Appendix A

Process development

Changing one parameter can sometimes require many adaptation in the initial process. That is what we faced in this project. In this annexe will be gathered evolutions of the process, problems and proposed solutions.

Fabrication

Electroplating

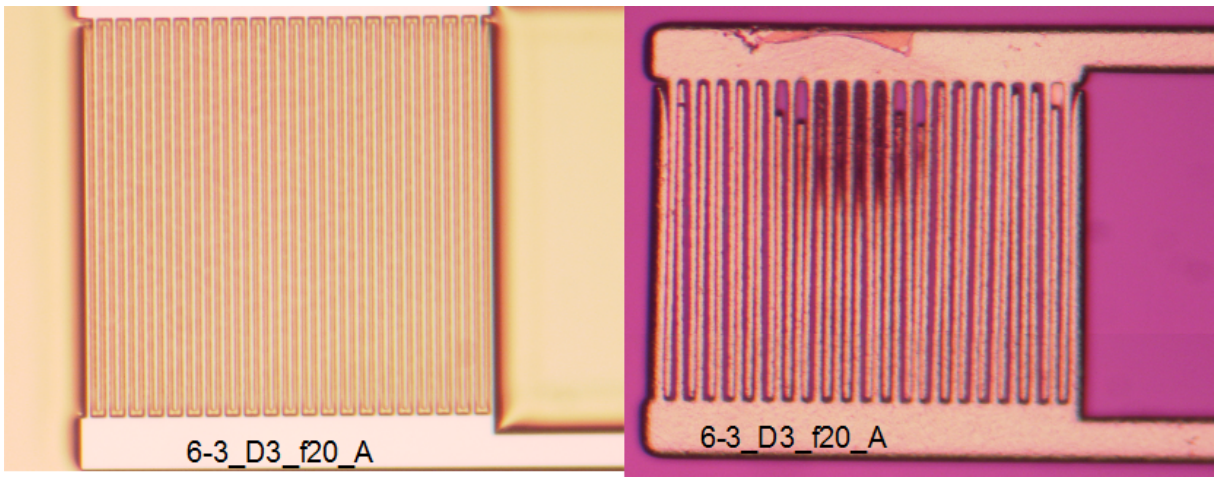

Figure A.1: These pictures show that even if the resist was very well developed, several problems could still happen.

The electroplating was the most difficult step to control. Many parameters could influence the final result. The resist obviously plays a key role on the final electrode geometry but a clearly defined resist pattern will not always results in operational sensors (Figure A.1).
Black areas kept appearing on each sensors right after the electroplating step. They were incomplete deposition spots were the electrode thickness was lower than on the rest of the sensor. To get rid of them, we tried to raise the plating solution temperature. We did see an improvement, almost no more black areas, however, electrodes stayed incomplete. This phenomenon is less likely to happen on thinner electrodes.
Sometimes, such black spots on nickel are mentioned in the literature as the result of an uneven deposition rate that would cause a current concentration and therefore a "burning" of the nickel locally. We supposed that an uneven deposition could be caused by air bubbles in which nickel

ions can't diffuse as well as in the nickel sulfamate solution. Therefore, we tried to systematically rinse each wafer before the electroplating step. This reduced significantly the apparition of those black areas.

Microfluidic : PDMS caps

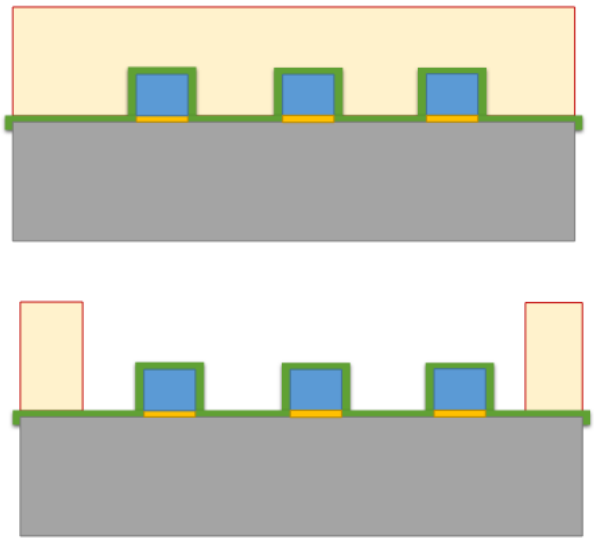

Figure A.2: Fabrication of KMPR walls to support the PDMS cap.

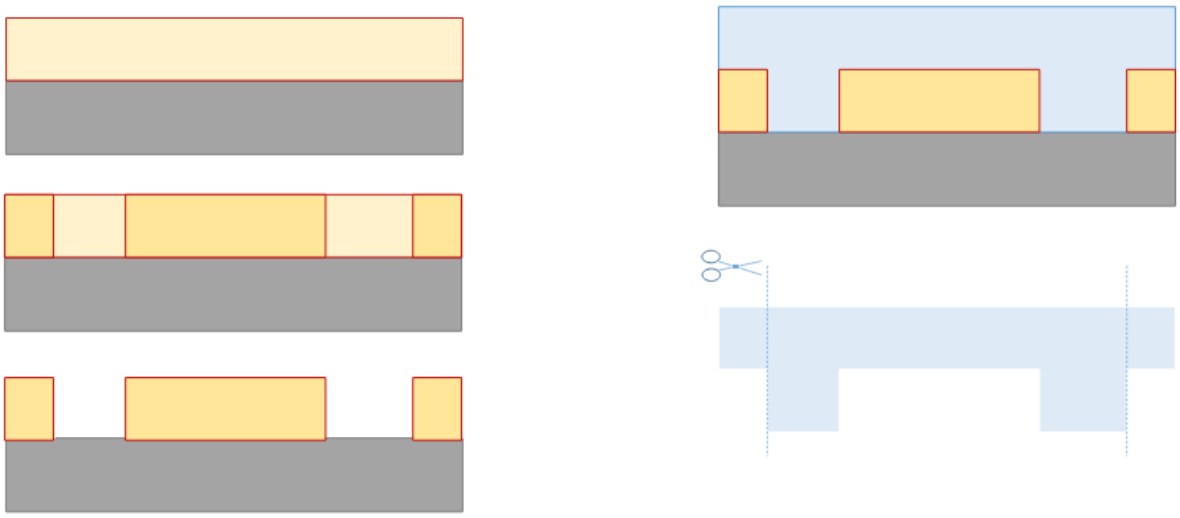

Figure A.3: Fabrication of PDMS caps on a KMPR mould.

Resist bonding

The standard oxygen plasma treatment to seal microfluidic cells was not really reliable. This is why we tried to use a resist-based bonding. SU-8 resist has quickly been abandoned, because of its too low viscosity it was not possible to spread it on a precise small surface. Moreover, its adherence to PDMS was almost null.

An other treatment has been tested involving the **ORDYL** resist.
After pre-heating the chip during 10 minutes at 80 °C, the resist is spread over the KMPR on the chip. It is then put back to 80 °C during 2 minutes. The PDMS cap is pressed on the chip after 5 minutes of cooling. Maintained under pressure, the chip is put back again in the heat chamber at 90 °C during 30 minutes.

Microfluidic tests were not conclusive. Despite appearances, the resist might not be deposited all over the KMPR surface in an uniform way. On such a small surface, a single discontinuity could lead to a leak.

Measurement system

PM8 PS vs PS5026 A

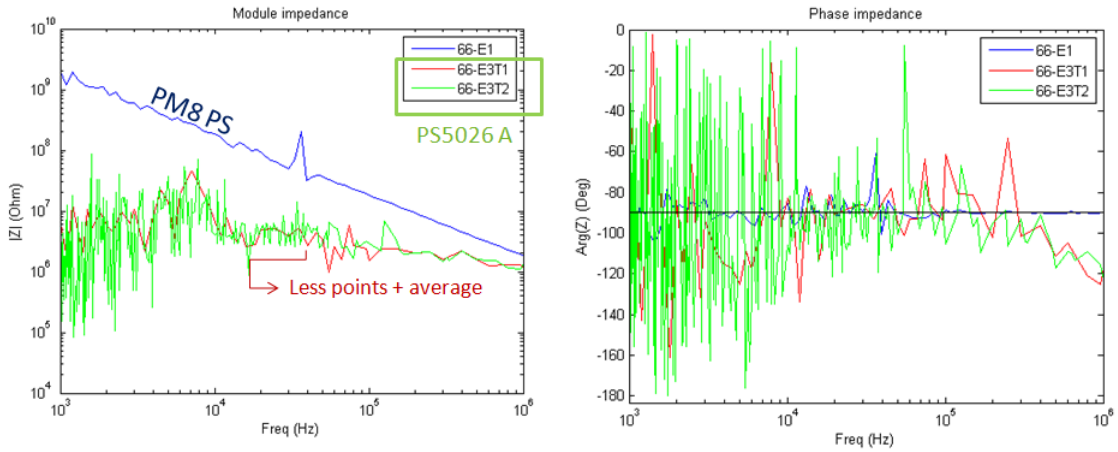

Figure A.4: Comparison of signals for the same sensor with different probe station.

Once our sensors fabricated, we still had to find an efficient way to test them. Our aim was to collect impedance signal from these sensors under various conditions, such as air, liquid (PBS) and bacterial solution.
To do so, microfluidic was an obvious choice.

A complete system of measure include a probe station connected to a LCR which send instructions and collect responses then send to a software (LabView) which will control the whole process. LabView has been used with some of the codes used by Numa Couniot, which were adapted for the desired application. The peristaltic pump remains under manual control.
The optimum use of the association of the probe station *PS5026 A* and LCR 4284 AII required several days of tests and improvements mainly because this probe station does not possess an efficient handling of the sample which means that scratching the pads' surface to remove the insulating layer is complicated and uneven.

PBS corrosion:

We run an another test in parallel with bacteria.

A chip of wafer 4-9 ($11\mu m$ thick), has been exposed to PBS for the same duration as a test cycle, so that is to say about 4 hours. The aim of this test is to observe the action of PBS on the insulator layer (alumina). Indeed, we have been told that PBS could attack this layer and

therefore cause a spontaneous change on impedance signal.

The PBS was dropped off on the chip with nothing else than the KMPR wall. To counteract the evaporation, the drop was renewed 3 times.
Measures were made 1 min after the first drop (= T0) and at the end of one cycle of experiment (=Tf) on two sensors with the same dimensions.

These results are worrying insofar as they show a quite large decrease in impedance modulus and change in phase. The modulus show a shift of 8% with frequencies above $10^{4.5}$ Hz.
However, we must keep in mind that this test has been done under different condition than the others in so that PBS drops were subject to evaporation and this means the salt concentration has been changed all along the test!

Therefore this result was not taken into account for the signals analysis but it shows that this type of sensors are very indeed very good conductance detector.

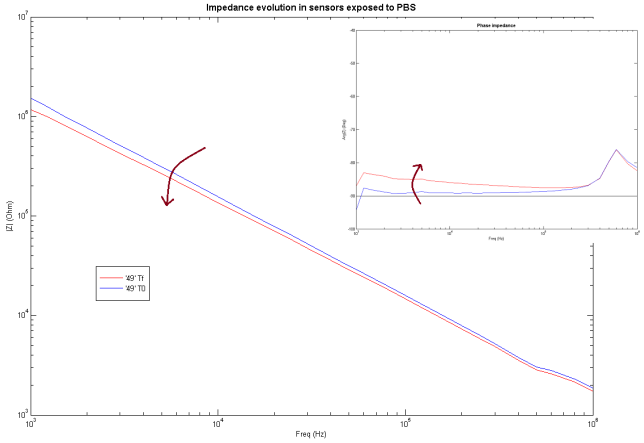

Figure A.5: Evolution of impedance measure on sensor exposed to PBS over time. The blue curve is the initial signal and the red one is an average of final data (after 4h).

Polydopamine aggregates:

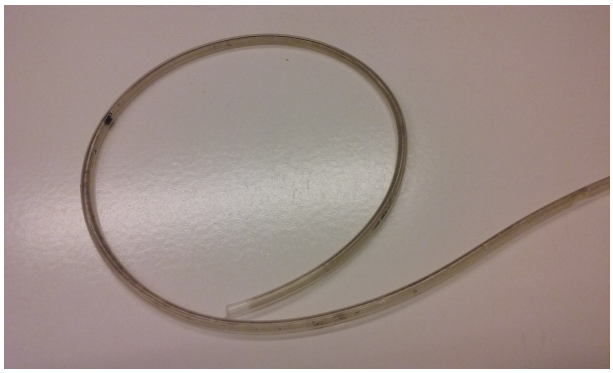

Figure A.6: Microfluidic tube after polymerisation of dopamine.

The Dopamine is polymerised directly on the chip. However, it also polymerize on the tubes' walls (Figure A.6 and creates aggregates which can detach and end up on the chip. Even if the

chip is washed with PBS before the bacteria entry, those polydopamine aggregates could still influence the final impedance.

A.1 Biological preparations

TSB used as culture media for *S.epidermidis* growth was prepared by mixing 30 g of powder (Biorad) in 1L of DI water. Then it is autoclaved 15 min at $121°C$ before being stored at room temperature.
TSA which has a solid aspect, is obtained by adding $1,4\%$ of Agar before the autoclave. At $50°$, it is poured in Petri dishes and stored at $4°C$.
PBS is used to prepare bacterial suspension and dilutions. It is composed of different salt and its pH is 7,4. It is prepared by diluting one dose in 200 ml. Autoclaved in closed bottles 15 min at $121°C$, PBS can be stored at room temperature.