SELECTIVITY ENHANCEMENT STRATEGY FOR CANTILEVER-BASED GAS-PHASE VOC SENSORS THROUGH USE OF PEPTIDE-FUNCTIONALIZED CARBON NANOTUBES

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ABSTRACT

A major challenge in the development of chemical sensors for volatile organic compounds (VOC) has been finding sensitive films, which selectively partition different volatile organics. This work presents a selectivity enhancement strategy using carefully chosen peptides to preferentially interact with different VOCs based on the polarity of the analytes. Carbon nanotubes (CNT) grown at low-temperature are used as a scaffold for the peptides. The CNTs are grown on top of mass-sensitive cantilever-based sensors and provide a large surface area for peptide binding, thus helping to increase the sensitivity of the sensors. Tests show that the peptides do in fact interact differently with VOCs based on the polarity of the compound. Achieved detection limits are in the low parts-per-million range.

INTRODUCTION

A significant research effort has been devoted to the development of MEMS-based resonant sensors for the detection of volatile organic compounds (VOC) in air [1-3]. A common approach has been the use of cantilever-type resonators coated with a chemically absorbent polymer. The polymer coating absorbs VOCs from the surrounding, increasing the mass of the resonator, and causing its frequency to drop [2,3]. This method can give detection limits in the low ppm range, but has the disadvantage that the sensitive polymers damp the resonator oscillation and exhibit only limited selectivity between different VOCs, thus making in-situ analysis of complex gas mixtures difficult [4]. Two possible solutions to this issue that have been investigated are (1) the use of molecularly imprinted polymers to differentiate between analytes [5], or (2) the use of an array of sensors with different, partially selective (polymer) coatings combined with signal analysis to analyze a mixture [4].

An alternative approach that addresses the selectivity issues while maintaining a highly integrated platform is pursued in this work: carbon nanostructures are grown on the cantilever surfaces and functionalized with selective biomolecules, in this case peptides. To demonstrate the feasibility of this approach, peptides with an affinity towards polar analytes were tested. Similar peptides have been shown to exhibit high selectivity to TNT in the gas phase [6]. In many applications where peptides are used, particularly in biosensing, the compounds are attached to the device surface in a single monolayer. For detection of VOCs this approach would be problematic given the small mass of the individual analyte molecules. In order to address this problem, a carbon nanotube scaffold is used in this work, which allows for protein attachment in three dimensions.

Carbon nanotubes (CNT) are ideal for sensing applications because they provide a large surface area for analyte binding compared to e.g. planar surfaces with ligands attached via self-assembled monolayers. As mentioned, while a single-layer functionalization might be sufficient for biosensing applications, because of relatively large analyte masses, 3-D CNT networks functionalized with sensitive peptides are more suitable for lighter analytes, such as VOCs. However, CNTs are generally grown at temperatures ranging from 600-800˚C, which makes their back-end integration with silicon-based sensing devices challenging, especially in case of aluminum metallizations. In the current work, a low-temperature CNT growth process has been adapted, which allows their integration with sensors fabricated using a CMOS-compatible bulk-micromachining process.

SENSOR FABRICATION

The transducers employed in this work are thermally excited, piezoresistively detected single-crystal silicon hammerhead structures (Figure 1), which are operated in their first in-plane flexural resonant mode [7]. The cantilever-based devices themselves are fabricated using a CMOS-compatible bulk-micromachining process [8] and have a silicon thickness of 8 μm in this work. As mentioned above, since the resonators use aluminum metallization, the commonly used high-temperature processes for CNT growth are not suitable if the CNT growth is to be performed at the end of the process. Instead, a low-temperature growth process was developed, allowing for a localized CNT layer to be deposited on the "wing" of each hammerhead structure.

To prepare for the CNT growth, two modifications were needed to the bulk micromachining process presented in [8]. First, the SiO_2/SiN_x passivation sandwich deposited by plasma enhanced chemical vapor deposition (PECVD) needed adjustment. Since this film sandwich is deposited at a lower temperature than the CNT growth, the subsequent higher temperature processing could potentially alter the stress distribution in the PECVD film, leading to bent cantilevers after the CNT processing. To prevent this from hap-

pening, the PECVD oxide and nitride layers were annealed at 450˚C after deposition. The stress in both films was measured after annealing using the wafer curvature method. Because oxide and nitride films have opposite stress signs, the layer thicknesses could be chosen to give approximately zero total stress in the passivation film. The second process modification was the deposition and patterning of a nickel film as catalyst for the CNT growth on top of the hammerhead structures (see below).

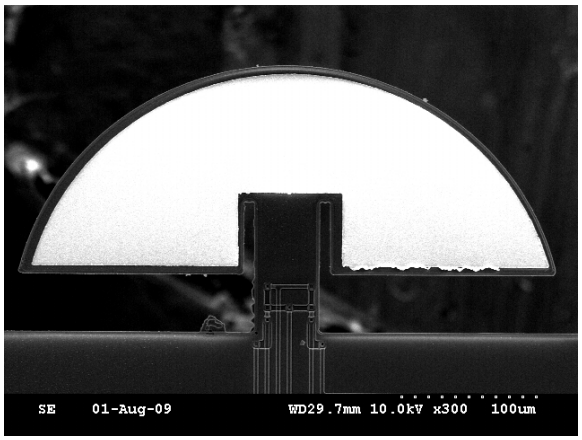

Figure 1: SEM image of a cantilever-based hammerhead resonator. The integrated resistive structures for thermal excitation and piezoresistive detection of in-plane vibrations are located close to the clamped edge. The devices tested have a resonant frequency of roughly 750 kHz.

The low-temperature CNT growth was performed in an AIXTRON Black Magic PECVD system using a nickel catalyst at 450˚C (Figure 2). The catalyst was deposited using e-beam evaporation and patterned using lift-off prior to the KOH etching step used to release the microstructures. Prior to CNT growth the wafer was diced, and individual dies were placed in the Black Magic PECVD, instead of the entire wafer. For CNT growth, individual dies were first annealed in an H_2 plasma at 450˚C; then the plasma was turned off and the CNTs were grown in a flow of pure acetylene at 450˚C for 75 minutes (growth process similar to [9]). The resulting CNT layer was roughly 0.5-1µm thick (Figure 3).

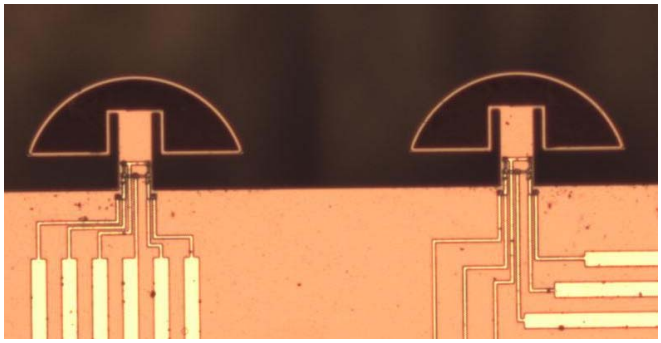

Figure 2: Photograph of hammerhead structures with CNTs deposited on the wing area (seen in black on the wings).

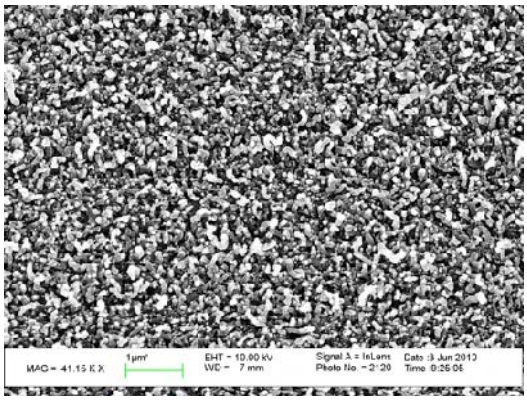

Figure 3: SEM image of carbon nanotubes grown using a low-temperature process at 450˚C.

After CNT growth, a two-section peptide was deposited onto the cantilevers using a Bioforce Nano eNabler. One end of the peptide (23 amino acids in length) is designed to attach to CNTs [6], while the other end contains an amino acid sequence that binds to the analyte of interest. In the present work, each peptide has eight repeats of specific amino acids, namely arginine, histidine, and threonine, to provide different functional groups to modulate their affinity to different VOCs. Figure 4 shows the amino acids arginine, histidine and threonine, whose side chains are either positively charged (arginine and histidine) or uncharged but polar (threonine). It is expected that e.g. the polar side group of threonine will preferentially interact with polar compounds, such as ethanol and methanol.

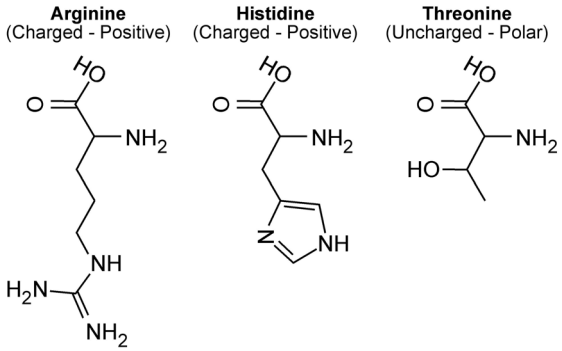

Figure 4: Structure of amino acids arginine, histidine and threonine (reproduced from [10]).

For CNT functionalization, the peptides were dissolved in distilled water at a concentration of 0.24 µg/µl. A nanoliter quantity of peptide solution was placed on a cantilever with embedded fluidic channel in the Bioforce dispenser. For peptide functionalization of the CNT film, the cantilever in the Bioforce dispenser deflects against the die surface depositing a drop of fluid. Because of the small quantity of peptide used, measures must be taken to prevent evaporation during the deposition process. These steps included using an atmosphere of 75% relative humidity (RH) and also adding 10% v/v glycerol to the peptide solution. To facilitate the chemical reaction between the section of the peptide that

interacts with CNT and the nanotubes themselves, the resonant cantilevers were annealed in 75% RH for at least 15 minutes after coating. Before use, each resonator chip was rinsed in pure DI water and dried. For testing, each die was wire-bonded into a 28-pin DIL package. The devices were used the same day that the peptide was applied in order to avoid possible issues with peptide degradation.

SENSOR TESTING AND RESULTS

For gas testing, the packaged resonator chips were attached to the measurement chamber of a customized gas manifold system. The system comprises three gas lines, an analyte line, a mixing line, and a reference line, with computer-controlled mass-flow controllers regulating the flow of nitrogen carrier gas through each line. The analyte line contains a bubbler with an analyte-soaked quartz sand matrix, yielding an analyte-loaded gas stream with an analyte concentration corresponding to the saturation vapor pressure of the analyte at the bubbler temperature. By mixing the analyte line with carrier gas from the mixing line, the analyte concentration can be further adjusted. In a typical measurement, the resonator under test is subjected to a sequence of 5-10-minute analyte exposures at different analyte concentrations, with 5-10-minute exposures to pure carrier gas from the reference line in-between. During testing, the resonator structure is embedded in an amplifying feedback loop and the resonance frequency is recorded by a frequency counter (Agilent 53131A) using a 1-second gate time. During the measurement, the nitrogen flow is kept constant at 80 ml/min and a four-way valve ensures fast switching between analyte-laden and reference gas flow.

Initially, a hammerhead structure with CNT coating but without peptide functionalization was tested. Figure 5 shows the response of this device, i.e. the measured frequency change as a function of time, upon exposure to different ethanol and toluene concentrations. In both cases, the frequency change increases roughly linearly with the analyte concentration, with a stronger response to toluene likely because of its lower vapor pressure.

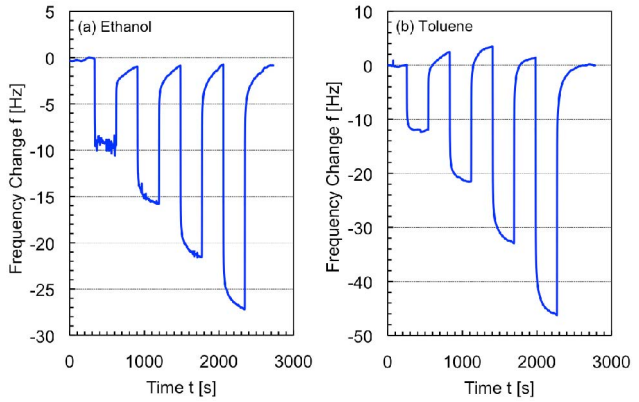

Figure 5: Frequency change of hammerhead structure with CNT coating (no peptide) to 4 subsequent injections of (a) ethanol (8125, 16250, 24375, 32500 ppm) and (b) toluene (4000, 8000, 12000, 16000 ppm) in dry nitrogen.

To investigate the affinity towards polar analytes, a hammerhead device was functionalized with a threonine-terminated peptide and exposed to different ethanol concentrations in dry nitrogen (see Figure 6). The response at the lowest tested ethanol concentration roughly triples compared to the measurement with a non-functionalized microresonator (Figure 5a), thus indicating the affinity of the peptide to polar analytes. However, the response is no longer linear and seems to slowly saturate at higher analyte concentrations. From the measured Allan variance (a measure of the short-term frequency stability of the resonator within the gas flow) of 1.2×10^{-8} (9 mHz @ 750 kHz), a detection limit of ≈ 11 ppm for ethanol is extracted.

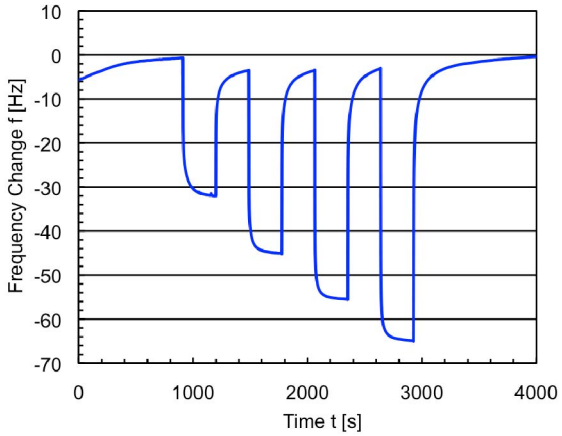

Figure 6: Frequency change of hammerhead structure coated with threonine-terminated peptide to 4 subsequent injections of ethanol (8125, 16250, 24375, 32500 ppm).

As an additional control, we functionalized an additional hammerhead structure with a control peptide, which contains the sequence binding to the CNTs but no repetition of a specific amino acid at the other end, and exposed it again to different ethanol concentrations. The device coated with the reference peptide showed an approximately three-times smaller sensitivity to ethanol, demonstrating again the affinity of the resonator coated with the peptide with polar amino acids to polar analytes.

Overall, the measured frequency changes of the CNT/peptide-coated devices upon exposure to ethanol and toluene in dry nitrogen were found to be substantially smaller than frequency changes obtained for polymer-coated microstructures. As an example, the same hammerhead geometry (however with a silicon thickness of 19 µm instead of 8 µm) coated with a 0.5-0.6 µm thick layer of poly(isobutylene) (PIB) exhibits a frequency change of approx. 1200 Hz when exposed to 16000 ppm of toluene, i.e. a roughly 20-times higher sensitivity. However, the polymer film introduces damping, which degrades the Allan variance and negatively affects the sensor's limit of detection.

Based on the comparably small sensor sensitivities obtained with dry nitrogen as carrier gas, we started investigating the effect of humidity on the response of the CNT/peptide-coated microstructures. It was expected that

humidity would aid in the interaction of each peptide sequence with the VOCs, since the charge and conformation of amino acids are known to be affected by the properties of the surrounding solution. Initial testing focused on peptide chains where the active part of the molecule contained either arginine or histidine, which are both amino acids with positively charged side chains.

To perform sensor testing with humidity, the mixing line of the gas testing setup was outfitted with a bubbler filled with water. As a result, the analyte-laden gas stream is mixed with humid carrier gas before entering the measurement chamber. Due to limitations of the current measurement setup, the relative humidity depends on the analyte concentration and decreases with increasing analyte concentration. After each analyte exposure, the measurement chamber is purged with dry nitrogen. Figure 7 shows the frequency change of two hammerhead structures, one functionalized with the peptide with arginine repetitions, the other one with the control peptide to different toluene concentrations. Clearly, the measured frequency changes are strongly increased compared to the measurements in dry environment, indicating the importance of humidity when working with peptide functionalizations rather than traditional polymer coating. The maximum measured frequency changes of 500 Hz are comparable with those obtained for polymer-coated devices and can likely be further improved by optimization of the CNT-peptide coating.

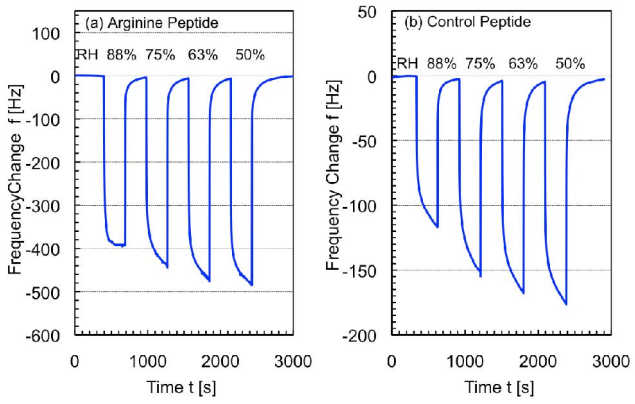

Figure 7: Response of hammerhead resonators functionalized with (a) arginine peptide and (b) control peptide to different toluene concentrations (4000, 8000, 12000, 16000 ppm) in humid carrier gas (the relative humidity is given in the graphs).

As seen in Figure 7, the frequency change does not scale linearly with analyte concentration. This is likely due to the fact that the relative humidity decreases with increasing analyte concentration as mentioned above. Thus, less water will be adsorbed by the microstructures at higher analyte concentrations. More importantly, the sorption of analyte at low analyte concentrations might be improved by the higher surrounding humidity. Further measurements with an improved setup to maintain constant humidity levels are needed to clarify these dependencies.

CONCLUSIONS

The presented work highlights a way to integrate three-dimensional CNT scaffolds with silicon-based microsensors, namely silicon-based cantilever structures, through low-temperature CNT growth. Using proper peptide functionalization, the affinity of the microsensors to volatile organic compounds can be modified based on their polarity. It is expected that the selectivity of the biomolecules to particular VOCs can be improved by proper peptide screening, as has been demonstrated for TNT [6]. Humidity promotes the interaction between biomolecules and target analytes. Further work is needed to look at the effects of the CNT growth time and functionalization solution concentration on the sensitivity of the sensors.

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