The Research Catalyst

PATHOGENS -ECON2022

Pathogens-eCon 2022

Abstract eBook

30 November 2022 (London GMT+0)
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Content

DAY 1 **30 November 2022 (London GMT+0)**

Pathogens-eCon2022

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DAY 2 **01 December 2022 (Tokyo GMT+9)**

PATHOGENS-ECON2022

VIRUSES AS LIVING SYSTEMS—A METACYBERNETIC VIEW

DAY 1

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The debate over whether viruses are living organisms tends to be paradigmatically determined. The metabolic paradigm denies that they are, while new research evidences the opposite. The purpose of this paper is to deliver a generic model for viral contexts that explains why viruses are alive. It will take a systems biology approach, with a qualitative part (using metacybernetics) to provide deeper explanations of viral contexts, and a quantitative part (using Fisher Information de- riving from the variational principle of Extreme Physical Information) which is in principle able to take measurements and predict outcomes. The modelling process provides an extended view of the epigenetic processes of viruses. The generic systems biology model will depict viruses as autono- mous entities with metaphysical processes of autopoietic self-organisation and adaptation, enabling them to maintain their physical viability and hence, within their populations, mutate and evolve. The autopoietic epigenetic processes are shown to describe their capability to change, and these are both qualitatively and quantitatively explored, the latter providing an approach to make measurements of physical phenomena under uncertainty. Viruses maintain their fitness when they are able to maintain their stability, and this is indicated by information flow efficacy. A brief case study is presented on the COVID-19 virus from the perspective that it is a living system, and this includes outcome predictions given Fisher Information conditions for known contexts.

DAY 1

PROMISING ANTIMYCOBACTERIAL ACTIVITIES OF FLAVONOIDS AGAINST MYCOBACTERIUM SP. DRUG TARGETS: A COMPREHENSIVE REVIEW

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Tuberculosis (TB) caused by the bacterial pathogen Mycobacterium tuberculosis (Mtb) remains a threat to mankind, with over a billion of deaths in the last two centuries. Recent advancements in science have contributed to an understanding of Mtb pathogenesis and developed effective control tools, including effective drugs to control the global pandemic. However, the emergence of drug resistant Mtb strains has seriously affected the TB eradication program around the world. There is, therefore, an urgent need to develop new drugs for TB treatment, which has grown researchers' interest in small molecule-based drug designing and development. The small molecules-based treatments hold significant potential to overcome drug resistance and even provide opportunities for multimodal therapy. In this context, various natural and synthetic flavonoids were reported for the effective treatment of TB. In this review, we have summarized the recent advancement in the understanding of Mtb pathogenesis and the importance of both natural and synthetic flavonoids against Mtb infection studied using in vitro and in silico methods. We have also included flavonoids that are able to inhibit the growth of non-tubercular mycobacterial organisms. Hence, understanding the therapeutic properties of flavonoids can be useful for the future treatment of TB.

Keywords: Mycobacterium tuberculosis; anti-tubercular compounds; drug discovery; flavonoids.

MOULDS - STILL WATERS RUN DEEP. HOW TO CONTROL THE FLOW?

DAY 1

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Moulds are microscopic fungi that exist throughout the environment. Some of them are useful; they are harnessed as cell factories for the production of a diverse range of organic acids, proteins, and secondary metabolites. On the other hand, some moulds can cause severe illnesses in humans, animals, and plants. Growing on a variety of different crops and foodstuffs including cereals, nuts, spices, dried fruits, apples, coffee beans, and the production of meat produsts, and cheese often under warm and humid conditions causes the production of mycotoxins. Mycotoxins are naturally occurring toxins produced by fungal genera such as Penicillium, Aspergillus, Fusarium, and Alternaria. Intake of food and feed containing mycotoxins can cause various adverse health effects and pose a severe health threat to both humans and animals. The adverse health effects of mycotoxins range from acute poisoning to long- term effects such as immune deficiency and cancer. Therefore it is essential to control grow of toxigenic and pathogenic moulds all way from field to storage.
Mycotoxins, are extremely difficult to remove due to their natural resistance to mechanical, thermal, and chemical factors. Modern methods of analysis allow the detection of the presence of mycotoxins and determine the level of contamination with them. Various food processes that can affect mycotoxins include physical, chemical, and biological methods. Most efficient techniques can be selected and combined on an industrial scale to reduce losses and boost crop yields and agriculture sustainability, increasing at the same time food and feed safety.

PLANT AND MICROBIALVOLATILOMES - A CURRENT-GENERATION PLANT DISEASE MANAGEMENT STRATEGY

DAY 1

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Plants and microbial-inducing volatiles have a rich source of antimicrobial properties for sustainable production of crop growth from the infection of plant diseases and thereby promoting the nutritional status of crops to avoid agricultural crop demand in the future. Volatilomes, a group of Volatile organic compounds (VOCs) produced by the plant origin or from microbial origin have been introduced to induce the host defense mechanism by suppressing the growth and spread of phytopathogens. The volatile bio-molecules from those origins act as an alternative to chemical fungicides and also could contribute to the field of plant disease management for developing disease-free crop plants. In our research study, we investigated the volatile inhibitory activity induced by Mentha spicata, Cymbopogon citratus, and Trichoderma sp. on the mycelial growth of Fusarium oxysporum f.sp. lycopersici, isolated from Fusarium wilt-infected tomato plant. Volatiles produced by M. spicata documented the complete suppression of pathogens under laboratory conditions. The field application of M. spicata volatilomes in the form of suitable vermiculite immobilized ball formulation supported the emerging concept by suppression of 98 per cent of Fusarium wilt pathogen in soil, which acted as eco-friendly based biocontrol management strategies due to the exposure of volatilomes of M. spicata in the tomato plants raised beds with relatively abundant control of F. oxysporum
f. sp. lycopersici.

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Antibiotic resistance is now a major threat to public health. Added to this, the increasing demand for "natural" and healthy products has boosted the search for new antibiotics and disinfectants that minimize the damage to or even stimulate a healthy gut microbiome. Lactic-acid bacteria (LAB) fermentation is known to produce bactericide (poly)peptides, which are generally accepted as safe (GRAS grade-1) and pose a high potential as health-promoting agents. This work aimed at evaluating the potential of whey LAB fermentation to produce antibacterial peptides and test their activities in vitro and in vivo approaches. Whey from different origins (ovine, caprine and bovine) were tested using different lengths of fermentation periods and combinations of LAB strains, aiming to optimize proteolysis and antibacterial activity. Lactic-acid production and protein variations were monitored through chromatographic and electrophoretic techniques. Low molecular weight polypeptides were isolated by ultrafiltration and RP- HPLC and tested for their bioactivities against an array of different bacteria and fungi. The effect of the isolated peptide on the gut microbiome and in TNBS-induced colitis in mice was also tested in vivo. Proteolysis and antibacterial activities induced by fermentation were significantly (P<0.05) dependent on the bacterial strain, type of whey and length of fermentation, being highest using a 6- day fermentation and with a consortium of L. lactis lactis, L. lactis cremoris, L. lactis lactis biovar. diacetylactis, S. thermophilus, and L. delbrueckii bulgaricus.

The isolated peptides presented high antibacterial activity against food-born Listeria monocytogenes and Escherichia coli with MICs of 3 and 6 µg.mL-1 respectively, being more effective as a disinfecting agent than chlorine and standard food disinfectants. The growth of non-food related bacteria Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas aeruginosa, also significantly (P<0.001) reduced in a dose-dependent manner. Antifungal activity against Botrytis cinerea and Phaeomoniella chlamydospore was also detected, with MICs of 180 and 22.5 µg.mL-1 respectively.

Unexpectedly, the selected peptides also shown ability to strongly inhibit gelatinases MMP-9, which are key-players in inflammatory bowel diseases, and they also significantly reduced TNBS-induced colitis lesions whilst maintaining a stable gut microbiota in mice models of colitis.

These whey-derived polypeptides present a dual antibacterial and anti-inflammatory activity, revealing potential to be used as nutraceuticals and alternatives to antimicrobials and disinfectants. With the increasing health concerns about antibiotic resistance, gut inflammatory diseases and microbiome, they could be a turning point towards a One Health Approach.

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The COVID-19 pandemic seems endless with the regular emergence of new variants. Is SARS-CoV-2 virus particularly rebel to the immune system or is it merely disrupting communication between the body and the brain, thus pre-empting homeostasis?
Retrospective analysis of COVID-19 and AIDS pandemics, as well as prion disease, emphasizes the pivotal but little-known role of the tenth cranial nerve in health.
Considering neuroimmunometabolism from the vagus nerve point of view, noninvasive bioengineering solutions aiming at monitoring and stimulating the vagal tone are subsequently discussed as the next optimal and global preventive treatments ... especially for vulnerable populations

SCREENING AND PROBIOTIC POTENTIAL EVALUATION OF MARINE SPOREFORMERS STRAINS IN VITRO

DAY 1

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Aquaculture industry is the world's fastest growing animal protein producer. However, frequent bacterial diseases occurring during fish farming can limit the worldwide growth of this sector and pose serious threats to public health. To avoid or minimize the loss in aquaculture due to disease; use of the chemotherapeutics, antibiotics, and vaccines has become a customary practice. However, the indiscriminate use of these substances is plagued with negative consequences, and now the situation lies where the chemotherapeutics are at risk of running out of efficiency. As an answer to this very concern, the probiotics have established their significance as an effective and sustainable biocontrol strategy in global aquaculture.

Keywords: Aquaculture, fish diseases, probiotics, sporeformers, biofilms, adhesion

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GUT MICROBIOTA MODULATION TO CONTROL INFECTIOUS DISEASES AND ANTIMICROBIAL RESISTANCE SPREADING

DAY 1

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The old notion of "organism" intended as a singular entity is being outclassed, to accept the novel view of the "superorganism". This innovative perspective integrates the functional districts of the animal body along with its associated microbiome, opening new avenues in the understanding of the physiologic processes and how these orchestrate the functions of diverse tissues, organs and even the whole organism. From a functional perspective, the overall number of bacterial cells harbored in the animal's intestine is approximatively ten times the number of host cells, comprising a microbiome that consists of more than five million genes of

fundamental importance in the myriad of interactions occurring between host- microbiota for maintaining the homeostatic balance. Here, the microbiota members establish mutualistic associations, as the mammalian intestine, is a nutrient-rich environment that is maintained at a constant temperature, ensuring thriving condition for the microbial growth. On the other side, harbored microbial community provide the animal host with a variety of processes ranging from organs and immune system development, to vitamines and micronutrient supplementation until the communication with long-distance organs such as the gut-brain axis and gut-liver axis.

Acknowledged the importance of the harbored microbiota and the dynamic interconnection they establish among each other and the hosting organism, a huge number of studies are ongoing to shed light on the gut microbiota and the molecular mechanisms undertaken by the microbial community to finely orchestrate the diverse physiological properties of the animal host, of course the main goal is understanding this network of interactions to better modulate the gut microbiota composition and activity for the active stimulation of the animal health, welfare and production.

In this meeting is provided a rappresail of the latest achievements scored in the study of gut microbiota and its tailored modulation to improve animal health and immunology.

DAY 1

LABORATORY TRIAL OF NAPHTHALENE AND ITS COMBINATION WITH KEROSENE AGAINST THE EMERGENCE OF ANOPHELES GAMBIAE

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The WHO African regions, especially Nigeria with a quarter of the global malaria burdens, cannot continue to rely on only insecticide recommended interventions. The issues surrounding insecticide resistance has prompted the search for nonchemical approach for mosquito larviciding. Thus, this study examined the efficacy of naphthalene and its combination with kerosene against the emergence of Anopheles gambiae. Immature stages of An. gambiae were collected from Abbi, Ndokwa West, Delta State, and left to acclimatize for 12 hours in standard laboratory conditions. Naphthalene measured in 2 grams and its combinations with kerosene in 50:50 were emptied in 400ml, 200ml and 100ml of water which resulted in 0.005%, 0.01% and 0.02% concentrations respectively. Water alone served as control for the experiment. Twenty third instar larvae and pupae were sorted into containers before exposure to treatments. Experiment was done in triplicates and observed for 10, 15, 20, 30, 40, 50, and 60, minutes coinciding with WHO protocol for Anopheles mosquito exposure. Mortality was highest in larvae exposed to 0.02% kerosene and naphthalene, and was also high in 0.02% naphthalene. Lowest mortality was recorded in pupae exposed to 0.005% of naphthalene. Significant differences in toxicity was recorded (p < 0.05). Increase in exposure time favoured mortality in larvae and pupae. Highest mortality in pupae and larvae was recorded in 0.01% kerosene and naphthalene mixture at 60 minutes post exposure time. LC 50 and LC 95 of naphthalene exposed to Anopheles larvae and pupae was between 0.002-0.021% and 0.004 - 0.491% respectively. More so, for naphthalene in kerosene mixture, was between 0.002 - 0.010%, and 0.002 - 0.052%. Pupae exposed to 0.005% naphthalene had more adult emergence than in others and the differences were significant (p< 0.05). Anticipated efforts are required to expand the focus of best larvicides in semi-field and field conditions.

Keywords: Anopheles gambiae, Emergence, Laboratory trial, Kerosene, Naphthalene.

DAY 1

DIFFERENT MECHANISMS BY WHICH SARS-COV-2 IS EVOLVING TO INCREASE ITS INFECTIVITY AND TRANSMISSIBILITY

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The emergence of new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VOC) are constantly threatening global public health that threaten the effectiveness of diagnostic tests and vaccines. SARS-CoV-2 Spike protein is evolving to evade the immune system causing an increased virus transmissibility. As mutations in the Spike surface protein of the virus are regularly observed in the new variants this may increase the probability of emergence of novel viruses with different tropism from the current ones, which may change the symptoms and severity of the disease. An effective way to postpone the emergence of SARS-CoV-2 VOC is the combination of immunization, wearing masks, hand hygiene, and keeping environments ventilated. During the evolution of SARS-CoV-2 the Spike protein uses different mechanism to increase effectiveness such as: (1) increase in Spike binding affinity to hACE2; (2) increase the time to be bound to the cell receptor to increase the probability to be cleaved by the proteases; (3) increase the RBD up-conformation state in the Spike ectodomain; (4) increase the amount of uncleaved Spike protein in the virion particles; (5) increment in Spike concentration per virion particles; and (6) evasion of the immune system by increase the structural conformational flexibility. These factors play key roles in the fast spreading of SARS-CoV-2 variants of concern, including the Omicron.

DAY 1

LATEX GLOVES PROVIDE FRONTLINE PROTECTION FOR HEALTHCARE WORKERS AND PATIENTS – BUT HOW EFFECTIVE ARE THEY REALLY?

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Until recently there has been no effective way of assessing the durability of latex gloves after they are removed from the dispenser, box or packet. All current glove standards only describe the mechanical performance and incidence of pinoles at point of packing, not while gloves are actually being used. Nonetheless, it is well recognized that natural latex gloves are more comfortable and durable than synthetic latices, even though the prevalence of Type I latex (protein) allergy in the 1990's encouraged a major shift to the synthetics, especially in examination gloves.

We have developed a testing device which can quantify the durability of different types of protective gloves, including those made from different elastomeric materials. For the first time, we can assess variability both within and between materials and manufacturers and determine the impact of different environments in which the gloves may be used. This ability becomes critical as new natural elastomers (e.g. circumallergenic guayule latex), and new curing packages (e.g. xanthates) are deployed.

Our data prove that gloves made from natural latices are more durable and so provide better protection against pathogens than synthetics. Guayule latex gloves outperform even Hevea natural latex gloves and do not pose an allergy risk. As more natural latices are used, these advantages enhance the prospects of essential geographical and biological diversification of the natural rubber supply as well as significantly improving the carbon footprint and sustainability of the glove manufacturing industry, as a whole.

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The role of viruses has been studied extensively for use as curative cancer therapies. However, the natural immunogenicity of viruses and their interaction with the host's immune system needs to be examined to determine the full effectiveness of the viral treatment. The prevalence of cancer is increasing globally, and treatments are needed to support the increasing body of patient care. Oncolytic viral therapies used existing pathogenic viruses, which are genetically modified to not cause disease in humans when administered using a vaccine viral vector. Immunotherapies are another avenue of recent interest that has gained much traction. This review will discuss oncolytic viral approaches using three DNA-based viruses, including herpes simplex virus (HSV), vaccinia virus, and adenovirus; as well as four RNA-based viruses, including reovirus, Newcastle disease virus (NDV), poliovirus, and measles virus (MV). It also examines the novel field of cancer-based immunotherapies.

DAY 1

IMMUNOREGULATION VIA CELL DENSITY QUORUM SENSING- LIKE MECHANISM: AN UNDEREXPLORED EMERGING FIELD WITH POTENTIAL TRANSLATION IMPLICATIONS

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Quorum sensing (QS) was historically described as a mechanism by which bacteria detect and optimize their population density via gene regulation based on dynamic environmentalcues. Recently, it was proposed that QS or similar mechanisms may have broader applications across different species and cell types. Indeed, emerging evidence shows that the mammalian immune system can also elicit coordinated responses on a population level to regulate cell density and function, thus suggesting that QS-like mechanisms may also be a beneficial trait of the immune system.In this review,we explore and discuss potential

QS-like mechanisms deployed by the immune systemto coordinate cellular-level responses, such as T cell responses mediated via the common gamma chain (γc) receptor cytokines and the aryl hydrocarbon receptors (AhRs). We present evidence regarding a novel role ofQS as a multifunctional mechanism coordinating CD4+ and CD8+ T cell behavior during steady state and in response to infection, inflammatory diseases, and cancer. Successful clinical therapies such as adoptive cell transfer for cancer treatment may be re-evaluated to harness the effectsof the QS mechanism(s) and enhance treatmentresponsiveness.

Moreover, we discuss how signaling thresholdperturbations through QS-likemediators may result in disturbances of the complex crosstalk between immune cell populations, undesired T cell responses, and induction of autoimmune pathology. Finally, we discuss the potential therapeutic role of modulating immune-system-related QS as a promising avenue to treat human diseases.

Keywords: T cell homeostasis; cytokines; immune system;macrophage; quorum sensing.

BACTERIAL GROWTH IN ELECTROSPUN NANOFIBERS

DAY 1

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Bacterial growth studies are one of the most important features that have to be analysed in samples that are proposed as biomedical devices. Electrospun nanofibers have been gaining much attention in the biomedical industry, because they are interesting tridimensional structures that resembles extracellular matrix in tissue and can be fabricated with a great variety of polymers that confers to the fibrous scaffolds certain interesting characteristics such as biocompatibility, biodegradability, high surface area, bioactivity, adequate mechanical properties, amongst others. These electrospun fibers can be proposed in drug delivery systems, tissue engineering, biotechnology, biosensors and other areas where cytotoxicity is the most important characteristic. In our group, cytotoxicity has been tested trough the MTT assay which test mitochondrial activity of viable cells (fibroblasts and polymorphonuclear leukocytes), several polymeric nanofibers such as poly (caprolactone), poly (vinyl alcohol) and poly (vinyl pyrrolidone) functionalized with bioactive molecules such as Ruthenium complex, graphene, silver nanoparticles, curcumin and some pharmaceutical drugs such as dexamethasone phosphate, sildenafil citrate and propranolol. Moreover, some in vivo analyses have been used to test irritability and toxicity in tissue of animal's models following the ISO-10993-1. Also, bioactive antibacterial activity has been tested using Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. Most of the electrospun fibrous scaffolds demonstrated biocompatibility and slight effect in all the cells tested and in vivo animal provides evidence of the potential of the samples to be use in biomedical applications. This work discusses cell response, viability and characteristics of the nanofibers made by the electrospinning technique, the parameter used and drawbacks.

DAY 1

GLUTATHIONE MODULATES THE HOST IMMUNE RESPONSES AGAINST MYCOBACTERIUM TUBERCULOSIS INFECTION

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Type 2 diabetes mellitus (T2DM) has been described as a global epidemic fueled by population growth,aging, urbanization, and increasing obesity. It is estimated that the number of people diagnosed with T2DM is expectedto grow from 171 millionin 2000 to 366-440 millionby 2030, with three-quarters of the patients living in low-income countries (1-11). It has been well- recognized that individuals with T2DM are increasingly susceptible to Mycobacterium tuberculosis (Mtb) infection (1-5). In particular, TB meningitis (TBM) is the most severe form of extrapulmonary TB, as it is associated with significant morbidity and mortality (61-69). Since the current anti-TB regimen is formulated for pulmonary TB, they are not optimal for treating TBM, particularly among T2DM cases. Thus, adjunctive immunotherapy is a promising approach to improving the clinical outcome of refractory mycobacterial infections in these cases. We have reported that individuals with active pulmonary TB exhibit a marked deficiency in glutathione (GSH), the principal non-protein thiol responsible for cellular homeostasis and maintaining the intracellular redox balance. GSH levels are significantly compromised in peripheral blood mononuclear cells (PBMCs) and red blood cells (RBCs) isolated from individuals with T2DM (12, 13, 14, 15), and this decrease correlated with increased production of pro-inflammatory cytokines and enhancedgrowth of Mtb (12, 14, 15). GSH has direct antimycobacterial activity in vitro and at physiological concentrations (5 mM) (16, 17). In combination with cytokines such as IL-2 and IL-12, GSH enhances the functional activity of natural killer (NK) cells to inhibit the growth of Mtb inside human monocytes (18, 19). Similarly, GSH activates the functions of T lymphocytes to control Mtb infection inside human monocytes (20). GSH levels have also been compromised in subjects with T2DM, who have increased risk of developing both pulmonary and extrapulmonary TB (9-12, 13, 14, 15). Importantly, in our recent clinical trial, we demonstrated that oral liposomal GSH (L-GSH) supplementation in individuals with T2DM for three months was able to maintain the levels of GSH, reduce oxidative stress, and diminish mycobacterial burden within in vitro generated granulomas of people with diabetes (75).L-GSH supplementation to T2DM cases for three months also modulated the levels of various cytokines (15). Furthermore, we also reported that L-GSH supplementation in conjunction with rifampicin (RIF) treatment achieved better control of Mtb infection in the lungs of diabetic (db/db) mice and significantly reduced the levels of oxidative stress compared to treatment with RIF alone (21).Put together, these findings 1) unfold a novel and potentially important innate defense mechanism adopted by human macrophages to control Mtb infection (12-21) and 2) indicate that GSH controls Mtb infection by functioning as an antimycobacterial agent as well as by enhancing the effector functions of immune cells (12-21).

STRONG & DURABLE STERILIZATION BY IMPROVED PHOTOCATALYST

DAY 2

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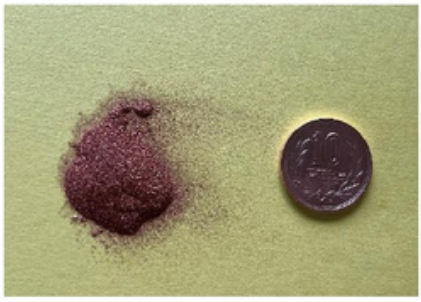
Background

Photocatalytic reaction has turned out to be too show and benign for practical sterilization purposes. Main reason is the unexpectedly low density of active oxygen arose from this reaction under the natural environment. Therefore some supplemental measures must be taken to make this reaction actually practical for the public hygiene or healthcare. We adopted metallic fine copper powder as the supplemental ingredient with perfectly sufficient results so far. Thus together with Nafion resin we have successfully invented new photocatalytic coating (Hereafter referred to "NFE2")

Theory & Outline

We have chosen copper ion Cu^{2+} to attain strong sterilization function in place of active oxygen such as hydrogen peroxide H_2O_2 and for this durable supply source adopted fine copper Cu powder.



Fundamental reaction of the photocatalyst TiO_2

Anodic reaction

$H_2O \rightarrow 2H^+ + 1/2O_2 + 2e$

Cathodic reaction

$O_2 + 2H^+ + 2e \rightarrow 2OH\cdot$

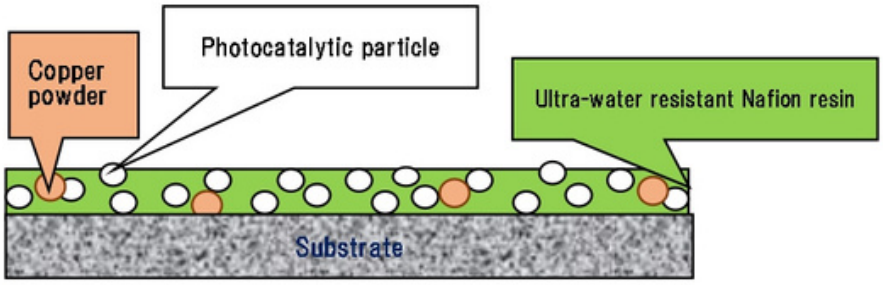
$2OH\cdot \rightarrow H_2O_2$

Our supplemental reaction

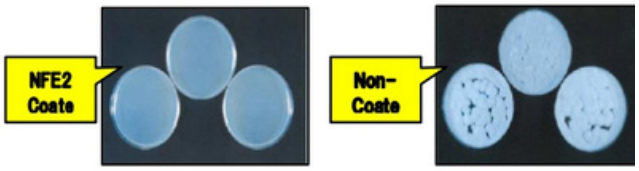
$$Cu + H_2O_2 \rightarrow Cu^{2+} + 2OH^-$$

Generated oxygen peroxide then reacts with metallic powder Cu thus arise strong Cu^{2+} slowly into the Nafion made photocatalytic layer. Copper ion concentration in the layer will become high enough in the cause of photocatalytic reaction to eradicate all kind of microorganism while only non-hazardous small amount will be dispersed in the environment thanks to the cation exchange nature of Nafion adopted as the binder of this coating. Only intermittent irradiation is necessary for this function to continue as copper ion Cu^{2+} will accumulate without any major consumption in the ordinary circumstance.

Cross Sectional Structure of this photocatalytic coating layer

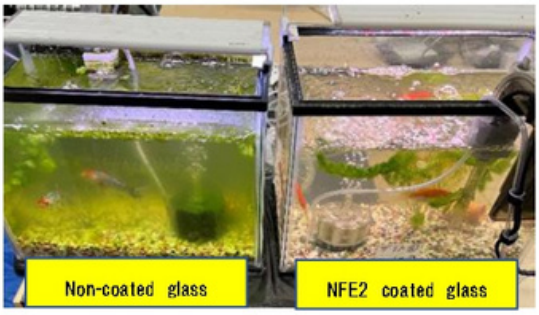


Case Study 1 Mold control



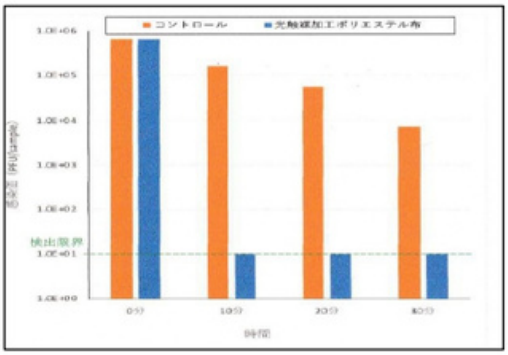
Coated surface shows strong mold control function against Penicillium, Cladosporium and even Trychophyton fungus. As already mentioned it can be detected immediately and with persistence unlike any conventional photocatalytic coating.

Case Study 2 Algae control



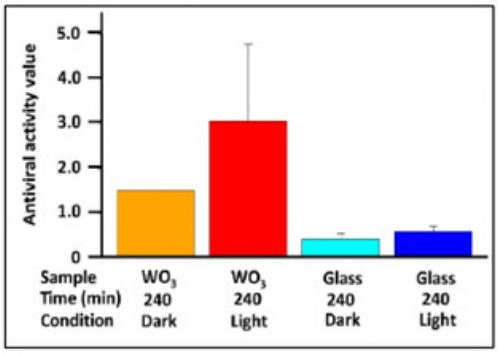
This effect can be expected from the beginning as copper ion Cu^{2+} has long been applied just for this purpose. But superb water resistance is derived from the peculiar nature of Dupont made fluoropolymer Nafion, with has been otherwise widely adopted currently as the solid electrolyte of fuel cells.

Case Study 3 General virus control



We have tested this NFE2 coated surface against various viruses including flu and norovirus. The latter is a notoriously resilient non-envelope type but disappeared within 1hour. We have already confirmed its effectiveness against initial COVID-19 by the cooperation of Nara Medical College, of which result shows the inactivation of this virus within 15min.

Case Study 4 Against Omicron variant



In the improving process of this coating we can fortunately accept the cooperation of University of Tokyo team to clarify the effectiveness against Omicron variant. We adopted this time WO_3 in place of TiO_2 to dramatically improve the activity under weak or dim irradiation, which should be necessary for the application to medical facilities in general. The result was quite satisfactory.

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Organoid cultures mimic all features and gene expression profiles of the original organ. Organoid technology is an important tool in regenerative medicine, precision medicine, cell therapy, gene therapy, and drug development and standardization.

Organoid technology provides novel human and animal organ models for the study of cancer, genetic diseases and other infectious diseases. Newly developed drugs could be first screened in organoids to assess organ response, eficacy, and dosage.

Organoid-pathogen coculture models also provide alternative and reliable methods of evaluating new drugs against infectious diseases and other neglected tropical pathogens. Additionally, genetic disorders and congenital abnormalities with no animal models are suficiently studied through the application of organoid technology.

SIALIDASE AND ATHEROSCLEROSIS

DAY 2

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BACKGROUND: Reduced sialic acid LDL content have been previously associated with atherosclerosis, but the mechanismunderlying this association has not been explored. In this study, the hypothesis that LDL desialylation contributes to development of atherosclerosis has been tested.

METHODS: For in vivo low-density lipoprotein (LDL) desialylation, neuraminidase from Vibrio cholerae immobilized on immunoglobulin G with N-ethyl-N-(3-dimethyl aminopropyl)carbodiimide was prepared. To induce LDL desialylation, 0.02 U immobilizedneuraminidase in 100 µl salinewas used as a singledose. The study involved two groups of laboratory animals(Apoe-/- deficient mice on the genetic background of the C57BL/ 6J line). The first group (n=10) included Apoe-/- deficient mice that did not receive the immobilized sialidase preparation. In the second group of Apoe-/- deficient mice (n=10) were animals that were injected with a drug for desialylation of lipoprotein. Both groups received a standard hypercholesterol diet for 12 weeks. After the animals were euthanized. The aorta was removed and stained with Oil Red O. Images of the aorta were taken with a digital camera. The lesion area was assessed using specialized image analysis software. RESULTS: When comparing the control group and the experimental group, it was shown that in the experimental group there was an increase in the area of accumulation of Oil Red O in the aortic intima by 42.7% compared with changes in the control group. There were no histological changesin the aortic tissue samplesof the control and experimental groups.
There were no morphological changes in the aorta in wild-type mice in groups, regardless of the diet and administration of the immobilized sialidase preparation. CONCLUSIONS: The result can be considered a confirmation of the importantrole of sialidase in the pathogenesis of atherosclerotic lesions.

MITOCHONDRIAL DNA EDITING: MECHANISM OF MICROHOMOLOGY-MEDIATED END JOINING

DAY 2

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Background and Aims:
We have previously found an association of some mitochondrial mutations with asymptomatic atherosclerosis in the carotid arteries of patients. The most direct way to elucidate the role of these mutations in atherogenesis is by editing the mitochondrial genome. The aim of this work was to develop an approach to eliminate mitochondrial mutations from mitochondrial DNA (mtDNA).

Methods: The mitoCAS9 vector was used to produce RNA complex, consisting of Cas9 nuclease linked to sgRNA. Mannose liposomes were used to deliver RNA complex in the THP-1 cells. The THP-1 cybrid cells that carried Cytb G15059A mutation. The efficiency of mutation elimination was assessed by T7E1, qPCR, and ddPCR.

Results: The elimination of Cytb G15059A mutation by MitoCas9 RNA complex was successfully confirmed by T7E1, ddPCR, and sequencing. We found that the MitoCas9-RNA complex can cleave up to 92% mtDNA, and the heteroplasmy level was reduced up to 3.7% from 68%. Moreover, we found that some double-strand breaks were repaired by the mechanism of microhomology-mediated end joining (MMEJ). The possible matrix for MMEJ was a part of the mitoCas9 vector, that was delivered to mitochondria together with the RNA complex. This mechanism might be used to incorporate mitochondria mutations of interest in “healthy” mitochondria.

Conclusions: The method to eliminate mitochondrial mutations was created. It might be possible to create a novel approach of mtDNA editing via the MMEJ mechanism. This study was supported by Russian Science Foundation, Grant # 22-15-00064

PHOTOCATALYTIC BIONANOCOMPOSITE ELECTROSPUN COATINGS FOR PATHOGENIC INACTIVATION TO CONTROL THE SPREAD OF COMMUNICABLE DISEASES

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Human civilization has contended with disease outbreaks throughout recorded history, from the Antonine Plague of 250 to the 1720s Bubonic Plague, and the 2019 ‘s COVID pandemic that are still infamous in our collective memory. For the first time, humanity has the ability to meaningfully use nanotechnology to combat a major infectious disease, namely the COVID-19 pandemic caused by SARSCoV-2. SARS-CoV-2 has spread to millions of people worldwide through a novel mechanism of binding to ACE-2 receptors in the human body using its spike protein .The author of this talk and his group of researchers have done some pioneering research towards the control of spread of COVID- 19 and reported in recent publication entitled “Nanoparticle Engineered Photocatalytic Paints: A Roadmap to Self-Sterilizing against the Spread of Communicable Diseases". In this talk, the author describes for the first time the potential of these novel paints for the self-sterilization of surfaces in hospitals and public places by merely exposing them to visible light. The purpose of this talk is to identify the root cause of this widespread sickness and propose a strategy for halting its spread. We've put up an alternative strategy based on the use of silk fibroin (SF) bio- nanocomposites with strong antipathogenic capabilities in this lecture .Additionally, electrospun bionanocomposites can be applied to metallic surfaces in a uniform manner. This technique is described in detail to control the spread of communicable diseases in combination with photocatalytic inactivation of pathogens.

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FACE MASK AGAINST COVID-19 – ROLES, REQUIREMENTS, EFFICACY AND SUSTAINABILITY

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Face mask prevents pathogen-containing aerosols and liquid droplets from being inhaled or exhaled in a public setting as a nose and mouth covering. Corona virus sizes ranging from 0.06 to 0.14 microns must be blocked by face masks, which can be ensured by maintaining the porosity of face masks, regardless of the type of cotton masks, surgical masks, and N95 respirator masks. However, nano-induced antibacterial advanced technology (electrospinning) on polypropylene (PP) and polyethylene (PE) polymeric non-woven fabrics (e.g., melt blown or spun bond) is used to improve the mask efficacy. Despite of the technologies, the use of low-cost, sustainable raw materials, avoiding air leakage, allowing normal breathing by improving fit and comfort, and blocking input and output bacteria to at least 95% are the main requirements. Waste generation, on the other hand, causes physical and chemical pollution as a result of the widespread use of disinfectants, chemical sterilization, and waste incineration, as well as a lack of research into new environmentally friendly biodegradable materials and techniques. Manufacturing industries taking an environmentally friendly approach and developing sustainable waste management policies with all stakeholders could help build a robust system to deal with current pandemic waves and future crises. This paper looks at the latest developments in face masks focusing on the requirements, types, and materials. Furthermore, manufacturing techniques, efficacy, challenges, and associated risks are also discussed in light of their effective applications and the consequences of the ever-increasing demand for local and global mandates.

Keywords: Coronavirus, Covid-19, Face Mask, Efficacy, Sustainability

IMPLICATIONS OF MICROORGANISMS IN ALZHEIMER'S DISEASE (REVIEW)

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Alzheimer's disease (AD) is a deadly brain degenerative disorder that leads to brain shrinkage and dementia. AD is manifested with hyperphosphorylated tau protein levels and amyloid beta (Aβ) peptide buildupin the hippocampus and cortexregions of the brain. The nervous tissueof AD patients also contains fungal proteins and DNA which are linked to bacterial infections, suggesting that polymicrobial infections also occur in the brains of those with AD. Both immunohistochemistry and next-generation sequencing (NGS) techniques were employed to assess fungal and bacterial infections in the brain tissue of AD patients and non-AD controls, with the most prevalent fungus genera detected in AD patients being Alternaria, Botrytis, Candida, and Malassezia. Interestingly, Fusarium was the most common genus detected in the control group. Both AD patients and controls were also detectable for Proteobacteria, followed by Firmicutes, Actinobacteria, and Bacteroides for bacterial infection. At the family level, Burkholderiaceae and Staphylococcaceae exhibited higher levels in the brains of those with AD than the brainsof the control group. Accordingly, there is thoughtto be a viscous cycle of uncontrolled neuroinflammation and neurodegeneration in the brain, caused by agents such as the herpes simplex virus type 1 (HSV1), Chlamydophila pneumonia, and Spirochetes, and the presence of apolipoprotein E4 (APOE4), which is associated with an increased proinflammatory response in the immune system. Systemic proinflammatory cytokines are produced by microorganisms such as Cytomegalovirus, Helicobacter pylori, and those related to periodontal infections. These can then cross the blood-brain barrier(BBB) and lead to the onset of dementia.

Here, we reviewedthe relationship betweenthe etiology of AD and microorganisms (suchas bacterial pathogens, Herpesviridae viruses, and periodontal pathogens) according to the evidence available to understand the pathogenesis of AD. These findings might guide a targeted anti- inflammatory therapeutic approach to AD.

Keywords: Alzheimer's disease;neuroinflammation; neurodegeneration; inhibitors; gut microbiota; beta-secretase; gamma-secretase; blood-brain barrier

DAY 2

POTENTIAL THERAPEUTIC APPLICATIONS OF L-ALANINE PRODUCED BY PEDIOCOCCUS ACIDILACTICI BD16 (ALAD +)

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L-alanine possesses extensive physiological functionality and tremendous pharmacological significance, therefore could be considered as potential ingredient for food, pharmaceutical, and personal care products. However, therapeutic properties of L-alanine still need to be addressed in detail to further strengthen its utilization as a viable ingredient for developing natural therapeutics with minimum side effects. Thus, the research work was aimed to explore the anticipated therapeutic potential of L-alanine, produced microbially using a lactic acid bacterial strain Pediococcus acidilactici BD16 (alaD +) expressing L-alanine dehydrogenase enzyme. The anticipated therapeutic potential of L-alanine was assessed in terms of anti-proliferative, anti-bacterial, and anti-urolithiatic properties.

Anti-bacterial assays revealed that L-alanine successfully inhibited growth and in vitro proliferation of important human pathogens including Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Streptococcus mutans, and Vibrio cholerae in a concentration-dependent manner. Current investigation has also revealed its significant anti- proliferative potential against human lung adenocarcinoma (A549; IC 50 7.32 µM) and mammary gland adenocarcinoma (MCF-7; IC 50 8.81 µM) cells. The anti-urolithiatic potential of L-alanine was augmented over three different phases, viz., nucleation inhibition, aggregation inhibition, and oxalate depletion. Further, an in vitro cell culture-based kidney stone dissolution model using HEK293-T cells was also established to further strengthen its anti-urolithiatic potential. This is probably the first in vitro cell culture-based model which experimentally validates the immense therapeutic efficacy of L-alanine in treating urolithiasis disease.

DAY 2

A CLICK BEETLE LUCIFERASE REPORTER SYSTEM FOR BIOLUMINESCENCE IMAGING OF LISTERIA MONOCYTOGENES

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Listeria monocytogenes is a Gram-positive intracellular pathogen that is widely used as a model organism for the analysis of intracellular parasitism. In this context, there is a current need to develop improved reporters for enhanced bioluminescence imaging of the pathogen in infection models. We have developed a click beetle red luciferase (CBR-luc) based vector (pPL2CBRopt) for L. monocytogenes and have compared this to a lux-based system for bioluminescence imaging of the pathogen using in vitro and in vivo models. The CBR-luc plasmid stably integrates into the L. monocytogenes chromosome and can be used to label field isolates and laboratory strains of the pathogen. Growth experiments revealed that CBR-luc labelled L. monocytogenes emits a bright signal that is maintained during stationary phase. In contrast, lux- labelled bacteria produced a light signal that peaked during exponential phase and was significantly reduced during stationary phase. Light from CBR-luc labelled bacteria was more efficient than the signal from lux-labelled bacteria in penetrating an artificial tissue depth assay system. A cell invasion assay using C2Bbe1 cells and a systemic murine infection model revealed that CBR-luc is suited to bioluminescence imaging approaches and demonstrated enhanced sensitivity relative to lux in the context of Listeria infection models. Overall, we demonstrate that this novel CBR reporter system provides efficient, red-shifted light production relative to lux and may have significant applications in the analysis of L. monocytogenes pathogenesis.

ADJUVANTICITY EVALUATION OF CERIUM NANOPARTICLES IN VETERINARY RABIESVACCINE

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Background and Purpose: Rabies virus is a fatal infectious disease in the mammalian host including humans, carnivores, and livestock which imposes a huge burden on the health of the community and the global economy. The development of new rabies vaccines is a critical step for controlling the disease; however, a safe, cheap, and effective vaccine against the disease remains unaffordable in developing countries. Nowadays, aluminum oxide (Alum) is used to improve the efficacy of rabies vaccines. However, alum improves immune responses, but some of its disadvantages limited its application in the formulation of the vaccine. Therefore, new adjuvants with low side effects, long-term immune stimulation, and simultaneous stimulation of humoral, cellular, and mucosal responses are necessary to increase the immunity of weakened antigens. Recent advances in the field of nanotechnology, especially in the production of the size and shape of metal nanoparticles, lead to the development of various types of applications. Cerium metal, with its special properties, especially low toxicity, has a unique ability to stimulate the immune system as an adjuvant. Given the above, the aim of the current study is adjuvanticity evaluation of cerium nanoparticles in veterinary rabies vaccine to use it to increase the potency rate of the vaccine.

Methods: Aqueous cerium nitrate was used for purification by sonification to synthesize cerium nanoparticles. Then we used X-ray and electron microscopy to confirm the nature of synthetic nanoparticles. In the next section, we examined in vitro toxicity, adjuvanticity and immunogenic properties, the eliciting of neutralizing antibodies, and the production of interleukin-4. Synthesized CeNPs were confirmed through XRD, SEM, and TEM analysis.

Results: The prepared CeNPs were spherical with a diameter of less than 50 nm. As determined by the dynamic light scattering method, the zeta potential was 26.6 mV. No significant cytotoxic effects were observed at any of the tested concentrations. The neutralizing antibodies (NAbs) measured by the RFFIT method in the fourth group (killed virus formulated with CeNPs) also had a statistically significant difference compared to the negative control groups ($P \leqslant 0.01$). There was no significant difference in the level of interleukin-4 in the CeNPs group compared to the negative control groups ($P \geqslant 0.05$).

Conclusions: The data showed that cerium nanoparticles have adjuvant properties in the rabies virus vaccine. In this regard, it seems that more studies are needed on the adjuvant property of metal nanoparticles, especially cerium, and their effect on the production of neutralizing antibodies by using them as carriers of the rabies virus nucleoprotein.

Key Words: rabies virus, cerium nanoparticles, Adjuvant