Fabrication of impedimetric sensors for label-free Point-of-Care immunoassay cardiac marker systems, with microfluidic blood flow delivery, and results telemetry to PDA.

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ABSTRACT

Recent work has demonstrated nanopatterning of gold, using a Focussed Ion Beam, and similarly, nanotemplating gold, for direct protein attachment, useful for alternative sensing techniques.

The impedance changes due to myoglobin capture by antimyoglobin immobilised on gold, have been measured, in a 3 electrode electrochemical cell. The impedance changes reach an end-point level after 15-20 minutes.

Two electrode planar sensors have been fabricated in gold/titanium on PI. Microfluidic delivery of blood/serum sample to the sensor was investigated, with HF-etched test structures in glass. Sensor microfluidic structures will be replicated in PMMA.

Point-of-Care cardiac marker systems are under development, incorporating the novel elements of RF data transmission to PDA, and label-free impedimetric immunoassay sensing.

Keywords: Impedimetric, cardiac, microfluidic, telemetry, nanopatterning.

1. INTRODUCTION

Impedimetric-based sensing systems are becoming important for rapid sensing of cardiac enzyme markers, in a point-of-care environment. Here, it is essential to determine the true levels of cardiac markers as soon as possible after a suspected acute myocardial infarction (AMI), commonly known as a heart attack.

The general clinical requirement for sensors in cardiac monitoring environments is to circumvent the reliance on large laboratory-based analysers, with their inherent delays in results production, via a simple-to-use, lightweight, accurate and rapid sensing method. Accurate, rapid and reliable cardiac enzyme level results are very important for monitoring the degree of recovery after AMI, as well as determining the severity and time of such, so that treatment can be optimised.

Impedimetric sensing is label-free, and so the sensor does not require the addition of fluorescent reagents, therefore simplifying sensor use. A passive microfluidic input of patient whole blood, or possibly filtered blood/serum, makes such devices easier and cheaper to manufacture than pumped systems, and simpler to use.

The utility of such sensors may be greatly enhanced by built-in RF transmission of the sensor data to a PDA, which the physician can carry from patient to patient. The use of a PDA enables much more powerful results processing and display than when the display device is built into the sensor. The processing software may be more easily upgraded on a single platform that handles multiple inputs. Generally, the use of RF transmission to a PDA should make the overall system much more flexible.

2. BACKGROUND INFORMATION

2.1 Impedimetric sensing.

Impedimetric sensing of cardiac enzymes depends on the use of a simple immunoassay method. An antibody (AB) to the cardiac enzyme to be detected, i.e. the analyte, is first immobilized on a conducting electrode, via the well-known technique of attaching it to self-assembled monolayer (SAM) - a SAM may, typically, be formed on a gold electrode by using thiol (sulphur) group bonding onto the gold. The opposite end of the SAM chains is formed by a molecular group that bonds easily to the antibodies, e.g. an amine (NH2) group.

The antigen in the sample then binds to the immobilized antibody, forming an extra layer on top – see Fig. 1. The sample essentially forms an electrolyte, through which a low level current passes under an applied voltage. The amount of the bound antigen can be determined by impedance sensing, using a suitable electrode configuration – the impedance changes will depend on the layer thickness (i.e. molecular length) and density of the bound antigen.

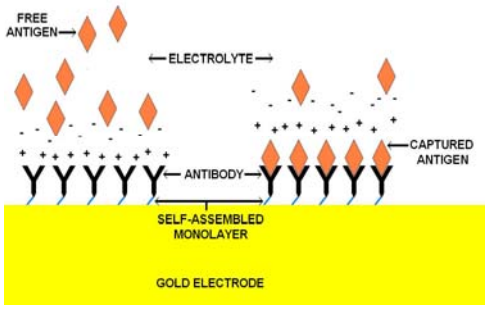

Fig. 1. Antigen capture to antibody immobilised on a self-assembled monolayer.

2.2 Electrode systems

There are a number of well-known electrode geometries for such purposes. Typically, a two electrode system may comprise either a set of interdigitated electrodes, or a working electrode/counter electrode configuration. For interdigitated electrodes, antibodies would be immobilized on both electrodes. For a working/counter electrode set-up, the working electrode only would normally be functionalized by AB immobilization – in this case, the area of the counter electrode is usually greater than 20 times the area of the working electrode, to ensure that the impedance of the smaller working electrode, with the captured antigen, dominates.

Three electrode systems are traditionally used in a conventional electrochemical cell. Here, gold forms the working electrode (for AB functionalisation), Pt the counter electrode, and Ag/AgCl is used as a very stable reference electrode. An electro-deposited AgCl coating on top of the Ag is used to render it resistant to electrochemical corrosion – Pt and Gold are, of course, very resistant to such.

Three electrode systems are also commercially available as planar sensors, where a central working electrode is largely surrounded by firstly a concentric Ag/AgCl reference electrode, and this in turn by a gold counter electrode - see Fig.2.

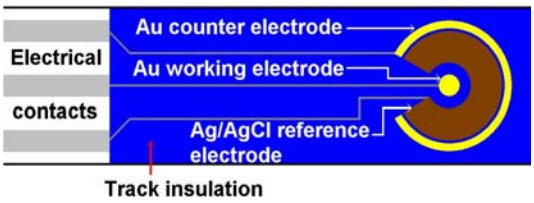

Fig. 2. Typical layout of a commercially available amperometric detector.

In this work, the final device will require a planar electrode sealed or deposited within a microfluidic structure. There may be further advantage gained in defining submicron-spaced interdigitated electrodes (IDEs), which can confine the electric field more closely to the surface, and, in principle, therefore, give larger impedance changes from the thin layer of captured antigen.

2.3 Microfluidics structures

For a microfluidic device to function with whole blood, it is necessary that the geometries of the channels used are large enough to permit the free flow of blood cells – this requires at least 10 microns depth and width. Pre-filtered serum can be used with smaller channels, but a larger volume of blood will be required, initially. Ideally, it would be optimum if the sensor could be configured to operate with a few drops, i.e. several microlitres of whole blood.

Passive microfluidic flow depends on capillary action to pull the sample through the device – this, in turn, means that the channel ahead of the fluid front must be dry, initially. Thereafter, if the fluid drains into a waste reservoir, it is possible to introduce a second fluid into the filled channel, and achieve flow-through without pumping, providing the first fluid is wicked out of the waste reservoir – the wicking action effectively draws the second fluid along by soaking up the first fluid.

Wicking action is relevant in the case where the immobilized AB is preserved by a dry coating within the microfluidic device. In this case it might be preferable to flood the device with buffered saline solution first of all, to dissolve the preservative coating, before introducing the patient sample.

3. EXPERIMENTAL METHODS

3.1 Impedance measurements

Initial impedance spectroscopy measurements were initially carried out using a standard 3 electrode electrochemical cell, comprised of a Au working electrode, Pt counter electrode, and Ag/AgCl reference electrode. The gold electrode was immobilized with anti-warfarin antibody, using a standard EDC/NHS coupling procedure.

A secondary antibody, goat anti-mouse, IgG, which acts as a warfarin-simulant, was captured by the primary antibody. Complex impedance plots before and after the antigen-simulant capture were measured over the frequency range of 0.1 Hz to 200 kHz. The impedance change due to Myoglobin capture by immobilised anti-myoglobin was then measured at 10 Hz, for 100ng/ml concentration.

3.2 Electrode manufacture

For basic electrode manufacture, an SF100 maskless photolithography system was used to expose a variety of different electrode designs in photoresist-coated Au/Ti on polyimide film. (The Au/Ti was previously deposited in an e-beam evaporator, the Ti interlayer of about 20 – 30 nm, providing good adhesion for the gold, the total thickness of both layers being about 200 nm.) KI/I2 Gold etch solution was used, which conveniently etched the Ti underlayer as well as the gold, in about 2 minutes.

Some of the electrodes produced had an extra photopatternable polyimide layer (DuPont PI2737) spun on top, to provide passivation of the electrode tracks between the connections, and the sensing elements. This negative acting polymer was, therefore, patterned in a second mask

process, to open holes over the sensing elements and contacts only. This prevents adhesion of the antigen to the connecting tracks, and ensures that impedance sensing is not degraded by such.

Nanopatterning of Au/Ti on glass using Focussed Ion Beam (FIB) equipment was investigated, and demonstrated for 200 nm widths and spacings, in an interdigitated pattern. Nanotemplating of gold for direct protein attachment, suitable for alternative sensing techniques, was also demonstrated.

It is intended to measure and compare the impedance change results for the planar electrodes with those from the electrochemical cell. This work is ongoing.

3.3 Microfluidic device study

3.3.1 HF etching

Test fluidic structures were projected onto positive photoresist-coated glass substrates using the SF100 maskless photolithography system. The photoresist was developed, and then hard-baked at 115°C, for 20 minutes. A coating of resist was then applied to the rear side, and hard-baked, to provide further protection.

HF solution, diluted at approx. 1 part to 10, HF:H2O, was used to etch the glass, over an approximate 2.5 hour period.

3.3.2 Lid bonding.

A variety of lidding methods were examined, including a study into thermal bonding. A set of soda-lime microscope slides were diamond-scribed into approx. 25 mm x 25 mm sections. These were thermally annealed at 400 °C for 2 hours to relieve residual stress. Bonding tests were carried out at 600 to 660 °C, to determine the optimum temperature - a set of alumina ceramic sheets were placed above and below the samples to be bonded, and a weight applied on top [1].

Bonding by use of UV-sensitive adhesive was examined. The glue was applied to the area outside the trenches and reservoir areas, the lid placed gently on top, and the adhesive was exposed to UV from the SF100 for about 10 seconds, which hardened it sufficiently.

3.4 RF data telemetry

A commercially available RF transmission chip was sourced and connected to the sensor output, having been set up to sample at a suitable frequency of 50KHz. The real-time live sensor signal levels were transmitted to a PDA, and displayed in graphical format.

4. RESULTS

Complex impedance plots of Z” vs. Z’ shown, in Fig. 3 are consistent with antigen capture occurring, as the imaginary part of the complex impedance, Z”, increased in the presence of the secondary AB antigen-simulant.

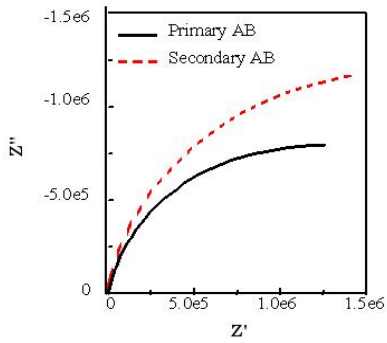

Fig. 3. Complex impedance plots for AB and antigen.

Z” vs. time for capture of myoglobin by antimyoglobin antibody immobilized on gold is shown in Fig. 4:

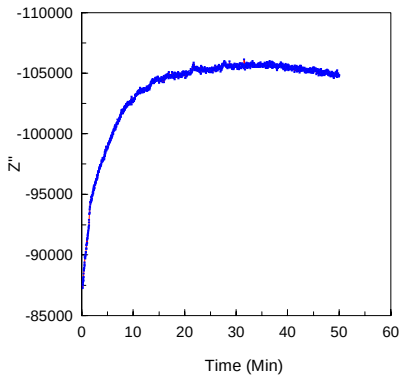

Fig. 4. Impedance change with time for myoglobin capture by antimyoglobin immobilised on gold, at 10 Hz.

Au/Ti on PI was used for planar electrode fabrication, as in Fig.5a, with Au/Ti on glass nanopatterned and nanotemplated by FIB, as in Figs. 5b and 5c, respectively:

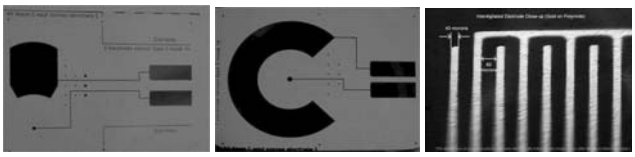

Fig. 5a. Fabricated Au/Ti/PI planar electrodes.

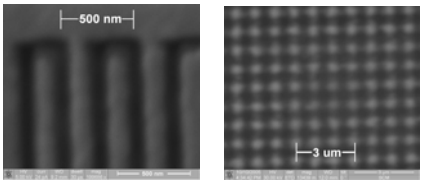

Fig. 5b. IDE-type FIB nanopatterning of Au/Ti/glass.
Fig. 5c. 500nm gold islands for direct protein attachment.

Microfluidic test channels HF-etched in glass are shown in Fig. 6. The channels were approx. 28 um in depth, while

the tops were broadened to about 1 mm, by HF undercutting the resist.

Fig. 6. Close-up image of microfluidic channels.

Thermal bonding of glass to glass is shown in Fig 7. The optimum temperature can be seen to be 660 °C.

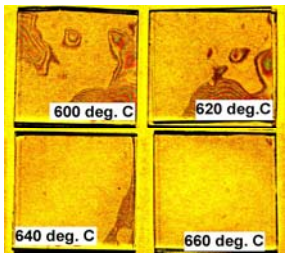

Fig. 7. Thermal bonding of glass to glass.

However, when attempting to thermally bond the 1 mm thick lids over the test device, it was found that the glass showed very significant reflow into the reservoir area, as in Fig. 8. This technique, therefore, is found to be only really suitable for thin channel devices, with small mixing/reservoir areas.

Fig. 8. Thermal reflow into reservoir area.

Microfluidic flow is shown in a test device having a lid bonded via UV-sensitive adhesive:

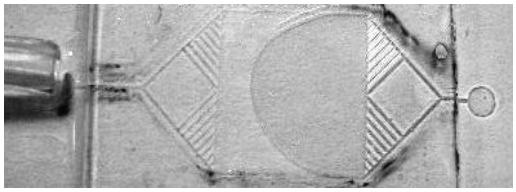

Fig. 9 Fluid flow in a microfluidic device.

A screen-shot of RF transmitted data shows data gathered in real-time, from the sensor.

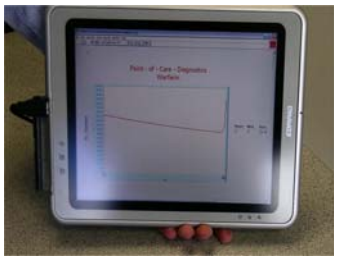

Fig. 10. PDA showing sensor data.

5. DISCUSSION

Most of the necessary elements to produce a suitable sensor system have been shown to work, including the impedance sensing method. There are some problems with implementing a microfluidic system in glass for a mass-producible device, namely how to achieve bonding over reservoirs without significant reflow, and how to functionalise enclosed electrodes. Therefore, it is anticipated that the final system will involve channels defined in pressure-sensitive adhesive (PSA), bonded between the planar sensor base, and a PMMA lid.

The fact that no raised temperature is involved in PSA bonding would allow the AB to be immobilized on the working electrode surface, and a preservative coating applied, before sealing via the PSA. If the preservative coating is thin enough to dissolve completely in whole blood/serum, then wicking of wash-fluid will not be required, and the sensor only requires patient sample addition.

6. CONCLUSIONS

Impedimetric sensing of cardiac markers using antibodies immobilised on planar gold sensors have been demonstrated to have the potential for commercialisation. Microfluidics sample delivery to the sensor has been shown to be feasible, as has RF data transmission.

7. FUTURE WORK

Ongoing work is intended to integrate planar electrode structures into a microfluidic device, together with live RF data transmission to a PDA, in a public concept demonstration in April '06.

Investigation of nanopatterned interdigitated electrodes for potential signal enhancement will continue, alongside nanotemplating for alternative sensing techniques.

8. ACKNOWLEDGEMENTS

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[1] Lin C H, Lee G B, Lin Y H, Chang G L, “A fast prototyping process for fabrication of microfluidic systems on soda-lime glass”, *J. Micromech. Microeng*. **11**, 726 – 732, 2001.