Technical notes towards an efficient experiment using droplets as incubators for the immunology study

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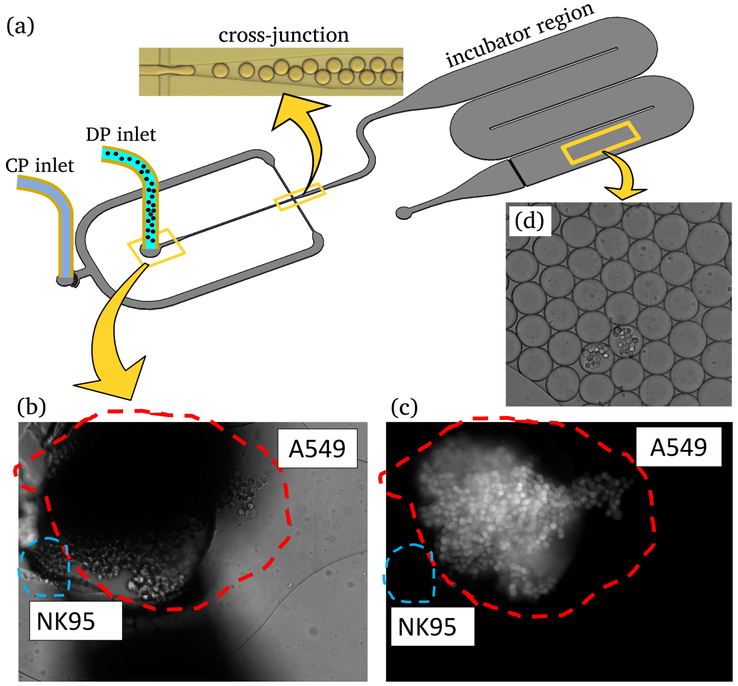
Droplets as micro-incubators is a common activity in the droplet microfluidic field. Here we will discuss some some technical challenges and its solution.
1. Cells supply problem (see image below)
2. Pervaporation of droplets: PDMS absorbs medium or water, similar to a sponge.
3. Immobilization of droplets inside the incubator region, difficulties for tracking the droplets (and what's inside) during image analysis

Objective of the experiment (engineering part only):
1. ~10k droplets per observation, volume 0.3~0.5 nL.
2. Observation of the cells within droplets for around 24 hr.



(a) Schematic of initial experiment setup. Agglomerates of (b) NK cells (NK95) and (c) the cancer cells (A549) at the DP inlet. (b) and (c) are bright field image and fluorescence microscopy image, respectively. (d) No cells encapsulated inside the droplets, or there is a clamping of cells within one droplets.

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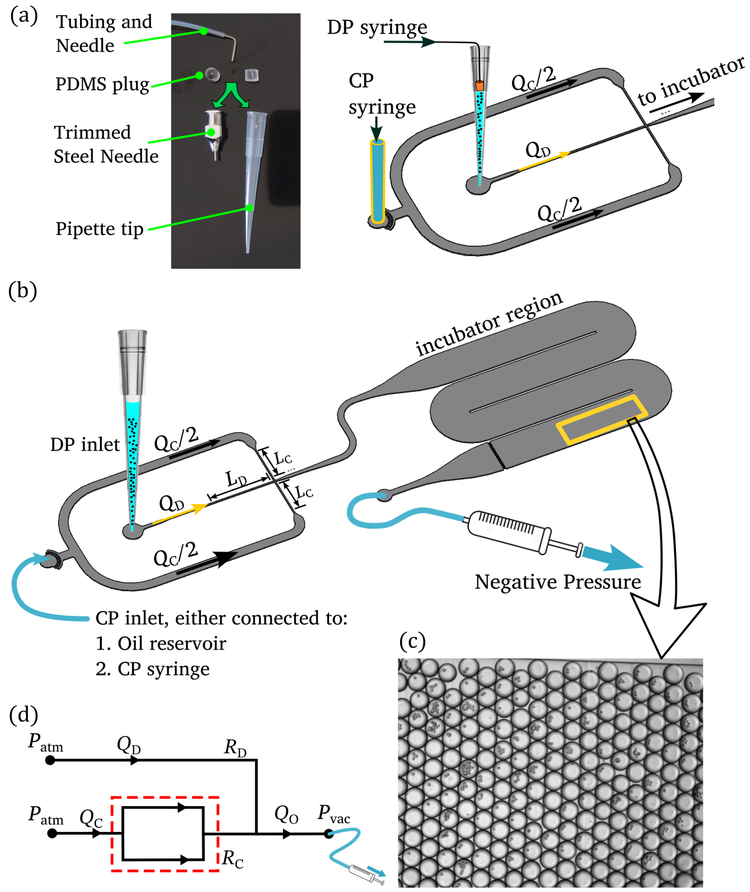
1. Using pippette-tip or steel needle assembly for cell-containing medium supply and negative pressure driven system (NPD).



a) The schematic of a pipette tip or steel needle assembly for cells and the modified experiment set-tup using positive pressure supply b) The negative pressure driven system. Only one syringe is required. The CP inlet can be connected to the oil reservoir or to a syringe. (c) The experiment results showing majority of droplets contains only few cells.(d) Flow circuit model of the Negative Pressure Device inlets.

Advantages of using NPD and a pipette tip in the medium inlet:
1. The use of the CP syringe is optional. The ratio of the flow rate can be embedded into the chip design: $q = L_C/2L_D$. Thus, only the outlet-connected syringe is required.
2. Allows easy bubble or air removal.
3. Mixing of cell-containing medium using a pipette just before cell loading
4. By plugging in the pipette tip and immediately starting droplet production, it prevents cell clamping.

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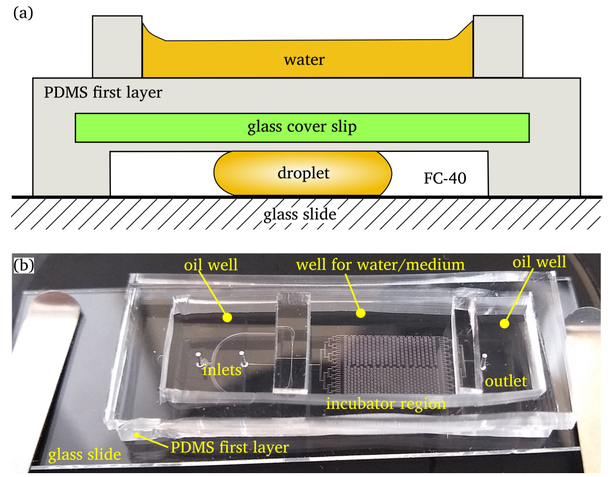
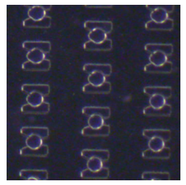
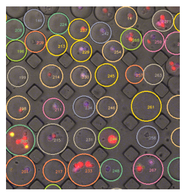
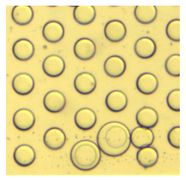
2. Implant glass cover-slip and use water well on top of the chip to reduce pervaporation



(a) Illustration of cross-section of the incubator region that uses implanted glass cover-slip near on top of the incubator region (b) the photo of a chip with reservoir made by bonding a PDMS structure on top of the PDMS first layer. The oil well above inlets and medium/water welll above the incubator region are necessary. The well above outlet is necessary only if the outlet tubing will be unpluged during incubation of cell.

3. Comparing different structure to immobilize the droplets inside the incubator region

Array Structure	Visualization	+/−
No structure (with barrier in the end)	(see images above)	+ The lowest total area of the incubator region + easy removal of unwanted droplets - Highest droplets mobility - High disruption to the droplet generation
Traps		+ Droplets are immobilized + No disruption to the droplet generation process - Requires the largest total area. - Very high rate of pervaporation. - Difficulties in removing unwanted droplets
Pillars		+ Droplets are immobilized. + moderate total area, minimum size depends on fabrication. + moderate difficulties in removing unwanted droplets - requires medium total area: increase time interval - high rate of pervaporation - disruption to the droplet generation, it requires a bypass channel.
Anchor wells		+ Droplets are immobilized. + easy removal of unwanted droplets + Low total area, it doesn't need a bypass channel. + Medium rate of pervaporation. - Requires two layer structure: fabrication challenge.