new areas. Also, included among the speakers are several brilliant scientists who have brought new perspectives to their respective fields. I strongly believe that this colloquium will have a significant impact on the current development of research and will also create opportunities for productive discussion and collaboration among the participants.

I sincerely hope that this colloquium will provide valuable insight to all participants, who will contribute to further development and collaborative research work in future. May I wish you all an enriching and memorable two days at the 3rd AMDI Postgraduate Colloquium 2017.

Thank you.

Assoc. Prof. Dr. Bakiah Shaharuddin

Deputy Director (Academic and Students Affairs)
Advanced Medical and Dental Institute
Universiti Sains Malaysia

Advisor
The 3rd AMDI Postgraduate Colloquium 2017

WELCOMING MESSAGE

Best wishes to all.

Welcome to the 3rd AMDI Postgraduate Colloquium 2017.

The Advanced Medical and Dental Institute (AMDI) is pleased to be able to continue to organize this scientific event that has been highly anticipated. I would like to express my special thanks to all members of the scientific and program committee, and Lifestyle Science Cluster for the dedication, thoroughness, and great effort in organising this colloquium.

The colloquium aims to provide a platform for all the postgraduate students and postdoctoral fellows at the AMDI to interact by sharing their research, discussing the current/future trends in their respective research areas, and exchanging ideas with other senior colleagues and academic members of the institute.

This meeting also seeks to foster dialog among the industry collaborators, academicians, and postgraduate students to address some of the challenges in translating the fundamental research finding in the laboratory (bench-side) into a potential treatment for the disease (bedside) or industrial application (community).

The 3rd AMDI Postgraduate Colloquium 2017 covers various topics including medical and health sciences, applied sciences and technologies, chemical and physical sciences. It features several invited lectures, the showcase of different research groups within the AMDI, and industry talks to provide the student with inspiration in the translation of research to practice, as well as the career beyond the academia.

I hope that this colloquium will be the most enjoyable, productive and informative academic event that participants have ever experienced in 2017.

Thank you.

Dr. Ooi Cheong Hwa

Senior Lecturer
Lifestyle Science Cluster
Advanced Medical and Dental Institute
Universiti Sains Malaysia

Organising Chair
The 3rd AMDI Postgraduate Colloquium 2017

ORGANISING COMMITTEE

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GUEST SPEAKERS

Invited Lecture

Prof. Dr. Liong Min-Tze | School of Industrial Technology, USM

Assoc. Prof. Dr. Siti Noor Fazliah Mohd Noor | AMDI, USM

Dr. Mohd Ghows Mohd Azzam | School of Biological Sciences, USM

Dr. Abdul Rashid Aziz | Singapore Sports Institute, Singapore Sports

AMDI Research Group Showcase

Assoc. Prof. Dr. Bakiah Shaharuddin
Eye Stem Cells Research Group

Assoc. Prof. Dr. Md Azman PKM Seeni Mohamed
ASG on Molecular Toxicopathology

Assoc. Prof. Dr. Badrul Hisham Yahaya
Stem Cell and Gene Therapy Group

Assoc. Prof. Dr. Ahmad Munir Che Muhamed
Thermal Physiology Research Group

Dr. Citartan Marimuthu
Aptanostics of RNA-Biology Group

Dr. Ng Siew Kit
Molecular Hydrogen Research of RNA-Biology Group

Industry Talk

Mr. Huang Chee Giap
Sterling Circle Sdn Bhd

INVITED SPEAKERS

Dr. Liong Min Tze obtained her Ph.D. from Victoria University (VU), Melbourne, Australia in 2006 at the age of 28. She joined Universiti Sains Malaysia in December 2006, was promoted to Senior Lecturer/Assistant Professor in August 2008, Associate Professor in July 2010, and Professor in October 2015. She has a H-index of 20 (as of 2016) and has published over 90 papers in international indexed journals, four book chapters and edited two scientific books. She is also on the Editorial Board of several international journals and has delivered keynote and plenary lectures both nationally and internationally. Dr. Liong has graduated both M.Sc. and Ph.D. students and is currently supervising two MSc and seven Ph.D. students, all in full research mode. Liong was the recipient of the Best Research Student Award presented by VU in 2006, top three finalists for the Malcolm Bird Award presented by AIFST in 2006, named one of the three top young women scientists in Malaysia in 2007 by L'Oreal-UNESCO, IAP Young Scientist Award in 2015, and Top Researcher Scientists Malaysia (TRSM) in 2016.

Dr. Siti Noor Fazliah Mohd Noor received Bachelor of Dental Surgery in 1997 and completed Master in Clinical Dentistry (Child Dental Health) in 2004 from Universiti Malaya. She obtained MFDS, from the Royal College of Surgeon of England in 2008, and has completed a Ph.D. in biomaterials and stem cells from Imperial College London, United Kingdom in 2015. Her current appointment is as Head of the Craniofacial and Biomaterial Sciences Cluster, cum Paediatric Dentistry Consultant, Advanced Medical and Dental Institute, Universiti Sains Malaysia. Dr. Fazliah has research interests in craniofacial development and anomalies, biomaterials and stem cells.

Dr. Mohd Ghows Mohd Azzam is currently a senior lecturer in the School of Biological Sciences, Universiti Sains Malaysia (USM). He obtained his DPhil from Department of Physiology, Anatomy and Genetics in University of Oxford in 2012 where he started work on miRNA biogenesis. Then he pursued a postdoctoral research training for another two years in the Weatherall Institute of Molecular Medicine, University of Oxford, continuing his research interest on miRNAs and employing genome engineering techniques (CRISPR and TALENs) to understand the functional miRNA-target interaction. Currently, one of his main laboratory research interest in on understanding the virus-host interaction between dengue virus and mosquito involving small RNA profiling and transcriptomics. Dr Ghows was also awarded the Young Investigator Award from the Young Scientist Network – Academy of Sciences Malaysia during the 11th Malaysia Genetic Congress in 2015.

Dr. Abdul Rashid Aziz has been involved in Singapore's sporting scene for over 20 years. Rashid is currently the Head of Strength and Conditioning Department at the Singapore Sports Institute (SSI) where he serves as an advisor and consultant to elite local athletes and their coaches from a broad range of high performance sports, on many aspects of sports-specific testing, training for fitness and preparation for peak performance. He was previously Head of the Sports and Performance Physiology Unit at SSI. Dr. Rashid's work in the areas of exercise science and sports performance training and testing has been published in international sports medicine and sports science journals. He is a pioneer in research investigating emerging Asian-dominated sports such as sepak-takraw and pencak-silat. His research interest lies in the practical applications of research findings to the improvements of the athletes' sporting performances. Rashid obtained his PhD at Nanyang Technological University, Singapore, where he examined the physiological effects of Ramadan fasting on competitive sporting performances and training of Muslim athletes, and designing ways to attenuate or circumvent the impact of Ramadan fasting on Muslim athletes' performance.

ORAL PRESENTERS

Alvin Paul (Infectomics Cluster)

Azali Azlan (Infectomics Cluster / School of Biological Sciences)

Chew Yik Wei (Oncological & Radiological Sciences Cluster)

Diyana Mazuin Ridzuan (Regenerative Medicine Cluster)

Goh Ying Hwa (Regenerative Medicine Cluster)

Janet Mary Anne Santhanam (Integrative Medicine Cluster / School of Chemical Sciences)

Kasturi Purushodman (Oncological & Radiological Sciences Cluster)

Lee Pei Chen (Regenerative Medicine Cluster)

Mouylin Chem (Integrative Medicine Cluster)

Muhammad Asyraf Abduraman (Lifestyle Science Cluster)

Nazilah Abdul Satar (Regenerative Medicine Cluster)

Ng Wai Hoe (Regenerative Medicine Cluster)

Nur Shuhaidatul Sarmiza Abdul Halim (Regenerative Medicine Cluster)

Ong Si Min (Regenerative Medicine Cluster)

Ooi Pei Wan (Integrative Medicine Cluster)

Prabu Siva Sankar (Infectomics Cluster)

Presela Ravinderan (Infectomics Cluster)

Rasmey Soeung (Integrative Medicine Cluster)

Rodziah Romli (Craniofacial and Biomaterials Sciences Cluster)

Rogini Sivalingam (Infectomics Cluster)

Ros Amizah Abdullah (Lifestyle Science Cluster)

Siti Maisura Azmi (Regenerative Medicine Cluster)

Tan Lee-Lee (Infectomics Cluster)

Tey Lee Hung (Infectomics Cluster)

Wasiu Balogun (Integrative Medicine Cluster)

PROGRAM – DAY 1

Time	Events on July 25, Tuesday
07:45-08:15	Registration
08:15-08:30	Opening Ceremony By **Dr. Norehan Mokhtar (Director, Advanced Medical and Dental Institute, USM)**
08:30-09:00	Invited Lecture by Research Fellow of AMDI **"Molecular Response of Dengue Virus Infection"** **Dr. Mohd Ghows Mohd Azzam (School of Biological Sciences, USM)**
09:00-09:30	AMDI Research Group Showcase #1 **Eye Stem Cells Research Group** By **Assoc. Prof. Dr. Bakiah Shaharuddin (Regenerative Medicine Cluster)**
09:30-10:30	Oral Presentation Session **Chairperson: Ros Amizah Abdullah** **1.** Small Non-coding RNA Profiles of DENV1-infected *Aedes albopictus* Cell – **Azali Azlan (Infectomics Cluster / School of Biological Sciences)** **2.** Human Umbilical Cord-derived Mesenchymal Stem Cells Improve Expression of Human Corneal Epithelial Cells in Spherical Culture – **Siti Maisura Azmi (Regenerative Medicine Cluster)** **3.** Murine Cardiac Stem Cell Growth, Differentiation and Resistance to Oxidative Stress on Human Mesenchymal Stem Cell-derived Extracellular Matrices – **Ng Wai Hoe (Regenerative Medicine Cluster)**
10:30-11:00	Tea Break / Exhibition / Networking
11:00-11:30	Invited Lecture **"Probiotics and Health: Research, Application and Commercialization"** **Prof. Dr. Liong Min Tze (School of Industrial Technology, USM)**
11:30-12:00	AMDI Research Group Showcase #2 **Aptanostics of RNA-Bio Group** By **Dr. Citartan Marimuthu (Infectomics Cluster)**
12:00-13:00	Oral Presentation Session **Chairperson: Ros Amizah Abdullah** **4.** In vitro Evolution Eventuates the Isolation of RNA Aptamer Against Dengue Virus 2 NS1 Glycoprotein – **Rogini Sivalingam (Infectomics Cluster)**

	5. Generation of an RNA Aptamer Against Human Pituitary Tumor Transforming Protein 1 (PTTG1) – **Prabu Siva Sankar (Infectomics Cluster)** **6.** Generation of RNA Aptamer Against Progesterone Receptor DNA Binding Domain Towards the Development of Aptahistostaining of Breast Cancer – **Presela Ravinderan (Infectomics Cluster)**
13:00-14:00	Lunch Break
14:00-14:30	Industry Talk **"The Emerging Market of Molecular Hydrogen Products: What Can a Marriage of Science and Industry Achieve?"** **Mr. Huan Chee Giap (Sterling Circle Sdn Bhd)**
14:30-15:00	AMDI Research Group Showcase #3 **Molecular Hydrogen Research of RNA-Bio Group** By **Dr. Ng Siew Kit (Infectomics Cluster)**
15:00-17:00	Oral Presentation Session **Chairperson: Harris Kamal Kamaruddin** **7.** Limiting Exercise-induced Muscle Damage with Hydrogen-rich Water Ingestion: The Potential Therapeutic Effect – **Ros Amizah Abdullah (Lifestyle Science Cluster)** **8.** Cervical Cencer and Pap Smear Screening: Knowledge, Attitude, and Practice among Working Women in Northern States of Malaysia – **Rodziah Romli (Craniofacial and Biomaterials Sciences Cluster)** **9.** Effect of Bifunctional Isocyanate Linker on Adsorption of 2,4-dinitrophenol onto β-cyclodextrin – **Janet Mary Anne Santhanam (Integrative Medicine Cluster / School of Chemical Sciences)** **10.** Electrokinetic Supercharging in Capillary Electrophoresis for the On-line Preconcentration of Seebumeton in Water Samples – **Rasmey Soeung (Integrative Medicine Cluster)** **11.** The Effects of Mitragynine on the P-glycoprotein mRNA and Protein Expression in Brain Endothelial Cell – **Muhammad Asyraf Abduraman (Lifestyle Science Cluster)** **12.** Development of Novel Exosome-based Delivery System for Proteins – **Chew Yik Wei (Oncological & Radiological Sciences Cluster)**

PROGRAM – DAY 2

Time	Events on July 26, Wednesday
08:00-08:30	Registration
08:30-09:00	Invited Lecture by Research Fellow of AMDI **"The Potential Health Benefits of Exercising in the Fasted State"** **Dr. Abdul Rashid Aziz (Singapore Sports Institute, Singapore Sports)**
09:00-09:30	AMDI Research Group Showcase #4 **Thermal Physiology Research Group** By **Assoc. Prof. Dr. Ahmad Munir Che Muhamed (Lifestyle Science Cluster)**
09:30-10:30	Oral Presentation Session **Chairperson: Ros Amizah Abdullah** **13.** Functional Evaluation of Flag-STAT1 in U3A Cells – **Tey Lee Hung (Infectomics Cluster)** **14.** Investigating the Effect of PPAR-γ Ligands on TIGIT and ICOS Expression on CD4+CD25+FoxP3+T Regulatory Cells – **Lee Pei Chen (Regenerative Medicine Cluster)** **15.** IRF9 Phosphorylation in Type 1 Interferon Response – **Alvin Paul (Infectomics Cluster)**
10:30-11:00	Tea Break / Exhibition / Networking
11:00-11:30	Invited Lecture **"Tissue Engineering in Dental Science"** **Assoc. Prof. Dr. Siti Noor Fazliah Mohd Noor (AMDI, USM)**
11:30-12:00	AMDI Research Group Showcase #5 **Stem Cell and Gene Therapy Group** By **Assoc. Prof. Dr. Badrul Hisham Yahaya (Regenerative Medicine Cluster)**
12:00-13:00	Oral Presentation Session **Chairperson: Ros Amizah Abdullah** **16.** Suppression of *AML1/ETO* via siRNA-mediated Gene Knockdown and Its Effects on *FOXO3* and *c-MYC* Expression in AML t(8,21) Cells – **Ong Si Min (Regenerative Medicine Cluster)** **17.** Downregulation of *SKP2* via siRNA-mediated Gene Knockdown and Its Effects on *FOXO3* and *c-MYC* Expression in AML t(8,21) Cells – **Kasturi Purushodman (Oncological & Radiological Sciences Cluster)**

	18. Study on the Adsorption of DNA on β-cyclodextrin Nanoparticles – **Goh Ying Hwa (Regenerative Medicine Cluster)**
13:00-14:00	Lunch Break
14:00-14:30	AMDI Research Group Showcase #6 **ASG on Molecular Toxicopathology & "What's Next After the PhD?"** By **Assoc. Prof. Dr. Md Azman PKM Seeni Mohamed (Integrative Medicine Cluster)**
14:30-16:50	Oral Presentation Session **Chairperson: Muhammad Asyraf Abduraman** **19.** Ultrastructural and Biochemical Responses of the Earthworm *Eudrilus eugeniae* Exposed to Contaminated Soil: Effects of Heterocyclic Amines – **Wasiu Balogun (Integrative Medicine Cluster)** **20.** A Simplified In-house Extraction Method Suitable for Food PCR Analysis – **Tan Lee-Lee (Infectomics Cluster)** **21.** Determination of the Marker Compounds in Adulterated and Non-adulterated Stingless Bee Honey Using GC-MS – **Mouylin Chem (Integrative Medicine Cluster)** **22.** Analysis of Phthalate via Spectrophotometry in Environmental Samples Using Non-ionic Silicone Surfactant-mediated Cloud Point Extraction – **Ooi Pei Wan (Integrative Medicine Cluster)** **23.** Curcumin Potentially Inhibiting and Preventing the Self-renewal Capability of Lung Cancer Stem Cells (CSCs) Derived from Non-small Cell Lung Cancer (NSCLC) – **Nazilah Abdul Satar (Regenerative Medicine Cluster)** **24.** Use of Fresh Frozen Plasma at Hospital Selayang: A Retrospective Study – **Diyana Mazuin Ridzuan (Regenerative Medicine Cluster)** **25.** In-vitro Co-culture Model of Mesenchymal Stem Cell Stimulate Airway Epithelial Repair and Regeneration via a Paracrine-mediated Reparative Mechanism – **Nur Shuhaidatul Sarmiza Abdul Halim (Regenerative Medicine Cluster)**
16:50-17:05	Award and Closing Ceremony By **Assoc. Prof. Dr. Bakiah Shaharuddin (Deputy Director, Academic and Student Affairs Division, AMDI)**

ABSTRACTS

Oral Presentation 1

Small Non-coding RNA Profiles of DENV1-infected *Aedes albopictus* Cell

Azali Azlan[1], Muhammad Amir Yunus[2], Ghows Azzam[1,2]

[1]School of Biological Sciences, Universiti Sains Malaysia, Malaysia. [2]Infectomics Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Malaysia.

Background: Small RNAs post-transcriptionally regulate gene expression, and are crucial in many biological processes such as development, immunity and infection, especially in *Aedes* mosquito. Three major small RNAs – microRNA (miRNA), small-interfering RNA (siRNA), and PIWI-interacting RNA (piRNA), are all present in mosquitoes, *Aedes albopictus* (*Ae. albopictus*) and *Aedes aegypti* (*Ae. aegypti*). Unlike *Ae. aegypti*, small RNA profiles of *Ae. albopictus* are not well-characterized and little is known about their biological functions. Dengue virus (DENV) is a single-stranded, positive-strand RNA virus that belongs to the family of Flaviviridae. DENV is transmitted by *Aedes* mosquitoes. and it can cause dengue fever. The alarming increase of dengue fever outbreak in Malaysia and other countries requires immediate attention. **Objective:** The aim of this study was to comprehensively characterize small RNA profiles in *Ae. albopictus* cells (C6/36) following DENV1 (one of the 4 serotypes of DENV) infection. In this study, DENV1 was chosen since there is no study that reports the relationship between DENV1 and ncRNAs in *A. albopictus*. **Method:** C6/36 cells were infected with DENV1 at multiplicity of infection of 0.5. After 3-days post-infection, total RNA was extracted. The total RNA integrity was checked, and RNA samples were sent for Illumina next-generation sequencing. miRNA discovery and expression were analyzed using mirDeep2 software, while analysis on piRNA was done using proTRAC. **Results:** A total of 363 mature and 447 precursor miRNAs were discovered. Following DENV1 infection, most miRNAs were downregulted. piRNAs in C6/36 cells exhibit canonical characteristic of piRNAs (5'U bias and ping-pong signature). It was found that C6/36 cells encode a significant number of smaller piRNAs which fall between 18-23 nt in size. They also showed similar characteristics as the canonical piRNAs. Upon DENV1 infection, it was observed that piRNA expression were not interrupted. It was found that certain predicted piRNA clusters only appeared following DENV1 infection; thereby, they were categorized as DENV1-specific clusters. **Conclusion:** DENV1 infection results in the rewiring the transcriptional pattern of miRNAs and piRNAs in C6/36 cells. Further functional assays of the miRNAs and piRNAs necessary to dissect the mechanisms underlying this observation.

Keywords: miRNA, piRNA, *Aedes albopictus*, dengue virus

Acknowledgement: This work was funded by University Sains Malaysia Research University Grant (1001/PBIOLOGI/811320) and ScienceFund (305/PBIOLOGI/613238).

Oral Presentation 2

Human Umbilical Cord-derived Mesenchymal Stem Cells Improve Expression of Human Corneal Epithelial Cells in Spherical Culture

Siti Maisura Azmi, Wai Hoe Ng, Jun Jie Tan, Rafeezul Mohamed, Bakiah Shaharuddin

Regenerative Medicine Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia.

Background: Transplantation of cornea limbal stem cells is the contemporary cellular therapy for cornea surface regeneration. The difficulty in maintaining the self-renewal capability of cells is due to the insufficient microenvironment. Cells in spherical culture exhibit extensive cell-cell contacts mimic the morphology and ultrastructure of the native environment thus improve stem cell functions and engraftment. Umbilical cord-derived mesenchymal stem cells (hUC-MSCs) which have great regenerative potentials were introduced to human corneal epithelial cells (HCECs) to improve its characteristics, proliferation and cellular migration. **Objective:** To investigate the effects of hUC-MSC on HCEC on cornea stem cell expression and cell proliferation. **Method:** Spheres were generated from HCECs alone or combination of both for four days in StemPro MSC CTS serum free medium. The spheres were harvested and characterised by immunocytochemistry. These spheres were indirectly co-cultured with HCECs using transwell and scratch migration assay was performed to assess the effect of the spheres on HCECs after 24 hours. Cellular proliferation was evaluated using Ki67 staining. **Results:** Generation of spheres has improved in HCEC-UC-MSCs co-cultures compared to HCECs alone. Co-culture with hUC-MSCs in generating spheres maintained corneal markers (p63, ABCB5, ABCG2 positive) and Cytokeratin 3 negative. Co-cultured spheres significantly enhanced the migration of HCECs and the cells migrated out showed significantly expression of Ki67. **Conclusion:** hUC-MSCs improved the generation of sphere in HCECs co-culture and maintained corneal stem cell markers. Spherical co-culture of hUC-MSCs and HCECs enhanced HCECs cellular proliferation. Secretion of paracrine factors by hUC-MSC may be the underlying key factors the beneficial effects of hUC-MSC in cornea repair.

Keywords: HCEC, UC-MSC, co-culture, spheres

Acknowledgement: This project was funded by Fundamental Research Grant Scheme (203.CIPPT.6711509).

Oral Presentation 3

Murine Cardiac Stem Cell Growth, Differentiation and Resistance to Oxidative Stress on Human Mesenchymal Stem Cells-derived Extracellular Matrices

Wai Hoe Ng[1], Rajesh Ramasamy[2], Yoke Keong Yong[3], Bakiah Shaharuddin[1], Jun Jie Tan[1]

[1]Advanced Medical and Dental Institute, Universiti Sains Malaysia. [2]Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. [3]Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

Background: Myocardial infarction remains the number one killer disease worldwide. Cellular therapy using cardiac stem cells (CSCs) are capable of regenerating injured heart. Previous studies showed mesenchymal stem cell-derived (MSC) extracellular matrices can provide structural support, and are capable of regulating stem cell functions and differentiation. **Objective**: This study aimed to evaluate the effects of human MSC-derived matrices for CSC proliferation, differentiation and its resistance to oxidative stress. **Method**: Human Wharton Jelly-derived MSCs were cultured in ascorbic acid supplemented medium for 14 days to full confluency prior to decellularisation using two methods: 1% SDS and 0.1% Triton X-100 (ST) or 20 mM ammonia buffer and 0.5% Triton X-100 (AT). Murine CSCs, isolated from 4-week-old C57/BL6N mice, were cultured on the decellularised MSC matrices, and were induced to differentiate into cardiomyocytes in cardiogenic medium for 21 days. Cardiomyocyte differentiation was assessed by immunocytochemistry and qPCR. All data were compared to CSCs grew on culture plastic without ECM, and analysed using ANOVA. **Results:** *In vitro* decellularisation using ST method caused matrix delamination from the wells. In contrast, decellularisation using AT method improved the matrix retention up to 30% ($p<0.05$). This effect was further enhanced when MSCs were cultured in cardiogenic medium, with up to 90% matrix retention rate. CSCs cultured on cardiogenic MSC matrix, however, did not significantly improve its proliferation after 3 days ($p<0.05$). Nevertheless, the viability of CSCs was augmented to 67.2 ± 0.7% after 24-h exposure to H_2O_2 stress, as compared to 42.9 ± 0.5% in control CSCs ($p<0.05$). Furthermore, CSCs cultured on cardiogenic MSC matrices showed 1.7-fold up-regulation in cardiac troponin I (cTnI) gene expression after 21 days ($p<0.05$). **Conclusion:** Cardiogenic MSC-derived matrices could protect CSCs from oxidative stress while improving cardiomyocyte differentiation, with potential application in myocardial tissue engineering or cell therapy.

Keywords: extracellular matrices, cardiac stem cells, mesenchymal stem cells, differentiation

Acknowledgements: This study was funded by Sciencefund from Malaysian Ministry of Science Technology and Innovation (02-01-05-SF0684). WHN thanks Malaysian Civil Service Department (JPA) for sponsoring his studentship under the Yang Di-Pertuan Agong scholarship.

Oral Presentation 4

In vitro Evolution Eventuates the Isolation of RNA Aptamer Against Dengue Virus 2 NS1 Glycoprotein

Sivalingam Rogini, Thean Hock Tang, Marimuthu Citartan

Advanced Medical and Dental Institute, Universiti Sains Malaysia.

Dengue Fever, a prevalent disease around the world especially in the tropical and subtropical regions. The endemic attribute of the disease evokes the requisite for an ideal dengue diagnostic test that can distinguish dengue fever from other clinically similar diseases. Tempted by the excellent specificity of the aptamer, we endeavoured to isolate RNA aptamer via SELEX against DENV-2 NS1. Antigen NS1 represents the potential diagnostic target as it was found to induce a strong humoral response and is present in high concentrations in the blood up to 9 days after primary and secondary infections. SELEX was performed up to 11 cycles. Sequencing manifested the appearance of 7 different classes of sequences. The most prominent class of sequence DENVI 3, appeared at 14.3 % (DENVI 1, 4 and 5 appeared at 14.3%, DENVI 2 and DENVI 6 appeared at 7.1%, and DENVI 7 appeared at 3.6%). Verification by gel shift assay substantiated the binding of the sequences DENVI 3, 4, 5, and 6 against the target protein. These putative aptamers harbour diagnostic potentiality towards the development of aptamer-based diagnostic detection of dengue fever.

Keywords: dengue, SELEX, NS1 antigen, aptamer

Acknowledgement: This work was supported by National e-Science Fund under the Ministry of Science Technology and Innovation (305/CIPPT/613235) and Research University Grant for Individual, RUI (1001 / CIPPT / 811317). Sivalingam Rogini was supported by Graduate Assistant Scheme.

Oral Presentation 5

Generation of an RNA Aptamer Against Human Pituitary Tumor Transforming Protein 1 (PTTG1)

Siva Sankar Prabu[1], Marimuthu Citartan[1], Peng Yeong Woon[3], Ji-Hshiung Chen[2,3], Thean-Hock Tang[1]

[1]Advanced Medical and Dental Institute, Universiti Sains Malaysia. [2]Institute of Medical Science, Tzu Chi University, Hualien, Taiwan. [3]Department of Molecular Biology and Human Genetics, Tzu Chi University, Hualien, Taiwan.

Human Pituitary Tumor Transforming Protein 1 (PTTG1) is a global transcription factor that drives the transcription of more than 700 genes such as c-myc, bGFH, and CXCR2 as well as possessing more than 80 different multiple binding partners such as SP1 and p53. The pleotropic interactivity of PTTG1 and its high level of expression in cancers cells such as breast, gastric and prostate cancer predispose the protein as an exceptional diagnostic and therapeutic target for the isolation of molecular recognition element (MRE). One of the most eminent classes of MREs is aptamers which are single stranded nucleic acids that bind targets with high specificity and affinity. The wide applicability of aptamers both in the diagnostic and therapeutic platforms spurred our interest to generate RNA aptamer against the PTTG1. In our previous study, we have successfully purified recombinant PTTG1 from a bacterial expression system and demonstrated its bioactivity with p53 and anti-PTTG1 antibody. We employed SELEX with a dual partitioning strategy using an initial degenerate RNA library that contains 60-nucleotide randomized region. Filter binding assay showed sequence enrichment was successfully improved throughout the progressively increasing cycles (Cycles 0, 5, 8, and 11). After the 11th cycle, sequencing was carried out and revealed the appearances of 4 main clusters of sequences. One class of sequence (9.61% frequency) showed prominent binding and was selected as the most potent PTTG1 aptamer. Using filter binding assay, the equilibrium dissociation constant was estimated at 8 ± 2.819 nM. In conclusion, we have successfully generated an RNA aptamer with a low equilibrium dissociation constant of 8 nM ± 2.819 nM which has potential to be employed in theranostic assays with PTTG1.

Keywords: aptamer, Human Pituitary Tumor Transforming Protein 1, SELEX

Acknowledgement: The project was funded by Ministry of Higher Education E-Science fund (305/CIPPT/613235), Fundamental Research Grant Scheme (203/CIPPT/6711441) and Research University Grant University Science Malaysia (1001/CIPPT/811319). Siva Sankar Prabu was funded by the USM Vice Chancellor's Award.

Oral Presentation 6

Generation of RNA Aptamer Against Progesterone Receptor DNA Binding Domain Towards the Development of Aptahistostaining of Breast Cancer

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The standard biomarkers for immunohistostaining-based diagnostics of breast cancer are estrogen receptor-α (ER-α) and progesterone receptor (PR), which are commonly identified using monoclonal antibodies. However, a conundrum arises regarding the detection of ER-α -ve/PR +ve breast cancer with immunohistostaining, which was previously reported as false positive attributed to non-specific binding of the anti-PR antibody. Of present, aptamer, single-stranded nucleic acid (DNA or RNA) that has high binding affinity and specificity towards the target, have its own advantages over antibody. Aptamers are stable despite denaturation, smaller in size and can be easily labelled and modified. Thus, in this study, we sought to isolate RNA aptamer against PR, more specifically against the DNA binding domain (PR DBD) which is highly conserved among all the isoforms of PR and exhibits DNA binding activity. In the previous study, we have overexpressed and purified recombinant PR DBD using a bacterial expression system. The recombinant PR DBD was subjected to 8 cycles of SELEX and the binding enrichment was monitored by filter binding assay. Sequencing following the completion of SELEX cycles revealed the appearances of several different classes of sequences. The equilibrium dissociation constant (K_d) of class PRapt-9 was estimated at 155.9nM. The generated RNA aptamer against PR can be applied in concert with immunohistostaining of ER-α in the future studies.

Keywords: aptamer, progesterone receptor, DNA binding domain

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Oral Presentation 7

Limiting Exercise-induced Muscle Damage with Hydrogen-rich Water Ingestion: The Possible Therapeutic Effect

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Background: Exercise-induced muscle damage (EIMD) is manifested by the delayed-onset of muscle soreness (DOMS) and loss of force generation capacity in the exercised muscles. However, the effective treatment strategy remains uncertain to date. Recently, the beneficial effects of hydrogen-rich water (HRW) have been described in experimental and clinical disease conditions. **Objective:** The purpose of this preliminary study was to investigate the protective effects of HRW against DOMS and strength loss following EIMD in a cohort of young, healthy and physically active male adults. **Method:** This interventional study was conducted in a randomized, double-blind, crossover manner with a 28-day wash-out period. Five young and healthy adult males (26.6 ± 4.0 years) were assigned into the experimental trial to receive a 14-day treatment of either HRW (H_2-water, ~1.0 ppm, -500 mV, 1.74 $L \cdot day^{-1}$) or similar amount of pure water (Placebo). On the day 7 of the treatment period, participants were subjected to a 30-minute bout of continuous downhill run (DHR) on a motorized treadmill at a speed corresponding to 65% of their $\dot{V}O_2max$ and a slope of −15% in an environmental controlled condition (~22°C, rH ~40%). Assessments on muscle soreness and tenderness, range of motion (ROM), circumference of thigh, and maximal leg extension strength were conducted before and repeated at various time points (0, 24, 48, 76, and 168 hours) after the DHR. **Results:** Muscle soreness as measured by using the 100-mm visual analogue scale were significant lesser in the HRW trial immediately on both thighs, and at 24 and 48 hours on the right thigh only, following the DHR (p=0.012). However, no significant differences were detected between the HRW and Placebo trials on muscle tenderness, ROM, thigh circumference, and maximal leg extension strength. **Conclusion:** Ingestion of HRW for 14 days may have a protective effect on DOMS induced by eccentrically-biased or muscle lengthening exercise such as downhill running.

Keywords: downhill running, delayed-onset of muscle soreness, molecular hydrogen

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Oral Presentation 8

Cervical Cancer and Pap Smear Screening: Knowledge, Attitude and Practice among Working Women in Northern State of Malaysia

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Cervical cancer is among the most common cancer in women worldwide and Pap smear test is the primary screening procedure used to detect abnormal cells that may develop into cancer. The objectives of this study were to determine the knowledge of working women about cervical cancer and Pap smear test and their attitude about the test. Their practice of the test and the association with socio-demographic factors were also investigated. This cross-sectional study was performed from 9th to 27th October 2016. We recruited 260 women entrepreneurs of *Sahabat Amanah Ikhtiar* in district of Northern state of Malaysia who were between 20 – 65 years old, married or previously married and had no history of being diagnosed with cervical cancer. The simple random sampling was used to select participants. Self-administered questionnaire was used as research tool which was consists of 18 items regarding knowledge of cervical cancer, 12 items of knowledge regarding Pap smear screening, 15 items of attitude towards Pap smear screening and latest practice of Pap smear screening. Only 37.3% participants had done Pap smear within the past 5 years. Less than half of participants knew that unpleasant discharge (41.5%), abnormal bleeding between periods (48.1%) and discomfort or pain during sex (41.9%) were the symptoms of cervical cancer. Additionally, only 18.5% knew that vaginal discharge was not a symptom of cervical cancer. Besides, only 33.1% of participants knew that Human Papilloma Virus infection is a risk factor for cervical cancer. In general, our participants showed positive attitude towards Pap smear screening. There was a significant relationship between age ($p<0.001$), level of education ($p=0.002$), family planning practice ($p<0.001$), history of been pregnant ($p<0.001$), knowledge regarding cervical cancer ($p=0.040$) with Pap smear uptake. In conclusion, the uptake of Pap smear screening among working women in Northern state of Malaysia is low even though most have positive attitude towards Pap smear screening. Their knowledge about cervical cancer and Pap smear test was also limited.

Keywords: pap smear screening, cervical cancer, working women

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Oral Presentation 9

Effect of Bifunctional Isocyanate Linker on Adsorption of 2,4-dinitrophenol onto β-Cyclodextrin

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β-Cyclodextrin was polymerized using aromatic toluene diisocyante (TDI) and hexamethylene diisocyanate (HDI) as an aliphatic linker to form insoluble βCD-TDI and βCD-HDI. Both polymer was characterized and the results obtained were compared with each other and native βCD. The Fourier Transform-Infrared (FT-IR) results showed that βCD-TDI and βCD-HDI were successfully synthesised by the absences peak of isocyanate group in spectrum, while the thermo gravimetric analysis(TGA) results showed that the presence of aromatic crosslinker (TDI) increase the stability of the polymer. Based on the Barret-Joyner-Halenda (BJH) pore size distribution for dry βCD-TDI and βCD-HDI, it exhibited that both polymer was a mesoporous polymer further proved with scanning electron microscope (SEM) with two different morphological structure. Batch adsorption study were carried out and the βCD-TDI polymer showed enhanced sorption capacity and high removal rather than βCD-HDI towards 2,4-dinitrophenol (2,4-DNP). The removal was optimum at pH 4 for both βCD-TDI and βCD-HDI. A kinetic analysis showed that the pseudo-second order equation provided better fit for the adsorption of 2,4-DNP by both with 120 minutes as the equilibrium time. While, Freundlich's isotherm were found to be fitted well for low temperature (298K). The thermodynamics results showed that the adsorption process of 2,4-DNP was exothermic since the negative value of ΔH° was obtained for both polymer. Moreover, additional of NaCl for sorption using βCD-TDI showed an increasing trend in removal efficiency. While 80 mg of sorbent dosage were chosen as it showed an optimum removal of 2,4-DNP and to check on the regeneration of the synthesized polymer, both βCD-TDI and βCD-HDI were used for seven cycles for the removal and it showed removal efficiency was still more than 70% for both polymer. At last, it was tested on real water sample for the adsorption of 2,4-DNP, and βCD-TDI proved as best adsorbent. In addition, adsorption mechanism between βCD and 2,4-DNP was characterized using ^{1}H NMR and 2D NOESY spectrum. As a conclusion, βCD-TDI can be considered as a promising sorbent for the removal of 2,4-DNP better than βCD-HDI.

Keywords: β-Cyclodextrin, toluene diisocyante (TDI), hexamethylene diisocyanate (HDI), 2,4-DNP, adsorption, pollution

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Oral Presentation 10

Electrokinetic Supercharging in Capillary Electrophoresis for the On-line Preconcentration of Secbumeton in Water Samples

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In this study, an on-line preconcentration method termed electrokinetic supercharging capillary zone electrophoresis (EKS-CZE) was developed for the determination of secbumeton (SEC) in environmental water samples. The effects of diode array wavelength, buffer pH and concentration, applied voltage, the type terminating electrolyte (TE) and injection time of leading electrolyte (LE), sample and TE were investigated thoroughly. The optimum EKS-CZE involved hydrodynamic injection of leading electrolyte (100 mM sodium chloride, 30 s, 50 mbar), electrokinetic injection of sample (250 s, 7 kV) and hydrodynamic injection of terminating electrolyte (100 mM Tris, 40 s, 50 mbar). The other effective parameters included background electrolyte comprising 100 mM formate (pH 2.5), a voltage of 25 kV for separation (+25 kV) and DAD wavelength at 214 nm. Under the optimum conditions, the sensitivity of SEC was enhanced by 3847-fold and 2267-fold when compared to normal hydrodynamic injection (HDI) and electrokinetic injection (EKI), respectively. Wide linear range (0.1–500 µg L-1) with good linearity, R^2=0.9997, satisfactory repeatability (RSD < 4%, n=5) and good recovery (85.7–105.6%) were obtained for SEC. The detection limit for SEC with this EKS-CZE system was 0.03 µg L^{-1}. The results demonstrated that the developed method has a great potential for the determination of SEC in complicated aqueous matrices.

Keywords: capillary zone electrophoresis; preconcentration; electrokinetic supercharging; secbumeton; water samples

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Oral Presentation 11

The Effects of Mitragynine on the P-glycoprotein mRNA and Protein Expression in Brain Endothelial Cell

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Introduction: Neurotoxicity of new chemical entities represents one of the major bottlenecks in drug discovery and development. The prediction of neurotoxic effects is an important key feature in the toxicological profile of compounds. P-glycoprotein (P-gp) is the 170-kD protein product of the *multidrug resistance-1* (*MDR1*) gene which is highly expressed on the luminal surface of brain capillary endothelial cells. P-gp acts as an ATP-dependent efflux pump which limits the entrance of various types of drugs in the blood brain barrier. Modulation of P-gp function in the brain reduces brain protection, resulting in neurotoxicity. *Mitragyna speciosa* Korth or Ketum is a medicinal plant that has been used for various ailments. However, Ketum is also known for its stimulant and euphoric effects, hence it is often misused by the local population. Multiple poisoning and fatal cases involving Ketum and mitragynine have been reported, but the underlying mechanism remains unclear. This study aims to investigate mitragynine constitute neurotoxicity risk. **Objective:** Our main objective is to determine the effects of mitragynine on P-gp mRNA and protein expression in brain endothelial cells. **Method:** The effects of mitragynine on the mRNA and protein expression of P-gp were carried out using optimal RT-qPCR and Western blot analysis, respectively. P-gp-overexpressing brain endothelial cells (hCMEC/D3) was exposed to various concentrations of mitragynine for 72 h. The relative expression of P-gp mRNA was determined using the ΔΔCt method, with β–actin and GAPDH as internal controls. The relative protein expression of P-gp was calculated based on protein band intensity with β–actin served as an internal control. All data analysis were presented as mean expressions of 3 independent experiments. **Results:** Mitragynine down-regulated the mRNA and protein expression of P-gp at concentrations of 4 µM and above. **Conclusion:** Mitragynine modulates the expression of P-gp in brain endothelial cells suggesting a possible risk of neurotoxicity.

Keywords: P-glycoprotein, mitragynine, blood-brain barrier

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Oral Presentation 12

Development of Novel Exosome-based Delivery System for Proteins

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Cellular proteins contribute to critical signal pathways that is require for the cell functions. Most diseases somehow result from the malfunction of one or more of such proteins. To palliate such dysfunction, therapeutic proteins are used to correct the cellular functions. Although still relatively expensive to produce, it is still considered the most direct and safe approach for treating diseases. A large number of today's big-selling drugs are protein-based biologics (>$60B annual in sales). There are more than 1,500 potential disease treatment targets that reside inside the cells, without a clear receptor pathway for their specific targeting. Therefore, the need for intracellular delivery and targeting select host cell compartment represents a formidable hurdle to the development of novel prospective intracellular targeting protein-based biologics. Exosomes, 40-120 nm membrane vesicles secreted by cells, have recently emerged as promising natural vehicles for the delivery of macromolecules. Exosomes interact and affect the function of neighbouring cells through intercellular transfer of mRNA, miRNA, receptors and enzymes. It is also found to play important roles in immune dysregulation. A number of viral proteins such as HIV-1 Nef, Vif and VSV G protein are reported to induce exosome production. These observations lead us to hypothesize that simultaneous over-expression (bicistronic vector) of exosome inducing proteins (ExIP) and a heterologous protein from the same plasmid vector will result in the production of exosomes that contain the desired protein. The main objective of this work is to construct novel mammalian expression vectors that can simultaneously induce exosome production and packaging of recombinant protein into produced vesicles. We have constructed bicistronic mammalian expression vectors that concomitantly express various ExIPs and GFP as a reporter protein. We have found that both HIV-1 Nef and VSV-G proteins induce the induction of GFP-containing exosomes. These observations may lead to novel possibilities for the efficient delivery of recombinant proteins (such as antibodies) and provide new avenues for the development of intracellular protein biologic drugs.

Keywords: exosomes, bicistronic vector, heterologous protein

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Oral Presentation 13

Functional Evaluation of Flag-STAT1 in U3A Cells

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Background: Viral infections have had massive socio-economic impact, at the costs of human health and invaluable lives. However, effective and broad spectrum protection against viruses is still lacking despite best efforts. Antiviral immune defense is crucial for host protection in the event of viral infections. Type I interferons (IFN), are key mediators for antiviral immunity, and are induced following recognition of viral-associated molecular patterns. Type I IFNs released to the extracellular matrix then help establish antiviral state at the cellular level in autocrine and paracrine manner. Signaling by Type I IFNs is transduced via Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, leading to upregulation of interferon stimulated genes. STAT1 is a key component of the JAK-STAT signaling pathway, where two major isoforms: STAT1A and STAT1B are naturally expressed during Type I IFN response. STAT1 is essential in antiviral immunity as *Stat1*$^{-/-}$ mice are more susceptible to virus infection. However, the possibly of distinct roles of STAT1 isoforms in antiviral immunity warrants further investigation for more comprehensive understanding of the JAK-STAT pathway towards developing protection against viruses. **Objective:** We aim to establish an assay involving the expression of a functional recombinant Flag-STAT1B in U3A cells, a cell line lacking endogenous STAT1 that is derived from 2fTGH cells. **Method and Results:** Expression plasmid for recombinant Flag-STAT1B was constructed by sub-cloning. The expression plasmid was transfected into U3A cells and Flag-STAT1B was successfully expressed. When the transfected cells were challenged with IFN-β, recombinant Flag-STAT1B partially restored the previously dysfunctional JAK-STAT signaling pathway in U3A cells. Protein expression levels of several interferon stimulated genes were semi-quantitatively analyzed through immunoblotting and ImageJ. From our results, overexpression of STAT1B without STAT1A in U3A cells could not fully restore the JAK-STAT signaling pathway. **Conclusion:** Partial restoration of JAK-STAT signaling pathway by Flag-STAT1B, in response to IFN-β induction, was observed. This suggests that STAT1 isoforms may have overlapping yet distinct roles at transcriptional control during Type I interferon response.

Keywords: STAT, JAK-STAT, antiviral immunity

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Oral Presentation 14

Investigating the Effect of PPARγ Ligands on TIGIT and ICOS Expression on CD4+CD25+FoxP3+ T Regulatory Cells

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Background: Natural CD4+CD25+Foxp3+ T regulatory cells (nTregs) are derived from the thymus and later migrate to the periphery. Natural Tregs represent approximately 8-10% of the total CD4+ T cell population. These cells are important for maintenance of immune homeostasis and to prevent autoimmune development. Apart from CD25 and Foxp3, immune co-stimulatory (ICOS) and the newly identified TIGIT molecules are of recent interest among researchers. This study aimed to identify the regulatory effect of PPARγ ligands on pro-inflammatory or anti-inflammatory decisions in nTreg cells. **Objective:** To examine the influence of PPARγ ligands (Ciglitazone & 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2) on co-inhibitory receptor TIGIT and co-stimulator receptor ICOS expression in nTreg cells from BALB/c mice. **Method:** Spleens of female BALB/c mice (56 weeks old) were harvested via cervical dislocation. Splenocytes were stained with anti-CD4-FITC and anti-CD25-PE. Natural Tregs (CD4+CD25+) were sorted by using Beckman coulter MoFlow cell sorter. Cells were analyzed for intracellular FoxP3 expression by Becton Dickinson FACS Calibur flow cytometry. Isolated CD4+CD25+ regulatory T cells were cultured for 72 hours in RPMI in the presence of anti-CD3, anti-CD28 antibodies at 1 µg/mL and IL-2 cytokine at 20 ng/mL. Cells were treated with or without PPARγ ligands (20 µM ciglitazone and 10 µM 15d-PGJ2). After 72 hours of *in vitro* culture, cells were stained with anti-ICOS and anti-TIGIT antibodies conjugated with fluorochrome APC to detect expression levels using MoFlow. **Results:** CD4+ CD25+ Foxp3+ cells were successfully isolated using MoFlow cell sorter and purity was 45.5%. After 72 hours of *in vitro* culture, results showed that there were no significant differences recorded in TIGIT and ICOS expression levels in nTreg cells treated with or without PPARγ ligands compared to untreated groups. Dot plot analysis showed that TIGIT and ICOS were relatively low on nTreg cells isolated from splenocytes of BALB/c mice. **Conclusion:** Both endogenous (15-deoxy-$\Delta^{12,14}$-prostaglandin J_2) and synthetic agonists (Ciglitazone) PPARγ ligands had no significant influence on the expression of co-inhibitor TIGIT and co-stimulator ICOS receptors on CD4+CD25+FoxP3+ Tregs isolated from BALB/C mice.

Keywords: T regulatory cells, PPARγ, TIGIT, ICOS

Acknowledgement: FRGS Grant from MOE (203.CIPPT.6711483).

Oral Presentation 15

IRF9 Phosphorylation in Type I Interferon Response

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Introduction: The innate immune antiviral response is a result of a network of pathways culminating in the suppression of viral pathogens. The detection of viruses or its viral-associated molecular patterns leads to the induction of antiviral mechanisms, mediated by type I interferons (i.e. IFN-α/β). Binding of type I interferons to cell surface receptors lead to a signalling cascade of tyrosine phosphorylation through the Janus kinase (JAK)-signal transducer of activators of transcription (STAT) pathway. Interferon Stimulated Gene Factor 3 (ISGF3) is a trimeric complex composed of STAT1, STAT2 and interferon regulatory factor 9 (IRF9) proteins and acts as a potent transcription activator of interferon stimulated genes (ISGs) in the nucleus following IFN treatment. Our aim is to elucidate the post-translational modification – specifically on phosphorylation – of IRF9 during the JAK-STAT signalling. **Method:** Two cell lines, HeLa and 2fTGH cells were used. Both cells were induced with IFN-β and whole cell lysates collected after 24 hours. Phos-Tag™ was added to polyacrylamide gels. Phosphoprotein column was used to enrich the phosphoproteins from 2fTGH lysate. In addition, a phosphatase assay using calf intestinal alkaline phosphatase (CIAP) was performed to de-phosphorylate IRF9 in lysates. Immunoblotting was used to visualize the proteins. **Results:** IRF9 was enriched in the phosphoprotein fraction after selective enrichment by phosphoprotein column. Phos-Tag™ reduces the migration rate of phosphorylated protein in SDS-PAGE. Hence, the observed doublet bands for IRF9 in Phos-Tag™ gels suggest that IRF9 was phosphorylated. This was not observed in polyacrylamide gels without Phos-Tag™. In phosphatase assay, there was a decrease in IRF9 signal in IFN-β – induced 2fTGH cells as visualized using immunoblotting. Thus, further confirmed the changes in the phosphorylation state of IRF9 during type I interferon response. **Discussion and Conclusion:** Phosphorylation of human IRF9 was not previously described. The function of different phosphorylation states of IRF9 is currently not clear and will require further examination to determine its effect in JAK-STAT signalling and type I interferon response. Collectively, our data suggests that the phosphorylation state of IRF9 is altered following induction with IFN-β.

Keywords: interferon regulatory factor 9, phosphorylation, innate immunity

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Oral Presentation 16

Suppression of *AML1/ETO* via siRNA Mediated Gene Knockdown and Its Effects on *FOXO3* and *c-MYC* Expression in AML t (8,21) Cells

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Background: Acute Myeloid Leukemia (AML) with t (8,21) translocation is one of the most frequent karyotypic abnormalities observed in AML that results in the formation of fusion onco-protein, AML1/ETO. siRNA is a commonly used RNA interference (RNAi) tool to induce short-term silencing of protein-coding genes. There are studies shows that silencing of *AML1/ETO* results in up- or down-regulation of various genes responsible for apoptosis, proliferation, and self-renewal of cells. *FOXO3* act as a tumor suppressor gene by triggering apoptosis with its upregulation while *c-MYC* is a proto-oncogene which is found to be upregulated in many types of cancers. Nonetheless, the correlation between these three genes is not known. **Objective**: The objective of this study was to study the suppression of *AML1/ETO* gene via siRNA-mediated knockdown and its effect on *FOXO3* and *c-MYC* gene expression in AML t (8,21) positive Kasumi-1 cell. **Method**: A siRNA-mediated gene knockdown by electroporation of *AML1/ETO* followed by the study of gene expression level of *FOXO3* and *c-MYC* using qPCR were carried out in this study. **Results**: The result of siRNA-mediated *AML1/ETO* gene knockdown experiments using siAGF1 reveal that there is a significant knockdown of more than 50% in AML1/ETO with $p<0.05$ for all three time points. However, the gene expression level of *FOXO3* and *c-MYC* after the knockdown of *AML1/ETO* does not showed any significant results for all three time points. **Conclusion**: The results of gene expression after *AML1/ETO* gene knockdown does not show any conclusive or significant correlation between *AML1/ETO*, *FOXO3* and *c-MYC*. A prolong knockdown is suggested to further verify the finding.

Keywords: siRNA, AML1/ETO, FOXO3, c-MYC

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Oral Presentation 17

Downregulation of *SKP2* via siRNA Mediated Gene Knockdown and its Effects on *FOXO3* and *c-MYC* Expression in AML t(8,21) Cells

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Background: Acute Myeloid Leukemia (AML) is a hematopoietic stem cell disorder caused by the inability of the cells to differentiate and proliferate in a normal manner. The chromosomal translocation t(8;21) is the most frequent translocation, accounting for 12-15% of *de novo* AML cases. One of the proteins that was identified to have high expression in AML is S-phase kinase–associated protein 2 (SKP2). SKP2 is responsible for recognizing and determining the degradation of the target protein. It is well known for its oncogenic property and as a therapeutic target in various cancers. FOXO3 protein was identified to present in AML and acts as tumor suppressor gene. *c-MYC* plays a key role as the proto-oncogene and is recognized to induce AML. Numerous experiments showed a correlation between *SKP2* and *FOXO3* as well as *SKP2* and *c-MYC*. However, no information is available on the relationship between *SKP2*, *FOXO3* and *c-MYC* gene in AML with t(8;21) translocation. **Objective:** The objective of this study was to investigate the relationship between *SKP2*, *FOXO3*, and *c-MYC* in AML with t(8;21) translocation. **Method:** Kasumi-1 cell line was used as a model of AML with t(8;21). SKP2 downregulation via siRNA-mediated gene knockdown was carried out to identify its effect on *FOXO3* and *c-MYC* gene at three different time points: 24, 48 and 72 hours. siRNAs were introduced into Kasumi-1 cell line via electroporation, and the expression level of each gene was identified using real-time PCR. **Results:** Results show a successful knockdown of SKP2 at all different time points with more than 50% downregulation ($p<0.05$). However, there was no significance between both *FOXO3* and *c-MYC* expression levels with or without *SKP2* knockdown. The expression of the *FOXO3* gene was identified to increase slightly from 24 hours to 72 hours after the *SKP2* knockdown. **Conclusion:** Although the results were not significant, *FOXO3* gene was noted to increase slightly up to 72 hours after the *SKP2* knockdown. This shows that there is an inverse correlation between *FOXO3* and *SKP2*. A prolonged gene knockdown may be necessary to obtain a significant value.

Keywords: AML, *SKP2*, *FOXO3*, *c-MYC*

Acknowledgment: This work is supported by the USM RUI Grant 1001/CIPPT/813064.

Oral Presentation 18

Study on the Adsorption of DNA on β-cyclodextrinFe_3O_4 Nanoparticles

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Background: To the best of our knowledge, β-cyclodextrin coated magnetic nanoparticles (β-CD-MNP-TDI) application in nucleic acid adsorption (DNA) has yet been reported in any studies. The establishment of new nucleic acid adsorption method from this research might contribute to the development of new nucleic acid extraction methods and specific therapeutic nucleic acid vector. **Objective:** To investigate the adsorption of DNA onto the surface coated magnetic nanoparticles (MNP) by β-cyclodextrin. **Method:** MNP and β-CD-MNP-TDI was characterized using fourier transform infrared (FT-IR) device and vibrating sample magnetometer (VSM). The amount of adsorbed DNA was measured using spectrophotometer at 258 nm. Different parameters and kinetic, isoterms and thermodynamic models were studied to describe the adsorption behaviour of DNA onto β-CD-MNP-TDI. **Results:** The characteristic spectra of β-CD-MNP-TDI was shown at 1657 cm^{-1} which is attributed to the emergence of carbamate linkage (NHCO). The presence of carbamate linkage signifies the completion of polymerization between TDI and β-cyclodextrin. The magnetic moment of β-CD-MNP-TDI (~ $\pm$40 emu/g) was lower than MNP magnetic moment (~ $\pm$60 emu/g) due to the present of the coating material which suggestively chaperone and reduced the inherently large surface energies. The optimum working parameter of β-CD-MNP-TDI was found to be at pH 6, 298 K, with adsorption process reaching equilibrium at 20 minutes, and efficient initial DNA concentration being 10 mg/L. Ionic strength effect created by sodium chloride at the range of 0 to 0.40 mol/L have minimal effect on the adsorption capacity of β-CD-MNP-TDI. The adsorption efficiency of β-CD-MNP-TDI was found to perform on a par of 70 ± 5 % after being recycled for three times. The adsorption isotherms and kinetics was found to be best described by Freundlich isotherm, pseudo-second order kinetics. The entropy and enthalpy change of DNA on β-CD-MNP-TDI were -12.1567 J/Kmol and -3.7371 J/mol respectively. The progression of Gibbs free energy from negative to positive value indicates that the adsorption process is thermodynamically feasible, spontaneous and chemically controlled. **Conclusion:** The findings from the sorption studies suggest the adsorption of DNA onto β-CD-MNP-TDI was feasible and that the interaction between adsorbent and adsorbate occur through inclusion complex, hydrophobic interaction, hydrogen bonding and Van der Waals force.

Keywords: β-cyclodextrin, nanoparticles, DNA, Fe_3O_4

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Oral Presentation 19

Ultrastructural and Biochemical Responses of the Earthworm *Eudrilus eugeniae* Exposed to Contaminated Soil: Effects of Heterocyclic Amines

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Heterocyclic amines are a group of carcinogens formed during the cooking of proteinaceous foods. They are carcinogenic to man, rodents, and non-human primates. However, the toxicological effects of heterocyclic amines on earthworm, an organism with high biodegradability is unknown. Therefore, in this study, the toxicity of heterocyclic amine on the intestine of the earthworm was investigated using biochemical and ultrastructural techniques. In the study, we exposed earthworms *Eudrilus eugeniae* to artificial soil contaminated by PhIP and MeIQx for 28 days using a modified OECD TG 207 guideline. Results showed that compared to the control, heterocyclic amine induces a significant increase in the activity of reactive oxygen species (ROS), inhibited the activity of catalase and significantly stimulated the glutathione S-transferase activity especially at the high doses of MeIQx and PhIP. Using transmission and scanning electron microscopes, ultrastructural observations revealed heterocyclic amines caused cytological changes. There was disruption of the membrane of the chloragosome, increase in the fusion processes of the chloragosome and vesiculation of the chloragosomal granules. Moreover, there were damaged mitochondria, smooth endoplasmic reticulum and loosened muscle fibres in the heterocyclic amines-exposed earthworms. In conclusion, our results showed that heterocyclic amines caused ultrastructural alterations in the intestine of earthworms and induced oxidative stress by overproduction cum accumulation of ROS and weakening of the antioxidant system of the intestine which may be important in understanding its toxicity to the earthworms.

Keywords: earthworms, Heterocyclic Amine, oxidative stress, intestine, ultrastructure

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Oral Presentation 20

A Simplified In-house Extraction Method Suitable for Food PCR Analysis

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Reliable DNA extraction method is an important part of molecular food analysis. A simple, safe and inexpensive DNA extraction method for pork DNA detection in meat-based food products has been reported previously. In this study, we optimized the overnight in-house DNA extraction method to be completed in 15 minutes with minimum preparation steps. The recovery of extracted DNA was determined by subjecting the crude lysate for PCR amplification. Applicability of the method was evaluated with 121 commercial processed food samples collected from the local market around Northern Peninsular Malaysia. Results obtained were then compared with a commercial food DNA extraction kit. Comparable results were obtained from both methods, proving that the in-house DNA extraction method is able to provide good DNA quality for PCR amplification from samples with various compositions. The proposed extraction method was proven to be applicable for high-throughput screening of food samples for halal food industry.

Keywords: DNA extraction, food analysis, PCR amplification

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Oral Presentation 21

Determination of the Marker Compounds in Adulterated and Non-adulterated Stingless Bee Honey Using GC-MS

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Honey has been adulterated since a long time due to economical reason. Generally, honey is adulterated by adding the foreign substances to pure honey or used for feeding the bee. The method of detection of honey adulteration is limited due to complex composition of honey compounds, which depend on the bee's flowers preference and geographical region. This study focused on the development of a simple method by using gas chromatography-mass spectrometry (GC-MS) to detect adulteration in stingless bee honey. Pure stingless bee honey samples were analysed and used as the reference in this study. Propionic acid, glycerol, and myo-inositol were selected as marker compounds. The three selected compounds were used to identify pure and adulterated honey as well as sugar products. The results revealed that storage of the samples had significant effects on the content of marker compounds in stingless bee honey. It was found that within 5 months of storage, glycerol and myo-inositol were significantly degraded. However, propionic acid concentration remained unchanged for over a 5-month period. In conclusion, a simple and fast methodology based on GC-MS analysis has been developed to allow the detection of marker compounds, in order to differentiate adulterated and non-adulterated stingless bee honey.

Keywords: stingless bee honey, glycerol, propionic acid, myo-inositol, marker compounds, adulterated honey

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Oral Presentation 22

Analysis of Phthalate via Spectrophotometry in Environmental Samples Using Non-ionic Silicone Surfactant Mediated Cloud Point Extraction

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Determination of phthalates in environmental compartments become crucial in recent years due to the growing international concern about the health effects of phthalates. In the present study, a greener method based on cloud point extraction procedure was developed for the extraction of selected phthalates in environmental samples using non-ionic silicone surfactant of DC193C. The parameters affecting the extraction efficiency, such as the surfactant concentration, salt types, salt concentration, temperature, and incubation time were evaluated and optimized. Good linearity (R^2) in the range of 0.9963 – 0.9988 for all calibration curves was obtained. The LODs were 0.95 µg L^{-1} (diethylhexyl phthalate) and 0.72 µg L^{-1} (dibutyl phthalate) and the LOQs were 3.16 µg L^{-1} (diethylhexyl phthalate) and 2.42 µg L^{-1} (dibutyl phthalate), respectively. The proposed method was successfully applied in extracting the diethhylhexyl phthalate and dibutyl phthalate in selected environmental samples under optimized conditions with satisfactory recoveries in the range of 82 – 98 %.

Keywords: cloud point extraction, water samples, silicone surfactant

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Oral Presentation 23

Curcumin Potentially Inhibiting and Preventing the Self-renewal Capability of Lung Cancer Stem Cells (CSCs) Derived from Non-small Cell Lung Cancer (NSCLC)

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Cancer stem cells represent a small subpopulation within a tumour that possesses the stem-like properties which become the major challenge in cancer treatment. Natural compound containing high polyphenol such as curcumin has an ability to suppress the formation of tumour. Therefore, this study is design to explore the potential effect of curcumin in suppressing the cancer stem cells characteristic such as self-renewal capability. Lung CSCs (CD166+EpCAM+) isolated from A549 and H2170 were cultured and maintained in complete medium RPMI 1640 throughout the study period. Once cells reach ~90% confluence, the cells were subjected to clonogenic and spheroid assay to access for the self-renewal capability by evaluating the formation of colonies and spheroid. At the end of experiments, mRNA level of stemnes genes including *sox-2, pou51f, klf4, and nanog* were evaluated using real time PCR. Our finding showed that curcumin alone has the ability in inhibiting the formation of colonies as observed in A549 CD166+EpCAM+ with 83 colonies and H2170 CD166+EpCAM+ with 39.5 colonies as compared to the untreated with 165.3 and 101 colonies respectively. However, a combined treatment between curcumin and cisplatin tremendously reduced the formation of colonies into 37 colonies both in A549 CD166+EpCAM+ and H2170 CD166+EpCAM+.We also conducted the tumorigenic model to observe the development of spheroid in A549 CD166+EPCAM+ and H2170 CD166+EPCAM+ which impressively showed that curcumin alone suppressed the spheroid development by shrinking the size at 135.6 µm and 31.8 µm respectively as compared to their untreated A549 CD166+EPCAM+: 381.4 µm and H2170:192.4 µm. Still, we found that a combination of curcumin and cisplatin resulted in better effect by suppressing the spheroid formation into A549 CD166+EPCAM+: 61.5 µm and H2170 CD166+EPCAM+:19.0 µm. Fascinatingly, our study also proved that curcumin has the capability in preventing the formation of colonies and spheroid in both population. Finally, the expression of *sox-2*, *nanog, klf5*, and *pou51f* were founded down-regulated in both population after treatment with curcumin hence indicating the self-renewal may regulated by this gene. Taken together, the study has revealed that curcumin was an effective agent potentially in inhibiting the self-renewal capability of lung CSCs which resulted in suppression of CSCs thus may provide a new strategy in eliminating the CSC populations.

Keywords: curcumin, cancer stem cell (CSCs), self-renewal, A549 CD166+/EpCAM+, H2170 CD166+/EpCAM+, combined treatment

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Oral Presentation 24

Use of Fresh Frozen Plasma at Hospital Selayang – A Retrospective Study

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Background: FFP transfusion has increased in demand over the years and the global trending of high FFP usage can be seen in many countries. It has been used since 1920's and if appropriately used, it can improve the quality of life. However, FFP transfusion is not without risk. It may cause adverse transfusion reaction such as allergic reaction, TRALI, TACO and transfusion transmitted infections. Thus, this study was conducted to assess the indication of FFP transfusion against the guideline available. **Objective:** To assess the appropriateness of the use of fresh frozen plasma at Hospital Selayang against the National Guideline for the rationale use of blood and blood products and international guideline by College of American Pathologist. **Method:** A retrospective study among adult patients receiving fresh frozen plasma from 1 January 2016 until 31 December 2016 by reviewing total hospital information system (THIS), blood order and transfusion reaction forms. The appropriateness of requests was assessed against existing guidelines. The percentage of indicated diagnosis (appropriate) and not indicated diagnosis (inappropriate) FFP transfusions was obtained. **Result:** 373 patients receiving FFP were included in this study. 73.2% of the patients were given FFP with indicated diagnosis and is considered appropriate while 26.8% was not indicated (inappropriate). HPB and Orthopaedic are the most departments to transfused FFP with not indicated diagnosis. Those with normal INR were 3.22 times higher odds of being not indicated for FFP compared to those with prolong INR readings. 4.3% patients developed transfusion reaction mainly mild allergic and febrile non hemolytic reaction (FNHR) **Conclusion:** Inappropriate transfusion exposes patient to unnecessary adverse event and thus should be monitored and prevented. The findings of this study are beneficial to justify the role of clinicians in making the right decision in transfusion practices.

Keywords: FFP usage, retrospective, Hospital Selayang

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Oral Presentation 25

In-vitro Co-culture Model of Mesenchymal Stem Cell Stimulates Airway Epithelial Repair and Regeneration via a Paracrine-mediated Reparative Mechanism

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Background: Endogenous repair mechanism of airway epithelial cells often fails to achieve sufficient cellular turnover and diminish with age, thus leading to permanent alterations in the structure and function of the airway epithelium. Being anti-inflammatory, immunomodulatory, and regenerative in nature, we proposed that MSC release stimulatory compound that play key roles in the repair and regeneration of the airway epithelium. **Objective**: To investigate the paracrine mechanisms of MSC-secreted compounds in stimulating endogenous airway epithelial cell proliferation and migration during repair process. **Method**: Ovalbumin-induced airway injury was performed according to previous protocol. Briefly, the rabbits were sensitized and challenged with both i.p and inhalation of ovalbumin. The rabbits were euthanized and the bronchus was collected. The indirect in vitro co-culture of the bronchus rings with MSC was performed using air-liquid interface chamber for 3 days. The bronchus and secretome were collected at day 1 and day 3 of incubation. The bronchus tissues were subjected to histopathological assessment including H&E and immunofluorescence staining while the collected secretome were subjected to LC-MS analysis. **Results:** Histopathological analyses revealed that pseudostratified epithelium in MSC-treated group had de-differentiated into mono and multi-layered flattened epithelial cells. These flattened epithelial layer were positive for airway epithelial markers (K5+8 and K14). Our LC-MS data showed that 352 proteins were successfully identified and 173 of these proteins were significantly differentially regulated ($p<0.05$). The gene ontology (GO) classification of these 173 protein showed that 64 (37 %) had a correlation with reparative process. Among these 64 proteins, 25 (39.1 %) were related to cell-cell adhesion, 13 (20.3 %) involved in focal adhesion, 11 (17.2 %) associated with regulation of actin cytoskeleton, 8 (12.5 %) related with collagen binding and 7(10.9 %) were associated in ECM-receptor interaction pathway indicating that MSC-secreted compound regulate the airway reparative process. **Conclusion**: Our data suggested that the secreted factors of MSC stimulated proliferation and migratory behaviour of flattened epithelial cells during repair process. These findings will provide an insight in understanding the mechanism of MSC in airway epithelial repair and regeneration for development of novel therapies focused on airway repair.

Keywords: MSC-secreted compound, paracrine, repair and regeneration, airway epithelial

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