Micro- and nanofluidic systems in devices for biological, medical and environmental research

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Abstract. The use of micro- and nanofluidic systems in modern analytical instruments allow you to implement a number of unique opportunities and achieve ultra-high measurement sensitivity. The possibility of manipulation of the individual biological objects (cells, bacteria, viruses, proteins, nucleic acids) in a liquid medium caused the development of devices on microchip platform for methods: chromatographic and electrophoretic analyzes; polymerase chain reaction; sequencing of nucleic acids; immunoassay; cytometric studies. Development of micro and nano fabrication technologies, materials science, surface chemistry, analytical chemistry, cell engineering have led to the creation of a unique systems such as "lab-on-a-chip", "human-on-a-chip" and other. This article discusses common in microfluidics materials and methods of making functional structures. Examples of integration of nanoscale structures in microfluidic devices for the implementation of new features and improve the technical characteristics of devices and systems are shown.

1. Introduction
Modern technologies allow to form repeatable micro- and nanoscale structures with desired characteristics in various materials. Proximity of the size of the nanostructures formed to the size of biological molecules (nucleic acids, amino acids, proteins) allows to make precision operation with them. The integration of nanostructures into compact microfluidic devices (chips) promotes creation of new devices and systems for biological and medical research, which are implemented the unique properties of nanomaterials, especially nanofluidics and the broadest opportunities of microfluidics.

The global market for microfluidic devices in 2015 was about $ 2.56 billion (according to Yole Development, Emerging Markets for Microfluidic Application). Experts estimate the growth of the market by 2020 to about $ 5.95 billion. The largest growth in the market is predicted for the near future for systems aimed at pharmaceutical research and on-site diagnostic systems (Point Of Care - POC). At the current time, more than 50% of microfluidic devices are used in pharmaceutical research (search for medicines) and in Life Sciences, about 14% in clinical and veterinary diagnostics.

The advantages of systems on microchip platform include are: a) the total control of all (or basic) stages of analysis or synthesis, b) a low reagent consumption, and c) the possibility of operations with a very small sample volume or the amount of analyte, g) the high sensitivity of detection analytes, d) the high speed of analysis or high throughput synthesis of materials. Manipulations with individual biological objects (cells, bacteria, viruses, proteins, nucleic acids) in a liquid medium may be performed on microchip devices. These advantages led to development of the devises on microchip platform for: electrophoretic and chromatographic methods of analysis; methods based on polymerase
chain reaction; sequencing of nucleic acids; immunoassay methods; cytometric studies [1,2], and others. Although studies have been lead in microfluidics for more than 25 years, only a few companies have been able to fabricate commercial instruments [3]. For example, based on the methods of "droplet" microfluidics are produced commercial systems for digital PCR: QX200™ Droplet Digital™ PCR System (Bio-Rad) and RainDrop™ Digital PCR System (Raindance Tech., Inc.). An emulsion PCR is used in the Genome Sequencer FLX (Roche).

It is possible to identify the main trends in the development of micro- and nanofluid systems in the analytical instrumentation, aimed at the creation of: a) automated systems for "full" analysis or of certain key stages («micro Total Analysis System») [4]; b) relatively cheap microchips and specialized devices for rapid diagnosis (including «Point Of Care» system [5]); c) devices that mimic the basic physiological functions of the "living" systems for biological, pharmacological, toxicological studies («Organ-on-a-chip», «tissue-on-a-chip», etc.). [6]; g) systems, embedded in the laboratory devices (eg, mass spectrometers, optical microscopes), to solve research problems («chip-in-a-lab») [7].

One of the fundamental advantages of microfluidic devices is the ability to integrate multiple chemical processing and fluidic manipulation operations into a small sizes. In many cases an automated sample-in/answer-out system is the ultimate goal. Such systems should provide accurate results in real time. The integration of automated sample collection, processing, and real time analysis on microfluidic platforms with mass spectrometry (MS) continued to show progress in analytical research. [8] For example, a microextraction system was designed to automatically extract samples from biological object without any pretreatments. This mechanical extraction system was integrated with both MS and a fluorescent microscope in order to monitor the time resolved extraction progress.

The POC applications continue to be popular. A number of new portable microfluidic devices were reported [9] with many built-in functions for creating simpler ones [10], faster and cheaper devices [11] with better detection limits. Microfluidic device consisting of 500 channels is intended for the rapid analysis (within 20 minutes) of the integrity of the sperm DNA using fluorescent imaging [11]. In experiments a significant increase in the viability of the bull and human sperm was demonstrated in comparison with existing methods.

The cost of developing medicines is growing every year. Preparations considered as drug candidates often fail in clinical trials. Today, the cost of developing medicines in the US has reached the level of $ 2.6 billion. The limited predictability of in-vitro results relative to the results in vivo and the small number of patients participating in phase I and phase II clinical trials do not allow an adequate estimate of the likely clinical effectiveness [12]. This situation led to the creation of systems "organ-on-a-chip" for the detail study of physical and biochemical processes at the cellular level using biomimetic research platforms [6]. Separate systems of "organs" can be combined into a "human-on-a-chip." Systemic interactions between of various organs can significantly affect the health of the organism as the functions of the organs are highly dependent on their interaction [13]. With regard to testing preparations, individual drugs may well influence the target organ, but his the metabolites can lead to toxic effects on another organ [14]. An important task has been formed now, which is to develop new high-performance toxicity tests for a wide range of substances for prediction of toxic effects in the human body. Biochemical processes in the human body under the influence of toxic substances are complex and not thoroughly studied. The efforts of many researchers [15, 16] focus on the development of "organ-on-a-chip" devices that mimic the cellular environment in the organ or several interrelated organs. Thus, devices have been created to simulate the functioning of organs under the influence of drugs or changing metabolism to determine the toxicity of the drug. Many devices try to reproduce the processes occurring in the liver, since many pharmaceutical problems are caused by interactions occurring in the liver [for example, 14]. Systems that simulate several interconnected "human" "organs-on-a-chip" can help in solving problems of identifying new biomarkers, determining the toxicity of substances, studying the body's response to viral or bacterial infections, which may be important for clinical tests.
2. Materials and methods for manufacturing of micro- and nanofluidic systems

Development and manufacturing of the prototype device is an important stage in the creation of micro and nanofluidic systems for analytical instruments. Polydimethylsiloxane (PDMS) is the most popular material for such prototypes. In 1997 group prof. G. M. Whitesides (Harvard) demonstrated the possibilities of: production of reproducible fingerprint patterns from the master-form in PDMS with nanometer resolution, the usage of PDMS replicas for microcontact printing and micro-transfer molding elastomer [17]. The method was called Soft lithography. PDMS chips have high optical transparency, elasticity, gas and water-permeability and good biocompatibility. However, because of its porosity and permeability of PDMS is unacceptable for some applications.

Low molecular weight oligomer chains in PDMS can leach out into solution, negatively impacting cellular studies [18]. PDMS is hydrophobic material and susceptible to nonspecific adsorption and permeation by hydrophobic molecules [19]. Chemical modification of PDMS can address these issues. Plasma exposure will hydrophilize the PDMS surface but only a short time [20]. Reaction of silanes with silanols formed by plasma activation or other methods slows this change in surface properties [21]. PDMS is a valuable material for rapid prototyping and is commonly used in microfluidics studies, but it is not suitable for commercial use. The efforts of researchers are aimed at finding other polymers (for example, variants of polyurethanes) because it has been found that they have the same benefits as PDMS but do not have permeability for small molecules [22]. Thermoplastic polymers: Poly (methyl methacrylate), Polycarbonate, Polystyrene, Cyclic-olefin copolymer are cheaper, and are considered as candidates for mass production of microchips. [23]. Technology micro / nano hot embossing and injection molding are promising for mass production of microchips. But to get appropriate the good reproducibility of the sizes and shape of elements necessary avoid structural damage due to thermal stress, adhesion and friction at the interface between the workpiece and the shape is necessary to improve the surface properties of the master-form [24].

For the direct formation of micro-dimensional structures in polymeric materials, laser ablation is used often. The ablation mechanisms are different for nano-, pico- and femtosecond laser pulses [25]. With femtosecond pulses, it is possible to achieve more accurate and clean laser processing of metals and other solid materials than in pico and nano-second generation regimes. This technology is convenient for the production of a small series of microfluidic devices because it allows flexible enough to change the topology and design of microchips, but has limited spatial resolution capabilities of structures. In addition, laser ablation creates a rough surface, which is unacceptable for some applications.

Micro- and nano-fabrication technology is beautifully designed for silicon and glass, also used in the creation of microfluidic devices. Silicon surface chemistry based on the silanol group (−Si−OH) is well developed, so modification is easily accomplished via silanes. For example, nonspecific adsorption can be reduced or cellular growth improved through chemical modification of the surface [26]. Silicon is transparent to infrared but not visible light and not used for optical detection. This issue can be overcome by having a transparent material (polymer or glass) bound to silicon in a hybrid system. Microstructures in glass are created by etching into the glass through wet or dry methods [27]. Formation of fluidic communication requires bonding or protection layer attachment to enclose. Glass has low background fluorescence, and as with silicon, modification chemistries are silanol based. Glass is compatible with biological samples, has relatively low nonspecific adsorption, and is not gas permeable. Glass microfluidic systems are currently available to us as commercial products of Dolomite, Micralyne, Agilent and Caliper. The glass microfluidic chip with a flow-through reaction chambers containing an array nanocells for clusters with high density, is used in devices for nucleic acid sequencing HiSeq (Illumina) (Chromatin Immunoprecipitation Sequencing technology - ChIP-Seq), another microfluidic chip with a system of interlocking microchannels cameras is used in Agilent 2100 Bioanalyzer system (Agilent Technologies) for the electrophoretic separation of the sample and flow cytometry.
The integration of nano-sized structures in microfluidic devices can improve the specifications and realize new possibilities for detecting an analyte. Among the requested features of nanoscale structures are the following: a) highly sensitive detection of the desired object / analyte (electric detection, plasmon resonance methods, detection with use nanopores, etc.) [28, 29]. b) transport (control the motion of molecules, particles - such as electroosmotic system) [30]; c) filtering and separation [31]; c) control (valves, gates) [32], and others. (heat exchangers, mixers, reactors). There are difficulties in detecting for nanofluidic devices due to: 1) a small number of analyte molecules at low signal / noise ratio; 2) small size detection region; 3) considerable time scanning study area for high spatial resolution, if necessary. Since the optical detection methods are highly compatible with microfluidic devices, they often try to adapt to nanofluidics, such as thermal lens spectrometry method [33]. Other techniques such as surface plasmon resonance spectroscopy, Raman spectroscopy, may be useful for the detection of non-fluorescent molecules [34, 35]. Methods of electrical detection of biomolecules are promising. When the size of the holding space is close to the size of the molecule, its intrusion into that space causes a change in electrical properties that can be registered.

In systems for nanopore sequences that are widely applied at present [36], two types of nanopores are used: biological and solid state. Biological nanopores created through the introduction channel protein (alpha-hemolysin (α-HL) and MSPA) into the lipid bilayer [37]. Solid-state nanopores are usually formed by a focused ion or electron beam in the dielectric membrane [38]. There are problems using nanopore in particular: a) necessity to ensure extremely fast rate of DNA translocation through a nanopore thread [39]; b) nanopores must have reduced thickness to achieve single nucleotide resolution (application of modified graphene membranes [40].

Other promising nanoscale structures are "nanowiskers» (nanowires - NW), which may be made of different materials (e.g., semiconductors or metals). Semiconductor NW have narrow fluorescence band and higher photostability than organic fluorophores, and other useful properties. The area of possible application of NW is very wide. For example, a group of prof. CM Lieber (Department of Chemistry and Chemical Biology Harvard University) is developing technologies and methods for design and synthesis of nanoscale components, which can be used for creating any functional structures or nanosystems: from ultrasensitive sensors to nano interface devices for the study of living cells and tissues [41, 42].

The research at creating devices on a microfluidic platform with highly sensitive optical detection systems for express analysis of biological samples is conduct in the Institute for Analytical Instrumentation RAS. One of the research direction is the creation of nanoscale functional structures integrated into a microfluidic chip. Together with the University ITMO, lithography technologies are developed, which oriented to the creation of nanoscale structures directly in a microfluidic chip for mechanical fixation of cells [43]. Integration of porous meso- and nanostructures (for example, porous glasses) into a microfluidic chip allows us to realize new possibilities in the study of biological samples. Porous structures can be used as: filtration and separation units, elements of electroosmotic pump, base for sensitive sensor, etc. The possibility of using porous sodium borosilicate glass of SBS-MAP as a sensory element when insulin detection in the sample at competitive immunoassay was shown [44, 45]. This approach can be applied when creating microfluidic devices with biosensor elements for the detection of other proteins.

4. Conclusion
Micro- and nanofluid technologies are widely used in the modern developments of systems for sequencing nucleic acids, devices for digital amplification of nucleic acids, systems for testing diagnostic and therapeutic approaches in medicine. Microfluidic chips are considered an ideal platform for biochemical assays, tissue engineering, drug screening, detection of biomolecules, research and analysis of cells, toxicology studies [46, 47]. The development of microfluidic devices with integrate functional nanostructures will create fundamentally new devices with unique capabilities for biological and medical research.
References

[1] Tetala K K R and Vijayalakshmi M A 2016 *Analytica Chimica Acta* **906** 7e21
[2] Yousuff C M, Ho E T W, Hussain K I and Hamid N H B 2017 *Micromachines* **8** 15
[3] Chin C D, Linder V and Sia S K 2012 *Lab Chip* **12** 21
[4] Kim J, Jensen E, Stockton A and Mathies R A 2013 *Anal. Chem.* **85** 16
[5] Kulinsky L, Noroozi Z and Madou M 2013 *Methods Mol. Biol.* **949** 3
[6] Bhatia S N and Inger D E. 2014 *Nature Biotechnology* **32** 760
[7] Streets A M and Huang Y. 2013 *Biomicrofluidics* **7** 011302
[8] Hu J-B; Chen S-Y; Wu J-T; Chen Y-C and Urban P L 2014 *RSC Adv.* **4** 10693
[9] Zhu Z, Guan Z, Jia S, Lei Z, Lin S, Zhang H, Ma Y, Tian Z-Q and Yang C J 2014 *Angew. Chem. Int.* **53**, 12503
[10] Trantum J R, Baglia M L, Eagleton Z E, Mernaugh R L and Haselton F R 2014 *Lab Chip* **14**, 315
[11] Nosrati R, Vollmer M, Eamer L, San Gabriel M C, Zeidan K, Zini A and Sinton D 2014 *Lab Chip* **14** 1142
[12] Kaushik G, Leijten J, Khademhosseini A 2017 *Stem Cells* **35** 51
[13] Ungererait M, Rana A, Rera M, Granie I J and Walker D W 2014 *Cell Rep.* **8** (6) 1767
[14] Polini A, Prodanov L, Bhise NS, Manoharan V, Dokmeci M R and Khademhosseini A 2014 *Expert Opin Drug Met.* **9** 335
[15] Roth A, Singer T 2014 *Adv. Drug Delivery Rev.* **69-70** 179
[16] Esch EW, Bahinski A and Huh D 2015 *Nat. Rev. Drug Discovery* **14** 248
[17] Zhao X-M, Xia Y, Whitesides G M 1997 *J. Mater. Chem.* **7** 1069
[18] Berthier E, Young E W K and Beebe D 2012 *Lab Chip* **12** 1224
[19] Roman G T, Hlauš T, Bass K J, Seelhammer T G and Cubertson C T 2005 *Anal. Chem.* **77** 1414
[20] Hillborg H, Tomczak N, Olah A, Schonherr H and Vancso G J 2004 *Langmuir* **20** 785
[21] Sui G, Wang J, Lee C-C, Lu W, Lee S P, Leyton J V, Wu A M and Tseng H-R 2006 *Anal. Chem.* **78**, 5543
[22] Domansky K, Leslie D C, McKinney J, Fraser J P, Sliz J D, Hamkins-Indik T, Hamilton G A, Bahinski A and Inger D E. 2013 *Lab Chip* **13** 3956
[23] Nge P N., Rogers C I and Woolley AT 2013 *Chem. Rev.* **113** 2550
[24] Saha B, Toh W Q, Liu E, Tor S B, Hardt D E and Lee J 2016 J. Micromech. Microeng. 26 013002
[25] Kuznetsov A I, Koch J and Chichkov B N 1996 *Appl. Phys. A* **63** 109
[26] Li L, Marchant R E, Dubnisheva A, Roy S and Fissell W H J 2011 *Biomater. Sci.* 22 91
[27] Iliescu C, Taylor H, Avram M, Miao J and Franssila S 2012 *Biomicrofluidics* **6** 016505
[28] Choi S, Goryll M, Yi L, Sin M, Wong P K, and Chae J 2011 *Microfluid. Nanofluid* **10** 231
[29] Lopez G A, Estezvez M-C, Solera M and Lechuga L M 2017 *Nanophotonics* **6** 1
[30] Wang X, Cheng C, Wang S and Liu S. 2009 *Microfluidics and Nanofluidics* **6** 2
[31] Levy S L and Craighhead H G 2010 *Chem. Soc. Rev.* **39** 1133
[32] Siwy Z S and Howorka S 2010 *Chem. Soc. Rev.* **39** 1115
[33] Shimizu H, Mawatari K and Kitamori T 2010 *Anal. Chem.* **82** 7479
[34] Lopez G A, Estezvez M-C, Solera M and Lechuga L M 2017 *Nanophotonics* **6** 1
[35] Oh Y, Park S, Kang M, Choi J, Nam Y and Jeong K 2011 *Small* **7** 184
[36] Bayley H 2015 *Clinical Chemistry* **61** 1
[37] Rincon-Restrepo M, Mikhailova E, Bayley H and Maglia G 2011 *Nano Lett.* **11** 2
[38] Storm A, Chen J, Ling X, Zandbergen H and Dekker C 2003 *Nat. Mater.* **2** 8
[39] Ying Y L., Zhang J, Gao R and Long Y T 2013 *Angew. Chem. Int. Ed. Engl.* **52**, 50
[40] Merchant C A, Healy K, Wanunu M, Ray V, Peterman N, Bartel J, Fischbein M D, Venta K, Luo Z, Johnson A T C and Drndic M 2010 *Nano Lett.* **10** 8
[41] Guihua Yu and Lieber C M 2010 *Pure Appl. Chem.* **82** 12
[42] Zheng G, Gao X and Lieber CM 2010 *Nano Lett.* **10** 3179

[43] Evstrapov A A, Mukhin I S, Bukatin A S and Kuhtevich I V 2012 *Nuclear Instr. and Meth. in Phys. Res. B* **282** 145

[44] Evstrapov A, Esikova N, Rudnitskaya G and Antropova T V 2010 *Optica Applicata* **XL** (2) 333

[45] Esikova N A, Evstrapov A A, Bulyanitsa A L and Antropova T V 2015 *Glass Physics and Chemistry* **41** (1) 89

[46] Wu J, He Z, Chen Q and Lin J-M 2016 *Trends in Analytical Chemistry* **80** 213

[47] Kolahchi A R, Mohtaram N K, Modarres H P, Mohammadi M H, Geraili A, Jafari P, Akbari M and Sanati-Nezhad 2016 *Micromachines* **7** 162