S-4 [14:00~14:30]

Assay development and HTS on microfluidic Lab-on-a-chip

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ABSTRACT

Microfluidic lab-on-a-chip (LOC) systems have enabled a new generation of assay technologies in chemical and biomedical sciences. Caliper's microfluidic LOC systems contain a network of microscopic channels through which fluids and chemical are moved in order to perform experiments. The main advantages of these continuous-flow devices are integration and automation of multiple steps in complex analytical procedures to improve the reproducibility of the results, and eliminated the manual labor, time and pipetting errors involved in analyses. The present talk is devoted to give a brief introduction of microfluidic basics and to present in applying continuous-flow microchips to drug screening with model enzyme assays.

INTRODUCTION

Some of the advances that shrunk the size of computers while dramatically boosting their speed are now at work in biomedical and biochemical research labs, accelerating biological research and the promise of life-saving cures. The notion of a cheap and reliable computer chip-like has been introduced as the concept of micro-total-analysis systems in 1989, which proposes the integration of the different steps of an analytical process into a miniaturized flow system. A size reduction of analytical separation devices onto a chip size of a fingertip has a number of advantages over traditional methods. First, it reduces the labor-intensive process of manually transferring and handling samples, saving time and reducing errors. Second, it requires smaller amounts of fluids. Third, fluids travel shorter distances in the chip, which translates to speedier results.

The use of microfabricated analytical devices is made extremely attractive when high sample throughput is needed. In recent years, it has become generally accepted that large-scale, high-throughput analytical methods are needed in various emerging application fields of the life sciences, especially in the fields of genomics and proteomics. The statement also strongly applies to the pharmaceutical field, where these analytical issues play a major role as well in regard to drug discovery. Recently, the trend towards assay miniaturization has spurred development of homogeneous, fluorescence-based assays in LOC devices. LOC technology is based on microfluidics,

a technique that allows samples of fluids to be prepared and analyzed within the confines of a microchip. The chip consists of a network of tiny channels manufactured in glass that serve as pathways for the movement of fluid samples. Such devices provide orders-of-magnitude reduction in reagent consumption, and offer the potential for implementing high-throughput screening in formats that integrate up-front compound handling with unique assay functionality. The purpose of this talk is to examine recent progress made in the field of microfluidic LOC systems, particularly with respect to assay integration and high throughput screening (HTS) applications.

MICROFLUIDICS

Microfluidic LOC systems are characterized by very small dimensions with continuous fluid flow at slow rates. Fluid channels of lab-on-a-chip devices are photolithographically etched into one of the pieces while the other piece is bonded to the etched layer. Typically channel depths and widths of lab-on-a-chip channels are on the order of 10 to 100 um, while channel lengths can vary from less than a millimeter to several centimeters. The precise control of channel dimensions combined with the control of fluid actuation allows for accurate dispensing, mixing and timed incubation of sample plugs. This enables the researcher to tailor the conditions to achieve the most effective result.

Streams and plugs of different solutions needed in a lab-on-a-chip experiment are manipulated by microfluidics. Microfluidic chip devices use several modes of fluid actuation including electrokinetics, pressure, electrohydrodynamics, capillary pressure, centrifugation, and surface tension gradients. Electokinetic movement is the most studied and widely used motive force in integrated assays. The term electrokinetics refers to the linear combination of electroosmosis and electrophoresis, which take place under the action of an electric field. Electroosmosis drives bulk-fluid movement and electrophoresis drives the movement of chemical species on the basis of their charge. Electrokinetics has the advantage of speed and accuracy; there is very rapid fluid switching and very rapid separation of chemical species with a charge-to-mass difference.

Electrokinetic movement can have the disadvantages that compound mixtures separate into components, test compounds move at a different speeds depending on their electrophoretic mobility, and highly charged molecules may not migrate at all. Therefore, vacuum or pressure, usually hydrostatic head, is used to perform certain functions on microfluidic devices. Despite the disadvantage of hydrodynamic dispersion, pressure movement has the advantages of being insensitive to chemical

properties of the buffers or surface reactivity of the reagents. Therefore, hybrid microchip designs that take advantage of both pressure-driven and electrokinetic flow offer the best of both worlds for some applications.

MICROCHIP DEVICES FOR HTSIn the first extensive description of the work by Harrison and Manz (1992), the microfluidic device included several platinum electrodes intended for electrochemical detection. However, they found that detection using fluorescence is much more powerful and convenient, and the vast majority of the work since then has used fluorescence detection. There are many examples in which researchers have demonstrated kinetic parameters of enzymatic reactions on chips employing fluorescence techniques.

In order to employ LOC systems for HTS, Caliper's LOC systems use an approach based on the concept of a continuous-flow assay. Continuous-flow processes are high throughput and easily integrated within microchip devices. For HTS application, this means that significant throughput should be achieveable even in relatively simple con-multiplexed devices, while system stability and performance should be improved by eliminating the need to swap and re-equilibrate chips, re-align optical detection devices, etc.

ASSAY DEVELOPMENT AND HTS

1. Fluorogenic enzyme inhibition assay

A continuous-flow enzyme inhibition assay is illustrated in Fig 1 (phosphatase assay). In the relatively straightforward case of fluorogenic enzyme assays, the microchip employs electroosmotic pumping to continuously deliver enzyme assay components into a common channel on the chip. Following an incubation period, the fluorescent product generated as a result of the enzymatic reaction can be monitored near the end of the reaction channel by a fluorescence detector. Individual compounds or mixtures of compounds can be tested for inhibitory activity by injecting them into the stream of enzyme and substrate. The presence of active inhibitors appears as a decrease in the magnitude of the fluorescent signal.

For the HTS application of the phosphatase fluorogenic assay, a four-sipper system with automated plate handling capability is used and sample injection cycle times are optimized to yield 10.4 min per a 384-well plate. 613,888 compounds are tested with a 10% threshold (~ 0.1%) and greater than 97 % "call rate" for compound hits is observed (2% false positive and 1% false negative). In addition, good

reproducibility has been observed for within-run replicate injections of known inhibitors as well as for chip-to-chip and inter-instrument performance.

2. Mobility shift assay

Kinases and other enzymes for which fluorogenic substrates do not exist can be assayed in a LOC format by taking advantage of the changes in electrophoretic mobility that occur upon enzymatic transformation of the substrate. Typically, kinase-catalyzed phosphorylation is accompanied by a significant change in the charge-to-mass ratio of the substrate since each phosphorylation event introduces two negative charges, while the concomitant increase in mass is relatively small. In LOC devices for running continuous-flow kinase assays (Figure 2), enzyme and substrate are place in different reservoirs and continuously mixed with test compounds through the attached capillary. The main channel consists of the upstream section of the reaction channel and the down stream section of the separation channel, where an electric field is applied and the electrophoretic separation of substrate and product takes place. Without an electric field, the fluorescence signal observed at the detector is independent of the ratio of product to substrate since both carry the same fluorescent tag. On the other hand, with an applied electric field, the injection of an inhibitor results in a perturbation of the fluorescent signal at the detection point due to the electrophoretic separation of a zone containing excess substrate from a zone containing reduced product compared to that present in the steady-state reaction mixture. These two zones are detected as a peak and a dip in the signal, whose relative positions depend on the orientation of the electric field and the relative charges of substrate and product. The amplitude of the signal perturbation is correlated with the potency of the injected inhibitor.

For the HTS standard mobility shift assay test, another four-sipper system with automated plate handling capability is used and sample injection cycle times are optimized to yield 18.9 min per a 384-well plate. Using 27648 test compounds, greater than 95 % "call rate" for compound hits is achieved. Since the equivalent experiment in a macro format requires time and then stopping the reaction and running the product on a separation device, the real promise for screening with a LOC is that it enables fast, flexible assay formats that require very little reagents.

CONCLUSIONS

The viewpoint of scientists on laboratory methodology and infrastructure in the past is on the verge of a major change. The use of microfabricated devices for chemical and biological applications represents a paradigm shift. Beyond this, these technologies well help open up new and previously unimaginable possibilities for

scientists to probe, study, and ultimately understand our chemical and biological world. Since it is apparent that progress in the attempt to integrate many or all functions of an assay on a chip is proving to be difficult, it is important to realize that a biochip is not a single product, but rather a family of products that form a technology platform. Therefore, only through multidisciplinary efforts, LOC systems could be continuing to evolve as a collection of assays that provide a technology platform.

Figure 1

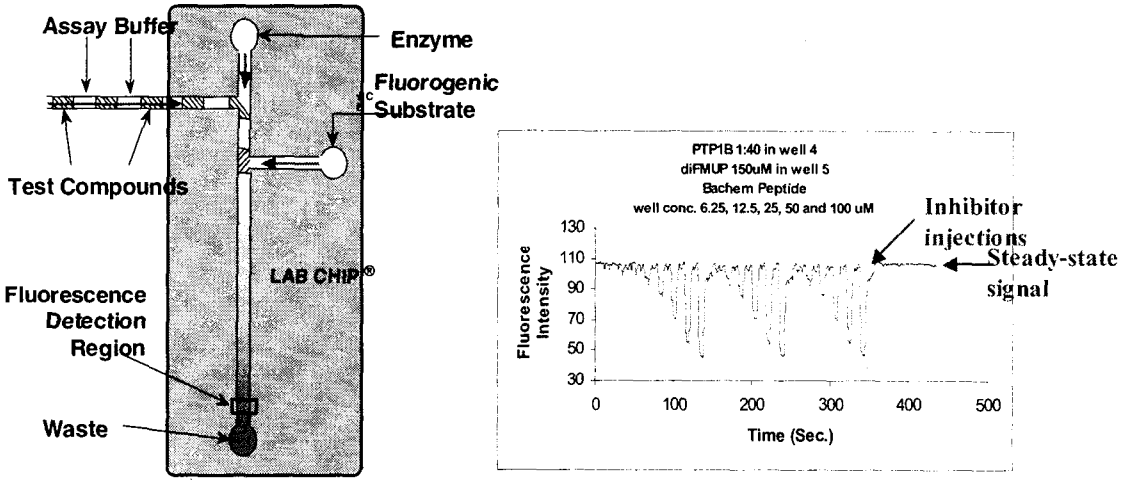

Figure 2