Integrated Microfabricated Biodevices

INTEGRATED MICROFABRICATED BIODEVICES

Advanced Technologies for Genomics, Drug Discovery, Bioanalysis, and Clinical Diagnostics

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MARCEL DEKKER, INC. NEW YORK • BASEL

Cover artwork by courtesy of Nanogen, Inc., San Diego, California, with permission.

ISBN: 0-8247-0606-4

This book is printed on acid-free paper.

Headquarters
Marcel Dekker, Inc.
270 Madison Avenue, New York, NY 10016
tel: 212-696-9000; fax: 212-685-4540

Eastern Hemisphere Distribution
Marcel Dekker AG
Hutgasse 4, Postfach 812, CH-4001 Basel, Switzerland
tel: 41-61-261-8482; fax: 41-61-261-8896

World Wide Web
http://www.dekker.com

The publisher offers discounts on this book when ordered in bulk quantities. For more information, write to Special Sales/Professional Marketing at the headquarters address above.

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

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Preface

Interdisciplinary science and technologies have converged in the last few years to create exciting challenges and opportunities that involve a new generation of integrated microfabricated devices. These new devices are referred to as DNA chips, Lab-on-a-Chip, microcapillary electrophoresis systems, microanalytical systems, and biosensors. Their development involves both established and evolving technologies, including microlithography, micromachining, microelectromechanical systems (MEMS technology), microfluidics, and nanotechnology. The development of these new devices and systems requires a high level of interaction and cooperation among engineers, computer scientists, materials scientists, chemists, molecular biologists, geneticists, and clinical scientists. Applications for this novel ''synergized'' technology will include genetic analysis, clinical chemistry (particularly DNA and immunodiagnostics), drug discovery, combinatorial chemistries, industrial process control, and portable, hand-held analytical instrumentation.

The first aim of this book is to provide a good overview of the key devices (DNA chips, Lab-on-a-Chip, etc.) and the basic interdisciplinary technologies (microfabrication, MEMS, microfluidics, etc.). The second aim is to give the reader a better understanding of how to utilize these interdisciplinary technologies and determine which will provide appropriate technical solutions to problems perceived as being limited to their own discipline.

Our contributors have identified important aspects of these broad interdisciplinary technologies that are particularly relevant to successful development of novel devices or systems. For example: DNA chips and arrays may be highly dependent on certain microfabrication or micromachining techniques and processes, whereas Lab-on-a-Chip and microcapillary electrophoresis systems are more dependent on so-called microfluidic processes. Successful development of a given device or system requires overcoming a variety

of problems. While this sounds self-evident, and generally does not represent a problem within a given discipline, it is indeed a problem in highly interdisciplinary areas. Thus, readers need to obtain a firm grasp of the underlying base technologies and/or applications to make better decisions about their own areas of research and development. The information presented here will help the reader make decisions as to whether a particular problem (often perceived as fundamental within a discipline) can be solved by using another technology. Finally, we would also like the reader to understand that as novel as these applications sound, the door is just opening to even more exciting advancements. Because of this, we complete this volume with a look at the so-called area of nanotechnology, which may herald the advent of molecular electronics and nanocomputing systems.

Because of the highly interdisciplinary nature of this book, it should be of interest and value to a relatively large audience. The text will serve a wide range of academic and industrial scientists and engineers, including molecular biologists, geneticists, and microbiologists; clinical and diagnostic scientists; nucleic acid, organic, physical, and analytical chemists; electronic and mechanical engineers; computer scientists and programmers; and physicists and mathematicians. This volume will also be valuable to many entrepreneurial and business people who are in the process of trying to better understand and evaluate these new, fast-moving high-tech areas.

Michael J. Heller
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Toward an Integrated Electrophoretic Microdevice for Clinical Diagnostics

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1.1 INTRODUCTION

Recent years have seen much effort invested in developing clinical diagnostic tools that provide both rapid analysis and accurate results. The growing literature on microminiaturized analytical devices has begun to delineate the potential applicability of the microfabricated chip platform for this task. While the enhanced analytical capabilities of microfabricated chip technology for expediting electrophoretic separations have been well established [1–8], the true power of this technology lies in the potential to miniaturize and integrate existing technologies in a manner that allows for sample preparation and analysis to be seamlessly carried out on a single device.

Many routine assays carried out in the clinical setting rely on slab gel electrophoresis for detection of DNA and proteins. Capillaries have catalyzed the first step in miniaturizing this process, having been shown to be an effective alternative to slab gel-based methods while reducing analysis times by roughly an order of magnitude [1,9–11]. In addition, the low volumes of reagents consumed by this electrophoretic process

combined with the miniscule sample requirements bolster the advantages it brings to clinical analysis. The disadvantage associated with the capillary format for electrophoretic analysis is that, while slab gel electrophoresis allows for the parallel processing of a number of samples simultaneously, capillary-based separations usually must be executed one by one. Consequently, any gain through reduction of analysis time is lost by serial analysis. While this yields an overall process time for a batch of samples that is roughly the same with both methods, capillary electrophoresis (CE) still has brought a number of benefits including semiautomation and online detection, both of which are advantageous in the clinical laboratory [12]. Moreover, newer CE instrumental platforms that incorporate multiple capillaries for the concurrent processing of a large number of samples [13–17] have solved the serial analysis problem.

While CE can reduce analysis times by orders of magnitude in comparison with slab gel systems, reduction in analysis time does not always translate to savings in total sample analysis time. Patient samples often must be processed in some manner before the electrophoretic separation step can be executed. Processing steps may include cell sorting, purification, DNA amplification, desalting, or labeling reactions; consequently, sample size reductions gained with the capillary format may be lost because of the material handling requirements of these initial steps. In addition to the time constraints of these steps, which are unaffected by the translation to capillaries, human intervention remains necessary between processing steps, as well as prior to the initiation of the separation step.

Early on, the electronics industry realized that miniaturization in the form of the silicon microchip represented an elegant solution to the problem of complexity and has successfully exploited miniaturization to reduce the size of devices while increasing their capacity and functionality. The value in applying this same concept to the analytical sector, in particular the separations field, has been known for some time. While the efforts of Terry et al. [18] in the late 1970s to improve gas chromatography through miniaturization on a glass substrate were not fruitful, it presented the possibility that microminiaturization could do for the analytical sector what microchips did for the electronics industry. Miniaturized analytical devices, fabricated in glass using the same

methodologies developed for the silicon microchip industry, have led to a wealth of literature that supports this concept. While glass-specific challenges exist as a result of its highly amophorous nature, the pioneering experiments of the Manz [7] Harrison [4], and Ramsey [19] groups demonstrated that, like fused silica capillaries, channels microfabricated in glass could be utilized effectively for highly reproducible separations. As a result of the channels being shorter than the typical capillaries used for electrophoresis, the field strength in a channel could be as much as 10-fold higher than in the capillary, yielding microchip-based separations that consume even less time than their capillary counterparts. For parallel processing of samples, arrays of microchannels can be etched into the glass device as simply as one channel is etched, providing a convenient solution to the parallel processing problem. However, the order-of-magnitude increase in analysis speed attainable with microchip devices over capillary-based systems highlights further the need to address the rate limiting nature of the preelectrophoresis processing steps.

The true advantage of microchip devices over their capillary and slab gel counterparts stems from the fact that, in addition to channels, other structures can be fabricated into a single device. Structures that function as reaction chambers for DNA amplification or protein labeling, filters for cell sorting, or extraction/purification domains based on solid-phase media, are all conceivable. While the miniaturized size, combined with a reduction in the number of any sample handling steps, will allow for even smaller sample volumes to be utilized effectively, the benefits extend beyond sample size and reagent consumption. It has been observed that for reactions such as PCR, a decrease in size has the potential to lead to an increase in speed [20,21], reducing the time necessary for the preelectrophoresis processing steps. Creating a device with integrated functionality would begin to approach the realization of the ''lab-on-a-chip'' concept which has been discussed in the literature for years but still doesn't exist in any concrete form. The development of a device that could accept a sample and, in an automated format, yield diagnostic results would take bold steps toward the robust ''sample-in/answer-out'' device that will be needed for the clinical sector to see value in this new technology. Such a device could function in a central laboratory environment for high-throughput analy-

sis or in a miniaturized single-analysis mode at the site of patient care, an area in clinical medicine that continues to grow.

The concept of a fully integrated microfabricated chip for clinical diagnostics will be discussed in this chapter in three sections. First, a brief description of the microchip manufacture process will be described. Second, the results of recent work using microchips as a diagnostic tool will be presented. These results will demonstrate the usefulness of this tool, simultaneously showing the need for a fully integrated device. Finally, we will present what we feel to be the important breakthroughs necessary to develop a fully functional and versatile clinical diagnostic tool.

1.2 CREATING MICROSTRUCTURES IN GLASS MICROCHIPS

The development of features in silicon or glass proceeds in a series of four steps. A metal layer is sputtered across the entire surface of the wafer followed by coating with a photoresist. The photoresist is exposed to UV radiation through a mask which was designed with the necessary features. The photoresist and metal in the feature areas are removed, and the wafer etched to produce structures in the wafer. While the idea is simple in concept, it is much more challenging in practice.

The mask is an important component in this series of steps, since the mask image is transferred first to the photoresist during UV exposure and then to the metal layer. The metal layer then acts as a secondary mask for the etching of the image into the glass. The lines and sizes on the mask set the feature sizes of the final structures in the glass wafer. Metal masks are advantageous, providing resolution in the nanometer range, but are expensive and take days to produce. Film-type masks are relatively easy to make and inexpensive, but features <10 μm in size are not yet possible using current film technology [22]. Producing an integrated device requires only that the chambers, channels, and connections necessary to carry out the analytical methods of interest be contained in the original mask.

Etching of the glass is carried out in a buffered solution of hydrofluoric acid. Different types of glass etch at significantly different rates, so the specifics of the etching step are determined empirically for the

desired depth and width of the final structures. Smooth walls are typically achieved, but V-shaped channels result because as the glass is etched downward, the etch solution also acts laterally. This lateral etching often extends under the metal layer; thus, the depth of the structures in the glass are set by the amount of lateral etching which is acceptable. Features on the mask are often made smaller than they need to be to allow for this type of spreading. The etched wafer is then bonded to a second piece of glass, into which reservoirs have been drilled, to enclose the chambers and channels of the device.

While most of the research on microchip devices involves glass substrates, the cost and effort necessary for current manufacture of microchips make them incompatible with the disposable-type assays that would be carried out in a clinical laboratory. There is some effort directed toward developing microchips out of polymeric-type materials. Polycarbonate, which can be injection molded, requires only that a single master mold containing the structures of interest be fabricated in metal. Microchips formed quickly and reproducibly by this process are now being investigated by ACLARA Biosciences [23]. Agilent Technologies has taken another approach, using polyimide as the material from which their microchips are constructed [24]. Polyimide is flexible, has good heat transfer properties, and is easily bonded to itself. Chambers and channels are easily formed in one piece by excimer laser ablation, which can then be bonded to a solid bottom layer and a top layer containing appropriate reservoirs. This type of technology is readily amenable to the production of three-dimensional microchips.

1.3 DIAGNOSTICALLY RELEVANT SEPARATIONS ON A MICROCHIP

One of the earliest illustrations of microchip analysis with potential clinical application was a competitive immunoassay for serum cortisol demonstrated by Koutny et al. [25]. The final design of the fused silica microchip, which was used only for the separation, is presented in Figure 1A. Known amounts of antibody and labeled antigen were added to the sample of interest where the labeled antigen and the native antigen competed for antibody binding sites. Following a ''pinched injection'' using a cross channel that is slightly offset, free labeled antigen was