Young Scientists' Symposium 2023

Jointly organised by



Singapore Society for Biochemistry and Molecular Biology



Sponsored by



Department of Biochemistry Yong Loo Lin School of Medicine

Young Scientists' Symposium 2023

ACKNOWLEDGEMENTS

The organisers would like to express their thanks to:

- The staff and student participants of the 5 polytechnics for their support and participation
- The oral and poster judges for their time and valuable comments
- Our Sponsor: the Department of Biochemistry, NUS
- The Emcees, Mr Zhao Junyu and Ms Lim Siew Kim (Republic Polytechnic)
- The Photographers, Ms Shruti Gadgil and Ms Caitlyn Tan Ying Hui (Republic Polytechnic)

Young Scientists' Symposium 2023



FOREWORD

Dear students,

Welcome to the Young Scientists' Symposium 2023. I am happy that you have decided to participate in this symposium and I really wish that it will be a fun and rewarding event for you.

Having completed your final year projects, you have probably realized that in order to succeed in the future, it will not be enough to just accumulate more knowledge. It is even more important to gain experience, independence, various skills, including the ability to write and present, and to be enthusiastic and show your own initiative. During your final year projects and internships you have probably already realized that it is in part your initiative that determines the outcome. Similarly, in the future after moving on from your Poly you will no longer be in the passenger seat, but driving your own route to become successful, happy and content. I believe that including the symposium in your journey is a good choice, as it will hopefully give you new experiences and be a stimulus to think about your future.

We have tried to make this conference interesting and rewarding for you. In our poster sessions we have given ample time to allow you to present your work. We have invited recent Poly graduates who are now in University or have entered their professional life. They will share some of their experiences and we hope that this will encourage you to consider about your own future.

We have also tried to make the program interesting and engaging. Ultimately, the success of the symposium is also dependent on you, for instance on your presentations and your participation in the Q&A. When it is not your turn to present a poster, you could also visit other posters and engage with other students. Doing this is really rewarding, for various reasons. You can learn about the topics and quality of other students' research or get to know students who have worked in related areas. Last but not least it can help you to overcome fears to discuss scientific topics that are new to you. Hence, I would encourage you to go through the program and find some other interesting posters.

Thank you for participating in this symposium! I would also like to take this opportunity to thank all the people who made this event possible, including our panel speakers, oral and poster presentation judges, your lecturers, the dedicated staff from Science Centre, in particular Ms Karen Koh and Miss Charissa Lin, the Emcees Ms Lim Siew Kim and Mr Zhao Junyu, the photographers Ms Caitlyn Tan Ying Hui and Ms Shruti Gadgil and the passionate members of the organizing team, including Dr Sun Guang Wen, Ms Koh Siok Im, Dr Yew Wen Shan, Dr Chua Yee Liu, Dr Albert Bo Xue, Dr Lim Yan Ping, Dr Carol Ping Han, Dr Choy Weng Keong, and Dr Leong Meng Fatt. Thank you!

Thilo Hagen

Vice President, Singapore Society for Biochemistry and Molecular Biology Associate Professor, Department of Biochemistry, National University of Singapore

Young Scientists' Symposium 2023

ORGANISING COMMITTEE

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Dr Padmapriya Sathiyanathan

Senior Research Fellow Institute of Molecular and Cell Biology, A*STAR

Dr Matias Autio

Research Scientist Genome Institute of Singapore, A*Star

POSTER JUDGES

Dr Mark Richards (NYP) Dr Henry Leung (NYP) Dr Tan Shixiong (RP) Dr Koo Ghee Chong (RP) Dr Suchitra Derebail (RP) Dr Chiradip Chatterjee (RP) Dr Lee Koon Guan (TP) Goh Miah Kiat (TP) Dr Tan Tuan Lin (SP) Wang Baoshuang (SP) Stephen Dinesh Raj (NUS) Hong Nguyen (NUS) Yow See Jie (NUS) Clara Koh (NUS) Previtha Dawn (NUS) Tay Hui Yi (NUS) Vivian Tan (NUS) Shermaine Thein (NUS) Leung Jia Yu (NUS) Ong Sze Min (NUS) Lee Rui Xue (NUS)

YOUNG SCIENTISTS' SYMPOSIUM 2023 Thursday, 2 March 2023

The Marquee @ Science Centre Singapore

SCIENTIFIC PROGRAMME

0830 Registration and Poster setup

0915 Welcome Address: Lim Tit Meng Chief Executive, Science Centre Singapore

0930 Oral presentation 1: <u>Akilaandeswari D/O Elangoh</u> *Republic Polytechnic* <u>Super diet can help reverse or reduce the severity of</u> <u>cancer, a Myth or Reality?</u>

0955 Invited speaker 1: Zhou Jie Fu Jeff National University of Singapore (Singapore Polytechnic Graduate from 2014)

1015 Breakfast

- 1035 Oral presentation 2: Mikail Adam & Chan Wei Wen Brian Ngee Ann Polytechnic Analysis of evolutionary and population dynamics of SARS-COV-2 variants and subvariants across different waves of infections
- 1100 Invited speaker 2: Vernice Long Nanyang Technical University (Temasek Polytechnic Graduate from 2022)

1120	Oral presentation 3: Ng Xin Xiu Nanyang Polytechnic Optimization of Lipid Nanoparticles (LNP) Formulation for Efficient Vaccine Delivery
1145	Poster session 1
1230	Lunch
1315	Changing of the posters
1320	Oral presentation 4: Lai Jia Yan Singapore Polytechnic USP15 inhibits ACCA & YAP to suppress breast cancer
1345	Invited Speaker 3: Daniel Chang Li Wei NTU alumnus and Republic Polytechnic Graduate from 2014
1405	Poster session 2 and tea
1530	Oral Presentation 5 Teo Yu Qing Temasek Polytechnic Phenotypic drug discovery in soft tissue sarcoma
1555	Concluding Speech Thilo Hagen <i>National University of Singapore, Vice President,</i> <i>Singapore Society for Biochemistry and Molecular</i> <i>Biology</i>
1615	Prize Presentation

YSS2023 Prizes

Poster Presentation Merit Awards	\$100 each
Oral Presentation Merit Award (first runner-up)	\$150
Oral Distinction Prize	\$200

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ORAL PRESENTATIONS

ORAL 1

Super Diet can Help Reverse or Reduce the Severity of Cancer, A Myth or Reality?

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Republic Polytechnic

Colorectal cancer (CRC) is one of the leading types of cancers in the world and its risks are linked to a diet high in red meat, processed meat, sugar, fat, refined carbohydrates and low in fruits and vegetables. In our study, we set up cell-based assays using HCT-116 cells and tested the effects of 'superfood' components Epigallocatechin gallate (EGCG) and curcumin in delaying or stopping the growth of HCT-116 cells in a dose and time dependent manner. The optimized based assay was on chemotherapeutic drugs 5-Fluorouracil and CGP 57380. It has shown that curcumin and EGCG are indeed effective in slowing down the growth of and killing HCT-116 cells. Curcumin showed high levels of apoptosis with increasing concentrations with a G2/M arrest while EGCG caused cell senescence and most concentrations and apoptosis at higher concentrations. Biphasic effect in EGCG cells observed is due for further investigations.

ORAL 2

Analysis of Evolutionary and Population Dynamics of Sars-Cov-2 Variants and Subvariants across Different Waves of Infections

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The emergence of numerous variants and subvariants of the novel coronavirus, SARS-CoV-2, since the start of the COVID-19 pandemic has caused concern due to their potential to alter the characteristics of the virus, including transmissibility, severity, and immune evasion. This study aimed to investigate the evolution and population dynamics of SARS-CoV-2 in the United States of America, using a dataset of 666 complete genomic sequences collected during various waves of infections in the country. Reference genomic sequences representing the Variants of Concern (VOC) such as Alpha, Beta, Gamma, Delta, and Omicron were used for comparative analysis. Bayesian phylogenetic analysis was conducted to infer the evolutionary relationships between SARS-CoV-2 viruses at the peak and trough of each wave of infections. The analysis revealed that multiple variants and subvariants of Alpha, Beta, Gamma, and Delta were widely circulating across multiple waves of infections in America throughout 2021, before the emergence of the Omicron variant. Notably, the Omicron variant formed a distinct monophyletic clade that is distant from other SARS-CoV-2 variants. The nucleotide diversity of SARS-CoV-2 virus in America fluctuated across different waves of infections, which aligned with the changing demographic history of the virus based on the Bayesian Skyline plot. The findings highlight the spread and explosive growth of SARS-CoV-2 virus in America, which may represent a microcosm of the larger trends in the pandemic's spread and impact worldwide. Understanding the SARS-CoV-2 virus evolution is critical for developing effective public health strategies to control its spread and for vaccine and drug development.

ORAL 3 Optimization of Lipid Nanoparticles (LNP) Formulation for Efficient Vaccine Delivery

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mRNA is a potential therapeutic agent for treating a variety of diseases and immunotherapies. However, the delivery of naked mRNA is inefficient due to the presence of enzymes in our bodies which degrades the mRNA before it reaches the target cells. As such, a more efficient and effective way of delivering mRNA is required. My project at ACM Biolabs involves the encapsulation and delivery of mRNA using nanoparticles (NP) where we optimised the lipid composition to yield stable and effective NP.

ORAL 4 USP15 Inhibits ACCA & YAP to Suppress Breast Cancer

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The Hippo pathway was found to be greatly dysregulated in various cancers such as breast cancer, which requires the discovery of new drug targets. The pathway is heavily regulated by ubiquitinases and deubiguitinases which are important enzymes controlling the stability of proteins in the cells and serve as potential drug targets. Through proteomics analysis, Ubiquitin Specific Peptidase 15 (USP15) was identified as one of the highly confident interactors of the Hippo pathway component, Kibra. Overexpression of USP15 reduces Yes-Associated Protein / Transcriptional co-activator with PDZ-binding motif (YAP/TAZ) levels and cell proliferation in the colony formation assay. Mass Spectrometry analysis of USP15-associated proteins revealed Acetyl-Coa Carboxylase A (ACCA) as an important interactor. ACCA is the first ratelimiting enzyme in lipid metabolism and shown to be an oncogene driving cancer progression. USP15 negatively regulates ACCA expression through its deubiquitinating activity. USP15 may act as a tumour suppressor and a possible therapeutic target for breast cancer.

ORAL 5 Phenotypic Drug Discovery in Soft Tissue Sarcoma <u>TEO Yu Qing¹</u>, TOH Tan Boon²

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The current standard of care for soft tissue sarcoma (STS) is doxorubicin. However, the use of doxorubicin has resulted in serious adverse effects in many patients. Therefore, there are increasing attempts in the repurposing of drugs for the management of STS. Through high throughput drug screening, carfilzomib and ixazomib were identified to exhibit strong inhibitory activity on STS cell lines, suggesting the exploration of proteasome inhibitors (PI) in STS. PIs target the ubiquitinproteasome pathway (UPP), a key pathway in the regulation of proteins through protein degradation. Inhibition of the UPP results in protein accumulation, eventually leading to cell death. This project aims to validate the functional therapeutic efficacy of PIs, specifically carfilzomib and ixazomib, in 5 STS cell lines. Carfilzomib and ixazomib were found to be most potent in LPS141 and LiSa-2 respectively. Activity of PIs was validated through detection of aggresome formation, which is the hallmark of proteasome inhibition. Further interrogation via protein analysis confirmed that PIs induced autophagy and ER stress in STS cells. Combination of doxorubicin and carfilzomib demonstrated an additive drug interaction. Through the various validation methods, both proteasome inhibitors mitigate sarcoma cell survival through induction of autophagic cell death at low doses of drug treatment. These data suggest that UPP is a potential therapeutic target in the treatment of STS.

POSTER PRESENTATIONS

P1 Formulation Strategies of Astaxanthin-Spirulina Supplements

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Republic Polytechnic Institute

The purpose of this study was to formulate astaxanthin-spirulina tablets which are rarely found in the market using rotary press. Each tablet should contain 2.5 mg of the active ingredient astaxanthin and meet all quality control tests. The important quality control tests for dietary supplements are weight uniformity test, hardness test, disintegration test and friability test. A total of 7 formulations were made and analysed. Most of the formulations passed every quality control tests except for the disintegration test. The study involved 3 phases. Phase 1 was the research of excipients based on commercially available products. Phase 2 was finding formulations that passed all quality control tests using direct compression. For the last phase, the formulation was put through rotary press but flowability was unsuccessful. Wet granulation was attempted and the flowability of formulation improved slightly.

P2 Water-Based Barrier Materials for Sustainable Food Packaging

TAN Jun Heng Louis, TAN Jun Kai Xavier, LOH Zhen Yuan, Gabriel LEE, FOO Kay Yang

School of Applied Science, Republic Polytechnic

Currently, the production of food packaging involves the use of synthetic polymers such as Low-Density Polyethylene (LDPE) and Biaxialorientated polypropylene (BOPP). The growth of the use of plastic packaging has increased over the past few decades at a rate of 5% per annum. The worldwide packaging industry has been valued at \$1.9 trillion in 2020 and is expected to reach \$3.4 trillion by 2030. With the constant increase in plastic packaging use, the extraction of natural resources to produce packaging and greenhouse gas emission from the manufacturing process would also increase.

To enable a more sustainable food packaging option, the objective of this project is to develop a water-based coating that has good barrier properties for paper/cellulose based materials. The coating's ability to act as a barrier against water vapour, liquid water and grease is investigated using different techniques such as Water Vapor Transmission Rate (WVTR), Cobb Test and Kit test. Rheological tests were also performed on the coating formulations to determine their viscosities. The results showed that the ability of the coating to act as a barrier against water vapour is improved with the inclusion of additives such as fumed silica and citric acid, which vastly decreases the amount of water vapour passing through the film. In addition, some formulations showed improved liquid and oil resistance. Further work is required for the coatings to compete with and replace conventional food packaging materials such as LDPE and BOPP.

P3 Encapsulation of Natural Photosensitizer to Develop Self Disinfecting Materials

LEO Yu Qing Cheryl, Abigail ANG, Cheryl TAY Ning Yue, Melody WUANG Jing Xuan, Syazwani Bte SULEIMAN

School of Applied Science, Republic Polytechnic

In the age of Covid-19, consumers all over the world are becoming more interested in self- disinfecting products to have a sense of reassurance of hygiene and safety. Self-disinfection can be achieved by incorporation of antimicrobial agents in various materials such as plastics, wood, fabric and metal for the application in clinical (PPE, hospital fabrics) and nonclinical (food packaging, paint & coatings) fields. However, the overuse of common antimicrobial agents in such materials may contribute to antimicrobial resistance which is a growing international concern.

Antimicrobial photodynamic inactivation (aPDI) is an alternative approach to kill pathogens. aPDI uses Photosensitizer dyes which produce highly reactive oxygen species (ROS) when activated by suitable wavelength of light. These reactive oxygen species display a broad-spectrum of antimicrobial activities that destroy microbes by a multi-targeted killing mechanism, which limits the development of antimicrobial resistance.

This project aims to encapsulate a natural, plant based, nontoxic photosensitizer, DGFX, to make it suitable for incorporation in different materials so that it can withstand the processing conditions. Formulation containing DGFX and different encapsulating materials are developed using different methods such as low shear granulation, extrusion spheronisation and high shear granulation. The encapsulated granules are characterised by particle size distribution and flow properties. A comparison study was performed to identify the best formulation and method tested in the current project.

P4 Synthesis of Silver Nanowires for Coating Applications

Haiqal Danial Bin FAISAL, Matthew LIN Ruirong, Rachel TOH Xin Juan, YONG Siok Cheng, Dr LAI Linke, FOO Kay Yang, Dr Gabriel LEE

School of Applied Science, Republic Polytechnic

Polydimethylsiloxane (PDMS) exhibits many unique physical and chemical properties, making it a good candidate for making products for different applications, such as car coating, microfluidics, lithography, etc. However, the insulative characteristic of PDMS makes it easier to attract airborne contaminants, due to the accumulation of surface static electricity. It also limits its application in products that require anti-static properties, such as electronics and medical devices. Silver (Ag) is wellknown for its superior electrical conductivity over all other materials. Based on literature, the nanowire (NW) form of silver with a high aspect ratio is the most suitable candidate for improving the electrical conductivity of PDMS. Polyol methods have been reported to successfully produce Aq NWs of different sizes, but discrepancy exists in the experimental conditions from different studies. Herein we present a comparative study on finding the suitable polyol procedure that produces Ag NWs and the optimization of synthetic parameters to produce Ag NWs with a high aspect ratio.

P5

Optimisation Study on 3D Printed and Compression-Based Vitamin B6 Tablet Formulation

<u>Shruthi D/O SHANGKAR</u>, <u>Arina Bte YUSRI</u>, <u>Alya Darwisyah Binte</u> <u>MOHAMAD ESA</u>, <u>Priyanka D/O ANPALAKAN</u>, ANG Yi Xin, Josefina SEOW, Felicia LIEW

Republic Polytechnic Institute

3D printing of tablets is described as a future use of tablet production. It is much cheaper to manufacture, easier to formulate and has many advantages as compared to traditional tablet manufacturing process. The study was conducted to determine ways to optimize 3D printed formulation into compression-based Vitamin B6 tablet formulation using various methods such as direct compression method, low shear wet granulation and high shear wet granulation. For wet granulation methods, various amounts of water were added to optimise the moistness and size of the granules formed. After the granules were formed, they were compressed into a tablet and the quality of the tablet made was further analysed by conducting various quality control tests such as weight uniformity test, friability test, hardness test and disintegration test. The conversion of the 3D-printed formulation into a compression-based formulation was successful using wet granulation methods. Based on the results, the high shear wet granulation method showed promising results in enhancing the adhesion of the granules formed, allowing the tablets to pass the quality control tests and meet industrial standards.
P6 LéRICE Laundry Detergent

<u>Karishma D/O RAM SINGASAN</u>^a, <u>TEO Chun Zhi</u>^b, XIE Qiqi, TAN Wie Jie, YOONG Caleb, CHEW Pei Xuan, Dr Subramaniam GURUSAMY

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Plastic waste is one of the major sources of marine pollution. The amount of microplastics produced by laundry washed with detergent is 86% higher than that produced by laundry washed with pure water. Lérice tackles the negative environmental effects of laundry washing while also addressing the issue of food waste. It achieves the goals of sustainability and customer-focus product. Our formulated detergent met all the needs of our customers and showed a market demand for it. Lérice consists of an anti-bacterial formulation that prevents the growth of bacteria which can cause a bad odour on fabric. The detergent is pH balanced to protect your hands while gently and effectively cleaning your laundry. This innovative invention is an eco-friendly sustainable detergent that uses rice waste as a primary ingredient, thereby saving costs. The methods of preparation are simple and easy to perform. It has been scientifically proven using UV/Vis Spectrophotometry to have the same effectiveness as a regular commercial detergent. It is a perfect remedy for those with skin sensitivity and does not lead to allergies during use which is a major social problem with commercial detergents. Its novelty and specialty lie in its ability to perform cleaning functions as efficiently as synthetic chemical detergents, as well as its sustainability, skin-friendliness, and ability to solve wastewater pollution issues by using rice granules and natural ingredients.

P7 Screening for Plant-Based Gelling Proteins <u>XIE Yi Ming</u>^a, Dr Prakash ARUMUGAM, Dr TAN Shi Xiong

^aSingapore Institute of Food and Biotechnology Innovation ^bRepublic Polytechnic

Gelling proteins such as gelatin used in food are from animal sources which are not sustainable and require large land spaces to produce which is a challenge for Singapore to achieve food security. To help achieve Singapore's "30 by 30" goal, we have begun the discovery of plant-based gelling proteins in place of conventional animal-based gelling proteins. In this project, I focused on transforming the plasmids of candidate gelling proteins into yeast *Pichia Pastoris* as it is regarded as safe and an established expression host to express and purify the target proteins that can be incorporated into food. Candidate gelling proteins were also expressed in *Escherichia coli* to obtain a high concentration for further downstream experiments to test its gelling abilities.

From my experiments, I have successfully integrated the candidate gelling proteins discovered from the plant proteome into *Pichia Pastoris*. However, the *Pichia* expression studies were not successful as there was not any visible target protein secretion observed. I was able to obtain protein 2_1 sample from *Escherichia coli* and after concentrating it, the sample became viscous and had a "gel-like" consistency.

Further experiments are required for protein 2_1 before it can replace conventional gelling proteins as only little amounts of protein 2_1 was obtained. We need to increase the concentration yield of protein 2_1 and successfully express it in a food-grade system before we can test its gelling abilities using rheology and incorporating it into food.

Validating Biomarker in Diffuse Large B-Cell Lymphoma

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Despite the standard-of-care treatment for diffuse large B-cell lymphoma (DLBCL), 40% of patients suffer from relapse or refractory disease, leading to significant morbidity and mortality (Sawalha, 2021). The need for alternative and specialised therapeutics can be discovered through mass spectrometry cellular thermal shift assay (MS-CETSA) technology. We identified a key biomarker, U2SURP, a protein involved in RNA metabolism in DLBCL using MS-CETSA. In this study, gemcitabine was used as an experimental drug as a new therapeutic agent against DLBCL. We aim to validate the biomarker responsible for DLBCL's sensitivity to gemcitabine. Validation of U2SURP biomarker was determined through western blot CETSA and cell proliferation assay. Our results show that U2SURP is a potential biomarker involved in DLBCL's sensitivity to gemcitabine. Collectively, our results underpin the potential for MS-CETSA to be used in the advances of knowledge in drugs and its effect on cellular mechanisms for better treatment of cancer.

Identification of Potential Substitutes for Foetal Bovine Serum (FBS) in Cell Culture Medium Using Plant-Based Materials

COMENDADOR Patrisha Gem Dalde^a, LEE Yun Hwa^a

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Foetal bovine serum (FBS) is regarded as the gold standard supplement in cell culture media worldwide. However, owing to a steady decline in availability, the search for alternative sources has been increasing in the industry. Medicinal plant extracts are potential substitutes, as they are more ethically sourced, readily accessible, and affordable. Medicinal plants, such as Aloe vera and Moringa oleifera, have been reported to possess wound-healing properties. This study investigated the potential of aqueous A. vera (AV) gel and M. oleifera leaf (MOL) crude extracts to support the growth and proliferation of adipose-derived stem cells (ADSCs) in vitro. The study was performed by separately preparing different concentrations of AV gel (blended and dried) and MOL extracts in basal media, in the presence or absence of FBS. The effects of the various extract cocktail media on ADSCs were investigated using cell viability and migration assays. Higher concentrations of both blended and dried AV gel extracts demonstrated high cell viability and enhanced wound-healing abilities. In contrast, MOL cocktail media showed dosedependent cytotoxicity, despite exhibiting wound-healing properties. The study concluded that AV gel extracts have the potential to substitute FBS in cell culture media, albeit still not as potent as FBS.

Knockdown of EGFR Expression Inhibits HepG2 Cell Growth

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Hepatocellular Carcinoma (HCC) is a highly aggressive liver cancer that is the most common type and has one of the highest death rates globally. The Epidermal Growth Factor Receptor (EGFR) is often over-produced in many cancers including HCC and acts as a transmembrane oncogenic receptor. Recently, EGFR has become a crucial target for treatment. This study aims to examine the biological roles of EGFR in HepG2 cells, which are derived from HCC. The goal is to eliminate EGFR in HepG2 to gain a deeper understanding of its impact on cell growth and survival. This study began by designing and creating shRNA targeting the EGFR gene. The EGFR-sh1RNA insert was cloned into the pLKO.1-Hygro vector through Gibson Assembly. EGFR expression in HepG2 cells was reduced by using lentiviral transduction of EGFR-sh1RNA through RNAi-mediated knockdown. The change in EGFR expression was confirmed through reverse transcription and real time qPCR at the mRNA level. Finally, the effect of EGFR suppression on cell growth was measured using a cell count assay. After 7 days of lentiviral infection with EGFR-sh1RNA recombinant plasmids, EGFR gene expression in HepG2 was reduced significantly by 86.7%, and cell proliferation was strongly inhibited as well. The results indicated that EGFR activity may upregulate cell proliferation and promote cell survival in HepG2 cells. As such, EGFR may serve as a potential therapeutic target for this malignancy and RNAi of the EGFR gene should be further investigated in vivo as a novel approach to treating HCC.

Studying the Functional Role of *Tp53* in Iron Regulation in Macrophages

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Based on current literature, there is no clear indication on how Tp53 is involved in the iron regulation pathway in macrophage behaviour. However, there is evidence suggesting that *p*53 has a relation with inflammation in the body, whereby inflammatory cytokines seem to connect p53, iron regulation, and macrophage polarization together. Hence, this project aims to determine the effects of *p*53 in iron regulation and its signalling pathway which in turn affects macrophage behaviour and polarization. To achieve the project objective, guantitative techniques such as RTPCR and qPCR, and qualitative techniques such as gel electrophoresis is utilized. Based on the data collated from the experiments done using said techniques above, this study provides in vitro evidence for the potential role of *p*53 in regulating iron metabolism in macrophages. The main takeaway from this study is that the p53 activation in macrophages contributes to changes in intracellular iron levels, which may alter the release of inflammatory cytokines that have tumor suppressive properties. Additionally, this study highlights the importance of optimal cell seeding density and concentration of drug treatments in the cell. More experiments and optimization will have to be carried out both *in vitro* and *in vivo* to further understand the functional role of p53-iron metabolism in macrophages behaviour.

Gene Y and Compound2 in Regulating T Cell Function and Exhaustion

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T-cell exhaustion is a common phenomenon observed in the tumour microenvironment, where persistent antigen exposure leads to a decline in T-cell function and efficacy. This, in turn, hampers the ability of the immune system to effectively combat cancer.

Using an in vitro culture system modelling persistent antigen exposure on human T-cells in the tumour microenvironment, gene Y was identified as a potential prognostic biomarker and therapeutic target, as it regulates T cell exhaustion under persistent stimulation. The results of the study showed that treatment with drug Compound2, which specifically inhibits the activity of Y, resulted in the suppression of T-cell exhaustion, leading to a prolonged anti-tumour immunity. This was identified by a decrease in exhaustion markers such as PD-1 and TIM-3 and an increase in anti-tumour cytokines such as IFNy and TNF α . These findings suggest that targeting activity of Y using drug Compound2 could be a promising approach for treating T cell exhaustion and improving the effectiveness of anti-tumour immunity.

P13 CRISPR Mediated Knockout of Gene X Improves CAR-T Function

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Adoptive T-cell therapy (ACT) has been increasing in prevalence in treating cancer. It involves the infusion of tumor specific T-cells into the patient. As recently as 2018, a type of ACT, known as Chimeric Antigen Receptor T-cell (CAR-T) therapy, has been approved for treating hematological cancers such as aggressive B-cell lymphoma. CAR-T cells are modified T-cells that better recognize tumor antigen. However, they face challenges such as exhasution and poor persistence in the harsh tumor microenvironment. Here, it is demonstrated that X knockout improves CAR-T function by increasing gene expression of interferon γ and increasing the proliferation and/or longevity of the cells.

Evaluating the Expression of Interferons in Diseased Human Lung Tissue

<u>Chroe TEOH Jia ling</u>, <u>Mary Florence VERGARA MANGIO</u>, Siti HAMIMAH

Republic Polytechnic

Interferon-alpha (IFN- α) is found to be a double-edged sword involved in mediating tumour suppressive effects while also promoting tumorigenesis through immunoevasion and immune suppression in tumour cells. However, information on the expression of IFN- α and how it changes with tumour progression is limited. This study aims to evaluate the expression of IFN- α on lung tumours – Adenocarcinoma (ADC) and Squamous Cell Carcinoma (SCC), to investigate if IFN- α can be used as a prognostic tool in lung cancers.

Hematoxylin & Eosin (H&E) staining presented poorly differentiated cells in both tumour types. Immunohistochemistry stain (IHC) was employed to evaluate IFN- α expression in normal and tumour lung tissue for comparison. The findings showed downregulation in IFN- α expression in lung tumour cells conferred by a weaker positive IHC staining for both ADC and SCC as compared to the normal lung tissues. In addition, both ADC and SCC were observed to be of high grade based on the H&E staining showing poorly differentiated tumour cells. The downregulation of IFN- α can therefore be associated with high grade lung tumour, thus providing insights to guiding prognosis of affected patients. Further studies can be conducted to include the comparison of low grade, earlystage tumours with high grade, late-stage tumours.

Evaluating Interferon-Lambda (IFN-L) in Diseased Human Lung Tissue

<u>SA'IT Rayyan, TOH Kai Kei, ABDUL RAZAK Nurul Nashrah,</u> <u>NURAMIN Khadijah Alyah, MANALO Alyssa Cada</u>

Republic Polytechnic

Type III interferons (IFN-Ls) are known to have antiviral and antiproliferative activities. IFN-Ls recently became a protein of interest for treatment of specific cancers, due to its limited signal at barrier epithelial surfaces. The use of Hematoxylin & Eosin (H&E) staining is essential in understanding the differences in structure of normal and diseased lung tissues. Immunohistochemistry (IHC) staining detects IFN-L expression by the antigen-antibody relationship. Primary antibody dilution of 1:100 has shown to produce optimal IHC staining. Based on the IHC grading system established in this study, HIER (Heat-Induced Epitope Retrieval) was optimal in unmasking the IFNLR1 epitopes for antibody binding. Lower expression of IFNLR1 was found in both lung and stomach adenocarcinoma. Findings of this study aids us in evaluating the possibility of high antitumor activity of IFN-L associated with downregulated IFNLR1, as well as potential IFNLR1 expression in asthmatic patients.

P16 Efficacy Evaluation of an Antimicrobial Surface Coating

LEE Mun Lok Timothy ^a, SINNIAH Divyarubini ^a, YEO Xue Ning Janine ^a, LEE Sin Rui Cheryl ^a, KOO Ghee Chong ^a

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The demand for antimicrobial surface coatings is rising partly due to the rapid emergence of drug-resistant pathogens because of the widespread use of antibiotics. Along with the COVID-19 pandemic, there is an increased awareness on maintaining clean or relatively sterile common surfaces on the common objects and facilities to reduce the transmission of pathogens and protect the general public from contracting any potential life-threatening infections. Antimicrobial surface coating is a promising alternative to antibiotics in prevention of transmissible diseases, as it has been shown to be highly effective in killing various pathogens through both the contact-dependent and contact-independent mechanisms.

In this project, the antimicrobial surface coating was applied on polyethylene terephthalate (PET) thin films and their antimicrobial effect Escherichia coli (Gram-negative) and was evaluated on both Staphylococcus aureus (Gram-positive) bacteria species, after 24-hour of incubation through the plate count test. Uncoated PET thin films were used as negative controls. Our results show that the coated PET thin films were remarkably effective against E. coli strain OP50 and S. aureus strain ATCC6538, with more than 99.99% reduction of viable counts as reflected through the complete absence of colony forming unit on LB agar plates for both species after overnight incubation. Reproducible and consistent results were obtained in our repeat experiments. These results collectively prove the effectiveness of the antimicrobial surface coating and support its potential deployment on common surfaces.

Evaluating the Aerosolization of Bacteria During Dental Procedures

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Aerosol generated in dental practice pose a hazard to patients and dentists increasing the risk of cross-infection. Procedures producing aerosols are known as aerosol generating procedures (AGP). This study quantified the spatial distribution of aerosols between high AGP and low AGP. Samples were collected over six weeks in a private dental clinic. Equal number of tryptic soy agar and blood agar were exposed for two hours after the start of the treatment at three different locations (1 m, 1.5 m and 2.5 m) from the dental treatment area. Surface samples were collected using tryptic soy agar contact plates. The agar plates were incubated at 37°C for 48 hours. Colonies were counted and differentiated based on gram status and bacterial shape using a light microscope at 1000x magnification. Statistical analysis was done using one-way ANOVA for mean values of bacteria count between high and low AGPs at all distances. The results showed a significantly higher bacteria count for high AGP (p<0.05), and no significant difference in the bacteria contamination at 1 m, 1.5 m and 2.5 m. Surface sampling results showed similarly high contamination on the dentist's gown, with the least contamination was found on the dental chair, where disinfection was done regularly. Microbial composition consisted of gram-positive cocci at 79.77%, gram-positive rods at 19.28% and fungi at 0.95%. This study established that more aerosols are generated during high AGP than low AGP, increasing the risk of cross-infection during high AGP. Thus, disinfection protocol should encompass the entire dental procedure room and not just the dental chair.

Alternative Culture Media Components for Cultured Muscle Cells

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Insulin is a hormone that can induce muscle differentiation via the Akt pathway in C2C12 myoblasts, a well-studied model for muscle differentiation, into myotubes for cultured meat research. However, insulin has now become costlier and inaccessible for research, hence there is a strong need to find a cheaper and accessible alternative to continue cultured meat research. Plant extracts RPE1 and RPE6 were tested for their potential in inducing muscle differentiation in C2C12 myoblasts into myotubes, and several quantitative and qualitative experiments were carried out to determine its potential compared to insulin, a positive control, and undifferentiated C2C12 myoblasts, a negative control. Data obtained showed that RPE1 and RPE6 do induce some form of muscle differentiation when C2C12 were exposed to them in the form of differentiation medium for 4 days in total. Two main experiments were carried out to determine how well RPE1 and RPE6 induce muscle differentiation in C2C12 myoblast via Quantitative Polymerase Chain Reaction (gPCR) against specific myogenic gene such as MyoD, Muscle Creatine Kinase (MCK), and myoglobin (Myg) and Western blot to detect for the presence of MyoD proteins. Results obtained showed that RPE6 showed greater potential as an insulin replacement as it showed increased gene expression compared to RPE1. The p-value for MCK and Myg was 0.0006 and 0.0003, which was below the accepted p-value <0.005, which showed significance. The results were therefore more similar to insulin controls.

Development of Spirulina-Astaxanthin Tablets For Manufacture of Health Supplements

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Spirulina and astaxanthin are natural foods derived from microalgae. Both contain rich nutrients such as β -carotene, proteins and antioxidants and are known to be beneficial for immune health. However, due to separate doses of each supplement needing to be taken, dosing compliance is an issue faced by consumers. To increase the nutritional value and convenience, the spirulina-astaxanthin tablet is formulated. Spirulinaastaxanthin tablets are not currently available in the market as health supplements, likely due to the poor flowability of the formulation powder which makes it hard to be directly compressed into tablets using the industrial tablet press. The main objective of this study is to develop a spirulina-astaxanthin tablet formulation by adding different excipients to optimise the tablet formulation such that the tablets produced would meet the pharmacopeia standards. The research was carried out using spirulina powder and 2% astaxanthin powder with different excipients. The addition of excipients such as anti-caking agents, glidant and lubricant, can help improve the flowability of the spirulina-astaxanthin formulation to make it more free-flowing. To ensure that the tablets are effective and safe for consumption, the formulation was put through quality control tests including powder flowability, weight uniformity, friability, hardness, and disintegration. Once the formulation has gone through the quality control tests, the formulation will undergo optimization and all quality control tests will be performed again. If the tablets have no significant difference in physical properties, it means they are of acceptable pharmacopeia standard for supplements and can be considered for mass production.

Characterisation of Senescent Human Umbilical Cord Vein Endothelial Cells (HUVEC) and Human Coronary Artery Endothelial Cells (HCAEC)

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The ageing population of Singapore is expected to rise to a guarter of the nation's population by 2030. Coupled with the increased healthcare costs and disparity in the hospitalisation duration between the ageing population and younger people, there is a need for interventions to promote healthy longevity in the individual. Cardiovascular diseases were determined to be a leading cause of mortality in the elderly worldwide with ageing as a major contributor. In this study, we aim to mimic ageing through replicative senescence via serial passaging of the following cells: Human Umbilical Cord Endothelial Cells (HUVEC) and Human Coronary Artery Endothelial Cells (HCAEC) from passages 5 to 15 and evaluate the gualitative and quantitative biomolecular changes in the cell secretome and the cellular phenotype. At the end of this study, we were able to observe trends in cellular morphological changes. Moreover, we also discovered a potential turning point in the effects of replicative senescence in Passage 10 for HUVEC, where significant changes were observed in the morphological evaluation. Additionally, increased protein secretion was observed in the HUVEC secretome. Further study will be required to investigate the biomolecular changes in the cellular secretome.

The Effects of Glycine on Ageing Cardiovascular System

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Ageing is one of the driving factors for the onset of cardiovascular diseases (CVDs). CVDs may result in inflammation of the aorta, leading to structural damage. Inflammation may also increase with age, resulting in CVDs. Glycine is a non-essential amino acid that is naturally present in the body and is known to have anti-oxidative and anti-inflammatory properties. Glycine level decreases with age and adversely impacts immune response. 20-month-old C57BL/6 male and female mice was fed ad libitum standard chow (0.8% glycine) or chow supplemented with 3% and 6% glycine for 8 months. A 31-parameter clinical frailty index scoring was performed at endpoint. Aorta was harvested and processed for RNA extraction and quantitative Polymerase Chain Reaction (qPCR). 8-month clinical frailty index score was non-significantly decreased in female supplemented 3% and 6% glycine. There was a non-significant increase in frailty index in males supplemented 6% glycine. p21 mRNA levels trends towards a decrease in males on glycine compared to controls while no differences were observed in females. IL-6, CD11b and MCP-1 mRNA levels was not statistically different between controls versus glycine groups in both male and female. Glycine may reduce frailty in aged female but not aged male mice. However, it did not significantly alter senescence or reduce inflammation in the aorta of aged mice. Future work includes processing aortic tissue samples for immunohistochemistry to examine its structural composition.

P22 Transforming Your Salad Bowl Using CRISPR-Cas9 Angela LEE Yu Xin ^a, DAISUKE Urano ^b

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Lettuce is one of the most consumed leafy vegetables worldwide, with the global trade value of approximately 22.3 trillion U.S. dollars in 2021 (Sabanoglu, 2022). However, local production of lettuce is difficult as it is a temperate crop that requires cool and dry conditions for its cultivation. Furthermore, seeds of most lettuce cultivars fail to germinate at temperatures above approximately 28°C (Yoong et al., 2016). Through the clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) system, we can edit genes in lettuce to generate genetically modified lettuce with desirable traits. Three genes, Gene-1 (G-1), Gene-2 (G-2), and Gene-3 (G-3) were edited in this project. Previous literatures reported the following functions of these genes: G-1 and G-2 causes dwarfness in maize, rice, and tomatoes. G-3 is associated with heat resistance in rice (Tiwari and Bisht, 2022). These crop traits are ideal for urban farming in tropical climates, especially in Singapore, where short and heat-resistance plants are favoured.

Novel Nuclear Receptor Regulatory Mechanism in Mouse Pluripotent Stem Cells and Synthetic Embryo

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Mouse expanded potential stem cells (EPSCs) possess the ability to selforganise and differentiate to form blastocyst-like structures, termed blastoids. However, this process has its limitations such as low blastoid yield. Currently, many scientific papers focus on the exogenous cues in blastoid culture media that can help to improve this differentiation and selforganisation process. The intrinsic factors that regulate this process remains understudied. Our study revealed that Gene X, a novel transcription factor, played an important role of intrinsic regulation in the differentiation process. Downregulation of Gene X expression level using siRNA caused a decrease in blastoid formation yield, while activation of Gene X through agonist addition increased blastoid formation yield. We further studied the mechanism of Gene X and uncovered that granulin (Grn), a downstream target of Gene X, is partially responsible for the phenotypes that were observed in blastoid formation. This study therefore elicits a Gene X-centric regulation of expanded pluripotency in EPSCs.

Comparing Cryopreserved and Fresh Mouse Feeders in Supporting the Clonal Growth of Human Epidermal Keratinocytes

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Cultured epithelial autografts are considered the gold standard for permanent wound coverage in patients with extensive burns and limited donor sites for graft harvesting. However, the production process is not only costly but also time-consuming. A possible solution would be to replace the fresh mouse feeder cells required with cryopreserved ones. This will reduce both the cost and time required, thereby improving treatment options for patients, and reducing the likelihood of complications arising.

In this study, we assessed the suitability of using cryopreserved feeder cells to produce cultured epithelial autografts. To do so, colony plating efficiency assays were used to compare the clonal growth of human epidermal keratinocytes supported by cryopreserved and fresh feeder cells. Terminally differentiated colonies and the total number of colonies that developed were then tabulated and statistically analysed.

Results showed that cryopreservation had no significant effect on both the ability of feeder cells to support the clonal growth of human epidermal keratinocytes and their proliferative capacity. This opens the possibility of using cryopreserved feeder cells in cultured epithelial autograft production. However, process evaluation will be required before this change can be applied to clinical practices. Further research on the effects of cryopreservation on the distribution of clonal types of human epidermal keratinocytes will also allow us to better understand the potential benefits and limitations of using cryopreserved feeder cells in cultured epithelial autograft production.

Enhancing Protein Thermal Stability with Protein Engineering Approach

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In vitro Point-of-Care (PoC) diagnostic test kits are gaining relevance in many industries due to their rapid and convenient properties. However, as PoC test kits are often limited by antibodies' biophysical properties that threaten result accuracy and product quality, the alternative of rcSso7d thermostable scaffold binders is developed. The rcSso7d binders are utilized in the reporter and capture protein constructs of this project to recognise target-specific biomarkers, which are easily adjustable to a wide variety of biomarkers. Fusion partner proteins are often added to protein constructs to enhance protein expression and solubility. The fusion partner Maltose Binding Protein (MBP) present in the reporter construct (BA-MBP-Sso) serves as a steric spacer to allow greater biotin accessibility and hence, higher signal detection. From previous studies and thermal stability tests, MBP in BA-MBP-Sso.TB construct was found to cause instability and denaturation under high temperature treatment, leading to loss in signal activity, as compared to BA-Sso.TB construct with minimal activity loss. This project aims to improve the stability and protein binding of the reporter reagent by exploring different fusion partner candidates with further optimization and in-depth stability checks to achieve a PoC test kit with thermostable and strong binder proteins.

Efficacy of Novel Anticoagulants in a Human *Ex Vivo* Thrombosis Model

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COVID-19, caused by SARS-CoV-2 virus, has been known to be linked to cardiovascular complications. In a previous study done on COVID-19 inpatients, 44.6% of patients had high D-dimer (≥0.50 mg/L). Amongst those, COVID-19-associated coagulopathy (CAC) is alleged to be associated with high fatality. Therefore, it is important to find high efficacy and safe anticoagulants for the treatment of CAC. Current therapeutics such as low molecular weight heparin (LMWH) and unfractionated heparin (UFH) have limited clinical evidence specific to the use in COVID-19. Two novel anticoagulants were developed, Ultravariegin and FXI038, which have shown superior efficacy to reference therapeutics such as UFH in the pre-clinical trial. The efficacy of these anticoagulants in a human ex vivo thrombosis model is currently being studied. Blood from study participants recently recovered from acute COVID-19 infection were driven directly through a series of ex vivo perfusion chambers containing endothelium-denuded pig aorta strips. The aorta strips provide thrombogenic surface for clot formation. Individual anticoagulants were infused into the blood as it passed through the perfusion chambers. Strips were collected, embedded, fixed, and processed into histological sections. Subsequent staining with antibodies against fibrin clots and platelets allowed for quantification of clots formed as an indicator of the efficacy of anticoagulants.

Recombinant Expression and Purification of Inhibitors of Coagulation Factor XIa

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Older generation of anticoagulants such as heparin and warfarin, has been shown to increase the risk of bleeding, which is an unfavourable side effect. Although new oral anticoagulants (NOACs) are effective and have a greater safety margin, there is still a risk of bleeding in individuals. The yearly occurrence rate of bleeding while taking oral anticoagulants is between 2-5% for major bleeding and 0.5-1% for fatal bleeding. Due to concerns about the potential haemorrhagic side effects of these anticoagulants, there is a need for the development of anticoagulants with low or no bleeding risk. This project aims to extract and purify novel inhibitors of coagulation factor XIa, P1395M and P1395M-Fc. P1395M was recombinantly expressed in E. coli and purified using His-tag purification followed by Reverse-phase High Performance Liquid Chromatography, while P1395M-Fc was recombinantly expressed in CHO cells and purified using Protein A affinity chromatography followed by anion exchange chromatography. The liquid chromatography methods were successful in purifying the protein, showing a single clear band after performing SDS-PAGE. Chromogenic assay showed FXIa inhibition by recombinant P1395M and P1395M-Fc, which suggest that they are folded in the right conformation during its synthesis process, with an average IC_{50} of 1.2 nM and 5.1 nM respectively. The successful production of these inhibitors will allow subsequent testing in pre-clinical animal models.

P28 Alternative Culture Media Components for Cultured Muscle and Fat Cells

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In a strive to meet Singapore's "30 by 30" initiative, advancements in food biotechnology are leading to the dawn of cultivated meat technology. The uprising technology promises healthier, cruelty- and disease-free meat products that can be environmentally friendlier and more efficient to produce, when matured. However, culture/differentiation media used by the R&D industry would prove non-transferable in the context of cultivated meat due to the use of animal-derived serum. Serum-free media supplemented with growth factors was introduced but was a major reason for the extremely high cultivated meat production costs. In hopes to lower production costs, the search for inexpensive alternative serum-free media components and growth factors began. Insulin, a differentiation inducing growth factor, emerged as a major target due to its rocketing price tag and vital role in differentiation media. RPE1, an inexpensive plant-derived extract, was discovered as a possible substitute for insulin in differentiation media. To study the effects of RPE1 in differentiation, it was used in place of insulin in differentiation media for myogenesis of C2C12 myoblasts and adipogenesis of 3T3-L1 preadipocytes. Morphological changes were documented throughout treatment. The expression of two myogenic and adipogenic genes were quantified using qPCR. The results of the project conveyed that RPE1-induced differentiation was a success, though less efficacious compared to insulin. RPE1's future remains bright and may prove a crucial discovery in bringing cultivated meat to the mainstream market. There is still much to study about RPE1; identification and purification of its active ingredient could be the next logical step.

A Comparison of High-Fat-Induced Depression Behavior Between C57BL/6 And BALB/c Mice

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Clinical studies suggest a strong relationship between diabetes and depression conditions. Mice model of diabetes induced by streptozotocin have been shown to display a variety of abnormal behaviors including cognition deficiency and depression, as tested in Porsolt's swim test, a putative animal model of depression. The high-fat-induced diabetic mouse model is the most clinically relevant and recognized animal model especially for human Type 2 diabetes. In the current study, high-fat-diet was used to induce diabetes in C57BL/6 and BALB/c mice. The time course of the development of diabetes and level of depression was assessed using the tail suspension test. The results show the difference between C57BL/6 and BALB/c mice in response to high-fat treatment in terms of fasting glucose and the level of depression at different time points following the high-fat treatment.

The Efficacy of Dietary Manipulation on Spontaneously Diabetic Torii Rats

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The prevalence of T2D continues to rise despite advances in medical interventions. To mimic the effects of TD2, we used SDT rats that have been shown to have similar pathophysiology to diabetic patients. In this study, we customized 4 dietary formulations (HP1, HP2, HP3 and LP), with varying GI and protein levels in an attempt to lower blood glucose levels and organ damage. To measure the extent of damage and compare the effectiveness of each diet group, we conducted physiological studies (BW, NA, BG, Treadmill), histological analysis on metabolic organs (Heart, Liver, Kidney, Spleen and Pancreas), and analyzed the serum by ELISA for inflammatory and diabetic biomarkers (Insulin, C-peptide, IL-18 and sTNFR-2). We employed the use of Microsoft Excel to perform ONE-WAY ANOVA and linear regressions to support the reliability and significance of our results. Through the regression tests, the diabetic and inflammatory. Biomarker results appear to be not significantly related. Through microscopy, we found that rats in the HP1 diet group's metabolic organs showed little damage while those in HP3 and LP diet groups showed exceptionally greater cellular impairment. The lack of sample size from HP2 made its results less reliable. Thus, this study has shown the potential of diet group HP1 stabilizing blood glucose levels and attenuating organ damage and inflammatory biomarkers in SDT rats.

Pilot Study of *Balantidium coli* in Piglets (*Sus Scrofa Domesticus*) and the Efficacy of Metronidazole

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The objectives of this pilot study were to access the prevalence of *Balantidium coli* (*B. coli*) in a large animal facility in Singapore, observe the clinical manifestations of the infection in piglets and to evaluate the effectiveness of Metronidazole as a primary treatment. The data collected from the piglets that were tested positive for *B. coli* from 2019 to 2021 was collated to calculate the prevalence of the infection yearly. Twelve piglets tested positive for *B. coli* were housed separately into 4 pens, A1, A2, B1, and B2 over 10 weeks. Piglets in pens A1 and A2 were experimental groups, where 33 mg/kg of Metronidazole tablets were given orally with 300 g of pig grower feed per piglet twice daily (BID) for 7 days. Piglets in pens B1 and B2 were control groups and were not provided with any treatment. Piglets were monitored and a Direct-Faecal Smear was performed weekly to observe any effect of infection and its parasitic load to evaluate the efficacy of Metronidazole.

Results showed an increase in the prevalence of *B. coli* in the large animal facility by 30% from 2019 to 2021 (p<0.05). Mildly infected pigs (CPG<100) were mostly asymptomatic, where some symptoms like diarrhoea and inappetence was observed. Trophozoites were mostly detected in diarrheic faecal samples, while cysts were present in normal ones. Efficacy of Metronidazole was limited as a long-term treatment and only acted as a temporary remedial measure to eliminate initial infection. This pilot study lays the groundwork for more extensive research in the future.

Investigating the Relationship Between the Gut Microbiome and Feeding Behaviour in Zebrafish Larvae

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The growing prevalence of obesity is a worldwide concern. As reported by the Ministry of Health in 2021, "Singapore's obesity rate has risen to the highest level since 2010". Surveys were conducted between July 2019 to March 2020 and results showed that 10.5% of Singaporeans were obese compared to 8.6% in 2017. Whilst exercise and proper diets are solutions to counter obesity, the rapid increase in obesity shows a lack of control in these factors.

However, an overlooked mechanism present in the gut could remedy the growing issue. The gut microbiome is a complex ecosystem which refers to all microbes present in the intestines, and they play key roles in metabolism. It influences appetite and dietary choices via the gut-brain axis, indicating potential gut microbiome outputs and therapeutics to manipulate the microbial diversity and composition. A study reported that the gut microbiome is an important environmental factor that affects energy harvest from the diet and energy storage in the host using a mice model.

This study complements the existing findings surrounding the benefits of the gut microbiome by using a new animal model, zebrafish (*Danio rerio*) and utilising feeding assays to explore impact of metabolites and microbes on the gut-brain axis, and the resultant satiating effects on feeding. Our findings suggest that the gut microbiome reduces feeding in the zebrafish larvae through the production of metabolites. Thus, investigating metabolites which induces a satiating effect may directly reduce overeating, leading to encourage healthier lifestyles.

Improving the Yield of Limonene in Engineered *Escherichia coli* by Investigating Different Carbon Sources

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Limonene is an essential, natural compound widely utilised in the flavour, fragrance, and pharmacological industries. Currently, engineering microbes for limonene production has been an attractive alternative to conventional plant extraction due to its higher yield, viability, and economic value. Central carbon metabolism (CCM) plays a key role in coordinating nutrient availability by transforming carbon through various pathways which in turn affects cell growth and limonene production. Regulating the CCM network via media formulation has posed as an alternative to pathway-specific inhibition methods since it does not perturb the tightly regulated circuit. Thus, this study aims to improve the yield of limonene in engineered *E. coli* by investigating different carbon sources i.e., glucose, fructose, sucrose, and glycerol. Though glucose has been established as the best carbon source for various *E. coli* strains, the use of dual carbon sources exhibits potential in improving limonene yield through the sequential utilisation of substrates which maximises nutrient allocation. Our results show that the best carbon source for limonene production was pure glucose since it was the main driver of glycolysis. Nonetheless, major bottlenecks and regions which exhibited potential for future investigations were identified.

Development of A Bioprocessing Workflow for the Biomanufacturing of Biologics following Good Manufacturing Practices

Devika PREM, Effa Maslynie Bte AHMAD, Kaisah Binte YUSOFF, Rabiatul Adawiyah Bte M A, Lloyd George

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Biologics are therapeutic products derived from living organisms or their components. Examples of biologics are stem-cells and monoclonal antibodies which are used in the therapy of cancers. A bioprocessing workflow in the biopharmaceutical industry refers to an ordered and consistent set of tasks, routines, and tools to produce a biologic product of high quality and safety. For therapeutic purposes, the critical quality attributes for safety are dependent on the integrity, strength, purity and efficacy of the biologic drug.

In our project, we developed a bioprocessing workflow for biologics production following ICH(Q7) also used by the biopharmaceutical industry. Our bioprocessing workflow can be broken down into three main stages which includes preparation, production, and purification. To model the preparation stage, we first performed cleanroom operations, and set up a Water for Injection (WFI) production system. We used this ultra-pure distilled water for all cleaning and sanitization of materials and environment; and also manufactured chromatography-grade buffers by tangential flow filtration (MWCO 1kDa) using a Minimate EVO TFF system.

Using recombinant IgD-producing HEK-293 cells as a model, we successfully set up a seed train to scale-up frozen cells from a 1mL cryovial to 200mL of healthy cells, for inoculation into a Bioreactor. We used human serum to simulate convalescent patient serum, and successfully purified IgG using ÄKTA start protein purification system by Protein G affinity capture. The identity of the simulated therapeutic antibody was confirmed by SDS-PAGE and uHPLC.

Our workflow may be used to conduct future R&D into biomanufacturing operations for new biologics.

From Raw Feedstock to Finished Product: Purification of Biopharmaceuticals

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Quality in equals to quality out, an important concept of Good Manufacturing Practices.

In our project, our goal was to replicate an industrial biomanufacturing process and scale it down to bioproduction within Republic Polytechnic for preparative levels of production of biopharmaceuticals. Our project involves the purification of polyclonal antibodies from human serum to model the bioproduction of therapeutic antibodies from convalescent patient serum. All work were carried out under GMP conditions according to ICH(Q7) in cleanroom conditions to obtain a high-quality biopharmaceutical.

Bioprocessing involves 3 stages: preparation, production, and purification. During bioprocessing of a raw feedstock, the preparation stage is essential to sustain the quality of the product. To create a quality workflow and obtain the final pure product, we utilised a variety of innovative strategies to get the necessary desired results.

To ensure environmental sterility and reduce contaminants during biomanufacturing, all work were performed in the Biosafety Cabinet (BSC) and laminar flow hoods within a cleanroom with 0.1µm filtered air. A distillation production area was set up to produce simulated water-for injection, which was used for all washing and cleaning; and to make high quality ultra-pure chromatography-grade buffers. Tangential flow filtration with MWCO 1kDa was used to produce all buffers for FPLC and uHPLC. ÄKTA start protein purification system was used to purify HulgG. The identification of the antibody was confirmed by SDS-PAGE. The identified antibody was then used as a reference analyte to optimise and develop an uHPLC methodology for future quality control (QC).

Microencapsulation Formulation for Natural Ingredients Using Natural Product-Based Materials for Cosmeceutical and Nutraceutical Application

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The term "nutraceutical" and "cosmeceutical" initially arose from the combination of "nutrition", "cosmetics" and "pharmaceutical". These were defined as a food or part of a food that provided a vast amount of health benefits. These supplements tend to deliver active compounds in isolation and in dosages that exceed what could be naturally obtained from common ingredients. Synthetic products that are used for food or in the form of supplements have its own drawbacks like stability issues, not able to be consumed by all due to health reasons and even not ideal to the environment. In this project, an alternative plant-based product is formulated, and its effectiveness is tested. The proteins, GA and GB, are mixed with Rosemary oil or Thyme oil with the addition of Stabiliser and Alkaline solution. After being homogenized and thereafter undergoing spray-drying, the powder product is assessed on different Quality Control tests. A total of 24 samples were formulated with differing unchanged variables. By doing so, it helped to determine the samples which will be the best possible formulation in all possible aspects. Although the characterization of the physical properties of encapsulated system offers meaningful information, it also helps to predict and accurately design tailored encapsulation systems and operating conditions for the "nutraceutical" and cosmeceutical" thereby keeping these activities intact.

Generation of Knock-out SLC25A22 and SLC25A18 Cell Line to Study Effect on Neuronal Function and Amino Acid Metabolism

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Neurological diseases encompass a wide range of disorder ranging from genetic dysfunctions to age related disease. Largely, the central nervous system (CNS) is controlled through tight regulation of neurotransmitter released, and dysfunction affecting the regulation causing imbalance can lead various neurological diseases. SLC25A22/GC1 and SLC25A18/GC2 are both considered to be mitochondrial carriers of glutamate, an excitatory neurotransmitter of the CNS, responsible for the translocation of cytosolic glutamate into the mitochondria. Through knocking-out of GC1 and GC 2 via CRISPR-Cas9 of wildtype H1 human embryonic stem cells (H1-hESCs) and subsequently Ngn-2 induced differentiation into neurons, we hope to investigate effects of the knock-out on electrophysiological activity and neuron development/growth. Our study showed knock-out SLC25A22 cell line showed decrease rate of growth and decrease excitatory postsynaptic current (EPSC) in induced neurons, while western blot of SLC25A18 showed no expression using the Ngn-2 differentiation system. Overall, we conclude no pathophysiological link can be established between SLC25A22 effect on neuronal function and associated diseases based on the limited data produced in this study. In the future, apart from collecting more data for a better representation, cytosolic and synaptic vesicle glutamate concentration can be quantified to investigate the effects on glutamate of GC1 and GC2 knock-out. Moreover, modification of current model or new model can be used to investigating the effects of SLC25A18 continue knock-out on electrophysiological activity and neuron development/growth.

Development of Patient Educational Materials for NKF Patients to Enhance Literacy

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Currently, National Kidney Foundation (NKF) does 1-to-1 patient counselling with their patients via a deck of PowerPoint slides to educate them on topics like anaemia, vascular access, fluid management and fall prevention. However, patients are not given any information leaflets to bring home to refer to. Therefore, this project aims to create educational materials (softcopy and hardcopy) for the NKF patients so that patients can read the topics on their own at home. Additionally, this project investigates the link between patient profile and their preference for the mode of educational materials (softcopy or hardcopy). From October to November 2022, data collection was performed in National Kidney Foundation (NKF) dialysis centres (DCs) in the north, central and east region to understand their preference for the educational materials. A total of 136 patients aged 30 and above were surveyed through face-to-face interviews. Among the 136 participants, patients aged 50-80 made up the majority (84%). 78% of the participants preferred hardcopy and 93% found the educational materials useful. Patient's level of educational qualification (p>0.05) and age group (p>0.05) did not have an impact on the type of educational materials preferred. The duration of dialysis that the patients were on did not affect how useful they found the materials to be (p=0.326>0.05). In conclusion, the implementation of hardcopy and softcopy educational materials for dialysis patients was deemed as successful based on the results gathered from the survey. Moving forward, educational materials created should still be in hardcopy and softcopy to cater to all patients.

Keywords: Health Literacy; Anaemia; Vascular access; Fluid management; Fall prevention; Hardcopy; Softcopy; Educational; Materials

Optimization of Chondrogenic Medium for Enhanced Chondrogenesis

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Mesenchymal stem cells (MSC) are multipotent adult stem cells that are able to differentiate into chondrocytes. Leveraging on this ability, researchers have developed chemically defined media to induce MSC differentiation into chondrocytes (e.g. in a pellet culture model) for implantation into cartilage defects to stimulate tissue regeneration. Over the years, there has been many developments in chondrogenic differentiaion media which include the supplementation of various cytokines and growth factors in order to enhance the efficacy of chondrogenesis. However, we found that conventionally used chondrogenic media compositions are inefficient, leading to poor collagen production which can impede cartilage regeneration. Therefore, our study aims to maximize the efficacy of chondrogenic medium by optimizing its composition and the concentrations of TGF-B3, BMP2 and vitamin C. We found that 75 µg/mL of vitamin C led to the greatest Col2A1 gene expression. The addition of BMP2 enhanced chondrogenic differentiation at both the mRNA and protein level. Furthermore, increasing the dosage BMP2 improved chondrogenesis of TGF-β3 or by enhancing glycosaminoglycan and glycoprotein production.

Computational Analysis of Ovarian Cancer Spheroid Invasion of the Mesothelium

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During metastasis, ovarian tumours invade the sub-mesothelial layer of the peritoneal cavity through clearance of the mesothelial lining protecting the peritoneal cavity. Timelapse imaging of fluorescent ovarian cancer spheroids invading the fluorescent mesothelial layer, and Traction Force Microscopy (TFM) were performed to quantify and analyse the traction stresses acting on the mesothelial basal substrate. Additionally, segmented labels of the cancer spheroid's nucleus using Star Dist Artificial Intelligence (A.I.) were used to track the movement of the spheroids invading the mesothelium. After generating the segmented nucleus images, they are applied to the original 3d videos in Imaris for nucleus movement tracking correction. Our results suggest that lateral migration of the mesothelial cells away from the spheroid, by generating basal traction stress, might be dispensable for the invasion process.

Bioengineered Siglecs for De Novo Discovery of the Sialoglycan Landscape in Cancer

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Hypersialylation, the overexpression of sialic acid, plays a major role in cancer progression and metastasis via the Siglec-sialic acid axis. In this study, we produced a series of recombinant Siglec-Fc proteins and employed high-plex cytometry on both blood cells and tissue samples to deep dive into cancer-specific regions. In particular, we used the latest imaging mass cytometry (IMC) to capture tumor-specific regions in Epstein Barr virus (EBV) positive diffuse large B cell lymphoma (DLBCL) and nasopharyngeal carcinoma (NPC) tissue sections. Flow cytometry was also used to compare pancreatic ductal adenocarcinoma (PDAC) cell line, AsPC-1, against peripheral blood mononuclear cells (PBMCs). To gain complementary insights into these high-dimensional datasets, both spatial and suspension analysis were done with R script programming and Flowjo respectively. Our results showed significant Siglec-X5 and -X6 ligand expressions on EBV-positive DLBCL and NPC, along with minimal expression of CD15s. On the other hand, AsPC-1 had a higher proportion of strongly positive Siglec-X2 ligand expression than PBMCs but no Siglec-X5 and -X6 ligand expressions were observed. More importantly, we demonstrated Siglec-X5 and -X6 ligand overexpression might be better prognostics than CD15s, a well-known cancer prognostic marker.
Transposable Element-X Enhancers Regulate Genes in Mouse Embryonic Stem Cells

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The interaction between mouse embryonic stem cells (mESC) and transposable elements (TE) in cell state-specific gene regulation is largely unknown. However, in a recent study TE has been found to mediate transcription in the mammalian genome (Fueyo *et al.*, 2022). Therefore, in this study we explore the interplay of TE-derived enhancers in mESC models using genome-wide sequencing data, CRISPR inhibition, luciferrase assay and chromatin immunoprecipitation. Through this, we found that the SINEs family is the more involved TE family associated with mESC, more specifically transposable element-X (TE-X), which is found to be co-opted by transcription factor-Y (TF-Y), and their interaction had been found to be crucial for the regulation of gene expression in mESC.

Expression of Proteins in the MHC Class I Antigen Presentation Pathway

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The MHC class I antigen presentation pathway is critical in the immune clearance of virus-infected and nascent transformed cells by CD8+ T cells. However, certain viruses harbour mechanisms to evade this pathway. For example, the Epstein-Barr Virus produces the immunoevasin BNLF2a, among others, that binds to and inhibits the transporter associated with antigen processing (TAP) whose function is essential for peptide loading onto MHC class I molecules. In this project, production of proteins involved in the MHC class I antigen processing pathway, namely, wildtype TAP, pMHC-I complexes, the TCR, and viral peptides, BNLF2a and ICP47, was attempted. To overcome the assembly of macromolecular complexes, the use of eukaryotic cell overexpression, Cre-LoxP plasmid recombination, and protein engineering techniques were employed. This project makes way for the study of the inhibitory binding mechanisms of the two viral peptides and the development of a soluble TCR probe for MHC-I downregulation assays which will be useful in testing the effectiveness of antigen rescue therapeutics such as a proposed watersoluble TAP protein. Wild-type TAP was successfully produced, while the production of the soluble TCRs, BNLF2a, and ICP47 needs to be further optimised.

P44 Genomic Studies on *Hericium erinaceus*

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The recent publication of the entire genome of *Hericium erinaceus* (LM), had gathered lots of interest in understanding the genome. Comparatively to the most consumed and well-studied mushroom *Pleurotus ostreatus*, LM genome is sparsely understood. In this study, the aim was to conduct an observational study on short tandem repeats (STR) changes over LM ageing process. The relationship between the STR and the phenotypic trait of the mushroom was also studied. Polymerase Chain Reaction (PCR) was conducted to amplify the target STR regions followed by 2% agarose gel-electrophoresis to visualise the PCR products. The PCR products undergo cycle sequencing and purification process before Sanger sequencing. The sequencing result of primer MK359 which amplifies CAG/CAA STR on chromosome 6 of LM has shown an inverse relationship between CAG and CAA STRs count as LM ages. Additionally, the CAG and CAA STR count inverse relationship is postulated to influence the phenotypic trait of LM. However, the LM STRs requires more LM sample sequences data for a definitive conclusion.

Conservation Genetics of Two Charismatic Butterfly Species of Singapore

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The Common Birdwing (Troides helena cerberus) and Common Rose (Pachliopta aristolochiae asteris) are listed as 'Vulnerable' in Singapore's Red Data book. It is believed that both species survived extirpation by utilizing the non-native Aristolochia acuminata as their larval host plant after the local extirpation of the native, Aristolochia jackii. Recent studies suggest that there are only 8 to 14 source populations across Singapore. It is further feared that some of these source populations may be disconnected, with important conservation implications such as inbreeding depression. Here, we present a pilot attempt to investigate the genetic variation within local populations of both the Common Birdwing and Common Rose. We report on the viability of DNA extracted from drypreserved museum specimens as well as 'freshly-dead' samples acquired from private-garden owners. The private-garden owners had been briefed on general specimen requirements for genetics analyses and how to properly preserve dead specimens of various life stages. Five primer pairs, targetting four genetic regions cytochrome c oxidase subunit I (COI), cytochrome c oxidase subunit II (COII), NADH dehydrogenase subunit I (ND1) and 16S ribosomal RNA, were utilized for gene amplification with varying success. Preliminary findings suggest that the degree of genetic variation amongst populations of both species is low. However, better geographic representation is needed for more accurate estimates. Consequently, the protocols and best practices generated through this project will inform future molecular surveillance efforts and the implementation of management strategies such as rear-and-release programs and the planting of host plants to improve habitat connectivity.

Determination of Possible Hybridization between Tilapia and Jade Perch through DNA Barcoding and Phenotyping

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The owner of Century Aquaculture Farm approached Republic Polytechnic to ascertain a potential hybridization event between female red tilapia (tilapia species hybrid) and male jade perch (Scortum barcoo), two non-closely related species, resulting in the production of viable and fertile progeny. Hybridization usually occurs between members of closely related species, such as species within the same family. When hybridization occurs between fishes of non-closely related species, the progenies are usually infertile due to the differing number of chromosomes that fail to pair up during meiosis. Hence, the aim of this study was to determine if the suspected hybrids are indeed hybrids of the red tilapia and jade perch. The progenies are of high interest as they appear to exhibit characteristics that are highly advantageous for aquaculture. To help determine the phylogeny of the suspected hybrids, DNA barcoding of the mitochondrial cytochrome c oxidase subunit I (COI) gene was conducted using the Promega DNA Extraction Kit, Promega PCR Kit, and Sanger sequencing. This study involved mitochondrial gene analysis which only allowed for the determination of the maternal line. Therefore, morphometrics was utilized to investigate if the suspected hybrids possessed any morphological similarities to both possible parent species and to gain insight into their paternal line.

P47 Recyclean Toilet Deodorizer Balls

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Recyclean is a toilet deodorizer ball product that came to life by the collaboration of both business and scientific ideas our during BioEnterprise and BioInnovation module in Year Three of our Polytechnic studies. Made from scratch with natural materials such as pectin, citric acid, glycerol, and lemon essential oil, we foresaw Recyclean to be a product that efficiently removes stains in toilet bowls. Through numerous interviews of our target customer segment (Household managers), and understanding what they desire in a cleaning product, we then gained direction for our idea (toilet deodorizer balls). After various prototyping phases that heavily involved trial and error coupled with putting our scientific knowledge to use, we managed to develop our final product that had proven effective through the testing phases. For instance, our final batch deodorizer balls (10 balls) were effective in removing Nutella and condensed milk stains per flush, while exhibiting prominent lemon scent for more than two weeks. Calculations also proved that our product could potentially eliminate microbes as the estimated mass released of citric acid per flush is 6.175 grams, which is only a marginal 2 grams away from the required mass of citric acid to effectively remove microbes (8.65 grams). Furthermore, by using modern-day social media spaces like Instagram and Facebook, we have garnered greater outreach for Recyclean by promoting our product and its efforts to be sustainable while maintaining cleaning efficiency. Through the customer feedback generated coupled with our team's hard work and vision, we harbour the belief that Recyclean is one step closer to becoming a bigger brand in the foreseeable future with enough time and dedication.

P48 Expression of Sars-Cov2 Spike Antigen in Insect Cells

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SARS-CoV2 virus is responsible for the COVID-19 global pandemic. The current recommended facility requirement for handling this virus by Centres for Disease Control and Prevention of USA is Biosafety Level 3 (BSL3). There are ongoing needs to study this highly infectious virus for detection of antibodies and development of small molecule inhibitors. The viral surface Spike protein (S) has been shown to play crucial during viral entry into host cells by interacting with angiotensin-converting enzyme 2 (ACE2) on the epithelial cell surface. The recombinant S-protein and mRNA have been used as vaccine successfully. In this project, we used BAC to BAC system to express SARS-CoV2 S-protein in insect cells. The aim was to generate S-protein expressing insect cells and pseudo-typed baculovirus to be used as safe research and testing platforms for SARS-Cov2 virus without the need for BSL3 facility. We first subcloned 4 four versions of S gene into pFastBac plasmid. These four versions of S gene are from Alpha and Delta variants of SARS-CoV2 with or without a functional furin cleavage site. Then the pFastBac plasmids were transformed into DH10Bac Escherichia coli competent cells to generate recombinant Bacmid. The recombinant Bacmids were then transfected into Sf9 cells for protein expression and generation of the baculovirus. The supernatant containing the generated baculovirus was harvested and transduced into sf9 cells for viral amplification. Immunofluorescent microscopy and ELISA were used to verify S-protein expression in Sf9 cells and baculovirus.

Identification and Characterization of Compounds that Inhibit TORC1 Complex to Delay Cellular Aging and Increase Lifespan

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Aging is the biggest risk factor for numerous human chronic diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases. Although a few drugs, such as rapamycin that directly address aging have been discovered, they cause substantial side effects. Thus, there is an urgent need to identify geroprotectors that can generally delay the aging process and prevent diseases associated with aging. Saccharomyces cerevisiae yeast is a powerful model organism that has been used to study the biology of human aging and identify anti-aging interventions. In this study, we discovered a small molecule Compound A that inhibits the TORC1 (Target of Rapamycin Complex 1), which is a conserved regulator of the aging process and master regulator of nutrient sensing complex from yeast to humans. Transcriptomics analysis was performed on mitochondrial genes, which showed the upregulation of tricarboxylic acid (TCA) cycle genes. In agreement with the expression profile of the mitochondrial TCA cycle, electron transport chain (ETC) gene expression and ATP levels were high in cells supplemented with Compound A. Compound A-mediated TORC1 inhibition Moreover. enhances mitochondrial function which is associated with beneficial functions during aging. Thus, our novel small molecule Compound A showed a potential anti-aging activity that could be utilized as a drug or food supplement to delay aging and increase lifespan.

Investigating the Effects of Mangosteen Peel Extract on Dermal Endothelial Cells

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Garcinia mangostana Linn. (GML) is known to possess plenty amount of polyphenol compounds such as phenolic acid and flavonoids, that have antioxidant and anti-inflammatory properties. Many regard the peel as waste, but there is much utilisation of such waste as a natural source of bioactive compounds. Investigation of their effects on endothelial cells (EC) is made possible due to their bountifulness. It has been reported that oxidative stress and inflammation are key causes of endothelial dysfunction and most skin-associated disease. Furthermore, most treatment method revolves around lifestyle changes and modifications made to the diet or medication which are usually costly and timeconsuming. Hence, in this study, we aim to use an alternative treatment by using GML peel. Trimethylamine N-oxide (TMAO), a gut microbiomederived metabolite, involved in CVD progression was used to induce inflammation and oxidative stress in the HMEC-1 cells. Extraction of GMP was done using pressurised hot water extraction (PHWE) system. Liquid chromatography-mass spectrometry (LCMS) was then used to identify the metabolic compounds and changes made in lipid profile of the treated cells. GMPE was then evaluated for their antioxidant capacity using DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay. qPCR was performed on the cells co-treated with TMAO and GMPE to identify the effects that GMPE has on TMAO-induced injuries. This study provides insight into the treatment of skin-associated disease by providing data that GMPE has antioxidant and anti-inflammatory properties which can help mitigate the effects of TMAO-induced injuries on EC. However, potential application of GMPE in mitigating endothelial dysfunction requires further research.

Feed Formulation and Nutrient Analysis of Fish Feed with Recycled Fish Guts and Fruit

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The growing seafood consumption industry has led to a need for creating more aquafeed to meet demands. Along with inflation across economies resulting in increased costs for ingredients like fishmeal, it is crucial to develop an environmentally and economically sustainable fish feed. As food wastage from unwanted parts of food has posed as a challenge worldwide, this study aims to simultaneously address both issues by developing a feed formulation that meets the nutritional requirements of fish using recycled fish guts and fruit waste. 5 different treatments consisting of fish guts and fruit waste with varying formulations of wheatstarch were made into pellets and compared against a commercial tilapia pellet feed. Pellets were subjected to solubility tests and nutrient analysis of fatty acid, protein, and amino acid profiles. The nutrient profile of the pellets and ingredients were quantified using High Pressure Liquid Chromatography (HPLC) and the Dumas compound separation method. Increased levels of fatty acids, including omega-3, 6, and 9 fatty acids, and similar levels of crude protein were found in our formulated feed in comparison to the commercial Tilapia feed, with essential amino acid requirements met. Solubility tests revealed the treatment with 15% wheat starch had the least variance in terms of weight loss of a pellet after soaking for 120 minutes. These results infer our feed formulated with recycled fish guts and fruit waste meets the demands for an omnivorous fish in an aquaculture system, and is feasible to be incorporated as the sustainable ingredients in a fish feed.

Generation and Differentiation of Human Sideroflexin-1 (*Sfxn1*) KO ES Cell Lines to Neurons for Neuro-Metabolism Study

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Previous research on Sideroflexin-1 (SFXN1), one of the major serine transporters in the mitochondria has been minimal. This study will therefore investigate the generation of human SFXN1 knockout (KO) ES cell lines for future neuronal differentiation to study their function(s) in neuro-metabolism. The involvement of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated 9 (Cas9) technology was utilized to KO SFXN1. 2 SFXN1 sgRNA constructs targeting different exon sites were successfully constructed and transfected into human H1 ES cells. Isolated ES cell colonies underwent KO/WT DNA screening. 1 KO and 27 WTs out of 65 clone candidates were identified. Generation of an SFXN1 KO cell line was successful though only 1 clone was knocked out. Therefore, it appears that these 2 chosen sites may not be efficient enough for Cas9 cutting or following repair within ES cells. Further experiments such as morphological analysis using immunofluorescence staining and electrophysiology will be required to understand the role of SFXN1 in neuro-metabolism better.

P53 Discovery of Genes Involved in Dendrite Pruning in Sensory Neurons

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Neurite pruning is an important neurodevelopmental process that occurs in humans during adolescence, and in insects such as Drosophila, during metamorphosis. Disruptions can lead to neurological disorders such as schizophrenia and autism spectrum disorder (ASD). The evolutionarily conserved CCT (chaperonin containing T-complex protein [TCP] 1) complex which consists of eight paralogous subunits (CCT1-CCT8), are chaperones that are required for protein folding, commonly known to fold cytoskeletal proteins actin and tubulin. CCT can regulate dendrite morphology in growing C4da neurons and autophagosome formation in Drosophila larval fat bodies. However, its roles in microtubule and dendrite pruning in remodeling neurons remain unknown. In a candidate forward genetic screen of 200 lines for genes known to regulate cell cycle, protein folding, and glycosylation, it is reported that *Drosophila CCT* is necessary for dendrite pruning in ddaC neurons. To characterize these genes, it was revealed that CCT is required for the regulation of Mical and the maintenance of microtubule structures in ddaC dendrites. Previously, CCT was found to regulate the folding of proteins with beta-propellers. This suggests that CCT may regulate dendrite pruning via folding of protein phosphatase 2A (PP2A) and/or Slimb, which were previously known to regulate dendrite pruning. However, depleting CCT6 or 7 subunits did not demonstrate ubiquitin accumulation; contradicting with the proposed model that CCT regulates Slimb. Overall, this study demonstrates that Drosophila CCT governs Mical expression and microtubule structures in ddaC neurons. Further studies are required to elucidate the mechanism of action of CCT during dendrite pruning of ddaC neurons.

Research, Singapore 138673

Genome-Wide CRISPR Screen on Paraquat-Treated Cells to Understand its Mode of Action

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Paraguat (PQ) is a widely used, non-selective herbicide. However, exposure to even minute quantities of PQ leads to severe health hazards, with more than 70% of cases of PQ poisoning resulting in death. Within the Asia-Pacific region, PQ poisoning causes an estimated 300,000 deaths yearly. PQ is banned in more than 30 countries, but its use is still prevalent in at least 130 countries, due to its affordability. PQ intoxication causes pulmonary fibrosis, which progresses into acute lung injury and death. The handful of patients surviving PQ poisonings will suffer irreversible respiratory damage. There is currently no treatment for PQ intoxication, other than removal of the compound from the body. This form of treatment is futile in most cases of PQ exposure due to its acute toxicity. The aim of this research is to identify the mode of action of PQ via a genome-wide CRISPR knockout screen. A pool of gene knockouts were created in A549 cells by transducing them with the Brunello sgRNA library. The transduced cells were treated with different concentrations of PQ. The gDNA of surviving cells were processed and sent for next-generation sequencing (NGS). The gene hits from the screen can identify significant genes and pathways involved in paraquat toxicity via gene mapping. Due to PQ's role in alveolar cell death, identification of apoptosis-associated significant genes such as TGF- β can be expected. The screen may also detect novel genes involved in lung injury. This information can aid in developing an effective treatment for PQ intoxication.

Oral Delivery of Nano-Encapsulated Hormone for Fish Spawning

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As hormonal injection for induced spawning in fish is physically tedious and invasive, a possible alternative would be the oral delivery of hormone. However, as hormone directly fed into fishes would be rapidly degraded by the fish's intestinal enzymes, causing a failure of hormone delivery, the nanoencapsulation of luteinising hormone releasing-hormone analogue (LHRHa) would be a feasible solution of protecting the LHRHa from degradation, thereby allowing a successful oral delivery for fish spawning. Hence, to obtain viable LHRHa encapsulated nanoparticles (NPs) for this study, the effects of different parameters on NPs' LHRHa encapsulation efficiency (EE%) were investigated, such as the materials used for NPs synthesis and the sonication and solvent removal parameters. Through this study, nanoencapsulation of the LHRHa and optimisation of the NPs' EE% was successfully performed, as an improved EE% of 51% was seen. As the size of NPs could affect its cytotoxicity in the fish, a safe NP particle size of 200 nm was chosen and successfully obtained for this study. This project successfully developed the approach of nanoencapsulation of LHRHa for orally induced fish spawning.

RNA Drug Development for Novel Therapeutics in Genetic Diseases

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Stargardt disease (STGD1) is an autosomal recessive macular degenerative disease which occurs due to pathogenic variants in the ATPbinding cassette transporter, alpha 4 subunit (ABCA4) gene. To treat this condition, we aim to use splice switching oligonucleotide (SSO). SSOs are synthetic, modified antisense nucleic acids that are usually 15-30 nucleotides long. They work by pairing with pre-mRNA through the Watson–Crick base-pairing and affect normal splicing by interrupting the interactions that occur. This ultimately leads to alteration to normal splicing in the target transcripts. In developing an effective SSO treatment, it is important to consider not only the SSO sequence, but also the method of delivery. As such, the experiment aims to select the best SSO to act as a positive control for the validation of a collaborator's delivery method for the SSO treatment. The SSOs (1944, 1945, 2022, 2024) designed for this experiment target exon 15 of the ABCA4 gene to induce splice out. SSO 2022 was found to be the most effective and specific in inducing the splice out of exon 15.

Production of Value-Added Lipids from Food Waste Streams

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In this current age, animal meat products are widely consumed globally but the production of these meat products are seen as unethical and not sustainable. Additionally, agricultural land for meat production is running scarce. The multiple threats and risks posed by the production of animal meat calls for an alternative way to produce and obtain these meat products. Current alternatives like plant-based meats are less palatable due to lacking taste and mouthfeel.

So, this project aims to produce animal free lipids using oleaginous yeast and food waste streams in order to mimic animal fat very closely in an affordable and scalable way. Yeast strains were grown on the food waste stream to enable waste valorization and a circular economy of food making it sustainable and cost effective. Subsequently, the CRISPR/Cas9 system was used to edit the genes in the yeast strains to modify them to have the lipid profile that mimics animal fats. This project has successfully established a protocol to alter the lipid profile of yeast strains through genetic engineering, to replicate the lipid profile of chicken and sheep.

P58 Management of Diabetes by Dietary Manipulation Jia Le Dexter TAN^a, Mohamed Shirhan MOHAMED ATAN^a

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The objectives of this research were to study 1) the morphological changes in gastrointestinal (GI) tissues and 2) the upregulation in inflammatory and diabetic biomarker levels of diabetic rats after being fed with four different customized diets. The customized diets were clinically formulated as optimal diets for human diabetes. The research was carried out in 20 male Spontaneously Diabetic Torii (SDT) rats. SDT rats are a robust inbred strain of non-obese Sprague Dawley rats that develops hyperglycemia in type-2-diabetes (TD2). In histomorphological analysis, samples of GI tissues, including the stomach, duodenum, jejunum, ileum, and colon, were sectioned and stained by Hematoxylin and Eosin. Inflammatory and diabetic biomarkers measurement from plasma samples were analysed using ELISA kits that uses a double-antibody sandwich enzyme-linked immunosorbent one-step process assay to quantify HbA1c, IL-6, and CRP biomarker levels. Among the four customized diets, LP, HP1, HP2, and HP3, both HP1 and HP3 were observed to be less than ideal in managing diabetes. HP1 and HP3 diets had shown extensive tearing and destruction of GI tissues with disordered columnar epithelial cell arrangement and sporadically positioned microvilli, significantly more seen in the small and large intestines. A moderately high white blood cell infiltration load was also observed, indicating severe inflammation. There were signs of eodema in the GI tissues when microscopically observed. LP and HP2 diet fed SDT rats were shown to have significantly managed diabetes. These were observed in the GI morphology of the LP and HP2 fed SDT rats. The GI tissues were observed to have similar morphology to that of non-diabetic rats. The levels of biomarkers in HP1 and HP3 had shown higher levels than LP and HP2. This study has shown that LP and HP2 customised diet fed SDT rats has the potential in managing diabetes. Therefore, customised diets have the ability in combating TD2.

Compound C Alleviates Non-Alcoholic Fatty Liver Disease (NAFLD) Phenotype in HFD/Aged C57BL/6 Mice

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Non-alcoholic fatty liver disease (NAFLD) is a prevailing liver disease that is strongly associated with metabolic comorbidities like obesity and insulin resistance. NAFLD development and progression is highly attributed to the formation of lipotoxic lipids, leading to a cycle of inflammation and insulin resistance. The objective of this study is to evaluate the efficacy of Compound C in alleviating high fat diet (HFD) induced NAFLD, specifically through the inhibition of lipotoxic lipid synthesis. The study was carried out in aged C57BL/6 mice that were split into three groups : (i) control diet fed group treated with vehicle, (ii) high fat diet fed group treated with vehicle and (iii) high fat diet fed group treated with Compound C. Body composition was tested with EchoMRI at the baseline, midpoint and endpoint of the study. Glucose intolerance was assessed with an oral glucose tolerance test at midpoint and endpoint. Lastly, histological analysis of hepatic steatosis was performed at sacrificial endpoint. Compound C was found to significantly suppress body fat gain, insulin resistance and hepatic steatosis in NAFLD development as compared to the high fat diet vehicle treated counterpart. Experiment results also showed the deleterious effect high fat diet has on NAFLD development through fat gain, glucose intolerance and lipid accumulation contrasted with the control diet group. Compound C is а promising pharmacotherapeutic in mitigating NAFLD steatosis and its relevant metabolic abnormalities.

P60 Scale Up of Fish Derived Fat Cells

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This project aimed to find the appropriate dynamic culture conditions to grow cells from the fish species *Pangasianodon hypophthalmus*, which could be used as a source of nutritious Omega-3 oils and fatty acids, as well as a unique flavoring agent. Multiple experiments were performed using spinner flasks and microcarriers to determine the most suitable conditions for scaling up the fish cells. The results showed that 30 rpm was the optimum speed to culture the cells of *Pangasianodon hypophthalmus* with Cytodex 1. As it had the highest yield of 2.892 x 10⁶ which is higher than the static control group by 36.1 folds and 2.89 folds higher than the cell seeded. This study contributes to the development of a more ethical, healthy, and environmentally sustainable choice of cultured meat to meet the increasing protein demand.

P61 A Sustainable Dog Food Solution

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The pet food industry has a significant impact on the environment, and there is growing concern about the sustainability of traditional dog food formulations. In this study, we explored the potential of using fish bones and scales, which are often discarded as waste, to develop a sustainable dog food. We solubilized the fish bones and scales using citric acid, a common food preservative, and mixed them with vegetable waste to create a nutrient-rich base. We then conducted feeding trials with a group of dogs to evaluate the palatability and nutritional value of the new dog food formulation. Our results showed that the dogs readily consumed the sustainable dog food, and we observed no adverse effects on their health or behavior. Additionally, the nutritional analysis of the dog food revealed a balanced macronutrient profile, with high levels of protein, omega-3 fatty acids, and other essential nutrients. Overall, our findings suggest that the use of fish waste and vegetable waste to create sustainable dog food is a promising approach to reducing the environmental impact of the pet food industry while providing a healthy and nutritious diet for our furry companions.

P62 Anti-Bacterial Surfaces that Prevent Bacterial Attachment <u>TAN Jun Xi Javier, Vicki YIM Jia Hui</u>, CHOY Weng Keong

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In light of the recent surge in healthcare-associated infections (HAIs), antibacterial textiles are becoming ever more important in combatting the spread of infectious diseases. In hospitals where the pathogen load is high, hospital essentials, such as cotton gowns, are becoming a potential source of cross transmissions. Cotton textiles provide an excellent substrate base for microbial attachment due to their porous structure and ability to retain human sweat. These textiles are often modified with antibacterial agents to resist microbial adhesion and reduce the potential for cross-transmissions. However, not all antibacterial agents have been proven to be effective against the more resilient, biofilm forming strains such as *Pseudomonas aeruginosa*.

This study will examine the antibacterial capacity of cotton fabric functionalized with silver nanoparticles (AgNPs) and hexadecyltrimethoxysilane (HDTMS). The end product is an antibacterial and multifunctional cotton fabric that can be applied to a plethora of process involves incorporating varying practical scenarios. The concentrations of silver nanoparticles onto cotton followed by a HDTMS coating to achieve superhydrophobicity. The treated cotton samples were then subjected to *P. aeruginosa* culture, and the resultant biofilms were guantitated using the Biofilm Assay.

Results indicated that cotton treated with 1 M AgNPs and HDTMS was most effective in resisting biofilm formation, with an 81.9% bacterial reduction when compared to untreated cotton fabric. Incorporation of HDTMS into the cotton samples was also a success as cotton samples treated with both AgNPs and HDTMS were able to achieve superhydrophobicity. However, HDTMS modified cotton samples were unable to significantly enhance the antibacterial properties of the cotton samples beyond their unmodified counterparts.

Impact of Viral Infection on *Agrobacterium Tumefaciens*-Mediated Transformation in *Malassezia*

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Malassezia spp. is the most prevalent fungal species found on human skin. They represent 90% of our skin microbiome and can cause various skin dermatoses such as dandruff, atopic eczema, and pityriasis versicolor. However, the mechanism of pathogenesis and exact interaction with the host is unknown. Recently, many Malassezia spp. were found to be infected with a mycovirus, specifically Totiviridae. Mycoviruses have long been found in other fungal species too, but their functions and effects remain elusive. Agrobacterium tumefaciens (A. tumefaciens) is a plant pathogen that relies on a host for their own propagation by inserting its own transfer DNA (T-DNA) into the plant genome. This mechanism, termed "Agrobacterium tumefaciens-mediated transformation (ATMT)", was coincidentally found to be the only method to study gene functions in Malassezia. Till date, there has been no reported successful ATMT in virus-infected Malassezia. In this study, we performed ATMT on virus-cured (SEC494) and virus-infected (KS012) *Malassezia sympodialis*, to determine if the mycovirus inhibits ATMT from occurring in virus-infected Malassezia, in order to block off competition from A. tumefaciens and have all resources to itself. Results showed that the mycovirus does not inhibit ATMT from occurring in virus-infected Malassezia, and further research is necessary to deepen our understanding of mycoviruses.

P64 Optimizing the Amount of Freeze-Dried Sample Required for Fatty Acid Profiling of Red Snapper

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Fatty acid analysis can be beneficial to Red Snapper Aquaculture in Singapore. This project aims to improve the technique by analysing the fatty acid content of freeze-dried Red Snapper (Lutjanus malabar) fillets at various weights to determine the minimum amount of sample required to obtain an accurate result compared to a 20g test portion. Analysing the fatty acid content of Red Snapper is useful to differentiate wild caught and farmed fish nutrition, helping to identify absent fatty acids needed to be supplemented in farmed fish diet. A total of 3 whole Red Snappers were filleted and freeze-dried to achieve below 5% moisture content. Fat extraction as per AOAC Official method 996.06 was performed on 3 Red Snappers at 6 different test portion sizes 1g, 2.5g, 5g, 10g, 15g and 20g. ANOVA analysis in terms of mg/g tested was measured between the different test portion sizes to evaluate the consistency of results. Results showed CFA value of RS3 5g and 20g observed to be an outlier, 5g test portion was replicated twice to confirm the outlier. However, 20g test portion was not replicated due to insufficient sample. The new replicated values replaced the original for comparison between different test portions. All 3 Red Snappers Total fatty acid concentration were found to be consistent between different test portion sizes, P value for ANOVA was 0.993. This concludes that optimal test portion size was 1g as it uses the least amount of sample to achieve accurate fatty acid content result.

P65 Sustainable Alcohol-Free Hand Sanitiser

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The demand for hand sanitisers surged in recent years due to the ongoing pandemic. Most hand sanitisers in today's market are alcohol-based which sees disadvantages due to their notorious effect on sensitive skin users and their environmental risks. Hence, the innovation for sustainable alcohol-free hand sanitisers is essential. Existing alcohol-free hand sanitisers in the market use benzalkonium chloride (BKC) as the main active ingredient. However, BKC was reported to be toxic to the environment. Therefore, an alternative to BKC was seeked. Hypochlorous acid (HOCI) as an alternative, is environmentally friendly, effective in eliminating microbes, and suitable for sensitive skin. Thus, HOCI was utilized in the formulation of our hand sanitisers. Additionally, the beneficial properties of food waste extract from okara, carrot peel and banana peel were explored and incorporated into our hand sanitiser.