ABSTRACT
Owing to the broad applicability of electrochemical sensors in the biomedical field, there has been considerable interest in incorporating electrochemical sensors into lab-on-a-chip platforms. Such sensors can be miniaturized easily and are relatively stable and robust. In this work, an effective method of incorporating fluid flow with nanoscale electrochemical sensors is presented.

KEYWORDS: Electrochemical nanogap sensors, integrated nanofluidics, silicon-glass adhesive bonding, SU-8 Bonding

INTRODUCTION
The suitability of electrochemical sensors for many biomedical applications has stimulated the development of miniaturized assays suitable for lab-on-a-chip devices. Previously, we have reported electrochemical nanogap sensors consisting of two parallel working electrodes separated by few tens of nanometers as shown in Figure 1a [1]. Redox-active molecules undergo repeated oxidation and reduction as they travel back and forth within the nanochannel between the electrodes, thereby providing a highly amplified electrochemical current at the two electrodes (as shown in Figure 1b). These devices count among the most sensitive electrochemical sensors built to date [2].

While a standard procedure has been developed for the fabrication of the nanogap devices [1], most measurements to date have relied on a polydimethylsiloxane (PDMS) reservoir filled with sample fluid placed in contact with the nanodevice, as shown in Figure 2. This approach relies solely on diffusion to exchange molecules between the reservoir and the nanogap device, limiting the response time of the sensor [1] and introducing additional noise due to the stochastic nature of random walks [3]. These limitations can be overcome by integrating the nanofluidic devices with a microfluidic network allowing advective mass transport, as was demonstrated using PDMS microfluidics directly bonded onto the nanogap chip [4]. While useful for early prototyping, this approach suffers from two limitations: the need to accurately align the PDMS to the device complicates assembly, and PDMS is prone to contamination over the course of measurements involving multiple analyte solutions due to its porous nature. Here we circumvent these issues by introducing an alternative fabrication method in which microfluidics is directly integrated at wafer scale via glass wafer bonding.
FABRICATION

Previously fabricated nanogap devices (Figure 3a) have been reported in detail [1]. In short, a sacrificial layer of chromium about 65 nm thick is deposited by evaporation between two platinum electrodes. The overlapping area between the two electrodes defines the active region wherein the redox cycling occurs. The entire structure is passivated by an oxide layer, and inlet/outlet holes are etched through the passivation layer by the deep reactive-ion etching (DRIE) process. In order to bypass PDMS based fluidics, we present a fabrication technique in incorporates fluidics. A silicon wafer bearing the nanodevices and a glass substrate were bonded. When structuring such microfluidic channels, sealing can be problematic and therefore there was a need for an appropriate adhesive layer. This was achieved by patterning microstructures directly on the Si substrate and then sealing using a glass substrate to form the roof of the channels. Due to the inert nature of SU-8 photoresist and its ease of fabrication, it was chosen as the adhesive.

A 100 mm silicon wafer, which was used as the substrate, was covered with a layer of SiO₂ of ca. 500 nm on its backside. The wafer was carefully aligned to create inlet/outlet holes for the microchannel. At first, the oxide was patterned by photolithography with careful alignment to the microchannel to be
structured on the front side. It was also etched using a DRIE process. The silicon oxide layer effectively acts as the mask for Si etch. Thereafter, the silicon was etched using another DRIE process (Figure 3b-c) to as well. These holes were etched until a few tens of microns of silicon were left over. This was done to prevent the wafer from having holes in the substrate front side. The wafer was then flipped over and SU-8 was spin-coated on the top of the nanogap structures. The resulting thickness of the SU-8, ~7 µm, defined the height of the channel. It was soft baked by ramping up the temperature to 95°C degrees for 10 min followed by cooling to room temperature. After exposure of the resist to UV light, the resist was post baked and subsequently developed (Figure 3d). The inlet and outlet holes of the reservoir of the SU-8 structures were placed directly around the etch holes aligned at the back side of the wafer. The structures were patterned such that the micro channels in SU-8 run parallel to the nanochannels. This patterned wafer was then bonded to a glass wafer at an elevated temperature and pressure (180 °C, 1.5 tons) for 1 hr. Finally the leftover silicon was etched away using the DRIE process once again (Figure 3f).

In order to make this design compatible with the existing experimental techniques in which the nanopores are directly connected to the contact pads, the electrical connection pads were needed to be exposed. This made it necessary to dice the glass and silicon/SU-8/glass stack separately. SU-8 was not patterned on the wafer that contained the connecting pads. Therefore, the glass covering these parts carefully diced out at first by dicing into the glass chips only by 350 µm (glass thickness of 500 µm). With this the glass could be easily removed without disturbing the structures made on Si wafer underneath. Finally, the entire wafer was diced into individual chips (Figure 3g). The resulting structures were tested for leakage.

**CONCLUSION**

We present nanogap devices with integrated microfluidic channels which were produced at wafer scale and used for pressure driven flows up to 2 bar. This approach offers a fast method for exchanging analyte solutions and minimizes the risk of contamination. In addition, fluidic access from the back side of the wafer combined with electronic access from the top side greatly simplifies interfacing of the devices with external instrumentation.

**ACKNOWLEDGEMENTS**

We gratefully acknowledge financial support from The Netherlands Organization for Scientific Research (NWO) and the European Research Council (ERC). This publication was further made possible by Grant Number 1R01HG006882-01 from National Institutes of Health (NIH); its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIH.

**REFERENCES:**


**CONTACT**

* S. G. Lemay; phone: +31-53-489-2306; s.g.lemay@utwente.nl