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Fabrication of impedimetric sensors for label-free Point-of-Care immunoassay cardiac marker systems, with passive microfluidic delivery.

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Abstract - **Miniaturised point-of-care cardiac marker sensors are being developed, based on impedimetric sensing of cardiac enzyme capture by an antibody layer immobilised on a planar gold electrode sensor. Gold/Ti-on-glass substrates have been used, in a 2 electrode configuration, with antibodies immobilised on the working electrode.**

Microfluidic structures have been fabricated by a CO_2 laser, in 25 um thick pressure sensitive adhesive (PSA), on a PMMA lid, and the structure bonded on top of the planar sensor. Microfluidic blood/serum delivery has been investigated using a visualisation dye. Some flow problems are observed if the sensor is exposed to air for several days, suggesting that flow channel nanopillars and hermetic encapsulation may be required to guarantee flow properties in commercially produced modules.

Work is ongoing to characterise the impedimetric signal changes for myoglobin capture by antimyoglobin, using these sensors. Fifty micron thick PSA, incorporating a robust spacer layer, will be used to give better definition of channel walls.

Keywords: Impedimetric, cardiac, microfluidic, antibody, planar, sensor, point-of-care.

I. INTRODUCTION

Rapid and accurate sensing of cardiac enzyme markers is becoming increasingly important in point-of-care settings. Here, it is necessary to measure accurately cardiac marker levels, as soon as possible after a suspected heart attack, or acute myocardial infarction (AMI).

Generally, small, lightweight, simple-to-use, rapid and reliable point-of-care sensors are beneficial because the reliance on laboratory-based analyzers, with their inherent measurement delays, is then circumvented. The same sensors may also be used for monitoring longer term recovery from AMI in the clinical setting, as well as determining the approximate time and severity of the initial event.

Impedimetric sensing is advantageous as it is label-free, not requiring the addition of any fluorescent reagents – it is, therefore, conceptually simpler in operation, and potentially, easier to manufacture in production quantities.

Passive capillary flow input of patient whole blood, or filtered blood/serum, makes such devices simpler to use, and easier and cheaper to manufacture than pumped systems. There are a limited number of commercially available systems (from e.g. Biosite Inc.) that use cartridges containing fibrous blood filters, to trap the blood cells, and passive capillary flow, thereafter, of the filtered serum throughout the device – at present, these known devices also use dried fluorescent reagents on-board, with optical rather than impedimetric sensing.

Some point-of-care cardiac marker monitoring systems, use a stand-alone base unit, with a built-in display and printer for results readout. However, the sensor unit may be miniaturized, and made cheaper, by using a proprietary RF chip to transmit sensor data to a PDA, which the physician can carry from one patient to another on the cardiac ward, logging data for each in turn. This aspect of the sensor development has already been reported, and a paper has been published as part of the conference proceedings of Nanotech 06, Boston, USA. [1].

This paper concentrates on further development of the sensors and microfluidics, where the original work [1], on this examined gold-on-polyimide sensors, with HF-etched glass microfluidics. The developments reported here examine fabrication of gold/Ti-on-glass planar sensors, with microfluidics replicated in pressure sensitive adhesive (PSA) on the underside of PMMA (poly methyl methacrylate) lids.

II. BACKGROUND INFORMATION

A. Impedimetric sensing.

Standard immunoassay techniques rely on the binding of the analyte (i.e. the antigen to be detected - here, cardiac enzymes) to an antibody (AB). In this case, if the antibody is first immobilized on the electrode surface, then it forms an insulating layer, therefore, increasing the impedance of the electrode. Binding of the analyte to the AB layer further increases the impedance of the electrode, therefore allowing measurement of the amount of captured antigen.

For AB immobilization on gold, the well-known technique of thioctic acid coupling of e.g. alkane thiols, to form a self-assembled monolayer on the surface is the first step. The termination group on the free end of the SAM molecules then allows chemical bonding of the AB's to the SAM, as in Fig. 1.

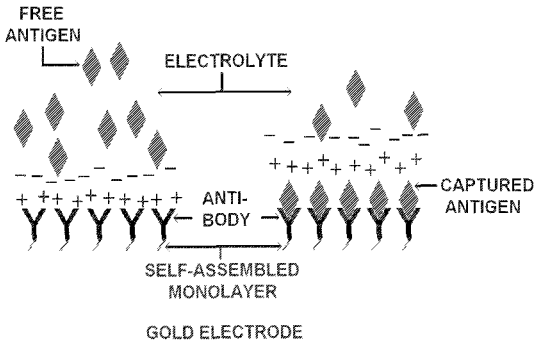

Fig. 1. Antigen capture to antibody immobilised on a self-assembled monolayer.

B. Electrode systems

Interdigitated electrodes have been used for measurements of captured antigen layers, as have three electrode systems, consisting of a working electrode, a counter electrode, and a reference electrode. For a 3 electrode sensor, the AB will, usually, be immobilized on the working electrode. The preferred reference electrode would be AgCl-coated Ag, known to provide a stable reference voltage, in electrochemical cells. It is possible to make the impedance of the working electrode dominate by making the area of the counter-electrode significantly larger, e.g. >20 times, the area of the working electrode.

Reference electrodes are often produced by screen printing silver paste, and then electrolyzing this to create a AgCl passivation layer. Alternatively, the reference electrode may be, for a technology demonstrator, dipped separately into the excess patient sample near the device outlet. The planar sensor, itself, would have the working and counter electrodes patterned in a gold layer, on a substrate such as glass, alumina ceramic, polyimide, etc.. The work reported here follows this latter course.

C. Microfluidics structures

Microfluidics devices that use whole blood need channel geometries of at least 10 um to allow the free flow of blood cells. In this work, it has been assumed that as a starting point, pre-filtered serum will be available, for technology demonstration purposes – however, the ultimate aim will be to produce a device that works with whole blood input.

Current commercial devices use fibrous or micropore filters, so that only the serum constituent of whole blood to flows through, at the input point. Alternative microchannel-based devices, utilizing a thin side-channel which filters off mainly the serum constituent from a broader input channel, have recently been reported [2]. These do allow a certain proportion of blood cells through, and so the device dimensions thereafter must still allow these to flow freely.

Passive microfluidic capillary flow is preferred for simplification of device design. On commercially available systems, the patient serum sample also acts as a wash, to dissolve onboard reagents, and AB preservative coatings. Onboard waste preservation is usually seen as important. Microfluidics systems necessarily incorporate an air escape point near the output, so that trapped air does not stop the fluid flow.

III. EXPERIMENTAL METHODS

A. Impedance measurements

Preliminary work on impedance has been reported as in [1]. Here, initial impedance spectroscopy measurements were performed using a standard 3 electrode electrochemical cell, with a Au working electrode, a Pt counter electrode, and Ag/AgCl reference electrode. An anti-warfarin antibody was immobilized to the gold electrode, using a standard EDC/NHS coupling procedure. Then a warfarin-simulant secondary antibody, goat anti-mouse, IgG, was captured by the primary anti-warfarin antibody, with the multi-frequency impedance plots being consistent with antigen capture.

Single frequency impedance change measurements have been carried out, as reported in [1], showing the impedance change due to myoglobin capture by immobilised anti-myoglobin, at 10 Hz, for 100ng/ml concentration, which is the upper limit of normal myoglobin levels. Work is ongoing to replicate the myoglobin capture results on the new electrodes being developed here, but a typical previous result from electrochemical cell work is shown in the results section.

B. Electrode manufacture

An SF100 maskless photolithography system, from Intelligent Micro Patterning, was used to define the electrode designs. The system projects a 1024 x 768 pixel bitmap into an image area of around 19 mm x 14 mm, giving an approximate resolution of 18.5 um per pixel. The bitmap images were prepared using standard Windows-based drawing packages.

A two mask process was required, as it is necessary to passivate the connecting tracks between the main electrical connector pads and the working and counter electrodes. If not passivated, the antibodies could immobilize on the tracks, as well as the sensing elements and distort the impedance signal. The passivation takes place by spin-on and exposure of a photopatternable insulating polymer, such as polyimide.

Gold-coated aluminosilicate glass slides, with a thickness of 100 nm of gold, on a 10 nm Ti interlayer (to provide good adhesion of the gold), were used to fabricate the sensors. The substrates were from a commercial source, with metal layers deposited by e-beam evaporation.

The basic electrode designs considered used a large, partial-annular section counter-electrode, with outer surfaces confocal on the smaller working electrode. The first design had the electrodes at the bottom of the short edge of the image, so that these could be used for immersion testing of antigen binding, in slot wells cut in a Perspex block. The second design was at right angles to this, to facilitate bonding of a microfluidic lid, for an integrated sensor design.

Standard Rohm and Haas S1818 SP16 positive photoresist was spun on to the pre-cleaned, and oven-dehydrated gold-coated substrates, at 3000 rpm. Mask 1 was exposed in the SF100, and developed in S351 sodium hydroxide developer, at a concentration of 1 part developer to 4 parts DI water. Hard bake in an oven, at 115° C for 20 minutes, followed.

Standard KI/I2 gold etch solution was used, to develop the resist pattern in the gold – conveniently, this also etched the Ti underlayer as well as the gold, in about 1 - 2 minutes.

DuPont PI2737 negative-acting photopatternable polyimide was spun on at 1000 rpm, to provide the necessary passivation of the electrode tracks, and the sensing elements. Soft-bake was carried out on a digital hot-plate (65°C, for 3 mins., 95°C for 3 mins.). The second mask was aligned in the SF100, exposed, and developed in DE9040 developer, then rinsed in n-butyl acetate. Hard-bake, ramped to 200°C and held for 1 hour, followed.

C. Microfluidic device study

In previous work [1], microfluidic flow was demonstrated in HF etched channels in glass, of about 30 um depth. Thermal and adhesive bonding of lids on top of the etched channels were, also, investigated. However, in this further development work, it was necessary to have a simple method of bonding a microfluidic lid to the solid planar glass substrate, at room temperature, after the antibodies were immobilized on the working electrode. Consequently, PSA was chosen for this task.

Initial test patterns were drawn out, in AutoCAD, at DCU, according to the schematic layout, as in Fig. 2.

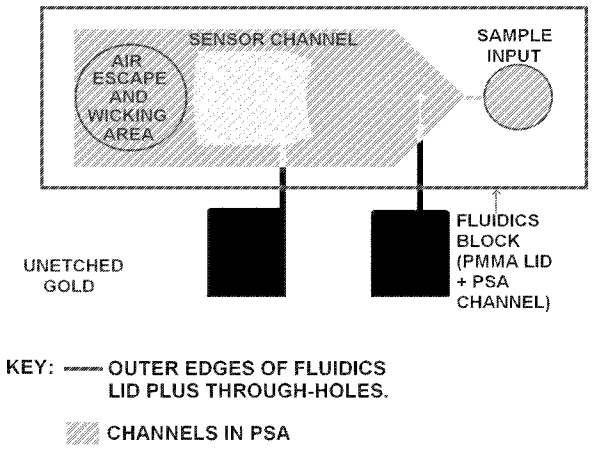

Fig. 2. Schematic of fluidics lid on sensor.

The PSA material, type 3M 8141, optically clear adhesive, of depth 25 um, was laminated onto the PMMA substrates. A transparent protective plastic backing layer protected the top-side of the adhesive lamination, while the underside of the PMMA was protected by a blue plastic backing layer. Through holes were conventionally drilled for the input aperture, and the air escape and wicking area.

The CO_2 laser used to replicate the AutoCAD design, was a Micromaster turnkey system, sourced from Optec. Laser characteristics were emission wavelength of 10.6 um, operated in CW mode (max. power, 100W), and effective focused spot size of approximately 200 - 300 um.

The laser, focused on the top surface of the PSA, burned through the covering film, and the adhesive itself, removing both simultaneously to create the channels. The blue backing layer on the PMMA underside was unaffected, as the laser was out of focus. Some carbonaceous debris is generated around the edges of the channels by the burn-off process – this is probably best removed by sonication in DI water, as many solvents, particularly acetone, could dissolve the adhesive.

Initially, a thin ~200 um width capillary, between the input and the sensor chamber, was fabricated, but it was found that, when bonded to a glass substrate, forced flow was required, to drive the fluid into the sensor chamber. Thereafter, the fluid flowed freely by capillary action, but the initial forced flow requirement limited the utility of the design. It was noticed that the channel edges in the adhesive layer are not perfectly smooth after formation, and this may have affected the surface tension of liquid in contact with the walls. Consequently, it was decided to significantly increase the width of the connecting channel to promote free fluid flow. On new test pieces, the connecting channel width was increased to 2 mm, which proved to provide satisfactory fluid flow into the sensor chamber.

Microfluidic lids were bonded to glass slides, by applying a suitable amount of pressure to the structure, using e.g. a vice clamp, or applied weight. Some care had to be exercised, as uneven application of pressure, or excessive pressure, caused the glass substrate to break. Filtered red ink provided a usable visualization fluid.

IV. RESULTS

Typical gold electrodes patterned on glass, and with a PI passivation layer, are shown in Fig. 3:

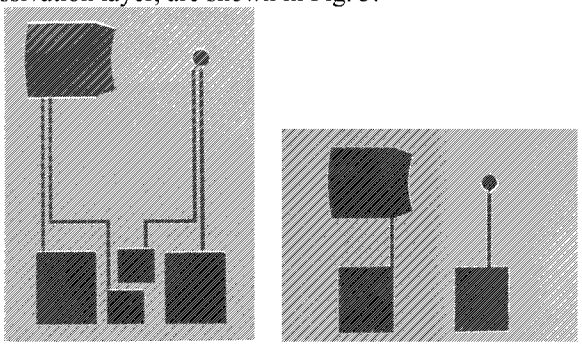

Fig. 3. Au/Ti sensors on glass, with PI passivation.

Some work has also been done on using FIB equipment to nanopattern gold, as in Fig. 4, which should allow the development of nanointerdigitated electrodes, which are of interest for increased signal to noise ratio, and reduced sample volume. Here a 20 nm thick gold layer has been

patterned with a set of parallel lines, of approx. 1.1 um separation, and 160 nm width.

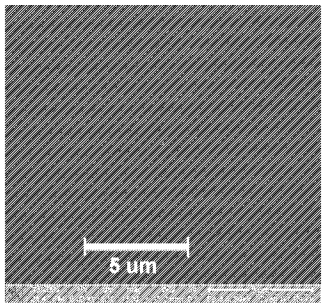

Fig. 4 Nanopatterning of gold by FIB.

Fluidic flow in a previous test device fabricated in glass, with a glued glass lid is shown for comparison with the new fluidics devices, in Fig. 5.

Fig. 5. Fluidic flow in a glass microfluidic device.

A typical microfluidic lid, as laser-etched in PSA on PMMA, is shown in Fig. 6.

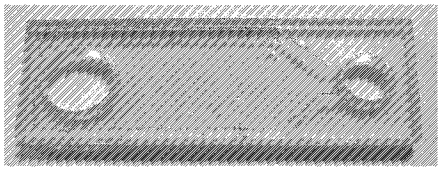

Fig. 6. Microfluidic chamber in PSA on PMMA lid.

A typical flow sequence, to a point approximately corresponding to the working electrode position, is show in Fig. 7. The flow can be seen to be proceeding reasonably evenly along the length of the patterned PSA chamber, with the time difference from first image to last being 4 seconds.

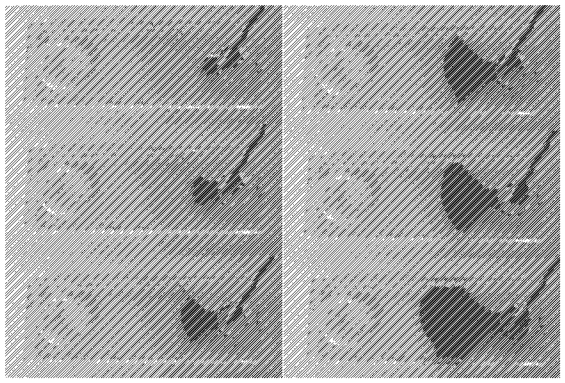

Fig. 7. Sequence of images showing microfluidic flow.

By contrast, a lid sealed to initially clean glass, where the structure has been left for several days, without any special sealing processes, exhibits changes in surface conditions, such that fill is not consistent at the edges of the adhesive layer, as in Fig. 8.

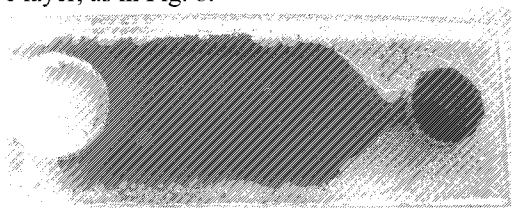

Fig. 8. Uneven fill for air-exposed structure, after several days.

A PMMA lid with PSA fluidic channel is shown adhered to an actual planar sensor base in Fig. 9.

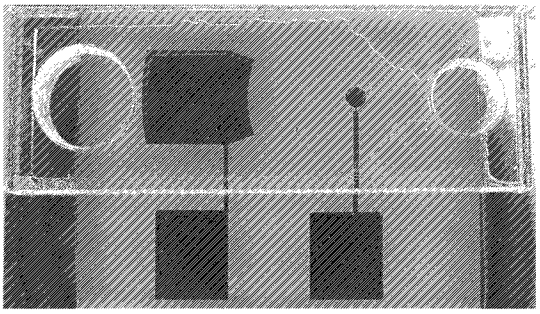

Fig. 9. Sensor with microfluidics lid attached.

To further control the capillary flow, nanostructuring of the surfaces can be used – this can give a very consistent even flow rate, across broad areas, many mms wide, and, also, has potential for trapping blood cells. A typical nano-structured Si surface, achieved using Focused Ion Beam (FIB), equipment is shown in Fig. 10 - the peak to trough height is about 100 nm.

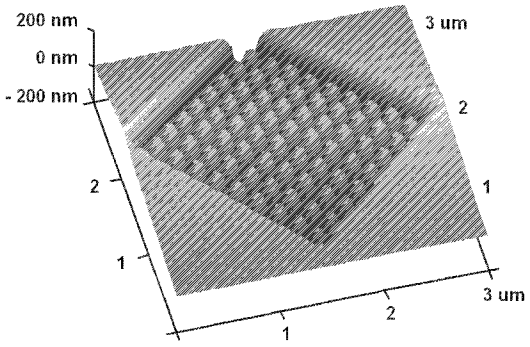

Fig 10. FIB patterning of nanopillars in Si.

The previous electrochemical cell impedance change result, which we aim to replicate for these new sensors, is shown in Fig. 11.

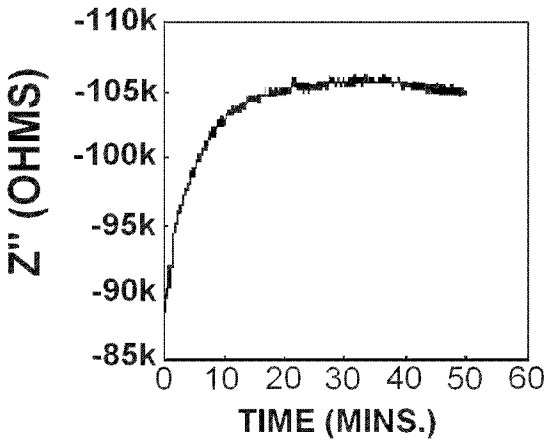

Fig. 11. Impedance change with time for myoglobin capture by antimyoglobin immobilised on gold, at 10 Hz.

V. DISCUSSION

Gold/Ti on glass was used for convenience, as it is available commercially in standard microscope slide format. There are some problems in achieving a perfect seal to the glass, due to fracture problems at high and/or uneven applied pressures. However, more robust substrates, such as alumina, can have Au/Ti deposited readily, and this would allow a more robust sensor, capable of withstanding the high pressures needed to spread the PSA evenly.

The PSA bonds at room temperature, which allows AB immobilization and preservation before the lid is sealed to the sensor. The PSA material is, also, available in 50 and 100 um thicknesses, where a double-sided adhesive coated spacer is used. This, potentially, gives better definition of the channel edges than the adhesive alone, promoting better flow characteristics.

Initially cleaned glass shows fast even flow, most likely because the glass is not only clean, but also dry, after oven evaporation of the surface moisture, before lid attachment. However, the quality of flow is observed to deteriorate with exposure to atmosphere over time, with bubbles being trapped along the edge of the adhesive, and within the channel structure.

The unevenness of fill demonstrable after several days exposure to air is probably due to a variety of factors, such as diffusion of contaminants, moisture absorption by the PMMA (known to be hygroscopic), formation of a layer of adsorbed water molecules on the glass, etc.. Therefore, as with all medical devices of this nature, it would be necessary to hermetically seal the sensor in a suitable package, possibly under dry nitrogen, to ensure consistent performance with time. Diamon-like Carbon coatings would be beneficial as regards controlling surface energies, and facilitating consistent flow.

Nanostructuring of the glass surface, and others materials can be achieved using FIB equipment. In commercial production, there are distinct advantages in using softer plastic type materials, such as PMMA, as a Nickel-coated Si hard master can be replicated on the nanoscale, in negative relief, in such thermoplastics, using hot embossing equipment.

VI. CONCLUSIONS

Fabrication of planar impedimetric sensors in gold/Ti on glass has been demonstrated to have the potential for commercialisation, although, it would be preferable, for robustness to use a stronger substrate such as alumina ceramic. Simple passive microfluidics capillary flow has been demonstrated to be reasonably effective for visualisation fluids, although hermetic sealing is probably required to preserve the flow properties. Serum flow is expected to be similarly successful, although whole blood input may need a special fibrous, or alternatively microfluidic filter input.

VII. FUTURE WORK

Further passive fluidic delivery may be investigated using thermal nanoimprinting of PMMA. Nanoscale electrode patterns, by FIB patterning of Au-coated substrates, may also be followed up, with a view to sample volume reduction. Thicker PSA material (50 um) consisting of a double-side adhesive-coated spacer, will be investigated as this is known to give more robust channel walls.

Incorporation of a whole blood filter, possibly a microchannel coarse filter, plus a nanopillar cell-trapping region, may be investigated. Diamond-like Carbon coatings for fluid control will also be assessed. Further impedimetric cardiac enzyme sensing experiments will be carried out.

ACKNOWLEDGEMENTS

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