A Human Cell-Based Model of Airway NO Production in Response to Inflammatory Stimuli: A Source of NO in Exhaled Breath to be Detected by a Hand-Held Device

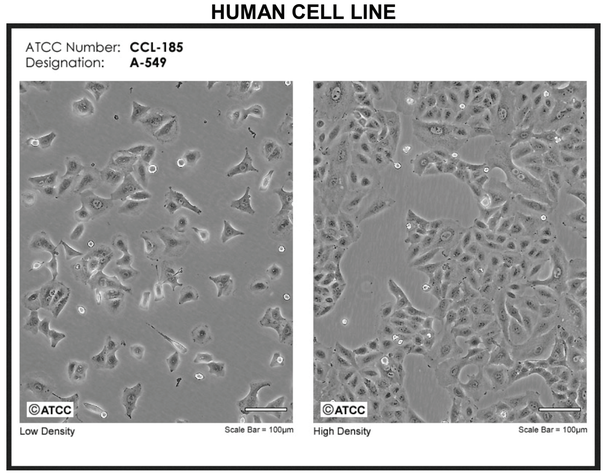
Sanford R. Simon[1], Katarzyna Sawicka[1], Elizabeth Roemer[1], Milutin Stanacevic[2] and Pelagia Irene Gouma[3]
Departments of Biochemistry[1], Pathololgy[1], Electrical and Computer Engineering[2], and Materials Science and Engineering[3]
Stony Brook University, Stony Brook, NY 11794

RATIONALE

The ultimate goal of our project funded by the Smart Health and Wellbeing program of the National Science Foundation is to develop a hand-held device to detect nitric oxide (NO) in exhaled breath as a quantitative indicator of inflammation in the lungs and airways, as well as in more distant sites throughout the body. NO is formed in human airway cells in response to direct stimulation by foreign particulates as well as the presence of cytokines released from remote tissues that reach the airway cells through the bloodstream. The presence of NO that diffuses out of stimulated airway cells has been used as a surrogate biomarker of asthma. In this exploratory project, we are using human airway cells in culture to generate NO rather than entering directly into clinical trials with human subjects. We maintain a human alveolar epithelial cell line in chambers from which the atmosphere above the cells can be collected for injection into our hand-held NO detector. The agents we are employing to trigger NO release from the airway cells have been implicated in asthma attacks in patients, providing a rationale for our model.

MATERIALS AND METHODS

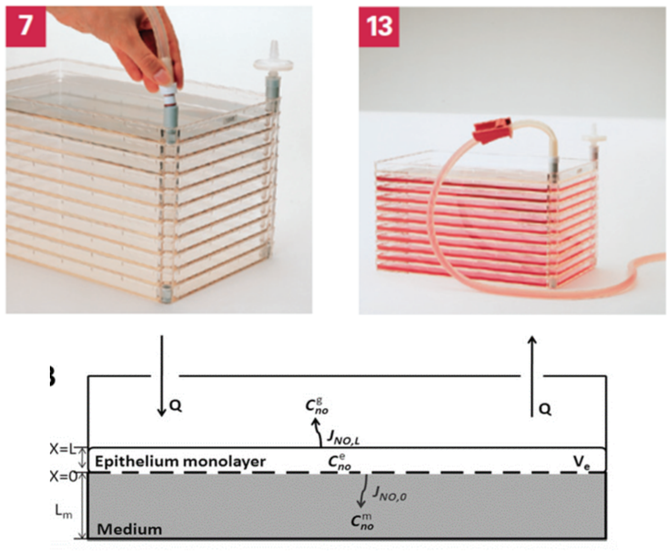
HUMAN CELL LINE



The A549 cell line was established by George Todaro's laboratory from a patient with lung cancer in 1973 and characterized as having characteristics of Type II alveolar epithelial cells (Lieber et al. 1976).

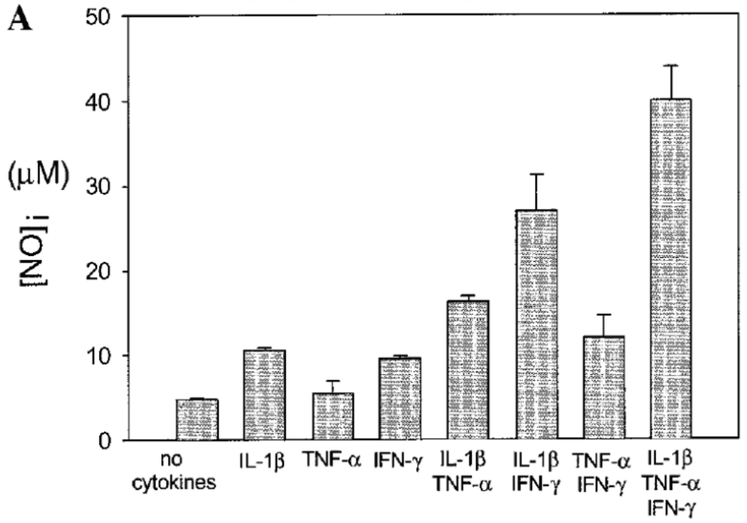
We culture A549 cells obtained from the American Type Culture Collection as monolayers in Ham's F-12K medium in the presence of 10% fetal bovine serum.

CULTURE METHOD



To approximate the air-liquid interface which lines the surface of human airways in vivo, we plate the cells onto enclosed polystyrene rectangular trays (335 mm x 205 mm; 632 cm^2) with ports to collect either the culture medium or the atmosphere above the monolayers (Nunc Cell Factory). A similar design has been employed by the laboratory of S.C. George at U.C. Irvine (Kwon et al., 2001; Jiang and George, 2011) to detect release of NO from A549 cells in response to the inflammatory cytokines Interleukin 1-β (IL-1β), Interferon-γ (IFN-γ, and Tumor Necrosis Factor-α (TNF-α).

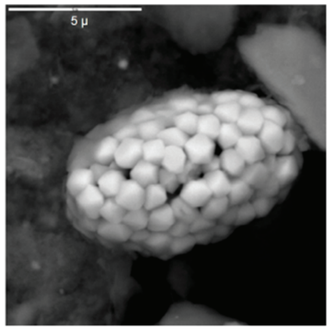
STIMULATION OF A549 CELLS WITH CYTOKINES
A MODEL OF AIRWAY INFLAMMATION



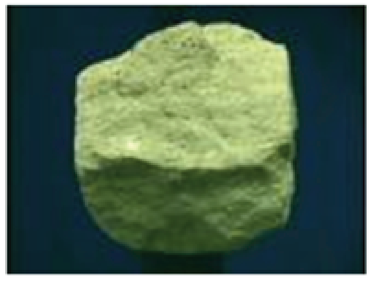
As previously reported by George's laboratory (Kwon et al., 2001), A549 cells release levels of NO in response to a cocktail of inflammatory cytokines that are about five times those released by cells cultured in cytokine-free medium. Circulating levels of these cytokines are also known to be elevated in patients experiencing an asthmatic attack or suffering from exacerbations of dieases marked by systemic inflammation. If these patients breathe into a mylar bag, the expired air can be subsequently anlyzed for NO using laboratory instruments. A more convenient strategy to facilitate increased patient compliance and better data collection is based on the hand-held device we are now developing, which can be employed to monitor such patients at home or workplace.

STIMULATION OF A549 CELLS WITH MINERAL POWDERS
A MODEL OF ENVIRONMENTAL AND OCCUPATIONAL ASTHMA

FRAMBOIDAL PYRITE (FeS)



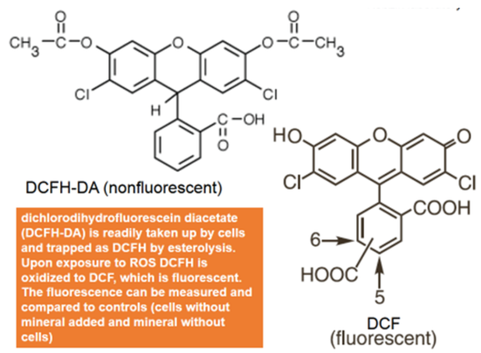
OLIVINE ($Mg_2Fe_2Si0_4$)



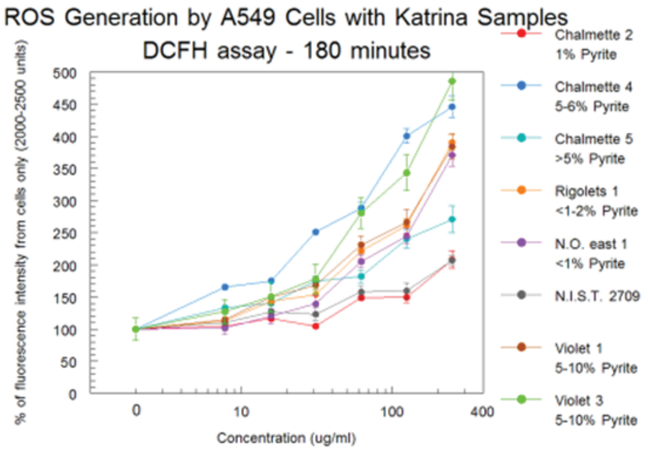
Airway epithelial cells can also be stimulated by direct exposure to dusts with different mineral compositions. Iron-containing minerals, such as pyrite and olivine, are present in dusts generated by mining operations, floods that leave quantities of mineral-rich mud and silt, and debris arising from construction or demolition sites. These dusts have been reported to trigger exacerbations of asthma in populations exposed to the mineral particulates, as has occurred in New Orleans after Hurricane Katrina and in New York City after the World Trade Center collapse.

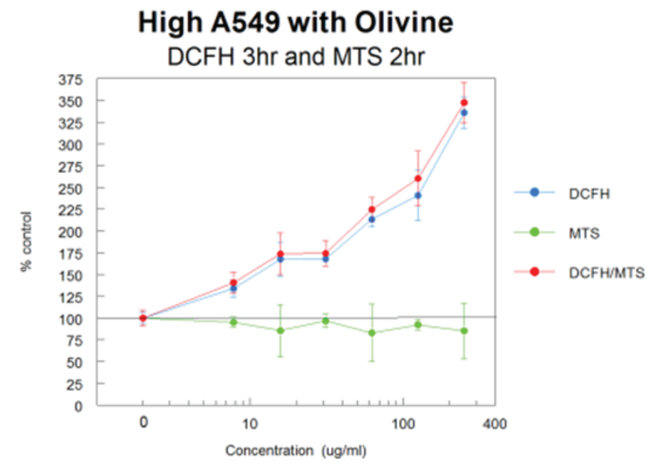
We have modeled the environmental exposure of airway epithelium to mineral dusts by incubating A549 cells with finely ground samples of pyrite and olivine. Before quantitating release of NO by these cells, we demonstrated that the mineral dusts induce other biomarkers of an inflammatory response. A reliable biomarker of inflammatory activation of A549 cells is the generation of Reactive Oxygen Species (ROS) that typically accompanies NO production.

STIMULATION OF A549 CELLS BY MINERALS



We detect the generation of ROS in A549 cells exposed to mineral particulates using the fluorogenic dye, dichlorodihydrofluorescein.





We will use these same incubation conditions to expose A549 cells cultured in Nunc Cell Factory units to pyrite and olivine and collect the headspace atmosphere to be injected into our hand-held NO detection device.

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