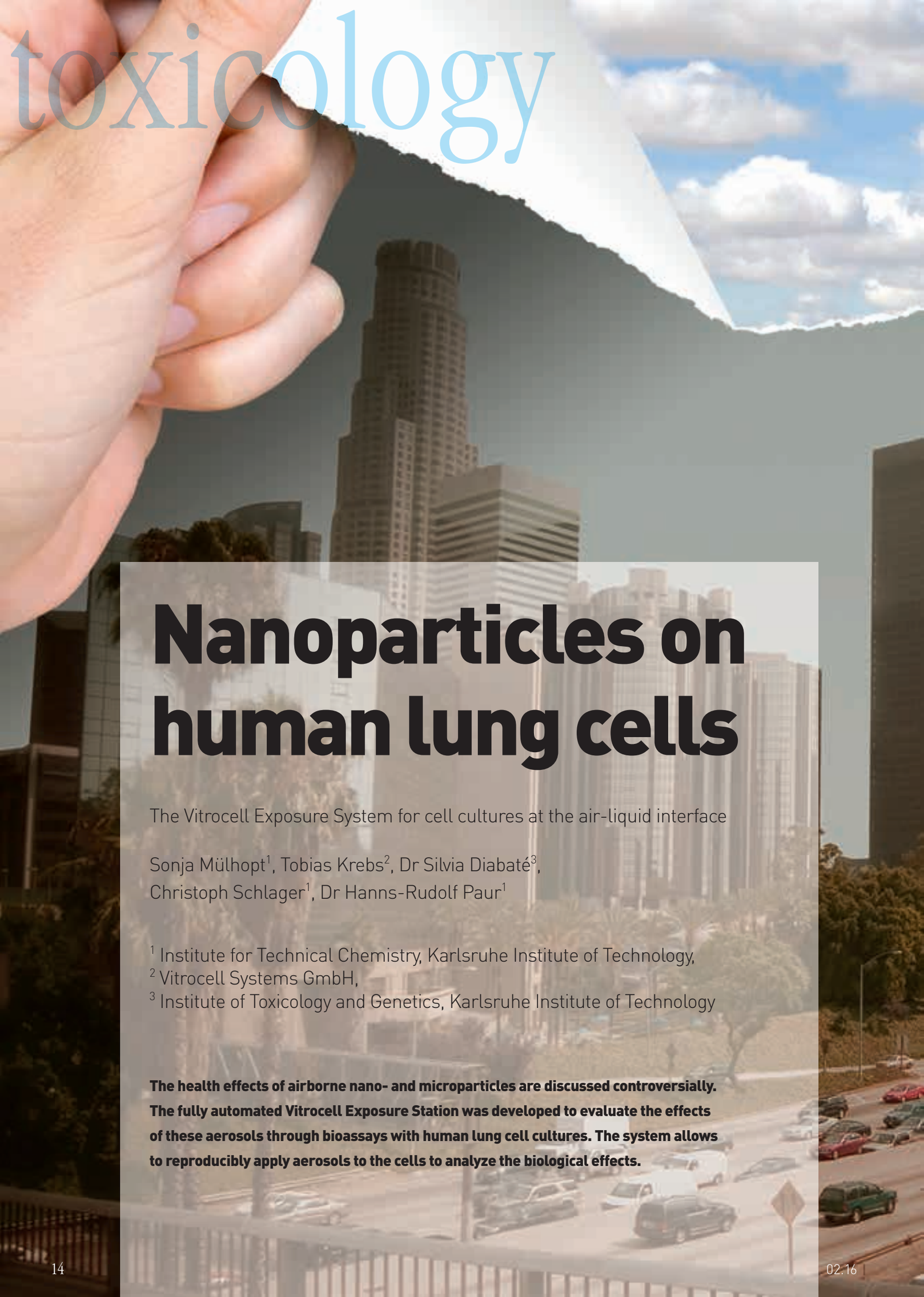

Nanoparticles on human lung cells

The Vitrocell Exposure System for cell cultures at the air-liquid interface

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The health effects of airborne nano- and microparticles are discussed controversially. The fully automated Vitrocell Exposure Station was developed to evaluate the effects of these aerosols through bioassays with human lung cell cultures. The system allows to reproducibly apply aerosols to the cells to analyze the biological effects.

New Materials – New Opportunities, New Risks? During the past two decades, measuring methods with ever higher resolutions and the resulting increasing understanding of the submicron regime have strongly influenced both the use and the risk assessment of nanoscale substances and systems: Whereas nanoparticle technology opens up new possibilities in the field of materials science, large-scale technical applications are generating new issues regarding occupational health and safety and environmental protection. The attractiveness of nanoparticles i.e., particles which according to EU standards are smaller than 100 nanometers (= $100*10^{-9}$ m) in at least one dimension, consists in the fact that most of their atoms are not located any more inside the molecules but on the surface and that the macroscopic properties, therefore, may change. The nanoparticles, for example, may have an increased solubility and chemical reactivity as well as reduced melting points. Besides, superparamagnetism and higher refractive indices and, hence, size-dependent chromaticity were observed. In addition to the often desired "new" physical properties, nanoparticles can have new biological properties i.e., in a biological system, they can cause so far unknown or untypical biological responses such as inflammations. Due to their small nanoparticle size, substances which so far have been classified as harmless hence can turn into potentially harmful products.

Undesirable nanoparticles

In spite of the above advantages, unwanted nanoparticles are becoming more and more of a problem: Although atmospheric loads have strongly decreased since the end of the eighties due to the improvement of combustion systems and filtering techniques in industry and traffic, particulate matter has become a quasi-measurable problem: The threshold value for particulate matter emissions of 50 µg/m³, which must not be exceeded on more than 35 days per year, was still often surpassed in 2014 in the big cities in spite of the introduction of low-emission zones and in spite of the fact that 50 µg/m³ still is far above of what has been recommended by the WHO. It was found in epidemiological studies by Dockery and Pope that environmental pollution with particulate matter correlates with the relative risk of diseases and death [1]. It was proved in several studies also by German scientists that the number of respiratory and cardiovascular diseases increases with the concentration of fine and ultrafine particulate matter [2]. Ultrafine particulate matter can deeply

penetrate the human respiratory tract and remain there for up to one year before being removed by the cleaning mechanism of the lung (Fig. 1).

The smaller the deeper

Whereas particles that are 1 to 10 µm in diameter are deposited mainly in the nasopharyngeal zone (green curve) and in the upper bronchia (yellow curve), particles smaller than one micrometer in diameter penetrate deep into the secondary and tertiary bronchia (blue curve) and the alveolae (red curve), where they have a mean residence time of ca. 400 days before they are removed by the cleaning mechanism of the lung. In adults, the alveolae, where the gas exchange from atmospheric oxygen to the blood and carbon dioxide to the respiratory air takes place, have a mean gas exchange surface of 140 m². Since there are hardly any air movements in this area, the gas and particle behavior is mainly characterized by diffusion.

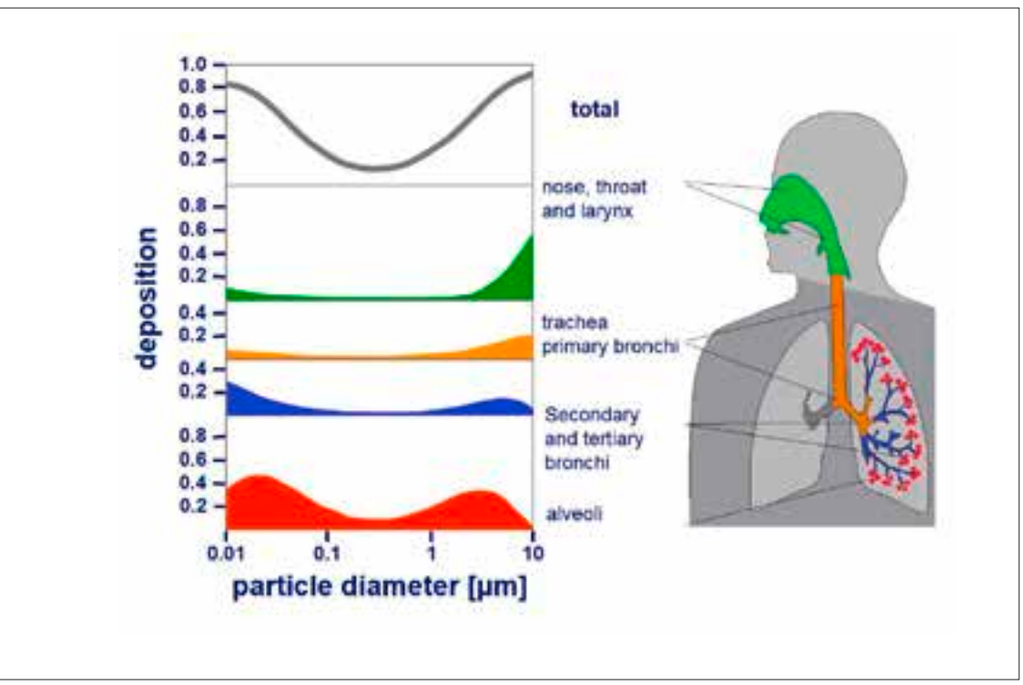

Fig. 1 Degree of separation as a function of particle size for the different regions of the human respiratory tract [9].

Fig. 2 Automated exposure system for reproducible exposure of bioassays at the air-liquid interface. Left: Schematic view of the main process components. Right: Photograph of Vitrocell system.
Picture: KIT, Vitrocell Systems GmbH

Investigation of nanoparticles

The correlation between particle emissions, residence time of particles in the human body, and biological effects of particles is the subject of intensive investigations. In addition to epidemiological studies, animal tests are carried out to be able to analyze systemic effects such as cardiovascular diseases. Screening tests and method development increasingly are carried out on the basis of cell cultures. During these in vitro studies, the cell cultures are exposed to the particles to be investigated and are analyzed for biological reactions after a defined incubation period. The responses can either be detected at a very early stage, for example metabolic changes in cells, or may occur only after some time, for example the release of cytokines (messengers) which are known as markers of inflammatory processes.

ALI processes

In the case of toxicological standard methods, particles are suspended in the culture medium, which is needed for cell cultivation, and are then applied onto the cultures. Whereas this so-called “submerged” (= covered with a liquid) method is well-suited for analysis of cells from organs that can be exposed to the particles without air admission e.g., intestinal cells, it is less suited for inhalation toxicology of air-borne particles. On the one hand, complete covering of the lung cells with liquid is not physiologic because the cells in the lung are covered only with a thin liquid film. On the other hand, the particles both during sampling and during application to the cells in culture medium are strongly influenced and hence the biological effectiveness may change considerably. Since the particles in the liquid are colloidal in character or partially agglomerated, the amount deposited on the cells cannot be determined precisely. A different technique where the cells are exposed at

Tab. 1 Survey of successively applied and analyzed aerosols, cell cultures, and biological effects

aerosols	**industrial nanoparticles**	titanium dioxide, silicon dioxide, silver, platinum	
	combustion aerosols	emissions from wood stoves, marine diesel engines, wood-fired boilers, pellet boilers, municipal waste incinerators	
cell cultures	**human lung epithelial cells**	A549, BEAS-2B, SK-MES-1	co-cultures from epithelial cells and macrophages and/or endothelial cells
	macrophages	THP-1, RAW264.7	
	human endothelial cells	HUVEC	
biological effects	**markers for inflammatory processes**	release of IL-8, IL-6, MCP-1, expression of ICAM-1	
	markers for cytotoxicity	release of LDH, reduction of AlamarBlue	
	markers for oxidative stress	expression of HMOX-1	
	markers for metabolism of foreign substances	expression of CYP1A1	

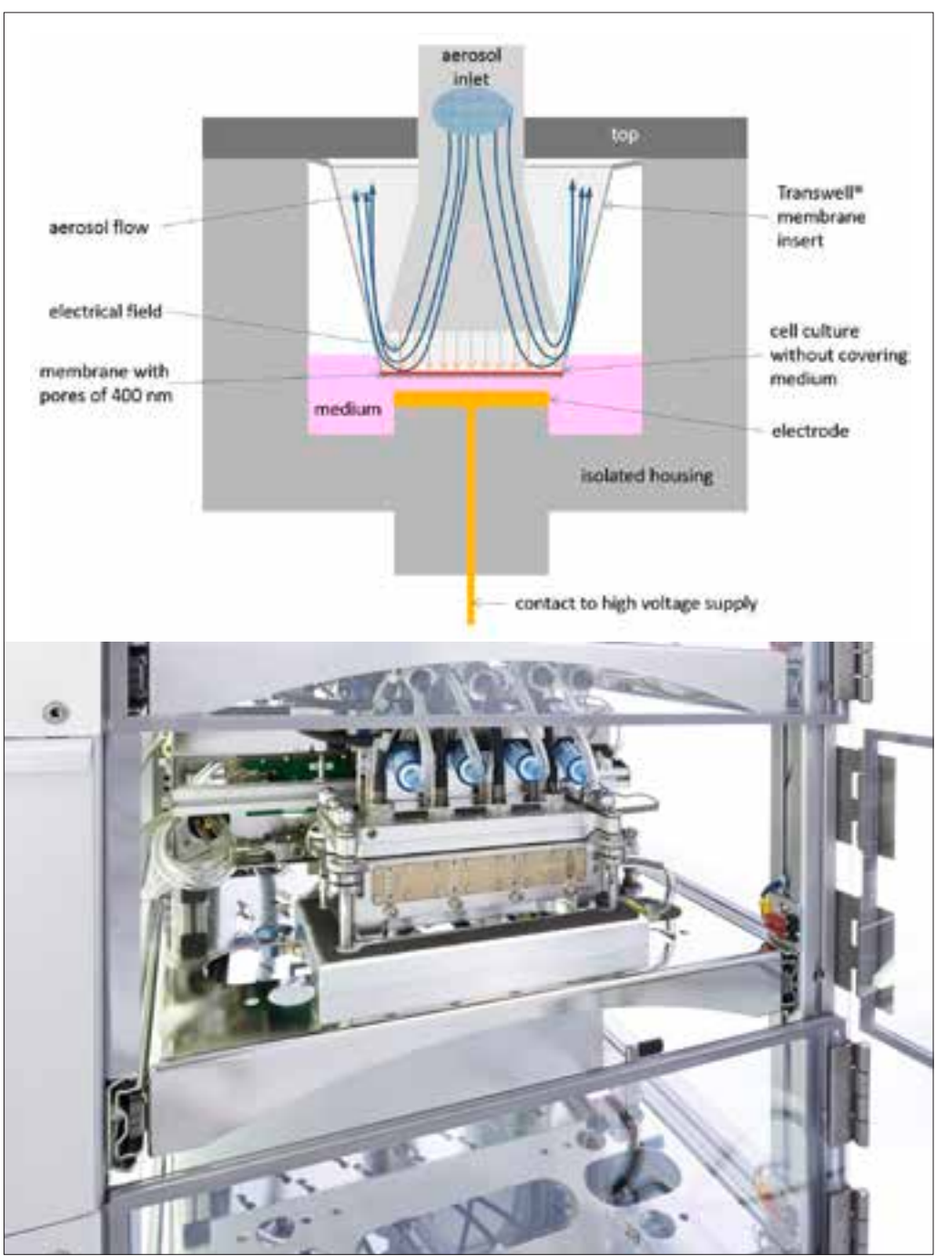

Fig. 3 Exposure chamber with Transwell culture dish. upper: Schematic diagram with electrode under the culture medium and flow lines of the aerosol flow above the cell culture. lower: Photograph of a quadripartite Vitrocell module for three 6-well inserts and a quartz crystal microbalance in the drawer of the exposure system. *Picture: KIT, Vitrocell Systems GmbH*

their air-liquid interfaces i.e., where the cells are covered only with a thin liquid film, has been used therefore for several years. This so-called ALI exposure (ALI = Air-Liquid Interface) is more realistic, can be reproduced more easily, and dose, in particular, is defined more precisely [3, 4].

The user-friendly automated exposure system

At KIT, an automated exposure system for ALI exposures was developed in cooperation with Vitrocell Systems GmbH (Waldkirch, Germany) (Fig. 2). This system allows both reproducible sampling and conditioning of aerosols and exposure of the cell cultures under conditions imitating those of the human lung. In addition, the relevant dose can be determined online [5]. For ALI exposure, bioassays were developed and used for toxicological analysis of particulate emissions from the industry [6, 7] as well as of nanoparticles [8].

Firstly, a sample of the aerosol to be analyzed is taken from the respective process at a volume flow of 1 m^3/h and is conducted through a PM2.5 low-volume impactor. The objective of the preliminary separation of larger particles is to simulate deposition in the upper respiratory tract and avoid that individual large particles make a non-reproducible contribution to the deposited mass and thus impede analysis by bioassays. Subsequently, the relative humidity is adjusted to 85 % r.H. through water vapor dosing to protect the cell cultures from drying out. Once stabilized, the humidified aerosol flows into a particle reactor. On each of the three levels of the reactor, there are isokinetic sampling probes from which the conditioned aerosol is conducted into the exposure chambers of the Vitrocell modules. Additional sampling points e.g., mobility analyzers, are available for external particle measurement or for filter-based sampling for electron microscopy. The Vitrocell modules are the heart of the system: Inside of them, the cell cultures that have been cultivated on the membrane inserts are apically exposed to the aerosol and are supplied basally with the culture medium (Fig. 3). All components are uniformly heated to 37 °C. To increase deposition efficiency, high voltage can be applied by an electrode below the culture medium. It is due to the electrical field, generated between aerosol inlet and cell culture, that charged particles are increasingly deposited on the cell culture by the electrical forces.

All flows are controlled by integrated mass flow controllers operated via touch screen

From left to right: Christoph Schlager, Sonja Mülhopt, Hanns-Rudolf Paur, Tobias Krebs *Picture: KIT*

Christoph Schlager He studied mechanical engineering / process engineering at Baden-Wuerttemberg Cooperative State University. During his studies and since their completion in 2012, he has been working on the development of the exposure method at KIT's Institute for Technical Chemistry and, in particular, has been supervising the use of the exposure system during large conjoint measurement campaigns conducted by the Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE) on e.g., wood-fired boilers and marine diesel engines.

Sonja Mülhopt She completed her studies in process engineering with a diploma degree and received her master's degree in chemical engineering in 2014. Since 2000, together with Vitrocell Systems GmbH and Institute of Toxicology and Genetics, she has been developing the method for aerosol exposure of cell cultures at the gas-liquid interface with integrated online dose measurement at KIT's Institute for Technical Chemistry. Since 2012, she has been heading the group Exposure Methods.

Hanns-R. Paur He obtained his doctorate in chemistry from LMU Munich and worked as a postdoc at UC Riverside in California. Currently, Dr. Paur heads the Division of Aerosol and Particle Technology at KIT's Institute for Technical Chemistry. His scientific fields of work comprise the formation, separation, and effects of ultrafine particles. Dr. Paur is vice president of Gesellschaft für Aerosolforschung – GAeF (Association for Aerosol Research) and appointed member of the VDI ProcessNet Gas Treatment Group.

Tobias Krebs studied industrial engineering. After having gained comprehensive entrepreneurial experience, he has been self-employed since 1997 in the development and marketing of technologically advanced products. In 1999, he started to work on in vitro inhalation toxicology and founded VITROCELL Systems GmbH as an independent company in 2007. Today, VITROCELL is a leading supplier of equipment for in vitro exposure of cells of the respiratory tract and of dermal tissue for research institutes, contract laboratories, regulatory authorities, and industrial companies throughout the world.

Silvia Diabaté She studied biology at Martin-Luther University Halle-Wittenberg and obtained her doctorate from Gießen University in 1984. Since 1998, Dr. Diabaté has been carrying out toxicological investigations of nanomaterials at Institute of Toxicology and Genetics at Karlsruhe Institute of Technology. In cooperation with Institute for Technical Chemistry, she developed the in vitro procedure for exposure of lung cells at the air-liquid interface.

monitors. The intuitive HMI (human-machine interface) surface for all control and data acquisition functions has been developed specifically for this device. The system can be integrated in a network.

The system is being used already in two EU projects (NanoMILE and QualityNano) and at the Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE), where considerable experience has been gained already with nano-aerosols.

Conclusion

The experience gained so far with the automated exposure system shows that the effects of nanoparticles on human lung cells can be analyzed reproducibly. Numerous groups from European laboratories have gained experience already in using the new technology. Since the new system allows realistic exposures, it is expected that a valid data base for evaluation of particulate matter emissions and nanomaterials can be created for the first time through in vitro experiments and that the number of animal experiments in that field can be reduced.

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AchemAsia 2016: Leading trade show for the process industries in China for the tenth time

From May 9–12, 2016, AchemAsia will open its gates in Beijing. For the tenth time, experts from all over the world will meet in order to present products and processes for plant engineering and chemical processing, get information on the latest developments in the process industries, and make new contacts. With exhibitors and participants from 17 countries, AchemAsia is the most international trade show for the process industries in China. It covers apparatus and plant engineering as well as process technology, petrochemistry, pharma and food processing, agrochemistry and laboratory and packaging techniques. Environmental technology and water treatment are also in the focus. The exhibition is accompanied by sseveral praxis-oriented symposia on process intensification, current challenges in the petrochemical industry, and single-use technologies.

The tenth anniversary of AchemAsia occurs at a time of economic change: China's economic growth is slowing down and the government strives for a structural change from an export-oriented economy to a strong domestic market. With the strategy "Made in China 2025" it has initiated an ambitioned program to transform China to a high-tech location. German and international companies have to prepare for more competition from China. On the other hand, the high-investment program opens great opportunities for German technologies in production, plant engineering and automation as well as in providing equipment for a wide range of sectors.

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