A hybrid microfluidic system for cancer diagnosis based on MEMS biosensors

Pedro Ortiz*, Neil Keegan*, Julia Spoors*, John Hedley*, Alun Harris*, Jim Burdess†, Richard Burnett†, Thomas Velten†, Margit Biehl†, Thorsten Knoll†, Werner Haberer†, Matthew Solomon‡, Andrew Campitelli‡ and Calum McNeil*

* Newcastle University, Newcastle upon Tyne, UK
† Fraunhofer Institute for Biomedical Engineering (IBMT), Sankt Ingbert, Germany
‡ MiniFAB (AU/ST) Pty Ltd, Victoria, Australia
E-mail: p.m.ortiz@ncl.ac.uk and neil.keegan@ncl.ac.uk (joint first authors)

Abstract — A microfluidic system for cancer diagnosis based around a core MEMS biosensor technology is presented in this paper. The principle of the MEMS biosensor is introduced and the functionalisation strategy for cancer marker recognition is described. In addition, the successful packaging and integration of functional MEMS biosensor devices are reported herein. This ongoing work represents one of the first hybrid systems to integrate a PCB packaged silicon MEMS device into a disposable microfluidic cartridge.

I. INTRODUCTION

The ability to diagnose cancer at its early stages has a profound impact on successful disease treatment. Such early detection in clinical diagnostics is now being realised through advances in analytical systems and instrumentation miniaturisation [1]. The more recent introduction of microfluidic technologies has shown that there is great potential to take this positive impact even further by enabling the development of point of care systems [2]. This has the important potential of significantly reducing the cost of health care [3]. The SmartHEALTH Integrated Project consortium is funded by the European Commission to address these issues [4]. The system presented in this paper is part of the clinical diagnostics instrument being developed within the SmartHEALTH project and its main aim is to address the necessity for accurate and early detection of several types of cancer.

II. DEVELOPMENT OF A MEMS-BASED CLINICAL DIAGNOSTIC SYSTEM

The diagnostic system being developed relies on a circular diaphragm resonator (CDR), MEMS mass sensor, as the central theme. This type of sensor is attractive as a new platform technology in the first instance due to the sensitivity it yields compared to current state of the art mass sensors. The published mass sensitivity being 100pg cm^{-2} Hz^{-1}[5]. In order to create a diagnostic system around this central theme a multi-disciplinary team was assembled to fabricate the CDR, functionalise the surface at the Nanoscale and package the whole concept into a proof of principle cartridge. The surface functionalisation research converts the basic CDR into a label-free BioMEMS analyte sensor; while the full packaged regime, realises a proof of principle test cartridge encompassing all electrical contacts and fluidic delivery.

As an introduction to the principles of the core technology the CDR device takes advantage of the degenerate mode resonant mass sensor principle [5]. In short, this principle consists of a vibrating circular diaphragm, which supports a pair of spatially independent modes of vibration sharing a common natural frequency. By functionalising the area corresponding to one of these modes with a biological capture species e.g. an antibody, but ensuring the remaining area remains inert, a biosensor is created. In basic terms, target molecules bind to the functionalised area creating a split in the resonant frequency of the two modes, which is proportional to a change in mass at the surface and, ultimately, analyte concentration. This type of structure presents the intrinsic advantage of having a reference frequency, to compensate for non-specific effects, within the same structure as the functional device thus improving the sensitivity of detection. The fact that a reference sensor is not required to compensate for pressure and temperature fluctuations is a significant advantage over the current state of the art. Fig. 1 presents a schematic diagram of this concept.

III. DISPOSABLE CARTRIDGE ASSEMBLY

The hybrid microfluidic cartridge realisation can be split into three strands. A. Microfabrication of the Si/glass CDR device. B. Surface functionalisation of the CDR device. C. Packaging the CDR onto a
custom designed PCB and integration of the downstream components into a polymer microfluidic platform. This has been successfully achieved and a step by step description follows:

A. MEMS device fabrication

In order to integrate the CDR device onto a microfluidic platform, a new device layout was designed. The current die layout of 2mm x 2mm is better suited to PCB packaging than the 15mm x 5mm die previously presented and designed for dual in-line packaging [6]. The CDR device layout and diaphragm details are shown in Fig. 2.

In the current fabrication process (shown in Fig. 3) the cavity underneath the diaphragm was created by reactive ion etching 2µm into a 4µm device silicon layer of a SOI wafer. As the diaphragm and the cavity walls are made of the same material, the diaphragm is perfectly clamped thus ensuring that the flexural displacement of the diaphragm at the outer edge is fully constrained - essential for the correct performance of the device. This step has been introduced to reduce quality issues encountered in earlier manufacturing runs, which were created during Si and glass bonding. The fabrication process design of the new layout devices was the result of collaboration between Newcastle University and Pusan National University (PNU), South Korea and was implemented by PNU.

Fig. 2 a) A CDR device (2mm x 2mm) with 20 electrical contact pads for drive electrodes, capacitive coupling screening and device silicon biasing. The detail shows a 2µm thick diaphragm with a 2µm high cavity which has not yet been functionalised. The drive/sense electrodes as well as the screening electrodes can be visualized through the diaphragm. b) Image of a diaphragm functionalised with 3-aminopropyltriethoxysilane (APTES) polymer.

B. Device functionalisation: surface chemistry and biopatterning

After much consideration, a hydrophilic polymer was developed as the basis for biological functionalisation [7]. Using novel conditions for deposition, APTES was shown to form a porous polymeric capture network covalently linked to silicon surfaces. The porous layer can be deposited with a controlled thickness between 100 nm and 5 µm, far exceeding the monolayer surface chemistry previously reported in the literature [8]. Firstly, the hydrophilic nature of the polymer helps to maintain the 3-D structure of biomolecules and therefore improves functionality at the sensor interface. Secondly, and most importantly, the polymer has demonstrated the ability to absorb biomolecules, which should increase the dynamic range of the sensor. Finally, the optimised chemistry on CDR patterns demonstrated that the immobilisation strategy produces highly site-specific and reproducible surfaces for both CEA antigen / HPV DNA target recognition.


Both species are important markers for cancer diagnostics and will be used in conjunction with the polymer and test cartridge in the analytical assessment of the proof of principle of the BioMEMS clinical diagnostic system. In addition, it has been demonstrated that the polymer has wide applicability to bio-molecule capture, signposting a new platform technology for generic immobilisation. Fig. 4 shows a step-by-step development guide to the surface chemistry and its capture capabilities. It is noteworthy that preliminary stability studies have indicated that the polymer is stable over a 50 day period and the probe antibody remains stable within the polymer matrix for over 50 days at room temperature.

C. Packaging and integration of the device into a microfluidic cartridge

One of the ultimate aims of the SmartHEALTH project is the full integration of the CDR biosensor into a disposable cartridge with suitable fluidic and electric interfaces, a challenge, which has been tackled by IBMT and MiniFAB in close collaboration. A schematic of the packaging concept for the MEMS biosensor is shown in Fig. 5. As added value to the device development process, the surface polymer can be packed with capture biomolecules after full CDR packaging is complete, so denaturation of bio-molecules is not a
limiting constraint on the packaging concept. This constitutes an advantage over conventional sol-gel polymers, which encapsulate the biomolecule within their matrix during polymerisation [9].
be used for prototype assay development. This final refinement of the embedding process will produce fully functional packaged devices, which will allow the production of microfluidic cartridges containing CDR devices with low cavity pressure under development. This will allow the development of the electronic readout in progress and will produce a viable signal amplifier and digital signal processing board.

In the first instance the fully packaged CDR devices (not functionalised) were mechanically tested. These tests were carried out in the fluidic reaction chamber under vacuum (0.2 mbar). Under these conditions, it was found that an unexpected attenuation was observed. This attenuation was attributed to the presence of air in the device cavity (cavity separating the diaphragm from the electrodes). Air was trapped because the epoxy embedding of the die was carried out at atmospheric pressure. As the embedding epoxy covers the electrical bond pads, the channels above the metal lines were sealed (channels result from the glass etch previous to the gold deposition Step 1 in Fig. 3). This effectively locks air inside the chamber beneath the diaphragm, making it impossible to evacuate the cavity when the air is pumped out of the rest of the fluidic network. In order to confirm this, a CDR was packaged without embedding the contact pads in epoxy and then tested at PCB level under vacuum (0.2 mbar) and at atmospheric pressure. This resulted in a clear contrast in the amplitude of the resonance peak as can be seen in Fig. 10. This was in accordance with what was previously observed during characterization of large die devices, in which severe vibrational damping was present in devices tested at atmospheric pressure [6]. Moreover, a similar damping effect has been reported by Pandey et al. [10].

In order to overcome this damping issue, a CDR device embedding process to be carried out under vacuum is currently under development. This will allow the production of microfluidic cartridges containing CDR devices with low cavity pressure (~20 mbar) which should have a positive impact on the sensor response as previously reported [6]. This final refinement of the process will produce fully functional packaged devices, which will be used for prototype assay development.

IV. PRELIMINARY TESTING OF THE CDR DEVICE IN A MICROFLUIDIC ENVIRONMENT

Although an ultimate aim of the SmartHEALTH project is to develop an instrument, which achieves an electronic readout of the signal, the preliminary tests of the CDR biosensor have been carried out using electrical stimulation and optical detection. The laser Doppler vibrometry test station used has been described previously [6]. For the purpose of optical access, a window was incorporated into the development prototype cartridge. It is worthy of note that development of the electronic readout is in progress and will produce a viable signal amplifier and digital signal processing board.

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