D_Cell Culture and Biomedical Engineering

D-1

Evaluation of Biological Activity of Lactic Acid Bacteria and *Bacillus* Isolated from Fermented Food

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Kimchi is a traditional fermented food in Korea and is known to contain various physiological activities such as carotene, dietary fiber, indicating various functions such as antioxidant, anticancer. We separated *Lactobacillus* and *Bacillus* from Kimchi. As a result, 89 *Lactobacillus* species and 101 *Bacillus* species were isolated, and we conducted experiments with eight of these strains, six *Lactobacillus plantarum* and two *Bacillus velezensis*. Cytokine is a protein immunomodulator secreted from immune cells. Tumor Necrosis Factor alpha (TNF-α) is a cytokine produced in macrophages that induces infection, inhibits tumor production and viral replication, and induces apoptosis of tumor cells. Interferon beta (IFN-β) is produced in virus-infected cells and acts on the cells themselves and surrounding them to increase resistance to viral infection. We measured the TNF-α, IFN-β secretion of selected *Lactobacillus* and *Bacillus* with RAW264.7, a mouse-derived macrophage cell line. So, we found *Lactobacillus* and *Bacillus*, which have the best secretion function. We hope that they can be applied as functional biomaterials in the future.

Keywords : *Lactobacillus, Bacillus*, cytokine

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D-2

Enterotoxin Express to Attenuated Bacteria Cancer Targeting and Therapy in Mouse Tumor Model

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Bacteria-mediated cancer therapy was capable of actively targeting and efficiently removing solid tumor. Also, tumor targeting bacteria could be used as mediators that can specifically deliver anticancer agents to tumors. To utilize these functions into bacteria, we engineered attenuated *Salmonella* typhimurium, which express to *Clostridium perfringens* enterotoxin (CPE). CPE is known as cytolysin, which specifically binds to claudin (CLDN)-4. CPE gene expression was regulated by pBAD promotor. We confirmed the expression and secretion of CPE induced by L-arabinose at engineered bacteria. *In vitro*, CLDN-4 positive breast cancer cell lines (BCCs) were treated with CPE to confirm binding to CDLN-4 and cytolysis of CPE. Consequently, CPE was co-localized with CLDN-4 and bring cell death of BCCs. *In vivo*, engineered bacteria administered to murine/human breast tumor-bearing mice intravenously, was successfully localized to tumor tissue and gene expression was induced by L-arabinose. The engineered bacteria significantly suppressed tumors. CLDN-4 expression level in the CPE-expressed bacteria group was down-regulated compared bacteria groups without L-arabinose. Therefore, engineered bacteria that carry a CPE gene for targeted CLDN-4 positive cancer therapy can be designed as a theranostic agent.

Keywords : *Clostridium perfringens* enterotoxin, Claudin-4 positive breast cancer, *Salmonella* typhimurium

D-3

Nanoperforator: A New Way to Defeat Enveloped Viruses

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Lipid-bilayer nanodiscs encircled by membrane scaffold proteins (MSPs) have been used to study membrane proteins of interest in a physiological environment. Here, we suggest novel nanodisc-based approach to deal with influenza virus infection. Mechanistically, nanodiscs carrying gangliosides bind to influenza virions and are co-endocytosed into host cells. At low pH in the endosome, the nanodiscs rupture the viral envelope, trapping viral RNAs inside the endolysosome for enzymatic decomposition. To improve stability and antiviral effect, we further established dimeric or large nanodisc with ganglioside receptors whose belt proteins were circularized. Through increased cooperativity of dimeric nanodisc or enlarged area of large nanodisc, they exhibited enhanced antiviral potency *in vitro*. We showed that ease of perforation involved in viral inhibition by analyzing membrane fusion and perforation between nanodiscs and viral envelope. Circularized dimeric nanodiscs were more thermally stable than conventional nanodiscs. In addition, PEGylation on cysteine residue of large nanodisc enhanced thermal stability and proteolytic resistance. With improvements in antiviral efficacy and stability, we expect *in vivo* potential of antiviral nanodiscs. Our results suggest a new class of antiviral that induces irreversible physical damage of enveloped viruses and its structural variations to improve *in vivo* efficacy. In conclusion, the lipid nanostructure provides new dimension for antiviral activity of decoy molecules.

Keywords : Nanodisc, enveloped virus, perforation

D-4

Development of Dual-Functional Aptamer that Inhibit the Pancreatic Cancer Cell Growth

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Pancreatic cancer is the 4th leading cause of cancer death in the world. It is hard to diagnose early and is not easily treated once it spread to other organs. Thus, novel therapeutic strategies are urgently needed. Here we developed an RNA aptamer against CTHRC1 and ALPPL2 protein for the prevention of pancreatic cancer. The RNA aptamer that has been treated with pancreatic cancer, will be targeted ALPPL2 expressed on the surface of pancreatic cancer cell and downregulated the CTHRC1 function by blocking the Smad signal for cell growth. We selected the RNA aptamer against CTHRC1 and ALPPL2 protein using in vitro transcription SELEX. Then, we analyzed the binding structure of the protein-RNA aptamer complex, which is confirmed the RNA aptamer bind to the active site of the protein using the MOE program. RNA aptamers of each binding to CTHRC1 and ALPPL2 were conjugated to produce an aptamer complex with two functions that specifically bind to the surface and inhibit the growth of pancreatic cancer cells. This dual-functional aptamer complex had a high affinity to each target protein active site.

Keywords : Pancreatic cancer, aptamer, SELEX

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D-5

Fabrication of pH-Responsive Nanoparticles Comprising Alginate and Chitosan with Bursting Release

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Drug delivery system using pH-responsive nanoparticles has been extensively explored due to the controlled release in the gastrointestinal tract with minimizing side effects. Herein, we fabricated nanosized vehicles comprising alginate and chitosan through the electrostatic complexation and investigated their pH-responsive bursting release of cargo protein. The size and absolute value of potential of nanoparticles at pH 2.0 were significantly higher than those at pH 7.4 indicating that the particles were aggregated at pH 2.0 while collapsed at pH 7.4. BSA, a model cargo protein was encapsulated with high efficiency ratio (about 100 μg/mL) and dramatically released at pH 7.4 (about 80%). However, the cargo protein was rarely released in the acidic (pH 2.0) condition resulting in only 1% of release ratio for 2 h. Results demonstrate that our pH-responsive nanoparticle system has a potential for the oral-administered drugs or vaccines.

Keywords : Nanoparticle, bovine serum albumin (BSA), bursting release

D-6

Diagnosis of Lung Cancer Using Odorant Markers and *Caenorhabditis elegans*

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Early diagnosis of cancer is important to reduce the mortality rate of patients and to increase the success rate of treatment. Along with the early diagnosis, an easy, economical, rapid and non-invasive diagnostic method is also required for reducing the number of severe cancer patients. Many researches have been recently studied to discover and identify an odorant molecule specifically from tumor tissues or cancer patients' blood, saliva and exhaled breath. A previous study has suggested that 2-ethyl-1-hexanol might be a biomarker or one of the specific volatile organic compound (VOC) occurring in the lung cancer tissue and patient. In this study, we investigated a novel lung cancer diagnosis system using *C. elegans*, a nematode which responded to cancer-specific odorant molecules with chemotaxis behaviors, attraction or avoidance. *C. elegans* strains showed either positive or negative preference depending on the strain species and the sample concentrations suggesting that a specific neuronal regulator genes execute the olfactory preference and locomtional behavior. Taken together, the *C. elegans* olfactory sensing of odorant molecules from cancer tissue has a potential for an easy, economical, rapid and non-invasive cancer diagnostic tool.

Keywords : *C. elegans,* diagnosis, biomarker

D-7

Cultivation of *Euglena gracilis* Using Nnoodle and Rice Cake Wastes as Nutrient Sources for Paramylon Production

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Paramylon, a β-1,3-glucan, is a commercially valuable polysaccharide as a nutirtional and medical supplement derived from *Euglena gracilis*. In paramylon production, the cost of microalgal cultivation is one of the important considerations. In this study, we evaluated cost-effective complex mediums for heterotrophic growth of *E. gracilis* utilizing various nutrient residues. Noodle and rice cake wastes were selected as major carbon sources accounting for about 80% of the medium cost. For the composition of the medium, an industrial by-product, corn steep solid (CSS), was additionally supplied as a nitrogen source. In addition, enzymatic hydrolysis was performed using three types of enzymes: α -Amlyase (AE), Amyloglucosidase (AGE) and their mixture (AE+AGE) to utilize glucose as a carbon source for accumulation of paramylon. As a result, it was shown that biomass and paramylon productivities were enhanced by up to 38.7% and 40.1% in complex medium compared to the synthetic (modified Hutner's) medium. This indicates that food residues can be a good supplement to the medium for economical production of paramylon.

Keywords : *Euglena gracilis*, paramylon, enzymatic hydrolysis

D-8

The Combination Effects of Zinc and Adiponection supplementation on Porcine *In Vitro* Embryo Development

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Zinc (Zn) is an important factor during oocytes in vitro maturation and many other physiological function. In addition, Adiponetine (ADQ) improved porcine somatic cell nuclear transfer embryo development during in vitro culture (IVC) in our previous study. The purpose of this study is to investigate the effect of combination treatment during IVM and IVC on porcine oocytes. Cleavage rate, blastocyst rate, and total cell number after parthenogenetically activation (PA) by electrical stimulation were studied. Zinc concentration added during IVM is 1 μg/ml, ADQ concentration supplemented during IVC after PA is 15 μg/ml, and the combination treatment was supplementation of Zn 1 μg/ml during IVM and ADQ 15 μg/ml during IVC respectively. Cleavage rate showed that Zn-ADQ (85.87 ± 0.65) resulted highest rate compared to control (79.1 ± 0.32) and Zn treatment (86.23 ± 0.34) (p<0.05). Moreover, the cleavage rate revealed Zn treatment significantly higher than control. Blastocyst rate showed significant different between all group treatment control (19.56 ± 0.42), Zn (25.32 ± 0.37), and Zn-ADQ (29.94 ± 0.56) (p <0.05). The Zn-ADQ treatment revealed highest blastocyst rate than other groups. Total cell number counting revealed that Zn-ADQ (71.22 ± 1.18) and Zn (68.91 ± 1.22) were higher than control (56 ± 0.97) (p <0.05), while Zn-ADQ and Zn treatment showed no significant different. We concluded that combination treatment during IVC and IVM utilizing Zn and ADQ has the best result among Control and Zn treatment groups to embryo development in porcine.

Keywords : Zinc, adiponectin, pig embryo

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D-9

Application of Polyethylenimine-Chitosan Composite Fiber to Induce Astaxanthin Accumulation in *Haematococcus pluvialis*

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The ability of *Haematococcus pluvialis*, a unicellular green microalga, to accumulate a high amount of astaxanthin has gained attention. Various stress-inducing factors were used to increase astaxanthin production in *H. pluvialis* cultures. Addition of a synthetic cationic polymer, polyethyleneimine (PEI), was recently suggested as a new way to increase astaxanthin accumulation. However, there is a disadvantage that the injection of the solution for stress stimulation cannot be recovered due to one-time use, and the stimulation is reduced by concentration dilution. In this study, we investigated the effects of reusable and recoverable PCF (PEI-Chitosan fiber) treatment on *H. pluvialis*. According to findings, PCF can cause astaxanthin accumulation. When comparing coating and composite methods for fabricating the PCFs, it was discovered that composite induced astaxanthin accumulation, while coating had little effect. PEI, chitosan, and cross-linker conditions were investigated in order to maximize PCF effect. Optimal PEI concentration was observed at 0.1M. In the case of chitosan, 2% small molecular weight chitosan was used for its role as a backbone and stability, and 0.1mL/L of Epichlorohydrin was used as a crosslinking agent. Content of astaxanthin was 324 ± 12 pg per cell, which was 4.4 times higher than untreated group. PCF should serve as a novel approach to enhance astaxanthin production.

Keywords : *Haematococcus pluvialis*, polyethylenimine, astaxanthin

D-10

Targeted Delivery of Immunogenic Cell Death Inducer and IDO1 siRNA *via* Anti-CD44/PD-L1 Aptamer-Conjugated Liposome

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Cancer cells have various immune evasion mechanisms to induce tolerance against immune cells by turning the tumor microenvironment (TME), such as overexpression of programmed cell death ligand 1 (PD-L1) and indoleamine 2,3-dioxygenase-1 (IDO1). To reverse and enhance the immune response in tumor site, we designed targeted co-delivery system of IDO1 siRNA and doxorubicin (DOX, immunogenic cell death inducer) using dual-aptamer conjugated liposome (aptamosome). Aptamosome was conjugated with dual DNA aptamers such as CD44 and antagonistic PD-L1 DNA aptamers to specifically deliver the drugs into targeted cancer cells as well as to inhibit PD-1/PD-L1 interaction between cancer cell and T-cell. We demonstrated that aptamosomes loaded with DOX and IDO1 siRNA were readily delivered into the cancer cells through an aptamer-mediated endocytic manner. Moreover, the aptamosome was shown to increase immunogenic cell death and suppress the expression of IDO1 in target cancer cells with a higher efficiency as compared to the plain liposome. We suggest that combinatory treatment of DOX and IDO1 siRNA with dual-aptamer conjugated liposome can exhibit synergistic anticancer effects by reversing immune evasive cancer cells into cancer cells with immune-favorable TME.

Keywords : Targeted delivery, liposome, aptamer

D-11

The Effect of Korean *Dendropanax morbifera* Extracts on the Alzheimer's Condition Cells

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Recent research suggests that the "cerebral lymphatic system", called the "glymphatic system", is responsible for the removal of extracellular waste proteins from nerve cells through perivascular pathways. And Aquaporin-4 channel protein (AQP4) is an integral part of this system and has been implicated in neuropathology such as Alzheimer's disease. As a result, it has been reported that the elimination of the Alzheimer-related proteins, amyloid β and tau are reduced according to the glymphatic system disturbances due to AQP4 deficiency. Dendropanax morbiferus is known to improve brain cognition by inhibiting AChE. However, there is no study between the relationship between the AQP4 activity and Dendropanax morbiferus. We tried to determine whether Dendropanax morbiferus leaf and branches extract increases AQP4 and brain-derived neurotrophic factor (BDNF), which is known as a cell aging marker, glial fibrillary acidic protein (GFAP) and low density lipoprotein receptor-related protein 1 (LRP1) or not. When 500 ug/ml of Dendropanax morbiferus extract was added, the protein expression levels of BDNF and GFAB were increased 20% and 17%, respectively and LRP1 and AQP4 were slightly increased. Our data suggest that Dendropanax morbiferus extract is helpful in suppressing Alzheimer's.

Keywords : Alzheimer, Dendropanax morbifera, AQP4

D-12

The Effect of the Red Grape Extracts on the Alzheimer's Condition Cells

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Glymphatic system is responsible for the removal of extracellular waste proteins from nerve cells through perivascular pathways. And Aquaporin-4 channel protein (AQP4) is an integral part of this system. It has been reported that the elimination of the Alzheimer-associated protein amyloid β is reduced by glymphatic system disturbances due to AQP4 deficiency. When the natural extract activates AQP4, it may be expected to suppress Alzheimer's. We tried to find the effect of red grape extracts, which are known to be good for brain cognitive function, on the Alzheimer's condition cells. We investigated the protein expression levels of aging marker protein BDNF (brain-derived neurotrophic factor), GFAP (glial fibrillary acidic protein) and the beta amyloid protein scavenging protein LRP1 (low-density lipoprotein related protein 1) by western blot. When 100~500 ug/ml red grape extracts were added, BDNF and GFAP increased 10% and 23-38%, respectively. And LRP1 and AQP4 were slightly increased compared to control. Our data suggest that red grapes extracts can be a natural food to help suppress the Alzheimer's disease.

Keywords : Aquaporin-4, Alzheimer's condition cells, glymphatic system

D-13

Expression of IGF1 and IGF1R in the Female Reproductive Organs in Dogs

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Insulin-like growth factor 1(IGF1) and IGF1R are implicated in the regulation of female reproductive function of granulosa and cell proliferation, but in dogs, our knowledge is limited to the possible role of the IGF1 system in ovary. Furthermore, the expression of IGF1 and its receptor (IGF1R) has not yet been described in dogs. In this study, we aimed to describe the protein localization of IGF1 and IGF1R in the female reproductive organs including ovary, oviduct and uterus of dogs. Female reproductive organs from three female dogs were collected by routine ovariohysterectomy. Cortex of ovary and uterine horn of bifurcation were cut into 0.5 cm^3 and fixed into 10% formalin. Oviducts were isolated from fat tissue with forceps and could be put into 10% neutral formalin. Detection of IGF1 and IGF1R proteins in ovarian follicle, oviduct and uterus was carried out with an indirect immunoperoxidase method using rabbit polyclonal antibodies. The IGF1 expression of follicular walls of ovaries were identified in all tested dogs. In oviduct, luminal epithelial cells and cilia showed positive signals and uterus also showed similar expressions for IGF1 and IGF1R. In conclusion, IGF1 and IGF1R may have a role in ovary as well as oviduct and uterus of dog.

Keywords : IGF1 and receptors, dog, female reproductive organ

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