



Microfluidics: its Impact on Drug Discovery

The benefits of microfluidics experienced by R&D chemists are now being extended to R&D biologists involved in life science technologies such as genomics, proteomics, high throughput screening and molecular diagnostics



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Drug discovery is a very complex and frustrating process, typically taking a team of hundreds of specialists between 10 and 15 years to identify a new drug candidate, with a large percentage of candidates failing to proceed beyond the clinical trial phase. With recent studies showing that the rate of new drug launches is decreasing despite increased R&D expenditure, technologies that have the potential to improve the efficiency and success of drug discovery are urgently needed.

One such technology is that of microfluidics – a multi-disciplinary field that involves the formation of micron-sized channels, electrodes, weirs and other novel features in chemically-resistant wafers. Fluids are then introduced into the channels and moved to regions of the wafer that perform functions such as mixing, filtering, reaction, product separation and analysis. Thus, the devices automate and integrate many steps that are currently performed in distinct, time-consuming steps by skilled and expensive laboratory technicians.

The application of microfluidic devices and instruments to the field of drug discovery offers many potential advantages – both for the R&D chemists involved in target identification, compound generation, lead identification and optimisation, and for the R&D biologists involved in life science technologies such as genomics, proteomics, high throughput screening and molecular diagnostics.

This paper will explore how some of the potential advantages that microfluidics offers over conventional analytical and process chemistry practices are being realised. In addition, one of the more recent exciting applications of microfluidics to structure-based drug

discovery will be discussed. Firstly though, a typical process for fabricating a microfluidic device suitable for use in the field of drug discovery and development will be described.

DEVICE FABRICATION

Key to the development of microfluidic devices is the use of microfabrication techniques capable of creating microchannels and complex structures in resistant and inert substrate materials, suitable for handling chemicals and biological samples. There is a wealth of expertise and a wide range of microfabrication tools available for processing complex structures in silicon. However, limited chemical stability and lack of optical transparency eliminate silicon as the material of choice for microfluidic devices in drug discovery applications.

It is these requirements for chemical inertness, optical transparency and biocompatibility that often lead to the use of glass for microfluidic devices within the pharmaceutical industry. Some of the tools and processes designed for fabricating devices in silicon can be adapted for use with this material, although some additional processing techniques need to be brought in from other disciplines or developed from scratch.

The inertness, biocompatibility and optical properties of glass make it ideal for an extremely broad range of applications in the field of drug discovery. Glass, however, being susceptible to breakage, cracking, scratching and chipping, is a difficult material to process and handle. This is one of the main reasons why there are so few suppliers of glass microfluidic devices in the world. A potential supplier requires expertise in three key processing areas:



- ◆ Conventional precision solids-processing of glass, including cutting, grinding, optical polishing, drilling and thermal bonding
- ◆ Photolithographic and wet etching techniques
- ◆ Microfluidic device and instrument design capabilities

As an example of the importance of the third skill set, the first step in producing a microfluidic device is usually to design the components of the device, including channels, mixers, valves, pumps, separators and heaters. This requires the use and, frequently, the development of software design packages capable of modelling the behaviour of fluids flowing in small channels.

Once the device has been designed, a chrome-on-quartz mask, suitable for use with standard photolithographic tools, can be produced. Since typical device features in microfluidic devices are of the order of 5-1,000µm, proximity lithography can be used to illuminate the layer of photoresist spun onto the metallised glass wafer. Conventional lithographic techniques of exposure, baking, development and etching are used to pattern the photoresist, and to provide a mask for the subsequent glass etching step that removes the exposed glass areas (see Figure 1).

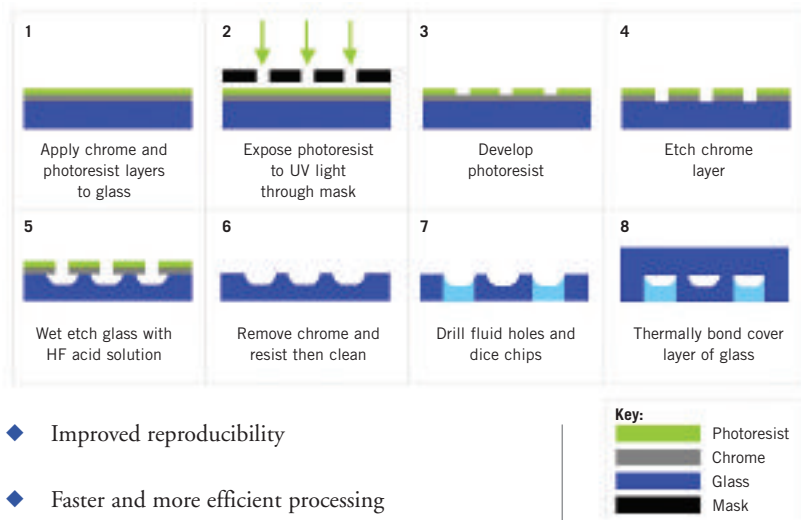
To ensure device quality, the whole lithographic fabrication process described above is performed in a clean room. Once the patterned glass wafer has been created, conventional solids-processing steps such as drilling, dicing, grinding and polishing are performed. The final step involves sealing the cleaned microchannels using a second glass wafer that is brought into contact and thermally fused with the patterned glass wafer.

To fabricate devices with internal fluid volumes above a few hundred microlitres, or to create more complex channel geometries, features can be etched into both glass layers. One of the challenges of fabricating such 'double-etched' devices is the need to align the features on the two patterned layers before bringing them into contact and thermally bonding them to create the final device.

REALISING THE POTENTIAL

The use of microfluidic devices and instruments by the R&D chemist involved in drug discovery, offers many potential advantages over conventional analytical and process chemistry practices. These include:

Figure 1: Process of patterning a glass microfluidic device



- ◆ Improved reproducibility
- ◆ Faster and more efficient processing
- ◆ Lower sample and reagent consumption
- ◆ More accurate and faster temperature control

However, achieving the benefits that microfluidics can offer requires much more than just the microfluidic device alone.

The typical R&D chemist has neither the specialist knowledge of fluid handling required to successfully introduce fluids into or remove fluids from the device, nor

Figure 2a: An automated, fully integrated micro flow system from Syrris Ltd



Figure 2b: A schematic illustration of the flow reactor system from Syrris Ltd

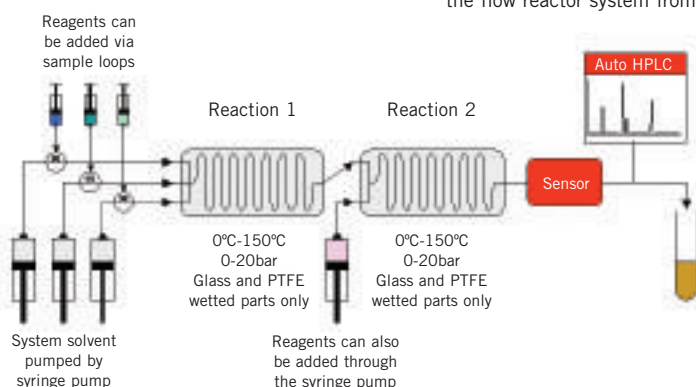


Figure 3a: A micro-reactor device

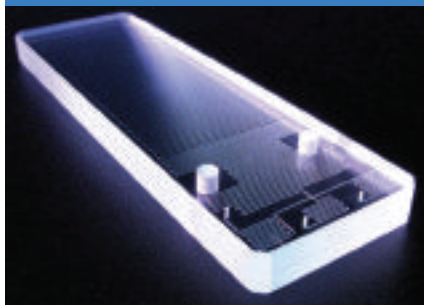


Figure 3b: A micro-mixer device

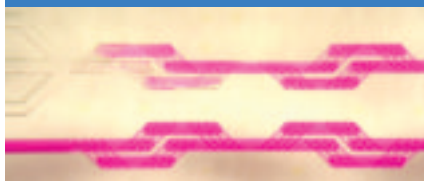
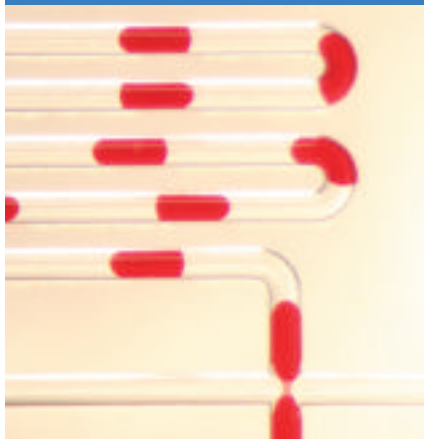


Figure 3c: Liquid-liquid extraction microfluidic component



Figure 3d: Slug flow within a microfluidic device



the knowledge of the pumps, detectors, heating modules and control systems typically required to create a fully functioning microfluidic system. It is only with the recent introduction of automated, fully integrated micro flow instruments that the true potential of microfluidics, for making productivity improvements in the areas of drug discovery and development, have started to be realised.

Figure 2a (see page 25) shows one such off-the-shelf instrument – the AFRICA flow reactor supplied by Syrris Ltd. It is useful to spend some time describing this microfluidic instrument in order to illustrate both the high level of functional integration required to make a successful microfluidic system, and also the wide range of functions that can be facilitated by the use of microfluidic devices. As illustrated in Figure 2b, reagents or samples are injected into the system using sample loops and valves. Syringe pumps move the solvent and reagents into the first microfluidic chip, a three-input micro-reactor device that is shown in Figure 3a. Fluid flow within the chip is designed to be non-chaotic and non-turbulent, so called laminar flow, whereby different reagents flow side by side in the fluidic channel, mixing by diffusion effects only. Since the channel dimensions are small – typically less than 1mm – mixing by diffusion can be fast – a few seconds – but at the same time much more controllable and reproducible than is typical of batch reactors with their chaotic, turbulent mixing.

Another advantage of performing the reactions in a microfluidic device is the large surface area-to-volume ratios in the chip that allow heat from exothermic reactions to be removed

rapidly, or alternatively heat to be applied to the reaction rapidly and controllably. To enable this efficient heat removal or addition, the micro-reactor in the AFRICA system is housed in a holder that places the chip in

intimate contact with a plate that is temperature-controlled between 0°C and 150°C. This holder also provides the means of introducing the fluids into the chip with a tool-free push-in connection for leak-free operation at pressures of 20bar.

By flowing the product of one reactor chip into another, as shown in Figure 2b, multi-step synthesis can be performed – with exact timing of reagent or quench addition – as a continuous process. All of this, coupled with fully integrated, on-line HPLC analysis, allows the R&D chemist to carry out high-quality synthesis and analysis.

An automated fully-integrated micro flow system, such as that described above, is ideally suited to automated reaction optimisation. Parameters such as time, temperature, stoichiometry and order of reagent addition, can be easily and accurately defined. Once the reaction has been optimised, the system's chip reactors can be replaced by tube reactors that enable flow rates to increase to produce the reaction product in sufficient volumes for the next phase of the drug discovery process. A completely scalable process can be developed for production at levels of up to kg per day.

Another means of increasing production can be to increase reaction temperature beyond the boiling point of the fluids involved by pressuring the micro-reactor. Although this may be undesirable in a batch reaction environment, the smaller volumes and greater control that can be achieved in a microreactor make this a viable option. It is interesting to note that the pressurised state of the microreactor in the AFRICA system is created and controlled by a back pressure regulator that itself contains a microfluidic component.

For most reactions, diffusion mixing within a microreactor is fast enough (5-10 seconds). In some cases, however, faster mixing is required. This can be achieved by designing more complex 'double-etched' chip geometries, such as the micromixer shown in Figure 3b, which can achieve mixing in a few milliseconds.

The AFRICA system also consists of a module that provides flow liquid-liquid extraction, a common process used to separate a mixture of molecules in solution which is a typical output from a microreactor chip. As illustrated in Figure 4, this module also contains a microfluidic component, as shown in Figure 3c. When the organic solvent containing the mixture of molecules is combined with an aqueous solvent, slug flow develops – as illustrated in Figure 3d

– that results in rapid partitioning of the molecules between the solvent phases. A microporous hydrophobic membrane sandwiched between two microfluidic layers is used to separate the solvent phases and hence the molecules.

The micro-flow system discussed illustrates how a number of functionally different microfluidic devices – integrated into a single, flexible automated laboratory instrument – can be used to potentially bring productivity improvements to the R&D chemist involved in drug discovery.

PROTEIN CRYSTALLISATION

In addition to the productivity improvements that microfluidics is bringing to the R&D chemist, similar benefits can be applied to other fields involved in drug discovery, such as structural and molecular biology. The application of microfluidics to one such area has received significant interest recently – and that is the field of protein crystallisation.

Proteins are critical components of many fundamental biological processes and their malfunction has been linked to numerous diseases. Understanding protein function is often a critical first step in designing drugs that can successfully interfere with the function of these proteins and combat the associated disease. One of the most powerful approaches to understanding a protein's function involves determining its three-dimensional structure in atomic detail – but this requires having pure crystals to study.

Protein crystals are particularly difficult to crystallise successfully due to their fragile nature, as well as the strong dependence of their crystalline quality on a wide variety of environmental factors such as protein purity, pH, concentration and temperature. Furthermore, the conditions required for successful crystallisation of one protein are often unique to that protein. Consequently, determining the optimum crystallisation conditions of any given protein is typically a slow and tedious process, and is one of the major bottlenecks in structure-based drug design for proteins.

Recent studies applying microfluidic technology to protein crystallisation are, however, showing great promise as a means to overcome this bottleneck. One approach in particular – crystallisation of proteins in nanolitre plugs – is worthy of note (1). In this approach, a continuous aqueous stream of proteins and precipitants are combined and injected into the flow of a carrier fluid within a microfluidic channel. Plugs,

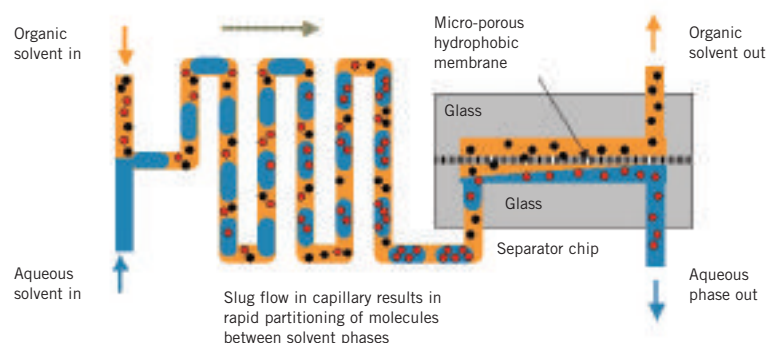


Figure 4: Schematic illustration of the function of the flow liquid-liquid extraction module from Syrris Ltd

similar to those shown in Figure 3d, of protein and precipitant can form spontaneously in the microfluidic channel. Since each plug can be subject to a slightly different experimental condition, thousands of crystallisation experiments can be effectively conducted within the microfluidic channel, each consuming only a few nanolitres of the protein solution. Each plug is examined using X-ray diffraction to determine the optimum experimental condition for crystal formation. Integration of this plug flow approach into an automated high throughput microfluidic screening platform, such as the system described above, could bring the advantages of reduced material consumption, improved control accuracy and high parallelism – already being exploited by R&D chemists – to the field of structure-based drug discovery.

CONCLUSION

This paper has looked at some of the revolutionary advances being made possible in the field of drug discovery and development through the application of microfluidic technology. We have discussed some of the ways in which both R&D chemists and biologists are starting to explore the benefits afforded by microfluidics. These benefits are common to both applications: reduced reagent consumption, improved accuracy and control, and the prospect of automated high throughput screening and optimisation. Although the field of microfluidics is still very much in its infancy, the keen interest currently being shown in this field can only help to drive this technology forwards to enable potential productivity improvements in drug discovery to be realised.

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Reference

1. *Current Opinion in Structural Biology*, 15: pp548-555, Elsevier, 2005