Microfluidic technologies in drug discovery

Johan Pihl, Mattias Karlsson and Daniel T. Chiu

Microfluidic systems are increasingly used as tools in various stages of the drug discovery process. Microscale systems offer several obvious advantages, such as low sample consumption and significantly reduced analysis or experiment time. These technologies raise the possibility of massive parallelization and concomitant reduction in cost per acquired data point. In addition, fluids in confined spaces display unique behaviors that can be used to acquire information not accessible using macroscopic systems. This article will focus on the implementation of microfluidic systems and technologies in the process of drug discovery.

During the past decade there has been tremendous development in the field of miniaturized fluid handling systems, known as microfluidic systems [1–3]. From being a specialized area of research, it has now grown to an interdisciplinary field engaging hundreds of research groups along with several companies. The development of microfluidic systems was made possible by the adoption of fabrication technologies developed within the microelectronics industry. This was initiated in the late 1970s, but received little attention until 1990, when Manz *et al.* [4] published the conceptual work on miniaturized total analysis systems, today known as μTAS. This initial work resulted in a tremendous increase in the research and development of chip-based miniaturized fluid handling systems, much like the revolution the electronic industry underwent upon the invention of the transistor and the integrated circuit.

Properly designed microfluidic systems can perform operations quicker than conventional macroscopic systems, at the same time consuming far lower amounts of chemicals and solvents. In addition, the low dimensions of microfluidic systems enable access to physical phenomena and mechanisms that are not accessible on the macroscopic scale. This results in entirely new ways of acquiring chemical, biological and physical information. When positioning microfluidic technology in the various stages of the drug discovery chain, the low volume consumption presents particular advantages, especially in HTS applications, because compound libraries tend to be limited and expensive. Furthermore, the unique physical behavior displayed by fluids in confined spaces enables the creation of completely new types of assays.

Although the majority of developments, thus far, has focused on miniaturization and integration, several commercial products have been launched recently that exploit the unique physics of microscale fluid flow. This review will discuss the physics of microfluidics, together with methods to control fluid flow in microfluidic devices. In addition, fabrication strategies for microfluidic devices will be covered. Finally, several products that take advantage of the physics associated with microfluidics will be presented, along with the problems they are capable of addressing in the drug discovery process.

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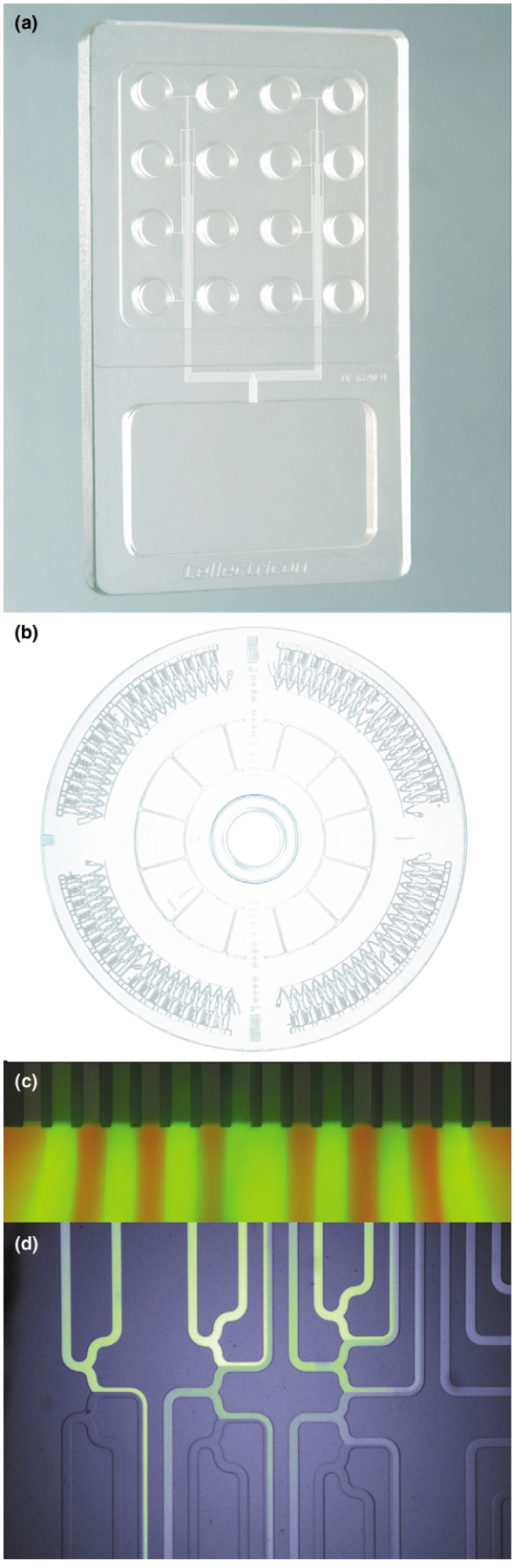

FIGURE 1

Examples of microfluidic devices. (a) Overview of the Dynaflow™ 16 chip from Cellectricon AB used in high throughput electrophysiology, comprised of 16 wells connected to an open bath volume through microchannels. **(b)** Overview of one of the Gyrolab™ CD microlaboratory from Gyros AB. Fluids introduced into the structures on the device are manipulated using centrifugal forces together with tailored surface chemistries inside the chip. **(c)** and **(d)** Fluorescence micrographs of microfluidic devices in operation. (c) Example of a microfluidic gradient generating device. These types of devices are capable of automatically generating gradients spanning several orders of magnitude using the unique physical properties associated with low *Re* flows [8–10]. (d) Dynaflow™ 16 chip in operation. The channels are alternately loaded with different dyes to visualize the fluid behavior. The fluid streams couple viscously, resulting in a single, patterned, laminar flow, containing 16 discrete solution environments.

Microfluidics

The term microfluidics refers to devices, systems and methods for the manipulation of fluid flows with characteristic length scales that are in the micrometer range [5]. Microfluidic systems display fundamentally different properties from everyday perceptions of how fluids behave. The fluid flow in this regime will be governed by viscous forces and pressure gradients with a low moment of inertia. The result is a truly laminar, turbulence-free flow and, as low Reynolds-number flows are time independent, flow patterns are reversible [6]. In addition to the unique behavior displayed by fluids at low Reynolds number, the short length scales and volumes involved in microfluidic systems result in other properties, such as high surface-to-volume ratios, small diffusion distances and heat capacities. Also, the surface tension of the fluid and the wetting properties of the system can present significant forces [7].

The behavior and relative influence of these phenomena will differ greatly, depending on the size of the system studied and, in microfluidic systems, the result can be highly counterintuitive. Specifically, the difference in physical behavior between microscopic and macroscopic systems allows for the construction of functionalities that are difficult or even impossible to access on the macroscopic scale. Taking all this into account, one arrives at the conclusion that rather than trying to design a microfluidic system that is just a downsized copy of a macroscopic system, one should aim to design microfluidic systems from the design rules obtained from the physics of fluid mechanics and diffusion in confined spaces. Examples of microfluidic systems designed in this manner include on-chip gradient generation [8–10], where solutions are diluted in an automated and highly reproducible manner, as well as sensors, taking advantage of the predictable diffusion behavior of molecules and particles in laminar flows [11,12].

The Reynolds number and laminar flow

The everyday perception of how fluids behave is derived from experiences in the macroscopic world. These macroscale fluidic behaviors, such as water flowing out of our faucets

or in rivers and streams, are governed by inertial effects. By contrast, the behavior of fluids in the microscale is typically dominated by viscous forces. One of the most useful parameters to determine whether fluid flow is dominated by inertial or viscous force is the Reynolds number, denoted *Re*, a dimensionless parameter defined as:

$$Re = \frac{\rho u l}{ç}$$

where ρ is the density of the fluid, u is the average velocity of the fluid flow, l is the characteristic length scale (e.g. the diameter of a channel) and η is the viscosity of the fluid. The Reynolds number is thus a ratio of inertial forces over viscous forces. At large *Re* inertial forces dominate, and at low *Re* viscous forces dominate. Therefore, reducing the characteristic length scale (i.e. dimension of the channel) has the same effect on fluid behavior in terms of *Re* as increasing the viscosity of the solution. The behavior of fluid flow dominated by inertia differs in a fundamental way from fluid flow dominated by viscous forces. Quantitatively, for a flow in a circular pipe, a *Re* of ~2300 marks roughly the transition from laminar to turbulent flow. In most microfluidic systems, flows are well below a *Re* of ten and often even below unity. For all practical purposes, fluids adopt laminar flow in microscale devices (Figure 1). This fact has important implications and forms the basis of many microfluidics-based technologies but, depending on the particular application, this feature can also pose significant challenges.

Diffusion and mixing

One of the consequences of laminar flow is that mixing of molecules in the fluid occurs solely through molecular diffusion. Diffusion is a non-linear process, where the time t required for a molecule to diffuse a given distance x scales quadratically with the distance, and can be described by the 1D random-walk equation according to:

$$\langle x^2 \rangle = 2Dt$$

where D is the diffusion coefficient of the molecule. Diffusion can be an efficient transport mechanism at the nano- and low micro-scale, but turns out inefficient at the higher micro- and macro-scale. The approximate diffusion coefficient for small molecules is typically 5×10^{-10} m^2/s and for proteins 5×10^{-11} m^2/s. Consequently, for a small molecule it will take 0.1 s to diffuse 10 μm, 10 s for 100 μm, and more than 27 h to diffuse 1 cm, which can in fact present an advantage if mixing is not desired. If efficient mixing is required, for example to carry out or study chemical reactions, different strategies must be employed to address the problem of slow diffusive mixing under laminar flow [13,14].

Fabrication of microfluidic devices

The development in the fields of microfluidics can be attributed to the adoption of the microfabrication technologies from the industry of microelectronics, which enabled the creation of complex miniaturized systems in materials such as silicon, glass and metals. As a consequence of this, microfluidic devices were initially composed of silicon or glass. These materials possess several advantages, such as excellent surface stability, solvent compatibility and optical properties. The key disadvantage of traditional microfabrication techniques resides in the high final cost of the device, dependent on the cost of the material itself together with the method of fabrication. This underlies the development of alternative fabrication technologies and materials for microfluidic systems, such as injection molding, hot embossing [15] and laser ablation [16] of polymeric materials, such as polycarbonate and polymethylmethacrylate, and casting techniques, such as rapid prototyping in silicone elastomers or epoxies. These methods rely on the replication of a master structure being a negative of the desired structure. The master structure is reusable, thus enabling the replication of microfluidic devices with a relatively low cost.

Silicon and glass micromachining

The fabrication of microfluidic devices was initially undertaken using micromachining technologies. These include several different fabrication techniques that selectively add or remove material from planar substrates. Once a pattern has been produced in a resist material by lithography, it can be transferred to the substrate by means of micromachining techniques. The most common micromachining techniques for the fabrication of microfluidic device include isotropic and anisotropic wet etching together with different types of dry etching, such as deep reactive ion etching. In addition, new techniques such as powderblasting [17,18] of silicon and glass are also emerging as alternatives for the fabrication of microfluidic devices. Micromachining techniques are presently not widely used for high-volume production, mainly for cost reasons, but they are frequently used for the fabrication of mold tools for microreplication.

Microreplication techniques

There is a wide range of methods to replicate microfluidic devices in polymeric materials. Well-established plastic companies (e.g. Greiner Bio One, and others) together with younger companies (e.g. Micralyne, Micronics, and Amic) are offering contract services to produce microdevices. However, because of the need to understand the boundary conditions imposed by the fabrication process as well as the need of the application, most established microfluidic companies manufacture their devices in house.

Injection molding is commonly used to fabricate macroscopic and microscopic objects in thermoplastic materials and has of late been employed for the production of microfluidic devices. The principle of operation is that a melted polymeric material is injected into a mold cavity under high pressure. The temperature is then decreased,

resulting in solidification of the polymer in the shape defined by the mold tool. Finally, the resulting structure is removed from the tool. Injection molding is the technology of choice for high-volume production of microfluidic devices mainly because of short cycle times, in some cases less than 10 s [19], together with the high degree of automation obtainable. However, injection molding is a non-generic technique in the sense that a limited amount of geometries can be successfully replicated.

Hot embossing has been extensively used for the replication of microfluidic devices, mostly in areas of R&D [20,21]. The principle of operation is that a microstructured stamp is pressed into a heated, thermoplastic substrate by means of a hydraulic press, thereby deforming the substrate to a negative of the stamp. The stamp is then demolded from the substrate and the substrate is cooled. Hot embossing is a flexible technique, suitable for prototype production or small–medium scale manufacturing. Cycle times are significantly longer than for injection molding, in the order of minutes instead of seconds, the upside being that it is possible to replicate a far wider range of geometries than, for instance, in injection molding.

Casting of epoxies, polyurethanes and elastomers is the technique that has gained most attention in research as well as in low–medium volume industrial production. Although the cycle times are long and generally the degree of automation is low, it can often present an attractive alternative because of the ease implementation together with the low investment required. In addition, by using elastomers it is possible to replicate geometries that are impossible to fabricate by other replication techniques.

The requirements on the mold tool are low; a one-tool fabrication alternative that has gained special attention is the rapid prototyping technique [22,23], developed by Whitesides and co-workers. In the rapid prototyping technique, the tool is fabricated directly in an epoxy-based photoresist in a single step process, which subsequently can be used in soft lithography for replication in elastomers, such as poly(dimethylsiloxane). This set of techniques has gained attention in a wide range of research areas as well as in commercial applications [24].

Microfluidics in the drug discovery process

As with almost any new technological breakthrough, it often takes many years before the technology reaches maturity and finally finds its niche application area. In this sense, microfluidics is no exception, the potential of microfluidics is far reaching and companies have early on focused on technology development, although they have aligned their technological platform with an application area. For example, Gyros has focused on proteomics and use microfluidics to prepare samples for mass spectrometry [25,26]. The main product of Fluidigm addresses bottlenecks in structural biology [27], such as protein crystallization. Nanostream has applied its technology to liquid chromatography [28] and the focus of Cellectricon is on cell-based assays, such as ion-channel screening and profiling [10,29]. Caliper has developed a more generic platform focused on increasing throughput in several traditional biochemical assays.

HTS

Caliper Life Sciences (Hopkinton, MA, USA) has developed a highly generic system that can be employed in various types of HTS applications [30]. The Caliper platform is capable of carrying out virtually any enzymatic assay, traditionally performed by conventional plate-based screening platforms. The key component of the platform is a glass microchip with integrated sipper capillaries that 'sip' fluids (e.g. compounds, dyes, buffer or negative controls) from plate wells, at the same time continuously drawing enzyme and substrate from wells-integrated on-chip. The resulting mixtures are transported downstream in a microchannel, also serving as an incubation chamber, to a detection point where fluorescence readout is performed. This is performed in a serial manner in the sense that each chip sips a large number of compounds and dyes, interdigitated with buffer to flush the system. However, to increase the speed of the system, each chip consists of 4 or 12 units and the system is capable of performing assays with considerably higher throughput and better reproducibility than conventional plate-based screening platforms. In addition, the enzyme, substrate and compound consumptions are far lower, typically 1/100, 1/40,000 and 1/500, respectively. The system integrates a majority of the steps that are typically carried out on different stations in conventional screening platform, resulting in a low footprint. The system has turned out to be a genuine success and is currently used in a large number of pharmaceutical companies in HTS applications, examples including everything from enzymatic assays to cell-based calcium flux assays.

Automated electrophysiology

Cellectricon AB (Göteborg, Sweden) has developed a platform intended to increase the efficiency in patch clamp. Ion channels are important drug targets and the patch-clamp technique is the gold standard in real-time investigations of ion-channel activity [31–33]. It can provide single-channel resolution, alongside with a temporal resolution in the microsecond range. The drawback is that it is a labor-intensive technique and notoriously low in throughput, rendering it difficult to generate statistically significant amounts of data for whatever purpose. The Dynaflow™ system from Cellectricon addresses this problem by increasing the number of data points obtained per cell through the implementation of microfluidics in conventional patch clamp [29,34]. The system is built around a microfluidic device (Figure 1a) fabricated in a silicone elastomer, a flexible technique that allows the creation of complex geometries. The devices comprise several sample wells, presently 16 or 48, connected to an open volume

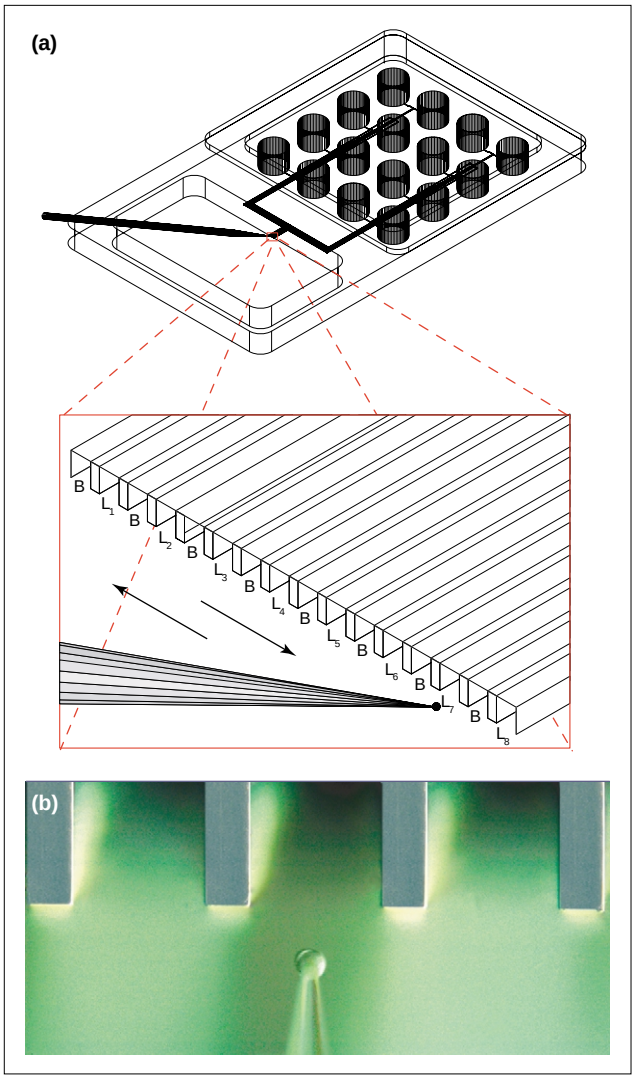

FIGURE 2

Representation of the Dynaflow concept. (a) Schematic of the Dynaflow™ 16 chip from Cellectricon AB, the inset showing the 50 x 60 μm (w x h) microchannels at the point of exit into the open volume. The loading pattern can be chosen in an arbitrary manner, and the cell is translocated through different solution environments by means of a motorized scanning stage, while the current response from the cell is recorded. **(b)** Micrograph of a patch-clamped cell positioned outside the channel outlets, during operation of the system. The center channel is loaded with a glucose solution to visualize the flow.

through microfluidic channels. At the exit into the open volume the channels are tightly packed. Fluids are pumped through the channels by application of pressure to the sample wells. When the device operates in the low *Re* regime, flows emerging from the channels will couple viscously, forming a single laminar flow propagating in the open volume (Figure 1d). This flow will contain several discrete zones with well-defined chemical environments corresponding to the loading of the sample wells (Figure 2a), and because of the low *Re* of the system during operation, mixing between adjacent environments takes place by molecular diffusion [35]. The device is placed on a computer-controlled scanning stage mounted on an inverted microscope. Cells are patch-clamped in the open volume, translocated to the channel exits and can then be scanned across the different solution environments with full control of transfer and exposure times. The system rapidly generates dose–response curves under extremely stable conditions, and several full dose–response scans can be obtained from the same cell under different experimental conditions. Examples of rapidly generated dose–responses can be seen in Figure 3. In addition, the well-defined force originating from the flow acting on the cell-pipette system stabilizes the patch-clamped cell, allowing for extended recording times from one cell, in some cases up to hours [34]. The throughput compared with conventional solution exchange systems is massive, the sample consumption is greatly reduced and, because the devices themselves are disposables, there is a zero risk of cross-contamination. The system has found use in the process of lead optimization as well as for different types of safety testings.

Proteomic applications

Gyros AB (Uppsala, Sweden) has launched a family of products that take advantage of the concept of compact disc (CD)-based centrifuge microfluidics. These are all based on a microstructured CD (Figure 1b) fabricated by injection molding, where the manipulation of fluid in the device is performed using centrifugal forces (i.e. by rotating the disc), a concept that can present numerous advantages. Manufacturing is performed using commercially available CD injection molding equipment, a method that possesses high-volume manufacturing capabilities, resulting in low unit prices. In addition, the centrifuge-based manipulation of fluids eliminates the need for multiple external connections apart from other methods for fluid manipulation, such as pressure-based or electrophoretic manipulation. One of the products, the Gyrolab™ MALDI SP1 CD microlaboratory, has been developed to facilitate matrix-assisted laser desorption–ionization (MALDI) mass spectrometry (MS) peptide mapping, a technique commonly employed in target identification and expression profiling. In the MALDI SP1 CD microlaboratory, protein digests are concentrated, desalted or derivatized and crystallized in a MALDI matrix, followed by MALDI MS analysis [25,26]. This system provides advantages in analogy with the Caliper platform, in the sense that it integrates several steps that are carried out on different stations using conventional techniques. In addition, the system processes large numbers of samples in parallel and handles minute volumes with excellent reproducibility. The platform has found application in the quantification and identification of proteins and can be applied for example in expression profiling.

Microparallel liquid chromatography

Nanostream (Pasadena, CA, USA) has developed a parallel platform for high throughput micro-parallel-liquid chromatography (μPLC), the Veloce™ system. The system is

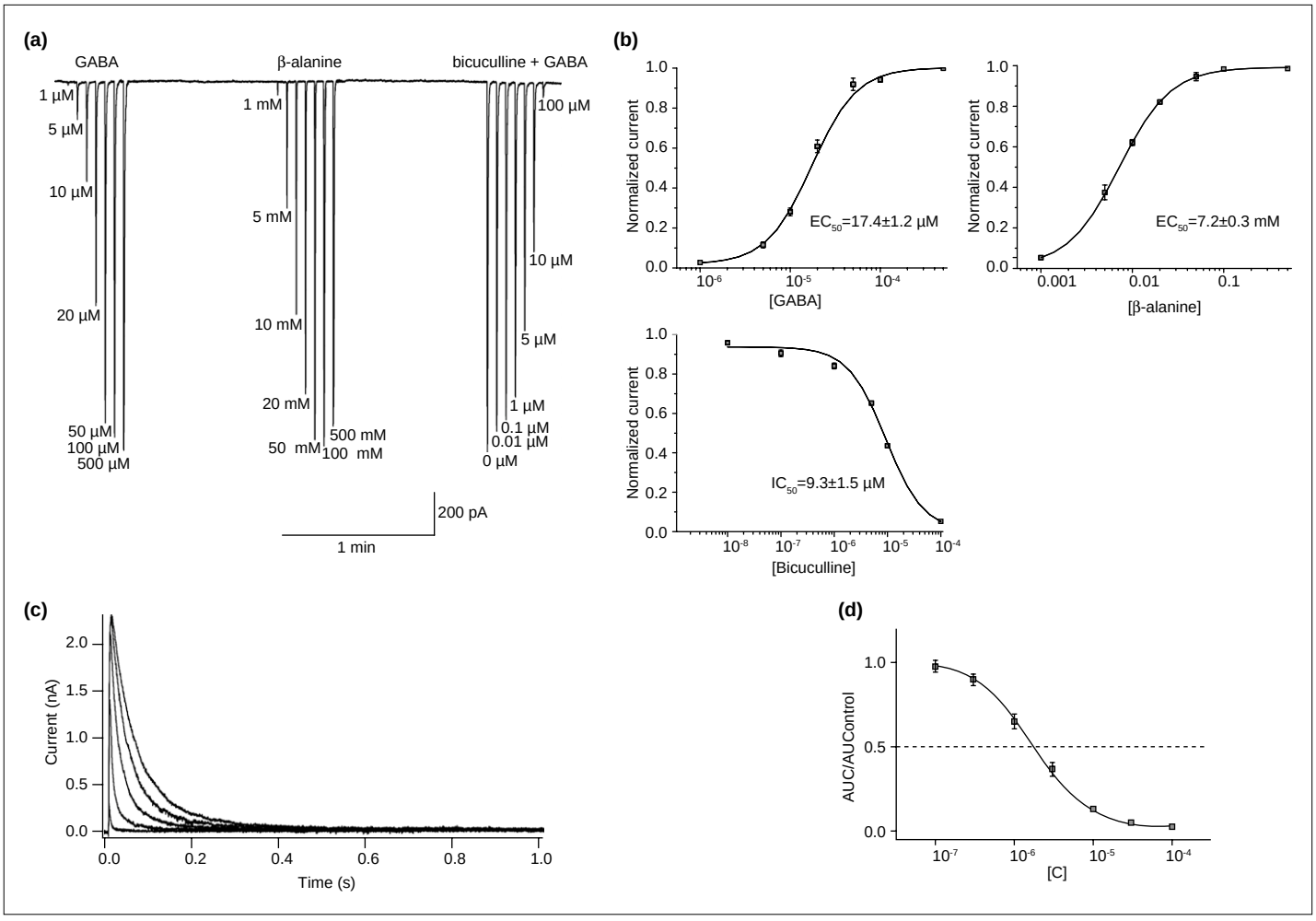

FIGURE 3

Examples of data acquired using the Dynaflow™ system. (a) Current trace recorded from dose responses of $GABA_A$ receptors to the two agonists GABA, β-alanine, and the competitive antagonist bicuculline together with GABA. **(b)** Corresponding dose–response relationships. **(c)** Effect of a blocker on A-type current from a voltage-gated ion channel from heart muscle, expressed in CHO-K1 cells. **(d)** Corresponding dose–response relationship for the determination of the IC_{50} value (n=5). Note the small standard error for this small dataset. The data contained in panel (c) and (d) are by courtesy of AstraZeneca.

built around the Brio cartridge, containing 24 individual separation columns, 80 or 30 mm in length. The system delivers solvents through a pump unit allowing gradient eluations, sample injections are handled by an eight channel autosampler and the system has the capability to perform ~1000 separations within 8 h time period [28]. The application focus of this technology primarily addresses some aspects of the absorption, distribution, metabolism, excretion – toxicology (ADME–Tox) process. Specifically, physiochemical profiling parameters, such as lipophilicity using log *P* or chromatographic hydrophobicity index determination, are important because the primary cause of drug failure in the development phase is due to poor ADME properties [36]. HPLC is well suited for this, except for the fact that it cannot be considered as a high throughput technique. Nanostream addresses this by parallelization along with miniaturized columns, resulting in a massive increase in throughput along with a decrease in sample and solvent consumption.

Protein crystallization

Fluidigm (San Francisco, CA, USA) is focusing on resolving bottlenecks in the field of structural biology. The company has launched the Topaz™ system, a protein crystallization tool, the heart of the system being a microfluidic device, the Topaz™ chip, fabricated in a silicone elastomer. The way that the device functions is highly interesting and relies on some of the intrinsic properties of the material itself, for example the fact that it is highly gas permeable and elastomeric. The gas permeability allows for channels to be dead-end filled, because the gas is expelled through the polymer matrix to the outside [27]. Furthermore, the elastomeric properties of the material are used to create monolithic valves, because microfluidic channels fabricated in elastomers can be compressed and, hence, closed by the application of a point pressure [27,37]. This is accomplished by applying pressure to a gas-filled microchannel running above and perpendicular to the channels to be controlled. This has led to the creation of a protein crystallization chip, where protein

crystallization conditions can be screened in a parallel manner. Protein and reagents are loaded through microfluidic channels into small, neighboring chambers separated by valves that can be opened to allow protein and reagents diffusively mix. In contrast to conventional robotic liquid handling systems commonly used for protein crystallization screening, the Topaz™ chip uses volumetric metering of fluid, resulting in accurate metering down to low picoliter volumes and minute protein consumption. The screening is performed in a truly parallel manner and screening trials can be performed for up to four different proteins with 96 different crystallization conditions. Once appropriate conditions have been established, the crystallization can be scaled up to produce diffraction quality crystals [38]. The highly parallel format together with the low sample consumption has made this method a tool for HTS of crystallization conditions, applicable in the field of structural genomics.

Future outlook

The field of microfluidics is still in its infancy and, until recently, has been mainly technology driven. The focus has been on the development of new functional components, such as pumps, valves and new cost-effective fabrication technologies, along with their functional demonstration. Presently, there is a large set of components and fabrication technologies available and, although new technology is emerging at a rapid rate, the focus in the future will most likely shift towards the implementation of the existing technology into new applications. Furthermore, existing concepts and technology platforms will most likely be further parallelized, therefore increasing throughput and efficiency. Microfluidics will undoubtedly assume a larger and more important part in the drug discovery process for the creation of more and higher-quality targets and leads.

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