An injection moulded microfluidic chip for polymerase chain reaction (PCR) thermo-cycling and imaging of droplets to detect food-borne pathogens Campylobacter spp

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An injection moulded microfluidic chip for polymerase chain reaction (PCR) thermo-cycling and imaging of droplets to detect food-borne pathogens *Campylobacter* spp

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**Introduction**

*Campylobacter* is a dangerous but common bacteria which each year account for an increasing amount of cases of campylobacteriosis[1]. The low bacterial dose required to infect and its potentially fatal consequences make this organism a serious threat to the public health in both the developed and developing countries[2]. In poultry, the infection is often asymptomatic and chickens may hence carry the disease to the age of slaughtering, effectively contaminating the slaughter house[2].

To date, most droplet microfluidic chips are fabricated in poly(dimethylsiloxane) (PDMS) bonded to glass slides due to the ease of fabrication and surface modification[3]. This fabrication method is, however, not applicable to mass production and the potential for commercialisation is therefore reduced. Alternatively, injection moulding and hot embossing of thermoplastics are of interest.

**Methods**

Water-in-oil droplets are produced by a flow focussing chip. A PCR sample is injected into the aqueous line using a HPLC injection loop. The droplets are collected in an injection moulded disposable all-polymer chip which has been bonded (sealed) using ultra sonic welding. This results in a turnaround time ~1 minute/chip.

The chips droplet incubation chamber is designed to exploit the positive buoyancy of the droplets to facilitate optimal droplet packing regardless of the droplet production rate and water-to-oil flow-rate ratio. The height of the chamber restricts the droplets to pack into a single layer. This monolayer enables fluorescence read out by fluorescence microscopy.

Droplet size and fluorescence intensity is extracted from the images using a custom MATLAB script which performs a circular hough transformation to identify droplets.

Thermocycling is conducted on a flat bed PCR machine controlled by a custom made LabVIEW script.

**Results**

A single image contains as many as 20,000 droplets which may be analysed all at once. The droplets are highly uniform and forms almost exclusively a single densely packed monolayer.

Amplification by on-chip droplet PCR was confirmed by gel electrophoresis.

**Conclusion**

A disposable all-polymer multipurpose droplet interrogation chip has been fabricated and demonstrated. Using a standard fluorescence microscope, thousands of droplets could be analysed using a single snap-shot. On-chip thermo-cycling of droplets for digital PCR was demonstrated and PCR product was detected.

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