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Cells to analyse air

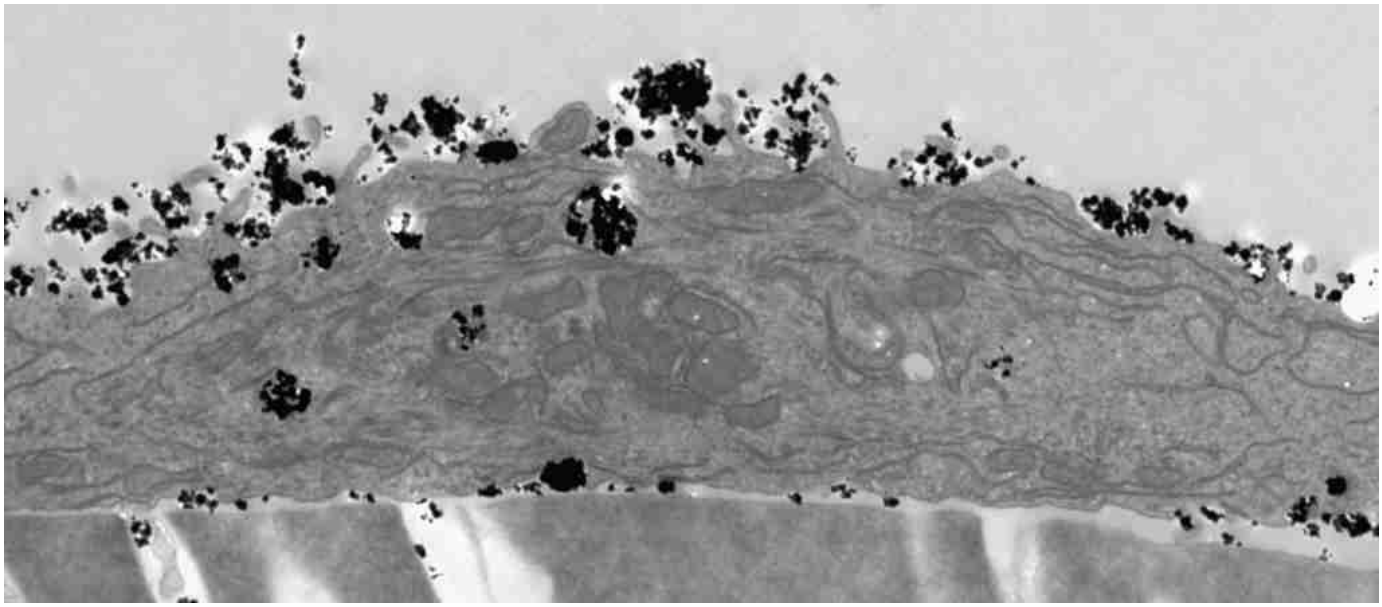
Exhaust gases, smoke generated by industry and private household combustion processes – every day we inhale a mixture of a broad range of particulate matter, potentially resulting in respiratory tract or cardiovascular diseases. Dr. Silvia Diabaté and her team from the Karlsruhe Institute of Technology (KIT) are examining the molecular mechanisms triggered by flue ash and other nanoparticles in the cells of the lung tissue. The researchers have developed an apparatus that simulates lung processes more realistically than cell cultures can. The so-called exposition chamber may also be used in the future as a biosensor for environmental monitoring.

Comprehensive epidemiological studies carried out in the 1990s have shown that increasing concentrations of particular matter in the air lead to an increase in respiratory tract and cardiovascular diseases. But what is in the air we breathe that makes us ill? The answer is the smallest particles created through combustion processes in engines, power stations or woodstoves; a complex mixture of soot, organic compounds and inorganic metal oxides that can be just a few nanometres in size. Nanoparticles are also produced in large quantities by industry; one example is titanium dioxide, which can be present in paint or sunscreens. It is now almost a decade since Dr. Silvia Diabaté and her team at the Karlsruhe Institute of Technology (KIT) started to focus on the pathogenic effect of flue ash. “Flue ash particles enter the lungs with the air we breathe, where they interact with the lung cells,” said Diabaté adding that it is only now that the researchers are beginning to understand the underlying molecular mechanisms.

A view into the cells

Out of all environmental ashes, the ultrafine particles have the strongest effect. Biologists from the KIT's Institute of Toxicology and Genetics (ITG) have focused on flue ash as well as industrially manufactured particles such as titanium dioxide. The researchers use cytotoxicity tests where they apply different concentrations of flue ash to the lung cells in order to determine which level of concentration leads to cell death. "Very high concentrations lead to cell death, but we do not yet know the exact mechanism that causes this." The researchers found that enzymes entered the extracellular space from which they concluded that the cell membranes become permeable upon exposure to high fine particle concentrations. The researchers also found that the cellular metabolism was reduced. Once the researchers have determined the particles that are particularly toxic and the dose at which they become lethal, they will then focus on the potential effect of the particles in the cells. "We will apply lower particle concentrations to our test cells and use different molecular biology methods to investigate the reaction of the cells," said Diabaté.

When the cells come into contact with environmental dust, they initiate mechanisms to reduce the



Human lung epithelial cell treated with titanium dioxide nanoparticles (black spots), seen under the transmission electron microscope.

oxidative stress resulting from the accumulation of reactive oxygen and nitrogen compounds in the cells. Such compounds can damage the DNA and other important molecules, at the same time as the cells are battling to prevent this. It would appear that environmental dust particles increase the quantity of such reactive compounds. In addition, certain lung cell types in the immune system generate an increase in proteins that trigger inflammation, for example cytokines. Cytokines are secreted by immune cells that have encountered a pathogen, thereby activating and recruiting further immune cells. This triggers an immune response in the lung. It is assumed that oxidative stress also triggers such processes.

A high-tech lung for street corners?

But can these results really simulate what happens in real lungs when they are exposed to dust particles? In order to get as close as possible to the real situation, Diabaté and her team have developed a special exposition chamber in addition to the traditional cell cultures they have always used. In cell culture, cells are covered with a liquid culture medium. However, lung tissue is normally only covered with a very thin film of liquid and is in direct contact with the aerosol phase. This situation is reproduced as closely as possible in the exposition chamber where the cells are directly "respirated" with a stream of air. The chamber looks like a cupboard, the difference being that it contains high-tech equipment. The aerosol is constantly moisturised, the temperature is kept at 37°C and the concentration of particles entering the chamber is closely monitored. All parameters are controlled by a computer. After exposure to the particles, the researchers determine the vitality of the cells and the concentration of cytokines secreted. In addition, they analyse whether increasing particle concentrations cause the cells to initiate a larger number of measures to counteract oxidative stress.

Diabaté and her team have already found that flue ash has considerable effects on the cells and that the cells react to the exposure to certain nanoparticles by releasing cytokines. "This is of major importance for environmental issues," said Diabaté. Will it be possible in future to place the exposition chamber by roads to analyse the effect of airborne pollutants? The apparatus has proved to be well suited to finding answers to certain environment-related issues. For example, the researchers from Karlsruhe, in cooperation with a research group from the University of Stuttgart,

The exposition system used by the KIT scientists to determine the biological effect of environmental dusts looks, at first sight, like a big cupboard.
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assessed the effects of particles generated by the combustion of wood pellets under different conditions. "The concentration of air-borne particles is too low at normal street intersections," said Diabaté. "The method used to analyse the effect of particles in the exposition chamber is not yet sensitive enough. We are currently working on improving the sensitivity."

Further information:

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