4.5.2. CAPPING ALTERNATIVES

Rubber capping

Another standard chip sealing alternative found in the literature is the use of rubber gaskets, plus clamping, to ensure a tight closure of the access holes ([Wilding1994], [Shoffner1996], [Cheng1996b]). This is carried out by mechanizing a hole onto the copper block and partially fitting a rubber gasket inside it, which is then squeezed by physical clamping of the chip. However effective at sealing, this method introduces again the problem of a physical gap between the chip and the underlying copper block if the gasket hole has been under-drilled, or the drawback of ineffective capping if it has been over-drilled.

Figure 95 - Sealing with rubber gaskets and problems arising from under-drilling (a) or over-drilling (b) of the gasket hole.

Since, at the time, and after a considerable amount of unsuccessful PCR assays, the present research had already attained the label of paranoia chain reaction regarding temperature accuracy (see p.82), the basic scheme of rubber gasket sealing was discarded beforehand, even though an alternative methodology was concocted to attain similar capping efficiencies while overriding the foreseeable problems of *Wilding's* team scheme.

The alternative rubber sealing method was to create a holding device to clamp the chip and use a small Peltier cell to heat the chip. The holding device was fabricated in Teflon (to avoid reaching the melting point of methacrilate, see *Materials and Methods*, p.297) and an oxygen-free copper block was also mechanized to fit the space between the cold side of the Peltier cell and the heat dissipater. The holding piece had two machined

indents that housed small rubber toric joints and could be tightly clamped with Allen screws, effectively sealing the chip access ports as illustrated in Figure 96. Temperature sensors were held into place using rubber-ended mini-tweezers.

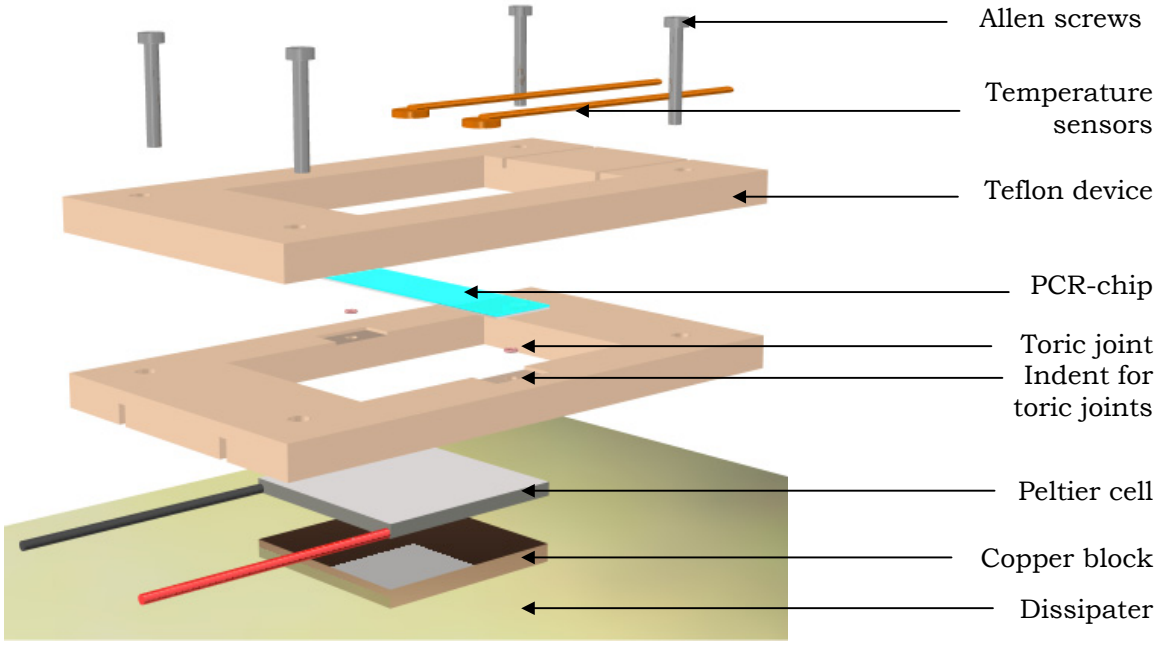

Figure 96 - Schematic view of the holding/clamping device for sealing with rubber torical joints.

Although the device worked well and effectively sealed the PCR-chip access ports, its management and positioning on the heat dissipater were a bit cumbersome, a fact to be noted when thinking about repeated experiments. Moreover, the system only worked with the available 30x30 mm^2 - 33W Peltier cells, which permitted the use of clamping devices (see Figure 69, p.150), but yielded poorer transient times (see Figure 85, p.172). Therefore, a last attempt was made to find a more versatile and efficient clamping system that fitted the present thermocycler design and could make use of the available 40x40 mm^2 - 68W Peltier cells.

Mineral oil capping

Capping the PCR mix with a thin layer of mineral oil has been, for years, the standard procedure when conducting PCR in a conventional

thermocycler that lacks an upper hot-plate (see p.68) and, although UV-irradiated mineral oil has been pointed out as a possible PCR inhibitor [Dohner1995], the technique using non-irradiated oil has become firmly established over the years. This method has also been approached in PCR-chips. *Burns et al.* [Burns1996] report using a drop of mineral oil to effectively seal their PCR-devices. However, in their approach, *Burns et al.* use chips with access holes drilled onto the glass wafer and, thus, they only need to fix a toric joint to the chip and cover the hand-made reservoir with mineral oil. The approach taken herein was substantially different. Mineral oil 50 µl drops were deposited with a micropipette on top of the access holes, and the chip was then flipped, positioned onto the copper block surface and clamped to secure the closure.

Mechanical setup

To allow effective clamping, ∅6 mm screw-matching holes were bored on the heat dissipater and ∅6 mm screws were inserted and used as pedestals for custom metallic pincers that were set in place between two nuts (see Figure 97). The custom metallic pincers ends were capped with Teflon film, in order to minimize heat transfer from the clamped chip to the metallic structure. A third pedestal-pincer pair was used to secure the Pt100 sensor to the base chip.

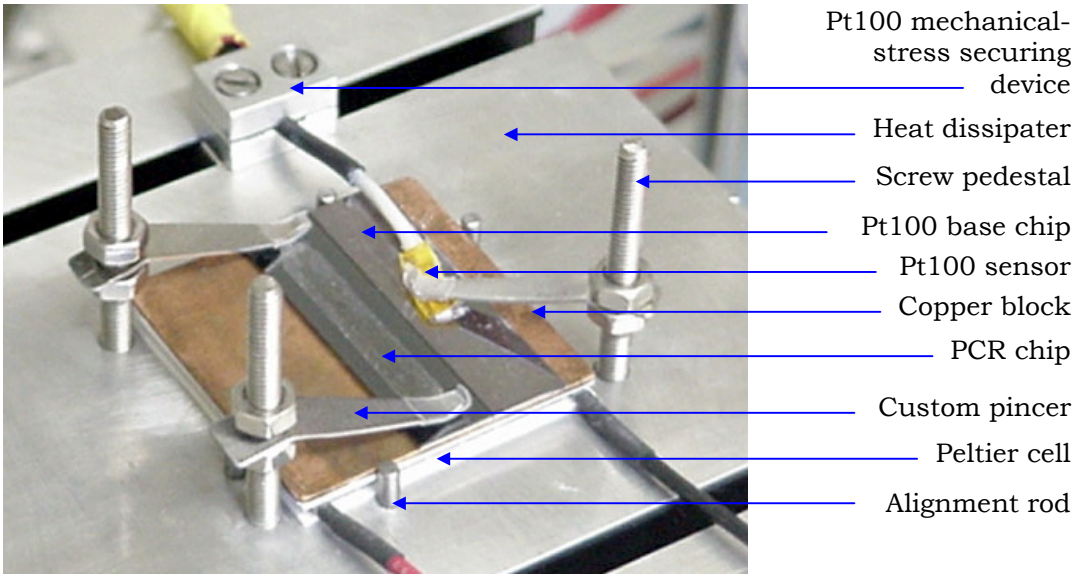

Figure 97 - Chip and Pt100 clamping using screw pedestals and custom pincers.

Using this setup, PCR experiments were carried out successfully, with no apparent evaporation of sample. The only drawback of mineral oil capping was that, over time and mostly during reagent extraction, small amounts of oil tended to enter the chip and were of difficult removal. Furthermore, attempts at cleansing them with organic detergents were not only unsuccessful, but they also introduced PCR inhibitors into the chip, rendering them useless for further PCR amplifications. With the setup and the experience gained from the mineral oil capping approach, direct capping (without mineral oil) was assayed to assess its feasibility as a sealing mechanism.

Direct capping

The principle of the direct capping method here implemented is quite straightforward. As in mineral oil capping, the chip is flipped, positioned onto the heat sink with its silicon side facing downwards and clamped with the custom made pincers. However, fearing that the copper block might free inhibiting ions into the PCR mix, the chip was not directly positioned onto the copper block surface. Instead, bare 300 µm silicon oxide-passivated chips were fabricated with the same dimensions of PCR-chips and used as base chips for clamping the PCR-chip (see Figure 98), thus ensuring that the PCR sample would only make contact with SiO_2-passivated silicon.

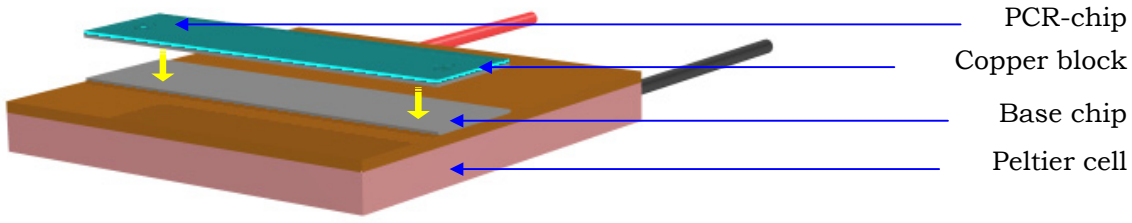

Figure 98 - Schematic view of the direct clamping technique using a SiO_2-passivated base chip for biocompatibility issues.

PCR experiments were successfully and repeatedly carried out using this scheme, and partial evaporation of the sample was only observed in sporadic cases. Therefore, it was clear that direct capping posed many advantages with regard to other capping strategies. On the one hand, it did not introduce any foreign materials in the setup (such as acrylic tape or rubber gaskets), the biocompatibility of which would have had to be separately assessed and thereafter independently accounted for. On the

other hand, direct capping did not require the presence of any underlying thermal conducting layers in direct contact with the chip (as in mineral oil capping), thus reducing the possibility of chip contamination with extraneous material.

Capping chips

To further enhance the reproducibility of the direct capping technique, a new batch of chips was fabricated making clever use of the existing photolithography masks. The new chips were insulated using the access hole-defining mask, but with positive, instead of negative, photo-curable resist. This meant that, after insulation, development and a hard bake to withstand ulterior processes, the resist would only be present in the holes area (see Figure 99). Silicon was then deep etched (100 µm) on one side with TMAH (see *Materials and Methods*, p.293) and, after washing away the remaining resist, the chips were passivated with a thin (380 Å) thermal silicon dioxide layer.

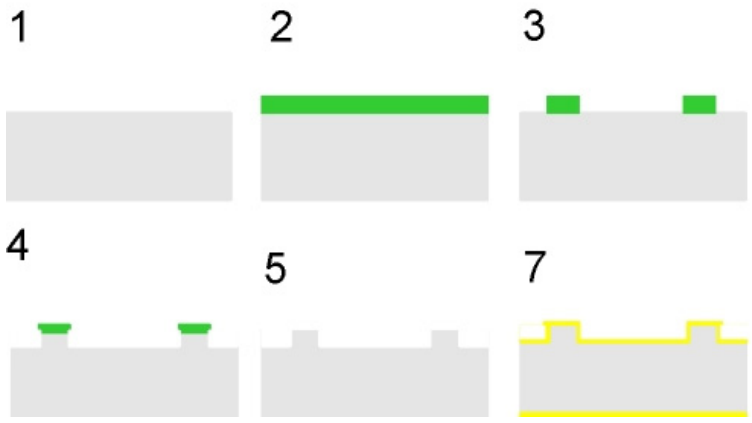

Figure 99 - Schematic view of the technological steps for producing capping chips. A thick positive resist is deposited (2) and patterned with hole defining masks (3) onto a 300 µm-thick silicon wafer (1). The wafer is then deep etched with TMAH (4) and the resist remnants washed away (5). Finally, the wafer undergoes thermal oxidation to create a passivation layer.

The resulting chips provided efficient direct capping, with no observed leakage or evaporation of the PCR mix after repeated experiments, and they, together with the direct capping technique, were subsequently used in all the further PCR experiments with PCR-chips that are described in the following sections.