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The Study of Microbiological Processes on Microfluidic Chips and Modeling

Diana R. Batyrgazieva, Illarion I. Khudeev*, Elena V. Guseva, Natalia V. Menshutina, Igor N. Dorokhov

Dmitry Mendeleev University of Chemical Technology of Russia, Miusskaya sq. 9, 125047 Moscow, Russia
illarionkhudeev@mail.ru

The development and improvement of micro- and nanotechnologies, microfluidic technologies, highly sensitive imaging systems and last advances in nanomedicine allow to creation of small-sized devices for the formation of different substances isolated droplets in the continuous liquid flow. Such droplets are kind of mini-reactors with a volume from 10 to 5 µL, surrounded by an inert medium, where different chemical reactions or other interactions can be carried out. Such reactions in small isolated volumes can be effective in various fields of researches, both at the molecular level and in the study of mammalian cells or other microorganisms. Two variants of directions related to microfluidics in the drop were considered: digital microfluidic technology allowing controlling the movement of individual drops on the substrate and droplets microfluidics allowing to form droplets in a liquid medium, to carry out various manipulations with them. Besides the different approaches to modelling of various interactions inside the lab-on a chip including different types of software packages were considered too.

1. Introduction

Microfluidic devices capable of manipulating and directing small volumes of fluid open new methodological approaches in the fields of biology, pharmacy and medicine. They have already proved to be of utmost importance for the cell analysis. The emergence of microfluid platforms paved the way for new methods of treatment and observation of living cells, for the creation of chemically defined liquid media, and for adapting biomechanical or physical conditions in small volumes. Modern trends are aimed at the miniaturization of processes, modernization of production and optimization of the production and economic systems – e.g. (Piemonte et al., 2016) on transport regimes and (Sarghini et al., 2015) on Protein/Pectin Complexes. The development of the integrated circuit allowed electrical devices to shrink from room sizes to pocket size, increasing all the time in speed and penetrating almost every aspect of our life. Similarly, it is to be hoped that many of the large, costly chemical and biological analyses that are currently being performed can be replaced by integrated microfluidic devices (MFPs), often called a laboratory on a chip. It can be led to a similar revolution. As a result, active development this direction the researchers do not have enough time and money to create and test successive prototypes in order to optimize the work of the laboratory devices on a chip. Rapid prototyping techniques are needed, that significantly help to reduce costs and development time after choosing a chip design. With the help of computational and analytical modelling of processes on a chip, it is possible to solve these actual current problems. Simulation allows researchers to quickly determine how the design changes will affect the performance of the chip, thereby reducing the number of iterations of prototyping. Perhaps more importantly, "numerical prototyping" used at the concept stage can provide excellent estimations of the potential characteristics of the chip, for example, surface hybridization rate of the target solution phases, thermal cycling rate for PCR, or separation efficiency in capillary electrophoresis. In (Erickson, 2005), the application of numerical simulation and computer programs used to design and developing laboratory devices on a chip has been reviewed. Currently, many studies related to microfluidic technologies are being conducted. For example, Piemonte et al. (2016) described the common modes in Microfluidic Bioreactors.

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2. Microdroplets

There are two main directions related to microfluidics in the drop:

a) Digital microfluidic technology, which allows controlling the movement of individual drops on the substrate;

b) Droplets microfluidics, which also belongs to the category of digital microfluidics technology, which allows to form droplets in a liquid medium, to carry out various manipulations with them and to move them through closed channels to various areas of the microchip – Figure 1 (Seemann, 2012).

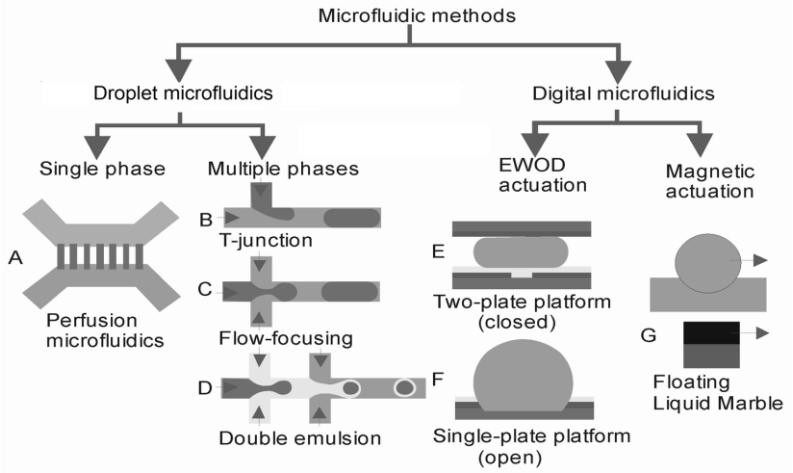

Figure 1: Two main directions associated with microfluidics in the drop: digital microfluidics and droplet microfluidics

Similar to droplet microfluidics it is the hydrodynamics of a segmented flow (Stanley et al., 2012). The difference between them, in particular, lies in the degree of sample interactions with the channel walls (Guo, 2012). Droplet microfluidics have an obvious advantage, since the test liquid sample does not interact with the channel walls, which prevents sorption of molecules and the possibility of cross contamination between discrete volumes.

Droplet microfluidics can be used to study individual biological microobjects. The advantages of microfluidic technologies include: high droplet formation rates, low consumption of reagents and samples, the possibility of complete control of the conditions for the droplets formation, the implementation of merger operations or "fragmentation" of drops, and etc.

The investigations in the field of droplet microfluidics indicate the possibility of creating not only microsystems for the chemical and biochemical synthesis of substances (Günther, 2005), micro- and nanoparticles (Shestopalov et al., 2004), but also high-performance molecular diagnostics by polymerase chain reaction (PCR) methods (Baret, 2008), study of individual cells and its functionalizations (Guo, 2012), drug screening, etc. The development and improvement of these technologies and methods based on the principles of droplet microfluidics is currently an urgent and demanded task.

2.1 Features of droplet microfluidics

The implementation of analytical operations (sample sampling, manipulation and measurement of the informative signal) by droplet microfluidics methods allows to significantly accelerate the analysis of the samples. MFPs can produce monodisperse droplets ranging from 0.05 pL to 1 nL or 5 µm to 120 µm in diameter, which saves reagents. Drops may contain cells, biomolecules, DNA or RNA and other particles or molecules in the aqueous phase (Bowman et al., 1998).

Substances or microparticles from different drops can be combined by coalescing droplets, which allows mixing their contents. After carrying out the necessary manipulations and reactions, the drops can be sorted by certain characteristics, and their components are extracted from the oil shell. The wide possibilities for various variants of manipulations with drops allow to realize practically any stages of analysis of studied object or synthesis of a complex substance. It was shown that a lot of analyses often used in biological studies can be implemented on the basis of the principles and methods of droplet microfluidics (Belousov et al., 2015).

In the case of droplet microfluidics for chemical synthesis, it should be considered that the reaction rate increases depending on the effective concentration, the reaction space in the droplet is small. Consequently, the reaction time can drop to several seconds or minutes in comparison with macrosystems where the reaction time can be about several hours. Moreover, the minimal concentration of reaction products to detect will be

achieved faster in small volumes, and, consequently, the detection can be performed more efficiently too (Belousov et al., 2015).
In many cases the formation of drops in various liquids is rather difficult task. To ensure the creation of stable drops (thermal stability, monodispersion, droplet form), it is necessary to modify the surface using wet chemistry, silane chemistry, plasma treatment and other methods. The hydrodynamic conditions for the droplets formation and construction of the emulsion generator also affect the size and shape of the droplets.
From the point of view of physics, the control of interfaces between media in dynamics is decisive in the formation of droplets in MFS. Nonlinear dynamic phenomena are observed, which are significant in a wide range of sizes - from hundreds of micrometers to nanometers. This range of nonlinear effects of almost five orders of magnitude, can play a decisive role, which mainly explains the difficulties encountered by researchers in creating droplet microfluidics devices.

3. Modelling of processes in droplet microfluidics

When studying the evolution of drops, the processes of its formation, transport, merger and stability are mainly considered. The problem of theoretical modelling of two-phase system is quite complicated in connection with the need to take into account the deformable boundary of drops, surface tension forces and their changes, that add a number of nonlinear effects and complexity (Belousov et al., 2015). An essential manifestation of the nonlinearity is that slight changes in the motion conditions can lead to the change in flow character. Similar transitions are possible, because changes in the droplets geometry in turn affect the velocity profiles of the flows, reinforcing initially small changes. Therefore, it is rather difficult to obtain exact analytical solutions, and a number of assumptions are used in their construction.
The nature of the droplet movement in the channels can be determined depending on the ratio of its diameter to the characteristic dimensions of channels. Due to this, there are some possible variants. The first one is when droplet diameter is smaller than dimensions of channel, and they move freely throughout its cross-section. The second one is when the channel has a large aspect ratio, and drops are limited only on one side. The third one is when drops occupy the entire channel cross-section limited in width and height. Thus, one can talk about three-dimensional, two-dimensional and one-dimensional drop transport systems.
Analysing three-dimensional systems, it is usually assumed that the droplets move with the rate of continuous phase. But with increasing droplet density, it turns out that they perturb the flow around themselves, creating (1/r) long-range hydrodynamic interactions. At a qualitative level, the droplet motion manifests itself in a strong long-range correlation and vortices that depend on the boundary conditions. However, the theoretical collective motion remains unclear due to the divergence of the forces sum (1/r) as the number of droplets participating in the interaction increases and the divergence of the resulting fluctuations increases too. The observed data have not yet been fully explained theoretically (Belousov et al., 2015).
In two-dimensional systems that are limited on the one hand, the hydrodynamic forces decrease as $1/r^2$, which relieves the system of the above described impassability. Thus, the interaction of drops with each other can be described as the interaction of hydrodynamic dipoles similarly to dipoles from electrostatics, which allows us to conduct the theoretical analysis of their behaviour. Thus, "acoustic modes" propagate along the droplet chain, which, like solid-state phonons, have corresponding dispersion relations, and which can lead to various instabilities. Taking into account the dipole interaction between droplets makes possible to predict the dispersion relations and the appearance of nonlinear instabilities (Belousov et al., 2015). In the one-dimensional case, when the droplets are confined from two sides, capillary effects and deformation of the droplet surface play a major role. In this case, the capillary number comes to the fore as the main criterion.
When the droplet moves in the channel, a thin layer of the continuous phase forms between it and the channel wall. With respect to the droplets, the channel walls move in the opposite direction and drag the carrier fluid into the space between the droplet and channel. On the other hand, the higher droplet pressure, in comparison with the surrounding liquid, compresses the layer located between the droplet and the channel.
Bretherton (1961) derived a nonlinear law which estimates the thickness of a layer for gas bubbles moving with a small capillary number (Ca <0.01) in a cylindrical channel. Similar dependences, differing only in the numerical coefficient before Ca, were obtained for viscous droplets and polygonal channel cross-sections. These theoretical results were experimentally confirmed and numerical modeling data including an empirical dependence were proposed to describe the layer thickness in the range of values up to $Ca \sim 1$.
The presence of thin layer directly affects the average velocity of droplet movement. It is noted in (Abiev, 2008) that the fluid flow rate in the layer can be calculated from the difference between the average droplets rates and carrier liquid. As the liquid in the thin layer flows at a rate lower then droplet rate, the droplet must move faster than the carrier fluid to maintain the mass balance.
In (Abiev, 2008), the more general solution was obtained for the bubble hydrodynamics in a cylindrical capillary, which makes it possible to calculate the velocity profiles in the bubble, in layer around it and in liquid between

bubbles. Besides the tangential stress distribution and longitudinal component of the pressure gradient, which well agrees with the experimental data can be calculated too.
For square microchannels, the picture changes somewhat, since the droplets do not fully fill the channel cross-section, leaving free the corners.
To predict the droplet and carrier fluid velocities, it is necessary to establish a relationship between them and the differential pressure ΔP in channel. In this case, the pressure difference occurs in the carrier fluid between droplets, in droplets themselves and in their curvilinear parts producing Laplace pressure. ΔP depends on the values of the capillary number, viscosities of the continuous and dispersed phase, their velocities, channel geometric parameters, and number of droplets. However, the relationship between pressure and flow velocity is too complex for general use, because the constants that depend on the channel geometry must be constantly recalculated the channel changes. In addition, the dependence on the capillary number ceases to be satisfied for medium and large *Ca*, and the presence of surfactant significantly affects the processes of droplet formation. For this reason, simplified empirical relationships are often used (Schaerli et al., 2009).
It is even more difficult to describe the process of droplet formation. There are a number of empirical formulas that allow to estimate the droplet size as a function of the capillary number and ratio of the flow values for different topologies (Chung, 2002). However, they are all private, thus, when creating new devices for droplet microfluidics, an important role is played by numerical modelling of processes in the created topologies of droplet generators.
The principle of numerical simulation consists in an approximate solution on the discrete space of the computational grid of equations describing the evolution of the system. There are two main approaches in modelling of multiphase system: with explicit separation of the interface tracking and interface capturing (Günther et al., 2006).
In methods with explicit separation of the phase boundary, elements of the computational grid lie directly on the boundary of the phases. Such methods include: the method of boundary integrals, immersed boundary method, finite element method with a deformable grid (Wootton et al., 2012).
In the method of boundary integrals, the grid is divided only over the interface, and expressions describing the motion of liquids are projected onto it, thus reducing dimensionality of the problem being solved.
In the finite element method, in contrast to the method of boundary integrals, the computational grid is present in the domains describing liquid phases, as a consequence, with deformation of the boundary, elements are deformed in the entire volume of modelling.
In the submerged boundary method, the interfacial forces are calculated at the boundary separated from main modelling area, where the flow equations are solved, which necessitates the cross-linking of solutions. These methods are fairly accurate and effective, as interphase boundary is part of the computational grid, and expressions with boundary conditions are precisely formulated. At the same time, while they are excellent at modelling the separation and merging of droplets, they have problems with further calculation of fluid motion.
In the count-through methods, phase boundary itself moves along the calculated grid, which remains fixed and does not deform. These methods include: Boltzmann lattice equation method, interpolation profile, volume of fluid (VOF method), phase field, level function. Discontinuities in the parameters of the medium at the boundary of the two phases (density, viscosity) are smoothed out, and surface tension force is distributed over a thin layer on the two-phase boundary, being a bulk force.
The Boltzmann lattice equation method considers liquid flow as the motion of an ensemble of pseudo-particles, averaging of the parameters of which gives an idea of the motion of entire fluid. In interpolation profile methods, fluid volume and level functions, the phase distribution is described using auxiliary functions, and the displacement of the boundary between them is determined by solving the mass transfer equations. The count-through methods are ideal for simulation large phase boundary displacements and designing the microchannel structure, however, an approximate boundary setting reduces their accuracy. To increase accuracy allows the use of an adaptive grid, which reduces its partitioning in the region of phase boundary (Hindson, 2011).

4. Software products for the modelling processes in microfluidic devices

There are many commercially available codes that have been very successful in modelling microfluidics processes (for example, Fluent, FEMLAB, CFD-ACE + from the Research Corporation CFD Coventor) (Figure 2). Although these excellent tools represent the path of least resistance to high-level numerical analysis, usually require the user to have some background in computational fluid dynamics (this skill not particularly common among chemists, biologists and physicians who dominate the development of chips). In addition, many of these codes tend to primarily focus on modelling the fluid flow and, to lesser extent, transport of species, which, as mentioned above, does not provide a complete picture of what is required to create a real laboratory on the chip.
The multifactor capabilities of FEMLAB, which facilitate communication and simultaneous solution of various fundamental equations along with its interface of points and clicks, make it a likely best candidate from widely

available tools for comprehensive modelling. In addition to these commercial packages, some research teams have developed their own codes (a much more time-consuming task), which allows them to be specialized for development on laboratory terms (Erickson, 2005).

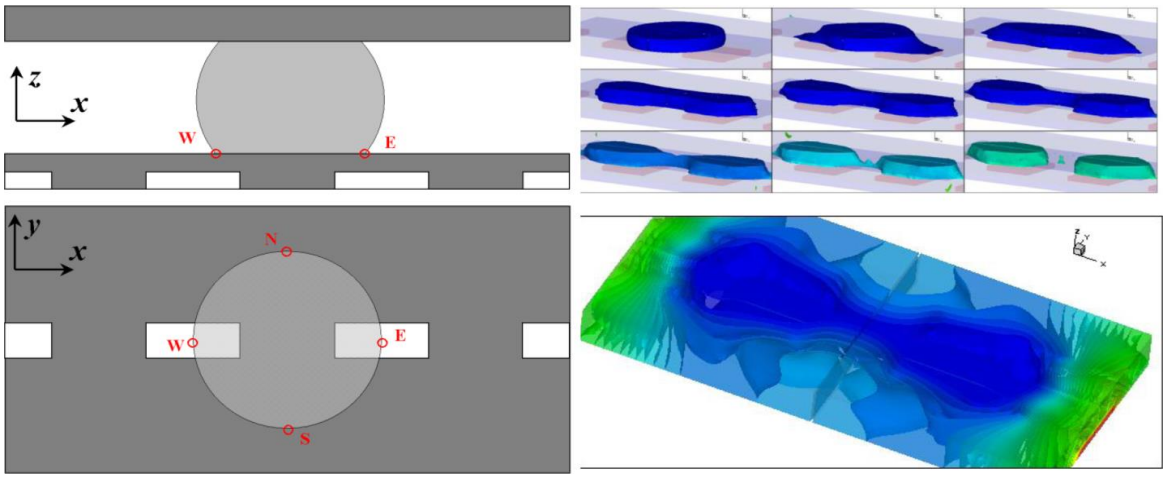

Figure 2: Droplet fission on driven lab-on-a-chip from Zeng and Korsmeyer (2004)

5. Conclusions

Microfluidic systems are highly efficient technological platforms that allow to automate and increase the productivity of laboratory analyses, accelerate the screening of medicines, and develop new diagnostic systems. The companies promoting and commercializing microfluidic technologies created over last few years argue that effective reagent consumption, high analysis speed, miniaturization of components, and availability of MFS made from inexpensive materials will reduce costs compared to conventional laboratory equipment. Although many effective and useful devices have been proposed in microfluidics, only a limited number of them have been brought to serial production. Even after acquiring a commercial MFS, users may encounter difficulties in compatibility with appropriate equipment, for example, with external pumps and pneumatic systems for managing fluid flow. It also requires basic user training for working with such systems. Additional equipment is necessary too, and in some cases, the analysis methods must be adapted. In this article, the presented overview of some various ways in which numerical modelling can be used to develop microfluidic processes and devices. In general, it is believed that there are two broad areas where the use of numerical methods can have significant growth. At present, numerical modelling is usually used as a tool for investigating or explaining previously observed experimental phenomena. As microfluidic devices become more complex, optimization of the fluid and transport structure is becoming increasingly difficult to do experimentally. As such, it is believed that in this area the future demands will be in highly integrated modelling tools that allow users without significant CFD experience of a "numerical prototype" to model whole microfluidic devices. It is assumed that a properly designed simulation program can shorten the time from concept to prototype and provide immediate evaluation of the potential performance of the chip (for example, the time required for complete surface hybridization or the thermal cycling rate for PCR), which allows the researcher to pass a fruitful path from the very beginning.
Microfluidic technology significantly expands the tools for single-chamber assays and it is of particular importance compared to cytometry or fluorescence microscopy, when more careful cell processing or tracking is required, as well as detection of the analyse. Encapsulation of cells in a specific chemical environment is important to reduce external noise and to ensure an accurate comparison between cells. Methods for rapid addition or removal of liquid allow kinetic studies of the reaction of cells in a shorter or longer period of time. The platforms described here for the creation of microdroplets and microballoons are complementary approaches, which differ, in particular, by the number of cells analysed and the feasibility of manipulation processes. While the droplet microfluidics is aimed at increasing the number of cells similar to conventional cytometry, the approach to the microchamber is more adaptable to various methods of stimulation and analytical analysis. Future researches can focus more intensively on selective capture and encapsulation of cells not only on the basis of physical parameters, such as size, but also because of biochemical phenotypes. In this respect, another interesting and highly relevant area is the selective capture of cells from tissue samples and the subsequent introduction into a platform for microfluidic analysis. Thus, single-chamber analytical tools are interrelated with

in vivo studies that allow the detection and mapping of heterogeneities within a particular tissue, including, for example, tumors, thereby overlapping the gap between in vivo and in vitro studies with a single cell.

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