This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Wafer-scale fabrication of glass-FEP-glass microfluidic devices for lipid bilayer experimentation

Johan G. Bomer, Alexander Prokofyev, Albert van den Berg, and Séverine Le Gac*

We report a wafer-scale fabrication process for the production of glass-FEP-glass microdevices using UV-curable adhesive (NOA81) as gluing material, which is applied using a novel “spin & roll” approach. Devices are characterized for the uniformity of the gluing layer, presence of glue in the microchannels, and alignment precision. Experiments on lipid bilayers with electrophysiological recordings using a model pore-forming polypeptide are demonstrated.

System design and fabrication

The device consists of two Borofloat™ glass substrates sandwiching a 12.5-µm thick FEP foil (Sabic BV, Enkhuizen, The Netherlands). The glass substrates comprise each a microfluidic channel (300-µm width and 100-µm depth), and the top substrate includes fluidic accesses. The FEP foil contains a 50-100 µm diameter aperture to support lipid bilayers. One wafer (4-inch diameter) comprises 21 identical microfluidic devices, with a footprint of 1 cm x 2 cm each (Fig. 2A).

The fabrication proceeds for the greatest part as published previously, although wafer-scale bonding brought essential modifications. The fabrication process, which is detailed in the supplementary information (See ESI), is illustrated in Fig. 1. First, as before, microchannels are wet-etched in both glass wafers, and reservoirs powder-blasted in the top wafer. In the next step, UV-curable glue (NOA81, Norland Product) is applied on the bottom glass wafer. To obtain a thin and uniform layer of glue across a whole wafer, we have developed an original “spin & roll” procedure, relying on spin-coating of NOA on a flexible substrate followed by its transfer by “rolling” the flexible substrate on the substrate to be bonded. As flexible substrate, a 1.6-mm thick layer of PDMS is employed, which is treated with oxygen plasma to increase its
wettability and secured on a dummy wafer before the spin-coating step. The PDMS slab is carefully rolled over the bottom wafer, and, after possible air inclusions have been wiped out, it is slowly removed, leaving a thin and uniform layer of NOA81 on the glass substrate (ca. 1.8 μm thickness). The FEP foil is placed over a dummy substrate, where it is slightly stretched to avoid formation of wrinkles, fixed by tape and treated with oxygen plasma to increase the wettability of glue on it. The NOA81-coated glass substrate is applied on the FEP foil, and, after removal of air inclusion, NOA81 is cured for 2 min (Electrovision mask aligner, EVG 620). Next, a shadow mask is carefully aligned with the wafer by hand under a stereomicroscope (with an accuracy of ca. 10 μm), and secured with the help of tape on the glass wafer-FEP stack. Next, apertures and fluidic accesses are dry-etched in the FEP layer as previously reported. Following this, the top glass substrate is glued on the glass-FEP stack using the same “spin & roll” procedure, a thicker gluing layer (ca. 3.1 μm) being used to ensure proper bonding. The layers are aligned using dedicated equipment (Electrovision mask aligner, EVG 620 and Electrovision Anodic Bonder, EV-501), before curing of NOA81. Finally, individual devices are released by dicing the wafer stack (Loadpoint MicroAce 3).

**Figure 1:** Process-flow of the wafer-scale fabrication of glass-FEP-glass devices. Schematic representation of one chip – not to scale. (1) Wet-etching of microchannels in two glass wafers (light grey) and powderblasting of reservoirs in the top wafer; (2) Application of NOA (black) on the bottom wafer using a PDMS slab; (3) Bonding of the FEP foil (dark grey) on the bottom glass wafer; (4) Dry-etching of micrometer-sized aperture and fluidic accesses in the FEP film using a shadow mask; (5) Application of a thicker layer of NOA on the top glass wafer; (6) Alignment and bonding of the top wafer with the rest followed by dicing into chips.

**Device characterization**

The characteristics of the gluing layer are essential in this bonding approach; the layer must be thick enough for proper bonding while avoiding glue in the channels. When assembling devices at the chip level, glue is found in the channels, along their edge, as shown in Fig. 2B, which affects the flow behaviour of solutions introduced by capillarity. On the contrary, wafer-scale bonding does not give rise to such issues (Fig. 2C). The thickness of the second layer of NOA is measured across a whole wafer (See ESI), and an average value of 3.14 ± 0.10 μm (n=8) is found, demonstrating excellent uniformity of the gluing layer. Imaging of the cross-section of a device confirms that the top layer of glue, applied in the second step, is thicker than the first one (Fig. 2D).

**Figure 2:** Picture of a wafer-scale fabricated glass-FEP-glass microdevice (A) with channels filled with ink for visualization purposes. Close-up on the channel intersection of devices fabricated at the chip level (B) and at the wafer-scale (C) showing the presence of glue in the channels (B) or not (C). Microscopy picture of a cross-section through the device (D) showing the FEP film with NOA81 glue on both sides, and the glass substrates. Microscopy picture of the channel intersection (E) revealing good alignment precision for wafer-scale fabricated devices.

The alignment precision is very good (Fig. 2E). Alignment at the wafer-scale is helped by the presence of dedicated marks and use of appropriate equipment, while at the chip level, alignment is more tedious, and simultaneous alignment of more than one feature is virtually impossible. Similarly, FEP etching is greatly facilitated, since the thin and fragile FEP foil is secured on a glass wafer during that step, and no dedicated tool is required to handle it. The wafer-scale approach is finally more efficient since 21 devices are assembled simultaneously within approx. 5 h, including etching of the FEP foil, while the chip level approach only for bonding, requires up to 1 h per device, depending on the experimenter.

**Lipid bilayer formation - electrophysiological recordings**

For lipid bilayer experiment, the device is placed in a dedicated chip-holder with reservoirs in which liquids are pipetted and Ag/AgCl electrodes inserted to connect the device to the headstage of a patch-clamp amplifier (Axopatch 200B, Molecular Devices, USA). Lipid bilayers are formed by first introducing buffer solution (1 M KCl, 10 mM HEPES, pH 7.0, 50 μL) in the bottom channel by capillary action, followed by lipid solution (25 mg/mL DPhPC in n-decane, 0.2 μL) in the top channel, which is finally replaced by buffer (50 μL). The lipid bilipot forms spontaneously in the aperture as soon as buffer reaches the aperture. Lipid bilayers exhibit excellent stability even under application of a voltage (up to 100 mV), as well as suitable properties for electrophysiological measurements. The seal resistance lies in the gigaOhm range (37 ± 9 GΩOhm), demonstrating the potential of the devices for single ion
channel recordings (Fig. 3). The capacitance is 29 ± 3 pF, from which the specific capacitance $C_s$ (0.59 ± 0.06 µF/cm²) is determined when taking into account the surface area of the bilayer. Noteworthy, $C_s$ values are in good agreement with previous reports.25

<table>
<thead>
<tr>
<th>$R_{SEAL}$ [GΩ]</th>
<th>$C_M$ [pF]</th>
<th>Area [µm²]</th>
<th>$C_s$ [µF/cm²]</th>
<th>n</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 ± 9</td>
<td>29 ± 3</td>
<td>62 ± 1</td>
<td>0.59 ± 0.06</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 3: Electrophysiological measurement in a wafer-scale assembled lipid bilayer microdevice. (Top) Characteristics of the lipid bilayers in terms of seal resistance ($R_{SEAL}$), capacitance ($C_M$), relative surface area (Area) and specific capacitance ($C_s$) (n=9 experiments in N=3 devices). (Bottom) Electrophysiological recordings of gramicidin (25 mg/mL DPhPC; 1 nM gramicidin; buffer: 1 M KCl, 10 mM HEPES, pH 7.0; 100 mV; sampling rate of 10 kHz; 1 kHz low-pass Bessel filter). Inset: picture of a lipid bilayer in a 100-µm diameter aperture.

Next, we perform single ion channel recordings using DPhPC bilayers and the gramicidin ion channel26 (Sigma-Aldrich, Zwijndrecht, The Netherlands), which is added to the phospholipid mixture prior to BLM formation (1 nM gramicidin in 25 mg/mL DPhPC in n-decane). The single channel current is recorded under symmetric conditions (1 M KCl, 10 mM HEPES, pH 7.0 buffer) by monitoring the current through the lipid bilayer (100 mV dc voltage; 10 kHz sampling rate; 1 kHz low-pass Bessel filter), showing continuous assembly and disassembly of gramicidin monomers in the membrane (Fig. 3). The average pore conductance is 34 ± 2 pS, which is similar to what we found with our chip-level assembled device.2 Finally, the noise level in these experiments is as low as 0.3 pA rms, which is comparable to manually assembled devices.

Conclusions

In this article we have presented a novel wafer-scale approach to assemble glass and FEP substrates using UV-curable glue. This wafer-scale approach is highly advantageous, not only by allowing larger scale production of devices, but also by providing higher control for the application of glue. This second aspect is essential to enhance the bonding quality through deposition of a uniform layer of glue, while reducing the risk of glue contamination in the channels. Furthermore, other steps of the fabrication process are greatly facilitated by the wafer-scale process, such as dry-etching of the FEP film and alignment of the different layers. Finally, the resulting devices yield similar performance for electrophysiological experiments and single ion channel recordings as their chip-level bonded counterparts.

Acknowledgements

The authors would like to thank Dr. E. X. Vrouwe and V. C. Stimberg for their critical comments on the manuscript. This work is supported by the Dutch-German Interreg IV program DIAMANT (MicroBLM project) and NanoNextNL, a micro and nanotechnology consortium of the Government of the Netherlands and 130 partners.

Notes and references

BIOS - Lab on a Chip group, MESA+ Institute for Nanotechnology, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands.

Electronic Supplementary Information (ESI) available: Details of fabrication process steps and on the device characterization (assessment of the thickness of the gluing layer). See DOI: 10.1039/c000000x/

We report a wafer-scale fabrication process for the production of glass-FEP-glass microdevices using UV-curable adhesive (NOA81) as gluing material, which is applied on the glass substrates using an original “spin & roll” approach.