Chapter 6

Electrochemical sensing with single nanoskived gold nanowires bisecting a microchannel

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Abstract

Nanoscale electrodes are of great interest as sensing elements in microfluidic devices, as they offer high sensitivity and efficient mass transport. Preparation of millimeter-long gold nanowires that can be easily manipulated has become possible using nanoskiving. In this research, single gold nanowires were suspended over a glass/PDMS microchannel. Through this channel, electroactive solution was pumped at different rates, and the nanowire was used as electrode to perform voltammetry in the center of the flow profile. Suspended nanowires exhibit improved current responses compared to nanowires placed at the bottom of microchannels due to efficient analyte transport towards the electrode surface. Furthermore, the dimensions of the electrode allow measurement in channels without disturbing the flow profile. Besides future applications of the suspended nanowires as sensors in microfluidic devices, a potential application in the study of fast electron-transfer kinetics is discussed.

PEO designed the study, developed and fabricated the system, performed all experiments and wrote the manuscript.
6.1 Introduction

The use of electrochemical detection techniques in microfluidics is of great interest to researchers in both analytical chemistry and biology. Due to the ease with which electrodes can be miniaturized, integration into microfabricated devices is often facile. Moreover, the sensitivity of electrochemical detection methods is not compromised when they are miniaturized to be used in such systems. They allow direct signal transduction, and electrodes with (sub)microscale dimensions often even outperform their macroscale counterparts due to improved mass transport towards the sensor. As radial diffusion of analytes to the micro- or nanoelectrode surface dominates, very fast mass transport occurs, allowing the study of fast electrochemical reaction kinetics with steady-state voltammograms as well as high current densities and signal-to-noise ratios. Furthermore, using micro- or nanosized electrodes allows measurements in very small volumes and integration in microfluidic devices. Interest in the use of nanowires as nanoelectrodes has recently increased, mostly due to improved fabrication techniques. Nanowires have been used as sensors for detecting DNA, glucose and foodborne pathogens. However, the flexible and precise placement of nanowires is generally challenging, as prevalent nanowire-fabrication techniques result in wires bound to a substrate, e.g., in lithography-based fabrication. Nanoskiving is a technique that allows the fabrication of (arrays of) millimeters-long wires with control over all three dimensions, although it can also be employed for the fabrication of many different planar, nanoscale structures. The technique is a form of edge lithography. Ultra-thin sections are taken from a cross-linked polymer block, which contains a layer of the material that the structures will be made of. Every dimension of the nanostructure can be precisely controlled. After the sectioning, each slab contains a nanostructure (e.g., a nanowire) surrounded by the polymer matrix, allowing easy manipulation and placement of one or more sections via a droplet of water. After placement of the slab and evaporation of the water, the polymer matrix can be etched off using oxygen plasma treatment, leaving only the nanowire. The reproducibility and ease-of-use of nanoskiving make it an ideal technique for the integration of single nanowires as sensing elements in microfluidic devices and channels. The technique is compatible with creating nanostructures in a broad range of materials, including polymers.

Dawson et al. showed that nanoskived gold nanowires can be employed as electrodes. Single wires were interfaced on a flat substrate using contact electrodes. The wires were subsequently used for cyclic voltammetry. We previously showed the bisecting of a glass-poly(dimethylsiloxane) (PDMS) microchannel with a single gold nanowire, thus positioning it in the center of the flow profile. The suspended nanowires were employed in the microfluidic channels for flow measurements by hot-wire anemometry. Furthermore, nanowires were used as substrate for DNA functionalization and used for the study of DNA-protein interactions under flow.

In this work, we used single nanoskived gold nanowires suspended over a glass-PDMS microchannel as electrochemical sensors, using a two-electrode setup. Positioning the wire in the center of a microchannel allows for a direct measurement. Due to the dimensions used in microfluidic channels, viscous forces dominate, resulting in laminar flow profiles. Hence, the flow velocity is zero at all channel walls, while at the center of a channel it is maximal, enabling fastest advective mass transport to the electrode surface. Cyclic voltammograms (CVs) of ferrocene in acetonitrile were obtained, with the electroactive solution flowing at various rates. The results were compared with CVs obtained from gold nanowires placed on the bottom of a microchannel at the same flow rates. This is the first report of the use of suspended nanowires at the center of the flow profile.
in a microchannel as electrochemical sensors.

6.2 Materials and methods

6.2.1 Nanoskiving
Gold nanowires were prepared by nanoskiving as described previously. In brief, 200-nm-thick Au films were deposited onto silicon wafers through a Teflon mask by thermal evaporation. The thickness of the Au film controlled the height of the nanowire, whereas the lateral dimension of the film controlled the length of the nanowires. Epofix epoxy resin (Electron Microscope Sciences, USA) was used to cover the gold film and silicon wafer. The epoxy containing the gold films was lifted from the wafer after curing using a razor blade. The epoxy around the films was cut out into blocks to fit into molds used for embedding samples for standard ultramicrotomy. The mold with epoxy blocks was filled with more epoxy and cured at 60 °C to completely embed the gold film. Using an ultramicrotome (Leica UC-6, The Netherlands), 200-nm-thick slabs were sectioned from the block and floated onto a water bath. Each slab contained a single nanowire surrounded by epoxy. The thickness of the slabs controls the width of the nanowires. Using a small loop tool, sections were transported via a droplet of water. The wires produced in this fashion were 200 nm x 200 nm x 1.5 mm.

Figure 1: Scanning electron micrograph of a nanoskived gold nanowire bisecting the etched glass microchannel.

6.2.2 Microfluidic device fabrication
The glass-PDMS devices used for the experiments with suspended gold nanowires bisecting a microfluidic channel were designed and fabricated in a similar way as described previously. In brief, 1-cm-long, 70-µm-wide and 30-µm-deep microchannels were wet-etched in prefabricated borosilicate (Schott Borofloat) wafers coated with chromium and photoresist (Telic, USA) using HF -etching solution (1:1 mixture of 49% HF and 19.6% HNO₃, HF and HNO₃ from Merck, Germany). Using a sandblaster, rectangular substrates (1 x 2 cm) were cut out of the wafer, with each substrate featuring one microchannel. The substrates were glued to larger square microscopy slides for facile handling. Nanoskived epoxy slabs containing gold nanowires were placed over the channels via a water droplet, making use of etched alignment marks in the glass for facile positioning. After the water was evaporated, small drops of conductive silver paste (Agar
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Scientific, UK) were added to check the wire integrity (conductivity) using a multimeter. The glass substrates were subsequently exposed to oxygen plasma for 1 h at 100 mTorr at 30 W in a plasma cleaner (Harrick Plasma, USA) to etch away the epoxy surrounding the gold nanowire. Complementary channels in PDMS (Sylgard, Dow Corning, USA) were made by casting uncured PDMS to glass wafers, patterned with SU-8 50 (Microchem, Germany) using soft lithography. Stainless steel pins (Ø 300 µm) were inserted through a 3D-printed template to provide controlled fluid inlets and outlets through the PDMS. After curing, rectangular parts (1 cm x 2 cm), each containing one 1-cm-long, 80-µm-wide, 40-µm-deep microchannel, were cut out and lifted from the molds, and the stainless steel pins were removed. Using a 3-mm-diameter biopsy puncher (Kai Medical, Germany) holes were made next to the channel to be fit over the conductive paste applied to the nanowires on the glass substrates. After exposure to oxygen plasma for 1 minute, the glass substrate and PDMS part were irreversibly bonded using the custom-built aligner described earlier. This sealed the microchannels, leaving the nanowire suspended in the microchannel. Additional conductive paste was added through the punched holes in the PDMS to facilitate connection of the wire to analytical instruments (i.e. a potentiostat, see Figure 2).

A similar device was fabricated to monitor the electrochemical response of nanowires placed on the bottom of microchannels, but in this case skived sections were placed on flat glass substrates. After the epoxy was etched away using oxygen plasma the wire was covered with an identical PDMS channel as used in the devices with suspended wires.

Figure 2: Top and cross-sectional schematic of the glass-PDMS device. The nanowire (NW) is depicted as a red stripe. Electrical contact is established with the NW through ports punched into the PDMS. The device for measurements with a nanowire positioned on the bottom of a microchannel did not feature a channel in the glass substrate, but was otherwise identical.
6.2.3 Electrochemical measurements

A solution of 1 mM ferrocene (VWR, The Netherlands) as electroactive species was prepared in acetonitrile (Biosolve B.V., The Netherlands) with 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF$_6$, Sigma-Aldrich, The Netherlands) as supporting electrolyte. The solution was flown into the channel from a 500-µL glass syringe through fluorinated ethylene propylene tubing (794 µm OD, 178 µm ID). The flow rate was controlled using a syringe pump (Harvard Apparatus, USA) initially set to 1 µL/min. Care was taken to remove any air bubbles from the solution before flowing it into the channel, as the high surface tension can result in breaking of the wire when introducing liquid. After submersion, the wire could withstand high flow rates (flow rates up to 20 µL/min were tested without failure of the wire). The solution flowed out of the channel into a waste container through a silica capillary (10 cm length, 250 µm ID; Supelco, USA) connected to the outlet (see Fig. 2 and 3). In this waste container, a Ag/Ag$^+$ reference electrode (BASi, USA) was submerged. The reference electrode was prepared by immersing a silver wire in a Luggin capillary (with Vicor tip) containing acetonitrile with 0.1 M TBAPF$_6$ and 0.01 M AgNO$_3$. CVs were recorded at a scan rate of 20 mV/s and different flow rates using a LabVIEW-controlled FEMTO DDPCA-300 transimpedance amplifier as a potentiostat. The nanowire was interfaced through the connection pads in the punched holes of the PDMS part of the microfluidic device. The experiments under flow were conducted both on suspended nanowires and nanowires placed on flat glass substrates. Figure 3 shows the experimental setup.

6.2.4 Simulations

We employed two-dimensional finite element modeling (COMSOL Multiphysics) to simulate CVs recorded at a bisecting nanowire electrode as a function of flow rate. The geometry of a 80-µm-long and 70-µm-high microchannel with a nanowire (square cross-section of 200 nm by 200 nm) was modeled. A flow velocity profile was determined by solving the Navier-Stokes equations for incompressible Stokes flow (Equation 1):
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The flow velocity profile $u$ was evaluated for various inlet flow rates, a pressure of $p=0$ Pa at the microchannel outlet and no-slip boundaries at the microchannel walls and nanowire surfaces ($\eta$ dynamic viscosity of 0.334 mPa.s)\(^{24}\). Convective transport of a fully reduced analyte at a 1 mM concentration (initially in the bulk and constantly at the inlet and outlet boundaries) was modelled in the determined Poiseuille flow profile, as well as by diffusion by using the drift-diffusion equation (assuming a high electrolyte concentration and no migration; Equation 2):

$$\nabla p = \eta \nabla^2 u, \quad \nabla \cdot u = 0 \quad (1)$$

$$u \cdot \nabla c_{R,O} = D \nabla^2 c_{R,O} \quad (2)$$

Here, $D$ is a diffusion coefficient of $2 \times 10^{-9}$ m\(^2\)/s\(^2\)\(^{25}\) and $c_{R,O}$ are the concentrations of reduced and oxidized species of initially 1 mM and 0 mM, respectively. CVs were determined by solving the Butler Volmer equation (Equations 3) for electrochemical electron-transfer kinetics\(^{23,26}\) at the nanowire surface:

$$i = nF \left( c_O k_f - c_R k_b \right),$$

$$k_f = k_0 \exp\left\{ \frac{-\alpha F (E-E_{\text{std}})}{RT} \right\},$$

$$k_b = k_0 \exp\left\{ \frac{(n-\alpha)F (E-E_{\text{std}})}{RT} \right\}. \quad (3)$$

Here, $i$ is the current, $n=1$ is the number of electrons exchanged per reaction, $F$ the Faraday constant (96485 C/mol), $R$ the gas constant and $T = 293$ K the temperature. $k_f$ and $k_b$ are the forward and backward rate constants. To model ferrocene transfer kinetics, we assumed a standard potential of $E_{\text{std}} = 0.089$ V\(^{27}\), a transfer coefficient of 0.5, a temperature of 293 K, and a low standard electrochemical rate constant ($k_0$) of 0.01 m/s.\(^{28,29}\) In the simulation, the standard potential for platinum was used instead of gold, as the parameters necessary for simulation of our system were only available for platinum electrodes. However, we do not expect large discrepancies between the two materials.\(^{29,30}\)

6.3 Results and discussion

6.3.1 Electrochemical measurements

CVs of 1 mM ferrocene were recorded with nanowires suspended in a microchannel and placed on a flat surface under different flow rates, ranging from stagnant flow to 20 µL/min. Figure 4 shows representative results.

The current generated by the oxidation of ferrocene on the nanowires increases with the flow rate in both cases. However, the current generated by suspended wires is higher as compared to wires placed at the bottom of the channel. The difference in current generated by the suspended nanowires and those placed on a flat substrate is, in part, a consequence of the fact that nanowires placed on a substrate only have 3 sides exposed to the electroactive solution instead of 4, and of the position of the sensing element in the flow profile. The electrode areas are approximately $5.6 \cdot 10^{-9}$ m\(^2\) for wires suspended in the center of the flow profile, and $4.2 \cdot 10^{-9}$ m\(^2\) for wires placed on the bottom of a channel. However, the majority of the difference exist because in the center of the flow profile, most direct sensing can be achieved due to the efficient advective transport of analyte towards the electrode, whereas at the walls of the channel the flow, and
therefore the analyte transport, is much slower. As the flow rate increases, all CVs with suspended nanowires, but not with nanowires placed on flat substrates, demonstrate a marked shift of the onset of the limiting current towards higher potentials. Furthermore, a progressive distortion of the sigmoid shape of the voltammograms can be seen as the flow rate increases. This can only partly be explained by a decoupling of the reference electrode (an iR drop of about 30 mV is expected for the highest currents generated). It indicates a dependency of the reaction on electron-transfer kinetics, as opposed to the mass-transfer of analytes towards the electrode. This is remarkable, as ferrocene exhibits fast electron-transfer kinetics.\textsuperscript{28,29} The relation of the limiting current (measured at 0.6 V) vs. the flow rate is shown in Figure 5. It can be seen that the limiting current increases sublinearly and monotonically with flow rate, as observed previously for measurements using conventional microelectrodes.\textsuperscript{22} Furthermore, following from the observed limiting currents, the detected current generated by suspended nanowires is on average 2.8 ± 0.4 times higher as compared to nanowires placed on the bottom of the channel.

Figure 4: CVs of 1 mM ferrocene in acetonitrile using single gold nanowires, suspended (A) or on a flat substrate (B), for different flow rates (scan rate of 20 mV/s for all CVs). The electrode areas are approximately 5.6·10^{-10} m^2 for wires suspended in the center of the flow profile, and 4.2·10^{-10} m^2 for wires placed on the bottom of the channel.

Figure 5: The average (n=3 for suspended, n=2 for on substrate) relation of the limiting current generated at 0.6 V by 1 mM of ferrocene on nanowires suspended in or placed at the bottom of a microchannel. Error bars represent the standard deviation.
Figure 6 displays an amperogram recorded at 0.6 V of a suspended nanowire with 1 mM ferrocene flowing past at different flow rates. Stable currents can be achieved. The increase of the generated current with flow rate that was observed earlier (see Figure 5) is reflected here as well. It can also be seen that the response is reproducible; when the flow rate is stepped back the current recovers to the previous values for the same flow rates. The current generated during chronoamperometry is slightly lower than the current generated during cyclic voltammetry at the same flow rates when compared to Figure 5. However, the nanowire used for this experiment showed lower current responses during cyclic voltammetry experiments as well. This is possibly a consequence of the positioning of the nanowire over the channel, insufficient removal of the epoxy slab during dry etching or an artifact introduced to the channel geometry during the etching of the glass channel. We aim to further explore chronoamperometric measurements with suspended nanowires in the future.

These results clearly show the improved response obtained by placing nanowire electrodes in the center of the flow profile in a microchannel, as opposed to the nanowires placed on the bottom. The reproducibility also indicates the suitability of this kind of nanoelectrodes as sensing elements in microfluidic devices.

6.3.2 Simulations

In Figure 7, simulated flow velocities in the channel around a suspended nanowire are shown for a flow rate of 20 µL/min. A gradient in flow velocities can be observed, ranging from about 0.1 m/s in the center of the channel (where suspended wires are situated), and approaching 0 m/s at the channel walls. This is in agreement with the lower current generated by nanowires placed on substrates, as experimentally assessed. The transport of analyte towards suspended nanowires is more direct due to the positioning in the flow profile. The disturbance of the flow profile caused by the nanowire itself is small.

In Figure 8, the concentration of oxidized product is shown in the channel at a flow rate of 20 µL/min, and at a potential of 0.6 V. This concentration profile...
shows that analyte transport is dominated by advection. This is further underlined by the approximate surface Péclet number for this system (Equation 4):

$$\text{Pe} = \frac{U h}{D}$$  \hspace{1cm} (4)

Here, $U$ is the average flow velocity in the channel (approximately 0.075 m/s), $h$ the height of the channel (70 µm), $D$ the diffusion coefficient of ferrocene in acetonitrile ($2 \times 10^{-9}$ m$^2$/s$^2$). This equation gives an approximate surface Péclet number of 2625. Oxidized product molecules move along with the flow due to Stokes forces and a very steep concentration gradient is established on the side of the wire facing the flow inlet; diffusion of product from the wire through the rest of the channel is limited.

Figure 7: Cross-section of a segment of the microchannel with a suspended nanowire, showing the flow velocity profile around the nanowire.

Figure 8: Cross-section of a segment of the microchannel with a suspended nanowire, showing the concentration of oxidized product around the nanowire (right: zoom).
In Figure 9, simulated cyclic voltammograms are shown for conditions comparable to those used experimentally (as shown in Fig. 4a; with $k_0 = 1 \text{ cm/s}$; $\alpha = 0.5$; $E' = 0.089 \text{ V}$), except for the nanowire material (Pt instead of Au). The CVs exhibit comparable limiting curvrents and current increase as a function of faster flow rates. Moreover, there is qualitative agreement in the shift of the onset of the limiting Faradaic current to higher potentials for fast flow. While not as pronounced as in the experiment, the finite element analysis also shows that the voltammograms exhibit a considerably distorted shape at high flow rates. We attribute this to the effect that at fastest flow velocities the electrochemical reaction becomes increasingly limited by the electron-transfer kinetics, instead of mass-transport to the electrode surface – even for a fast analyte such as ferrocene. An identical, but much smaller effect, is observed for fast-rotating disk electrodes used for Koutecky-Levich analysis. These results show that bisecting nanowire electrodes might have a potential electroanalytical application in the determination of fast electron-transfer kinetics.

![Figure 9: Simulated cyclic voltammograms for a suspended wire as a function of the liquid flow rate. The vertical gray line indicates a standard potential of 0.089 V.](image)

### 6.4 Conclusions

We demonstrated the use of nanoskived, suspended Au nanowires in microchannels for electrochemical sensing of ferrocene at different flow rates. Due to the positioning of a nanowire in the center of the channel, direct sensing was achieved without disturbing the flow pattern. Compared to nanowires placed on the bottom of channels in a similar microfluidic system, suspended nanowires generated a higher current during cyclic voltammetry. The use of suspended nanowires for chronoamperometric sensing will be further explored in future work.

The shape of the voltammograms obtained with suspended nanowires distorted reproducibly with faster flow rates, but not with nanowires on flat substrates. The onset of a limiting current shifted to more positive potentials and the shape of the voltammogram became more asymmetrical as the flow rate increased. This phenomenon, which we attribute to an increased dependency on fast electron-transfer at higher flow rates, can be exploited to study electron-transfer kinetics of electroactive compounds, which is traditionally done using rotating disk electrodes. Recently, Kim and Bard reported the assessment...
of kinetic parameters using ultramicroelectrodes of different sizes. Using our approach, a microfluidic device with only one suspended nanowire could be employed for this, instead of multiple electrodes. Besides this improvement of the experimental method, analysis of the obtained data will be simpler and thus faster. Our future work will focus on the study of this potential application, as well as decrease of the iR drop at higher flow currents.

6.5 References

29. A. M. Bond, T. L. E. Henderson, D. R. Mann, T. F. Mann, W. Thormann and C. G.