ACOUSTICALLY-DRIVEN DIRECTED ASSEMBLY IN THREE DIMENSIONAL AND MICROFLUIDIC ENVIRONMENTS

A Dissertation Presented

By

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to

The Department of Mechanical and Industrial Engineering

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in the field of

Mechanical Engineering

Northeastern University
Boston, Massachusetts

July 2018
ABSTRACT

Directed or selective assembly can create ordered microsystems for a broad range of applications from sensing to the life sciences. One example is templated assembly by selective removal (TASR), which has been shown to be able to selectively assemble microscale objects and biological cells by size into predetermined locations on a 2D surface based on the surface’s geometry and acoustic excitation. Although directed assembly excels at ordering structures on exposed 2D surfaces, it is less well developed for forming 3D and hierarchical architectures such as are typically required for metamaterials, tissue engineering or medical diagnostic devices. One potential solution to the challenge of creating 3D and hierarchical systems using directed assembly is to integrate the assembly techniques with other microscale technologies such as microfluidics or the folding of 2D surfaces into the third dimension. However, the physics of directed assembly processes are not independent of their context, and applying directed assembly to folding and microfluidic systems requires that their physics be understood and taken into account. This research examines the engineering principles that underlie the integration of the TASR process into folding and microfluidic systems and demonstrates TASR’s successful integration into these systems.

The first part of the research examines the capabilities and limits of the TASR process in folding systems. Because folding rotates planar surfaces through non-horizontal angles, the key requirement for integrating TASR into folding systems is that TASR must function effectively on non-horizontal surfaces. An analytical model was developed for predicting the performance of the assembly process on non-horizontal substrates. The model predicts that TASR-based assembly will be both effective and selective for low angles of tilt, below about 45 degrees, and that it will lose its effectiveness and selectivity as the surface rotates to higher angles. Experiments were carried out to demonstrate the effects of angle of tilt on the assembly of different sizes of microspheres into correct (well-matched) and incorrect (poorly-matched) assembly sites. The experimental results show good agreement with the model predictions, with microspheres successfully assembling into their correct assembly sites and successfully avoiding incorrect assembly sites at low angles. At higher angles, both the assembly and its selectivity are lost as predicted by the model.

The second part of the research examines the ability of TASR to guide selective assembly inside of microfluidic channels as a function of channel height, width, geometry, and operating conditions. The results demonstrate that microchannel dimensions are primarily important insofar as they affect the number of assembly components that are available relative to the number of assembly sites to be filled. However, the width of the microchannels is found to be a second and independent factor in determining the assembly results, with assembly being unsuccessful below a characteristic length scale that is determined by the acoustic excitation. The assembly is found to be largely independent of the microchannels’ layout and the placement of the assembly sites within the channels.
ACKNOWLEDGMENTS

I would like to appreciate Prof. Carol Livermore for providing me this research opportunity in her Lab, Micropower and Nanoengineering Laboratory (MNL). I am truly thankful for her tremendous guidance, mentorship, and support which significantly helped me to finish this research project and dissertation.

Besides my adviser, I would like to thank my committee members Prof. Andrew Gouldstone and Prof. Thomas Webster for their contributions and suggestions to my research.

I would like to acknowledge to NSF and AFOSR for funding this research since without their contribution, no part of this research could be completed.

I would like to thank Prof. Carlos Hidrovo and his lab members who permitted me to use his plasma cleaner tool for fabrication of microfluidic devices. Also, I appreciate Prof. Randall Erb for his valuable advice on suspension making.

I am grateful from George J. Costas Nanoscale Technology and Manufacturing Research Center director, Dr. Somu Sivasubramanian, fab manager, Scott McNamara and cleanroom specialist, David McKee for their extensive trainings, hands-on guidance and troubleshooting in the cleanroom throughout this research.
I am thankful to my current and former labmates Drs. Xin Xie, Chenye Yang, Tian Liu, and Sanwei Liu, as well as Chastity Kelly and Philipp Mehner, for their friendship and help.

I am also thankful to our summer time student, James Carroll who contributed to experimental setup design and its fabrication. Also, I thank summer time teachers, Ayo Awobode, Huseyin Turkoglu and Diana Cost for their contributions in early experimental works for microfluidic devices.

And finally, I would like to deeply appreciate my wife, Nasim Massoudi and her family and also my own family, Narges Razmara, Mehdi Bigdeli and Saeed Bigdeli for their unconditional love, support and encouragements. This journey would not have been possible without you all.
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<tr>
<td>$A$</td>
<td>Actual contact area</td>
</tr>
<tr>
<td>$A_n$</td>
<td>Nominal contact area</td>
</tr>
<tr>
<td>$c$</td>
<td>Velocity of sound in the assembly medium</td>
</tr>
<tr>
<td>$C_r$</td>
<td>Surface roughness coefficient</td>
</tr>
<tr>
<td>$d_c$</td>
<td>Hydrophobic Characteristic length</td>
</tr>
<tr>
<td>$f$</td>
<td>Frequency</td>
</tr>
<tr>
<td>$F_{\text{added mass}}$</td>
<td>Added mass force</td>
</tr>
<tr>
<td>$F_{\text{acoustic, drag}}$</td>
<td>Acoustic drag force</td>
</tr>
<tr>
<td>$F_{\text{Basset}}$</td>
<td>Basset force</td>
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<tr>
<td>$F_{\text{rad}}$</td>
<td>Radiation force</td>
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<tr>
<td>$F_{\text{ret}}$</td>
<td>Retention force</td>
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<tr>
<td>$F_{\text{viscous}}$</td>
<td>Viscous force</td>
</tr>
<tr>
<td>$g$</td>
<td>Acceleration of gravity</td>
</tr>
<tr>
<td>$h_h$</td>
<td>Maximum peak heights of the well</td>
</tr>
<tr>
<td>$h_s$</td>
<td>Maximum peak heights of the well</td>
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</table>
\( I \)  
Intensity of the incident acoustic waves

\( m \)  
Mass of the particle

\( M_{\text{added mass}} \)  
Added mass moment

\( M_{\text{acoustic, drag}} \)  
Acoustic drag moment

\( M_{\text{Basset}} \)  
Basset moment

\( M_{\text{rad}} \)  
Radiation moment

\( M_{\text{ret}} \)  
Retention moment

\( M_{\text{viscous}} \)  
Viscous moment

\( r \)  
Radius of the particle

\( R_{\text{eff}} \)  
Effective radius of curvature between the microsphere and well

\( R_m \)  
Radius of microsphere

\( R_w \)  
Radius of well

\( R_o \)  
Etched depth at the center of the microfabricated well

\( S \)  
Selectivity ratio

\( \text{SAMs} \)  
Self assembly monolayers

\( \text{TASR} \)  
Templated assembly by selective removal

\( V \)  
Volume of the particle
\( \bar{v} \)    Streaming velocity

\( v_T \)    Speed of the particle

\( U \)    Interfacial surface energy of the assembly component

\( Y_b^a \)    Assembly yield of microspheres of diameter \( a \) into wells of diameter \( b \)

\( z \)    Separation distance between the microsphere and the surface

\( \alpha \)    Radius change coefficient of curvature

\( \gamma_{SV} \)    Solid-vapor interfacial surface energy

\( \gamma_{SL} \)    Solid-liquid interfacial surface energy

\( \gamma_{LV} \)    Liquid-vapor interfacial surface energy

\( \eta \)    Viscosity of the fluid

\( \theta \)    Contact point angle of microspheres inside the well

\( \lambda \)    Wavelength of acoustic waves

\( \rho \)    Mass density of the assembly medium

\( \rho_l \)    Mass density of the liquid

\( \rho_p \)    Mass density of the particle

\( \mu \)    Viscosity of the medium

\( \sigma_{eq} \)    Equivalent root mean square roughness
\( \phi \)  Rolling angle of the microsphere inside the assembly site
Chapter 1

INTRODUCTION

Many real world applications are enabled by systems with features at the small (micro or nano) scales. In many cases, conventional micro or nanofabrication technology is well suited to make these systems a reality; one example is computer chips. In other cases, however, the understanding of the structures that are needed can be far ahead of the technologies for making them a reality. For example, engineered structural materials may require precise orientation of nanoscale elements (Edel, 2013; Xu, 2013); metamaterials may require micro or nanoscale features to be defined not only in the plane but also out of plane; and tissue engineering can require precise replication of a three-dimensional (3D) arrangement of cells and biomaterials (e.g. extracellular matrix). Creating these types of systems becomes increasingly challenging when the dimensions are small, out of plane organization is required, and the intended structure is hierarchical and includes different types of order at different length scales.

One promising set of approaches to address these types of challenges fall under the heading of self assembly, directed assembly, and selective assembly. Self assembly refers to the spontaneous organization of initially-disordered objects into some kind of order, where the order is determined by the interactions among the objects themselves. Directed self assembly is a type of self assembly approach whereby the assembling process is guided by an external agent, force or template (Grzelczak, 2010). Selective assembly refers to any assembly process in which the assembly outcome depends on the types of objects that are assembling.
Assembly processes can offer many advantages, such as the ability to create highly precise arrangements of components, tunable control to induce various types of long and short range order among the components, functionality across a wide range of length scales from nanometers to microns, and applicability to many kinds of materials, both individually and in composite forms. These advantages of assembly approaches offer significant benefits for the fabrication of complex microscale and nanoscale systems. Directed assembly techniques in particular have been shown to be able to impose order on two dimensional systems (Morris, 2011; Varghese, 2006; Yap, 2007). However, in many cases the underlying physics of the assembly process limits the lateral extent of the imposed order (Y. Lee, 2015; Lodge, 2003). To a lesser extent, self and directed assembly techniques can impose order on systems in three dimensions. Hierarchical system architectures can expand the complexity that can be achieved using assembly techniques (Kachouie, 2010).

Hence, one promising approach to build complex three dimensional systems such as can be required for tissue engineering or for metamaterials is to integrate the formation of hierarchical architectures with assembly approaches. In other words, the assembly process can structure the system at the smallest length scales, while some other larger-scale process introduces structure or order at larger length scales. For example, the assemblies may be incorporated into or onto structures that exhibit some larger scale geometry or into microfluidic networks that control system behavior (in that case fluid flow) at larger length scales.

When the goal is to introduce three-dimensional structures with small-scale assembled features, there are two general approaches that one may consider. In the first, the assembled
structures are first defined in a simpler planar system, and then the larger-scale geometry is introduced by creating curvature or by folding, layering or laminating the initial planar system into the third dimension. Because handling is involved in the folding, curving, or layering processes, this approach is best suited for systems in which the assembled components are not overly sensitive to handling. For example, this approach would be better suited to the organization of inorganic components that are not sensitive to contamination and would be less well-suited to the organization of biological specimens or cells. In the second approach, the larger-scale geometry is created first, and the assembly process takes place thereafter. Defining the microstructure after the larger-scale geometry has been created can enable complex hierarchies with long-range order to be created without risking adverse effects from the handling involved in creating the larger-scale geometry. However, the ability to create assembled microstructures on curved, folded, or laminated surfaces can be limited by the underlying physics of the assembly process, including the delivery of the assembling objects to where they need to assemble and the capture of the assembling objects once they reach the locations where they are to assemble.

When the goal is to introduce assembled objects into other types of systems, such as microfluidic networks, similar challenges arise. For example, either the small scale structure must be assembled before the assembly of the microfluidic network or the assembly of the small scale structure must still be possible after the creation of the microfluidic network. For the second (and more promising) option, the physics of the assembly process can again affect the potential for successful manufacture of the system as a whole.
The main motivation of this research is to determine and understand the abilities and limits of integrating assembly processes into hierarchical architectures. Although this research was motivated by the challenges of creating hierarchical architectures for tissue engineering, these questions are equally relevant for building other types of complex advanced functional systems at small length scales. In particular, the present research focuses on understanding the capabilities and limits of a particular assembly technique that offers selective assembly based on size and shape for both inorganic objects (which can be either rigid or deformable) and biological cells. This assembly approach is known as templated assembly by selective removal (TASR) (Jung, 2005). The TASR technique is chosen for study because it offers a high degree of assembly selectivity at the length scales of interest, enabling the assembly of various cells or microcomponents. 

The present research uses a combination of analytical modeling and experimental characterization to predict and validate the limits of the TASR process under conditions that are relevant for the creation of hierarchical systems that include both small-scale assembly and larger scale structures. In the first part of the research, the ability of the TASR process to drive assembly onto the kinds of angled (non-horizontal) surfaces that would be found in curved or folded system architectures is analytically predicted and experimentally demonstrated. In the second part of the research, the ability of the TASR process to drive selective assembly inside previously created microfluidic networks is experimentally characterized. Although this research represents one portion of a broader program to replicate the three-dimensional complexity of layered, living tissue via a combination of directed assembly and origami folding (Mehner, 2015), the potential applicability of the present work is not limited to that context.
1.1 Thesis goals

In this research, the ultimate objective is to predict and validate the limits of the TASR process under conditions that are relevant for the creation of hierarchical systems that include both small-scale assembly and larger scale structures. Although the capabilities of TASR have been demonstrated for assembly onto wide, flat, horizontally-oriented surfaces in previous work (Eid, 2008), no research has been done or reported on the potential of TASR for driving selective assembly in more complex environments such as onto three dimensional geometries or into microfluidic channels.

To achieve this overall objective, this research focuses on three smaller goals. The first two are to develop an analytical model for predicting the performance of TASR-based assembly on angled (non-horizontal) substrates and to validate its predictions through experiments. Achieving these goals will set the stage for assembly onto 3D curved, folded, or layered systems. The model should be able to predict the angles of substrate tilt at which the assembly process will be effective and to identify whether there are any conditions under which the assembly will either cease to function selectively or cease to function at all. The experiments should test and ideally validate the analytical model’s predictions to provide design guidelines for the use of TASR-based assembly in future 3D hierarchical assembly systems.

The third goal is to evaluate the capabilities and limits of TASR-based assembly inside of microfluidic channels. In particular, the third goal aims to determine how TASR-based assembly inside of microchannels is affected by the microchannel design (e.g. the widths and heights of the channels and their in-plane layout) and the conditions under which the assembly is carried out.
1.2 Thesis roadmap

This dissertation comprises seven chapters. Chapter 1, Introduction, discusses the importance of this research in broad terms, explains the contribution of this work to the field, and presents the thesis roadmap.

Chapter 2: Literature review. This chapter defines the self assembly process and explains its fundamental working mechanisms in general. It introduces directed assembly and briefly reviews previous work done using directed assembly. It then focuses on directed assembly of micro- and nanocomponents for applications in tissue engineering, biomedical diagnostic devices, and building metamaterials and colloidal structures. It presents several attempts of how self and directed assembly approaches were used by researchers and engineers to fabricate three dimensional scaffold tissues or study the biological behavior of cells in three dimensional environments. It also provides information on techniques for the capture of tumor cell for drug screening and diagnostic purposes. In addition, it elaborates on the advantages and disadvantages of different assembly methods and their future challenges within each section. At the end, it provides an overview of the role of directed assembly in building metamaterials and colloidal structures.

Chapter 3: Templated assembly by selective removal (TASR). This chapter focuses on the specific selective assembly technique that forms the basis for the current research. The technique, its constitutive parts, and its fundamental working mechanisms are introduced in general terms. The chapter then continues by providing a detailed explanation of each constituent part of the process, including the elements that promote assembly and the elements that promote selective removal of incorrectly-placed components. Finally, the chapter ends with reviewing the previous work done on the TASR process.
Chapter 4: Experimental procedure. This chapter provides the full details of the experimental steps that are required to carry out this research. Since the research focuses on two sets of experimental questions, one pertaining to assembly onto angled surfaces and the other pertaining to assembly inside of microfluidic systems, the content is divided into two corresponding sections.

Chapter 5: TASR-based assembly in non-horizontal systems. This chapter first clarifies the importance and scope of this phase of the work. Then an analytical model is developed to describe the physics of TASR-based assembly onto angled substrates. Finally, the experimental results are presented and compared with the model predictions to validate them. Finally, the findings of both parts are summarized.

Chapter 6: TASR-based directed assembly inside microfluidic devices. This chapter elaborates on the potential implementation of TASR inside microfluidic systems. It first describes the role of microfluidic systems in bioengineering applications such as cell isolation and capturing cancer tumor cells. The experimental results showing the impact of different variables such as the microchannels’ geometries and the assembly conditions are presented. In the closing section, the experimental findings are interpreted in the context of the physics of the microfluidic assembly system, and useful hints for TASR-based microfluidic devices are summarized.

Chapter 7: Conclusions and future works. This chapter summarizes all of the important conclusions from both phases of the research and suggests future challenges and potential interesting work to be addressed in the future.
Chapter 2

LITERATURE REVIEW

2.1 Assembly techniques

Overcoming the engineering, technology and life science challenges in our world often requires building advanced multifunctional systems or products, which in turn can require integration of diverse engineering methods and concepts. This is particularly true as the target technology’s characteristic dimensions approach the micro and nano scales, at which control and precision play a vital role. One particularly useful set of manufacturing techniques for creating micro and nanoscale functional materials and systems is assembly techniques. Assembly techniques, which can include self assembly and directed assembly can provide versatile and powerful tools for structuring small-scale systems.

Self assembly is defined as a process in which individual components, such as micro- and nanoparticles, automatically arrange themselves under direct and/or indirect specific interactions, across their environment (Grzelczak, 2010). Fundamentally self assembly can be explained by thermodynamic equilibrium, in the sense that after a self assembly process, the free energy of system reaches its minimum (Whitesides, 2002). Generally speaking, in assembly processes the building blocks organize into ordered, macroscopic structures, either via direct interactions among components (self-assembly), or indirectly using external forces or patterned templates (directed assembly). Self assembly techniques offer benefits in a broad range of applications from sensors, actuators, and metamaterials to medical devices, tissue engineering, drug delivery and cancer therapy.
2.2 Directed assembly techniques

Directed assembly works according to similar principles as self assembly but with the addition of a greater degree of externally-induced control of the assembly process. The building constituents may be carefully chosen and constructed, and the assembly process may be facilitated by modulating the thermodynamic forces without applying sophisticated techniques. Molecular interactions can be tailored and utilized to create ordered assemblies. Interparticle interactions, morphological and functional features can potentially help the system in directed interactions. External directing fields or directing surfaces can create more specific and selective assembly interactions if components of specific physical properties are designed for that purpose (Grzelczak, 2010).

From a geometric point of view, templates can be considered as modified substrates containing predefined active locations, and they can selectively catalyze micro and nanocomponents’ deposition (Grzelczak, 2010). Using templates in assembly processes enables us to create structures that are not possible or at least not practically feasible to make in other fashions. Generally, there are three different classes of methods for templated assembly on substrates: chemically modified patterned surfaces, charged patterned surfaces, and topographically patterned surfaces (Winkleman, 2005). Each approach uses a specific mechanism to guide the assembly; for example, there is a preferential adsorption of components onto the treated regions in the chemically modified surface approach (Fan, 2004; Masuda, 2003). Charged patterned substrates usually have not produced high quality periodic well-ordered lattices, while patterned topographic surfaces such as wells, microfluidic channels and lithographically patterned substrates can effectively control and confine colloidal assemblies (Winkleman, 2005).
External influences and fields are strong candidates to control micro and nanocomponent suspensions in order to tune the properties of assembled materials for mechanical, optical, and electronic applications ranging from electro-mechanical systems to electronic inks (Grzelczak, 2010). Because of the mismatch between the dielectric properties of particles and their surrounding environment, most micro and nanocomponents get polarized under the influence of electric fields (Jackson, 1999). Glass microspheres have been directly assembled onto patterned gold electrodes aided by an electric field, but drawbacks such as yield percentage and the two-dimensionality of the assembled structure could limit the versatility of this method (Winkleman, 2005). Mobile charges can also respond to the electric fields and contribute to the polarization process during the assembly (R. W. O'Brien, 1978). AC electric fields are more frequently used than DC ones due to the presence of electro-osmotic and electrochemical effects under DC current (Lumsdon, 2004). Magnetic and capillary forces have been utilized to self-assemble microscale silicon building blocks into interconnected structures to make 3D MEMS architectures with relative success (Morris, 2011). An assembly technique called “chemically directed contact electrification” has been used to make 3D structures out of charged polystyrene microspheres; however, the initial microspheres’ size is the limiting factor of this method due to aggregation of the spheres (McCarty, 2007). Overall, external fields are promising for directing the assembly of components, particularly microscale ones. However, these external fields would face some challenges and difficulties for assembly of parts at the nanoscale.

Viscous flow fields have been also used to directly assemble particles from a suspension onto ordered crystals (Panine, 2002; Y. Yan, 1994). In another attempt (Grego,
2005), a double layer of polystyrene microspheres was successfully assembled into a patterned silicon wafer via an evaporation technique, and that structure was used as a template for further self assembly of other microspheres to fabricate multilayer self assembled structures. In (Varghese, 2006), a size-selective assembly of microspheres via centrifugal forces has been introduced, where templates were prepared using laser ablation to form trenches and circular pockets in 2D and the assembly was done using a spinning method. The results were promising, except that the size selectivity was limited.

Due to the versatility of directed assembly approaches, and also their ability to produce multicomponent structures, these methods can be used or implemented in many applications such as tissue engineering, microfluidic devices, metamaterials and colloidal assemblies. The next subsections summarize some of the progresses and improvements in the aforementioned fields using different directed assembly methods. At the end of each section, a short summary of the achievement, challenges and potential future works are presented in the scope of the current research.

2.3 Directed assembly for tissue engineering

Tissue engineering combines biological science principles and engineering laws to mimic artificial tissues or organs as a whole or in part for replacement in the human body or for applications like drug screening. In other words, in tissue engineering the goal is to understand the structure-property-functioning relationships in healthy or infected tissues to develop new biomaterials and suitable techniques to build artificial tissues (Lutolf, 2005; Naderi, 2011; Wobma, 2016). Biomaterials play crucial roles in the field of tissue engineering and regenerative medicine (Hubbell, 1995). One of the main constituents involved in tissue engineering is the scaffold (F. J. O'brien, 2011). Scaffolds are designed
to function as in vitro extracellular matrices for cell culture to promote cell differentiation, proliferation and migration. They typically gradually degrade upon implantation in the patient and are replaced by new tissue (Yahya, 2014). Generally, based on its application, a scaffold structure should exhibit the below properties (X. Liu, 2012; Patel, 2008; Yahya, 2014):

- Mechanical properties to support and sustain the scaffold’s preferred shape;
- Highly interconnected porous network to transfer nutrients and oxygen and also to promote cell proliferation and differentiation;
- Three dimensional (3D);
- Biodegradable and a rational degradation rate in the target tissue to be replaced;
- Biocompatibility with the host tissue; and
- The surface properties and the ability to mold the scaffold into a specific construct.

Scaffolds can be bifurcated into natural and synthetic ones. Natural scaffolds are built on protein or carbohydrates with specific biochemical, mechanical, and structural properties, and their sources can be from plants or animals. Collagen, chitosan, hyaluronic acid, fibrin, and gelatin are some examples of natural scaffolds (Asti, 2014; Patel, 2008). These scaffolds have been used for many tissue engineering purposes, but the ability to control or modify their chemical and biological properties is limited. Hence, the focus has been placed on synthetic and blended biomaterials. Synthetic scaffolds are favorable since their physical and biological properties can be tailored and they are producible in large scales. Glycolic acid derivatives, lactic acid derivatives, and other polyester derivatives are the major classes of synthetic scaffolds (Patel, 2008; Place, 2009). Hydrogels are made up of polymer chains that swell in an aqueous solution, and they can be natural or synthetic in
Hydrogels are typically fabricated with thermally, chemically, or photo-chemically induced radical initiators, forming a covalently bound polymer network. These gels are often designed to be biodegradable, as they can be degraded within the body by water or natural enzymes and ultimately absorbed. Hydrogels are typically biocompatible, owing to their large water content, as they do not provoke an immune response or cause inflammation. Thus, hydrogels have been found to possess tissue-like properties as they can be successfully used to encapsulate cells and create scaffold environment for cells. Hydrogels can also be used in an injectable form to repair irregularly-shaped defects and for cosmetic surgery (Patel, 2008; Ullah, 2015).

A key factor for the success in engineering and fabricating functional living structures is to fully understand the fundamentals of cellular self assembly and the ability to employ them (Jakab, 2010). All biomolecules (e.g. peptides and proteins) naturally interact and organize themselves to produce well-defined functional structures. As a result, it is possible and useful to employ self assembly methods to synthesize new materials or advanced functional systems (S. Zhang, 2003). Currently, a common strategy for scaffold fabrication is the assembly of molecules and/or microscale species into structures at the macro scale. A ‘bottom-up’ method works based on the directed or self assembly of a scaffold from tiny components or modules designed to deliver different specific tasks in the final artificial tissue (Elbert, 2011; Truskey, 2016).

Microtissues can be fabricated in large scales by co-culturing of preformed cell aggregates such as was done in (Elbert, 2011), where uniform size tissue spheroids were formed by cutting cylinders of a cell slurry followed by overnight culture in solution. These spheroids are useful for cell printing as bioink. However, the self assembly mechanism of
cells into hydrogel has not been fully understood plus the process needs other considerations for different types of cells. A significant advancement in 3D bioprinting of heterogeneous microtissue of multiple materials with defined vascularized systems has been shown in the efforts of Kolesky and co-workers (Kolesky, 2014), in which the research developed a new approach for constructing 3D tissue. This technique may potentially benefit in drug screening, wound healing, stem cells and angiogenesis. In another work (Rago, 2008), Hep2G liver cells were seeded onto the bottom of a micro-patterned gel, formed 3D microtissues, and finally were encapsulated using alginate. As claimed, this approach may resolve some issues about conventional microcapsules. Similarly, Fukuda and co-workers (Okuyama, 2010) used a microfluidic device to self assemble Hep2G cells inside microwells, and then the spheroidal microtissues were surrounded by fibroblast cells (not fully coated or encapsulated).

Tissue engineering using modular self assembly was introduced for the first time by McGuigan et al. (McGuigan, 2006). In this approach, cylindrical modules are cut from a tabular cell-loaded collagen gel. These modules are then coated with endothelial cells and exposed to blood flow in a chamber. In this research, it is proven that 3D cell culture models and experiments more reliably and accurately mimic the in vivo cell behavior and microtissue architecture (Abbott, 2003). Dean et al. studied the potential of directed assembly of human and rat cells embedded in microscale hydrogels and successfully cultured them onto several patterned geometries such as honeycombs, tori and rods (Dean, 2008; Dean, 2007). 3D cardiac microtissue spheroids of cardiac myocytes (CMs) and/or cardiac fibroblasts (CFs) have been fabricated and used as building blocks to generate larger microtissues with different distributions of CMs and CFs (T. Y. Kim, 2018).
Vascularized functional cardiac tissue was also fabricated through microscale modules made of collagen embedded with rat heart cells. These modules were assembled onto a porous sheet scaffold, and then a number of those scaffolds were immobilized in alginate gel matrix (Leung, 2010). The functionality and behavior of cardiac myocytes and fibroblasts were studied using a scaffold-free technique with 3D microtissues. These microtissues have been generated with self assembly inside a nonadhesive hydrogel substrate containing big wells of 800 µm deep and 400 µm in width. It is claimed that this method is inexpensive and easy to use (Desroches, 2012).

In another work from the Whitesides group (Bruzewicz, 2008), microfluidic channels and soft lithography were merged to create a 3D mammalian cell culture process. The extracellular matrix was mainly made of biopolymers, and each module had at least one dimension below 300 µm, which was said to be sufficient for cell metabolic actions such as oxygen flux and delivery of nutrients. Soft lithographic approaches have also been used to encapsulate cells in 3D gels of biopolymers such as collagen, agarose and Matrigel. These modules were fabricated in several shapes, including cylinders, crosses, rectangles and prisms in the size range of 40 µm to the millimeter scale. The authors claimed that this technique provides a good understanding of 3D tissues of high cell densities.

In a recent work (Jia, 2014), alginate hydrogels have been used as bioink to investigate the effect of alginate viscosity and density on the printability of the materials. However, the self assembly mechanism of cells into hydrogel has not been fully understood plus the process needs other considerations for different types of cells. 3D aggregates and modules are common methods of promoting differentiation of embryonic stem cells in vitro. Khademhosseini’s group has developed a novel hybrid microgel platform (GelMA/PEG)
with asymmetrical extracellular matrices to mimic the anisotropic stem cell niche. This technique can be a strong and versatile fashion to modulate the stem cells and create patterned tissues to build sophisticated organs in tissue engineering field (Qi, 2010).

The interaction between hydrogel units for self assembly of 3D microtissues using hydrophobic/hydrophilic forces has been simulated with Monte Carlo model and Lennard-Jones potential in (Shi, 2009), and the model agreed well with their experimental findings. Hence, arranged tissue packages or structures can be formed with help from hydrophobic forces between gel units and the liquid environment. Cell-laden microgels (with NIH-3T3 cells) have been used as the building blocks for fabrication of 3D microtissue structures. The hydrophilic microgels have been directly assembled onto glassy patterned substrates rendered to be hydrophilic and hydrophobic in different regions (Du, 2010). In a similar follow up work, these microgels (100-200 µm) have been assembled into a pre-defined architecture on a hydrogel substrate in a mineral oil environment; endothelial and smooth muscle cells were then added to mimic a 3D vasculature network (Du, 2011). In another parallel work (Fernandez, 2010), Fernandez and Khademhosseini have accomplished the directed self assembly of microgels onto high-affinity PDMS molds. In their approach, microgel modules of the desired shape are mixed with a pre-biopolymer and are poured onto the PDMS mold. Then the pre-polymer is exposed to UV light to crosslink it, and eventually the PDMS mold is separated from the fabricated microtissue. In fact, this technique is a combination of photolithography and directed assembly of microtissue modules.

Generally speaking, the creation and study of artificial tissues tends to follow two main pathways. The first involves fabricating tissue modules and delivering them into chambers
or scaffolds, and the second involves preparing scaffolds and perfusing the cell culture medium or microtissues into them. The work described above has contributed to advancing the field of tissue engineering, but challenges remain. For example, in modular tissue engineering, microtissues are randomly mixed together and there is no control over their placement. This could cause inconsistency in tissue properties and in the final product. It can be difficult to arrange or place microtissues of different kinds in a specific architecture that may be biologically relevant. Another problem is that the majority of these techniques cannot efficiently create 3D complex artificial tissues such as are found in the liver. Recreating the complex 3D microstructures of native tissue in engineered tissue needs an approach that precisely controls and manages the placement and ordering of microtissues or cells of different kinds via a high throughput process. The directed assembly of microtissues or modules to create the required 3D structures would offer a potential pathway to tissue engineering that could address these underlying challenges. Hence, one goal of the present research is to create the knowledge necessary to bring the control of selective, directed assembly via the TASR process into the manufacture of more 3D or hierarchical systems.

2.4 Directed assembly inside microfluidic devices

Microfluidic devices provide outstanding capabilities and performance because of their controllable shape and composition, and they show significant advantages and potential in the fields of biomedicine and biosensing (J. Ma, 2017). Microfluidic devices used in studies of biology have been fabricated via different techniques such as micromachining, soft lithography, in situ construction and micromolding, etc. (Beebe, 2002). These devices serve many purposes, such as mimicking the cell-cell and cell-extracellular matrix
interactions of tissues by creating gradient concentrations of biochemical signals such as growth factors, chemokines, and hormones (Tehranirokh, 2013).

In self and directed assembly processes, the driving forces can be internal ones such as hydrogen bonds, electrostatic interactions, hydrophobic interactions, and Van der Waals interactions, or they can be external forces such as electrical, magnetic, acoustic forces. Hence, to guide self assembly and directed assembly processes, it is required to control both the internal factors and external environment. Microfluidic devices are useful tools for controlling both the physical and chemical properties of a fluid medium inside a restricted volume (Dou, 2017). 2D cell culture in microfluidic devices can be beneficial in the study of cellular responses and viability of cells while 3D cell culture offers stronger methods for cell-cell and cell-matrix interactions, stem cell studies, more in-vivo like physiological behavior, responses and growth for cells. 2D cultures are unable to mimic the tissue structures in many cases (except for endothelial) because cells show their physiological behavior and responses 3D microenvironments (Tehranirokh, 2013).

There have been various efforts in which microscale objects such as cells or hydrogels have been fabricated and/or directly assembled using microfluidic devices with diverse driving forces or mechanisms. For example, live mouse cells have been assembled with the help of the stimuli-responsive hydrogel alginate. This method could program the assembly of cells of different kinds to attach to desired electrodes under physiological pH conditions (Cheng, 2011). Monosized peptide microparticles were fabricated via a combined procedure using a droplet microfluidic devices and biocatalytic self assembly in Bai’s work (Bai, 2014). Also, two and three phase particles were fabricated using microfluidic devices and selectively functionalized at their surfaces (Nie, 2006). In another
work, scaffolds in the form of porous 3D honeycomb structures and also spherical hydrogels have been fabricated using two concentric micropipettes as simple microfluidic devices. The cell culture results proved that chondrocytes were able to proliferate inside this porous scaffold structure (Chung, 2009). In Whitesides’ group (Martinez, 2008), 3D microfluidic devices were made out of papers (defined as fibrous materials) in different geometries for fast prototype and new designs. It is worth mentioning that paper materials can be selected from a wide range of materials such as glasses, polymers, and cellulose.

Capturing circulating tumor cells (CTCs) is a critical step in understanding the physiology of them and how to treat different diseases. Tumor cells can be present in the bloodstream after escaping from the primary tumor mass, and these propagated cells are the source of eventual lethal metastases. CTCs are observable in the peripheral blood of patients with epithelial derived cancers at ultra-low concentrations of 1 in $10^6$ to $10^7$ peripheral blood mononuclear cells (Marrinucci, 2007). Identifying circulating tumor cells in blood is a non-invasive and useful approach in cancer diagnosis, prognosis, prediction, and drug delivery system studies (J. Myung, 2015). Analyzing and assessment of these cells can prevent patients from requiring tissue biopsy. CTCs can potentially be beneficial in personalized therapy, therapeutic efficacy monitoring, and potentially early diagnosis of cancer (Arya, 2013). Microfluidic devices are promising means for performing CTCs because they use a very small amount of samples and reagents, can be integrated with other technologies for improvement of devices or tools, and enable the capture and analysis of multiple infected cells all at once (J. Myung, 2015). Using a broad range of microfluidic devices has helped and improved CTC capture strategies. One of these strategies relies on
size separation, which works according to the fact that the sizes of cancer cells and healthy cells are different.

Cancer cells have been extracted from leukocytes based on morphological and size shape differences using a series of filters. Zheng et al. (Arya, 2013) have fabricated a microfluidic device based on parylene polymer membranes to separate CTCs from LNCaP cells (epithelial). It seems that this method could be a fast, cheap and effective process compared to conventional approaches of capturing cancer cells. However, a full study on the effects of pore size, shape and membrane thickness has not been done yet. In a parallel work from the same group (Lin, 2010), a parylene-based membrane microfluidic device was used to capture carcinoma cells, cancer cells developed from epithelial cells, and the analyses proved that the method can usually capture and recover more cancer cells compared to the CellSearch method, which is another FDA approved technique. Filters can be prepared or designed with different sizes, geometries and pore densities and also can be distributed inside microfluidic devices (Attard, 2011; J. Myung, 2015).

A physiologically-inspired design was introduced in (L. Y. Wu, 2008) whereby a microfluidic device could trap cancer cells in pre-defined geometries in such a way that self assembly of spheroids and post characterization are completed at once. Also, epithelial cancer cells have been captured by micropatterned streptavidin coated magnetic beads in wide strips along with in situ cell culturing inside microchannels with the help of magnetic fields and electrostatic self assembly methods (Sivagnanam, 2010). Silicon wafers can also be used as a template for microsieve CTCs platforms. This technology benefits the CTC field with high throughput device production and the capability of creating complex structures and features, creating effective post analysis due to the small size of the device.
It has been shown that devices built on this technology can end up with rapid CTC separation with high flow rates such as what Lim and et al. accomplished for CTC of Hep2G (liver cells) and MCF-7 (breast cells) (J. H. Myung, 2015). CTC separation microfluidic devices have been also fabricated from metal filters. A 3D palladium filter of two different size of 8 and 30 µm were fabricated with electroforming process. The device functions based on the gravity and needs about 20 minutes with a tumor cell recovery of more than 85%. This filter was examined on different cell lines such as gastric and pancreatic cells and successfully performed the CTC separation process on the tested cell lines (Yusa, 2014). It seems that this technique can be helpful in isolation and genetic analysis of CTCs from blood in both preclinical and clinical settings. However, some disadvantages (namely using upright florescence microscopy, a lengthy ejection process for single cells, and pore size of the filter) can limit its application. In another attempt, a strong binding surface made of dendrimers has been utilized as a CTC platform. It was used for capturing lung cancer cells in both in vivo and in vitro model environments (J. H. Myung, 2015). The obtained results of this study were promising for potential translational studies, but further investigation and research is needed. It is known that CTCs clusters could be more invasive than single CTCs and hence, the cluster capturing method has been proposed and investigated (Aceto, 2014; Molnar, 2001). Sarioglu and colleagues (Sarioglu, 2015) have created microfluidic devices called “Cluster Chips” to capture CTC clusters with high output. This technology contains a 2D array of pillars with rectangular base shapes that can even capture a cluster of two CTCs with good performance. This technique was shown to successfully detect breast, prostate and melanoma cancer cells in several patients.
In summary, microfluidic devices have been used for different purposes in the bioengineering field such as fabrication of microtissues and modules, 2D and 3D cell culturing, cell isolation and entrapment and etc. Looking at most works done via microfluidic devices for fabrication of 3D tissues constructs, there is no comprehensive or efficient approach for architecting 3D tissues out of microtissues or modules of different kinds due to lack of control over micro components or modules. Arrangement of different cells or microtissues based on the needs of the target tissue is crucial in the study of biological behavior of the tissue, and this can be addressed using techniques in which the ordering of micro objects is possible. Given the need for greater control in tissue engineering while maintaining parallel manufacturing processes, it is a good time to study the possibilities of implementing selective assembly techniques such as TASR for controlled tissue engineering environment such as microfluidics.

Capturing tumor cells has been also done with several techniques, but none of them was completely successful since CTCs have heterogeneity properties which means that the different tumor cells can show distinct morphological and phenotype behavior. Capturing and entrapping such cells based on size selectivity offers potential for this application. CTCs are usually in the range of 15-40 µm and most healthy cells are below 10µm, hence size-selective assembly techniques such as TASR could work efficiently for separating cells in this size range. In the present research, a highly selective directed assembly method, TASR, is investigated for implementation in microfluidic devices, and this could provide valuable information for potential CTCs applications.
2.5 Directed assembly for colloidal particles and structures

Photonic crystals are spatially periodic structures made of alternating regions of dielectric materials with different refractive indices. Photonic crystals are artificially made and the challenge of fabricating photonic band-gap structures is significant because the lattice constant of the photonic crystal must be comparable to the wavelength of the light passing through the crystal. In many applications such as optical communications systems, the photonic crystal-lattice constant must have dimensions of roughly a micrometer, and self assembly techniques have showed potential for fabricating huge single crystals (Joannopoulos, 2001).

Colloidal domains can be described by the dimensions of their constituents, which can be in the range of a few nanometers to micrometers. These constituents can be organized in complicated hierarchical architectures. The colloidal assembly structures can be described by three main characteristics, namely order, length scale and porosity. One of the biggest challenges in this field is how to implement these structural hierarchies from mesoscopic length scales (nano- and micro scale) to macroscopic dimensions with the highest possible efficiency and precision. Colloidal hierarchies can be made in different configurations such as closed-packed, open-packed, binary, planar patterns and even on topographically-patterned templates in 2D or 3D shapes. Generally speaking the acting forces in colloidal assemblies can be categorized into three major classes: attractive interparticle forces, repulsive interparticle forces, and external forces (Vogel, 2015). 2D close-packed colloidal monolayers have been synthesized via several direct assembly methods such as controlled evaporation (Micheletto, 1995), vertical and horizontal depositions (Goldenberg, 2002; Kumnorkaew, 2008), spin coating (Mihi, 2006),
sedimentation (Park, 1998), electrostatic deposition (X. Zhang, 2010), electrophoretic deposition (K.-Q. Zhang, 2004). Each of these techniques suffers from some drawbacks. For example, in controlled evaporation large area patterning is a serious problem due to meniscus pinning, or in sedimentation a complex design is needed to prevent multilayer formations. Non-close packed monolayers of colloidal structures of can be built via self assembly directly on oil/water interfaces (Law, 2013) and spin coating (Jiang, 2006). 2D Hierarchical structures of colloidal crystals have also been accomplished via assembly on curved surfaces (Vogel, 2011), on surfaces with charge contrast (Jonas, 2002) or wettability contrast (Gu, 2002), on micro-contact printed surfaces (X. Yan, 2004), via optical tweezers (Jonáš, 2008), and via pre-assembly at the air-water interface (Retsch, 2010).

3D colloidal structures have been made through various techniques, including sediment (Zhu, 1997), centrifuge (Wijnhoven, 1998), electrodeposition (Trau, 1996), microfluidic channels (Zeng, 2007), filtration (Velev, 1997), static vertical deposition (M. H. Kim, 2005), spin coating (Arcos, 2008) and Sessile drop (Huang, 2012). Also, 3D hierarchical structures made of colloidal crystals have been introduced via several approaches such as photolithography (H. S. Lee, 2013), microimprint lithography (Ding, 2014), surface functionalization (Fustin, 2003), printing (Wang, 2013).

2.6 Directed assembly for metamaterials

Metamaterials are artificial composite structures that exhibit fantastic materials properties. They are studied in several fields such as materials science, physics, chemistry and engineering. In principle, the concept of metamaterials could be applied to any wave at any scale. Since these materials or structures can produce amazing properties which cannot be seen in natural materials, they are called metamaterials and they have many
potential applications in construction of complex spatial or frequency domain devices and can be characterized for either 2D or 3D structures (Yongmin Liu, 2011; Turpin, 2014). These materials can be made as a composite or hybrid structure from a broad range of materials such as metals, semiconductors, graphene, carbon nanotubes, etc. (Zheludev, 2012).

For example, Alù and co-workers (Alù, 2006) have shown that with proper arraying of non-magnetic nanospheres in a loop configuration, it is possible to produce magnetic resonant behavior at optical frequencies; this could be useful in imaging and optics applications. Also, magnetic resonance properties were induced in a nanofabricated structure decorated with gold pillars (~ 100 nm) at the visible-light frequencies in (Grigorenko, 2005). A relatively new concept called “artificial plasmonic molecules” (Urzhumov, 2007) has been introduced via building a cluster of metallic nanoparticles in a tetrahedral configuration. This work proved that a colloidal solution of plasmonic tetrahedral nanoclusters can act as an optical medium in the visible or near infrared bands with strong magnetic and electric responses. In other research, it has been shown that a composite made of magnetodielectric spherical particles arranged in a 2D background dielectric matrix can reflect a negative index which is suitable for fabrication of metamaterials (Padilla, 2006). It is worth mentioning that different geometries of nanoparticles can be used for creating plasmonic effects such as are seen in (Ekinci, 2008), where ordered nanoparticle arrays of aluminum nanospheres, nanorods, and ellipsoidal particles were arranged on lithographically-processed quartz substrates and eventually exhibited strong and sharp plasmonic resonances around the ultraviolet range. Similar works have been completed with several other materials such as gallium nanoparticles (P.
C. Wu, 2009) and silver nanoparticles (Hilger, 2001; Oates, 2006). It is believed that one of the challenges of the future is to develop 3D isotropic metamaterials (Soukoulis, 2011). Metamaterials have been used in many terahertz applications such as sensing, super lensing, on chip optoelectronic and optoelectronic devices, energy harvesting and plasmonic structures (Walia, 2015).

As a summary, the properties of metamaterials are crucially dependent on the scale and architecture of their features in both 2D and 3D designs since the feature size and layout significantly affect the electromagnetic waves’ reflection. Different techniques and materials (polymers, metals, and ceramics) have been used to fabricate such devices but yet some issues are not fully addressed. One of the issues is to create mechanical flexible structures especially on polymeric materials. Another issue is the resolution of structures or features such as the arrangement of micro or nanoparticles or tiny objects on the device. Hence, it is highly relevant to make improvements in the mentioned areas. Since TASR has special capabilities in creating arbitrary arrangements of small components such as micro and nanoparticles, it seems that it would be a potential candidate to utilize this technique for fabrication of such structures. Hence, the outcome of this research could have a great potential for the fabrication of metamaterials and plasmonic structures.
Chapter 3

TEMPLATED ASSEMBLY BY SELECTIVE REMOVAL

Templated assembly by selective removal (TASR) is a powerful directed self-assembly technique in which components can be placed from fluid into pre-defined assembly sites on a substrate. TASR can effectively assemble a diverse set of components, in the range of nano to macro scale, into an arbitrary arrays of features. The arrays do not need to necessarily present periodic architectures, and the assembly process can work for components with an array of different shapes, as long as they have curvature in at least one direction. Using TASR, it is possible to precisely position objects of different kinds into a desired arrangement without requiring interlayer alignment (Eid, 2008; Jung, 2005). TASR has the potential to be useful for many applications such as tissue engineering (Kachouie, 2010), diagnostic devices (Sarioglu, 2015), biosensors (W. Ma, 2016), chemical sensors (J.-H. Lee, 2009), and electronics (Stebe, 2009).

The TASR technique works in part based on the size and shape match between assembly components and pre-defined features on the substrate. For example, consider the case in which the component is a sphere and the pre-defined assembly site is a hemispherical well in the substrate. Locally, they both have spherical curvature, and if their radii are sufficiently similar, they will be well-matched in shape and size. Generally and simply, if there is a good size and shape match between the component and the assembly site, assembly can happen at that location. If there is not good shape and size matching, then even if the component randomly visits that site, it will be ejected from that location (Jung, 2005).
An important question is how the components are retained in the assembly sites. When the components and the assembly template are in a fluid environment, the system’s free energy changes when a component contacts the surface of the assembly template. This change occurs because area is lost at the liquid-solid interface and is gained at the solid-solid interface. If contact between the components and the template is more energetically favorable than contact between the components and the liquid and/or between the template and the liquid, then assembly is energetically favored. The greater the degree of shape and size matching between the components and the template, the more energetically favorable the assembled configuration will be. These effects give rise to adhesive forces, which are discussed in greater detail below.

A second important question is how the components are ejected from the assembly sites when they are not well matched in shape or size. Even a poorly-matched assembly configuration will be somewhat energetically favorable if replacing the liquid-solid interface with a solid-solid interface lowers the system’s free energy. To overcome the small adhesive forces that arise in poorly-matched assembly, fluid forces are applied to the system. These forces arise from high frequency acoustic waves, which produce both primary oscillatory forces and steady secondary flows. The selective assembly in TASR is driven by a competition between adhesive effects and fluidic removal effects. The adhesion forces tend to hold the assembly components inside the assembly sites, and the removal forces act to take the component out of the site (Eid, 2008).

The assembly occurs in a small beaker located above a high frequency ultrasonic transducer; both the transducer and the small beaker are placed in a larger, water-filled outer beaker so that the water can transmit the excitation. The transducer creates acoustic
waves in the megahertz frequency range. The waves propagate through the water, to the
inner beaker, and through to the assembly template, arriving with normal incidence at the
assembly template (See Figure 3.1). The transducer runs for several minutes while the
assembly template and the components are immersed together in the assembly beaker so
that assembly can occur. During this time, the acoustic excitation both removes
components from mismatched sites and drives circulation that delivers components to
assembly sites in the first place.) At the end, the template is lifted up without turning off
the acoustic transducer, and the template is dried by a gentle stream of pressurized air
(Agarwal, 2016).

![Diagram of conventional experimental setup of TASR.](image)

**Figure 3.1.** Schematic of conventional experimental setup of TASR.

### 3.1 Component retention mechanism and parameters

In TASR, components are retained in place because of adhesion between the assembly
components (in this case polystyrene microspheres, as explained in further chapters) and
the substrate. The components and the assembly template (the substrate) can adhere when
the total free energy of the system decreases in the adhered state as compared with the case
in which the components and the substrate are separated from each other. The free energy
change is frequently described in terms of free energy difference per unit area and is known
as the interfacial energy. Adhesion occurs when contact between the components and the assembly sites is more energetically favorable than contact between the components/substrate and the assembly medium. In this research, adhesion is driven by the hydrophobic force between hydrophobic components and substrate in a water-based assembly medium. (Pure water is not used to prevent the hydrophobic force from being too strong and causing problems, as is discussed in subsection 3.1.1.)

In order to determine how strongly a component is retained in an assembly site, it is important to know how much contact area there is between the component and the assembly site. The contact area may be multiplied by the interfacial energy to determine the total reduction in free energy upon assembly of the component into the site. The contact area depends on two factors. The first is the nominal contact area, or the microscale area over which the component and the assembly site appear to be in contact when surface roughness is not considered. The second is the fraction of the nominal contact area over which the surfaces are actually in contact with each other, where “actually in contact” is defined as “separated by a shorter distance than the range of the hydrophobic force”. The fraction of the area over which the surfaces are actually in contact can be estimated using the actual surface roughness of the assembly sites. The product of the nominal contact area and this fraction is the actual contact area. The amount of water in the water-based assembly medium may be adjusted to ensure that the assembly is promoted by adequate adhesion given the roughness of the surfaces. In this research, polystyrene microspheres are chosen as the assembly components. As a result, the best shape for the assembly sites is hemispherical wells. Subsection 3.1.2 elaborates on how the nominal contact area may be calculated for this case and how it affects the retention force.
3.1.1 Surface properties and assembly medium

To facilitate the adhesion between the assembly components and the assembly sites, some parameters should be chosen correctly or optimized. Depending on the materials chosen for assembly substrate and components, the type of adhesion force can be selected. In this research, hydrophobic polystyrene microspheres are chosen as assembly components. Hydrophobic surfaces are not attracted to water (see Figure 3.2). The assembly substrate is silicon. Although silicon itself is also hydrophobic, its surface is covered with a very thin layer of naturally-grown oxide that makes it hydrophilic (i.e. it is attracted to water).

Hence, to utilize hydrophobic interaction forces as the adhesion agent between the components and the assembly sites, the surface of the assembly substrate needs to be treated in such a way that it becomes hydrophobic like the assembly components. Hydrophilic surfaces can be chemically treated to render their surfaces hydrophobic instead of hydrophilic. This chemical treatment is often done using self-assembled monolayers (SAMs), and it is called surface functionalization. In this research, trichlorosilane-based SAMs are used to functionalize the silicon wafer surface. The oxide surface is covered with OH^− groups and surrounded with water molecules before any treatment. After

![Figure 3.2. The schematic definition of a hydrophilic and hydrophobic surfaces](image-url)
functionalization of the surface, the trichlorosilane groups react with their oxide surface and replace their Cl\(^-\) terminations with OH\(^-\) groups. Then the OH\(^-\) groups condense to form water molecules, and the surface becomes hydrophobic due to formation of CH\(_3\) terminals (Ulman, 1996). These terminals are electrically neutral and cannot participate in hydrogen bonds. This means that if water molecules are close to the functionalized surface, the interfacial energy will be high. In this case, the water molecules start to arrange themselves with surrounding water molecules to maximize the number of hydrogen bonds, creating the hydrophobic effect (Eid, 2006).

The assembly medium also needs to be optimized to promote the appropriate degree of attraction between the assembly components and the substrate. Too strong of an interaction would prevent the acoustic excitation from removing mismatched components from the surface, but too weak of an interaction would prevent even correctly-matched components from being retained in the assembly sites. In addition, if the assembly medium were pure water, the assembly components (polystyrene microspheres) would make large drifts or extensive aggregates since they are hydrophobic and preferentially adhere to themselves rather than being in contact with the water. This excessive aggregation dramatically lowers the number of individual collisions of components with the assembly sites and makes it very unlikely for components to separately hit the assembly sites. However, the assembly medium should not be a pure solvent because in that case the medium does not provide enough change in free energy upon assembly to enable the selective assembly process. Solvents such as ethanol and acetone are polar, but they do not form hydrogen bonds with other ethanol or acetone molecules. However, they can participate in polar interactions with water molecules and form hydrogen bonds. Hence, one solution is to control the assembly
mixture composition to control the interfacial energy. The solvent-water mixture should be tuned in such a way that its interfacial energy is not as high as in the case of hydrogen bonds in pure water and not as low as in the case of Van der Waals interactions in a pure solvent.

The common method for determining the interfacial energy is via a contact angle measurement. The calculation of interfacial surface energy is done using Young’s equation (Israelachvili, 2011):

\[ \gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta \]  

(3.2)

where \( \gamma_{SV} \) is the solid-vapor interfacial surface energy, \( \gamma_{SL} \) is the solid-liquid interfacial surface energy, \( \gamma_{LV} \) is the liquid-vapor interfacial surface energy, and \( \theta \) is the wetting contact angle. These calculations have been done for water-ethanol mixtures in (Eid, 2006) and for water-acetone mixtures in (Jung, 2007). In this research, the assembly medium is a mixture of ethanol and water, and the corresponding interfacial energy value is extracted as described above in Chapter 5. The interfacial energy values of water, ethanol and energy of formation of solid were extracted from (Israelachvili, 2011) and the wetting contact angle of the mixture was experimentally measured as explained in Chapter 4.
3.1.2 Nominal contact area definition and roughness

The contact area between a microsphere and the assembly site directly affects the adhesive force between them. The nominal contact area is defined as total area over which a microsphere’s surface would be within a certain adhesive distance of the substrate’s surface if both the component and the substrate’s surface were ideally smooth. The adhesive distance is the one over which the attraction forces act. As mentioned, in this research the relevant attraction force arises from hydrophobic interactions. The adhesive distance for the hydrophobic forces is commonly reported as 1.5 nm (Jung, 2005). It is worth mentioning that this definition of nominal contact area makes the contact force behave like a step function, so that the force is modeled as being zero for separations over the hydrophobic adhesive distance and a constant value for separations below the adhesive distance. In reality these forces change in an exponential fashion, and this assumption considerably simplifies the assessment of nominal contact area. However, this approximation is sufficient for the present purposes.

The nominal contact area reflects the shape of the assembly wells, which in turn reflects the microfabrication processes by which the assembly wells are created. In the present research, the assembly sites are created by creating a tiny opening in a layer of photoresist on a flat substrate. The substrate is then etched isotropically (uniformly in all directions). As long as the opening in the resist is much smaller than the etch depth, this produces a nearly hemispherical well in the substrate. The details of this process are explained in Chapter 4. The deviations of the well from a perfectly hemispherical shape reflect the design (the relative sizes of the initial opening in the resist and the etch depth) as well as the fabrication process itself, including factors such as photoresist delamination, variation
in the local etch rate, etc. Hence, analytical evaluation of the contact area of a spherical component with a particular microfabricated well is relatively complex; a precise approach for this calculation is explained elsewhere (Eid, 2008; Jung, 2007). An alternative is to create a simplified model of the well’s shape and to use that as the basis for the analysis of the contact area.

Nominal contact area is defined as the contact area that would be observed if both the components and the assembly sites were perfectly smooth, so that there were a continuous contact between both parts up to the periphery of the contact area. However, these surfaces are not ideal, and their surface roughness reduces the actual contact area by creating locally larger separations between the surfaces. Surface roughness could stem from variations in the microfabrication process, the surface quality of the assembly components, and potential nonuniformities of the monolayer coating on the substrate. The discontinuous contact between assembly components and sites caused by surface roughness dramatically lowers the actual contact area compared to its nominal value. Mathematically, the effects of surface roughness can be taken into account by adjusting the contact area by a multiplicative factor called surface roughness coefficient. The analytical and numerical calculations for this coefficient were done in (Jung, 2005) and the relationship between these factors can be expressed as:

\[ A = C_r A_n \]  

(3.3)

where \( C_r \) is the surface roughness coefficient, \( A_n \) the nominal contact area, and \( A \) the actual contact area.
### 3.1.3 Retention force and moment approximation

The reduction in free energy when the surfaces of the component and the assembly site are in contact gives rise to a force that opposes any effort to lift the component from the assembly site as well as to a moment that opposes any effort to roll the component out of the assembly site. This force and this moment are called the retention force and the retention moment, respectively. Because the retention force and the retention moment increase with increasing contact area, these retention effects are responsible for keeping the component inside the assembly site when there is a good size and shape match between the component and the assembly site. In general, the system resists any change in contact area because lifting the component or rolling the component out of the assembly site would increase the contact with the energetically unfavorable water-based assembly medium. At the same time, these retention effects also enable the selectivity that allows components to be released from assembly sites in which their size and shape matching are poor. When size and shape matching are poor, the retention effects are smaller in magnitude and are less effective at retaining the component in the assembly site. This tradeoff can be described mathematically. The retention force that opposes direct liftoff of the component from the surface and the retention moment that opposes the rolling of the component out of its assembly site can be calculated as follows.

\[
F_{\text{ret}} = \frac{du}{dt} = -\gamma \frac{dA}{dt}
\]

(3.4)

\[
M_{\text{ret}} = \frac{du}{d\phi} = -\gamma \frac{dA}{d\phi}
\]

(3.5)

where \(F_{\text{ret}}\) is the retention force, \(M_{\text{ret}}\) is the retention moment, \(U\) is the interfacial surface energy of the assembly component, \(A\) is the real contact area between the microsphere and the assembly site (including the effects of surface roughness), \(l\) is the perpendicular
distance between the component and the surface of the assembly template, and $\Phi$ is the rotation angle of the component within the assembly site. The rotation angle equals to zero when the component surface touches the bottom of the well and it gets 90 degrees when it touches the flat surface of the assembly well. Assuming that the well is axisymmetric, the free energy varies only with the angle $\phi$ that the microsphere’s contact with the wall makes with the horizontal. As described above, the nominal contact area will for simplicity be approximated based on a mathematical model of a typical well shape. Although this approximation will provide a less accurate model of the shape of any given, as-fabricated assembly site, it makes up for that in its ability to predict the behavior of assembly sites across a range of size scales. The use of this approximation continues in Chapter 5, where the calculation of the retention forces and moments is described in detail.

3.2 Removal forces and moments approximation

If there were no forces or moments acting to oppose the retention forces and moments, then even the small retention forces and moments that act on a component in a poorly-matched site would be sufficient to prevent the component from exiting the site. The high frequency acoustic excitation creates fluid forces that oppose the retention forces and moments, enabling the selective removal process to take place. Whereas the retention forces and moments depend on whether the component occupies a well-matched site or a poorly-matched site, the removal forces and moments are largely independent of where the component is located. The balance between the two therefore depends on where the component is located, enabling the selective removal process to take place.

The removal forces and moments are created by the high frequency ultrasonic waves impinging on the components. Ultrasonic waves are sound waves with frequencies higher
than the upper audible limit of human hearing (Agarwal, 2012). The transducer produces sound waves at normal incidence to the assembly medium and template; their direction of travel is vertical. First, the waves propagate into the water inside the outer beaker. Then they hit the assembly beaker, pass through the assembly medium and the assembly template (the substrate), and finally impinge on the components.

This process creates various forces that act on the components. These forces can be taken to act at the component’s center of mass. In contrast, the retention forces can be taken to act at the center of the contact between the component and the assembly site. When there is a horizontal moment arm between the point of component-substrate contact and the center of mass of the component, the vertical forces that arise from the vertically-incident acoustic waves also create a mechanical moment that acts to roll the component out of the assembly site. This removal moment is opposed by the retention moment described above, and it is the tradeoff between these two effects that dictates the outcome of the assembly process.

The various fluid forces that act on the components fundamentally differ from each other and include both linear (time dependent) and non-linear (time independent) forces. Some of these forces act normal to the surface and produce mechanical moments because of the horizontal moment arm described above. Other forces act parallel to the surface and produce mechanical moments because of the vertical moment arm between the component-surface contact and the component’s center of mass. Table 3.1 summarizes the fluid forces that can contribute to component removal and their characteristics.

The fluid forces and their associated moments serve two main purposes. First, they circulate the assembly medium to ensure that there are enough collisions between the
components and the assembly template. Since the initial assembly process is quasi-random (that is, the selectivity primarily arises from the selective removal process), adequate collisions are critical to achieve the necessary initial assembly states so that selective removal may occur. Second, the fluid forces also act as removal agents to reject the components from their assembly sites when there is a poor size and shape match between the components and sites, as described above.

Table 3.1. Summary of acting removal forces in TASR

<table>
<thead>
<tr>
<th>Force</th>
<th>Type</th>
<th>Direction to the surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basset</td>
<td>Time dependent</td>
<td>Vertical</td>
</tr>
<tr>
<td>Added mass</td>
<td>Time dependent</td>
<td>Vertical</td>
</tr>
<tr>
<td>Viscous</td>
<td>Time dependent</td>
<td>Vertical</td>
</tr>
<tr>
<td>Radiation</td>
<td>Time independent</td>
<td>Vertical</td>
</tr>
<tr>
<td>Acoustic streaming</td>
<td>Time independent</td>
<td>Horizontal</td>
</tr>
</tbody>
</table>

Three of the most important fluid forces are the Basset force, the viscous force, and the added mass force. The relative magnitudes of these three forces varies with the size scale of the components because the forces depend on the size scale of their targets in fundamentally different ways. For the purposes of the present work in which the components are microspheres, the size scale is described solely by the radius of the sphere. All three of these forces are primary forces, which means they all act normal to the assembly template and are linear, with zero time average. These forces attempt to lift the components straight up off of their assembly sites in one half cycle of the acoustic wave and act so as to return the components to their sites in the other half cycle. In other words, the primary forces are time-dependent. The moments resulting from these primary forces
are also time-dependent; hence they are characterized by a magnitude and a phase. The magnitudes of the primary forces and moments acting on the microspheres are expressed as follows (Eid, 2006; Jung, 2007):

\[ F_{\text{added mass}} = \left( \frac{2}{3} \pi \rho R_m^3 \right) \left( 2\pi f \frac{I}{\sqrt{\rho c}} \right) \]  
(3.6)

\[ F_{\text{Basset}} = \sqrt{\frac{72\pi^3 \mu f}{c}} R_m^2 \]  
(3.7)

\[ F_{\text{viscous}} = 6\pi \mu R \frac{I}{\sqrt{\rho c}} \]  
(3.8)

\[ M_{\text{added mass}} = F_{\text{added mass}} R_m \sin \phi \]  
(3.9)

\[ M_{\text{Basset}} = F_{\text{Basset}} R_m \sin \phi \]  
(3.10)

\[ M_{\text{viscous}} = F_{\text{viscous}} R_m \sin \phi \]  
(3.11)

where \( M_{\text{added mass}}, M_{\text{Basset}}, \) and \( M_{\text{viscous}} \) are the moments resultant from the forces \( F_{\text{added mass}}, F_{\text{Basset}}, \) and \( F_{\text{viscous}}, \) respectively; \( R_m \) is the radius of microsphere; \( \mu \) is the viscosity of the medium; \( f \) is frequency; \( c \) is the velocity of sound in the assembly medium; \( \rho \) is the mass density of the assembly medium; \( I \) is the intensity of the incident acoustic waves; and \( \phi \) is the rolling angle of the microsphere inside the assembly site. The radius of the component and the angle \( \phi \) that the center of the component-substrate contact makes with the vertical (the rolling angle) together determine the horizontal moment arm of the vertical forces. The resultant magnitude of the primary moments taking into account their relative phases, \( M_{\text{Prim}} \), is then given by (Eid, 2008):

\[ M_{\text{Prim}} = \sqrt{(M_{\text{added mass}} + M_{\text{Basset}} \sin(45^\circ))^2 + (M_{\text{viscous}} + M_{\text{Basset}} \cos(45^\circ))^2} \]  
(3.12)

The non-linear fluid forces are the acoustic streaming force and the force that arises from radiation pressure. The acoustic streaming force acts parallel to the assembly template
surface (that is, horizontally). It originates from the loss of acoustic momentum caused by the attenuation of the sound waves in the viscous fluid medium. The radiation pressure force happens due to the scattering of the acoustic energy by the microspheres inside the assembly medium. This force has a nonzero time average, and it acts normal to the assembly template (that is, vertically). These forces and their resultants moments can be approximated as follows (Jung, 2005):

\[ F_{\text{acoustic, drag}} = 6 \lambda \mu R_m \bar{v} \]  

(3.13)

\[ F_{\text{rad}} = 64 \rho \left( \frac{2 \lambda f}{c} \right)^4 R_m U^2 \]  

(3.14)

\[ M_{\text{acoustic, drag}} = F_{\text{acoustic, drag}} \cdot R_m \cdot \cos \phi \]  

(3.15)

\[ M_{\text{rad}} = F_{\text{rad}} \cdot R_m \cdot \sin \phi \]  

(3.16)

where \( F_{\text{acoustic, drag}} \) and \( F_{\text{rad}} \) are the acoustic and radiation forces; \( M_{\text{acoustic, drag}} \) and \( M_{\text{rad}} \) are their resultant movements, respectively; and \( \bar{v} \) is the streaming velocity. Finally, the maximum value of the total removal moment is the summation of all primary and secondary moments expressed by (Eid, 2006):

\[ M_{\text{removal}} = M_{\text{acoustic drag}} + M_{\text{rad}} + M_{\text{prim}} \]  

(3.17)

Depending on the experimental conditions and the size of (Eid, 2006) the components, different moments can prove to dominate component removal. The assessment of the dominant forces and moments for the present experiments is described in Chapter 5.

As described above, the tradeoff between the retention moments and the removal moments determines whether a component will be retained in a given assembly site. The ratio of the removal moments to the retention moments can quantify this tradeoff and provide a base for predicting the assembly outcomes. If the ratio \( \frac{M_{\text{rem}}}{M_{\text{ret}}} \) for a component in
a given assembly site is larger than 1 by a sufficient margin, the component will not be retained in that assembly site and selective assembly will not happen at that location. If the ratio is less than 1 by a sufficient margin, then the component will be retained in that location and selective assembly will happen at that location. When the ratio is near 1, then the outcome is uncertain; the assembly system should be designed to avoid this eventuality. Generally, when there is a good size and shape match between the microsphere and assembly site, the ratio will be sufficiently below 1 to enable assembly. Similarly, in a well-designed system, the ratio will be sufficiently above 1 for components that sample mismatched (undesired) assembly sites.

3.3 Previous research on TASR

TASR was first introduced by Jung and Livermore (Jung, 2005). In that work, a proof of concept for size-selective self-assembly was proposed using an analytical model and then experimentally validated. It was demonstrated that the TASR technique can effectively enable selective assembly of rigid microspheres of functionalized silica near and somewhat below the 1 micron length scale. It was claimed that the method could potentially work for nanoscale components as well. In follow up research, Eid and his co-workers (Eid, 2008) extended the research, demonstrating simultaneous, selective directed assembly of nano- and micro components into the pre-defined sites on a template. They used functionalized silica spheres and did the assembly using an etch, functionalized silicon dioxide film as the template. Their results were in excellent agreement with the analytical model, suggesting that this approach can be used for real applications such as building functional devices or systems. Agarwal et al (AgarwalServi, 2011) augmented the analytical model to show that TASR can assemble deformable polymer structures onto
rigid assembly substrates. This model evaluates the onset of plastic deformation in systems comprising deformable spheres on a surface of given geometry. The assembly data on the deformable system resembles that from more rigid systems, indicating that deformability need not interfere with the potential for selective assembly, as long as the conditions are appropriately controlled. In another effort (Agarwal, 2016) to prove the versatility of TASR, deformable polymer microspheres were successfully assembled onto low-cost deformable polymer substrates. The relevant considerations for substrate deformability were also added to the analytical model. Again, it was found that the presence of substrate deformability need not prevent TASR-based selective assembly.

As a complementary work for the analytical model of TASR, the role of mean flows (steady-state, non-linear flows) in the TASR system was investigated for spherical assembly components (Jung, 2012). The effects of the mean flows and the shapes of the components and assembly sites on the initial assembly process (before selective removal) was assessed. It was predicted in (Agarwal, 2014) that the assembly should function for other components with uniform curvature (such as cylinders) as well as for objects with nonuniform curvature. In other research (Agarwal, 2016), TASR’s ability to achieve directed assembly of non-spherical components on rigid assembly substrates was been demonstrated. In (Agarwal, 2016), cylindrical microcomponents were successfully assembled into hemicylindrical wells onto silicon substrate with high local assembly yields in agreement with the theoretical model.

Finally, in 2011 it was shown that TASR is capable of sorting non-mammalian cells (SF9 cells) into designated assembly sites with both good size-selectivity and high assembly yield (Agarwal & Livermore, 2011). On the strength of their results, Agarwal and
Livermore claimed that TASR has the potential to structure systems for biological applications such as diagnostic devices for cell sorting or cell isolation or for tissue engineering purposes.
Chapter 4

EXPERIMENTAL PROCEDURE

This chapter describes the experimental procedures for the current research. The content is organized into two sections. The first section describes the microfabrication process steps for creating assembly templates and the experimental details for characterizing templated assembly by selective removal on angled substrates. The second section describes the details of the microfabrication process for assembly templates that are integrated into microfluidic devices and the experimental details for TASR-based directed assembly inside micro channels. The two sections are referred as “TASR on angled substrates” and “TASR inside micro channels”, respectively.

4.1 TASR on angled substrates

For all of the present experiments, an assembly template that contains suitable assembly sites is required. As was described previously, the assembly sites that are required to assemble small spherical objects are hemispherical (or nearly hemispherical) wells with radii that are similar to the radii of the assembly components. Because the components and assembly sites for the present experiments are on the scale of tens of microns, microfabrication methods are an appropriate means to create the necessary features.

Generally, the basic idea in microfabrication is to create features or structures of interest via a series of processes that are carried out on substrates (typically silicon wafers). Complex structures are created by carrying out each process in certain regions of the substrate and not in others. This is accomplished by first patterning a photosensitive “resist” or “photoresist” on the surface of the substrate. Light shines through a
photolithographic mask, which is a glass plate on which a pattern of chrome has been created. Where the light impinges on the resist, the resist is chemically changed. In the present research positive resist is used, which means that resist can be removed wherever it has been exposed to the light. This light-exposed resist is removed through a process known as "resist development". Once the pattern has been transferred from the mask into the photoresist, subsequent processes may take place. For example, the material in the exposed regions may be etched away using a dry (e.g. plasma) or wet etch process.

In general, these photolithographic processes create structures that are similar to 2D extruded shapes. A feature can have an arbitrary outline in the plane (within certain limits), but its height will be uniform. This poses a challenge for the fabrication of the hemispherical wells that serve as the assembly sites for the present research because they require more complex shapes in the out-of-plane direction. To create assembly wells with the required out-of-plane shape, the following process is used. Figure 4.1 briefly presents the schematic of microfabrication process in sequence. Further details are provided in the following subsections.

One or more masks are designed with CAD or technical drawing software and created by a mask vendor. For the case of microfabricating hemispherical wells for the assembly process, the mask contains an array of very small openings that permit light to pass through the mask at each location where an assembly well is to be located. These openings are as small as is possible while still allowing the light that passes through the mask to expose (pattern) the underlying photoresist on the wafer. For the present research, the openings are 2 µm across. Then photoresist is spin-coated onto the wafer and exposed through the mask. Once the photoresist has been patterned (exposed and developed), the wafer is entirely
covered by photoresist except for tiny openings in the photoresist at the places where the assembly wells are to be created.

Assembly wells are created in the locations where there are openings in the photoresist. The wells are created using an etch process. Etch processes typically either etch nearly straight down into the substrate (for an anisotropic etch) or etch in all directions equally (for an isotropic etch). An anisotropic etch would create a cylindrical hole in the wafer with a diameter that would be roughly equal to the diameter of the opening in the resist. In other words, it would not create a hemispherical well. However, an isotropic etch will etch in all directions approximately equally, so that the substrate is etched as far in the in-plane direction as it is in the out-of-plane direction. If the opening in the resist had a diameter of zero (but was still an opening), a perfectly isotropic etch would create a perfect hemispherical well. In practice, the opening will always have a diameter larger than zero, so that the isotropic etch approximates a hemispherical well when the etch depth is significantly larger than the diameter of the initial opening in the resist. The approximation is not perfect because the width of the initial opening in the photoresist results in a flattened region at the bottom of the well. In addition, any non-uniformity in etch rates will result in deviations from a perfectly rounded well shape. After the etch is complete, the photoresist is removed and an array of assembly wells is complete. To create a second set of wells on the same template, a similar process is repeated on the same wafer, but with small modifications to protect the first array of wells during the manufacture of the second array of wells.
Figure 4.1. Schematic of the microfabrication process for preparing templates with wells of two different sizes for TASSR experiments on angled substrates. The steps shown are as follows: 1) a bare silicon wafer after cleaning steps, 2) a thin layer of photoresist is spun onto the wafer surface, 3) the photoresist is exposed with UV light, developed with a developer and the wafer is isotopically dry-etched for creating the first set of wells, 4) a very thin layer of silicon dioxide is grown onto the wafer surface using thermal oxidation technique, 5) the photoresist is spun onto the silicon dioxide, exposed with UV light, developed by the developer, and then the silicon dioxide is wet-etched using buffer oxide etch (BOE) to create small openings in the oxide layer, 6) the wafer is isotopically dry-etched for creating the second set of wells (bigger ones), and 7) the photoresist and oxide layer are removed with acetone and BOE, respectively.

This section and its subsections (4.1.1 to 4.1.3) elaborate on the details of a) the microfabrication process for the assembly substrate, b) the experimental setup for the directed assembly, c) the execution of the assembly process, and d) the characterization of the assembly results after the assembly is complete.
4.1.1 Mask design for the angled substrate

The first need for the microfabrication process is to design a mask. The mask design was done using AutoCAD Software 2014. There are two different arrangements of arrays in the assembly templates. (Each template is a single die from a silicon wafer.) In one arrangement, there are eight arrays of features divided into two similar groups of four that are located in two opposite corners of the assembly template. In the second arrangement, all eight arrays are located near the center of the die (Figure 4.2 a and b). Arrays are labeled with numbers from 1 to 8 for simplification of the experiments and analysis. In each group of four arrays, one array targets components with a nominal diameter of 10 µm with pitch size of 55 µm; a second array targets components with a nominal diameter of 20 µm with pitch size of 55 µm; and two arrays target a mixture of 10 µm and 20 µm components with a pitch of 120 µm between identical components and 60 µm between different-sized wells. This mask is designed for 3” wafers, each of which contains a total of 44 assembly templates for a total of 352 arrays (Figure 4.2 c). The mask features from which the assembly sites are ultimately created are circular clear openings in a dark-field mask pattern with various diameters (1.5 µm, 2 µm, and 2.5 µm), depending on the particular design.
Figure 4.2. The mask layout designed with AutoCAD 14 Software showing a) the first arrangement in which four arrays occupy two opposing corners, b) the second arrangement in which eight arrays occupy the center of the die together, c) the 3” wafer size pattern, and d) a zoomed in view of the circular clear openings.

4.1.2 Assembly template microfabrication

As mentioned earlier in Section 3, the concept of TASR is to assemble the components of interest into the corresponding assembly sites that have similar shape and size. Hence, it is important to create the proper assembly sites for the target components. Since the assembly components used in this research are polystyrene microspheres with two different
nominal diameters of 10 µm and 20 µm, the proper shape for the assembly sites on the template is hemispherical wells with radii of approximately 5 µm and 10 µm, respectively. As was described above, one of the best methods to create these assembly sites is to use silicon-based microfabrication techniques. In addition to creating the required small features, microfabrication enables the creation of many features in parallel with high precision. Silicon wafers are therefore used as the substrate material. They are suitable for the TASR process, and the subsidiary processes for creating the desired features in silicon wafers are well-established. (However, the combination of these processes to produce the required structures was determined in this research.) The silicon wafers were purchased from University Wafer, Inc. The microfabrication process steps are performed as described below:

1. A three-inch silicon wafer is thoroughly cleaned with piranha (H₂SO₄:DI water, 2:1).
2. The wafer is rinsed and dried with pressurized air.
3. The wafer is heated on a hotplate for 5 minutes to get rid of possible moisture or water.
4. The wafer is spin-coated with HMDS adhesion promoter at 4000 rpm for 45 s.
5. The wafer is heated on the hotplate for 2 minutes and then cooled down to room temperature.
6. The wafer is spin-coated with photoresist AZ1813 at 4000 rpm for 45 s.
7. The wafer is baked on the hotplate for 1 minute at 115 °C.
8. The photoresist on the wafer is exposed with UV light using the Quintel 4000 Mask Aligner. The exposure lasts 1 minute and is carried out under vacuum mode contact. “Mask 1” is used, which contains the alignment marks and die numbers.
9. The photoresist is developed by immersing the wafer in MIF 726 Developer for 1 minute.

10. The wafer is rinsed in water for 5 minutes to remove any photoresist residues.

11. The wafer is inspected with an optical microscope to make sure that the photolithography process has produced the correct result.

12. The wafer is anisotropically etched for 1 minute in the Unaxis PlasmaTherm 790 tool, which can etch silicon.

13. The wafer is sonicated with acetone for 1 min and then sprayed with IPA to remove the photoresist. It is then dried with pressurized air.

14. The etched features are observed with an optical microscope to confirm that the etch process has produced the correct result.

15. The etch depth is measured via profilometry, using the Dektak 3030/3ST tool.

16. Steps 4 and 5 are repeated.

17. The wafer is spin-coated with Photoresist AZ1805 at 4000 rpm for 45 s.

18. Steps 7 to 11 are repeated except than the mask used is “Mask 2”. Mask 2 will define the openings in the resist that, when the silicon is subsequently etched, will define the first set of hemispherical assembly wells.

19. The whole wafer is isotopically dry-etched for 8 minutes using xenon difluoride (XeF₂) gas at a pressure of 3 Torr in the Xactix e1 machine, with a target depth of 5 μm

20. The wafer is sonicated with acetone for 1 min and then sprayed with IPA to remove the photoresist. It is then dried with pressurized air.
21. The etched features (hemispherical wells approximately 5 µm deep) are examined using optical microscope. The etch depth is estimated after measuring the diameter (the radius minus the opening window in photoresist) of wells.

22. If a second set of wells of a different size are to be created on the same wafer, the wafer is then cleaned with an RCA clean in preparation for deposition of a protective oxide layer.

23. A 500 nm-thick thermal silicon dioxide layer is grown on the wafer in the Bruce oxidation furnace.

24. The wafer is spin-coated with photoresist P4620 at 5000 rpm for 45 s.

25. The wafer is soft baked on the hotplate for 5 minutes at 100 °C.

26. The wafer is exposed with UV light for 30 s under vacuum mode contact through the mask called “Cr Mask”. This mask creates the pattern for the subsequent etch of the protective oxide layer.

27. The wafer is post baked on the hotplate for 2 minutes at 115 °C.

28. The wafer is developed by immersion in a 1:3 mixture of AZ400 and deionized water for 2 minutes.

29. The wafer is rinsed in water for 5 minutes to remove any photoresist residues.

30. The oxide layer is wet-etched using buffered oxide etch (BOE) with a ratio of 1:6 for 8 minutes to remove the exposed portions of the oxide layer.

31. The wafer is sonicated with acetone for 1 min and then sprayed with IPA to remove the photoresist. It is then dried with pressurized air.

32. Steps 4 to 11 are repeated.
33. The wafer is isotopically dry-etched for 17 minutes using xenon diflouride (XeF₂) gas with pressure of 3 Torr in Xactix e1 machine, with a target depth of 10 µm.

34. The etched features (hemispherical wells approximately 10 µm deep) are investigated using optical microscope. The etch depth is estimated after measuring the diameter (the radius minus the opening window in photoresist) of the wells.

35. The wafer is sonicated with acetone for 1 min and then sprayed with IPA to remove the photoresist, and then dried with pressurized air.

36. The wafer is wet-etched using BOE (1:6) for 8 min to fully remove the oxide layer.

37. At the end, the wafer gets cut using a dicing machine to separate the dies into individual assembly templates.

4.1.3 Chemical modification of the template surface

As discussed in Section 3.1, there are various types of driving forces that can be used in TASR to promote adhesion between the assembly components and the assembly sites. In this research, it is decided to utilize hydrophobic forces because the assembly components are hydrophobic and it is rational to use this force as the adhesion force. The selection of the assembly components and their preparation are presented at length in the next subsection in this chapter. This subsection will focus on the preparation of the template surface to ensure that it functions as a hydrophobic surface.

The surface of a silicon wafer is hydrophilic due to the presence of a thin layer of naturally-grown silicon dioxide. It must therefore be chemically modified to render it hydrophobic before it can be used as an assembly substrate. One approach to change the surface property of the silicon dioxide from hydrophilic to hydrophobic is to chemically treat the surface with self-assembled monolayers (SAMs). They form ordered molecular
assemblies on the targeted surface and eventually alter the apparent chemical properties and interfacial energy of those surfaces (Ulman, 1996). In the current work, trichlorosilane-based SAMs are used to modify the surface properties of the assembly template. The process involves a couple of chemical reactions, after which the surface is covered by a set of organic tails that are nonpolar without any ability to participate in hydrogen bonds. As a result, the interfacial energy between these organic tails and the solvent/water assembly medium is high, so that the template has a hydrophobic surface (Agarwal, 2016).

The chemical surface modification is carried out as described here. First, the contact angle of water on the Si wafer is measured using the Phoenix 150 Contact angle measurement system. A typical value is 83.1°. A mixture of 80 ml of toluene (Fisher Chemical, Lot#158663) as a solvent and 5 droplets of trichloro(octadecyl)silane or OTS (trichloro(octadecyl)silane, Aldrich, Lot# MKBT9625V) as a precursor are mixed in a capped glass bottle (L=10 cm × D=3 cm), and the mixture is allowed to sit for 40 minutes to mix it very well. The mixture should not sit for a longer time because that could adversely affect the final coating quality. Simultaneously, the assembly template is piranha cleaned for 30 minutes to remove any organic or greasy debris from the surface and then is well dried with pressurized air. It is important to ensure that the template is free of moisture or water before the chemical treatment because moisture would facilitate the polymerization of the SAM and would reduce the formation of the intended monolayer coating. The template is placed inside the toluene-OTS mixture in the same capped glass bottle and is sonicated in an ultrasonic bath (UL Transonics, 3510R-DTH Bransonic) for 30 minutes. The ultrasonic excitation prevents the coating mixture from locally coagulating at some regions on the template and increases the uniformity of the SAM coating. The
template is taken out and thoroughly cleaned by spraying it first with dichloromethane and then with acetone to ensure that none of the mixture remains on the surface. Finally, the contact angle on the template surface is measured. A typical value for contact angle after coating is $97.5^\circ$.

### 4.1.4 Assembly components selection and medium preparation

Assembly components are another required element to perform TASR-based assembly. As explained in Chapter 2’s discussion of the background and motivation, TASR possesses the potential to be implemented in different applications such as bioengineering and tissue engineering as well as in non-medical engineering systems such as sensors. Hence, cells can be a candidate as assembly components. However, cells are alive and need more care and attention in terms of contamination and effective life span. Hence, it is useful to choose an experimental material that is not only a good model for cells in terms of size and density but also stable and predictable at the micro and nano scales. Polystyrene microspheres are chosen because they have been shown to behave similarly both to biological cells and to nanoscale elements. The microspheres were purchased from Phosphorex Inc. (1020KR and 1010KB), with two different nominal diameter sizes of 10 µm (blue-dyed) and 20 µm (red-dyed), respectively. The microspheres are provided in 2.5% concentration aqueous suspension. The actual diameters of the microspheres are $20.150 \pm 1.668$ µm and $21.4 \pm 3.6$ µm for nominally 20 µm microspheres and $10.370 \pm 1.270$ µm for nominally 10 µm microspheres, respectively.

The assembly medium is a mixture of ethanol and 8% wt. deionized water. Pure water cannot be used as the base assembly medium since it causes extreme aggregation or drifting.
of the hydrophobic polystyrene microspheres, which would prevent the microspheres from colliding individually with the assembly template. One solution is to use a solvent to prevent this aggregation. Ethanol is a good candidate among solvents due to its being nontoxic and polar. By controlling the ratio of ethanol and water in the mixture, it is possible to tune the interfacial energy between the liquid and hydrophobic surfaces such that it is small enough to prevent drifting of microspheres and large enough to provide the driving force for individual assembly of microspheres into the assembly sites. Therefore, the ratio of ethanol and water mixture is selected according to the study done (AgarwalServi, 2011; Eid, 2006)

Since the assembly medium is ethanol-based with a low percentage of water, and because the microspheres were purchased in aqueous form, the excess water must be removed from the microsphere suspensions. The suspensions are poured into a Falcon 15 mL conical centrifuge tube (Fisher Scientific) and centrifuged in an Eppendorf Centrifuge 5702 for 5 minutes at a speed of 4000 rpm. The water is then separated from the microspheres using a pipette, leaving the microspheres settled at the bottom of the tube. Then a mixture of pure ethanol 99.5% (Sigma Aldrich, Ethyl alcohol 200 Proof) and 8% wt. deionized water is prepared in another Falcon tube. The mixture is added to the Falcon tube containing the microspheres such that the assembly concentration reaches approximately 15 mg/mL.

4.1.5 Assembly onto angled surfaces using TASR

TASR assembly is conventionally performed in a setup as follows. There is a large beaker called the “outer beaker” that contains water. A high frequency (1.7 MHz)
ultrasonic transducer is placed into the outer beaker at the bottom of it. The power from the transducers comes from a variable voltage AC supply. The lower part of a smaller beaker (called the “assembly beaker”) is immersed in the water inside the outer beaker so that the smaller beaker is located above the ultrasonic transducer. This assembly beaker holds the template and assembly medium (with the components) during the experiment. The ultrasonic transducer plays two important roles. First, it transforms the drive voltage into acoustic waves in the MHz frequency range, with an amplitude that depends on the driving voltage. These waves propagate through the water inside the outer beaker and impinge on the bottom of the assembly beaker. The transmission of the ultrasonic waves into the assembly beaker produces fluidic forces in the assembly medium as discussed in Chapter 3. It also circulates the assembly components (microspheres) inside the assembly medium and increases the rate of collision between the microspheres and the assembly sites. The assembly is performed as follows. When the voltage supply is turned on at a given voltage, the transducer’s acoustic waves propagate first into the outer beaker and then into the inner beaker. The resulting fluid motion drives the microcomponents to move in the fluid, sometimes impacting the surface of the template. When the components reach an assembly site, the previously-described physics of TASR determines whether the component is ejected or remains assembled in that location. The process described here is the baseline TASR process. However, considering the research objectives, in the current research the assembly was done with some modifications as described below.

One objective of this research is to study how the TASR process takes place on angled surfaces such as may be found in a folding system or on complex surfaces that are not entirely flat and/or horizontal. In contrast to the previous research in which the assembly
template was always placed horizontally in the bottom of assembly beaker, in this work, it is needed to rotate the template to a wide range of angles. Hence, a sample holder or template stand is designed in SolidWorks software to be able to hold the template at certain angles. The template stand is built using a 3D printer (MakerBot Replicator 2) to hold the template at different angles of 0, 15, 30, 45, 60 and 75 degrees.

Another difference between this research and previous work is the components’ size. As described in previous sections, polystyrene microspheres of 10 and 20 µm are selected as model components for this research since the most cells are in this size range. However, in previous work the components are mostly sized between the submicron scale and the few micron scale (Eid, 2008; Jung, 2005). Large components with densities greater than the density of the assembly fluid sink faster in the fluid under the influence of gravity than the smaller spheres do. Therefore, as the components get larger (e.g. 20 µm microspheres), gravity starts to dominate and the megasonic transducer cannot provide adequate circulation. To approximately calculate the settling time of a microsphere in the assembly fluid, the Stocks drag equation (Panton, 2006) can be used. The simplified equation is given by:

$$
\bar{v}_T = \frac{\Delta x}{\Delta t} = \frac{-m.g}{6\pi.\eta.r} = \frac{-V(\rho_p-\rho_l).g}{6\pi.\eta.r} = \frac{2r^2(\rho_p-\rho_l).g}{9.\eta}
$$

(4.1)

where $m$ is the mass of the particle, $g$ is the acceleration of gravity, $\eta$ is the viscosity of the fluid, $r$ is the radius of the particle, $v_T$ is speed the particle, $V$ is the volume of the particle, and $\rho_p$ and $\rho_l$ are the mass density of the particle and liquid, respectively. Making the assumption that all microspheres start at the assembly medium-air interface with zero initial velocity at the beginning of the experiment, the calculations show that the
approximate settling time for 20 µm polystyrene microspheres can be two orders of magnitude shorter than that for 2 µm polystyrene microspheres. This rapid settling requires more energetic component circulation to combat its effects. Hence, large microspheres such as 20 µm ones need a boost for circulation. Otherwise, the number of collisions between microspheres and the template would dramatically drop.

Figure 4.3. Schematic diagrams of the TASR setup on the angled substrate; a) the template holder and the cross section view of the template, b) the whole experimental setup.

Therefore, a magnetic stirrer is used as an extra source of circulation. In addition to controlling the angle of the template, the sample holder also holds the template in an elevated position. The space beneath the elevated template is occupied by a magnetic stirring bar. During assembly, a magnetic stirrer (HANNA Instruments, HI 310 Autospeed), rotates the magnetic stirring bar for better circulation of the polystyrene microspheres. Figure 4.3 shows a schematic of the assembly setup and the CAD design of the sample holder.
Before an assembly experiment is carried out, the assembly medium is sonicated in the Branson 3510-DTH Ultrasonic Cleaner for 15 minutes to separate any polystyrene microsphere drifts or colonies. Then, the assembly medium is mixed for 5 minutes with the Vortex Genie 2 Lab Mixer. The template is cleaned with IPA and ethanol before assembly and placed into the stand inside the assembly beaker. The assembly beaker is placed 2 ± 0.1 cm into the water inside the outer beaker, 1 ± 0.1 cm above the ultrasonic transducer (Advanced Sonics, MMDIT-1.7 MHz). A total volume of 6 ml of assembly medium is poured inside the assembly beaker, the voltage supply with an output of 0-130 V (Power Stat, L10C, 60 Hz) is turned on and the experiment runs for 4 minutes without interruption. After the designated assembly time, the assembly template is removed from the assembly beaker by lifting up its sample holder, and then the power to the transducer is turned off. The sample is dried using a very gentle air stream and removed from the sample holder to be imaged under an optical microscope. The TASR experiments are completed at the voltage range of 20-70 V with an interval of 5 V at template angles of 0, 15, 30, 45, 60 and 75 degrees. Figure 4.4 shows the experimental setup and all equipment used for this set of experiments.
4.2 TASR inside microchannels

This subsection describes how assembly is performed when both the assembly sites and the components are located inside microfluidic channels. The mask design,
microfabrication process, microfluidic device prototype, and TASR experiments are all described in detail in the next subsections.

4.2.1 Microfluidic device design

The research objective is to understand whether TASR can perform inside tiny spaces while preserving its characteristics such as selectivity. This study case is very important since in many bioengineering, tissue engineering and even non-bio applications, microchannels (i.e. small-volume spaces) are used to control the behavior of fluids and small-scale objects within those systems. TASR will be most useful if its capabilities may be combined with the existing capabilities of microfluidic systems. Capturing cancer cells (Millner, 2013) controlling the arrangement of cells or microtissues for building tissue scaffolds (Kachouie, 2010) or even chemical sensors (S. Lee, 2016) are a few examples of aforementioned applications. Hence, the idea behind this part of the research is to implement and perform TASR-based assembly inside microchannels and investigate how different aspects of the microchannels’ design such as their shape, the ratio of their width to their, their cross sectional area, the concentration of components in the assembly medium, and the positioning of the assembly sites inside the microchannels affect the assembly results.

The microfluidic devices are designed with two different types of shapes (serpentine and spiral), and all of the microchannels have rectangular cross sections. Also, the dimensions (width and height) of microchannels are designed in such a way that they span a broad range from a few hundred microns to the millimeter scale.
The microchannels must include assembly sites that are contained within microchannels. The assembly sites and the microchannels are fabricated on two different substrates, and they are bonded together to build the final microfluidic device. However, it is not practical to build the devices out of silicon wafers because the devices must have large out-of-plane dimensions and in some cases high aspect ratios. In addition, the fabrication of silicon wafers is time consuming, and it is not easy to re-use the wafer for many experiments if silicon bonding technologies are used. It is more efficient and practical to make master patterns (which may be silicon-based) and to replicate them using polymer molding technology. This benefits the process in the sense that disposable devices can be built from bonding two different polymer replicas. The devices can be nominally identical to ensure repeatability. A given master pattern may be used many times, and the replicas can be disposed of after one experimental run.

A silicon-based organic polymer called polydimethylsiloxane (PDMS) is used for these experiments. PDMS is the most common type of polymer for replica molding of microfluidic devices, and it has been utilized in many applications such as medical devices and micro analysis systems (McDonald, 2000). The microfluidic device building process starts with the microfabrication of assembly wells on one silicon wafer and the fabrication of master patterns for microchannels on a second silicon wafer as master mold or template. It continues with a series of PDMS molding steps that create replicas of the silicon-based master molds. Finally, the PDMS replicas of assembly sites and microchannels are bonded together under an optical microscope to build the final sealed microfluidic devices.

The assembly site master patterns are fabricated into silicon wafers in a way that is very similar to how they are created for the previously-described experiments that demonstrated
TASR on angled templates. The primary difference lies in the size of assembly sites. Namely, there is only one assembly site size, and it is nominally 10 µm in diameter. Because there is only one size of assembly well on the substrate, the microfabrication process described previously terminates after step 21.

The master patterns from which microchannels are molded could be conventionally made out of a high contrast, epoxy-based negative photoresist called SU-8. SU-8 is useful when a thick, chemically and thermally stable structure is desired. However, in the current work, since the channels have heights of up to 1 mm, the microfabrication process can be challenging and susceptible to errors if the master mold is made of conventional SU-8. Instead, this research utilizes a state-of-the-art, dry-film laminated form of SU-8 that is sold under the commercial name of SUEX Epoxy Thick Film Sheets (TDFS) from DJ Devcorp. SUEX TDFS sheets are thick epoxy sheets capable of being photo imaged for wafer level packaging and MEMS applications. This product is prepared and applied to the substrate using a highly controlled solvent-less process, which provides uniform coatings. It also shows high compatibility with and adhesion to many materials, including silicon wafers. Hence, SUEX TDFS sheets are utilized to fabricate the master molds for microchannels of several heights in this part of the research. Figure 4.5 briefly depicts the schematics of the microfluidic device fabrication process. In the further subsections, more details of device fabrication is presented.
4.2.2 Mask design for microchannels

The microchannel devices include two sets of features, each of which is fabricated on a separate substrate before they are bonded together to form the complete device. In the lower half of the device, hemispherical assembly wells are created; this process is conceptually similar to what is described above for the fabrication of templates for the angled assembly experiments, although it varies in some details. In the upper half of the device, microchannels are created. The upper and lower halves are then bonded together.
The mask designs for this set of experiments are therefore bifurcated, with masks that define microchannels and masks that define assembly sites. One mask defines the layout of the microchannel master mold; this one is named “Microchannel Mask”. The other mask, called “Assembly Sites Mask” includes the features needed for fabrication of the assembly site master mold. The assembly sites must be located within the microchannels, so they are designed together. In some designs, the assembly sites and microchannels are arranged in a back-and-forth, serpentine layout. In other designs, the assembly sites and microchannels are arranged in a spiral layout. Different designs also have different microchannel widths, so that the pitch of the columnar and spiral features also varies. Figure 4.6 depicts the CAD layouts for the different microchannel designs.

4.2.3 Microfabrication for assembly sites and microchannels

The master pattern for the assembly sites is fabricated on 4-inch silicon wafers using a procedure that is similar to that explained in Sec 3.1.2. In Sec. 3.1.2, the purpose of the microfabrication process is to create arrays of assembly wells that can include two different sizes of wells. The microchannel assembly experiments only require wells of a single size (nominally 10 µm in diameter), so their fabrication process is shorter than the one that is described previously for the angled assembly experiments. For the case of assembly into microchannels, the microfabrication process by which the assembly sites are created is the same as the previous process up through step 21 except than in the last step, which is a dry-etch, just half of the wafer is etched. Then the process ends, yielding assembly sites that are all nominally 10 µm in diameter.
Figure 4.6. a) The CAD layout of microfluidic devices of serpentine and spiral geometries, b) top view of a serpentine microfluidic device after alignment, c) side view of the device before aligning of both parts.
The microchannels are created by molding polydimethylsiloxane (PDMS) over a master pattern that is the inverse of the desired microchannel shape. To build the master pattern for the microchannels, the process steps are as follows:

1. The four-inch silicon wafer is thoroughly cleaned with piranha (H₂SO₄:DI water, 2:1).

2. The wafer is dried with pressurized air.

3. The wafer is heated on a hotplate for 5 minutes to remove possible moisture or water.

4. The wafer is pre-baked at 85 °C for 10 minutes and cooled down for 2 minutes.

5. The hot roll laminator machine (Sky Machine, Model: 335R6) is set at 40 °C and speed of 1 mm/s.

6. The SUEX sheet with different thicknesses of 100, 200, 300, 500 and 1000 µm is put on top of the wafer and rolled into the laminator machine, with one SUEX thickness per wafer.

7. Using the Microchannel Mask, the SUEX sheet is exposed with UV-light according to the Table 4.1 depending on the SUEX sheet thickness (the thinner the sheet, the shorter is the exposure time).

<table>
<thead>
<tr>
<th>SUEX Sheet Thickness (µm)</th>
<th>UV-Light exposure time (second)</th>
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<td>200</td>
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<td>500</td>
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8. The wafer is post-baked at 85 °C for a given duration that is based on the SUEX sheet thickness (Table 4.2). Generally, the thinner the sheet, the shorter the baking time.
Table 4.2. The SUEX sheet post bake time for different thicknesses

<table>
<thead>
<tr>
<th>SUEX Sheet Thickness (µm)</th>
<th>Post-bake time (minutes)</th>
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<tbody>
<tr>
<td>100</td>
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<td>200</td>
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<td>1000</td>
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9. The SUEX is developed in MicroChem SU-8 Developer (PGMEA) for different time periods depending on the sheet thickness (the thinner the sheet, the shorter the developing time) as described in Table 4.3. It is also recommended that the wafer be frequently shaken while developing because this action accelerates the residue removal process.

Table 4.3. The SUEX sheet developing time for different thicknesses

<table>
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<tr>
<th>SUEX Sheet Thickness (µm)</th>
<th>Developing time (minutes)</th>
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4.2.4 Microfluidic device prototype

To build the microfluidic devices for experiments on selective assembly within microchannels, the topography of the microchannel master pattern and the topography of the assembly sites are replicated in a polymeric material (PDMS). The polymer copies are then bonded together to form the complete device. Prior to making any PDMS copies of the microchannels or of the assembly sites, the surfaces of the master patterns are chemically treated to facilitate the release process. For chemical treatment, the wafers are
placed upside down on a crystallization dish (Kimble) approximately 5 cm above the bottom of it. A disposable plastic weighing dish is placed inside the beaker below the wafer. Then 60 µL of Trichloro(1H,1H,2H,2H-perfluorooctyl)silane purchased from Sigma-Aldrich is poured into the dish. Trichlorosilane chemical groups are used as precursor for surface treatment to render hydrophobic properties into the exposed surface. The whole setup is placed inside the vacuum oven with a pressure less than 20 mTorr for roughly 2 hours at room temperature. Then, the pressure is increased to atmospheric pressure, the vacuum oven is turned on and set at 60 °C, and the wafers are baked overnight.

After surface treatment, the PDMS (SYLGARD® 184, Dow Corning Corporation) is prepared from its two components, the elastomer and the curing agent, with a ratio of 10:1. The mixture is degassed in a desiccator for roughly 30 minutes and then poured on top of both master patterns. The PDMS-coated master patterns are placed in an oven at 90°C for about 45 minutes and then are allowed to cool down. Following the cool down, the PDMS replicas are released by peeling them off the master pattern. The microchannel replica that is fabricated in this manner can be directly used as the upper half of the microfluidic device because a single replication step converts the inverse master pattern into the correct channel structure. However, the assembly template master pattern contains assembly wells that must be replicated to again form wells. As a result, two replications are required, the first to produce an inverse pattern and the second to create assembly wells. This is accomplished by molding a PDMS copy of the initial master pattern for the assembly template and then molding a second PDMS copy of the first copy. To make the second replica, the same surface treatment and PDMS molding procedure are repeated.
The microfluidic devices require inlet and outlet ports that are not defined in the original microfabrication process. The inlet and outlet are instead created using a 0.5 mm biopsy punch. The inlet and outlet are punched in the microchannel piece (the upper half of the device). The PDMS microchannel replica must then be assembled onto the PDMS assembly site replica. To do this, both replicas are plasma treated under 300 mTorr of oxygen for 2 minutes using a plasma cleaner manufactured by Harrick Plasma. Using a stereomicroscope (Zeiss, Discovery V20), the assembly site replica is placed under the microscope light face up such that the assembly sites are visible under the microscope objective. After that, the microchannel replica is carefully aligned onto the other replica to assure that the assembly sites are placed along the center line of the microchannels. It is worth mentioning that manual alignment of these two replicas is challenging since not only are the assembly sites very small (10µm), but also the focal planes of the two replicas are different. Therefore, one optional approach to improve this alignment is to use some solvents as a surfactant to protect the active surface of PDMS replicas. This will extend the lifetime of the PDMS active surface under the microscope. This process can be done by adding a droplet of ethanol or methanol onto the assembly site replica surface immediately after plasma treatment (Jo, 2000). The bonding process is completed by heating the device (that is, by heating two replicas aligned on top of each other) on a hotplate at 200 °C for 90 minutes. After cooling down the microfluidic device, the device is bonded to a piece of microscope glass using PDMS to protect it from tearing apart due to the acoustic waves that hit the bottom of the device while doing the experiment. The glass was added because the initial experiments showed that if the device is placed into the water over the ultrasonic transducer, it tears apart from the bottom due to the high energy acoustic waves. Figure 4.7
shows the actual PDMS replicas and microfluidic device prototypes before the TASR experiments.

4.2.5 TASR inside microfluidic devices

The TASR experiments are completed for different dimensions (widths and heights) for both serpentine and spiral shapes. The assembly medium has the same chemical composition, ethanol and 8% Wt. deionized water, as was used for angled experiments except that the concentration of the components is 9 mg/mL. The medium is shaken for 2 minutes before each experiment using the Vortex Genie 2 Lab Mixer. After pipetting enough of the medium inside the microchannels, the inlet and outlet are secured using PEEK tubes with inner and outer diameters of 0.76 and 1.58 mm, respectively, purchased from McMaster-Carr Company. The microfluidic device is positioned 1 cm above the ultrasound transducers in the outer beaker using a sample holder. The TASR experiment is run with the same setup used for angled assembly experiments at 45 V for 4 minutes, except that no magnetic stirring is possible within the enclosed channels. After that, the device is removed from the outer beaker without turning the voltage off until after the device has been removed. Then the device parts are separated from each other for microscopy observation and calculation of the assembly yield. The experimental setup is shown in Figure 4.8.
Figure 4.7 Photographs of a) SUEx layer containing the inverse of the microchannel pattern, b) the PDMS replica of the microchannels, c) the first PDMS replica containing the inverse of the assembly wells (bumps), d) the second PDMS replica containing assembly wells peeled off the first replica, and e) the serpentine and f) the spiral microfluidic devices bonded to the microscope glass with inlet and outlet tubes containing the assembly medium.
Figure 4.8. The top view of the actual experimental setup of TASR for microfluidic devices. There is a big beaker filled with water and the device is placed on top of transducer at a constant distance using a sample holder.
Chapter 5

TASR-BASED ASSEMBLY IN NON-HORIZONTAL SYSTEMS

As was explained in previous chapters, the TASR technique is chosen for this study because it offers a high degree of assembly selectivity at the length scales of interest, ensuring that different types or sizes of cells or microcomponents may be assembled into pre-determined locations on a surface. When TASR-based assembly is carried out on flat, horizontal substrates, the competition between the retention and removal moments acting on the microcomponents drives the assembly’s selectivity. These moments have various origins and can be tailored by changing the experimental variables or the materials selection.

TASR can be used to dictate component adjacencies in 2D on a flat, horizontal surface (Eid, 2006; Jung, 2012). However, to structure microscale features in out-of-plane, curved, or layered architectures, TASR must be extended to permit assembly on surfaces that are not uniformly oriented perpendicular to the waves’ incidence. This is particularly important for tissue engineering, in which cells and/or microtissues must be positioned appropriately to ensure that the final engineered tissue has the right structure and demonstrates the desired biological responses. Although essentially 2D tissues such as skin and bladder do exist and often require simpler cell organizations than fully-3D tissues such as the liver do, many of the most critical challenges for tissue engineering lie in recapitulating the structure and function of fully 3D tissues. Although it is possible to create 3D structures directly through techniques like 3D printing or bioprinting, creating them through hierarchical manufacturing (e.g. assembly followed by folding) offers a potentially more parallel, scalable approach. However, integrating TASR-based assembly into these types of
hierarchical architectures will require the ability to carry out the assembly deterministically on complex, non-horizontal surfaces.

Hence, the present chapter focuses on analytical modeling and experimental characterization to predict and validate the limits of TASR onto angled surfaces such as are found in curved or folded system architectures (Mehner, 2015). The target is to demonstrate the effects of the substrate’s angle (both with respect to the horizontal and with respect to the angle of incidence of the acoustic excitations) on the effectiveness of the directed assembly process. By capturing the effects of angle variation on the effectiveness of the assembly process, design rules may be created that describe the angular range over which the folding elements may be tilted without undue reduction of the assembly’s selectivity and yield. This phase of the research should answer two questions. The first is whether TASR can successfully drive the assembly of components on a template tilted to a given angle with respect to the horizontal. The second is whether TASR faces any limitations in its characteristics (i.e. its ability to drive correct assembly and its ability to prevent incorrect assembly) while tilting the template to different angles.

Nominally monodisperse polystyrene microspheres are used in the experiments as accurate, stable models for the aforementioned object of interests (C. Liu, 2010; Yuanfang Liu, 2005; Yap, 2007). At the end of this chapter, the model predictions and experimental findings are compared, and the outcome reveals the important aspects of TASR on angled surfaces and the potential design rules for implementing TASR in non-horizontal systems. The size of the model components was chosen to resemble the typical sizes of most biological cells and microtissues, as well as the building blocks for many metamaterials.
5.1 Modeling TASR-based directed assembly onto angled surfaces

An analytical model is created to describe the functioning of the assembly process when the acoustic excitation impinges on the assembly surface at a non-perpendicular angle. The model describes the surface adhesion and fluid forces, predicting the magnitudes of the moments that drive component adhesion and component removal. The ratio of the retention and removal moments predicts trends in the assembly yield for well-matched components and predicts trends in the system’s success at preventing assembly of poorly-matched components. The following discussion presents how to estimate or calculate the retention and removal moments for TASR-based assembly on angled surfaces considering the governing and conventional equations described in Chapter 3.

To calculate the retention moment resulting from surface adhesion forces, Equation 3.3 is implemented into Equation 3.5, and the retention moment is expressed as:

\[ M_{ret} = -\gamma C_r \frac{dA_n}{d\Phi} \]  \hspace{1cm} (5.1)

The surface roughness coefficient can be calculated according to (Jung, 2005):

\[ C_r = 1 - \int_{-\infty}^{0.5(h_s+h_h)-d_c-z}/\sigma_{eq} \frac{1}{\sqrt{2\pi}} e^{-w^2/2} dw \]  \hspace{1cm} (5.2)

where \( h_s \) and \( h_h \) are the maximum peak heights of the microsphere and well surface, \( \sigma_{eq} \) is the equivalent root mean square roughness of both surfaces, \( z \) is the separation distance between the microsphere and the surface, and \( d_c \) is the hydrophobic characteristic length.

The surface roughness values of the assembly well and polystyrene microsphere were measured using AFM machine (Parks Scientific, Model XE7). The root mean square roughness and maximum peak height, were measured as 4.99 and 18.14 nm for assembly
wells and 17.10 and 67.05 nm for polystyrene microspheres, respectively. Finally, the coefficient was calculated as 0.01 for the current combination of wells and microspheres.

As explained in Chapter 3, the analytical estimation of the contact area is very complicated due to deviation of the well from a perfectly hemispherical shape, hence, a simplified model of the well’s shape would be the best choice for the current analysis. Since the component is a microsphere of (positive) radius $R_m$, and the well is a larger hemisphere of (negative) radius $R_w$, the radius $R$ of their nominal contact area $A_n$ is given by $R = \sqrt{2\frac{R_{eff}}{}}$, where the effective radius of curvature between the microsphere and the hemispherical well is given by

$$\frac{1}{R_{eff}} = \frac{1}{R_m} + \frac{1}{R_w}.$$  \hfill (5.3)

Microfabricated wells exhibit a radius of curvature that is larger near the top of the well than it is deeper in the well (see Figure 5.1 and also refer to the discussion in Section 3.1.2). For simplicity, the radius of curvature of the well is approximated here as

$$R_w = R_o (1 + \alpha \Phi),$$  \hfill (5.4)

where $R_o$ is the etched depth at the center of the microfabricated well and $\alpha$ is a coefficient that quantifies the change of the radius of curvature with angle. In the current work, $\alpha$ is estimated from scanning electron microscope (SEM) images as being 0.05 for approximately 10 µm diameter wells and 0.09 for approximately 20 µm diameter wells. By implementing Equation 5.4 into the radius of nominal contact area’s term, $A_n$ can be estimated and the retention moment is then approximated as
The next step is to estimate the removal moment. There are different removal forces acting on the microsphere inside the well (see Section 3.2), however, not all removal forces are dominant in the current experimental setup. Considering the length scale of interest, which is the microsphere sizes (10 µm to 20 µm), it is found that the added mass and Basset forces are the dominant ones in this case. With all else held constant, the added mass and Basset forces increase as the third and second power of the microsphere’s radius, respectively. Hence, the total removal moment presented in Equation 3.17 should be equal to just the resultant of the primary moments from both mentioned forces. The magnitudes of these forces are given by Equations 3.6 and 3.7. However, the resultant moments of these forces cannot be directly calculated from Equations 3.9 and 3.10 since in the current experimental setup, the template is supposed to be tilted to different angles and this makes a significant difference in the magnitude of the moments as described below.
Figure 5.2. SEM images of cross section of assembly wells with nominal size of, a) 20 and b) 10µm in diameter which shows the slight variations of radius at the surface and bottom of the wells.

Figure 5.3 shows schematics of templates at two different angles. First, the template is flat (parallel to the horizontal) and the acoustic waves are perpendicularly hitting the template (Figure 5.3a). The template tilting angle $\theta$ is measured with respect to horizontal and it equals to zero in this case. This configuration of the experiment is exactly the same as in the previous works on TASR, and the moment equations presented in Chapter 3 are derived for this case. However, the story is different for the second case in which the
template is tilted at an angle with respect to the horizontal (Figure 5.3b). In this case, the template is tilted to a given angle but the acoustic waves are still coming in the same direction. This causes the lever arm between the acting force, which passes through the center mass of the microsphere, and the point of contact of microsphere inside the well to get smaller. To reflect this change in the moment equation, term \( \sin \Phi \) needs to be replaced by term \( \sin(\Phi - \theta) \). Then the total removal moment can be expressed as:

\[
M_{\text{rem}} = F_t R_m \sin|\Phi - \theta|
\]

where \( F_t \) is the magnitude of total removal force which can be calculated from Equation (5.7) since the added mass and Basset forces are commonly considered to be out of phase by 45 degrees (Eid, 2008).

\[
F_t = \sqrt{(F_{am} + F_B \sin 45^\circ)^2 + (F_B \sin 45^\circ)^2}
\]

This change in the sine’s argument reflects the fact that when the template’s tilting angle gets bigger, the lever arm gets smaller. This would significantly change the removal moment magnitude compared to what was calculated in previous work on TASR. The most extreme case of this difference arises when the tilting angle of the template \( \theta \) is equal to the angle \( \Phi \) of the microsphere’s contact point within the well. In this case, the removal moment will go to zero, rendering the selective assembly process entirely ineffective. Another important point is that the sine’s argument is an absolute value. This means that if the template’s tilting angle passes the attachment angle of microsphere, the magnitude of the removal moment would not change but the rolling directing of the microsphere inside the well would be reversed. This can be interpreted as meaning that the microsphere would
roll to the other side of the well and be ejected from the other corner if ejection were supposed to happen.

Figure 5.3. (a) A mismatched microsphere inside the assembly well when the template is a) horizontal, and b) tilted at an angle. Note that $\theta$ is the template tilting angle, $\phi$ is the contact point angle of the microsphere inside the well, the green arrow is the lever arm and $F_{rem}$ is the fluid removal force.

It is worth mentioning that the contact point of the microsphere inside the well is assumed to be independent of the tilting angle of the template. The contact point angle of the component can be estimated using numerical calculation using surface roughness and the larger-scale profile of the well as was done in (Eid, 2008; Jung, 2007). However, since the well’s size in this research was so large that AFM machine could not measure or image the whole surface profile inside the well, it would be challenging to exactly predict the contact point angle of the microsphere in the well. However, the contact angle may still be estimated. In this research, the well’s curvature was slightly bigger than the microspheres’
curvature. (In other words, the substrate was etched slightly smaller than the radius of the microspheres). The curvature is therefore expected to be best-matched partway up the wall of the assembly well rather than at the base of the well. Using the model described previously, this corresponds to a contact angle $\Phi$ of approximately $60^\circ$, reflecting the shape matching with the surface and independent of the substrate’s tilt angle.

Figure 5.4 depicts the predicted moment ratios versus the tilting angle of the assembly template for two cases in which the microsphere size is well-matched to its assembly well (10 µm/10 µm diameter and 20 µm/20 µm diameter) and for one case in which the microsphere size is much smaller than its assembly well’s size (10 µm/20 µm diameter). This analytical prediction is created using the measured experimental parameters for the implemented system described above.

At zero tilt angle, the assembly of spheres into well-matched wells is predicted to be effective (with a moment ratio less than one), and assembly of spheres into much larger wells is predicted to be ineffective (with a moment ratio of greater than one). As the angle of tilt increases, the moment ratio that predicts the selective removal behavior of spheres in well-matched wells decreases, indicating that they will continue to be retained in the wells. However, the ratio that predicts the selective removal of spheres from much larger wells also declines, decreasing from 5.24 for zero tilt angle to 4.72 for $45^\circ$ and to less than one at an angle of $60^\circ$. In other words, for sufficient tilt angle, the assembly becomes indiscriminate. Both 10 µm spheres and 20 µm spheres can assemble into a 20 µm well, and this loss of selectivity prevents the selective removal mechanism from functioning. The specific details of this prediction depend on the microsphere size, the well size and
shape, the surface roughness, etc. The loss of selectivity as the tilt angle approaches the angle $\Phi$ is robust to the experimental details, however.

Figure 5.4. The ratio of retention to removal moments ($M_{\text{rem}}/M_{\text{ret}}$), versus the tilting angle for 10 and 20 $\mu$m polystyrene microspheres assembled into the corresponding-sized assembly wells and 10 $\mu$m microspheres assembled into 20 $\mu$m assembly wells. These ratios have been plotted using the following values: $\rho_{\text{medium}} \equiv 810 \frac{kg}{m^3}$, $c \equiv 1.194 \times 10^3 \frac{m}{s}$, $f = 1.7 \times 10^6$ Hz, $\mu \equiv 1.07 \times 10^{-3}$ Pa.s, $u = 4.2 \times 10^{-3} \frac{m}{s^2}$, $\gamma = 5.77 \times 10^{-3} \frac{L}{m^2}$, $C_r = 0.01$, $d_e = 1.5 nm$ [5], $I = 17.12 \frac{W}{m^2}$ [9].

### 5.2 Results and Discussion

Figure 5.5 presents SEM images of the assembly wells fabricated into the silicon wafer. The cross sectional view depicts how two different size wells are placed next to each other and shows that they are almost hemispherical in shape. The high magnification image shows the top view of an assembly well indicating the short wavelength roughness on the sidewalls. Figure 5.6 shows a dark field optical microscope image of an interleaved array
of 10 and 20 µm wells after 10 µm microspheres have been assembled into the template at a 30° tilt angle with a transducer driving voltage of 50 V. The dark blue spots are wells into which the 10 µm microspheres are assembled. The bright spots are empty 20 µm wells into which the 10 µm spheres have not assembled.

Figure 5.5. The SEM images of a) cross section view of both approximately hemispherical wells and b) the top view of an assembly well.
Figure 5.6. Dark field optical micrograph of an assembly array after TASR-based assembly of 10 µm blue-dyed polystyrene microspheres at 50 V into a template that is angled at 30 degrees. The large bright spots are empty 20 µm assembly wells, and the small dark blue spots are 10 µm wells assembled with the corresponding microspheres.

The assembly results are presented as the measured yield percentage versus various experimental parameters. The yield percentage, $Y^a_b$, is defined as the ratio of the number of wells of nominal diameter $b$ that are filled with microspheres of nominal diameter $a$ to the total number of wells of diameter $b$ in the array. Figure 5.7a plots measure the yield versus applied voltage for 20 µm microspheres. Each plot includes the measured yield for different angles of substrate tilt from 0 to 75 degrees in increments of 15 degrees. The assembly is characterized over a voltage range of 20-70 V, which brackets the voltages at which measured yields are maximized. Looking at the plots, it is found that by increasing the voltage, the yield percentage first increases to a peak and then drops to lower values, consistent with the observations of (Eid, 2008; Jung, 2012). At high voltages (i.e. for voltages above the voltage at which peak assembly yield is observed), the drop in yield can be attributed to the increasing ratio of removal moments to retention moments as described previously. The low assembly yields measured for low acoustic transducer voltages (i.e. for voltages that are lower than the voltage at which peak assembly yield is observed)
cannot be merely attributed to the selective removal mechanism. Instead it is more dependent on the circulation of the microspheres in the suspension, as described in (Agarwal, 2016; Eid, 2008; Jung, 2012). In other words, at low applied voltages the excitation strength is not sufficient to drive enough collisions between the microspheres and the wells across the surface. The angle of tilt of the template may also affect the number of collisions by increasing the height of some of the assembly wells, especially for low applied voltages. Overall, the assembly process is under the control of a competitive process between the selective removal effects and the circulation conditions. At the peak voltage, there is a good balance between advantageous moment ratios (where the retention moment is stronger than the removal one as explained previously) and strong circulation effects so that the yield hits its maximum value. For example, the maximum yield $Y_{20}^{20}$ (80%) for the assembly of 20 µm spheres into matched wells at a tilting angle of 45 degrees appears at 45 V, whereas the corresponding yields at 20 and 70 V are 20% and 33%, respectively. Figure 5.7b plots the measured assembly yield of 10 µm microspheres assembled into their corresponding-sized wells. These plots are very similar to those of 20 µm microspheres in terms of how the yield depends on the applied voltage. All plots at different tilting angles indicate a maximum yield at a certain voltage, and the yield drops at lower and higher voltages as explained above.

The yields in Figures 5.7a and 5.7b are in good agreement with the model that predicts that a removal moment to retention moment ratio smaller than 1 will permit microspheres to be assembled into their corresponding wells. (See Figure 5.4 plots “$Y_{10}^{10}$”, “$Y_{20}^{20}$”). These results prove that TASR-based assembly can successfully assemble micro-spherical objects into well-matched wells even when the template is tilted at an angle with respect to
the horizontal (for vertically-directed acoustic excitations). However, assembly yields generally drop to lower values once the tilting angle passes 45 degrees. One cause of this may be that the steep angle of the template makes it hard for the microspheres to circulate to and enter the assembly wells. It is also seen that the greater the tilting angle, the lower the voltage at which the maximum yield is achieved. (The peak value of the yield declines as well.) This stands in contrast to the predictions of Figure 5.4, in which the moment ratio is minimized when the angle of tilt equals the angle of the microsphere’s attachment within the well and increases for both larger and smaller angles of tilt. This discrepancy can be explained by the fact that the sphere’s angle of attachment within the well is in practice not entirely independent of the substrate’s angle of tilt. In fact, the sphere’s optimal rest position within the well will depend on surface forces, fluid forces, and gravity, but only surface forces are considered in the calculations of Figure 5.4. At zero angle of tilt, the microsphere’s rest position is expected to be lower in the well (i.e. at a smaller angle) than it is at higher angles of substrate tilt. At higher angles of substrate tilt, gravity can bias the optimal rest position of the microsphere to be closer to the edge of the well. Because the radius of curvature is not constant in the well (see equation 5.4), less reduction in interfacial energies and smaller retention moments will occur as a result of gravity shifting the microsphere further up the wall of the well. The premature ejection of the microspheres from their wells at lower voltages is attributed to this effect. These results indicate that angle of tilt should be maintained below about 45 degrees (depending on the required yield values) in order to prevent the loss of assembly yield of well-matched microspheres.

Figure 5.7c plots measured assembly yields for the assembly of 10 µm microspheres into 20 µm assembly wells. Except when the angle of tilt is 60 degrees, the measured
assembly yield is very low (close to zero) for all applied voltages and there is no noticeable peak. This means that the selective removal of smaller microspheres from larger wells is successful for most tilting angles, similar to what is observed for zero angle of tilt. If the relevant moment ratios for these tilting angles (See Figure 5.4, plot “$Y_{20}^{10}$”) are estimated, it is found that the ratios are a few orders of magnitude larger than 1, meaning that the removal force considerably exceeds than the retention one. This suggests that the 10 µm microspheres should be ejected from the larger wells, and this is in a good agreement with the measured results. However, at an angle of tilt of 60 degrees, a yield peak of 14% is observed even though the microsphere has a 2X smaller radius than the radius of the wells. In other words, when the substrate’s angle of tilt matches the angle at which the microsphere is initially at rest in the well, mismatched microspheres can assemble and the selective removal of TASR does not function properly. Consistent with the model predictions for this angle of tilt, the removal moment becomes zero and there is no removal agent to eject the mismatch microspheres from the larger wells. These results therefore demonstrate good agreement between the model predictions and the experimental results, and this phenomenon can be interpreted as dysfunction in the selectivity of TASR at this angle of tilt.
Figure 5.7. Assembly yield percentage versus the applied voltage for: a) 20 µm microspheres assembled into 20 µm wells, b) 10 µm microspheres assembled into 10 µm wells, and c) 10 µm microspheres assembled into 20 µm wells.
Figure 5.8a plots the maximum yield versus the angle of tilt for both microspheres assembled into their corresponding-sized wells and for 10 µm microspheres assembled into 20 µm wells. For both well-matched microspheres, the yield decreases gradually up to 45 degrees and then suddenly drops to lower values. However, when 10 µm microspheres are assembled into 20 µm wells, the yield is very low (close to zero) for all angles of tilt except for 60 degrees. To better understand these trends, the selectivity ratio, $S$, is defined as the ratio of the maximum yield percentage of the well-matched microspheres, $Y_a$, to that of the mismatched microspheres, $Y_b$. The higher the ratio, the more selective is the assembly. $S_1$ is the ratio of $Y_{20}^{20}$ to $Y_{20}^{10}$, and $S_2$ is the ratio of $Y_{10}^{10}$ to $Y_{20}^{10}$. Figure 5.8b plots the selectivity ratios $S_1$ and $S_2$ versus the angle of tilt. Looking at the $S_1$ value, the ratio is high (around 18) for tilting angles of 0 and 15 and drops to somewhat lower values (around 14) for 30 and 45 degrees. The dependence of $S_2$ on angle of tilt at lower values of angle is similar. However, the ratios drop abruptly with $S_1$ of around 5 at tilting angle of 60 degrees. At an angle of tilt of 75 degrees, the selectivity ratios are similar to those at 60 degrees, with $S_1$ increasing slightly and $S_2$ decreasing slightly. These ratios show that the selectivity ratio is greatly reduced when the angle of tilt of the substrate is similar to the angle of attachment of the sphere within the well, in good accordance with the model predictions.
Figure 5.8. a) The maximum assembly yield versus the angle of tilt for both 10 and 20 µm microspheres \( Y \) assembled into their corresponding-sized and 10 µm microspheres assembled into 20 µm wells, and b) the ratios of selectivity, \( S_1 = \frac{Y_{20}^{10}}{Y_{20}^{10}} \) and \( S_2 = \frac{Y_{10}^{10}}{Y_{20}^{10}} \), versus the angle of tilt.

5.3 Summary

The capability of templated assembly by selective removal on non-horizontal surfaces was investigated in this chapter. The analytical model for zero angle of tilt and for low angles of tilt estimates that TASR should be able to successfully assemble the microspheres.
into their corresponding assembly wells if they are well-matched in size and shape. However, it also predicts that TASR will lose its selectivity as the template’s angle of tilt approaches the angle of the microsphere’s point of contact inside the assembly well. At this particular angle, the fluid removal force coincides with the line connecting the microsphere’s point of contact and its center of mass, resulting in zero lever arm. When the removal moment is zero, the assembly process has no functioning mechanism to remove a mismatched microsphere from an assembly well. The experiments demonstrate that these predictions are correct. TASR is able to successfully assemble a given size of microsphere into its corresponding-sized assembly wells at low tilting angles as predicted. The experimental findings also demonstrate that at a critical angle that depends on the details of the microspheres and wells, the assembly loses its selectivity. The results show that the critical angle of tilt for the present case is about 60 degrees, in very good agreement with the model predictions. In summary, this research highlights the ability of TASR in self-assembly of micro components onto angled surfaces and clearly proves the relationship between the selectivity mechanism and the template tilting angle. The achievements of this work establish a robust understanding for implementing TASR in the self-assembly of multi components onto angled surfaces such as are found in curved or folded system architectures in bioengineering and other engineering applications.
Chapter 6

TASR-BASED DIRECTED ASSEMBLY INSIDE MICROFLUIDIC DEVICES

Microfluidic devices offer benefits in many bioengineering applications such as tissue engineering (Chung, 2009), biomedical diagnostic devices (Sackmann, 2014), development of cancer therapies (Boussommier-Calleja, 2016), drug delivery and medicine studies and research (Y. Zhang, 2013), and even engineering applications (Ohadi, 2013). In bioengineering applications, microfluidics are one of the most prominent platforms in the field of functional biomaterial synthesis (J. Ma, 2017). Microfluidic devices can replace conventional analysis and diagnostic methods using smaller volumes of samples and reagents, and they offer advantages such as a high degree of control, potential for automation, high-throughput processing, low cost, high reliability and sensitivity, and personalization and disposability (Sarioglu, 2015). There is a tremendous amount of work demonstrating the potential of microfluidic devices in the aforementioned areas. A few examples include the detection of circulating tumor cells (CTCs) (J. Myung, 2015), the fabrication of tissue scaffolds with controlled microfluidic networks (Choi, 2007), and single cell isolation in nanoliter droplets (Macosko, 2015).

As was discussed in the previous chapters, TASR possesses a valuable and unique set of characteristics, including high directed-assembly throughput, selectivity of the assembly, and the ability to simultaneously assemble multiple components or cells of different sizes and shapes with high scalability, simplicity, and a low cost procedure. If the benefits of microfluidics were combined with the advantages of TASR-based directed assembly, it would be possible to create new types of systems for bioengineering, ones that
combine microfluidics’ ability to control flows precisely with TASR’s ability to control cell locations precisely. For example, microfluidics have been used to create organs on a chip (Bhatia, 2014); by introducing TASR to such systems, it could become possible to control the cellular organization at the microscale within these organs on a chip to increase their biomimetic structure and performance. However, before such a thing can become possible, it is necessary to first investigate the behavior of TASR inside microchannels to identify the capabilities and limits of the TASR process within laterally and/or vertically constrained spaces. Therefore, this phase of the research aims to study the performance of TASR in tiny spaces such as microfluidic channels. The objective of the research is to first understand if TASR can be integrated into a microchannel environment, and second, to predict how the dimensions of the microchannels and the operating conditions of the TASR process affect the assembly outcome. The relevant parameters of the microchannels include their width, their height, and the in-plane shape of the microchannel network (e.g. straight, curved, with sharp corners, etc.). The TASR assembly variables are the microspheres’ concentration in the assembly medium, the applied voltage that controls the intensity of the acoustic excitation, and the locations of the assembly sites within the microchannels.

The experiments on TASR in microchannels were carried out in polydimethylsiloxane (PDMS) devices. As is common in soft lithography, the devices are formed by joining two layers (at least one of which includes topography that defines the sides and upper surfaces of a set of microfluidic channels) together to form enclosed microchannels. In the present case, the lower layer is made of PDMS that has been molded so that it includes sets of TASR assembly wells. The upper layer is also made of PDMS, but this layer has been molded to include the structure of the microchannels. When they are aligned and joined
together, they form a set of microchannels that include assembly wells in their bottom surfaces. Varying the design of the molds from which the devices’ constituent layers are patterned ensures that the resulting devices can demonstrate the effects of varying the parameters described above.

The mask design was completed such that it covers a broad range of dimensions for both width (W) and height (H) of the microchannels. The dimensions were chosen with respect for microfabrication considerations and limitations as well as to produce the desired device variations. The mask also assigns two different classes of in-plane shapes to microchannels; these are serpentine and spiral geometries (as depicted in chapter in Figure 4.6). In the serpentine geometry, the smallest channel design has a cross section of W=100 µm and H=100 µm, and the largest channel is as large as W=7000 µm and H=1000 µm. In fact, the mask is designed such that as the small channels transition to larger channels, they ultimately resemble a relatively large (nearly macroscale) chamber at the multiple millimeter size scale. For the spiral geometry, the smallest channel design would correspond to a cross section of W=50 µm and H=100 µm, and the largest one is the same as the above (W=7000 µm and H=1000 µm). However, in practice the smallest microchannel that was able to be consistently and accurately fabricated was larger than the nominal dimensions described above. For the microchannels with widths below 200 µm, the aspect ratio (height to width ratio) of the SUEx mold material was so high that the SUEx mold could not keep its integrity and broke into pieces during the process of creating PDMS replicas. Hence, the smallest cross sections in practice were W=200 µm and H=100 µm for the serpentine geometry and W=175 µm and H=100 µm for the spiral geometry. For ease of discussion, a designation system is defined to specify microchannels with
different dimensions. The nomenclature is in form of $W\alpha H\beta$, which would represent a microchannel with a width of $\alpha$ and a height of $\beta$, where the dimensions are given in microns. For example, a microchannel with a width of 200 $\mu$m and a height of 100 $\mu$m would be named $W200H100$.

This chapter continues with a discussion about the effects of varying applied voltage (acoustic excitation intensity) on assembly inside of microchannels with various dimensions. Then it moves forward with the investigation on the impact of width and height change of microchannels on the assembly output to discover the correlation between these variables. In the following subsection, the effect of the assembly sites’ positions inside microchannels on the assembly yield is studied. Finally, the selectivity characteristics of TASR inside microchannels are examined and verified.

### 6.1 The effect of microchannels’ dimensions on the optimum applied voltage

This series of experiments addresses the impact of the dimensions of the microchannels on the applied voltage (which is related to acoustic intensity) that is required to achieve successful TASR-based directed assembly in microfluidic devices. In other words, these experiments are designed to understand whether changes in the aspect ratios or widths of the channels causes the optimum applied voltage to change, or whether optimum acoustic intensity is independent of channel dimensions within the examined range. The optimum applied voltage is the voltage at which the assembly yield hits its peak while all other experimental variables are held constant in TASR-based directed assembly.

Three different device geometries are considered: $W200H100$, $W1000H100$ and $W4000H100$, which correspond to aspect ratios (H/W) of 0.5, 0.1 and 0.025, respectively.
These combinations of widths, height and their resultant aspect ratios are chosen because they cover a large range of sizes and aspect ratios. For example, device W200H100 has the tallest aspect ratio and smallest dimensions while device W4000H100 has the flattest aspect ratio while having a large width (millimeter scale) and small height (hundred micron scale). The experiments are done at different applied voltages ranging from 25 to 65 V with a 10 V increment between conditions. The experimental results are presented in Figure 6.1 in form of the assembly yield percentage plotted versus the applied voltage. (The intensity of the acoustic excitation increases as the square of the applied voltage.) Looking at device W200H100, it is found that the assembly yield peaks at a voltage of 45 V with an assembly yield of 50%. The yield drops to lower values at both lower and higher voltages. The trend is similar for the other two devices, with the peak yield occurring at 45 V. However, the value of the yield at a given voltage increases with the size of the microfluidic channel, reaching a peak value of 71% yield at 45 V for device W4000H100. These results suggest that the acoustic intensity (and the applied voltage) at which the highest yield is obtained is not affected by the aspect ratio or the dimensions of the microchannel. Therefore, all subsequent experiments in this chapter are carried out at a constant applied voltage of 45 V. The causes of the channel-dependent peak yield values are comprehensively discussed in coming subsections.
6.2 The effects of microchannel dimensions on assembly yield

If TASR is to be used to create microstructure (of cells or functional components) inside of microfluidic channels, it is important to understand which channel dimensions (width and height) are most conducive to successful assembly and how changing dimensions affect the assembly outcome. To pursue this goal, a set of experiments is designed to systematically explore variations in a range of parameters, including channel width, height, aspect ratio, and area, as well as certain aspects of the assembly conditions. Two sets of microfluidic devices were created, one with constant heights and varying widths and the other with constant widths and varying heights. Each set of microfluidic devices was fabricated with two basic types of geometry in the plane: a serpentine (back
and forth) channel layout and a spiral channel layout. Figure 6.2 summarizes the assembly results for both serpentine and spiral devices in 3D format where the assembly yield is plotted on the vertical axis as a function of the cross sectional area of the microchannels and either the width (Figure 6.2a) or the height (Figure 6.2b). Marker type and color code for device layout (serpentine or spiral) and either microchannel height (Figure 6.2a) or microchannel width (Figure 6.2b). The data in Figure 6.2 represent devices with three different microchannel heights (100, 200 and 1000 µm) and with microchannel widths that vary from 200 to 7000 µm in uneven increments. The maximum width for the spiral geometry is capped at 1630 µm because from a layout point of view, it is not practical to make wider microchannels in a spiral configuration in the die area that is available. Also, looking at the designed mask, it is found that both serpentine and spiral channels can be very similar in shape at large widths such as 1630 µm and wider. These 3D plots are shown here to provide an overview of the trends of the assembly yields for both geometries. However, to better understand the phenomena that govern the assembly yield, 2D representations of the dependence of assembly yield on various parameters are presented in coming paragraphs.

Figure 6.3 depicts the assembly yield versus the width of the microchannels for both serpentine and spiral geometries at three different channel heights. For serpentine devices, the maximum assembly yield of 90% is observed for a microchannel height of 1000 µm (Figure 6.3a) and a microchannel width of 7000 µm. (Note that for a microchannel width of 7000 µm, the channel’s design is not strictly a back-and-forth serpentine pattern, but rather a single wide channel that crosses from one side of the die to the other.) With 1 mm height and 7 mm width, the geometry of this W7000H1000 device approaches that of a
conventional chamber, i.e., it may act similarly to a small assembly beaker containing assembly medium confined with a low-height cap (1mm). The yield percentage declines by a relatively small amount when the width is reduced to 3000 µm, with W4000H1000 and W3000H1000 having assembly yields of 85% and 82%, respectively. The relatively small decline in these yield percentages suggests that these two devices behave very much like the 7000 µm-wide device and also mimic the behavior of a conventional assembly chamber. However, further decreases in width result in further declines in assembly yield, and the drop becomes precipitous at a channel width of approximately 400 µm. The trends in assembly yield vs. width are similar for the microchannels with smaller heights of 200 µm and 100 µm, but the smaller heights result in smaller values of yield for a given channel width. For a microchannel height of 200 µm, the maximum yield of 78% is observed for device W7000H200, which is the widest microchannel in this set. For a microchannel height of 100 µm, the maximum yield of 71% is observed for device W7000H100, which is the widest microchannel in this set.

The yield data for the microchannels with a spiral geometry (Figure 6.3b) are very similar to what are observed for serpentine ones. Note that the data plotted for 7000 µm width are the same “straight across the die” data as are plotted for the serpentine channels; this provides a common reference point for channels that are too wide to have a truly serpentine or a truly spiral layout in the available space on the die. As for the serpentine devices, the precipitous decline is observed at a microchannel width of about 400 µm for all heights.
Figure 6.2. The assembly yield percentage as function of microchannels’ a) width and cross sectional area and b) height and cross sectional area for both serpentine and spiral geometries.
Figure 6.4 depicts the assembly yield versus the height of the microchannels for both serpentine (Figure 6.4a) and spiral (Figure 6.4b) geometries at three illustrative channel widths (4000 μm, 500 μm, and 200 μm for the serpentine channels and 930 μm, 350 μm, and 175 μm for the spiral channels). In Figure 6.4a, each width demonstrates a gradual reduction in yield with microchannel height; the reduction in yield appears to accelerate slightly for channel heights below 300 μm. The difference in yield between the maximum and minimum channel heights is at the scale of approximately 15 percentage points, which is relatively small as compared with the greater than 30 percentage point differences that have been observed as a result of width variations. A similar descending trend in yield percentage is seen in data points attributed to the spiral geometry (Figure 6.4b).

One potential interpretation of the data in Figures 6.3 and 6.4 could be that the internal volume of a given length of microchannel plays a role in determining the yield percentage that will be observed; larger width and larger height both result in larger internal volumes when the other parameter is held constant. Larger internal volume per unit length of microchannel (i.e. larger cross-sectional areas) could increase assembly yield for various reasons, such as by affecting the circulation of assembly components within the channels, by impacting the volume of the assembly medium or the oversupply of assembly components, or a combination of these. It is important to examine the assembly results as a function of cross-sectional area in order to identify the extent to which either width or height has an independent effect on the assembly success apart from its role in determining the cross-sectional area. These results are described below.
Figure 6.3. The assembly yield percentage versus microchannel width at different constant microchannel heights for a) serpentine and b) spiral geometries.
Figure 6.4. The assembly yield percentage versus microchannel height at different constant microchannel widths for a) serpentine and b) spiral geometries.
6.3 The effects of cross sectional area and aspect ratio of microchannels

In this section, the assembly yield data are examined as a function of the cross-sectional area and aspect ratio of the microchannels. Figure 6.5 plots the assembly yield percentage versus the cross-sectional area of each device for both device geometries; the markers code for the height of the channels. If the cross-sectional area were the only parameter that were relevant to the assembly process, all of the data should fall on a common curve. If the microchannel height were a significant determinant of assembly yield independent of cross-sectional area, then one would expect the measured assembly yields to be consistently higher for some heights than for others. Instead, it is observed that the results show little dependence on height for large cross-sectional areas, but that the results diverge from the common curve as the cross-sectional area decreases. Note that the results diverge first for the channels with the largest height (which also means that the results diverge first for the channels with the smallest widths). For smaller heights, the divergence of the measured yield from the common curve occurs later. This suggests that width rather than height may be a significant factor in determining the assembly yield. On each plot, there are points at which the results for different channel heights diverge from each other. For instance, in serpentine geometry (Figure 6.5a), when the cross section area is 0.2 mm², the yield percentages for W200H1000, W1000H200 and W2000H100 are 53%, 63%, and 64%, respectively. The observed trends are similar for the spiral geometry (Figure 6.5b).

Figure 6.6 plots the assembly yield percentage versus the cross-sectional area of each device for both types of device layouts, but this time the markers code for the width of the channels. Similar to the discussion of Figure 6.5, if channel width affects assembly yield in a way that is independent of its effect on cross-sectional area, several observations are
expected. First, the data should not all collapse onto a common curve as a function of the cross-sectional area of the channels. Second, the measured assembly yields should be consistently higher for some channel widths than for others. This is exactly what is observed, suggesting that width is a separate factor in determining the assembly yield independent of its effect on cross-sectional area. Similar to Figure 6.5, the same cross-sectional area highlights the effects of varying width. These results are similar for devices with a serpentine layout and devices with a spiral layout (Figure 6.6b). These results show that if the cross-sectional area is identical, the wider microchannel can be expected to achieve a higher yield, at least up to a certain value of width. Figure 6.6a suggests that this critical width is below 500 μm, and Figure 6.6b suggests that the critical width is above 350 μm. These results are consistent with what was observed in Figure 6.5.
Figure 6.5. Assembly yield percentage versus cross section area of microchannels for a) the serpentine and b) spiral geometries with the results coded by channel height. Note: The cross sectional area axis is centered on small values of area to magnify the region of interest.
Figure 6.6. Assembly yield percentage versus cross section area of microchannels for a) the serpentine and b) the spiral geometries with the results coded by channel width. Note: The cross sectional area axis is centered on the small values of area to magnify the region of interest.
One can also ask whether and how assembly yield depends on the aspect ratio (H/W) of the microchannels. The yield percentage data are plotted versus aspect ratio for both types of microchannel layouts in Figure 6.7 (in which the data are coded by height) and in Figure 6.8 (in which the data are coded by width). In Figure 6.7, an increase in aspect ratio at given channel height corresponds to a decrease in channel width. For all three heights, the assembly yield decreases with increasing aspect ratio for given channel height. It is also observed that when the height is smaller, the yield drops at smaller values of aspect ratio and more steeply than for larger channel heights. Both of these trends are consistent with the previously-observed dependence of assembly yield on the width of the microchannels; a higher aspect ratio indicates smaller width for given height, and a given aspect ratio indicates a smaller width for smaller height.

In Figure 6.8, the yield increases with increasing aspect ratio for a given value of channel width. However, the assembly yield’s increasing trend plateaus at a lower value of yield for smaller channel widths. These results are consistent with the previously-observed dependence of assembly yield on the cross-sectional area of the microchannels. For fixed width, a larger aspect ratio corresponds to a larger height and hence to a larger cross-sectional area in the microchannel.

Combining all of the results from Figures 6.5 to Figure 6.8, it can be concluded that both a smaller channel volume and a narrower channel width can independently reduce the assembly yield. For a given channel cross-sectional area, a horizontal microchannel can offer higher yields as compared with a vertical one. These trends could reflect inefficient circulation of the components within the channels due to the channels’ small dimensions, particularly their small widths. They could also reflect the fact that a smaller channel area
contains fewer components per unit length of channel, thereby reducing the oversupply of assembly components inside microchannels relative to the number of assembly sites. Determining which factor plays the most prominent role and which one is less important requires further data analysis to better understand the results and to assess the relative importance of the above parameters.
Figure 6.7. Assembly yield percentage versus aspect ratio of microchannels for a) the serpentine, and b) spiral geometries when the width is the variable.
Figure 6.8. Assembly yield percentage versus aspect ratio of microchannels for a) the serpentine, and b) spiral geometries when the height is the variable.
6.4 The effects of component oversupply on assembly yield

In previous sections, the assembly yield data were examined as a function of channel width, height, cross-sectional area, and aspect ratio. However, as described above, these parameters are not independent of an important operational parameter, namely the oversupply of components in the assembly space relative to the number of assembly sites. Oversupply is defined as the ratio of the number of components to the number of assembly sites, or for the case of enclosed microchannels, as the ratio of the number of components in a given length of microchannel to the number of assembly sites in that same length of microchannel. The importance of oversupply has been identified in prior research, with typical component oversupply values set at $10^3$ (Eid, 2006; Jung, 2007) and reductions in average assembly yield being observed for lower oversupply values of $10^3$ and below (Agarwal, 2012; Agarwal, 2016). Oversupply is important because it affects the probability that some component will randomly sample each assembly site in a given period of time. The selective removal process requires initial assembly to take place before selective removal can occur, and the rate of initial assembly increases for higher values of the oversupply. For a given value of component concentration in the assembly fluid, the number of available components per unit length will be proportional to the cross-sectional area of the channel. If the number of assembly sites per unit length is held constant, than oversupply will also be proportional to the cross-sectional area of the channel.

In practice, the number of assembly sites per unit of channel length is not held precisely constant. Although the concentration (number density) of the components in the assembly medium is the same in all experiments and devices described above, the number of assembly sites is not identical in all microchannels. This means that the number of
microspheres available for each assembly site might differ somewhat from one device to another, so that oversupply has some correlation but not a perfect correlation with the cross-sectional area of the channel.

To understand the extent to which the dependence of assembly yield on channel geometry simply reflects the effects the role of oversupply on the assembly yield, the oversupply is varied by changing the concentration of the components in a device with fixed geometry. Because another objective of this research is to see how channel width affects the circulation of the microspheres and the assembly yield, a device with a large channel size (in terms of width, height, and cross-sectional area) that demonstrates large assembly yields at the standard component concentration is chosen for these experiments. Given this, device W4000H1000 is selected for the experiments that explore the effects of varying oversupply via changes in concentration. The experimental procedures are exactly as described previously except that the oversupply is varied between 65 and 4650 by changing the components’ concentration in the assembly medium.

Figure 6.9 plots measured assembly yield vs. oversupply both for devices with varying geometries and fixed concentration and for devices with fixed geometry and varying concentrations. The varying-concentration data are shown as red diamond markers in the plots. When concentration is varied directly, the yield percentage gradually drops from its maximum, which is observed when the oversupply is 4650, to a lower value of 59% at an oversupply of 140. When the oversupply reaches 65, the yield drops more precipitously to its lowest value of 25%. These results suggest that there may be a critical oversupply (probably below 100) at which the yield drops more dramatically.
Figure 6.9a plots the varying-concentration data together with the varying-geometry results for the serpentine channels; different marker types represent different channel heights. For the majority of the device geometries, the yield vs. oversupply data fall along the same curve as the variable-concentration data, indicating that the variation in oversupply with device geometry explains much of the variation in assembly yield between devices. For some device geometries, however, the yield vs. oversupply results diverge from the variable-concentration data, with lower measured assembly yields. Figure 6.9 shows that for each channel height, the assembly yield vs. oversupply data for variable-geometry measurements coincide with the varying-concentration data for some of the device geometries and not for others. Because channel height does not systematically dictate whether a given device geometry produces assembly yields that match what would be expected solely on the basis of oversupply, height is seen to not be a dominant factor in determining the outcome of the assembly process.

Instead, width appears to play a dominant role. The assembly yield vs. oversupply data for variable-geometry measurements coincide with the variable-concentration data for devices with channel widths between a few millimeters (the maximum tested, with no observed upper length scale limit) and a lower length scale limit of 400 µm. The outliers from the 1000 µm channel height data correspond to devices with channel widths of 200 µm and 300 µm, and the outliers from the other two channel heights correspond to devices with channel widths of 100 µm and 200 µm. In each case, these outliers are observed for devices with channel widths that are smaller than 400 µm. These results demonstrate that small channel width (below 400 µm) is a separate factor, independent of oversupply that can cause lowered assembly yield. Figure 6.9b plots similar data for the spiral geometry.
The results are similar to those observed in 6.9b, except that the outlier points (highlighted with ovals on the plot) are observed at channel widths of 350 and 200 µm.

The question arises of why small channel width below 400 µm leads to reductions in assembly yield. It is hypothesized that the circulation of the microspheres becomes ineffective at such small widths, limiting the number of collisions between the microspheres and the assembly sites and therefore also limiting the final assembly yield. For microchannels with widths larger than 400 µm, however, the oversupply of the microspheres appears to be the dominant factor in determining the assembly yield percentage.

To confirm the independent importance of channel width in determining assembly yield, the assembly yields for varying-geometry and varying-concentration are plotted vs. oversupply in Figure 6.10. This time, however, the various marker types indicate different channel widths. In Figure 6.10a, the widest serpentine channels (4000 µm) show assembly yield percentages that coincide well with the varying-concentration data, which are again shown in red. The 500 µm wide channels demonstrate slightly lower yields, but they remain relatively close to those of the 4000 µm wide channels and the of the varying-concentration data. The 200 µm wide channels, however, show much lower assembly yields than those that are seen for the wider channels and for the concentration data. These results are in harmony with what was seen in the other plot of assembly yield vs. oversupply for serpentine channels (Figure 6.9a), in which the devices with widths of 300 µm and below deviate from the general trend.
The microfluidic devices with a spiral layout show a similar trend. In Figure 6.10b, the widest spiral channels (930 µm) show assembly yield percentages vs. oversupply that coincide well with the varying-concentration data, which are again shown in red. However, the narrower channels (350 µm and 175 µm) deviate significantly from the data for wider channels and for variable concentrations. The gap between the narrower channels and the wider channels/varying-concentration data becomes larger at lower values of oversupply. Again, this finding is in line with the results of the previous oversupply plots (Figure 6.9b), in which the yield values attributed to narrow channels (< 400 µm) deviate from the majority of data points.

Understanding the dependence on channel width requires understanding why component circulation becomes ineffective at small values of width. Unlike selective removal, which is driven by primary (oscillatory) fluid forces, component circulation is driven by secondary (steady) fluid flows. Two secondary flows that can potentially drive circulation arise from acoustic streaming and radiation pressure, which are both nonlinear flows with non-zero means (Jung, 2012). The radiation pressure results in forces that are vertical (i.e. in the direction in which the acoustic waves travel), but their magnitude is negligible compared to gravitational forces. Hence the potential flow field arising from the radiation pressure will be ignored in the current discussion.

In contrast, the larger magnitudes of the acoustic streaming forces and their resulting flows enable them to drive significant component circulation within the assembly fluid. Acoustic streaming flows may provide two types of flow fields in the assembly system. One is called Eckart streaming (Eckart, 1948; Spengler, 2000; Wiklund, 2012) and may act at the large scale such as > 1mm. The other one was demonstrated by Kolb, Jackson and
Nyborg (Jackson, 1999; Jung, 2012) and works at smaller length scales, in the range of around the acoustic wavelength. It was discussed in (Jung, 2007) that Eckart streaming functions in a bulk fluid medium (i.e. with the large dimensions of width and height of the assembly beaker), and it should not be a dominant streaming flow in the conventional TASR setup. Since the current experimental setup contains the assembly fluid within even smaller spaces, it can be said that this streaming should not be effective in the smaller volumes of the microfluidic system as well. Hence, the only functioning streaming flow that plays a significant role would be the one demonstrated by Jackson and Nyborg.

In the flow field of Jackson and Nyborg, there are two characteristic flows. One is induced at the periphery of the central driven area where the intensity of the ultrasound is relatively high, and the other is associated with standing waves outside the driven area. As a result the circulation of microspheres varies by location and is divided into a concentric structure. According to Jung’s study (Jung, 2007; Jung, 2012) on the circulation of components, the components that are on or near the surface of the assembly substrate tend to be distributed in a concentric pattern that follows the circular nodal lines. The components are swept away along the surface in locations where the vorticity of the flow drives horizontal fluid motion, and they tend to collect on the surface between neighboring vortices where the flow is nearly zero. The horizontal component of this flow plays an important role in delivering the components to the assembly sites in the first place so that the selective removal process can occur, and the locations of the vortices and nodes can affect the uniformity of the assembly results in the conventional, open-beaker TASR setup. It is expected that these concentric regions will be separated by one half of the wavelength of the standing wave. The wavelength of the acoustic waves is calculated by:
\[ \lambda = \frac{c}{f} \] (6.1)

where \( c \) is the speed of sound in the assembly medium (1190.9 m/s for the current liquid) and \( f \) is the frequency of the sound waves (1.7 MHz for the current transducer). Hence, in this experimental setup, the distance between the concentric regions is approximately 350 \( \mu \)m. In other words, the lateral dimension of a single flow vortex is also 350 \( \mu \)m.

If a comparison is made between the widths of microchannels with yields that deviate from what would be predicted from the effects of oversupply alone and the characteristic dimensions of a single flow vortex, there is an interesting match between them. Conceptually, this can be explained by observing that for smaller channels below the 350 \( \mu \)m width threshold, a full cycle of the circulation as described by Jackson and Nyborg cannot be completed in the available space. Hence, it can be concluded that when the microchannels’ widths are smaller than 400 \( \mu \)m, the circulation of assembly components is negatively affected, the initial assembly that is necessary to enable selective removal is suppressed, and the final yield percentage deviates from the predictions of the varying-concentration experiments.

Overall, the dependence of assembly yield on oversupply is a very useful way to demonstrate how TASR-based selective assembly depends on component concentration, component availability, and circulation within the microchannels. The main factor affecting yield percentage is shown to be oversupply. The more concentrated the assembly medium and the larger the number of components inside the channels (which in turn scales with fluid volume within the channels), the higher the yield percentage will be. However, the dependence of assembly yield on oversupply appears to show two different regimes.
(see Figure 6.11). In the first regime, at higher values of oversupply, the yield gradually drops to lower values while concentration is decreasing with a linear relationship as shown with a fitted line in the plot. In the second regime, as the oversupply drops to below about 140, the yield deviates from the first regime and afterwards it suddenly drops to a very low value that does not fit the trend of the first regime. Although the yield variation with channel dimensions primarily reflects the effects of channel dimensions on oversupply, small channel widths at or below 400 µm are identified as a secondary factor that can reduce yield below what would be predicted based on oversupply alone. This dependence on yield reflects the effects of microchannel widths on component circulation. It is confirmed that the width change has more influence on the assembly yield than the height does. In fact, since all of the outliers in the assembly yield plots have small width (below 400 µm) and just a couple of them have tall heights (high aspect ratio, H/W), it is reasonable to conclude that the width has more impact than the height does. This is consistent with the fact that Eckart streaming is supposed to be effective in heights at least larger than 1 mm, and the tallest channel is 1 mm in height in this research. Figures 6.12 and 6.13 present micrographs of assembly sites after TASR experiments inside microchannels in different devices.
Figure 6.9. Assembly yield percentage versus oversupply inside of microchannels for a) the serpentine, and b) the spiral geometries when the width is the variable. Note: the red data points belong to oversupply (concentration) experiments.
Figure 6.10. Assembly yield percentage versus oversupply inside of microchannels for a) the serpentine, and b) the spiral geometries when the height is the variable. Note: the red data points belong to oversupply (concentration) experiments.
Figure 6.11. Assembly yield percentage versus oversupply inside one device to study the impact of oversupply under other constant experimental variables.
Figure 6.12. Dark field optical micrographs show an area of two serpentine microfluidic devices after TASR experiments were done at 45 V for 4 minutes with polystyrene microspheres of nominally 10 µm in diameter. Three images from a) to c) belong to device W4000H1000 and the other three images from d) to f) belong to device W200H100, respectively. Each group of three images show one column of assembly sites inside microchannels. Note: The shiny blue dots (marked with green circle) represent the filled sites and bright spots (marked with red circle) are empty sites. It is worth to mention that in device W4000H1000, the microchannels walls cannot be seen due to wide distance of the channel.
Figure 6.12. Continued.
Figure 6.13. Dark field optical micrographs show an area of two spiral microfluidic devices after TASR experiments were done at 45 V for 4 minutes with polystyrene microspheres of nominally 10 µm in diameter. Three images from a) to c) belong to device W930H1000 and the other three images from d) to f) belong to device W350H100, respectively. Each group of three images show one column of assembly sites inside microchannels. Note: The shiny blue dots (marked with green circle) represent the filled sites and bright spots (marked with red circle) are empty sites. It is worth to mention that in device W930H1000, the microchannels walls cannot be seen due to wide distance of the channel.
Figure 6.13. Continued.
6.5 Spatial dependence of assembly yield

The experiments above have primarily assessed the success of TASR-based directed assembly into assembly sites that lie near the centerlines of the microchannels. The experiments were designed this way to maximize the chances of successful device microfabrication. However, one can also ask whether the assembly yield depends on the locations of the assembly sites within the microchannels. For example, it is possible that the flow vortices induced by the acoustic transducer might prove more successful at transporting the components to the centerline of the microchannel rather than to its edges.

Answering the question of whether assembly yield depends on the locations of the assembly sites within the microchannels is important for two main reasons. First, in many applications there may be multiple components that need to be arranged in a particular spatial organization with respect to each other and to the microchannel walls. For example, in tissue engineering applications, multiple cell types may need to be assembled across the entire lower surface of the microchannel. This means that some components may need to be placed closer to the edges of the channels, and some may need to be placed closer to the centerline. Hence, it is crucial to know whether the assembly behaves differently at the channels’ edges compared to the center line. The second reason is that in practice, manufacturing tolerances may prevent precise alignment of the assembly sites on one surface of the chamber with the top and sides of the chamber. For example in the present experiments, the microfluidic devices are made out of two PDMS replicas as described in the experimental procedure chapter. One replica has the imprint of the channels, and the other one contains the assembly sites. The device is fabricated by aligning the two pieces and bonding them together. Since this alignment is done manually under an optical
steromicroscope, there is a good chance that the column of assembly sites may veer toward
the sides of the channels and not be placed right on the centerline of the channel. Therefore,
it is necessary and useful to know if the positioning of the assembly sites has had any
impact on the obtained results explained in previous sections, or if it may have an impact
on future research that utilizes TASR-based assembly in microchannels.

To answer this question, devices are intentionally fabricated in which the assembly
sites lie off the centerline of the channels. These devices are otherwise identical to the
devices in which the assembly sites lie on the centerline of the channels. This experiment
is done using device W500H1000 since it is large enough to differentiate the center line
from the edges and yet it is still at the sub-millimeter scale. The experiments are carried
out in the normal manner as described, except that the assembly sites are aligned close to
the edges of microchannels. Three devices are made with assembly sites positioned 50 to
120 µm away from channel edges. An average assembly yield of 68.3% ± 2.5% is achieved
for these devices, whereas the average yield percentage of the devices with center-aligned
assembly sites is 71%. These results suggest that the location of the assembly sites within
the channels has a negligible (at most a few percent) impact on the yield outcome. In other
words, this experiment provides strong evidence that TASR-based assembly results are
independent of the locations of the assembly sites within the channels. In addition, these
results confirm that the previous results can be considered to be accurate since they would
not have been significantly affected by the inevitable variations in the alignment process
during the fabrication of large numbers of devices. Figure 6.14 shows optical micrographs
of TASR-based assembly into sites close to the channels’ edges.
Selectivity refers to the ability of TASR or any other assembly process to locate components in different assembly sites based on which sites best match the components’ sizes and/or shapes. All of the experiments described above consider only the assembly of components into well-matched sites. To determine whether the assembly process in microchannels offers the same high degree of selectivity as is obtained in conventional TASR-based assembly, assembly is also attempted with components that are smaller than the assembly sites. If the assembly is appropriately selective, the smaller, more poorly-matched components will show a lower assembly yield than is observed for the larger, more well-matched components.

The selectivity experiments were carried out using nominally 6 µm diameter polystyrene microspheres (Corpuscular, Lot: 9601) in the same assembly medium using W200H100 devices. The average yield percentage is achieved as 6.7% ± 1.7. These near-zero yields demonstrate that the assembly of poorly-matched components can be prevented inside of microchannels, confirming that TASR offers good selectivity inside microchannels as well as in open environments.
Figure 6.14. Dark field optical micrographs show an area of serpentine microfluidic device W500H1000 after TASR experiments were done at 45 V for 4 minutes with polystyrene microspheres of nominally 10 µm in diameter. The column of assembly sites are positioned close to the microchannels wall edge (roughly 60 µm). These three images show one column of assembly sites inside microchannels. Note: The shiny blue dots (marked with green circle) represent the filled sites and bright spots (marked with red circle) are empty sites. Note: Bright blue drifts are remainder of microspheres inside assembly suspension after the experiment.
6.6 Summary

The potential implementation of TASR-based assembly inside microchannels was studied via a series of experiments inside microfluidic devices. The objective of this phase of the research was to investigate the effects that the shape, width, and height of the microchannels have on the assembly results, as well as to demonstrate how the results relate to more general considerations such as the component oversupply. Each device was made by bonding together two PDMS replicas, one that defines the channels and one that defines the assembly sites. The devices are designed with a range of microchannel widths and heights in two different microchannel layouts, serpentine and spiral.

The experimental results show that the assembly yield is largely independent of the microchannels’ layout (serpentine or spiral). The dominant factor that determines the assembly yield is shown to be the oversupply of assembly components. The oversupply is related to the channel geometry insofar as larger channel volumes (which correlate with larger channel widths and heights) can contain larger numbers of components at a given concentration. The assembly yield drops as the oversupply decreases, and in most cases this drop is independent of how the oversupply is achieved (by varying channel height, channel width, aspect ratio, or component concentration).

However, two deviations from this trend are observed. First, at very small values of oversupply value (below around 100), the drop in assembly yield is very dramatic. Second, small microchannel widths of <400 µm also produce a drop in assembly yield that deviates from what would have been predicted based on the dependence of oversupply on component concentration. This width dependence is attributed to inefficient circulation of
the components because the characteristic length scale of the channels (the width) is smaller than the length scale of the flow vortices that arise from the acoustic excitation. This observation is supported by the experimental and analytical findings of Jung (Jung, 2007) about the circulation patterns and the effective length scales of the circulation flows (≈ 350 µm). As a result, the microchannel width can be considered to be a second and independent factor that can reduce assembly yield relative to what would be expected in a macroscale assembly space. It is also confirmed that the assembly yield is independent of the locations of the assembly sites within the channels (i.e. centerline vs. edges), indicating that there is a significant design space over which different channels with diverse arrangement of assembly sites for potential applications may be created. Finally, the selectivity characteristics of TASR were demonstrated inside microchannels.

If the goal were to carry out TASR-based assembly in narrower (less wide) channels, it would be important to decrease the value of the minimum width below which the assembly becomes unsuccessful. There are some variables in the experimental setup that may be adjusted to modify the characteristic concentric flow pattern to achieve an efficient circulation of components. For example, looking at Eq. 6.1, one possible approach is to increase the frequency of the transducer since the wavelength gets smaller with increasing frequency. Decreasing the wavelength is expected to decrease the characteristic dimension of the flow circulation loops as well as the distance between successive rings in the concentric pattern. Another option would be to tune the medium’s composition such that the sound waves travel at lower speeds. This way, the numerator of Eq. 6.1 would become smaller, thereby reducing the characteristic sizes of the flow circulation loops and the distance between successive rings in the concentric pattern. The assembly fluid may need
to be selected to accommodate other requirements, such as keeping biological specimens alive. In addition, acoustic transducers may be designed for frequencies within a relatively large frequency range. It is therefore anticipated that modifying the transducer’s frequency will in practice offer a greater ability to reduce the minimum channel width below which assembly will become unsuccessful.

These results strongly support the potential for TASR to be implemented in microfluidic devices, with potential impacts for bioengineering application such as tissue engineering, cell trapping and isolation, and drug delivery and screening.
Chapter 7

CONCLUSION AND FUTURE WORK

As was discussed in previous chapters, one promising approach to build complex three dimensional systems such as can be required for tissue engineering is to integrate the formation of hierarchical architectures with assembly approaches. In other words, the assembly process can structure the system at the smallest length scales, while some other larger-scale process introduces structure or order at larger length scales. For example, the assemblies may be incorporated into or onto structures that exhibit some larger scale geometry or into microfluidic networks that control system behavior (in that case fluid flow) at larger length scales.

To that end, in this research the potential of a 2D directed assembly technique called templated assembly by selective removal (TASR) for creating more complex 3D and hierarchical systems was studied. The first phase of this research focused on the performance and limits of TASR-based assembly on non-horizontal surfaces such as may be found when a 2D surface is folded into the third dimension. The second phase evaluated the potential for implementing TASR-based assembly inside of microfluidic devices to enable the creation of more complex, hierarchical architectures. The resulting knowledge and achievements are summarized here.

TASR-based assembly on angled surfaces:

1. The analytical model of the TASR process was modified to capture the different physics that come into play for cases in which the substrate is tilted to a non-horizontal angle. The model predicts that for low angles of tilt, the selective assembly of components that are
well-matched in size and shape should be successful and should function much as it does for zero angle of tilt.

2. The analytical model also predicts that TASR will lose its selectivity as the template’s angle of tilt approaches the angle of the microsphere’s point of contact inside the assembly well. When the angle of tilt and the angle of contact align, the vertically-oriented fluid forces do not create a mechanical moment that promotes the removal of components from the assembly wells. Without a moment that acts to eject mismatched microspheres from the wells, the TASR process loses its selectivity. The selectivity is predicted to decline as the angle of tilt approaches the angle of contact, and it is predicted to be extremely low when the two angles are equal.

3. The experimental findings are in very good agreement with the model predictions. The results prove that TASR can successfully assemble microspheres into their corresponding-sized wells and can prevent assembly of microspheres into wells that are poorly-matched to the size of the microspheres, as long as the angle of tilt is small enough. (In the present experiments, performance declines by about 45°). In addition, the observed critical tilting angle of about 60° is consistent with the model predictions.

4. The combination of analytical models and experimental outcomes will enable predictions of TASR-based assembly to be made for the design of systems that require directed self assembly of diverse components into systems with complex geometries at larger size scales. These systems may include angled surfaces, folding surfaces and layer-by-layer architectures, and more complex 3D architectures such as curved substrates, which may found in bioengineering and metamaterials applications.
**TASR-based assembly within microfluidic channels:**

1. The ability to carry out TASR-based assembly inside of microfluidic channels was successfully demonstrated using microfluidic devices with a variety of different shapes (serpentine and spiral), widths, heights, and well locations. TASR was also successfully demonstrated under a range of different assembly conditions, including different ratios of the number of available components to the number of assembly sites to be filled (i.e. different values of oversupply).

2. The experimental results demonstrate that the assembly yield is strongly affected by the oversupply inside the microfluidic channels. Larger values of oversupply result in higher values of assembly yield, with the assembly yield reaching approximately 90% for oversupply values of 5420. The assembly yield drops gradually to 64% as the oversupply drops to about 450. When the oversupply falls below 100, the assembly yield drops precipitously.

3. The width of the microfluidic channels is shown to be a second determinant of assembly yield, and its effects are independent of the effects of oversupply. When the width of the microchannels is smaller than 400 µm, the assembly yield drops precipitously. This critical width is equal to half of the wavelength of the acoustic waves that both drive component circulation and provide the removal moments that dislodge components from mismatched assembly wells. The abrupt drop in assembly yield when the width drops beneath half of the wavelength of the acoustic excitation is attributed to the fact that the microchannel can no longer accommodate the full width of the out-of-plane flow vortices that drive component circulation. The resulting insufficient circulation limits assembly yield. To successfully carry out TASR-based assembly in narrower microchannels, experimental
variables such as the acoustic frequency and assembly medium composition can be tuned to shorten the characteristic length scale of the flow circulation loops.

4. It is also shown that the assembly yield is largely independent of the microchannels’ in-plane geometry (serpentine or spiral), their heights (for given value of oversupply), and location of the wells within the microfluidic channels. These findings offer promising opportunities for structuring hierarchical systems in which small scale components or cells are organized within the larger scale structure of a microfluidic system. This ability offers strategies for potential tissue engineering, cancer cell isolation and drug delivery systems and screening applications.

Based on the findings of both phases of this research, several promising avenues for future work can be identified:

- The research described here has examined the capabilities of the TASR process using polymer microspheres as convenient models for how biological cells will assemble in the future. Although the ability to selectively assemble biological cells using the TASR process has been demonstrated previously (Agarwal & Livermore, 2011), the present results should be confirmed using cells prior to their use in designing biological systems.

- Origami folding offers the opportunity to convert 2D arrangements of structures into 3D arrangements of structures, for example for the creation of metamaterials or for the engineering of 3D tissues. Combining TASR’s ability to organize cells in 2D (including on angled surfaces) with the ability to form larger-scale 3D architectures via origami folding offers the opportunity to engineer 3D tissues. One potential future path is to design origami-folded 3D polymer scaffolds and to use
TASR to seed them with the appropriate cells types in biomimetic arrangements. For example, TASR-based assembly of cells onto a scaffold from liquid may be combined with self-actuated (e.g. residual stress driven) folding of the scaffold to create a final 3D tissue. The ability of TASR to assemble onto angled surfaces would permit the seamless integration of directed assembly with liquid-triggered self-folding to create complex tissues.

- The proposal above would create tissues with two levels of hierarchical structure: cell placement and folded architecture. However, many tissues require a third level of hierarchical structure in which cells interact directly and closely through contact with their nearest neighbors. To meet this requirement, the assembly plus folding approach described above may be adapted to assemble microtissues (small spheres comprising cells and extracellular matrix) instead of individual cells.

- Systems such as optical metamaterials can benefit from incorporating components that are not spherical (e.g. cylinders or ellipsoids). The assembly of these types of components onto the angled surfaces of a multilayered folding system may be a useful direction for future study.

- A TASR-based microfluidic device may prove valuable for capturing tumor cells from flow. A useful direction for future study would be to examine how the research on TASR-based assembly within microfluidic devices may be adapted to the capture of CTCs and to assess the level of sensitivity and specificity that may be expected from such a system.

- The research demonstrated to date has focused entirely on assembling structures onto robust materials that do not readily degrade in the human body. However, it
would also be valuable to examine the potential for TASR-based structuring of biological systems in which the scaffold is intended to biodegrade over time, leaving only the cells and extracellular matrix. For example, one could design TASR-based microfluidic devices or folding systems out of biodegradable polymers such as poly(glycerol sebacate) PGS. Then one could demonstrate TASR-based assembly of microtissues or cells onto these structures and study the evolution of the system as the polymer degrades to leave behind the structured arrangements of cells.

### 7.1 Transition from the current research to practical tissue engineering

Both phases of this research showed the potential of using TASR and folding together to fabricate 3D tissue constructs. According to our findings in this research, if one wants to use this process for tissue engineering, the below items should be considered as general steps.

1. **Scaffold materials:** It is needed to have a substrate, to be able to organize the architecture of the cells, cell aggregates, microtissues or tissue modules. In fact, a surface is needed to arrange the cells on to control their adjacencies and mimic the final target tissue. There is a broad range of biodegradable polymers that can be chosen. However, it is important to know that any candidate material should have the process ability to have small features or shapes created in its surface.

2. **Preparation of tissue modules or cells:** Based on the target tissue chosen, it should be decided whether the assembly should be done with individual cells or with tissue modules. Once the decision is made, it is also required to know the shapes and sizes in which those tissue modules are prepared. This would directly impact the
microfabrication processes required to create the scaffolds with the necessary feature sizes and shapes.

3. Fabrication of microscale features on the scaffold: It is required to create small features (mostly at the microscale) in the chosen scaffold material since predefined locations for cells and tissue modules are required. Depending on the shape and size of the cells or modules (which may have different sizes or shapes, e.g., spherical, cylindrical, ellipsoidal and etc.), the fabrication method can be different. In general, it is suggested to use microfabrication processes such as photolithography and etching techniques on silicon, quartz, silica and/or PDMS or any other substrates because this family of techniques may create a wide range of assembly well sizes and shapes. These techniques are also very powerful in creating large-scale, arbitrary organizations of features. Other important features to be created in the microfabrication process are folding lines or creases that will guide the folding of the scaffold. This means that whatever the folding plan is, the crease features should be created at some point during the microfabrication process. This is true whether the fold is going to be done manually or via self folding.

4. Making replicas of scaffold materials from the microfabricated substrate: Once the required features are created in the substrates via microfabrication processes, the features can be imprinted into the scaffold materials by making replicas of the master substrates in biopolymers. Depending on what scaffold polymer is chosen, the replication process may be different. For example, the chemical or physical cross linking process might be different for different biopolymers.
5. Folding the scaffold: According to the designed folding process, this step can be completed either manually or automatically (self-folding polymers or processes).

6. Performing directed assembly of tissue modules on scaffold: To assemble tissue modules into the predefined features on scaffolds, there should be a large enough number of assembly components (cells or tissue modules) suspended in the medium. Also, this process can be done at different applied voltages (i.e. different acoustic excitation intensities), but there is an optimal voltage at which the assembly yield or outcome reaches its maximum. This optimal voltage can vary between different systems depending on the medium composition, tissue or cell module size and shape, etc. As a result, it is highly recommended that this voltage is experimentally found through some preliminary tests for a given system (module shape and size and medium composition). The assembly usually takes a few minutes to reach high yield, but this can be also considered a variable that can be adjusted to reach the highest possible assembly yield.

7.2 Case study: Fabricating artificial 3D heart tissue

This section provides the outline of a platform for fabrication of a target tissue, namely heart tissue. The process outlined here takes into account the effects of varying the process conditions studied in this work and makes a recommendation for how appropriate process conditions may be combined to produce the desired tissue output.

Heart muscles are anisotropically elongated in one direction to perform their functions. Also, heart has a dense and well-packed structure of muscles layered atop each other. Hence, it seems an interesting idea to use the directed assembly to define the location of cardiac cells in the desired direction and also to use the folding process to stack those
muscles on top of each other to create a hierarchical 3D structure. Table 7.1 summarizes the required materials, cells and assembly parameters that are suggested to carry out this particular tissue engineering example process. These selections are suggested as starting parameters to ensure the best final performance in the targeted tissue (heart). However, depending on the biological or physiological response of the tissue, these parameters should be experimentally optimized accordingly. In other words, the table information initially provides a set of values for the variables (materials, type, size, and etc.), but based on the tissue engineering process development and observations, the parameters may need to be adjusted. For examples, parameters such the scaffold material, the tissue spheroids’ size, the architecture of the tissue spheroids, the oversupply of the spheroids in the suspension, and the applied voltage (which controls the acoustic intensity) can be tailored for each case.
Table 7.1. The suggested list of required parameters and their specifications for cardiac tissue engineering.

<table>
<thead>
<tr>
<th>Work step</th>
<th>Suggested materials or process</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scaffold selection</td>
<td>PGS, PLGA</td>
<td>Biodegradable, suitable for soft tissues, can be molded and cured for polymerization.</td>
</tr>
<tr>
<td>Microtissue module</td>
<td>There are two types of cells,</td>
<td>Each cell type or a combination of them (with different ratios) can be embedded inside hydrogels using common microfluidic devices which are being used for fabrication of spheroids of cells with different sizes (e.g. sizes mentioned in Figure 7.1). It is recommended to make cardiac spheroids with 30 µm in diameter which can contain 2-3 cardio myocytes. Also, cardiac fibroblasts can be made in spheroids of 15 µm or in individual cell form (Kang, 2014). It is also recommended to provide an oversupply of 460 at minimum. Considering the nominal area of each scaffold plane (1cm²) and total number of planes (10), there is a need of $2.8 \times 10^6$ and 650,000 spheroids for cardiac myocytes and fibroblasts, respectively.</td>
</tr>
<tr>
<td>preparations</td>
<td>cardiac myocytes (MCs) and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cardiac fibroblasts (CFs)</td>
<td></td>
</tr>
<tr>
<td>Fabrication of microscale features</td>
<td>Microfabrication on silicon wafer, then, making master mold with PDMS</td>
<td>Create small features according to the suggested pattern in the schematic shown in Figure 7.1 and repeat it in a large area such as 1 cm². Two different sizes of features may be nominally 15 and 30 µm in diameter with pitch size of approximately 5 µm.</td>
</tr>
<tr>
<td>-----------------------------------</td>
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<tr>
<td>Folding process</td>
<td>A number of folds can be tried and adjusted according to tissue engineering or physiological behavior of the constructed cardiac tissue.</td>
<td>The folding lines can be designed in microfabrication steps to create simple accordion type folding. For example, to accordion fold of 10 planes, there should be 9 folding lines in large scale.</td>
</tr>
<tr>
<td>Scaffold replicas made from the PDMS master mold</td>
<td>The PDMS has the negative features of the final scaffold. By molding the scaffold polymer on PDMS replica, the final hemispherical shapes are imprinted on the scaffold.</td>
<td>The prepolymer is molded on the PDMS master mold, and it can be cured in different ways depending on the chemistry of the bio polymers (e.g. UV light curing or heating, or adding curing agent chemicals).</td>
</tr>
<tr>
<td>TASR assembly</td>
<td>Use ultrasound transducer of 1.7 Megahertz acoustic waves for 3 to 5 minutes duration, at applied voltage of 45V.</td>
<td>The folded scaffold structure can be placed inside a beaker containing the microtissue spheroids and the test is run. The duration and applied voltage can be adjusted depending on the size of microtissues (bigger spheroids need higher voltages) and dimensions of the scaffold (larger areas, need longer assembly time to assure the high yield)</td>
</tr>
</tbody>
</table>
Figure 7.1. This carton shows a possible design for mimicking the heart (cardiac) tissue. There are two types of features attributed to two different microtissues (or cells), cardiac myocytes and cardiac fibroblasts. The current sizes shown in the figure are suggesting to use hydrogel spheroids containing those cells and then doing the TASR with the microtissues. This architecture can be repeated in plane with similar pitch size. This configuration of cells covers almost 50% of the surface at the start of assembly but the cells inside spheroids are supposed to grow and proliferate after the assembly and connect to each other to not only cover a bigger surface area but also form a similar pattern as real cardiac tissue. The suggested area of each scaffold plan is 1 cm² and this architecture can be approximately repeated for 33 times (3 vertically by 11 horizontally) to cover such an area.


cardiac fibroblasts. *PLOS ONE, 13*(5), e0196714. doi:10.1371/journal.pone.0196714


plasmonic gallium nanoparticles. *Journal of the American Chemical Society, 131*(34), 12032-12033.


