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REVIEW

Application of microreactors in medicine and biomedicine

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Summary
Microreactor technology is an interdisciplinary field that combines science and engineering. This new concept in production, analysis and research is finding increasing application in many different fields. Benefits of this new technology pose a vital influence on chemical industry, biotechnology, the pharmaceutical industry and medicine, life science, clinical and environmental diagnostic. In the last few years, together with microplant development, a great part of research investigation is focused on integrated micro-systems, the so called micro-total-analysis-systems (μ-TAS) or lab-on-chip (LOC). They are devices that perform sampling, sample preparation, detection and date processing in integrated model. Cell sorting, cell lysis, single cell analysis and non-destructive single cell experiments on just one microreactor, makes the LOC platform possible. Clinical diagnostic devices are also leaning towards completely integrated, multiple sophisticated biochemical analyses (PCR amplification, cell lysis, separation and detection) all on a single platform and in real time. Special attention is also paid to the usage of microdevices in tissue. Tissue engineering is one of the most promising fields that can lead to *in vitro* tissue and organ reconstruction ready for implantation and microdevices can be used to promote the migration, proliferation and the differentiation of cells in controlled situations.

Key words: microreactors; microfluidics; medicine; biomedicine; micro-total-analysis-system; lab-on-chip

INTRODUCTION

In the past few years, microreactor technology has demonstrated numerous advantages in different fields of application. It is presented as a novel and break trough technology on which the new concept of production and research will be built upon. The chemical industry, biotechnology, pharmaceutical industry and medicine, life science, clinical and environmental diagnostic are just some of the small fields where this new concept in production, analysis and research could find its place of application. By decreasing the equipment size by several magnitude levels, substantial economical benefits, improvement of intrinsic safety, and a reduction of environmental impact can be achieved (Rebrov et al. 2003). In biotechnology and biochemical processing, the need to manipulate fluids moving in a narrow channel has stimulated several new research areas such as the development of new microfabrication methods for fluidic systems, the study of the fundamental behaviour of fluids etc. (Chován and Guttman 2002). Microreactors can be used both as research instruments in a laboratory scale to enhance the development of new catalysts and processes and for

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the determination of reaction kinetics as well as real production units in a large scale (Hernandez Carucci et al. 2009). From the medical and biomedicine point of view, clinical diagnostic devices using microreactor concepts are leaning towards completely integrated, multiple sophisticated biochemical analyses (PCR amplification, cell lysis, separation and detection) all on a single platform and in real time. The ability to miniaturize entire biomedical systems has the potential to reduce the cost of health-care management. There is also special attention paid to the usage of microdevices in tissue engineering and microengineering development. In this manuscript an intensive overview of the recent developments in the field of microfluidic technology in medicine and biomedicine will be given.

MICROREACTORS IN GENERAL

Microreactors are defined as miniaturized reaction systems fabricated by using, at least partially, methods of microtechnology and precision engineering (Ehrfeld et al. 2005). The term "microreactor" is the name that is generally used to describe a great number of devices that have small dimensions. Other names that are rarely used are nano-, mili- and mini-reactors. Most of the currently used structured microreaction devices take advantage of microfluidics and nanofluidics, which enables the use of micro and nanolitre volumes that ensure high efficiency as well as repeatability of biocatalytic processes (Urban et al. 2006).

Structure

Microreactors, in their simplest form, consist of a network of microchannels (Fig. 1a), in the range of 10 μm to 500 μm etched in a solid substrate. They may be fabricated from different materials including glass, silicon, quartz, metals and polymers such as polydimethylsiloxene (PDMS). Optimal material selection depends on chemical compatibility with solvents and reagents, costs and detection methods used in price control. The most commonly used material is glass since it is chemically inert and transparent which allows the visual inspection of microchannels (McCreedy 2000). Metal devices are often used in fast exothermic, heterogeneously catalyzed reactions and in different separation processes. Different fabrication techniques are also included in microchannel production. Photo-lithography, hot embossing, powder blasting, injection moulding, ultrasonic technologies and laser microformation are just some of them. It is also possible to combine different techniques. A combination of lithography, electroplating and modelling, called LIGA, was successfully used for the production of microreactors. Selection of a fabrication technique has a great impact on the flow in a microchannel where a rough surface can have a negative effect on fluid movement (i.e. on flow stability) so it is necessary to select an appropriate production technique. Microchannels or microchannel networks, if the device consists of several connected channels, are connected to series of reservoirs containing reagents by fused connectors. Reagents can be brought together on a specific sequence, mixed and allowed to react for a specific time in a controlled conditions using (most commonly) electrokinetic and/or hydrodynamic pumping. Combination of microchannels and supporting base material form a chip (Fig. 1b). A combination of a chip, supporting base material and connecting fluid lines form a unit (Fig. 1c). By a combination of other microdevices (micro mixers, micro heat exchangers, micro separator, micro absorbers etc.) and different element configurations, more complex devices, like some mentioned in this paper, are being developed. Should the throughput be increased, units with the same parameters and characteristics are being combined into a series or in parallel (see "numbering-up").

Basic characteristics and advantages

Microreactors exhibit numerous practical and performance advantages when compared to traditional, equivalent macroreactors. The small dimensions of microreactors allow usage of minimal amounts of reagent under precisely controlled conditions and make it possible to rapidly screen reaction conditions and improve the overall safety of the process (Gerey et al. 2006). Beside that, excellent mass and heat transfer, shorter residence time, a smaller amount of reagents, lightweight and compact system design, catalyst and waste products comparing to equivalent macroscale reactors, laminar flow, effective mixing, better process control and small energy consumption are just some of the microsystem advantages (Ehrfeld et al. 2005). Furthermore, they could be easily coupled with numerous detection techniques together with the pretreatment of the samples on the one single chip. But one of the main motivations for the use of microreactor technology is the gain in the yield and safety.

As mentioned, given the small dimensions, the flow regime is typically laminar. This type of flow favours the control and modelling of the reaction and provides high surface to volume ratio and interface areas, which is very important especially for multiphase systems. Flow in the microreactor can be

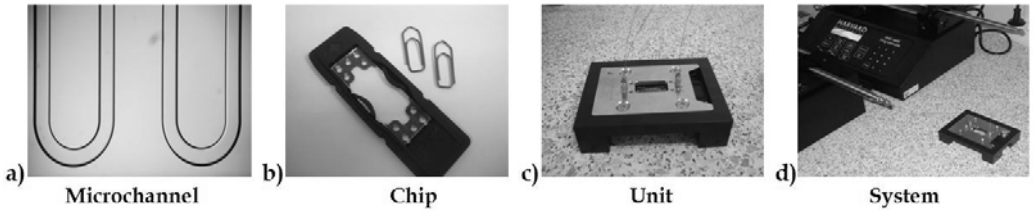

Fig. 1. **Basic structural units of microreactor system.**

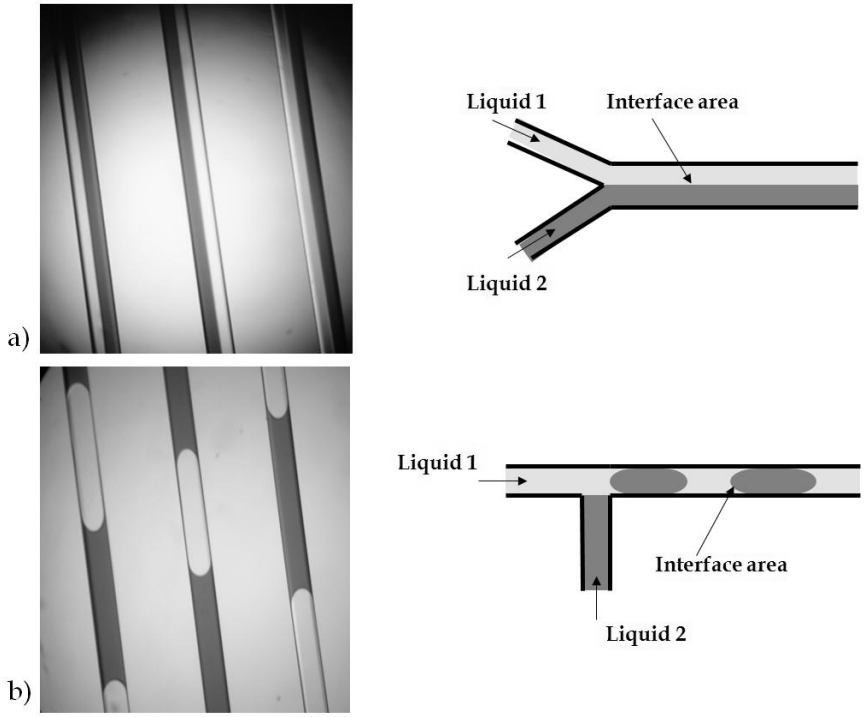

Fig. 2. **Flow patterns;** (a) parallel flow, (b) slug flow**.**

additionally divided into a single phase or a multiphase flow. When a multiphase flow is developed, two or more phases are separately added to the reactor where they form different flow patterns. When using a liquid-gas system in the microreactor a number of different flow patterns may be distinguish: bubble flow, slug (Taylor) flow, annular flow, spray flow, foam flow and transitional regimes (Sobieszuk et al. 2010). For the liquid-liquid system the most typical are a parallel and slug or segmented flow (Kashid 2007, Dessimoz et al. 2008) (Fig. 2). Slug flow in a microchannel is a flow characterised by a series of a liquid slugs of one phase separated by the other. The flow pattern formation depends on linear velocity (Burns and Ramshaw 2001), ratios of the phases, fluid properties, the channel geometry (Kashid 2007, Kashid and Agar 2007) and the construction material of the microreactor; all these parameters have to be considered when controlling the flow pattern.

Also, one of the most important properties of a microreactor is the high surface-to-volume ratio. Because of it, on the microscale it is possible to perform extremely rapid and high exothermic reactions as well as making the mass transfer distance very low. Specific surfaces of the microchannel

Table 1. **Comparison of macro- and micro-heat exchange systems.**

Parameter	Shell and tube heat exchanger	Compact heat exchanger	Microchannel heat exchanger
Surface-to-volume ratio [m^2/m^3]	50–100	850–1500	>1500
Heat transfer coefficient (liquid) [$W/(m^2\ K)$]	~5000 (tube side)	3000–7000	>7000
Heat transfer coefficient (gas) [$W/(m^2\ K)$]	20–100	50–300	400–2000
Approach temperature [°C]	~20	~10	<10
Flow regime	Turbulent	Turbulent	Laminar

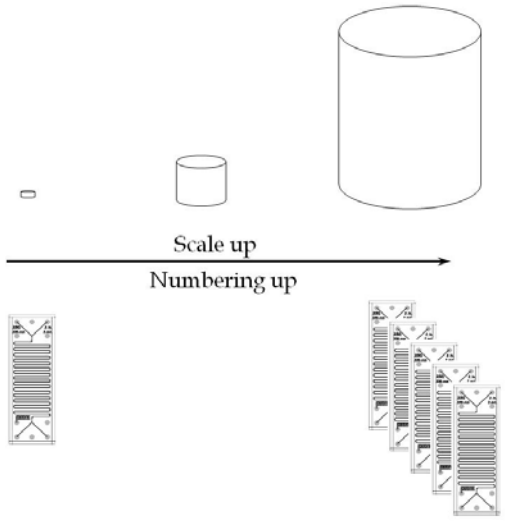

Fig. 3. **Comparison of scale-up methodology in macroreactor and microreactor system.**

amount from 10,000 to 50,000 m^2/m^3. In comparison to that, typical laboratory and production vessels usually do not exceed 1000 m^2/m^3 and 100 m^2/m^3 (Ehrfeld et al. 2005). This significant increase in the surface area relative to the volume will dramatically affect the transfer of mass, momentum and energy. Because of the high surface to volume ratio in microchannels, heat transfer is very efficient and reaction temperatures in microreactors can be regulated by very effective heat removal or application (Pohar and Plazl 2009). As a result, the heat transfer coefficient measured in a microreactor goes up to 25,000 $W/(m^2\ K)$. This exceeds those of conventional heat exchangers by at least one order of magnitude (Ehrfeld et al. 2005). A comparison between the micro and macro heat exchange system is presented in Table 1.

The excellent heat transfer characteristics of microfabricated devices also avoid the risk of potential significant industrial accidents caused by a thermal runaway (Chován and Guttman 2002).

Numbering up

Numbering-up or scale-up (Fig. 3) was among the major predictions on microreactors benefits made in pioneering and was later a topic of in-depth industrial analysis on process intensification (Schenk et al. 2004). Connecting microreactors to operate in parallel or in a series of compact microplants could be built-up (Löwe et al. 2002, Carpentier 2005).

There are two ways to perform numbering up: external and internal. External numbering-up refers to a parallel connection of many devices. The advantage of this type is the possibility to use standard process-control equipment but because of the disadvantages like fluid equipartition, high costs of fabrication and material, internal numbering-up is proposed as an alternative. Internal numbering-up means the parallel or the serial connection of the functional elements only, rather than of the complete devices (Fig. 4). These elements are usually packed in a housing that contains one flow manifold and one collection zone (Schenk et al. 2004). When to use one or the other principle, depends on the reaction or process performed in the microchannel.

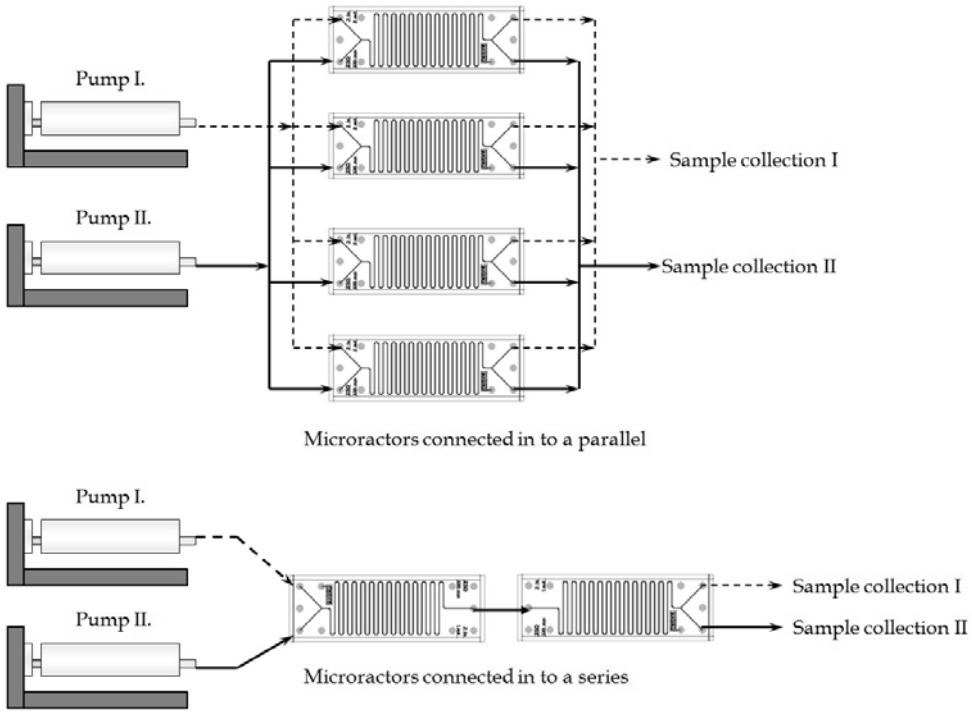

Fig. 4. **Simplified methodology of serial and parallel numbering-up.**

In comparison to scale-up, one of the biggest advantages of numbering-up operation systems is that continuous operation is uninterrupted if one of the units fail, because it can easily be replaced without effecting the other operating units. Another advantage is that unit operation development meaning timeframes needed for the setup, testing and turnaround are much smaller than in the traditional technology development. Once the process is described in a single chip, by combining the same units we can increase the capacity.

Micro-total-analysis-system or lab-on-chip

In the last few years, together with numbering up and microplant development, a great part of research investigation is focused on integrated micro-systems, the so called micro-total-analysis-systems (μ-TAS) or lab-on-chip (LOC); μ-TAS includes pumps, valves, mixers, reactors and separators (Santini et al. 2000) so those devices can perform sampling, sample preparation, detection and data processing in an integrated manner (Fletcher et al. 2002, Tanaka et al. 2006). Performing biochemical reactions within

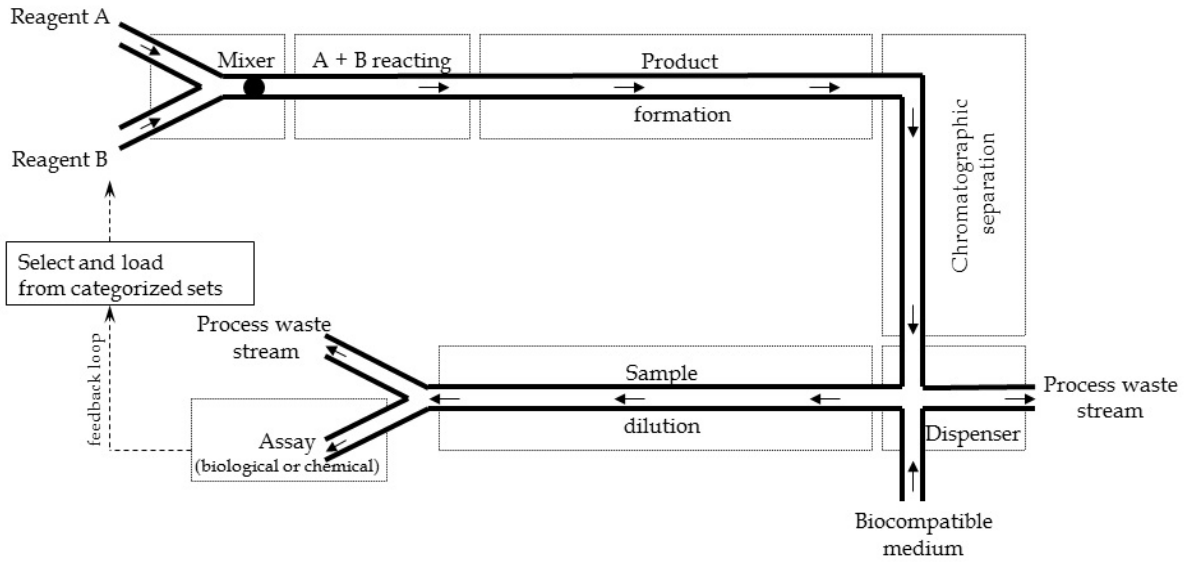

Fig. 5. **Micro-total-analysis-system diagram (μ-TAS).**

microfluidic systems also provides the opportunities to perform real time separation (Watts and Haswell 2003).

Optimally, such devices would automatically perform sampling, sample preparation, separation, detection and data processing in a fully integrated manner (Fig. 5). In addition these devices offer potential as remote controlled systems, which could be placed in inaccessible locations for continuous monitoring of processes (Fletcher et al. 2002). The most popular area of μ-TAS research has been in the biomedical field, particularly in genomics and proteomics where it has been used in the analysis of both DNA and proteins. The miniaturization of chemical reactors offers many advantages of relevance to the pharmaceutical industry, which is constantly searching for high-throughput methods of producing products with a high degree of chemical selectivity (Watts and Haswell 2003). To enable this technology to function, different evolving technologies like microlithography, micro-electro-mechanical-systems (MEMS) technology, micro-fluidics and nanotechnology are being developed in parallel (Zhang et al. 2006).

Micro-electro-mechanical-systems (MEMS) technology refer to the miniaturization process of biological and chemical analytical devices and biosensors that show promising results in many fields of application from detection of pathogens, to environmental pollutants detection. A general approach of these systems is based on microcomponent unit operations such as micromixers, micro heat exchangers, microadsorbers, microchannel catalytic reactors, microseparators etc. The main advantage is that these systems can provide a high level of control over temperature and the consequent reaction, in addition to thermal integration and parallel operation (Chován and Guttman 2002). In the biomedical field, microseparation and microfiltration systems, used for sample pre-treatment are of special interest.

Microreactor disadvantages

Taking into consideration all mentioned microreactor advantages, it is obvious that they have many areas of application but those systems are still not perfect. There are some problems that occur when working with microreactors. Frequently quoted disadvantages of microreactors are high fabrication cost, low throughput, incompatibility with solids and the omission of cost reduction by scale up effects which lead to still poor industrial acceptance (Westermann 2009). To insure stable flow in a microreactor it is necessary to use low pulse or pulseless pumps; pumps are one of the most expensive parts of the microreactor apparatus. Due to the small dimensions of microchannels one of the biggest problems is clogging (Poe et al. 2006) when working with small diameter solids (for example enzyme dispersion) in a microreactor or with highly viscous solvents clogging can occur. It is also important to mention that when working with microreactors a very short residence

time can be achieved, so it is necessary to perform a fast reaction. Fast reactions also require very active catalysts that are stable in the microreactor. So microreactors still, cannot be used as a replacement for all traditional processes. Another very important problem is analytics. By the number of applications, off-line analytics is used so sometimes a very long time period is necessary for gathering a sufficient amount of samples for performing the analysis. Therefore, there are many efforts in developing on-line analytical techniques for microstructured devices.

APPLICATION OF MICROREACTOR IN MEDICINE AND BIOMEDICINE

A great number of papers have been published on the application of microreactors not only in the field of fundamental laboratory research but also in the field of medicine and biomedicine. They have been developed in order to facilitate production and routine work in analysis. The general opinion is that the pharmaceutical and fine chemical industry could benefit from this technology approach, especially since it is important to develop new technologies that enable rapid synthesis, screening, production and metabolic studies of novel chemical entities and to reduce the development costs that are extremely high.

From the medicine and biomedicine point of view, clinical diagnostic devices are leaning towards completely integrated, multiple sophisticated biochemical analysis (PCR amplification, cell lysis, separation and detection) all on a single platform and in real time. The ability to miniaturize entire biomedical systems has the potential to reduce the cost of health-care management. There is also special attention paid to the usage of microdevices in tissue engineering and microengineering development. Tissue engineering is one of the most promising fields that can lead to *in vitro* tissue and organ reconstruction ready for implantation. Tissue scaffold is essential framework that provides an initial cellular support necessary for an appropriate cell density and functionality in tissue engineering. Implementing microreactor scaffolds cell migration could be altered together with proliferation and differentiation to achieve functional tissue replacement. Necessary nutrients can also be provided together with the removal of cellular waste through a microfluidic network (Leclerc et al. 2006). On the other hand, microengineering neural development is taking in advantage of all microfluidics properties to achieve better interaction between target cells. Researchers hope that this investigation will give better and precise insight of the nervous system, form neurogenesis and neuronal migration to axonal path-finding and synapse forming (Gomez 2000).

High throughput screening (HTS)

One of the most important applications of the microreactor system especially the LOC is the high throughput screening (HTS) for combinatorial chemistry, gene and protein analysis etc. The polymerase chain reaction (PCR) microchip/ microdevices, together with the capillary electrophoresis (CE) microchips and the hybridization microchips are studied rapidly. They could be used for fast DNA replication, microbial detection, biological agents and diseases diagnostics including infectious diseases like the human immunodeficiency virus (HIV), the human papillomavirus (HPV), the hepatitis virus and other (Zhang et al. 2006). To enable this technology to function, different evolving technologies like microlithography, micro-electro-mechanical-systems (MEMS) technology, microfluidics and nanotechnology are being developed in parallel (Zhang et al. 2006).

Target molecules separation

Devices that are used for separation in microreactor technology are based on extraction, filtration and diffusion processes. Usually they are designed as parts of different analytical systems and not as single instruments.

In biotechnology and chemistry, extraction of target molecules from a primary liquid and concentration of these molecules in a secondary liquid is an essential operation process performed before further analysis. Extraction processes, performed in microextractors, are based on the contact of two immiscible fluids (usually a combination of organic and inorganic fluids) and the resulting solute between the two phases. Up to now different solutions have been proposed like immiscible liquids co-flowing in groves and separated by micro-ridges, microporous membranes, co-flowing immiscible liquids separated by interfaces anchored on micro-pillars and more recently droplets continuously flowing through the concentrating liquids (Berthier et al. 2009). The very important field of microsepation is sorting specific biological targets from complex mixtures. Adams et al. (2008) reported the design and fabrication of the multitarget magnetic activated cell sorter. The mentioned device incorporates microfabricated ferromagnetic stripes to generate a magnetic field within the microchannel.

Microfiltration is performed by micromembranes and microfilters that are typically used to remove

particles in the range of 0.1–10 μm from a suspension. This action is usually taken before the suspension is pumped into microchannels. The main reason is that microreactors inherently suffer from clogging if particle solutions are employed or generated. A simple filtering step in advance of the reaction may help to prolong the operational life-time of a microreactor.

Different processes based on diffusion are developed on a microscale. They are mainly used in biotechnology and system biology. Some of them are capillary electrophoresis, ultra-thin layer gel electrophoresis, micro column liquid chromatography etc. Capillary electrophoresis is based on the difference in mobility of analytes in the electric field. After the development of non-gel sieving based capillary zone electrophoresis and after overcoming the problems like washing the capillary and the refilling the gel, this methodology became crucial for DNA sequencing. Besides that, it is now widely utilized for analysis of genotyping such as single standard conformation polymorphisms, single nucleotide polymorphisms and various kinds of mutations, metabolic studies (Cheng et al. 2006), proteomic analysis, single cell analysis etc. (Liu et al. 2006).

Drug discovery and testing

There has been great interest is using microfluidic systems as a valuable tool for discovering of many drugs. When compared to the equivalent bulk reactions, reactions performed in the microreactor invariably generate purer products in much shorter time. As mentioned, many chemical reactions that have been demonstrated improved reactivity, product yield and selectivity when performed in microreactors in comparison to those produced by traditional laboratory practice (Watts and Haswell 2003). The microfluidic channel can also be used for testing target selection, leading identification and optimization and preclinical testing and dosage developments (Kang et al. 2008). Roberge et al. (2005) claims that 50% of the reactions in the fine chemical or pharmaceutical industry could benefit from a continuous process based mainly on microreactor technology, and for the majority (44%) a microreactor would be the preferred reaction device. System integration is also one of the most important roles of miniaturization; it will lead to better process control and intensification (Chován and Guttman 2002). Once a microreactor is optimized it can easily be introduced into an industry. One of the most challenging tasks in drug discovery is predicting pharmacokinetic behaviour in humans. For this purpose cell based microfluidic devices are used. They can be useful for accessing the interaction within normal and diseased cells together with simulating and recreating cell-cell interactions that are present in living organisms. Another application is to test the synergistic effects of combinatorial drugs. Combinatorial drugs offer new hope for a number of diseases, but the range of possible combinations is too large to be investigated in expensive clinical studies (Kang et al. 2008). Today the isolated human hepatocytes are used for first drug screening. To ensure hepatocytes stability microdeviceds are used to test nutrient, oxygen, metabolic waste and new drug concentration gradients (Maguire et al. 2009). Microscale devices have the potential to be used for the analysis of the desired *in vivo* system.

Cell investigation and single cell experimentation

Cell sorting, cell lysis, single cell analysis (Huang and Rubinsky 2001) and non-destructive single cell experiments on just one microreactor, the LOC platform is now possible. The success of LOCs was initially determined by their enhanced analytical performances: their fast, highly sensitive and reproducible analysis, with low consumption in chemical energy. Cell sorting based on the fluorescence path of the cells, cell size, specific proteins expressed on their surface or electrical properties are usually used (Kovac and Voldman 2007). For the next step, cell lysis, chemical, electrical, optical, mechanical or thermal is incorporated into a platform. The possibility of controlling the spatial and temporal cues to which cells are exposed, necessary for non-destructive single cell experimentation and analysis (Le Gac and van der Berg 2009) in combination with the dimensions of the microchannel that is highly compatible with the size of the biological cells is what makes LOC so interesting. Using this technology it is possible to get information about the behaviour of a single cell which is not possible during study at a large scale (Dragavon et al. 2008, Le Gac and van der Berg 2009) where the main problem is the long diffusion time that is necessary for a reagent to access the cell and then to provide feedback information (Tanaka et al. 2006). This problem is overcome in microreactors where diffusion impact is negligible. Microchannel devices can be useful in imitating the biological reaction apparatus, such as cellular surface and vascular systems, by providing the advantages of reduced space and laminar flow (Miyazaki and Maeda 2006). By using a microfluidic approach (using microinjections) the problem of delivery of molecules across the cell membrane for many experimental biological protocols can be solved (Adamo and Jensen 2008).

Since the scale of liquid microspace inside the LOC devices is fitted to the size of the cells, microchip technology has several advantages for cellular biochemical analysis. The small internal dimensions found in LOC devices enable the trapping of individual cells and the investigation on long-lasting processes. In comparison, conventional cell studies on large scale require a large cell population and they don't reveal the behaviour of a single cell. A cell grown in a culture is used in a variety of contexts such as cell biology, tissue engineering, biomedical engineering, and pharmacokinetics for drug development. Micro-technology facilities the study of cell behaviour *in vivo* because it provides the necessary tools for recreating *in vivo*-like microenvironments (Chiu 2001, Walker et al. 2004). Microfluidics cell culture platforms can be divided into: (i) a single cell-type culture and (ii) a multiple cell-type culture (Huang et al. 2010).

In its simplest form a microchip for cell cultivation consists of two small access holes for an inlet and outlet of reagents and a large hole for cell introduction (Fig. 6).

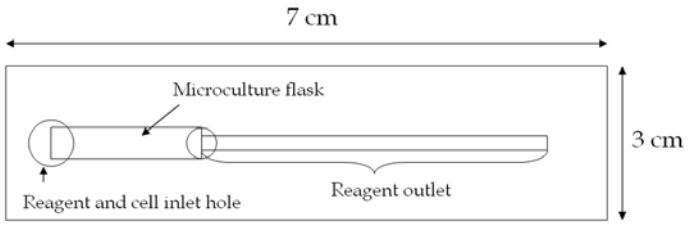

Fig. 6. **Illustration of the microchip for cell culture** (Tanaka et al. 2006)**.**

Combining this simple form with different LOC technologies cell sorting, cell lysis and single cell analysis and non-destructive single cell expe-rimentations could be performed as previously described in the section on LOC application. El-Ali et al. (2005) described the microfluidic device with rapid stimulus and lysis of mammalian cells for resolving fast transient responses in cell signalling networks.

The next new step is the concept of a "lab-in-a-cell" (LIC) where the single cell is used as an experimental unit. The concept relies on the basic idea of utilizing a powerful cellular factory for experimental purposes. The first advantage of this system is that a cell performs many more operations and functions that could ever be integrated into a single LOC device. The second is that all occurring processes are ruled by diffusion so there is request for additional mixing and pumping devices. To make this system work, three main tasks should be fulfilled: cell handling (placement of cells in precise locations), cell characterisation (ability of tools for real time characterisation of a single cell) and accessing intracellular content (the possibility of crossing cellular content and inside monitoring). Different principles and methods crucial for LIC expe-rimentation are mentioned in Table 2.

Biomolecule analysis

A great number of microeractors application refers to protein and peptide mapping together with nucleic acid analysis. In protein analysis the most frequently used enzyme is trypsin which catalyses the process of protein digestion through the hydrolysis of peptide. The analysis of nucleic acids relays on the polymerase chain reaction (PCR) and amplification of DNA on the microscale. By combining these principles together with preparation, screening, detection etc. more complex devices called micro-total-analysis-systems (μ-TAS) or lab-on-chip (LOC) are developed.

Using microfluidic devices, it is also possible to perform high throughput analysis of different biomolecules like proteins, nucleic acid, amino acids, DNA, peptide mapping etc. The analysis of genetic material typically calls for first the amplification of the DNA sample and then its detection. The amplification of DNA is usually performed by a polymerase chain reaction (PCR) based on the reconstruction of each double helix. A major advantage of miniaturizing PCR systems is the fact that a much lower thermal mass of the reaction chamber allows for much rapid heating and cooling and thus a much faster process overall. Beside that, it is possible to integrate heaters and temperature

Table 2. **Micro- and nano-fluidic tools and protocols essential for LIC experimentation** (Le Gac and van der Berg 2009)**.**

	Technique	Principle	Reference
Cell handling	Mechanical and chemical trapping	Mechanical trapping includes lateral and planar trapping using side structures or microapertures or microwells located under the cells. Chemical trapping relies on formation of extracellular matrix proteins on which cells are immobilised.	Kurosawa et al. 2006 Thery and Bornens 2006 Dragavon et al. 2008 Skelley et al. 2009
	Electrical manipulation	Method involves electrophoretic or dielectrophoretic methods which explode negative charges at the cell surface and its dielectric properties.	Ozkan et al. 2003 Tresse and Takeuchi 2004 Taff and Voldman 2005
	Optical manipulation	Laser generated force is used for precise 3D displacement of cell.	Piggee 2009
	Alternative techniques	Magnetic trapping in based on cell attachment to magnetic beads. Usage of droplets where the substrates are scattered by cells.	El-Ali et al. 2005 Adams et al. 2008
Cell characterisation	Optical characterisation	Fluorescent proteins like green fluorescent protein, due to their stability have been employed for cell tracking. Other approaches are nanoparticles used for high resolution imaging and thermal lens microscopy that relies on the molecular adsorption properties in the visible and UV range.	Lidke et al. 2004 Valero et al. 2008
	Biochemical sensing	Using electrical sensors or combination of electrical and optical measurements, various parameters like pH, temperature, O_2 concentration can be obtained. Also with the possibility of fabrication sensing structures on the nanoscale opens the possibility to subcellular and intracellular measurements.	Cheng et al. 2006 Guenat et al. 2006 Krommenhoek et al. 2007
	Patch-clamping	Patch-clamping involves ion channels that become interesting because of their accessibility on the cell membrane and possibility to be automated and parallelized.	Fertig et al. 2002 Ionescu-Zanetti et al. 2005
	Mechanical sensing	Information is derived from the mechanical properties of a cell membrane because these are altered in response to specific stimuli during the cell cycle and development.	Waconge et al. 2008
Accessing intracellular content	Crossing the cell membrane	Combination of different paermeabilization technique on the membrane (electroporation, pore-inducing chemicals, high energy laser pulses) and LOC methodology enhances poration performances. Another approach for crossing membrane obstacle is piercing cell membrane with a sharp structure.	Huang and Rubinsky 2001 Adamo and Jensen 2008
	Nanochanneles and nanopumping	Nanochannles and nonopumping are essential for the manipulation of subcellular liquid volumes.	Tas et al. 2002 Hoang et al. 2009
	Cell fussion	Method is based on fusion with a vesicle or another cell to achieve material introducing in to cell. Electrofusion benefits from LOC technology because it brings a higher degree of control over the different steps of cell alignment, pairing and fusion.	Chiu 2001

sensors into the same chip to improve temperature control and process efficiency (Slyadnev et al. 2008). Microtechnology is also used for the determination of a precise DNA molecule code.

For gene microarray experiments, probe DNA or oligomer chains are immobilised to a solid surface. Different chains of DNA or oligomer sequences are localised to separate a specific molecule. The target (free DNA or RNA) is labelled with fluorescent markers and hybridized to the microarray. The array is scanned and images are interpreted. Microarray systems vary in the type of polynucleic acid immobilised, the methods of immobilisation, nucleic acid formation (on chip or *ex situ*), hybridisation and reading of the hybridised array (Sanders and Manz 2000). Microfluidic DNA amplification can be performed in continuous flow microPCR and microfluidic in PCR droplets (Fig. 7). Continuous flow microPCR strongly depends on optimisation of the device design and operating flow rates (Zhang and Ozdemir 2009). Chip based microPCR devices can be classified into two types: well based PCR chips and continuous flow PCR chips. In well-based PCR, the PCR mixture is injected into the well and then the whole chip, including the sample, is heated and cooled through specific thermal-cycling temperatures. Continuous-flow microPCR moves the sample through fixed temperature zones to achieve the required thermal-cycling (Sanders and Manz 2000). Recently, the microdroplet technology has been applied in continuous-flow microPCR, where the PCR effectively occurs within droplets, so that not only PCR inhibition but also carryover sample contamination will be eliminated. In comparison with the conventional continuous-flow microPCR devices, which use a single aqueous phase, the droplet technology can further reduce thermal mass and thus shorten the thermal-cycling process. Lately single molecules studies are being performed on an even smaller scale than micro, using nanochannels (Hoang et al. 2009). Interactions of single DNA and protein molecules were studied in 120 nm × 150 nm fused silica channels (Wang et al. 2005).

Recent expansion in the use of DNA chips and capillary electrophoresis has had an effect on the further development of immunoassay chips. Microchips open new possibilities in immunoanalysis applications. Most steps of the immunoassay procedure can be integrated within a simple chip (Přibyl et al. 2004). Immunoassay is the main analytical technique used in the study of infectious diseases and clinical endocrinology, due to the high sensitivity and selectivity of the antigen-antibody reaction. With development of lab-on-chip technology, miniaturisation of the immunoassay has become very interesting (Xiang et al. 2006).

Peptide mapping in which proteins are identified and characterised, is another interesting field of microreactor application. The mapping finds applications in different fields from quality control to proteomics.

Most of the microreactors described in this field are built as detached biocatalytic chambers. Enzymes in them, for protein digestion are chemically immobilised, trapped or physically adsorbed to a support (Bossi et al. 2004). The most frequently used enzyme is trypsin, the enzyme catalyzing the process of protein digestion through hydrolysis of peptide bonds at a basic residue.

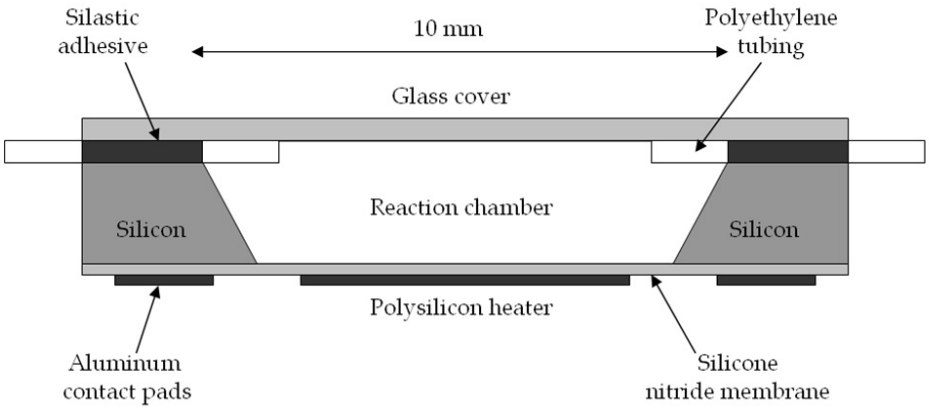

Fig. 7**. Micromachined PCR chamber** (Northrup et al. 1993).

Metabolic studies

In the last decades there has been great interest in syntheses of new molecules as potential drugs. These potential drug substances need to be tested for their adsorption, distribution in the metabolism, elimination and toxicity (ADMET). These properties are usually studied using cell cultures of live animals, but this method is quite expensive and requires long time periods. To eliminate the mentioned problems some microfluidic devices have been developed. Zguris et al. (2005) described a microreactor with microsomes entrapped in a polymer matrix of poly(ethylene)glycol for determination of metabolic products of a potential drug substance. The microsomes were derived from ultrasonic homogenisation of the human liver, and they retained the properties of the liver. They showed that fewer microsomes than hepatocytes are needed to generate the same level of its activity. Mass transfer of drug delivery was characterized by calculating the mesh size of the hydrogel, and metabolic activity was measured using a fluorescent reporter for cytochrome P450.

Metabolic measurements of glucose consumption and production of ammonia via glutamine metabolism were estimated using enzymatic reactions and direct and indirect absorbance measurements, respectively (Kraly et al. 2009).

There is a large interest in the development of microfluidic devices as a tool for metabolic profiling, metabolomics and other metabolite related biological studies. Many approaches for metabolomic analysis utilize high-resolution separation techniques such as capillary electrophoresis. As other microfluidic devices, microchip capillary electrophoresis ensures rapid analysis times, small reagent and sample consumption, portability and low costs (Kraly et al. 2009). Liquid chromatography is also commonly used for metabolic studies; it ensures highly effective separation. The biggest advantage of microchip capillary electrophoresis (CE) is the ability to perform parallel-array or multi-dimensional analysis (Loughran et al. 2005). Guenat et al. (2006) presented a generic platform for cell culture able to monitor extracellular ionic activities for real time monitoring of cell-based responses. Measurement of different ion concentrations in cell cultures is important for understanding cellular signalling and metabolism.

Ion channels are an important field of research due to the fact that those places in the cell serve as true targets. Using micro technology the research is becoming more and more simplified; automated microstructured devices for whole cell patch clamping is one of the advancements in that field (Fertig et al. 2002, Ionescu-Zanetti et al. 2005).

Clinical diagnostics

One of the major applications of microfluidic devices is for disease diagnostics. For example, cell sorting is a method included in many diagnostics tests. Sorting can be based on physical parameters (cell motility, size, deformability, biochemical markers) (Rivet et al. 2011). Traditionally flow cytometry, employing fluorescence detection [high-speed fluorescently activated cell sorting, FACS (Wlodkowic and Cooper 2010)], is used for measurement of the physical and chemical characteristics of cells. Lately many microfluidic flow cytometers using a variety of detection instruments have been developed (Yi et al. 2006). Cell surface markers can also be used for detection and diagnosis of a variety of diseases. Many microfluidic devices used surface coated with antibodies specific to cell surface markers to capture the cell of interest. Microfluidic devices are used for detection of circulating tumour cells, for cancer biomarkers as an epidermal growth factor and $CD4^+$ T-cells (Rivet et al. 2011). There has also been interest in developing microdevices for detecting protein concentrations in plasma (plasma protein profiles are often associated with human diseases). DNA analysis on-chip can be used for diagnosis of genetic basis. PCR base amplifications and analysis can ensure good insight in gene expression. PCR microfluidics insure a large number of parallel amplification analysis and can lead to more accurate information and a better understanding of the analysed processes (Zhang et al. 2006).

Lin et al. (2010) describes the specific use of microdevices for immunoassay tests. Immunoassays have a variety of formats, all of which make use of the sensitivity and specificity of this AB (antibody)-AG (antigen) interaction, which allows the quantification and monitoring of small molecules, such as drugs and metabolites, large proteins, nucleic acids, and even whole pathogens. The immunoassay tests include a series of washing, mixing and incubating steps, therefore they are quite time consuming. Also chemicals are very expensive, so miniaturisation can ensure expenses reduction. Microfluidic devices for immunoassays can be fabricated from a variety of materials. The most commonly used substrate materials are silicon, glass, and polymers. Liquid transport in those systems can be electroosmotic, pneumatic, electrowetting, centrifugal and piezoelectric.

The process of immunoassay on microchips is based on the immobilisation of ABS or AGS on the microchip surface. A scheme of the disc design microchip for immunoassay is given in Fig. 8.

Sun et al. (2009) used a microfluidic device to determine the level of antibodies in the serum of

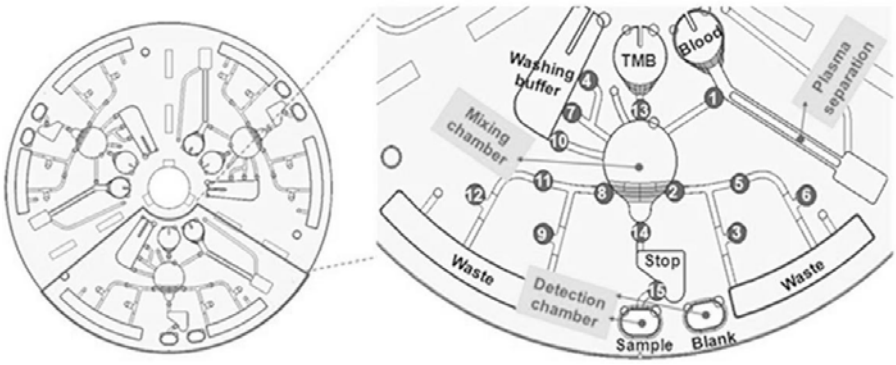

Fig. 8. **Schematic illustrations of the disk design** (Lin et al. 2010).

HIV-positive patients. The developed microfluidic network, replaced the 96-well plate. To improve the sensitivity of microfluidic assay for HIV, an ES membrane was integrated into the microchip improving proteins adsorption. Lawi et al. (2009) presented the microfluidic cartridge system for conducting complex heterogeneous proteomics and genomic assay experiments. The complex systems composed of a disposable microfluidic cartridge and a sensing and controlling instrument are used for clinical studies.

Another example is the use of a microchip for human papilloma virus (HPV) diagnosis (Vecchio et al. 2010). HPV infections are widely considered to be an important risk factor for the genesis of cervical cancer. Molecular biology methods are used for HPV virus detections (mainly polymerase chain reactions and hybridization tests). Due to a high parallelism of analysis miniaturized biochips are recognized to be suitable for HPV analysis. Vecchio et al. (2010) developed plastic, disposables, modular chip suitable for HPV diagnostics, by two sequential steps performed on the same device.

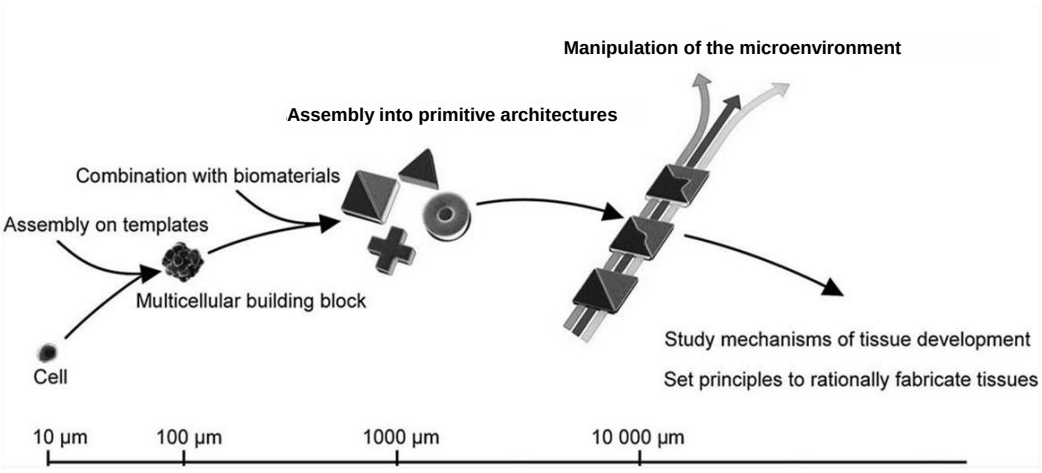

Fig. 9. **From tissue assembly to tissue development** (Rivron et al. 2009).

Tissue engineering

While a large number of patients succumb to multiple organ failure in their final days, the majority of diseases in the current population result from damage, failure, or loss of a single organ or tissue component (Bhatia and Chen 1999). Tissue engineering is one of the promising fields; it may lead to *in vitro* tissue and organ reconstruction. Cartilage, bones and other organs are complex 3D tissues. Tissues scaffold ensures initial cell support to provide the development of cell population. Microchannels can be used for physical orientation of cell migration. Due to the nature of microfluidic devices, the continuous operating mode, nutrients and cellular waste can be removed. Engineered tissue should resemble as much as possible the *in vivo* structures (Leclerc et al. 2006). Microfluid devices provide a well organized microenvironment for multiple cell types (vascular systems and organ systems) (Marimuthu and Kim 2011).

Tissues are often a combination of small repeating units assembled over several scales. The rapid development of complex microfluidic systems, microbioreactors and detecting tools allows the long term culture of microscale tissues in a precisely defined microenvironment (Rivron et al. 2009). It is still great challenge; how to orchestrate the development mechanism *in vitro*. A scheme representing the process from tissue assembly to tissue development *in vitro* is shown in Fig. 9.

Leclerc et al. (2006) described the use of rectangular polydimethylsiloxane microchannels for growth of liver and kidney organotypic cultures. The study demonstrated the positive effect of a rectangular microchannel on an embryonic liver development on a hydrophobic surface. The authors mentioned that the advantage of microchannel scaffolds is that all the dimensions can be precisely controlled. Condie et al. (2007) presented the use of microchannels for immortalized osteoblast precursor adhesion. The idea stemmed from the problem that success and the lifetime of a bone implant depends largely on the degree of osteointegration at material-bone interaction; layer of soft tissue can accumulate at the surface of implanting materials, this can result in many problems. A microchannel was used to test the cells- materials complex interactions, suggesting that minimal width of a flat engineering biomaterial surface insures optimal integration with bone cells.

CONCLUSION

Microreactor technology has seen exponential growth over the last decade with applications in the felds of analytical chemistry, chemical synthesis, chemical engineering, biotechnology and biomedicine. The growth of microreactor technology could be attributed to their merits and unique operating characteristics compared to conventional technology.

The general opinion is that microfluidic and microreactor devices will bring revolution to the world of medicine. The combination of biomedicine and microfluidic technologies provides a unique opportunity to fully exploit the potential of microfluidic technologies and to expend our knowledge of biological systems through precise control of the reaction environment. Cell sorting, cell lysis and single cell analysis and non-destructive single cell experiments on just one microreactor, clinical devices, tissue engineering on a microscale are already our reality and the future developments and expectations look even more promising.

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