Chapter 1

Introduction

1.1 Background

Microfluidics is increasingly being used to scale down and automate laboratory procedures in the fields of biotechnology and chemistry [218, 205]. The small dimensions of microchannels tend to reduce reagent consumption and waste production, leading to cost savings and enabling precious samples to be divided up among larger numbers of screening assays [97, 25]. Furthermore, many identical reactions or assays can be replicated on a single microfluidic chip to harness parallelism and increase throughput [170, 112, 48], or many different stages in a complex process can be integrated into a single chip to improve ease of use and reduce human error—for example, in medical diagnostic devices. It has also been reported that microchannels can improve the speed and accuracy of chemical reactions [286, 62], as well as the speed, sensitivity, and repeatability of many assays.

Microfluidic devices based on elastomeric materials such as polydimethylsiloxane (PDMS) are rapidly becoming a ubiquitous platform for applications in biotechnology [218, 205]. Recent growth in the field of PDMS microfluidics has far outpaced that in alternative device technologies based on glass and silicon, due in large part to significantly simpler and less expensive fabrication procedures as well as the possibility of easily incorporating integrated mechanical microvalves at extremely high densities [272, 268].

This trend is limited to applications involving aqueous solutions, however. Glass and silicon devices are still preferable to PDMS devices in many areas of microfluidic chemical synthesis and

analysis, where acids, bases, and organic solvents are frequently used. PDMS is incompatibile with many such solvents [160], and exposure can lead to adverse effects (including swelling) that are especially pronounced in microscale channels due to the high surface to volume ratio. On the other hand, glass, silicon, and other rigid materials, such as ceramics and metals, are relatively inert.

Despite this inertness, there are several drawbacks to the use of rigid materials in microfluidic devices. In particular, mechanical valves are difficult and expensive to fabricate [218], and devices of high complexity have been impossible due to the large size of these valves—typically several millimeters [305, 218]. To circumvent these problems, non-mechanical fluid manipulation techniques such as electrokinetic pumping have frequently been used in these devices. Such methods do not scale well to complex channel networks, however, and unlike mechanical valves and pumps, their operation depends sensitively on the physical and chemical properties of the fluid [134]. This latter disadvantage is particularly problematic for applications in organic chemistry due to the huge variety of solvents with different properties that are commonly used.

We believe that the field of chemistry could benefit tremendously from the development of an elastomeric microfluidic device technology that offers the same advantages as PDMS microfluidics with the additional feature of high solvent-resistance. Efforts to develop this new enabling technology are the subject of the first half of this thesis.

With the advantage of simplified device fabrication, it is expected that solvent-resistant elastomeric microfluidics will become more accessible to a greater number of chemists than glass and silicon fluidics will, therefore accelerating explorations in this field. In addition, the use of mechanical microvalves could eliminate the dependence of reactor and assay designs on fluid properties, leading to greater design re-use and more rapid development of new applications. Unlike their glass and silicon predecessors, devices based on solvent-resistant elastomers possess the property of gas permeability, which allows device designs to be simplified through the use of dead-end channels. Permeability also allows evaporation to be used as a means to dry out reagents or to exchange solvents on-chip, thus providing valuable new tools for accessing a broader range of reactions than was previously possible with microfluidics. Finally, the ability to fabricate solvent-resistant devices with

thousands of individual valves and reaction chambers will likely lead to novel applications such as combinatorial organic synthesis and high throughput screening that were not possible in glass and silicon devices. Combinatorial techniques are widely used in industry to discover and screen novel compounds for properties such as catalytic activity or therapeutic effects in a high-throughput brute force manner.

Microfluidic combinatorial chemistry is the subject of the last half of this thesis, with particular emphasis paid to the synthesis of combinatorial peptide and DNA arrays, and their applications in the areas of genomics and bioinformatics.

1.2 Organization

This thesis is organized as follows. Chapter 2 provides a brief overview of microfluidics, with particular emphasis on PDMS device fabrication. The chapter concludes with a discussion of the advantages of PDMS microfluidic technology that have led to its rapid adoption for many sophisticated biotechnology applications.

In Chapter 3, I argue that highly-integrated applications in the areas of chemical synthesis and analysis have not yet been realized due to the lack of solvent-resistance of PDMS and due to the many limitations of alternative technologies. The bulk of this chapter describes our efforts to fill this gap by developing new microfluidic device technologies that combine the advantages of PDMS devices with the property of solvent-resistance. Results are discussed for many different directions of investigation, some of which met with moderate success. Two additional approaches are described in Chapters 4 and 5, both of which culminated in the successful demonstration of solvent-resistant devices with functional microvalves. Chapter 4 discusses the fabrication of devices from fluorinated norbornene polymers in collaboration with Materia Incorporated, and Chapter 5 discusses fabrication from perfluoropolyethers in collaboration with Joseph DeSimone's group at the University of North Carolina at Chapel Hill.

A novel technique for fabricating microfluidic devices from three-dimensional molds is described in Chapter 6. While the approach was originally pursued merely as a means to eliminate bonding steps

during device fabrication—steps that proved particularly problematic in solvent-resistant materials—our molding technique may find other uses in 3D device fabrication due to its many advantages compared with alternatives.

Chapter 7 deals with combinatorial synthesis, an important branch of chemistry that can benefit from the high integration densities that are possible with solvent-resistant elastomeric microfluidic devices. In particular, we have designed microfluidic devices that have the potential to synthesize *in situ* arrays of compounds at much higher densities and with greater purity than other methods. As examples, we demonstrated several principles of the synthesis of DNA and peptide arrays by solid-phase methods.

In Chapter 8, I argue that combinatorial arrays of DNA could be used for genome-wide expression analysis and could offer many advantages—such as universality—over the targeted arrays that are currently used for such studies. We developed a mathematical model to determine the required value of n such that an array of all possible DNA n-mers could provide meaningful results in experiments with complex organisms such as mouse or human. We show that the minimum useful value of n is technically feasible in terms of array fabrication and readout. It was this result, in fact, that originally motivated our pursuit of microfluidic array synthesis and our development of solvent-resistant microfluidic device fabrication technologies.

High-throughput gene expression studies have helped to deduce the functions of unknown genes and to identify the interconnections among genes in the complex genetic networks of many organisms. Chapter 9 motivates and describes a new algorithm that we developed for mining the vast wealth of published gene expression data to determine pairs of genes that are likely to be related. Our algorithm uses a non-metric probability measure that can in principle detect a wider variety of relationships than other approaches.

1.3 Contributions

The work described in this thesis represents significant original contributions in several fields.

First, we developed several novel materials and fabrication procedures for elastomeric microfluidic devices to confer the property of high solvent resistance (Chapters 3, 4, and 5). This new property will enable elastomeric microfluidic devices—with their intrinsic advantages—to be used in numerous new applications, including chemical synthesis and analysis. Devices with functional microvalves were demonstrated using a variety of resistant materials and coatings. The work on perfluoropolyether devices (Chapter 5) has been published [230], and a patent application has been filed.

Second, we devised a new method to fabricate three-dimensional microfluidic networks based on replication molding from sacrificial wax molds (Chapter 6). With this technique we have demonstrated the first complex 3D fluidic networks containing integrated elastomeric microvalves. A manuscript is in preparation.

Third, we designed microfluidic devices that can be used for high-density combinatorial solid-phase synthesis and demonstrated several aspects of their operation for the synthesis of DNA and peptide arrays (Chapter 7). A patent has been granted on these concepts [274]. However, this work has not yet been published due to the scarcity of solvent-resistant materials, which has prevented the demonstration of a large scale microfluidic synthesis.

Fourth, to our knowledge, we were the first to contemplate the use and advantages of combinatorial n-mer arrays for universal gene expression analysis (Chapter 8). The algorithm we developed enabled us to quantify the minimum value of n that is theoretically necessary to construct a *useful* array and to show that it is within the realm of technical feasibility. This work has been published [275], and a patent application has been filed [220]. Gene expression analysis using (slightly different) universal arrays has recently be experimentally demonstrated by Roth *et al.* [233].

Fifth, our probabilistic analysis of gene expression ratio data (Chapter 9) is a significant extension to the approach of Walker *et al.* [282, 281, 280]. Our modification fundamentally changes the type of data that can be analyzed, opening up a vast wealth of published microarray data to analysis by this approach. A manuscript describing this work is in preparation, and a patent has recently been

awarded [219]. We have implemented this algorithm in computer code and have made the computed probabilities available in an online database.

Finally, I helped Matthew Reese to demonstrate the effectiveness of novel microarray pens that he microfabricated from stainless steel foil. These pens can be used on standard microarrayers to deposit cDNA probes or other biomolecules onto arrays at significantly higher densities than conventional pens. This work is described briefly in Chapter 7. Our results have been published [225], and a patent application has been filed [276].