From Microfluidics for Biotechnology to Biomicrofluidics

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ABSTRACT

Microfluidics solutions for biotechnology have considerably evolved with the years. At first, biotechnology was following a downscaling approach and the associated microfluidic solutions were the reduced scale image of macroscopic netwoks. In a sense, the approach was conducted by engineers and physicists who have seen the immense potential of this scaling down. However, progressively with the maturity of the technique, the bridge between biology and biotechnolgy has narrowed down and now the two domains are highly connected. The other important factor in this shift towards biology is the increasing interest in cell behavior analysis.

In this paper we illustrate this convergence by some characteristic examples of recent developments.

Keywords: digital microfluidics, inertial microfluidics, droplet microfluidics, open and capillary microfluidics

1 INTRODUCTION

In today's biotechnology, microfluidics is constantly adapting to the goals of biologists. Once a precise goal has been identified, biotechnology is searching for the best approaches to meet the demand of biologists. These approaches encompass different scientific domains besides biology, such as microfluidics—for transporting the targets, materials—for the fabrication of the chips, and chemistry—for the functionalization (Fig.1).

Microfluidic solutions have considerably evolved along the years: at first, a downscaling approach from macroscale to microscale has been followed leading to the so-called microflows lab-chips where microflows are circulating in confined microchannels at the downscaled image of macroscopic networks [1,2]. This approach was first motivated by genomics and the need for DNA recognition. As the goals of biologists diversified and progressively turned to protein recognition and cell culture and/or analysis, new microfluidic solutions have been developed. Progressively a large panel of solutions has emerged, which depart from the mere downscaling approach. A panel of the different microfluidic solutions is shown in figure 2.

In this presentation, we illustrate through some selected examples the diversity of today's microfluidic approaches.

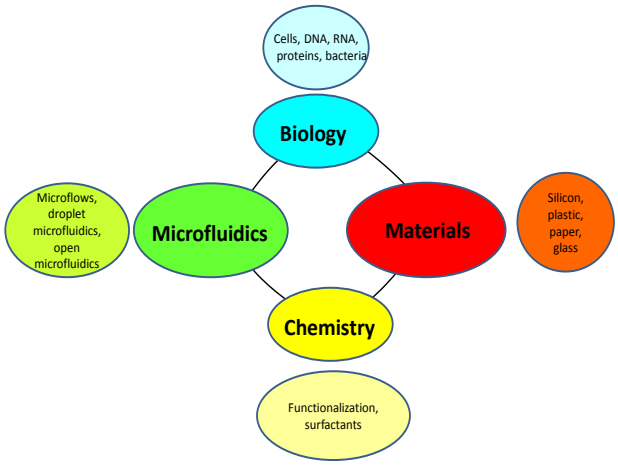

Figure 1: Biotechnology is a composite science in which microfluidics is a fundamental sub-domain.

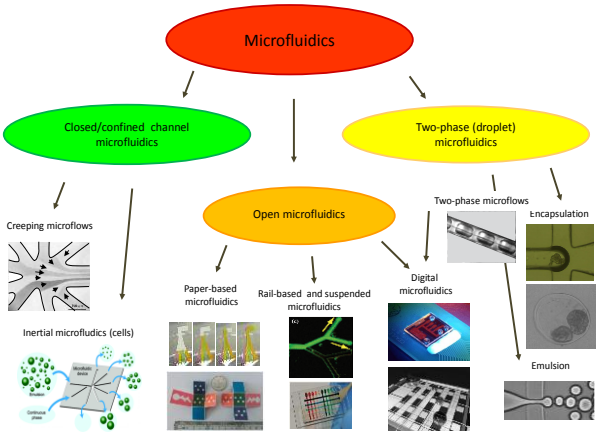

Figure 2: Different forms of microfluidics and their different applications

2 DIGITAL MICROFLUIDICS AND EWOD

Utilizing droplets instead of microflows reduces the volume of sample liquid. It constitutes an additional miniaturization step. Hence less costly reagents are needed,

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reaction times are shorter, handling is easier, sensitivity is higher. Besides, it is more convenient to work with a device closer to the size of the objects of interest. A very interesting solution is brought by the use of digital microfluidics, especially electrowetting and its EWOD configuration (electrowetting on dielectric) [3,4].

2.1 Principles of EWOD

In electrowetting, a droplet has its apparent contact angle reduced by application of an electric potential [5-7]. Basically, EWOD makes use of electrodes coated with a hydrophobic layer (parylene, teflon, SIOC, etc.) on top of a dielectric layer which role is to avoid electrolysis. Figure 3 shows typical EWOD micro devices. There are two possible configurations, open (one-plate) and covered (two-plates) systems [8]. It appears that the covered system are often preferable to open systems. In such systems, the basic operations like droplet motion, merging, division, and dispensing are easily done by the applications of electric potentials of the order of 40 to 60 V.

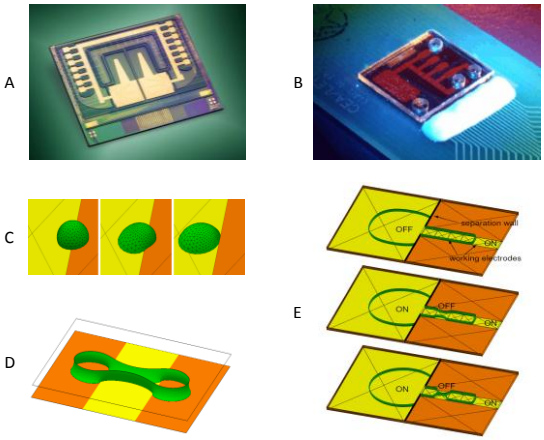

Figure 3: A and B: closed or covered EWOD systems showing the reservoirs and the lines of electrodes; C: motion from a non-actuated electrode to an actuated electrode; D: droplet division by elongation and pinching; C: droplet dispensing from a reservoir.

2.2 EWOD for DNA recognition

EWOD digital systems have proved to be very useful for the biorecognition of DNA. It has been shown that PCR can be achieved in digital microfluidic devices with identical results than conventional devices using much more sample liquid [9,10].

2.3 EWOD and cell culture

Recently, Fiddes and colleagues have shown that EWOD can be conveniently used for cell culture [11]. The principle is shown in figure 5. A hydrogel disc is first immobilized between the EWOD plates and loaded with cells transported by a moving droplet. Successively nutrients are brought to the disc, again using moving droplets.

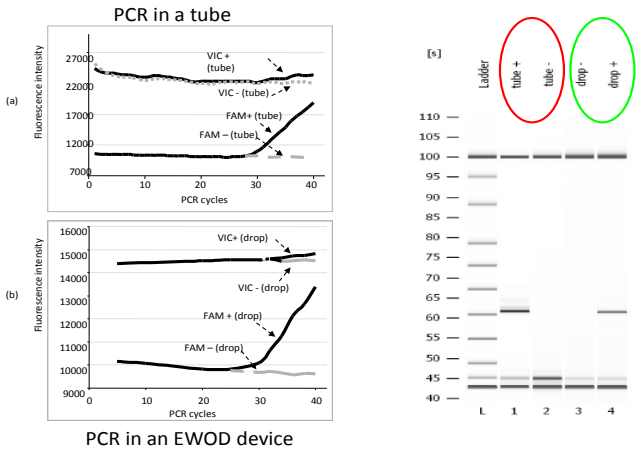

Figure 4: Comparison between PCR in a tube (conventional) and in a droplet.

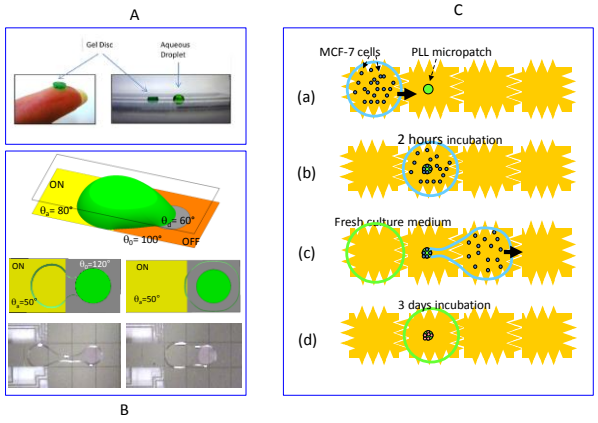

Figure 5: A, view of a hydrogel disc used for cell culture; B, modeling with Surface Evolver of the liquid motion around a hydrogel disc; C, principle of cell culture in a hydrogel disc placed in a covered EWOD device. From [11].

3 INERTIAL MICROFLUIDICS FOR CELL TRANSPORT AND SEPARATION

Cells (and bacteria) are now the most studied targets in biology. However, they are heavier and larger biologic objects than DNA and proteins. Hence, they require more energy to be transported, and recently the term “inertial microfluidics” has appeared, referring to faster microflows, *i.e.* microflows circulating at medium Reynolds numbers (between 20 and 100) [12]. Such microflows have special and interesting properties (Dean effect, lift effect, etc.) that

can be used for cell separation, trapping, and storage in cell chips (Fig.6) [13].

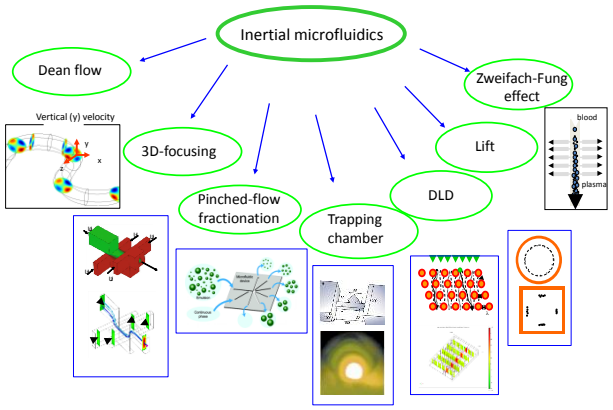

Figure 6: Different forms of inertial microfluidics.

The different devices shown in the figure 6 can be used or assembled to find solutions to problems such as cell manipulation (transport, separation, etc.) or cell immobilization for cell culture.

4 DROPLET MICROFLUIDICS FOR LIVE CELL ENCAPSULATION

Live cell encapsulation is seeing a growing interest in biology and medicine. For example, pancreatic cell encapsulation in alginate capsules is the most promising way to treat type I diabetes [14]. However, the success of the technique depends on the viability of the grafted capsules, and a droplet-microfludic solution is well adapted to this task. It will be shown that a combination of a micro flow-focusing device (FFD) with a phase-transfer device (PTD) can produce the capsules containing the live cells ready to be implanted in the human body.

The principle of an on-line live-cell encapsulation device is shown in figure 7 [15]. It comprises a FFD for the formation of alginate droplets containing live cells, a pre-gelling capillary tube for the pre-gelling of the capsules, and finally a phase-transfer device (PTD) to extract the capsules from their organic phase towards an aqueous phase containing calcium. Well calibrated droplets of alginate containing the cells are formed in the FFD. The two external additional branches are delivering oleic acid for the gelation of the capsules. However, too much oleic acid would kill the cells, and a rapid contact during the transport of the capsules in the pre-gelling capillary tube is sufficient for the partial gelling and the formation of a small shell around the capsules. The capsules are then sufficiently resistant to be introduced in the PTD where they cross the oil/water interface. Indeed the capsule crossing of the interface without resulting in a large deformation is a challenge. Pre-gelling and adequate interface position is the key to the passage to the other phase.

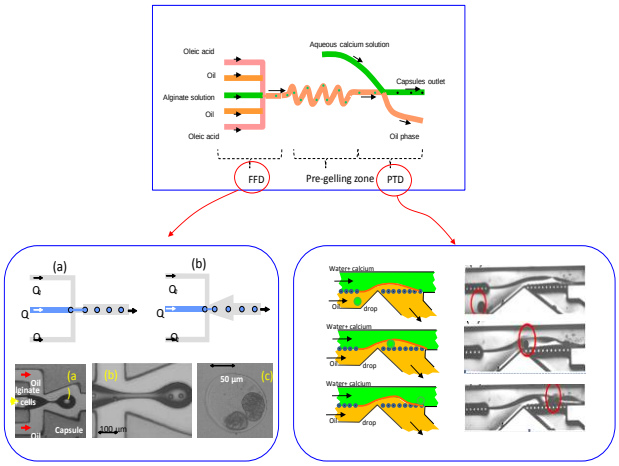

Figure 7: Top, principle of the device; bottom left, encapsulation in a FFD; bottom right, transfer of a pre-gelled capsule from the oil phase to the water/calcium plase in the PTD.

5 OPEN (CAPILLARY) MICROFLUIDICS FOR BLOOD TESTING

Another fast growing field in biotechnology is that of capillary-based microfluidics in open environments (*i.e.* partly in contact with the air). This is the case for labs-on-paper—or thread-based systems—where the sample fluid progresses by wicking the fibers [16,17]. Labs-on-paper have lately gained in complexity without abandoning their original advantages. Applications to cancer or HIV detection are already well advanced. This is also the case of rail-based or suspended microfluidics where a spontaneous capillary flow (SCF) is established between rails or vertical walls [17-19].

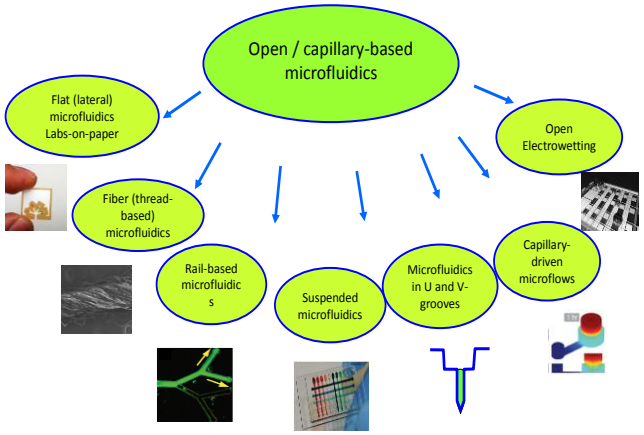

Figure 8: Different forms of open/capillary microfluidics

The theory of spontaneous capillary microflows (SCF) is now well advanced [20], and open designs using SCF are now proposed that allow the flow in the system without requiring any moving parts—such as pumps—or external energy sources—such as electric or acoustic actuation.

On the other hand, SCF of liquid polymeric solutions can be of high interest to compartementalize new types of cell-chips (Fig.9).

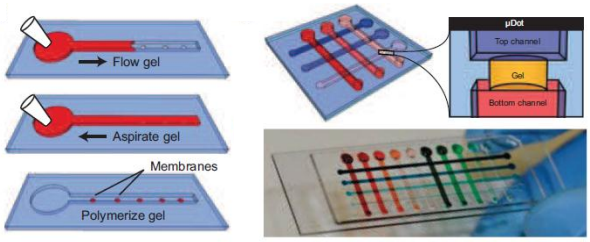

Figure 9: Formation of gelled membranes by SCF in a U-groove pierced with circular holes. These solidified plugs act as porous membranes for the study of cellular communication and/or migration (from [19]).

6 PERSPECTIVES

At the same time the conventional biotechnological microsystems are reaching maturity, and the focus of biotechnology is shifting towards cell behavior investigations. These evolutions call for new microfluidic solutions for the lab-chips of the future.

On the one hand, for detection and recognition purposes, the notions of portability, low cost, low energy, accessibility and recyclability are increasingly important. Hence new open systems, such as labs-on-paper or capillary-flow driven systems, are progressively gaining ground. On the other hand, the emergence of cell-chips are promoting new techniques such as inertial microfluidcs to manipulate, separate, sort out and immobilize cells.

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