Development of a Novel Nanotube Array Biosensor for Real-Time Detection of Volatile Small Molecule Biomarkers Directly Released from Cell Cultures

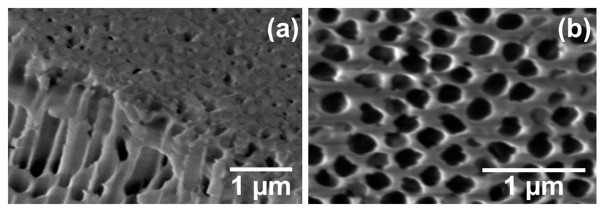
NIST researchers are developing a novel conductometric nanotube array sensor for analyzing whole-cell gas output, thereby enabling direct and near real time measurements of volatile biomarkers. Important classes of these biomarkers are reactive oxygen and nitrogen species, which are important indicators of inter- and intracellular signaling and of oxidative stress. As envisioned, the nanotube array sensor platform should permit, for the first time, the monitoring of entire cell populations in a spatially sensitive manner.

K.D. Benkstein, R. Cavicchi, S. Semancik, M. Tarlov (Div 836), J. Elliott, and H. Rodriguez (Div. 831)

The novel conductometric nanotube array sensor under development at NIST is made by directly culturing cells on the sensor platform. This process greatly enhances the sensitivity and speed of the sensor for reactive species, since the reactive species need only to diffuse short distances before detection. In addition, the sensor platform is designed such that entire cell populations can be monitored while enabling localization of the sensing so that single cells may be monitored as necessary.

The first achievement in this project was the coating of a nanopore template with a functional metal oxide layer sensitive to the targeted biomarkers. A porous alumina membrane was used as the supporting template structure, which features pores with an average diameter of 200 nm and a membrane thickness of 60 µm. The overall diameter of the templates is 21 mm with a porosity of 25% to 50% (≈500 times more internal surface area when compared to the external surface area). After pre-cleaning the alumina membranes, they were dip-coated for 10 s in a tungsten (VI) chloride/ethanol sol-gel and then fired to 525 °C in air to yield a WO_3-covered template. The nanopore platforms were characterized by scanning electron microscopy and by energy dispersive spectroscopy (EDS). Scanning electron micrographs of the WO_3-coated membrane (see figure) show the broken edge of a template near the top (a) where the internal structure of the pores is visible running down from the top as tubes. It appears that the pores remain open after the dip-coating process, and (b) confirms this with an image taken perpendicular to the template surface demonstrating that the WO_3 deposition does not fill the pore entrances. The EDS studies confirm the presence of tungsten at the top of the membranes, as well as inside. As expected, the elemental mapping of the template shows significant amounts of Al, but the mapping also shows that W is found wherever the Al has intensity, indicating that, indeed, the WO_3 conformally coats the membrane template. Based upon these studies, significant internal surface area in the nanotubes will be available for small molecule sensing.

Below are scanning electron micrographs of a WO_3-coated alumina template produced via sol-gel dip coating. (a) The top and interior of a WO_3-coated template, with tubes running through the membrane. (b) The top of the same template, probing perpendicular to the surface, showing that the pore entrances are not blocked by the WO_3 coating.



Preliminary tests of the sensing behavior of the WO_3-coated membranes are encouraging. Sensing experiments were performed by sandwiching the template between an aluminum-coated glass slide and a stainless-steel screen. The resistance measured through the films is quite high (≈600 MΩ) in air at room temperature; however; upon exposure to volatile compounds such as ethanol, the resistance quickly decreases by greater than two orders of magnitude. Work is also in progress to deposit thin film metal contacts on the top and bottom of the template to enhance electrical contact and flow of gas-phase analytes to the nanotube interior. Future studies will examine the response of the platform to targeted relevant analytes (e.g., H_2O_2, NO), and will develop and characterize, by X-ray photoelectron spectroscopy (XPS), the hydrophobic self-assembled monolayer (SAM) that will enable operation in aqueous cell culture environments.

The schematic below shows a simplified (1 tube) nanopore conductometric sensor platform as envisioned for whole-cell gas sensing.