epithelial cells (16hbe14o-) were cultured on transwell plates and exposed to 5-25 µM PFOS. PFOS exposure reduced transepithelial electrical resistance (TEER) and increased 4 kDa FITC-dextran permeability up to 72 h. Reductions in the tight junction organization rate (TiJOR) and protein levels of occludin and ZO-1 were observed following 15 µM PFOS exposure. Interestingly, the mRNA abundance of *OCLN* and *TJP1* were elevated 24 h post exposure. Furthermore, pro-inflammatory mediators, IL-8 and IL-6, were unchanged. Protein kinase D (PKD) activity, a key regulator of pulmonary epithelial barrier dysfunction was also evaluated. PKD phosphorylation was increased 3 h post PFOS exposure. Co-treatment with the PKD inhibitor CID755673 restored TEER and FITC-dextran permeability to vehicle levels, and partially attenuated occludin protein levels. Overall, our data demonstrate that PFOS exposure compromises pulmonary epithelial integrity by downregulating tight junction proteins, potentially in part through a PKD dependent mechanism. Additional studies are needed to clarify the mechanisms underlying PFOS induced barrier dysfunction and the impacts of PFOS exposure on respiratory disease risk. *Supported by T32 ES007026.*

3059 Evaluation of Tumor Markers and Immune Parameters among Chicken Husbandry, Grape Orchard, and Rose Greenhouse Workers

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Exposure to occupational hazards such as biologically active dust, inorganic dust, pesticides, gaseous pollutants, or physical hazards in the agricultural sector is recognized to cause various respiratory illnesses and immune modulation. Although farming has been considered at low risk of lung cancer, exposures during specific tasks in different crop or livestock farm have been found to increase the risk of lung cancer. The immune status alteration stands as a major determinant in tumor development and progression, but little has been explored on the association of immune status with tumor markers in the farming population. Blood samples collected from 51 chicken husbandry workers, 19 grape orchard workers, and 21 rose greenhouse workers were subjected to plasma pro-gastrin releasing peptide (Pro-GRP) and cytokeratin fragment 19 (CYFRA 21-1) level. Peripheral blood mononuclear cells (PBMC) were used for natural killer (NK) cell phenotyping and stimulated for cytokine profiling of interferon-gamma (IFN-γ) and interleukin-13 (IL-13). This study was authorized by the Institutional Review Board at Daegu Catholic University. Plasma Pro-GRP level and CYFRA 21-1 level, representing the small cell and non-small cell lung cancer biomarker respectively were found significantly higher in orchard (Pro-GRP: 2572±204.4 pg/ml, CYFRA: 4.27±0.28 ng/ml) and chicken husbandry workers (Pro-GRP: 2709±202 pg/ml, CYFRA: 4.04±0.13 ng/ml) than the greenhouse workers (Pro-GRP: 957.3±189.7 pg/ml, CYFRA: 3.34±0.22 ng/ml). Low proportion of NK cell was revealed in the orchard workers (19.78±3.29 %) compared to chicken husbandry (26.08±1.77 %) and greenhouse workers (26.88±2.59 %). Regarding the immune skewedness towards type-1 (T_H1) or type-2 helper T cell (T_H2), higher IFN-γ: IL-13 ratio was observed in the greenhouse workers (632.1±126.6) than the orchard (117.9 ±22.1 %) and chicken husbandry workers (167.6±18.5 %). The correlation between age and study parameter levels was not found. Hence, it can be depicted that orchard and chicken husbandry workers are apparently at high risk of lung cancer and disturbed immune homeostasis in comparison to the rose greenhouse workers. Owing to the multiple exposures in farming environment, the necessity of lung cancer risk assessment and immune status evaluation in the agricultural sector has been highlighted. *Supported by the Korea Rural Development Agency (Grant#PJ014269022020), Ministry of Environment-Educational training program for the management of information on the hazards and risk of chemicals substances.*

3060 Elucidating the Mechanism of Pleural Fibrosis following Anti-Plasminogen and Mesothelial Cell Interaction

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Pleural Fibrosis (PF) is an untreatable condition in which the pleural cavity becomes thickened with collagen and fibrin. PF develops after prolonged exposure to asbestos fibers or other irritant materials. Our prior research demonstrated that mesothelial cells have a binding site for the enzyme plasminogen (Plg) on the outer surface of the cellular membrane. We also demonstrated presence of anti-Plasminogen antibodies (anti-Plg) in asbestos exposed people and mice, and showed positive correlation between antibody presence and PF. Therefore, we hypothesized that anti-Plg contribute to PF development following interaction with mesothelial cells, the main cellular component of pleural tissue. Using flow cytometry, cell activity assays, and ELISAs, we demonstrate that anti-Plg block Plg conversion to the active enzyme plasmin, resulting in increased collagen deposition by mesothelial cells. We also show that Plg does not increase cellular proliferation or differentiation of mesothelial cells. Lastly, we examine the ability of Plg to activate matrix metalloproteinases (MMP), as these enzymes are known to contribute to collagen and fibrin reorganization; altered MMP activity may perpetuate PF by increasing collagen deposition. Elucidating the mechanisms of anti-Plg-mesothelial cell interaction may reveal therapeutic targets in PF development, thus aiding in treatment and prevention of this disease.

3061 Characterization of Different Consumable Wires in a Novel Thermal Spray Coating Aerosol Generator and Inhalation Exposure System

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Thermal spray coating (TSC) involves spraying a metal coating product that is melted by extremely high temperatures and then applied under air pressure onto a surface. Large amounts of a complex metal aerosol comprised of Fe, Cr, Ni, and Zn are formed during the process, presenting a potentially serious risk to the operator. Information about the health effects associated with exposure to aerosols generated during TSC is lacking. Even less is known about the chemical and physical properties of these aerosols. The goal was to construct and characterize an automated TSC aerosol generator and inhalation exposure system that would simulate workplace exposures. An electric arc wire-TSC aerosol generator and exposure system was designed and separated into two areas: (A) an enclosed room with a closed spray booth where the spray coating occurred; (B) an exposure chamber with different particle characterization devices. The physicochemical properties of aerosols generated during electric arc wire-TSC using five different consumable wires were examined. The metal composition of each was determined by ICP-AES, including two stainless-steel wires [PMET720 (82% Fe, 13% Cr); PMET731(66% Fe, 26% Cr)], two Ni-based wires [PMET876 (55% Ni, 17% Cr); PMET885 (97% Ni)], and one Zn-based wire [PMET540 (99% Zn)]. The generated particles, regardless of composition, were poorly soluble, complex metal oxides and mostly arranged as chain-like agglomerates of primary particles in the ultrafine range (<100 nm). The MMAD as determined by a MOUDI was similar when comparing the generated particles from the different wires and ranged from 290-378 nm. To allow for continuous, sequential coating within the spray booth during a 2-hr testing period, a motor rotated the metal feedstock pipe to be coated in a circular and up-and-down direction. The spray gun was controlled by computer software and fired at programmed 1-sec intervals for up to a total of 12 sprays during the testing period. A targeted exposure chamber concentration of 25 mg/m^3 was achieved and maintained during the 2-hr period, along with minimal fluctuations in relative humidity, temperature, and CO_2 levels. This exposure system will generate continuous, controlled metal spray coating aerosols for future animal exposure studies.

3062 Using FBS-Free Media in *In Vitro* Cell Cultures: A Case Study in Transitioning and Characterizing A549 Cells

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Replacing the use of fetal bovine serum (FBS) in cell culture media bolsters the reproducibility of *in vitro* research and overcomes the ethical and legal challenges associated with its use. Increasingly, scientists are focusing on replacing the use of FBS as a supplement in cell culture media with animal-component-free media. Using A549 cells—an immortalized human epithelial alveolar-like cell line commonly used in respiratory research—as a case study, we demonstrate the process of transitioning cells cultured in medium containing FBS to commercially-available media without FBS. To determine whether the transition was successful, cellular morphology and functionality were assessed by imaging (scanning electron microscopy); calculating cell doubling time, cytokine release (Bio-Plex), and cell viability (Alamar blue assay); monitoring the expression of relevant genes; and determining surfactant production (surfactant droplet test). Our results show that, while success varies based on the transition process and type of media, animal-derived components can be replaced in the culture of A549 cells. Because FBS-free media can replicate the phenotype of A549 cells similar to that observed in FBS-supplemented medium or a phenotype more similar to normal alveolar epithelial cells, the medium should be chosen based on the objective of the study. This case study can be used as a guide to transition other cell types to FBS-free media.

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