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A hydrogen peroxide sensor for exhaled breath measurement

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Abstract

An increase in hydrogen peroxide concentration in exhaled breath (EB) of patients, who suffer from some diseases related to the lung function, has been observed and considered as a reliable indicator of lung diseases. In the EB of these patients, hydrogen peroxide is present in the vapour phase together with water, thus one of the approaches of monitoring hydrogen peroxide in the EB is to condense it and then to perform the hydrogen peroxide measurement in the condensate.

Earlier, a hydrogen peroxide sensor based on an electrolyte metal oxide semiconductor field-effect transistor (^{E}MOSFET) has been investigated. The sensor shows the possibility to measure hydrogen peroxide at a concentration of micro-molar level. Due to its miniaturizability, the sensor is able to detect hydrogen peroxide in a small volume and is thus especially suitable for monitoring of hydrogen peroxide in the EB. In this paper, a simple set-up for condensation of hydrogen peroxide in the EB is introduced. The set-up consists of a cooling tube using a Peltier element for condensation of the exhaled breath air and the ^{E}MOSFET-based hydrogen peroxide sensor. Using this cooling tube, primary results on the collection and measurement of hydrogen peroxide in artificial EB are given.

Keywords: Hydrogen peroxide sensor; ^{E}MOSFET; Exhaled breath; Lung diseases

1. Introduction

Exhaled breath air consists of traces of many volatile compounds as nitric oxide (NO) and carbon monoxide, and a water vapour saturated phase that contains aerosol particles with non-volatile compounds, such as H_2O_2, nitrite, chloride, isoprostane and proteins. These compounds reflect the composition of the bronchoalveolar extracellular lining fluid in lung, which is continuously subjected to many noxious agents presented in a polluted environment [1]. At healthy people, the concentrations of the non-volatile compounds in the exhaled breath are quite low [2], but it will dramatically increase at patients who suffer from certain diseases. When a person has lung inflammation, white blood cells (granulocyte type) release enzymes, hydrogen peroxide and other chemicals to kill bacteria.

An increase in the hydrogen peroxide concentration in the exhaled breath has been considered as a main and reliable indicator of lung diseases, such as asthma and chronic obstructive pulmonary diseases [3,4]. Increase in the hydrogen peroxide concentration in the breath condensate is also recognized when the human lung becomes in contact with oxidative gases like tobacco smoke. Some studies presented in [5] show a difference in the hydrogen peroxide concentration in the exhaled breath condensate of smokers and non-smokers.

Therefore, the monitoring of the hydrogen peroxide level in the exhaled breath can provide reliable information about lung injury that is important for diagnostics and further treatment of mentioned diseases.

Up to now, a common method to collect the exhaled breath condensate is to use a special cooling collector including a freezing cooling tube by ice [5–7] or liquid nitrogen [8] and cooling machine, which has a refrigerator's circuit. Further, the obtained condensate is analyzed off-line by other techniques, such as spectroscopy, with assistance of peroxidase due to the lack of a miniaturized hydrogen peroxide sensor. For spectroscopic analysis, a relatively large volume of the condensate, in the order of hundreds micro-liter, is required while sample dilution is not possible due to a low hydrogen peroxide concentration in the condensate, which is typically in the range between hundred nano-molar and micro-molar

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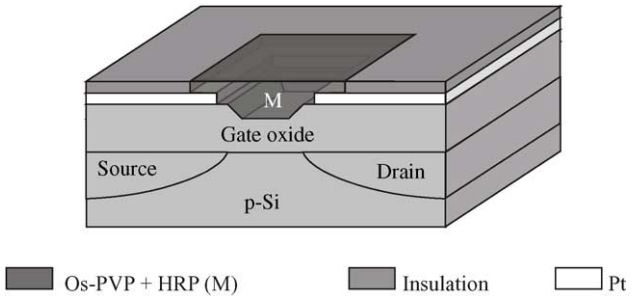

Fig. 1. The ^{E}MOSFET-based hydrogen peroxide sensor.

level. Therefore, the required time for obtaining one sample is rather long.

In this paper, we present a set-up, which allows to directly condensate the exhaled breath on a hydrogen peroxide sensor and then to perform the H_2O_2 measurement in the condensate. This set-up consists of a cooling tube using a Peltier element for condensation of the exhaled breath and an electrolyte metal oxide semiconductor field-effect transistor (^{E}MOSFET)-based hydrogen peroxide sensor (see Fig. 1).

Earlier, the hydrogen peroxide sensor, which is based on the ^{E}MOSFET having an Os-polyvinylpyridine (Os-PVP) gate containing peroxidase (HRP), has shown a possibility to measure hydrogen peroxide in solution at a concentration of micro-molar level due to a high catalytic property of peroxidase [9–11]. The sensor shows a typical high sensitivity to hydrogen peroxide when an external nano-current is applied between the gate electrode and measuring solution (constant current potentiometric mode). The threshold voltage of this ^{E}MOSFET, which is seen as the signal of the sensor depends logarithmically on the concentration of hydrogen peroxide and the applied current [12]. Due to its miniaturizability, the sensor is able to detect hydrogen peroxide in a small volume of solution that is especially suitable for monitoring of hydrogen peroxide in the exhaled breath.

2. Experiments

The cooling set-up presented in this paper consists of the cooling tube maintained by a Peltier element and a sensor. The cooling tube consists of three separate parts: a Peltier element, cooling tube body and condensate collector as shown in Fig. 2.

One side of the Peltier element contains a set of metallic cooling fingers coated with hydrophobic material, where the aerosol particles in the exhaled breath are condensed. The body of the cooling tube has a double wall to ensure a good isolation from the environment. The tube has a plastic inlet and outlet for blowing the exhaled breath air in and out.

During an experiment, artificial breath air containing hydrogen peroxide is made by blowing pressurized air through a hydrogen peroxide aqueous solution contained in a washing bottle. The solution in the washing bottle is kept at an

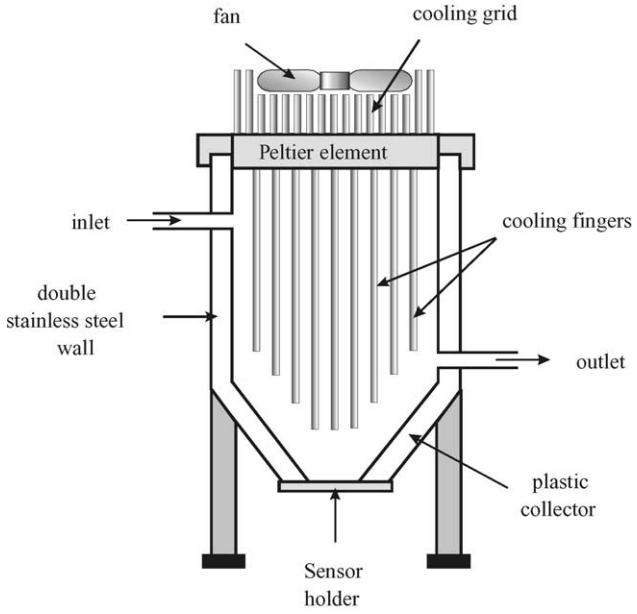

Fig. 2. Cross-section of the cooling tube.

elevated temperature of 45 °C by a heater. This temperature is chosen higher than the body temperature to compensate for the heat loss while the air travels from the washing bottle to the cooling tube. In addition, the elevated temperature of the solution ensures high humidity of the air that is close to the practical value. When the pressurized air is blown to the hydrogen peroxide solution through a grid, small air bubbles are created which increases the contact surface between solution and air. After every hour of condensation, the mass of the collected condensate is weighted. The concentration of hydrogen peroxide in the artificial breath is varied by changing the hydrogen peroxide concentration in the washing bottle. Calibration of the hydrogen peroxide in the artificial breath is done by amperometric measurement in the artificial breath condensate.

When the humidified air containing hydrogen peroxide is blown to the cooling tube, the aerosol particles in the air are condensed into droplets containing hydrogen peroxide and collected at the sensing area. The sensing part consists of the ^{E}MOSFET-based hydrogen peroxide sensor, which has an Os-polyvinylpyridine containing peroxidase (from BioAnalytical System) gate, and a counter electrode made from platinum. This electrode is used for applying an external nano-ampere current between the gate electrode and the condensate during hydrogen peroxide measurement [12]. In this design, a minimal required sample volume is calculated to be ca. 450 μl.

In all experiments, the external nano-ampere current is supplied by a battery-operated current source. Two types of hydrophobic coating have been used: commercial coating FC722 and poly(iso-propylene). The poly(iso-propylene) coating has been prepared from 20, 30 and 40% poly(iso-propylene) solutions as described in [13]. All chemicals used

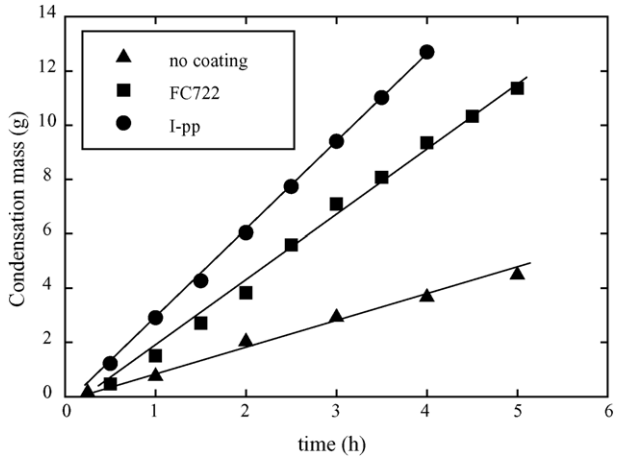

Fig. 3. Mass of the condensate as a function of time, which is shown to depend on the hydrophobicity of the inner surface of the cooling tube.

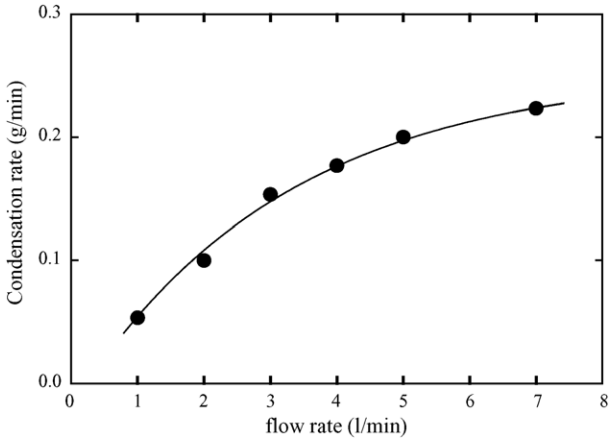

Fig. 5. The condensation rate depends on the airflow rate (poly(iso-propylene)).

in experiments were of analytical reagent grade from Merk and Fluka.

3. Results and discussions

3.1. Exhaled breath condensator

Prior to the investigation of the condensation of the artificial exhaled breath containing hydrogen peroxide, the cooling process of pure artificial breath using the cooling tube, has been studied.

It can be seen in Fig. 3 that the mass of the condensate increases proportionally to the cooling time. From this curve, if there is no coating on the inner surface of the cooling tube, the condensation rate is calculated to be about 0.9 g/h or 0.9 ml/h. However, at given water temperature and air flow rate of 1 l/min, the expected amount of the condensate is calculated to be about 3.4 ml/h, much higher than the obtained value. A reason for the low condensation rate can be explained by the poor hydrophobicity of the cooling surface. In fact, the condensation occurs much faster, but water droplets do not easily come off from the surface of the cooling fingers. To improve this, different types of hydrophobic coatings, such as poly(iso-polypropylene) (I-pp) and commercial coatings FC722 have been applied to the inner surface of the cooling tube as well as the cooling fingers.

Among these coatings, the highest hydrophobicity has been achieved when the coating is made from a 40% I-pp solution. With the I-pp coating, the contact angle with deionized water increases to 140°, while for the commercial FC722 coating the contact angle is only 105° (Fig. 4).

Consequently, as plotted on the same Fig. 3, with the FC722 coating, the condensation rate of the cooling tube is 2.6 g/h while with the I-pp coating, about 3.3 g of condensate is collected after 1 h. Especially, for the I-pp coating, the delay time, which is needed to wait until the first droplet is collected in the collector, is minimal. Therefore, the I-pp coating has been chosen for the cooling tube in further experiments. With this condensation rate, according to the geometric design of the collector, the time, which is required for one measurement, is estimated to be about 15 min.

Using the presented collector with the I-pp coating, the condensation rate of the artificial breath can be increased at a higher flow rate as shown in Fig. 5.

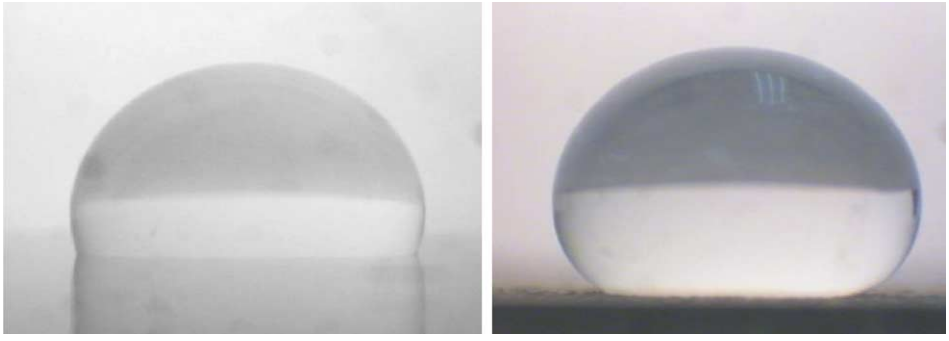

Fig. 4. Hydrophobic coatings: commercial FC722 (left) and poly(iso-propylene) (right) are applied on the inner surface of the cooling tube to enhance its hydrophobicity.

3.2. Set-up for making the artificial breath containing hydrogen peroxide

Generation of the artificial breath air containing hydrogen peroxide has been described in Section 2. The H_2O_2 concentration in the air depends on the H_2O_2 concentration in the washing bottle according to the Henry's law:

$$K_H = \frac{[(H_2O_2)_g]}{[(H_2O_2)_s]} \quad (1)$$

where $[(H_2O_2)_g]$, $[(H_2O_2)_s]$ and K_H are the partial pressure of the gas, molar concentration of the gas in the solution and the dissolution constant, respectively.

After condensation of the artificial breath air containing hydrogen peroxide, the H_2O_2 concentration in the condensate is measured by amperometry.

Fig. 6 shows that the H_2O_2 concentration in the condensate is proportional to the H_2O_2 concentration in the artificial breath air and can be varied depending on the H_2O_2 concentration in the washing bottle. At a flow rate of 1 l/min, the H_2O_2 concentration in the condensate is found to be ca. 600 times lower than the one in the washing bottle because of the difference in evaporation rates of water and hydrogen peroxide. This correlation coefficient is almost independent on the H_2O_2 concentration in the washing bottle.

3.3. Hydrogen peroxide sensor

Before measurement, a calibration curve of the sensor is performed. As earlier explained in [12], the threshold voltage of the sensor depends on the oxidation ratio of the ^{E}MOSFET's gate Os-polyvinylpyridine that is influenced by the H_2O_2 concentration and an external reducing current applied between the gate electrode and the solution:

$$V_T = \text{const} + \frac{2.3RT}{2F}\log[H_2O_2] - \frac{2.3RT}{F\alpha}\log\frac{I}{I_0} \quad (2)$$

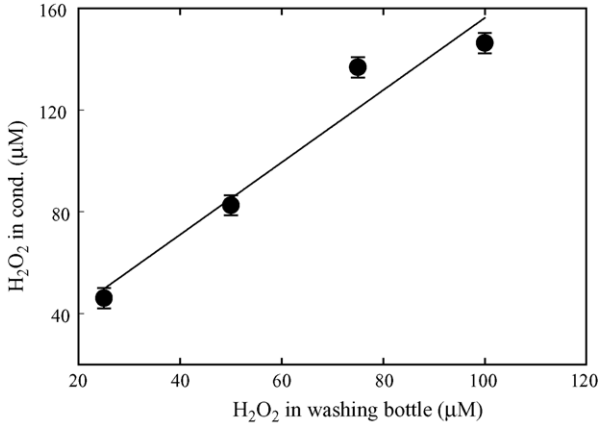

Fig. 6. Hydrogen peroxide concentration in the condensate, which is measured by amperometry, linearly depends on the hydrogen peroxide concentration in the washing bottle.

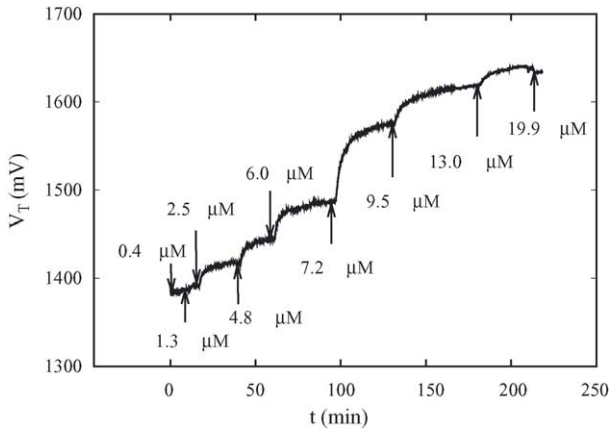

Fig. 7. Threshold voltage of an Os-PVP containing HRP-based sensor at an applied reducing current of 10 nA as a function of time when small amounts of the H_2O_2 solution are added.

where I and I_0 are the external applied current and exchange current between the gate electrode and solution, respectively; α is the dimensionless coefficient of the redox reaction between the gate and hydrogen peroxide.

The response of the threshold voltage of the ^{E}MOSFET to changes in a hydrogen peroxide concentration in solution is shown in Fig. 7, while small amounts from a stock hydrogen peroxide solution are added. Because the expected H_2O_2 concentration in medical applications is in micro-molar range, a reducing current of 10 nA has been applied between the gate electrode and the platinum counter electrode to achieve a low detection limit of the sensor.

From this figure, the calibration curve of the sensor at the applied reducing current of 10 nA is presented in Fig. 8. The influence of the value of the external reducing current on the threshold voltage is studied in a condensate obtained from a 13.7 mM hydrogen peroxide solution. According to the results shown in Fig. 6, the concentration of hydrogen per-

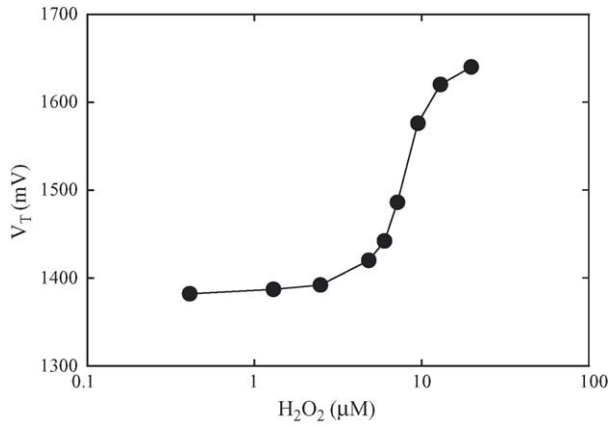

Fig. 8. Sensitivity of an Os-polyvinylpyridine containing peroxidase based sensor to H_2O_2 at the applied reducing current of 10 nA.

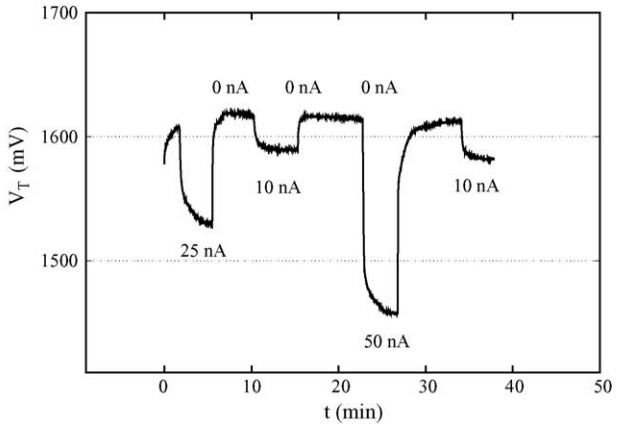

Fig. 9. Threshold voltage of the ^{E}MOSFET measured in the condensate obtained from a 13.7 mM hydrogen peroxide solution as a function of time at different values of the applied currents (0, 10, 25 and 50 nA).

oxide in the condensate is estimated to be about 25.1 μM that shows a good agreement with the calibration curve presented in Fig. 8.

At this concentration the gate of the ^{E}MOSFET is almost fully oxidized even when the reducing current of 10 nA is still applied. If the current increases, the threshold voltage decreases due to decrease in the oxidation ratio of the gate material. At a higher applied external reducing current, the threshold voltage of the ^{E}MOSFET is more reduced as shown in Fig. 9. When the current is reduced to 0, the gate material becomes oxidized and the threshold voltage rises, respectively.

Next, when the H_2O_2 concentration in the condensate is reduced, the threshold voltage decreases as presented in Fig. 10 that shows a result reaching the expected value of hydrogen peroxide concentration in the artificial exhaled breath. At an applied current of 10 nA, the sensor shows a detection limit close to 0.8 μM.

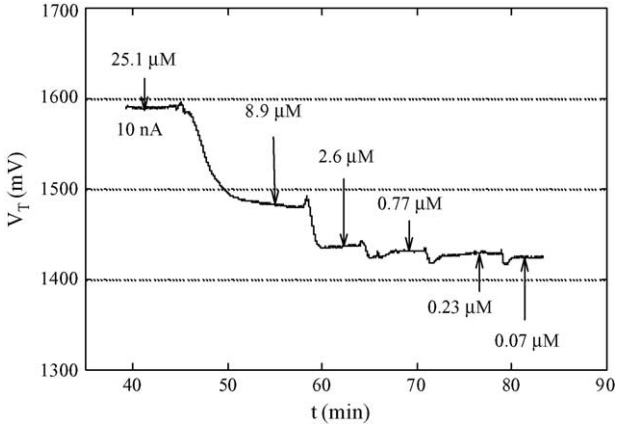

Fig. 10. Threshold voltage of the ^{E}MOSFET as a function of time when the H_2O_2 concentration in the condensate is decreased at the applied reducing current of 10 nA.

4. Conclusions

In this paper, we introduce a simple cooling condensator using a Peltier element to condense the exhaled breath. The portable set-up consists of the Peltier-based collector, which requires a low consumption power, in combination with an miniaturized ^{E}MOSFET-based hydrogen peroxide sensor, that allows the condensation of the exhaled breath and in situ measurement of the hydrogen peroxide concentration in the condensate.

The experimental results show that the condensation rate of the cooling collector depends on the hydrophobicity of the inner surface of the cooling tube and the cooling fingers. By applying the poly(iso-polypropylene) coating on the inner surface of the cooling condensator, the condensation rate increases to 55 μl/min. With this condensation rate, the required time for one measurement is estimated to be about 15 min.

Furthermore, artificial breath containing hydrogen peroxide has been made by blowing pressurized air with a constant rate, through an initial hydrogen peroxide solution. A correlation between the H_2O_2 condensation in the condensate and the initial solution has been found to be independent on the H_2O_2 concentration in the initial solution, but is dependent on the airflow rate. Measurement of hydrogen peroxide concentration in the condensate by the ^{E}MOSFET-based sensor has been performed and shows results close to the calibration curve.

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Biographies

Dam Thi Van Anh received the MSc degree in chemistry from the Byelorussia State University, Minsk, Byelorussia in 1994 and the PhD degree from the Faculty of Electrical Engineering, University of Twente, the Netherlands in 2003. The subject of her PhD dissertation was the development of hydrogen peroxide sensor based on the EMOSFET concept. Since 2003, she has been working as a postdoc in the Lab-on-a-Chip group, MESA^{+} Research Institute, University of Twente. Her research relates to the field of redox materials, nano-electrode array and electrochemical sensors for single cell analysis and medical and environmental applications.

Wouter Olthuis (1960) received the MSc degree in electrical engineering from the University of Twente, Enschede, the Netherlands in 1986 on the subject of thermally excited resonating silicon membrane pressure sensors. In that year, he joined the Center for MicroElectronics, Enschede (CME) doing research on inorganic electret materials for subminiature silicon microphones. In 1987 he started his PhD-project and received the PhD degree from the Faculty of Electrical Engineering, University of Twente, in 1990. The subject of his dissertation was the use of iridium oxide in ISFET-based coulometric sensor-actuator devices. Since 1991 he is working as an assistant professor in the Laboratory of Biosensors, the Lab-on-a-Chip group, part of the MESA^{+} Research Institute, of the University of Twente and as such co-supervising many projects on both physical and (bio)chemical sensors and sensor systems for medical and environmental applications.

Piet Bergveld was born in Oosterwolde, The Netherlands, on January 26, 1940. He received the MS degree in electrical engineering from the University of Eindhoven, the Netherlands, in 1965 and the PhD degree from the University of Twente, the Netherlands, in 1973. The subject of his dissertation was the development of ISFETs and related devices, the actual invention of the ISFET, since then also investigated by many international research groups of Universities as well as industry.

Since 1965 he has been a member of the Biomedical Engineering Division of the Faculty of Electrical Engineering (University of Twente) and was in 1984 appointed as Full Professor in Biosensor Technology. He is one of the project leaders in the MESA Research Institute. His research subjects still concern the further development of ISFETs and biosensors based on silicon technology as well as physical sensors, both for biomedical and environmental applications, resulting up to now in more than 350 papers and 25 PhD theses. He was research dean from the Faculty of Electrical Engineering from 1994 to 1998 and received in 1995 the Jacob Kistemaker Award. He was founder and chairman of the International Steering Committee of the Annual MicroTAS Symposia from 1994 to 2000. In 1997 he was appointed as a member of the Royal Netherlands Academy of Arts and Sciences. He received the Microsystems Leadership Award during MicroTAS 2002 in Nara, Japan, retired from the University of Twente in February 2003 and received the knighthood of the Dutch Lion in April 2003.