Microfluidics – Lab on Chip for Life Sciences

D2.1 Comparison of flow field in superhydrophobic and hydrophilic microchannels with micro PIV
(K. Tsougeni, K. Ellinas, Th. Christoforidis, A. Tserepi, E. Gogolides and external collaborators)

Plasma processing is used to randomly roughen and tailor the wetting properties of polymeric PMMA trapezoidal microchannels to superhydrophobic or superhydrophilic, depending on the plasma duration and chemistry: After oxygen plasma nanotexturing, microchannels are superhydrophilic, while following a short fluorocarbon plasma deposition step, they become superhydrophobic with three superhydrophobic walls. De-ionized water velocity profiles are measured under using micro PIV, and significant slip lengths are estimated for the slip case.

D2.2 Microfluidic devices for DNA amplification (μPCR) integrated with microheaters
(D. Moschou, G. Kaprou, G. Kokkoris, G. Papadakis, S. Chatzandroulis, A. Tserepi)

The development of low-cost and low-power μPCR devices integrated with microresistors on commercially available substrates was continued this year further improving the device design and the fabrication process, to yield robust and reliable devices. Numerical computations led the design so that there was no thermal crosstalk between the 3 zones of our chip and excellent temperature uniformity within each zone. In addition, the total power consumption during the chip operation is calculated to be in the order of a few Watts, verified experimentally. An external, home-made temperature control system was utilized to maintain the heater temperatures in the designated values (±0.2 °C). The PCR chip was validated by a successful amplification of a 90 base-pair DNA template.

![Fig.1](image) (left) Temperature profile across each temperature zone of the μPCR chip. (middle) The unit cell of the fabricated μPCR chip. (right top) Optical image of the fabricated chip with integrated microheaters and (bottom) demonstration of on-chip DNA amplification, compared to a commercial thermocycler.

D2.3 Passive micromixers for enzymatic digestion of DNA
(V. Papadopoulos, I. Kefala, G. Kokkoris, G. Kaprou, D. Moschou, G. Papadakis, A. Tserepi)

A passive micromixer with zig-zag geometry is designed through simulation and used for the enzymatic digestion of DNA. The micromixer is fabricated on a PCB substrate using flexible circuit board (FCB) technology and allows easy integration of the microdevice in more complex Lab-on-a-Chip platforms. When heated at 37 °C, the micromixer achieves
complete DNA digestion with restriction enzymes within 2.5 min, and is proved a valuable component of LoC systems for diagnostic purposes.

**Fig.2** (left) Simulation of the mixing efficiency of the designed zig-zag micromixer. (middle) Optical images of the fabricated micromixer. (right) Images of the gel with uncut and digested DNA after mixing with enzymes in the micromixer.

**D2.4 Polymeric lab on chip for wetting and fouling control and cell patterning**  
(K. Tsougeni, A. Tserepi, E. Gogolides and external collaborators)

We propose a planar technology for fabrication and surface modification of disposable, polymeric, microfluidic devices, and show applications in cell patterning. We demonstrate significantly increased cell attachment on the plasma treated PMMA areas compared to untreated ones, and highly selective cell attachment (on–off) onto hydrophilic versus the superhydrophobic areas using a particular cell line (Fig.3).

**Fig.3** Fluorescence image of HT1080 cells cultivated on a microchannel with variable wetting characteristics (superhydrophilic, CA < 10°, and superhydrophobic, CA~150°) formed in PMMA by O₂ plasma etching and deposition of a hydrophobic teflon-like film in the middle zone. The cells were stained with phallolidin-Atto 488 to visualize the cytoskeleton and DAPI for staining the nucleus.

**D2.5 Microfluidics for bacteria cell capture and DNA purification: Towards microchips for food safety**  
(K. Tsougeni, A. Kastania, G. Papadakis, V. Constantoudis, A. Tserepi, E. Gogolides and external collaborators)

We design, fabricate, and successfully demonstrate sample preparation modules comprising bacteria cell capture, thermal lysis, and DNA extraction on chip. Plasma nanotexturing of the polymeric substrate allows increase of the surface area of the chip and the antibody binding capacity. The modules have achieved 100% efficiency for bacteria capture and DNA extraction below a certain concentration (10⁵ cells/ml). These modules will be used in an integrated chip for bacteria capture, lysis, DNA extraction, PCR and SAW detection.
D2.6 Microfluidic devices with integrated microheaters for isothermal amplification of DNA
(G. Kaprou, D. Papageorgiou, G. Kokkoris, G. Papadakis, S. Chatzandroulis, A. Tserepi)

We have developed low-cost microfluidic devices to enable isothermal nucleic acid (DNA) amplification methods, such as Rolling Circle Amplification (RCA, ligation step @ 65°C) and Helicase Dependent Amplification (HDA @ 65°C) on PCB substrates with embedded microresistors (driven by an external home-made temperature controller to maintain the desired temperatures). The observed DNA amplification efficiencies (Fig.9b) are comparable with those on bench-top devices (conventional thermocyclers).

![Microfluidic device with an integrated copper microheater for isothermal amplification of DNA. (b) Gel images of isothermally amplified DNA on bench-top (tube) and chip devices.](image)

D2.7 Continuous Flow vs. Static Chamber μPCR Devices on Flexible Polymeric Substrates
(V. Papadopoulos, I. Kefala, G. Kokkoris, G. Kaprou, G. Papadakis, A. Tserepi)

Two types of micro-PCR (polymerase chain reaction) devices, a continuous flow (CF) and a static chamber (SC) device, with specifications imposed from flexible printed circuit technology, are compared through a computational study. Performance is quantified in terms of DNA amplification, energy consumption, and total operating time. The outcome of the calculations, contrary to what is generally believed, is that the SC device fabricated on thin substrates requires (2-4 times) lower energy consumption compared to the CF device.

![The logarithm of DNA amplification at the middle height of the microfluidic channel of the CF device for 10 cycles. (b) Energy consumption for 10 cycles depending on the protocol, the heat transfer coefficient, and the type of μPCR device (CF or SC).](image)

D2.8 Passive micromixer for enzymatic digestion of DNA
(I. Kefala, V. Papadopoulos, G. Kaprou, G. Kokkoris, G. Papadakis, A. Tserepi)

Three passive micromixers for bioanalytic applications with different geometries, i.e. zigzag, spiral, and a split and merge (SAM) with labyrinthine channels, are compared with respect to their mixing efficiency by means of a computational study. Compared to a linear micromixer, the spiral micromixer improves the mixing efficiency by 8%, the zigzag by 11%, and the SAM by 92%. The best of the three designs is realized by FPC technology. Finally, its mixing efficiency is validated for enzymatic digestion of DNA.
D2.9 Superhydrophobic, passive microvalves with controllable opening threshold: Exploiting plasma nanotextured microfluidics for a programmable flow switchboard
(K. Ellinas, A. Tserepi, E. Gogolides)

Plasma processing is used to create passive superhydrophobic on-off valves with tailored opening pressure inside microfluidic devices. First, anisotropic O2 plasma etching on polymeric microchannels is utilized to controllably roughen (nanotexture) the bottom of the microchannel. Second, the nanotextured surfaces are hydrophobized by means of a C4F8 plasma deposition step through a stencil mask creating superhydrophobic stripes or patches. The superhydrophobic patches play the role of on/off valves with predesigned opening pressure threshold (in the range 40-110 mbar), determined by the microchannel dimensions and the size of the nanotexture on the patch. These valves are integrated inside microchannel networks paving the way to autonomous microfluidic devices.

Fig. 7 Operation of a passive superhydrophobic microvalve. Photograph of the microchannel inside the chip holder. The hydrophobic patch is placed in the middle of the microchannel and its SEM image is shown as inset. (a) Stopped fluid flow (red dye) at the nanotextured hydrophobic patch (b) Pressure rise curves versus time in microchannels containing superhydrophobic patches a 20μm deep microchannel for three different etching times 1 min, 2 min, 4 min is given as insert.