LABS ON A CHIP FOR HEALTH CARE APPLICATIONS

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ABSTRACT

Three examples of Lab on Chip devices for medical applications are presented. First, a disposable, prefilled capillary electrophoresis chip for monitoring lithium in blood of manic depressive patients is discussed. It is demonstrated that the same chip-platform can also be used to measure sodium in urine which is relevant for kidney patients. In a second example, a simple chip for counting sperm cells in semen is presented that is used to determine male fertility. Finally, the development of silicon nanowires for a nanopill usable for early cancer diagnostics is presented, and its electrochemical functionality is demonstrated. These three examples show that the first lab on chip devices are coming close to commercial application, and that future healthcare may strongly benefit from extensive point-of-care diagnostics capabilities.

KEYWORDS: point of care diagnostics, lithium, fertility, nanowire sensing

INTRODUCTION

Over the past 2 decades the field of microfluidics and lab on a chip has experienced a spectacular growth. Initiated originally by improvement of chemical separation techniques (in particular capillary electrophoresis [1]) using micromachining, the ability to precisely manipulate and experiment with biological material (DNA/RNA, proteins, cells, tissue), made possible with simple PDMS technology [2], generated a huge research interest from biological and medical fields. Apart from this, there is an enormous market potential for labs on a chip in a variety of areas, of which (bio)medical microdevices are generally considered as being both most promising and closest to realization. There are several reasons for the high expectations from Lab on Chip microdevices for health care:

1) **Personalized medicine.** It is generally accepted that drug prescription and use should be more adapted to the individual patient's needs and response to therapy. Therefore, both monitoring of the level of medication, as well as the effect of medication (on certain biomarkers) is of great importance for improved therapeutic efficiency. In addition, patient monitoring provides information about patient compliance.
2) **Early diagnostics.** For most diseases, early diagnostics gives the most dramatic improvement of prognosis, while in many cases the surgical intervention is also strongly limited (minimal invasive surgery).
3) **Extended patent lifetime for drugs.** Finally, a commercial motivation to couple drugs prescription to a monitoring device is that it allows pharmaceutical industry to develop new patents on the basis of existing drugs in combination with point of care monitoring devices (which are 10-100 times cheaper, and definitely less risky, to develop than a new drug).

Finally, the reduced costs of such medical microdevices (typically < 1$ fabrication costs, and in the case of paper-based diagnostics [3] < 0.1$) allows for the use in less-developed countries, although the costs of diagnostics should be compared with the costs of therapy which is usually much higher. In the following we will describe a device for measuring lithium in blood of patients suffering from bipolar disorders, a chip enabling testing of sperm-fertility in the home situation, and nanowire sensors that enable early diagnostics for colon cancer.

LITHIUM CHIP

Patients with bipolar disorder are often treated with oral lithium, an efficient drug that has a limited therapeutic window, making a regular determination of the lithium concentration necessary. Approximately 1-2% of the population suffers from this disease and in the Netherlands 20-30.000 patients currently use oral lithium therapy. A glass-based capillary electrophoresis chip was fabricated as described earlier [4,5] and is currently commercialized by the company Medimate. The chip is vacuum-prefilled with buffer solution, and the inlet channel is protected with a polymer seal that has to be removed shortly before application of the blood sample. The glass chip is placed in a plastic holder to facilitate handling. The chip contains features to compensate for sample conductivity (fig. 1a), temperature induced internal pressure (fig. 1b), and to avoid bubble formation (fig. 1c) and buffer evaporation (fig. 1d). After bringing a blood sample (serum, whole blood or finger stitch blood) on the inlet channel, the sample ions are electrokinetically driven to the inlet-cross section, after which they are separated and detected at the outlet by an integrated conductivity detector electrode-pair. The lithium value is calculated using an algorithm based upon regression analysis of a large set of test data. Results obtained from over 1500 measurements of whole blood, serum and finger stitch blood show that measured values are within a +/- 10% error range for >95% of the chips without any further external calibration needed. It is also found that the same device can be used to determine calcium and magnesium in cow blood as indication of milk fever.

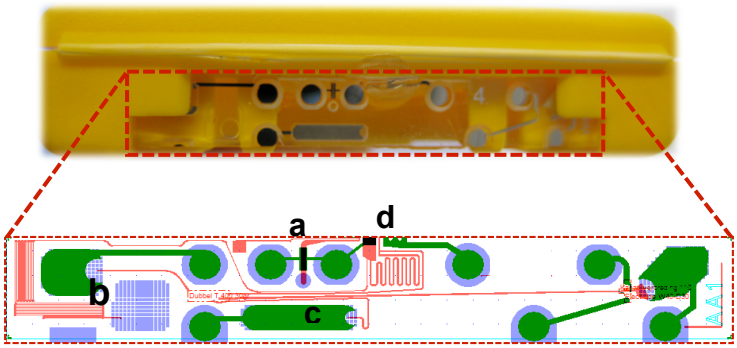

Figure 1. Plastic yellow holder with a lithium glass chip, containing compensation structure for sample conductivity (a), micro expansion vessel (b), large electrode surfaces (c) and evaporation chamber(d).

Figure 2. Picture showing the application of blood droplet from a finger stitch.

FERTILITY CHIP

Approximately 10% of all couples worldwide suffer from an unfulfilled desire to have children, and in these cases the first step to investigate this is the assessment of the semen quality. The most important parameter assessed with a semen analysis is the spermatozoa concentration, whereby the generally accepted lower limit for fertile men is $20*10^6$/ml. Because the current golden standard, visual counting of the spermatozoa under the microscope, suffers from subjectivity and is time consuming, computer assisted counting still is rather expensive, and often at least three consecutive samples need to be analysed, there is a strong need for a test that can be used by the man himself at a convenient moment at home. We developed a glass-based microfluidic chip that enables the determination of the concentration of spermatozoa using electrical impedance measurements without knowing the actual flow speed. The electrical impedance is measured between two planar electrodes at a single frequency (96 kHz), allowing differentiation between 6 µm polystyrene beads, added to calibrate the volume, spermatozoa and leukemia white blood cells (HL-60) [6].

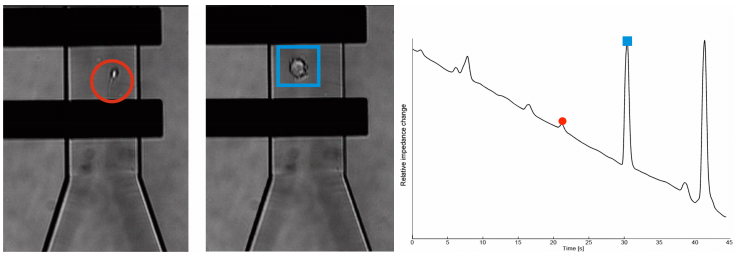

Figure 3. Picture of microchannel with two platinum electrodes in the presence of a spermatozoon (red) and white blood cell (blue).

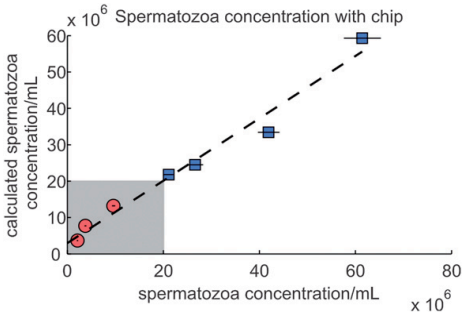

Figure 4. Sperm concentration determined using 6 µm beads for volume calibration.

While the actual measurements were carried out while counting app. 600 sperm cells and took about 10 minutes, the setup allows for a throughput of >100 cells/s thus bringing the measurement time down to < 10 s. The grey area in fig. 4 indicates the subfertile region, and fig. 4 clearly indicates that the device covers both subfertile and fertile concentration ranges. Future development focuses on the integration of an arrangement to determine sperm motility, as well as using the same detection device for rapid diagnosis of infections.

NANOWIRE SENSOR FOR CANCER DIAGNOSTICS

One of the largest problems with intestinal cancer, the second most occurring cancer for men and women, is it's late discovery. Early diagnosis is generally considered as the most efficient way to improve life expectancy, and one very challenging idea for this is the realization of a disposable nanopill that is able to detect the presence of malign intestinal tumors in an early stage and communicate it to an external receiver. For this, a crucial and necessary element to be developed is a small and integratable detector that can detect cancer biomarkers at very low concentrations. One such an early cancer biomarker is hypermethylated DNA, and there are specific probes, methyl binding domain (MBD) proteins, that can recognize and bind specifically to such biomarkers. Nanowires are known to be able to very sensitively detect binding events, but so far were mostly realized using bottom-up fabrication techniques, or expensive fabrication technologies such as E-beam lithography, making it difficult to realize disposable devices. We have developed a cheap top-down nanofabrication technique to make functional silicon nanowires, based on edge-lithography [7]. The technique

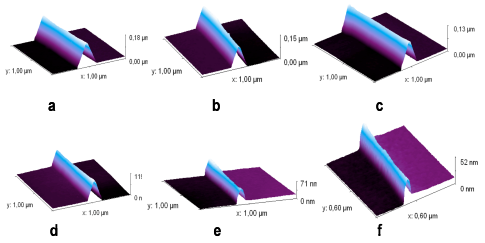

Figure 5. AFM images of silicon nanowires scalable heights. a: 135 nm; b:110 nm; d: 70 nm; e: 30 nm; f: 5 nm

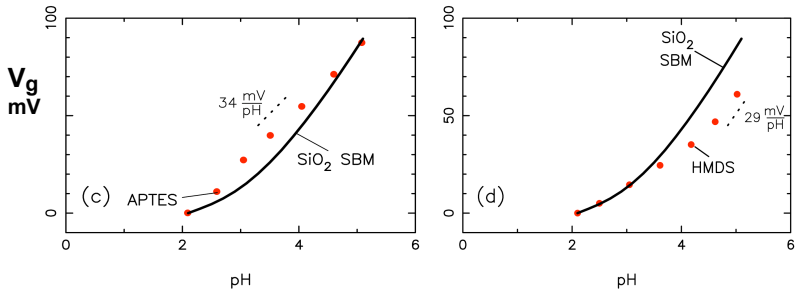

Fig. 6. pH sensitivity of silicon nanowires treated with APTES with (left) and HMDS (right). Black line: response of unmodified SiO2. c:90 nm;

allows for easy scalable nanowire dimensions, as illustrated in fig. 5. The fabrication procedure ensures low resistance contacts without the need for local implantation, and enables easy integration in a microfluidics setup.
In order to test the nanowires' functionality, we have chemically modified the silicon oxide surface with monolayers and evaluated their sensitivity. We found that modifying the surface with pH-insensitive surface groups using hexa-methyldisilazane (HMDS) lowered the original pH sensitivity of the nanowires, while modification with amino-groups using aminopropyltriethoxysilane (APS) resulted in a larger pH sensitivity around the point of zero charge of the silicon oxide (pH 2) (see fig.6).

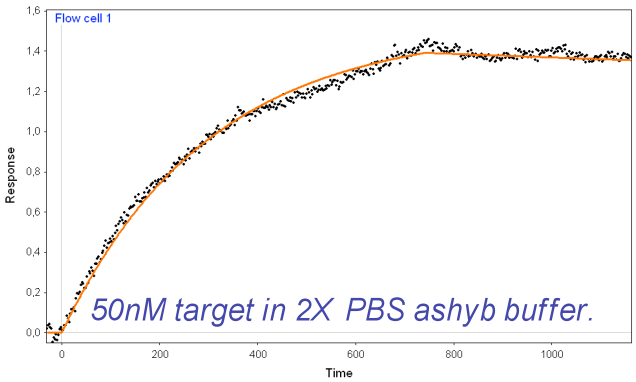

Figure 7. SPR binding curve for hybridization of 50 nM target DNA.

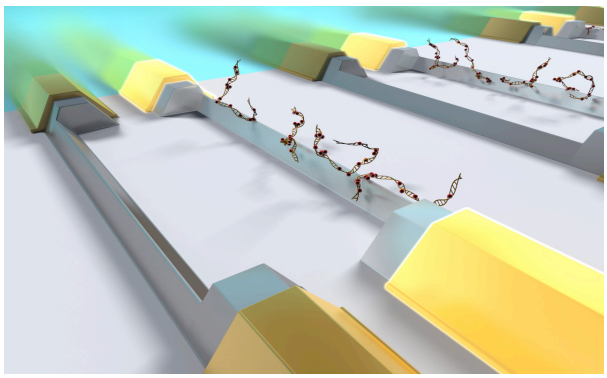

Fig. 8. Artist's impression of functionalized nanowire in a differential setup.

The APTES surface chemistry used to bind receptors to the surface was tested using hybridization of single stranded PNA using an SPR sensing surface. For this purpose, the SPR gold surface was functionalized with aminated SAMs, reacted with SSMCC cross-linker and then coupled to thiolated single stranded PNA. A hybridization curve of the reaction with 50 nM target DNA is shown in fig. 7. Figure 8 shows an illustration of a locally functionalized nanowire array in a differential setup, as will be utilized in the nanopill cancer diagnostics device.

CONCLUSIONS

For the first time a disposable point-of-care device based upon a capillary electrophoresis chip has been realized. Integrated electrical detection offers a method that can be used both for a sperm count chip as well as for sensitive detection of DNA. This opens the way to develop a variety of cheap and disposable point-of-care devices.

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