Invited Paper

Multi-unit Integration in Microfluidic Processes: Current Status and Future Horizons

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Abstract: *Microfluidic processes, mainly for biological and chemical applications, have expanded rapidly in recent years. While the initial focus was on single units, principally microreactors, technological and economic considerations have caused a shift to integrated microchips in which a number of microdevices function coherently. These integrated devices have many advantages over conventional macro-scale processes. However, the small scale of operation, complexities in the underlying physics and chemistry, and differences in the time constants of the participating units, in the interactions among them and in the outputs of interest make it difficult to design and optimize integrated microprocesses. These aspects are discussed here, current research and applications are reviewed, and possible future directions are considered.*

Keywords: *Microfluidic processes, Lab-on-a-chip integration, Design considerations, Applications, Future directions.*

Introduction

In recent years there has been a phenomenal increase in both research and applications of microfluidic (and nanofluidic) processes. The large number of recent reviews [1-6] indicates the spiralling growth of microfluidic devices and processes. A recent survey [7] has shown that the world-wide market for microfluidic technologies was around £1.98 billion in 2008, and it was expected to increase by 15% every year. This encouraging forecast has understandably motivated commercial interest, as evident from the large number of patents [8] and from products and processes actually in use [9-11].

The growth of microfluidic processes has been not just in terms of patents, publications and products, but also in the variety of areas and reaction systems they cover. Microfluidic processes or devices have been applied in chemical syntheses [12], electrochemical processes [13], enzymatic and cellular reactions [3, 4], quantum dots for semiconductor devices [14], controlled drug delivery systems [15], and the synthesis of nanoparticles for special applications [16]. The reaction systems cover reverse micelles, microemulsions, layer-by-layer films and polyelectrolyte capsules [16].

Most studies in microfluidics have focused on microreactors, which are often the heart of a process. These reactors have many advantages over conventional reactors. They are less costly, more efficient and have greater reproducibility of successive batches. Since the processes are carried out in narrow tubes with high aspect ratios, it is possible to have efficient heat dissipation and accurate control [17, 18]. These benefits are realized strongly in

two areas – exothermic reactions and biological processes – which have the most profitable microreactor applications.

Even though a microreactor is the heart of a microprocess, a successful process needs to integrate this with other micro- or macro-devices. Considering the scales of operation and the complexities of the reactions and transport processes, this integration is not easy. Hence, to develop commercially viable microdevices, current research is increasingly focused on integrated lab-on-a-clip devices rather than on single units. This shift in emphasis is seen from the trend in the number of patents for microreactors, which rose until 2005 and then declined; there was a corresponding increase in patents for integrated micro-chips and micro-processes after 2005 [8], which today account for 40% of microfluidics patents. Since integrated systems are poised for rapid growth and commercial applications, this is an opportune time to evaluate their current status and future prospects.

Rationale for integrated microdevices

Like any physical, chemical or biological process, a successful microdevice also has to be process-oriented. This means several unit operations should be integrated in to one microchip so as to function efficiently to achieve a descried objective. This concept of a coherent assembly of many units has given rise to the term "integrated lab-on-a-chip" (ILOC).

Different architectures have been proposed for ILOCs, according to the participating microunits and the final objective. However, one basic design concept underlies all ILOCs. This is the idea of multiplexing. Multiplexing addresses the problem of controlling flow through a large number (F) of flow channels by manipulating only a small number (C) of control channels. If F is small, then each flow channel may be controlled independently. However, when F is large, such independent controls become unpractical. Thorsen et al. [19] showed by analogy with the multiplexing used in electronic circuits that $C = 2\log_2 F$ achieves control over all F flow channels. This provides a highly significant reduction in control policy; for example, for F = 1024, only 20 control channels are sufficient.

Which multiplexing reduces the control load, it still leaves open the exact topology of the network. It is difficult to propose a universal optimum topology for a given value of F because the choice will depend on the nature of the components of the ILOC, the interactions among them, the processes involved and the outputs. Hence heuristic rules have been proposed as guidelines to avoid random searches. Erickson and Li [20] and Melin and Quake [21] have discussed these heuristics and some common ILOC designs. It emerges from their studies that many designs are inspired by biological applications. This is not surprising since biological systems are complex, require carefully selected upstream and downstream units, and have to be stringently monitored and controlled. These authors, and also Ismagilov [22], emphasize the inadequacy of present-day heuristics and discourage the use of a bottom-up approach in which individual units are optimized and then latched on to a microchip. They suggest instead a top-down approach which allows both architecture-level synthesis and geometry-level synthesis [23], thus accommodating functional optimization in place of unit-wise optimization. The top-down approach also allows the complete ILOC to be decomposed into modular sub-ILOCs, each of which may be functionally optimized and then integrated with the others. However, the authors caution that explicit design rules for both ILOC decomposition and functional optimization are yet to be formulated.

Applications and discussion

We consider here some representative applications that illustrate the strength and versatility of integrated microfluidic processes. Since a major fraction of the applications are for biological or microbiological systems, these are considered separately. We also briefly discuss some manufacturing aspects of integrated microchips through illustrative applications.

Non-biological applications

The complex reaction network and the critical role of temperature in determining the reaction pattern and the outputs have made steam reforming an attractive candidate for lab-on-a-chip studies. Fazeli and Behnam [24] studied the autothermal reforming of methane to produce hydrogen. A microreactor with special geometry was considered and the reactions were considered to follow Langmuir-Hinshelwood kinetics. CFD simulations predicted hot spots at the entrances of the microtubes. A proper distribution of air inflow through the tubes eliminated the hot spots and the resultant performance exceeded that of a commercial reformer.

Arzamendi et al. [25] used methanol in place of methane but for the same purpose. In this study, as well as in that of Fazeli and Behnam [24], the key integration step was between the endothermic reforming step that produces CO and H2 and the exothermic water-gas shift reaction for the finial products, CO_2 and H_2. With microreactors, Arzamendi and associates reported selectivities in excess of 99% for H_2 at 270-290ºC, whereas conventional reformers achieve about 70% selectivity at temperatures twice as high.

Since the reforming process has two key reactions, of which the first step (reforming) is endothermic with $\Delta H = 206$ KJ·mol^{-1} and the second (shift reaction) is exothermic with one-fifth the heat of reaction, recovery and recycling of the exothermic heat is a critical factor in the success of a steam reforming process. Shah [26] therefore addressed this issue and recommended suitable scale-up methods and a packaging scheme to reduce convective and radiative losses.

Other investigators have analyzed applications very different from steam reforming. Khan et al. [27] have described an integrated microprocess for continuous flow silica synthesis and subsequent titania overcoating. Titania (TiO_2)–coated silica (SiO_2) particles are used in photocatalysis and in pigments for photonic crystals. Khan et al.'s microreactor consisted of two stacked reactor layers, the first having microchannels for silica synthesis and the second for titania overcoating. Nissila et al. [28] and Fidalgo et al. [29] combined a microreactor with a mass spectrometer for different applications. The former authors digested bovine heart cytochrome c by trypsin in a microreactor, and the products were analyzed directly by an integrated on-chip electrospray ionization unit linked to a mass spectrometer. Fidalgo and co-workers also had a similar arrangement to monitor the time-varying composition of microdroplets generated insides microchannels. They suggest that their proof-of-principle experiments indicate the possibility of application to on-chip protein evolutions and chemical syntheses.

Not all applications contain a microreactor. An example is that of Roman and Kennedy [30], who discussed lab-on-a-chip designs for product recovery and analysis. Conventional methods rely on microdevices to generate products, which are purified and analyzed by macroscale equipment such as the mass spectrometers mentioned above. Such "integration" reducs portability and autonomy, so Roman and Kennedy considered methods to either

fabricate analytic equipment directly on to a microchip or develop plug-in modules that can be connected according to individual requirements.

Biological applications

Biological applications of microfluidics have dominated largely due to the huge potential benefits of improved assay methods and processes for the synthesis of new products. Among these, methods related to DNA analysis have produced the most highly integrated microdevices [31]. These are broadly of two kinds: (i) polymerase chain reaction (PCR) devices and (ii) DNA hybridization chips. Two examples illustrate the power and usefulness of chip-level-integrated PCR devices. In one application, Lagally et al. [32] presented a glass microunit that performed eight PCR and capillary electrophoretic (CE) analyses on one chip. The unit possessed very rapid thermal cycling and demonstrated the potential for single template PCR analysis. The other PCR device [33] incorporated on-chip PCR and CE in a cross microchannel chip by attaching a pair of thermoelectric heating/cooling elements over a reactant reservoir and separating the products through on-chip CE.

Like the PCR, different microchip designs have been employed for DNA hybridization devices also. Liu et al. [34] introduced a disposable microchip made of polycarbonate, which integrated PCR amplification with DNA hybridization. However, the high temperature (139°C) required for polycarbonate bonding necessitated that oligonucleotide detection be done separately. Polycarbonate was also preferred by Lenigk et al. [35], whose biochip consisted of a single polycarbonate channel coupled with a Motorola E-sensor chip, thus enabling continuous monitoring of the rate of hybridization.

It is also possible to design ILOCs for separation-based detection of biological molecules. Paegel et al. [36] described a microchip-based electrophoretic bioprocessor for DNA sequencing, sample desalting, template removal, preconcentration and CE analysis. The high degree of integration is evident from the fact the microchip had 384 separate lanes for CE alone. An improvement on this design has been the recent use of contactless electrodes with a standard CE chip. This allows isolation of the detector from the separation voltages, with concomitant benefits. Lichtenberg et al. [37] separated the electrodes from the buffer by a 15 μm thick glass wall. Such devices are often based on monitoring of conductivity as a measure of the magnitude of the variable of interest [38, 39].

Proteomics is a rapidly growing area of ILOC applications, especially in view of the lucrative market for therapeutic proteins. Gao et al. [40] developed a PDMS-based microdevice that carried out protein digestion, peptide separation and subsequent protein identification. Pressure driven flow was used to drive the protein solution through the reactor and regulate the extent of digestion. Recently, Ranquin and co-workers [41] investigated the feasibility of employing triblock copolymeric nanoreactors as carriers for prodrug activating enzymes. Inosine-adenosine-guanosine preferring nucleoside hydrolyse of *Trypanosoma vivax*, a potential prodrug activating enzyme, was encapsulated in nanometer-sized vesicles of triblock copolymers. The multi-functional nanoreactor could cleave efficiently three natural substrates and one prodrug, 2-fluoroadenosine.

Owing to the large number of repetitive steps involved, immunoassays are good candidates for the automation of reaction analyses [42]. Different workers have used different principles to construct their devices. While Rossier et al. [43] used electrochemical detection in their ELISA set-up, Stokes et al. [44] used photosensors. More recently, Kim and Park [45] developed on immunoassay system based on the magnetophoretic mobility of microbeads

labeled with magnetic nanoparticles. This device could simultaneously detect very low (sub-femto molar) concentrations of a number analyses.

While the applications described above pertain to bimolecules, integrated microfluidic chips have also been applied to cells themselves. Cytometry is a notable area for these applications, which include Gawad et al.'s [46] micro-Coulter-based cell separator, Wolff et al.'s [47] fluorescent-activated cell sorter and Kim et al.'s [48] shape-deformation-based device to differentiate between normal and cancerous cells

Conclusions and outlook

Microfluidic devices have taken rapid strides forward in recent years. From the initial focus on single equipment, the emphasis has moved to integration of a number of units on one microchip. These lab-on-a-chip devices function as either a full process or a significant part of it, with many advantages over conventional processes.

Chemical and biological processes form the bulk of integrated microdevices. However, effective integration is not always easy, especially when the participating units have different time constants or there are many interacting microchannels or an ILOC has to function in consonance with a macroscale instrument. Some of these issues have been addressed in recent studies. Melin and Quake [21] analyzed a basic problem in multiplexing: cross-contamination between flow channels because of dead volume at the outlets. Their solution was a modified multiplexer based on a binary tree design. Park [49] has cited many new designs from his laboratory in optoelectrofluidic manipulations, hydrophoretic separations and magnetophoretic assays.

Wu and co-workers [50] addressed a fundamental weakness limiting the scope of microfluidic processes: their integration with macroscale devices. Wu et al.'s approach is to use integrated circuits based on printed circuit board technology in place of large instruments. This method is economical and has been implemented for the on-chip monitoring of nucleic acids and amino acids. The expanding research and the growing number of patents and applications thus augurs well for the future of integrated microprocesses.

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