



New prototype assembly methods for biosensor integrated circuits

Anthony H.D. Graham^{a,*}, Chris R. Bowen^b, Susan M. Surguy^c, Jon Robbins^c, John Taylor^a

^a Department of Electronic & Electrical Engineering, University of Bath, Bath BA2 7AY, UK

^b Department of Mechanical Engineering, University of Bath, Bath BA2 7AY, UK

^c Receptors & Signalling, Wolfson CARD, King's College London, London SE1 1UL, UK

ARTICLE INFO

Article history:

Received 15 December 2010

Received in revised form 14 March 2011

Accepted 16 March 2011

Keywords:

Biosensor
Integrated circuit
Prototype
Assembly
Packaging
Partial encapsulation

ABSTRACT

Two new prototype assembly methods have been evaluated for biosensors that combine an integrated circuit (IC) sensor with a culture chamber. The first method uses a poly-ethylene glycol (PEG) mould to mask the IC sensor during application of a room temperature vulcanising (RTV) silicone elastomer used to insulate the bondpads and bondwires. The second method utilises the 'partial encapsulation' service offered by Quik-Pak, USA. Both methods were shown to provide good electrical insulation and demonstrated biocompatibility with the NG108-15 cell line. These methods are particularly useful for the assembly of low-cost ICs with a small (<4 mm²) sensor area.

© 2011 IPEM. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Biosensor integrated circuits (ICs) often require demanding packaging solutions that are not readily available through the semiconductor industry. Whilst micro-electro mechanical systems (MEMS) have driven the need for new forms of packaging [1,2], there are still many applications where suitable solutions are sparse. This is discussed in [3] where it is stated, 'new or alternative packaging and assembly methods and materials are necessary for biosensors and bio-MEMES applications'. An example is cell-based biosensors based on integrated circuit technology. The unique difficulties arise from the need to enable cell culture media and buffers to contact the electrode (sensor) areas of the semiconductor die whilst simultaneously providing biocompatible electrical and chemical isolation from the bondpads and bondwires at the edge of the die. With the industry in its infancy, several prototyping solutions have been developed by researchers to meet their specific needs. Of particular merit is the method optimised by Offenhäusser et al. [4] where a customised epoxy ring is adhered between the sensor and bondpads before a potting resin or room temperature vulcanising (RTV) silicone elastomer is used to cover the bondwires. This popular approach has been further developed by Heer et al. [5] to extend lifetime up to at least three months by using EPO-TEK 302-3M (Epoxy Technology Inc., USA). These solutions appear to

be well suited to ICs that have a fairly large die area compared to the sensor area. This allows for a large distance, e.g. greater than 2 mm, between the central sensor area, such as an array of micro-electrodes, and the bondpads. For example, Frey et al. [6] had a die of 48 mm² with sensor area of 6.4 mm². This allows for relatively easy/ low-tolerance placement of the epoxy ring. ICs with such geometries typically have amplifier and logic circuits in the area between central sensor and the bondpads at the IC periphery. Alternatively, Hammond and Cumming [7] and Delille et al. [8] have developed packaging solutions based on SU-8 and Loctite photo-patternable adhesives where a thick coating (~1.5 mm) exposes the sensor area whilst leaving the bondpads and bondwires coated. These photo-patternable methods are also attractive due to their simplicity and have reported to be a repeatable assembly process for cell-based sensors with a short lifetime of up to 7 days. Beyond this timescale it is reported in [7] that the SU-8 suffers excessive electrical leakage. Similarly, in [8] they found that the useful lifetime of the Loctite 3340 adhesive was one week and was incompatible with ethanol sterilisation.

This paper reports new techniques for prototype assembly in application areas that require small die sizes (e.g. <20 mm²) and the distance between the edge of the exposed sensor and the bondpads is also small (i.e. <2 mm). With these geometries it becomes increasingly difficult to fabricate and place a thin epoxy wall between the sensor and the bondpads. In addition, the assembly method must be suitable for devices with a lifetime longer than one week. For example, our CMOS IC biosensor, featuring a reusable multiple electrode array (MEA), has a square array of 48 electrodes of 30 μm diameter with just 700 μm between the edge of the

* Corresponding author. Tel.: +44 01225 386071; fax: +44 01225 826305.

E-mail addresses: abmahdg@bath.ac.uk, abmahdg@agraham.me.uk (A.H.D. Graham).

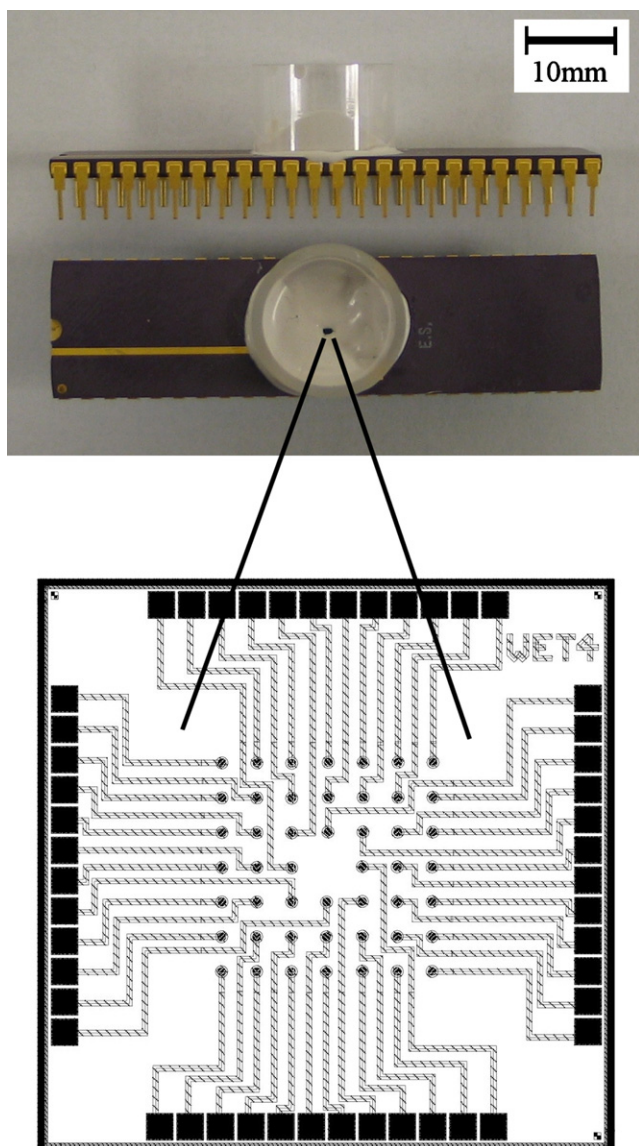


Fig. 1. Biosensor and die schematic. The photograph shows our established assembly method by manual application of Silastic 9161 elastomer. The central void in the elastomer contains the sensor shown in the IC schematic. The side dimension of this square IC is 3.16 mm. The central area of the IC is an array of 48 circular electrodes of 30 μm diameter. The square bondpads are at the periphery of the IC. The width of the electrode array is 1.2 mm with only 0.7 mm between array and bondpads.

sensor area and the bondpads. The device (Fig. 1) is being used for neuronal recordings and as an electric cell-substrate impedance sensor (ECIS) which require a cell culture duration of up to 14 days *in vitro*. We have therefore developed a method that builds on our experience using the biocompatible elastomer Silastic 9161 (Dow Corning, UK) combined with a simple mould to protect the sensor array. We have also evaluated a packaging solution based on the commercial ‘partial encapsulation’ process provided by Quik-Pak (San Diego, USA). The potential of the Quik-Pak method for biosensor applications was illustrated in [9] where an 8 pin dual-in-line (DIL) package was demonstrated to be stable for the two hours required to measure oxygen and nitric oxide release from fibroblast cells. We have extended this work by evaluating the biocompatibility of this packaging method and performing these tests at 14 days *in vitro*.

2. Materials and methods

Two processes were evaluated: ‘mould-based assembly’ and ‘partial encapsulation’. The principle of the mould-based process developed in this work is to initially shield the sensor array with a water-soluble mould. An RTV elastomer is then applied over the whole chip so that it fills the cavity, covering bondwires and bondpads. The water-soluble mould is then dissolved to leave the exposed sensor electrodes. The principle of the partial encapsulation method is to adhere a frame to the IC surface that defines the sensor window, then back-filling the void behind the frame with mould compound to cover the bondwires. The following sections describe the mould-based assembly materials, the mould-based assembly process and the partial encapsulation assembly process. The electrode post-processing and cell culture methods common to both assembly processes are outlined.

2.1. Materials for mould-based assembly

MEA ICs were fabricated by austriamicrosystems AG, Germany. Thirty devices were supplied in 48-lead ceramic DIL packages with removable die-cavity lids. For our work, a 10 mm tall glass cylinder culture chamber (QB Glass, UK) is adhered to the top of the ceramic package so that it encircles the open die cavity. A cyanoacrylate adhesive can be used for a permanent bond, or the glass adhered using Silastic 9161 (Dow Corning, UK) so that the packages can be more easily disassembled, e.g. for scanning electron microscopy (SEM) analysis.

To form the water-soluble mould, a reusable aluminium mould template was prepared using basic machine-shop tools (Fig. 2). The critical dimension is the size of the aperture at the base of the mould. Above the aperture, a conical taper was formed using a 45° countersink bit – the resulting open well shape is preferred for good cell plating, ease of microscopy and possibly better diffusion of media into and out of the well. The angle must be sufficient to ensure that the well sides cover the knee of the bondwires where they rise away from the bondpads. The mould itself was formed of polyethylene glycol (PEG) with average molar mass of 35,000 g mol^{-1} (Sigma Aldrich, UK), supplied as flakes a few millimetres in length. The mould was formed by placing the aluminium template on a glass microscope slide, heated on a hotplate to approx 100 °C and then flakes of PEG were melted into the mould. A solid core wire ‘handle’ was then inserted into the mould and held in position with cross-grip tweezers whilst the PEG was allowed to cool. By using a template of two halves, the mould (Fig. 3a) can more easily be released. Fillets of excess PEG, resulting from the template joint, were removed with a modelling knife.

Preliminary experiments found that the solid PEG-35,000 does not form a seal with the sensor surface which is sufficient to keep out the fluid elastomer. This was resolved by applying a thin layer of waxy PEG to the centre of the mould base (Fig. 3b). As the mould is lowered onto the IC surface the waxy PEG is squeezed and forms a tight interface. By assessing a range of compositions, a 1:1 weight ratio of PEG-1000 and PEG-1450 (Sigma Aldrich, UK) was found to be a suitable formulation, where 1000 and 1450 are the respective average molar masses (g mol^{-1}). PEG was chosen due to its good biocompatibility [10] and its low melting temperature ($\sim 64^\circ\text{C}$ for the Sigma Aldrich PEG-35,000). This enabled easy removal of the mould by melting at a temperature that is also compatible with the cured elastomer.

2.2. Mould-based assembly process

No specific equipment is necessary for the PEG process, but the mould must be positioned accurately over the sensor array during the application and curing of the elastomer. This requires some

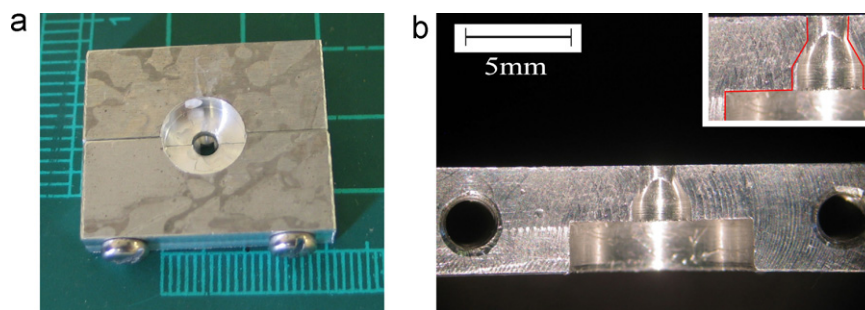


Fig. 2. (a) Reusable aluminium mould template (minor scale units at bottom of the image are 1 mm). (b) Side view of half mould. The die aperture (width 1.6 mm) is at the top of the image. The inset shows the same view but with the mould outline highlighted.

form of three dimension micro-manipulator and for this work we used an optical bench. The following is the process for assembling the MEA illustrated in Fig. 1:

1. Adhere a glass culture chamber to the top of the IC package with exposed die cavity.
2. Apply waxy PEG to base of the mould. Remove excess waxy PEG from the periphery of the PEG mould with a modelling knife.
3. Lower the mould into position on the die surface, causing the waxy PEG to be squeezed to form a tight interface.
4. Fill the die cavity with Silastic 9161 RTV elastomer (drip from the end of a wire).
5. Allow the elastomer to cure (~4 h).
6. Submerge the IC in a dish containing de-ionised water and place in an oven at ~80 °C for 15–20 min until the PEG mould melts and dissolves (indicated by the wire handle falling from its vertical position).
7. Remove the remnants of the PEG mould by jetting with water at ~80 °C using a disposable pipette.
8. Dry the package using a compressed air line (air duster).
9. Inspect for a clean surface. Repeat steps 7–8 until the sensor well is clear of PEG.

2.3. Partial encapsulation assembly process

Twenty bare die MEAs fabricated by austriamicrosystems were shipped to Quik-Pak (San Diego, USA) for partial encapsulation. Their standard manufacturing process was used except that bio-compatible compounds were specified: Silastic Medical Adhesive Silicone Type A (Dow Corning, USA) for placement of the window frame and Hysol CB064 (Loctite, USA) for encapsulation. The following is a summary of Quik-Pak's process:

1. Select an open-cavity package with suitable lead frame to complement the size of the die.
2. Die-attach (to bond the die to the lead-frame). A conducting die-attach was used, but non-conducting die-attach can also be specified, depending on the IC substrate biasing requirement.
3. Wire bond (i.e. attach bondwires between lead-frame and IC bondpads).
4. Design and laser cut the epoxy window frames. The frame wall thickness used was only 0.13–0.15 mm. The frame height can exceed the final IC height as the frame is milled flush at step 9.
5. Attach the frame to the die using a low-stress epoxy (e.g. Silastic Medical Adhesive Silicone Type A).
6. Epoxy cure (72 h at room temperature).
7. Back-fill the void with moulding compound (Hysol CB064) to cover the bondwires.
8. Mould compound cure (2–3 h at 110 °C followed by 2–3 h at 150 °C).
9. Mill the top of the package to planarise the package, the mould compound and the frame, and also to minimise the height of the cavity.
10. Remove any milling debris using a compressed air duster.

After completion of the Quik-Pak process, the devices were finished by adhering the glass culture chambers using Silastic 9161 elastomer.

2.4. CMOS electrodes post-processing

For all devices (i.e. ICs assembled using both methods), post-processing was required to make the aluminium sensor electrodes biocompatible and low impedance, as explained in [11]. Briefly, this entailed anodisation of the aluminium electrodes for approximately 40 min in 0.4 M phosphoric acid, followed by 20 min plating

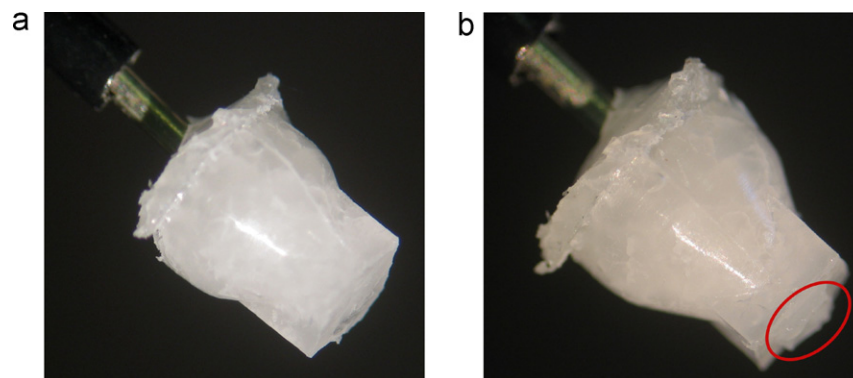


Fig. 3. (a) A PEG mould on a wire handle after release from the template; (b) waxy PEG has been placed on the base of the mould with a modelling knife (as highlighted). Excess waxy PEG from the periphery of the mould base has been removed, leaving waxy PEG only in the central area. For scaling, the square base of the mould has sides of 1.6 mm.

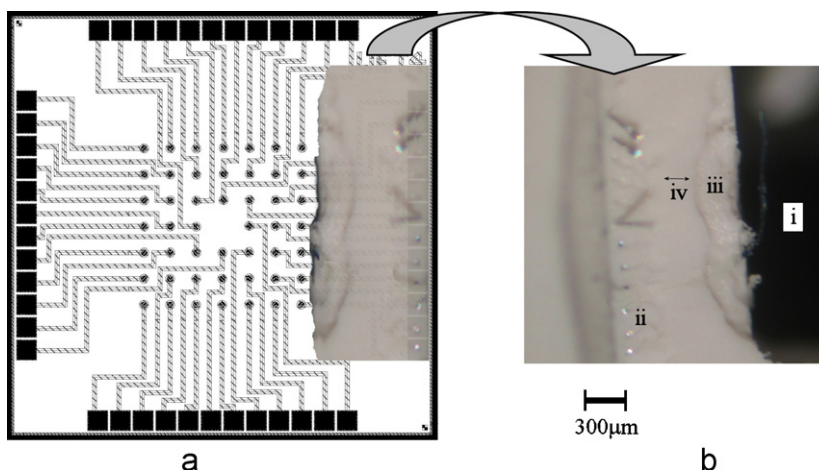


Fig. 4. Disassembled elastomer removed from between the bondpads and the sensor well. Image (a) is only to assist with orientation, showing part of the elastomer overlaid onto the IC schematic. This elastomer was removed from the die and flipped over to give the underside view (b) which is therefore looking *upwards* from where the die was positioned. During assembly, the PEG mould would have filled the void (i) on the right side of the image which is the sensor well opening. Detached bondwires (ii) are visible on the left hand side of the image and indicate the location of the bondpads. A cavity (iii) has been created in the elastomer where waxy PEG had extruded from the sides of the mould when lowered onto the die. The elastomer between the cavity and the bondpads provides electrical and chemical isolation: the minimum measured width ($\sim 200\ \mu\text{m}$) is highlighted (iv).

in a 59 mM gold chloride $\text{H.AuCl}_4 \cdot 3\text{H}_2\text{O}$ bath and approximately one minute for platinum black deposition using chloroplatinic acid (CPA) $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ with $264\ \mu\text{M}$ Lead(II) acetate trihydrate.

2.5. Cell culture

Our experience using Silastic 9161 elastomer for packaging has previously established its biocompatibility [12,13]. However, the Quik-Pak process used materials new to cell culture and therefore required an evaluation of biocompatibility. The choice of the NG108-15 mammalian neuronal cell line for these tests was governed by the proposed applications, ease of use and past experience. Cells were cultured in 50 ml flasks (Nunc, Thermo Fisher Scientific), each containing 9 ml of growth medium (Dulbecco's Modified Eagle's Medium (DMEM) with GlutaMax (2 mM L-glutamine, Gibco), 5% foetal calf serum (FCS, Invitrogen), HAT supplement (30 μM hypoxanthine, 0.12 μM aminopterin, 4.8 μM thymidine, Sigma), 10 units ml^{-1} Penicillin and 0.1 $\text{mg}\ \text{ml}^{-1}$ Streptomycin (Sigma)) and incubated at 37°C , 10% CO_2 . Passaging was performed with a 3:1 division when cells were approximately 70% confluent.

Sterilisation of the substrates was performed in the laminar flow hood by submersing in ethanol for 30 min followed by air drying for approximately 15 min. The ICs were coated with poly-L-lysine (poly-L-lysine hydrobromide 15–30 kDa, 0.01% (w/v) (P7890, Sigma Aldrich, UK)), incubated for 1 h at 37°C , then rinsed with growth medium. The packaged ICs were plated with cells (seeding density of 30,000–60,000 ml^{-1}) and incubated at 37°C , 10% CO_2 . Growth medium was replaced after 24 h with differentiation medium (DMEM with GlutaMax (2 mM L-glutamine), 1% FCS, HT supplement (30 μM hypoxanthine, 4.8 μM thymidine, Sigma), 10 μM prostaglandin E1, 50 μM IBMX in 50 mM DMSO, Penicillin-Streptomycin as above) and replaced after a further 24 h with plating medium (as differentiation medium but excluding the prostaglandin E1 and IBMX). The cells were then incubated for a further 13 days, refreshing the plating medium every 3–4 days, prior to an evaluation of cell vitality using standard methods described elsewhere [14]. Biocompatibility was tested by one-way ANOVA (Analysis of Variance) with a null hypothesis ($\alpha < 0.05$) that the Quik-Pak partial encapsulation devices have the same number of cells per unit area as the devices assembled using the mould-based process.

3. Results

3.1. Mould-based assembly process

The devices assembled using the PEG mould-based method were subsequently used to post-process CMOS aluminium pads to form biocompatible electrodes, as discussed in [11]. The process requires the application of 60 V to the pads and this was also adopted as an electrical leakage to detect assembly defects. The expected current during this processing was an individual pin leakage, I_{in} , of $< 2\ \text{nA}$. During this time, any excessive electrical leakage between bondwires or bondpads to the electrolyte in the culture chamber would have been recognisable as $I_{in} \gg 2\ \text{nA}$. The assembly yield was 93% (28/30) demonstrating that the elastomer insulation was functioning correctly. Disassembly of a functional device showed a good width ($\geq 200\ \mu\text{m}$) of elastomer between well and bondpads: Fig. 4a shows the location of the elastomer removed from the die which was flipped over to give the underside view (Fig. 4b). A marginal device was found where – although functional – a single electrode in the array of 48 was almost covered by the creep of elastomer under the PEG mould. This was probably due

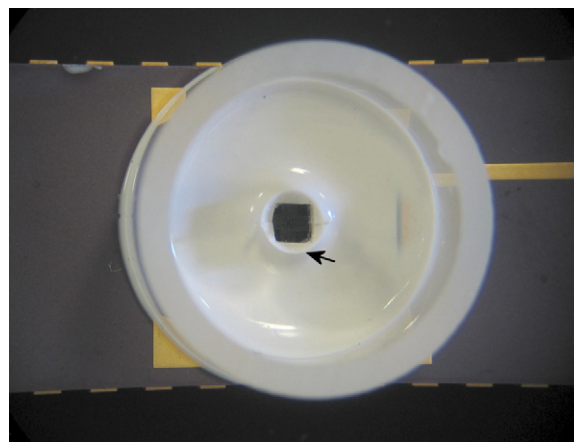


Fig. 5. A completed biosensor using the PEG mould-based process. The square die cavity opens to the conical aperture. The arrow highlights the elastomer meniscus, visible as a circular ring around the edge of the aperture.

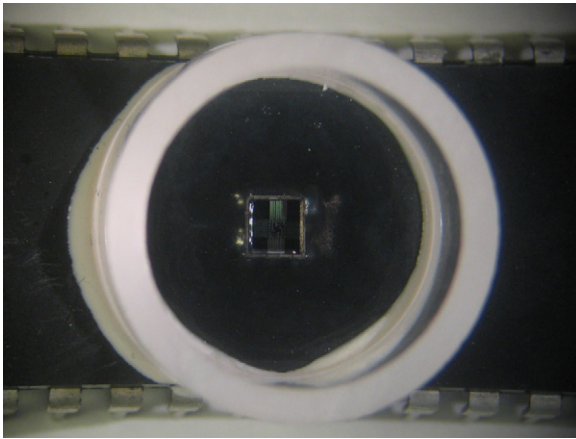


Fig. 6. A completed biosensor assembled using partial encapsulation by Quik-Pak. Adhesion of the glass culture chamber was the only manual assembly step.

to the mould not being lowered square to the die surface and confirms that the placement of the waxy PEG and the lowering of the mould require care. One of two failures did fail the leakage test (with $I_{in} \geq 1 \mu\text{A}$ and the set limit of the test) which analysis showed was due to misplacement of the PEG mould that had caused the bondpads to be exposed to the sensor well. The other assembly defect was due to mis-processing. Although not attempted, the process does allow an IC to be reworked where a mould is incorrectly placed: the mould can simply be lifted and the die cleaned of PEG in hot water. Such rework is not possible with an epoxy ring process.

It can be seen from Fig. 5 that during application of the moulding compound a meniscus is formed around the PEG mould. This leaves a 'spout' at the top of the well which is unlikely to have an effect on the cells cultured within the well. Attempts to modify the properties of the elastomer, such as by addition of 10 wt% silicone fluid, did not reduce the meniscus. It may be possible to eliminate it by a second application of elastomer to top-up its level to the lip of the spout, but this has not been attempted.

3.2. Partial encapsulation assembly process

The assembly yield for the Quik-Pak partial encapsulation method was 90% (18/20), although this can be considered worst-case since the two failures were considered by Quik-Pak as process setup samples. All 18 devices passed leakage tests. The partially encapsulated devices had consistent and regular windows (Fig. 6).

3.3. Biocompatibility

Fig. 7 shows cells in culture on the biosensors. After 14 days, cell vitality tests (sample size, $n \geq 11$) showed significantly (Welch's t test, $p \leq 0.001$) more cells on the Quik-Pak devices versus the devices assembled with the Silastic 9161 elastomer (Fig. 8). Visual inspection of the devices showed no evidence of compound incompatibility or corrosion problems. This statistical analysis treated the package type as a single factor. However, since there are many differences between the two package types (e.g. well shape and mould compound), these might be expected to result in non-parametric variances. It was for this reason that the non-parametric Welch's t test was chosen as a test of significance. This test is applicable to these samples where the *absolute* variances of cell count cannot be considered equal. It is also noted that, relative to their respective means, the variances are not so dissimilar as might be suggested by Fig. 8, i.e. the *relative* standard error (RSE) for the mould-based and partial-encapsulated devices was 82% and 75%, respectively.

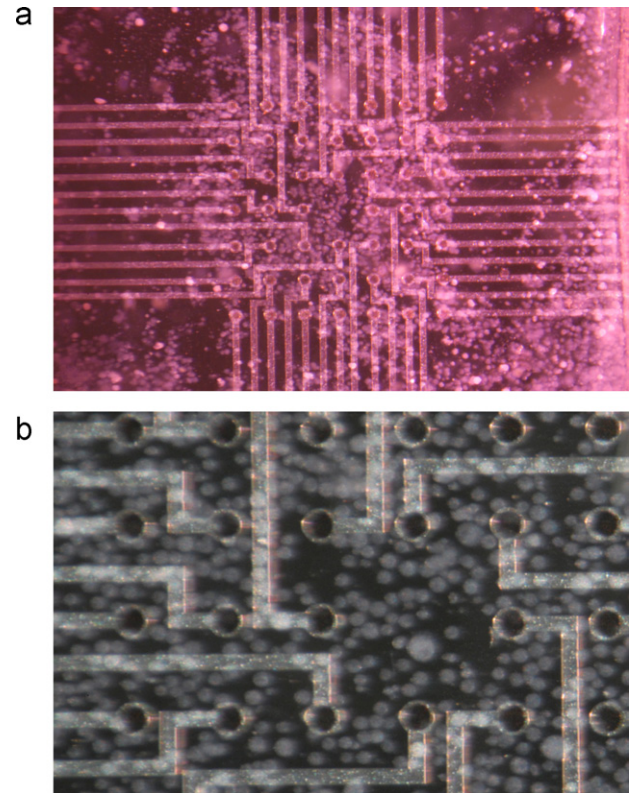


Fig. 7. Cells in culture on the MEAs: (a) NG108-15 cells plated on an IC (Quik Pak). The poor image resolution is due to the optical interference of the pigmented cell culture medium; (b) for improved resolution, the culture medium was replaced by a clear recording buffer prior to imaging for cell counting, as described elsewhere (Graham et al., 2009b). Resolution remains limited due to imaging under physiological conditions (i.e. through the solution). For scale, both images show circular electrode diameters of 30 μm .

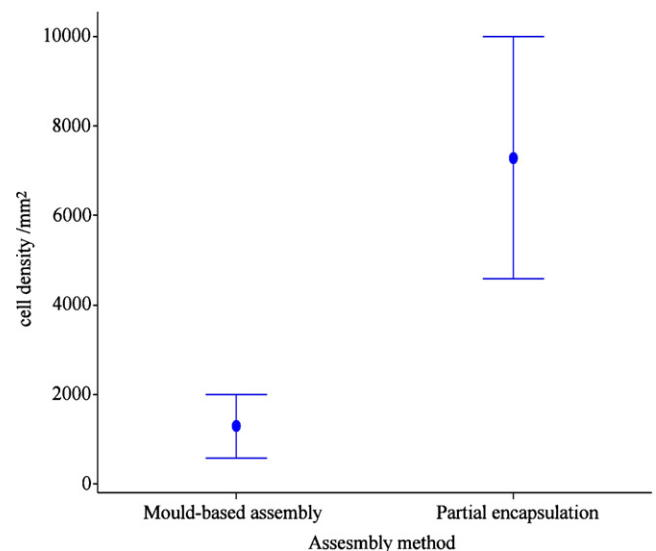


Fig. 8. NG108-15 cell density after 14 days in culture on the biosensors assembled with the mould-based process (mean, $\bar{x} = 1280 \text{ mm}^{-2}$; standard deviation, S.D. = 1054 mm^{-2} ; sample size, $n = 11$) and the partial encapsulation process ($\bar{x} = 7283 \text{ mm}^{-2}$, S.D. = 5445 mm^{-2} , $n = 14$). These results show there were significantly more ($p < 0.001$, Welch's t -test) cells on the Quik-Pak partial encapsulation devices. Error bars represent 95% confidence intervals with markers indicating means.

4. Discussion

Both assembly methods were demonstrated to produce the desired packaging structures, suitable for the culture of cells but also for a range of other biosensor applications including bioreceptors such as antibodies, enzymes, proteins and DNA and other forms of electrochemical transducer [15]. However, the two methods differ in performance both objectively (i.e. cell vitality results) and subjectively (e.g. ease of use): as discussed below, these aspects have influenced the authors' preferred method that has been chosen for use in future work.

4.1. Appraisal of assembly processes

A benefit of the simple mould-based method is that it allows small and compact ICs to be assembled in the research lab, requiring only a general metal workshop for tooling and some form of jig for accurate positioning of the mould prior to the application of elastomer. However, unless multiple jigs are available, the method is limited by the serial nature of the process – i.e. only a single device can be assembled at a time, each requiring four hour's curing before the jig can be reused. Further, the formation of the meniscus (Fig. 5) was undesirable and, whilst we do not believe it to be a problem with this particular application of cell culture, it may pose a problem for other biosensor applications. Neither of these limitations exists with the Quik-Pak process.

The authors also had an initial concern of using PEG for the mould since it may modify the nature of a substrate and reduce cell adhesion [16]. However, the PEG is dissolved during the assembly process and the die surface undergoes considerable further processing in the anodising acid and plating solutions which should ensure the PEG is removed. Additionally, the aluminium electrode surfaces are modified by the anodisation and plating processes and so any remaining PEG monolayer should be removed by these steps.

The Quik-Pak process has several advantages over the mould-based process. Firstly, the Quik-Pak process is available not only for DIL but also for other package outlines (e.g. utilising any open cavity package, such as QFN, QFP, SOIC and SSOP, as supplied by Sempac Inc., Sunnyvale, USA). Secondly, it avoids the precision manual processing required when assembling these devices in the research laboratory. Thirdly, it leverages the process quality typically achieved on a commercial manufacturing line. The Quik-Pak approach is therefore more conducive to commercial biosensor applications, providing good yields and consistent positioning and formation of the sensor windows. Lastly, the total cost for packaging of 18 die by Quik-Pak (£1100) was comparable to having the die attached and wire-bonded in ceramic packages (£880) ready for the PEG mould-based process. However, these ceramic packages then require the further processing involving manual elastomer assembly and therefore the combination of these tasks must represent a higher overall cost compared to the Quik-Pak process.

4.2. Biocompatibility

The reason for the improved vitality on the Quik-Pak devices is not clear, but three differences are noted: firstly, the use of PEG as a mould; secondly, the shape and size of the sensor well; and thirdly, the Silastic 9161 is not present on devices assembled by partial encapsulation. These are discussed below:

1. As previously mentioned, the PEG is likely to be removed during the CMOS post-processing (anodisation and plating of electrodes) followed by subsequent ethanol sterilisations and poly-L-lysine coatings. Each of these steps also includes several rinse cycles. However, it is perhaps conceivable that residue of the waxy PEG could have persisted in the voids shown in Fig. 4b (iii) as these areas require lateral dissolution of the waxy PEG

once the bulk of the PEG mould has been almost completely dissolved. Under these circumstances, further dissolution of any PEG residue could be expected to affect the cell culture. However, examination showed no evidence of PEG after disassembly. Further work, e.g. chemical labelling of the PEG and spectroscopy, could be performed to explore this.

2. The shape of the well formed with the mould-based process would, if anything, be expected to increase the number of suspended cells guided to the IC sensor rather than decrease the number since the elastomer meniscus forms a funnel. This is therefore inconsistent with the observation.
3. The final factor is the packaging compound which, due to the different nature of the surfaces, seems to be a good candidate to explain the observed differences in cell vitality [17]. For example, surface chemistry and morphology are known to affect cell-substrate adhesion [18,19]. However, this requires further work to confirm whether this is indeed the primary reason for differences in cell vitality.

4.3. Overall assessment

The Quik-Pak approach demonstrated good biocompatibility, avoided many of the drawbacks of the mould-based approach and was more cost-effective. For these reasons, the Quik-Pak process is the authors' preferred solution and provides an efficient solution to the problems of assembling biological sensors for the research laboratory.

It is acknowledged that the results are not generalisable beyond the evaluation of these two packaging methods. This is because the two methods encompass several differences that each can be considered as separate factors and each potentially affecting cell vitality. If a deeper understanding of these factors is required then further experiments must be performed. This could be a multi-factorial experiment or, for increased generalisation, substrates coated with each material (i.e. Hysol CB064, Silastic Medical Adhesive Silicone Type A, Silastic 9161 RTV, PEG-1000, PEG-1450, PEG-35,000) could be evaluated.

5. Conclusion

We have evaluated two new assembly methods for prototype biological IC sensors which meet our requirements of a 14 day lifetime and where die are small (<20 mm²). The method of manual encapsulation of bondwires using a PEG mould-based process and the method using commercial partial encapsulation by Quik-Pak both yielded functional sensors. Cell vitality tests showed the partially encapsulated devices were biocompatible, with cell densities exceeding those of the devices assembled with the Silastic 9161 RTV elastomer. The mould-based method allows small and compact ICs to be assembled in the research lab with relatively simple equipment. However, overall, the Quik-Pak process, available for DIL and other package outlines, provides a more efficient solution for assembly of biological sensors and avoids the precision manual processing required when assembling these devices in the research laboratory. The observed biocompatibility bodes well for commercial assembly of biosensor ICs.

6. Conflict of interest

No conflicts of interest are known to the authors.

Acknowledgements

The authors would like to thank Dr Andrew Dent and Pete Thomas for their helpful suggestions relating to moulding

technology, Dave Parker for his workshop assistance, Frank Hammett and Dr Siva Sivaraya for their technician support and Dr Duncan Allsopp and Dr Federica Causa for the loan of apparatus. This work has been partly funded by the UK Engineering and Physical Sciences Research Council (EPSRC) via a doctoral training grant and by the University of Bath's Enterprise Development Fund.

References

- [1] Darveaux R, Munukutla L. Critical challenges in packaging MEMS devices. In: Proc IEEE/SEMI adv semicon mfg conf. 2005. p. 210–7.
- [2] Chen CH, Yao DJ, Tseng SH, Lu SW, Chiao CC, Yeh SR. Micro-multi-probe electrode array to measure neural signals. *Biosens Bioelectron* 2009;24:1911–7.
- [3] Ardebili H, Pecht MG. Encapsulation technologies for electronic applications. Netherlands: Elsevier; 2009.
- [4] Offenhäusser A, Sprossler C, Matsuzawa M, Knoll W. Field-effect transistor array for monitoring electrical activity from mammalian neurons in culture. *Biosens Bioelectron* 1997;12:819–26.
- [5] Heer F, Hafizovic S, Ugniwenko T, Frey U, Franks W, Perriard E, et al. Single-chip microelectronic system to interface with living cells. *Biosens Bioelectron* 2007;22:2546–53.
- [6] Frey U, Sanchez-Bustamante CD, Ugniwenko T, Heer F, Sedivy J, Hafizovic S, et al. Cell recordings with a CMOS high-density microelectrode array. In: Proc 29th intl conf IEEE EMBS. 2008. p. 167–70.
- [7] Hammond PA, Cumming DRS. Encapsulation of a liquid-sensing microchip using SU-8 photoresist. *Microelectron Eng* 2004;73–74:893–7.
- [8] Delille R, Urdaneta MG, Moseley SJ, Smela E. Benchtop polymer MEMS. *J Microelectromech Syst* 2006;15:1108–20.
- [9] Patel BA, Arundell M, Quek RGW, Harvey SLR, Ellis IR, Florence MM, et al. Individually addressable microelectrode array for monitoring oxygen and nitric oxide release. *Anal Bioanal Chem* 2008;390:1379–87.
- [10] Alcantar NA, Aydil ES, Israelachvili JN. Polyethylene glycol-coated biocompatible surfaces. *J Biomed Mater Res Part A* 2000;51:343–51.
- [11] Graham AHD, Bowen CR, Robbins J, Taylor J. Formation of a porous alumina electrode as a low-cost CMOS neuronal interface. *Sens Actuator B-Chem* 2009;138:296–303.
- [12] Chauvel-Lebret DJ, Auroy P, Bonnaure-Mallet M. In: Dumitriu S, editor. *Polymeric biomaterials*. New York: Dekker; 2002. p. 311–60.
- [13] Peterson SL, McDonald A, Gourley PL, Sasaki DY. Poly(dimethylsiloxane) thin films as biocompatible coatings for microfluidic devices: cell culture and flow studies with glial cells. *J Biomed Mater Res Part A* 2004;72A:10–8.
- [14] Graham AHD, Bowen CR, Taylor J, Robbins J. Neuronal cell biocompatibility and adhesion to modified CMOS electrodes. *Biomed Microdevices* 2009;11:1091–102.
- [15] Velusamy V, Arshak K, Korostynska O, Oliwa K, Adley C. An overview of food-borne pathogen detection: in the perspective of biosensors. *Biotechnol Adv* 2010;28:232–54.
- [16] Heuberger M, Drobek T, Spencer ND. Interaction forces and morphology of a protein-resistant poly(ethylene glycol) layer. *Biophys J* 2005;88:495–504.
- [17] Chauvel-Lebret DJ, Pellen-Mussi P, Auroy P, Bonnaure-Mallet M. Evaluation of the in vitro biocompatibility of various elastomers. *Biomaterials* 1999;20:291–9.
- [18] Williams DF. On the mechanisms of biocompatibility. *Biomaterials* 2008;29:2941–53.
- [19] Glasgow KC, Dhara D. An overview of the biocompatibility of polymeric surfaces. In: Kulshrestha AS, Mahapatro A, editors. *Polymers for biomedical applications*. Washington: American Chemical Society; 2008. p. 268–82.