Chapter 1

General Introduction

1.1 Microfabrication Technology

Microfabrication

Photo-microfabrication became a valuable and reliable technique of manufacturing integrated electronic systems in electronics in the early 1960s. The milestone events in electronics were the invention of the transistor in 1947 and the development of the integrated circuit (IC) in 1958. Photo-microfabrication (Fig. 1.1) primarily consists of photolithography and chemical etching, enabling one to precisely fabricate micron-scale structures (Koch *et al.*, 2000; Maluf, 2000; McCreedy, 2000; Madou, 2002a). Silicon and silicon compounds are used as the substrate in photo-microfabrication. However, due to the drawbacks of traditional wet (liquid-phase) etching, photo-microfabrication had been used to fabricate two-dimensional (2D) microstructures. Recently developed deep reactive ion etching (DRIE) in dry (plasma-phase) etching (Maluf, 2000) realized the precise cutting of channels and holes with high aspect ratios (height-to-width ratio), which is applicable to fabricate three-dimensional (3D) microstructures. Some of the other most important fabrication techniques are molding, embossing and micromachining with laser, electrochemical or ultrasonic technologies (Chován and Guttman, 2002). Besides the commonly used silicon and glass wafers, polymer-based materials, such as polydemethylsiloaene (PDMS), polymethyl-methacrylate, polycarbonate, and teflon are currently applicable to microfabrication (Chován and Guttman, 2002).

Another microfabrication technology is LIGA (Lithographie Galvanoformung Abformung, in German), which combines X-ray lithography, electrodeposition, and molding, as illustrated in Fig. 1.2 (Becker *et al.*, 1982; Koch *et al.*, 2000; Maluf, 2000; Madou, 2002b). The capacity of LIGA for creating a wide variety of shapes from different materials makes it akin to the preceding microfabrication techniques with the added benefit of unprecedented aspect ratios and absolute tolerances. The final moulding step in LIGA process eased the replication of microstructures and made their manufacturing low cost (Becker *et al.*, 1982, Madou, 2002b).

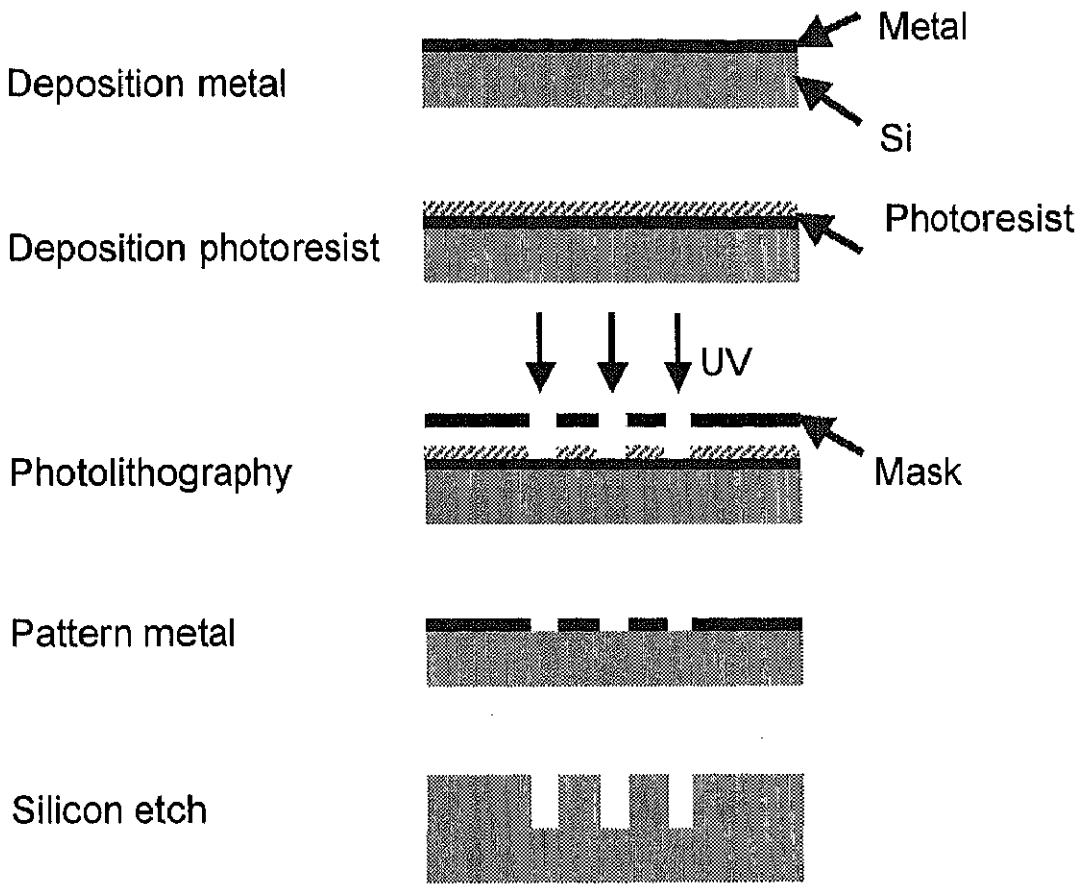


Fig. 1.1 Illustration of a basic photofabrication process.

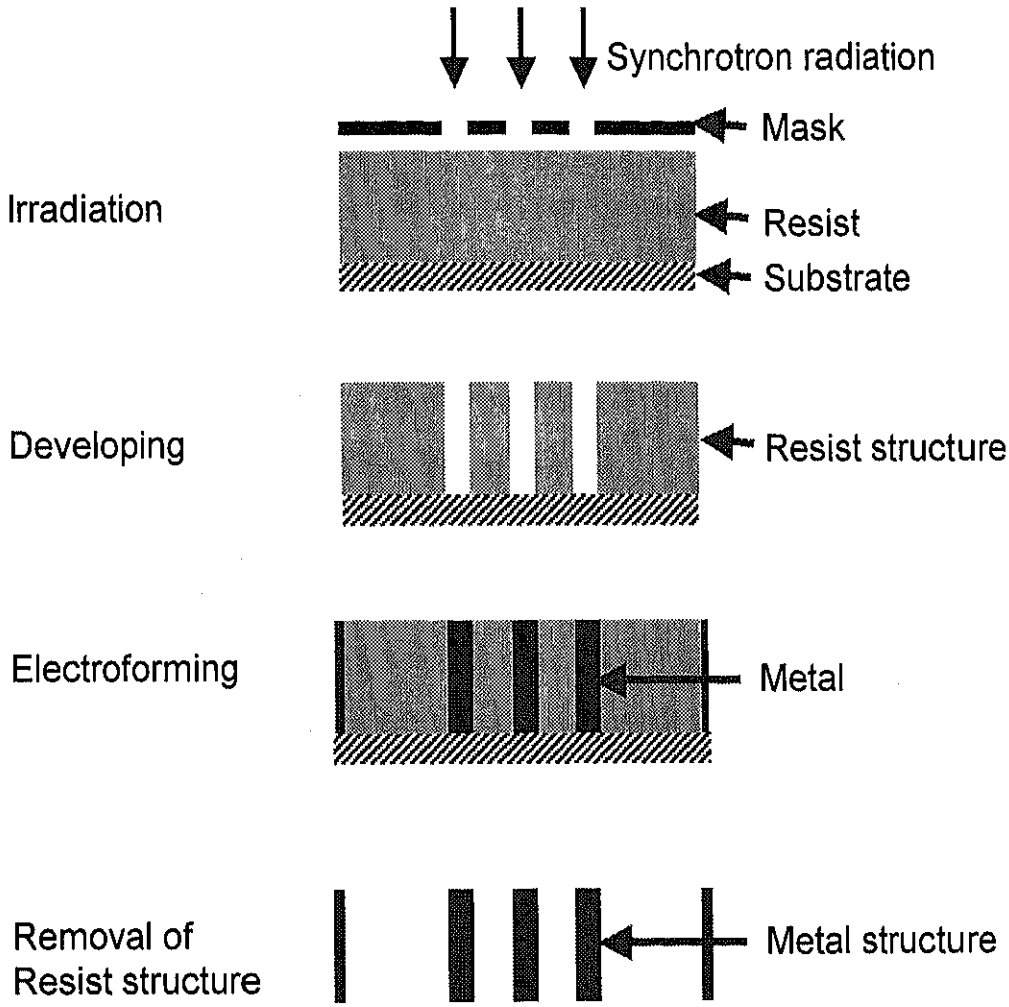


Fig. 1.2 Illustration of a basic LIGA process.

Microelectromechanical systems (MEMS)

Micromachining is the set of design and fabrication tools that precisely machine and form micron-scale 3D structures and elements (Maluf, 2000). The birth of the first micromachined element dates back many decades, e.g., a micromachined pressure sensor (Tufte *et al.*, 1962), whereas integration of the micromachined elements was not so easy. Remarkable progress of micromachining over the last decade allowed the manufacture of embedded microsystems involving one or many micromachined elements or structures, referred to as microelectormechanical systems (MEMS) (Koch *et al.*, 2000; Maluf, 2000; Gad-el-Hak, 2002). The earliest MEMS devices are microsensors, microactuators, and micromotors (Sugiyama *et al.*, 1983; Trimmer and Gabriel, 1987; Bart *et al.*, 1988; Fujita *et al.*, 1989). The science and technology of MEMS have been explosively grown during the last decade. Promising MEMS applications in microelectronics and micromechanics include high-resolution display, high-density data storage devices, and valves (Maluf, 2000). Worldwide market projections for MEMS devices in multidisciplinary fields tend to be optimistic, reaching 30$ billion by the year 2004 (Gad-el-Hak, 2002).

MEMS applications in (bio)chemical, biotechnological, and medical fields

Microfludic devices manufactured by MEMS fabrication techniques have applications in multidisciplinary fields (Koch *et al.*, 2000). In particular, a number of promising microfludic applications has been proposed in (bio)chemical, biotechnology, medical fields during the last decade. The typical microfludic applications in the fields include DNA amplification by polymerase chain reactions (Kopp *et al.*, 1998), DNA and protein analysis (Burns *et al.*, 1998; Chou *et al.*, 1999; Rocklin *et al.*, 2002), capillary electrophoresis (Harrison *et al.*, 1993; Jacobson *et al.*, 1994), liquid/gas chromatography (Manz *et al.*, 1990), inkjet printer nozzles (Koch *et al.*, 2000; Tseng, 2002), and (bio)chemical reactions (Ehrfeld *et al.*, 2000; Haswell and Skelton, 2000). The potential benefits of microfludic devices are reduced consumption of expensive samples and reagents, reduced amount of wastes, shorter reaction and analysis times, portability that allows in situ and real-time

analysis, greater sensitivity, disposability, and scale-up by numbering-up that reduces the cost of redesign and pilot plant experiments. Microfludic devices also have distinct properties as a result of their small dimensions as follows. Surface tension and viscosity become dominant instead of gravity and inertia. Laminar flow and high surface-to-volume ratio lead to fast diffusion-based mixing. Fluidic manipulation in a microchannel was conducted by elctro-osmotic driven force, capillary action, and elctrocapillary pressure (Sato *et al.*, 1999; Schasfoort *et al.*, 1999; Prins *et al.*, 2001). New microfludic applications, which exploit the preceded properties, have been developed, e.g., blood rheology measurements (Kikuchi *et al.*, 1992, 1994), transfers of small volumes (0.5 to 100 nl) of materials (Hosokawa *et al.*, 1999; Sammarco and Burns, 1999), micromixing (Ehrfeld *et al.*, 1999, 2000), chaotic mixing in a microchannel (Stroock *et al.*, 2002), and diffusion-based separation and detection (Weigl and Yager, 1999). The preceded microfludic devices are currently fabricated using metals, ceramics, and polymers (Ehrfeld *et al.*, 2000; Duffy *et al.*, 1998; Unger *et al.*, 2000; Beebe *et al.*, 2000; Knitter *et al.*, 2001).

1.2 Emulsion

Definition

An emulsion is a dispersion of small spherical droplets of one of the immiscible liquids (usually oil and water) in the other. Emulsions and emulsion-based products have been utilized in various industries, which include food, cosmetics, pharmaceuticals, and chemical industries. The droplet diameters of emulsions generally range somewhere from 0.1 to 100 μm (Dickinson, 1992; Walstra, 1996). Emulsions systems can be conveniently classified according to the distribution of the oil and aqueous phases. The simplest systems are an oil-in-water (O/W) emulsion composed of oil droplets dispersed in an aqueous phase (e.g., mayonnaise, milk, cream, and sauces) and water-in-oil (W/O) emulsion composed of water droplets dispersed in an oil phase (e.g., margarine, butter, and fat spreads). The substance that makes up the droplets in an emulsion is called the

dispersed or internal phase, whereas the substance that makes up the surrounding liquid is referred to as the continuous or external phase. Besides the preceded simple emulsions, it is also possible to prepare multiple emulsions, e.g., (W/O/W) and (O/W/O) emulsions (Dickinson and McClements, 1995). For instance, a W/O/W emulsion consists of water droplets dispersed within larger oil droplets, which are themselves dispersed in an aqueous continuous phase (Evison *et al.*, 1995). Recently, research has been conducted to create stable multiple emulsions which can be used to control the release of certain ingredients, reduce the total fat content of emulsion-based food products, or isolate one ingredient from another (Dickinsion and McClements, 1995; Matsumoto, 1987, 1989; Pays *et al.*, 2001).

Emulsions are thermodynamically unstable systems (Israelachivil, 1992), so that emulsions prepared by a pure oil/pure water system rapidly separate into a system, which consists of a layer of oil (lower density) and a layer of water (higher density) (Taylor, 1998). Kinetically stable (metastable) emulsions can be prepared by incorporating surface-active agents and/or thickening agents prior to emulsification. Surface-active agents, such as small molecule surfactants and protein, are amphilic molecules containing both a polar region with an affinity for water (hydrophilic) and a nonpolar region with an affinity for oil (lipophilic). Adsorption of surface-active agents at the phase interface enhances emulsion formation by diminishing interfacial tension and stabilizes the formed emulsions by inducing a repulsive force between droplets (Dickinson, 1992).

Droplet size and size distribution

The droplet size and size distribution of an emulsion determines many of the most important properties of emulsion-based products (e.g., shelf life, appearance, texture, and flavor in foods). Stability and resistance to creaming, rheology, chemical reactivity, physical properties, and physiological efficiency depend on their droplet size and size distribution (McClements, 1999; Mason *et al.*, 1996; Orr, 1996). If all the droplets in an emulsion are of the same size, the emulsion is referred to as monodisperse, but if there is a range of sizes present, the emulsion is referred to as polydisperse. Most of

emulsion-based products are polydisperse emulsions. Ideally, one would like to have information about the full droplet size distribution of an emulsion. However, knowledge of the most useful numbers, which are the average droplet size of emulsions and the width of the distribution, is sufficient in many situations (Hunter, 1986). The average diameter is a measure of the central tendency of the distribution, and the standard variation is a measure of the distribution. The coefficient of variation as a percentage is a measure of the distribution relative to the average diameter. And it is defined as one hundred times the standard deviation divided by the average diameter. The above average is also referred to as the average length diameter because it represents the sum of the length of the droplets divided by the total number of droplets.

Monodisperse emulsion

Monodisperse emulsions are of important in both scientific and industrial fields. They are useful for fundamental studies because the intepretation of experimental measurements inherent polydisperse emulsions can be avoided (McClements, 1999; Mason *et al.*, 1996a,b). For example, the stability of monodisperse emulsion droplets can be monitored very simply, since changes in the droplet size are easily discernible. In addition, resultant properties of a monodisperse emulsion are most easily studied due to the same Laplace pressure difference of the droplets. Their same Laplace pressure difference contributes to reduce Ostward ripening, which broadens the size distribution (Taylor, 1998). The Laplase pressure is defined as follows (Adamson, 1982):

$$\Delta P = 4\gamma / d_{av} \qquad (1.1)$$

where ΔP is the Laplace pressure in N/m^2, d_{av} is the average droplet diameter in m, and γ is the interfacial tension in N/m.

The better stability and simplified physicochemical properties of monodisperse emulsions have also attracted various industrial fields that produce valuable emulsion-based products, such as functional microparticles, and microcapsules and multiple emulsions. Microparticles and microapsules in the emulsion-based products are

prepared through the processes, such as a melt dispersion method (Reithmeier *et al.*, 2001), suspension polymerization (Omi *et al.*, 1995), and solvent evaporation (O'Donnell and McGinity, 1997; Cleland, 1998). Their particle size distribution prepared through the above-mentioned processes was greatly influenced by the droplet size distribution of the emulsions as precursor. It is therefore required to develop an emulsification device for the production of monodisperse emulsions.

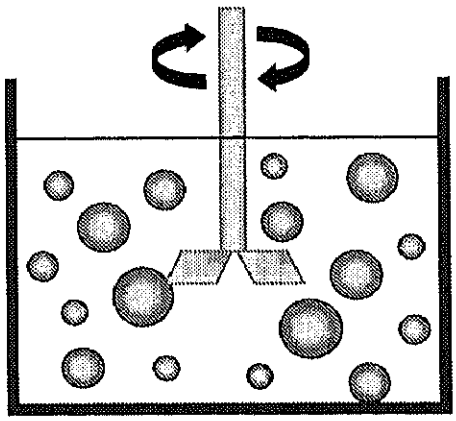
1.3 Emulsification Devices

Mechanical emulsification devices

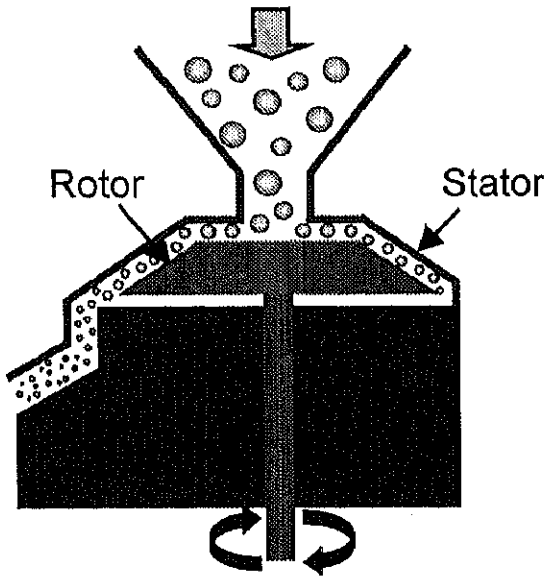
Many different types of mechanical emulsification devices have been developed to produce emulsions. Laminar, turbulent, and cavitational flows in the dispersing regime of the emulsification devices caused by the large shear forces disrupt one of two immiscible fluids into smaller droplets, which disperse in a continuous phase fluids (Schubert and Armbruster, 1992; Walstra, 1993; Karbstein and Schubert, 1995; McClements, 1999). Droplet disruption occurs when the deforming forces locally exceed the shape-preserving interfacial forces, which is characterized by the Laplace pressure. The re-coalescence rate of disrupted droplets, which is a parameter in affecting the resultant droplet size, depends on the surfactant adsorption and droplet-droplet collision kinetics (Stang *et al.*, 1994; Karbstein and Schubert, 1995). The most important types of emulsification devices are reviewed below.

High-speed blenders (Fig. 1.3a) are the earliest type of devices, which directly emulsify oil and aqueous phases (Schubert and Armbruster, 1992; McClements, 1999). High-speed blenders are particularly useful for preparing coarse emulsions with low or intermediate viscosities. The rapid rotation of the blade at typical speeds of 20 to 2,000 rpm in vessels of a few cubic centimeters to several cubic meters generates a combination of longitudinal, rotational, and radial velocity gradients in the liquids which breaks up an oil/water interface into large droplets and the larger droplets into smaller droplets (Fellows, 1998; McClements, 1999). The applied average energy input per unit volume, i.e., energy

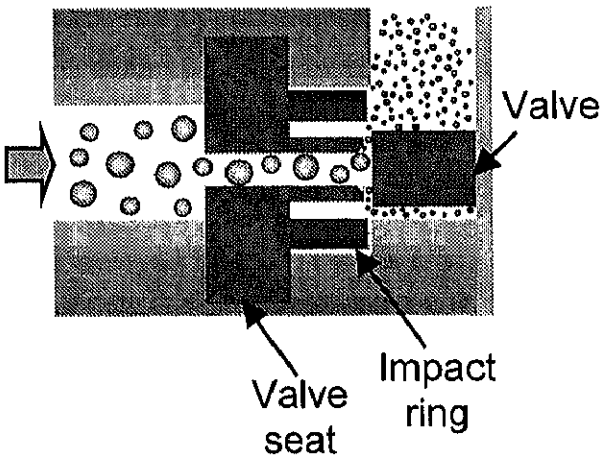
a. High-speed blender



b. Colloid mill



c. High-pressure homogenizer



d. Ultrasonic homogenizer

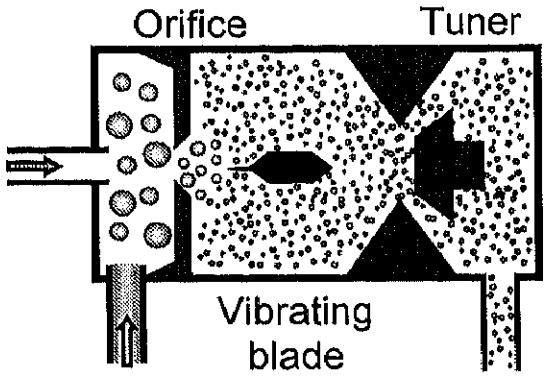


Fig. 1.3 Illustration of typical mechanical emulsification devices.

density, in the high-speed blenders is very low (Walstra, 1993). The emulsion droplets prepared by a high-speed blender typically have diameters between 2 and 10 μm.

Colloid mills (Fig. 1.3b) are the most suitable devices for emulsifying medium- and high-viscosity fluids with viscosities greater than 20 mPa s (Schubert and Armbruster, 1992; McClements, 1999). The course emulsions usually produced by a high-speed blender are fed into the colloid mill, and flow through a narrow gap between the rotor and the stator (typically from 50 to 3,000 μm). The rapid rotation of the rotor at speeds from 1,000 to 20,000 rpm generates a shear stress in the gap which caused the larger droplets to be disrupt into smaller ones. Colloid mills can produce emulsions with droplet diameters between 1 and 5 μm and throughputs between 4 and 20,000 l/h.

High-pressure homogenizers (Fig. 1.3c) are the most commonly used emulsification devices for producing fine emulsions with droplet diameters as small as 0.1 μm (Schubert and Armbruster, 1992; Pandolfe, 1995; McClements, 1999). They are most suitable for low- and intermediate-viscosity materials particularly when a small droplet size is required. A coarse emulsion is forced to flow into the radial space between a valve piston and a valve seat (typically from 10 to 300 μm) through a center hole of the valve seat. During the emulsification process, the larger droplets were broken down into smaller ones attributable to a combination of intense shear, cavitational, and turbulent flow conditions. Industrial high-pressure homogenizers allow the throughput of 100 to 50,000 l/h at homogenization pressures between 3 and 20 MPa.

Ultrasonic homogenizers (Fig. 1.3d) have been widely used in both industry and research (Sharman, 1968; McClements, 1999). High-intensity ultrasonic waves in the device generate intense shear and pressure gradients within a material, then disrupting the droplets mainly due to cavitational effects. Laboratory-scale piezoelectric transducers and industrial-scale liquid jet generators are commonly used to generate high-intensity ultrasonic waves (McClements, 1999). The minimum droplet sizes of emulsions produced by ultrasonic homogenizers are about 0.1 μm for piezoelectric transducers and 1.0 μm for liquid jet generators, respectively. Commercial devices enable one to produce

emulsions with throughput of 60 to 300,000 l/h using ultrasonic waves with frequencies of 20 to 50 kHz.

Membrane emulsification

Membrane emulsification developed by Nakashima *et al.* (1991) has received much attention as a potential technique for producing monodisperse emulsions with the lowest coefficients of variation of about 10%. A microporous glass membrane, called Shirasu porous glass (SPG) membrane, of a uniform pore size was originally used as the emulsification device (Nakashima *et al.*, 1987, 2000). An emulsion is formed by forcing the dispersed phase into the continuous phase through the membrane pores (Fig. 1.4). The droplet size of emulsions produced by membrane emulsification depends primarily on the membrane pore size. Uniform porous glass (Nakashima *et al.*, 1991; Kandori *et al.*, 1991; Vladisavljevic and Schubert, 2002), ceramic (Schröder and Schubert, 1999; Joscelyne and Trägårdh, 1999), and polypropylene hollow fiber (Vladisavljevic *et al.*, 2002) membranes have been used to produce O/W and W/O emulsions with droplet sizes of 0.3 to 30 μm under suitable operating conditions. The membrane surface must be sufficiently wetted by the continuous phase fluid in order to produce monodisperse emulsions by membrane emulsification (Nakashima *et al.*, 1993, 2000; Yuyama *et al.*, 2000). For example, a SPG membrane with hydrophilic surface due to silanol group can stably produce an O/W emulsion without any membrane treatment. In contrast, the surface treatments of a SPG membrane by silane coupler reagents (Kandori *et al.*, 1991) or by immersion in the oil phase (Katoh *et al.*, 1996) have to be conducted to produce a W/O emulsion.

Membrane emulsification requires small shear stress and low energy input to prepare emulsions (10^4 to 10^6 J/m^3) than conventional emulsification devices (10^6 to 10^8 J/m^3) (Schröder *et al.*, 1998). The droplet formation process from membrane pores has been analyzed by several research groups (Schröder *et al.*, 1998; Peng and Williams, 1998). They identified the resultant droplet size by calculating overall forces acting on the

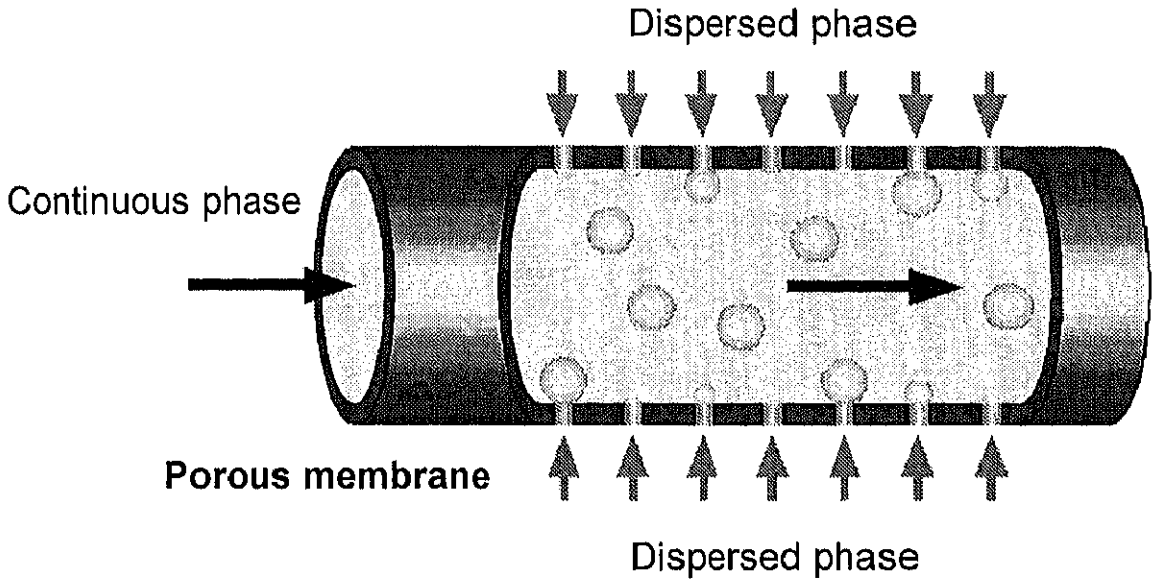


Fig. 1.4 Illustration of a membrane emulsification process.

droplets that grow at the pore tip. They also reported that the resultant droplet size depends on the continuous-phase flow velocity, the pore size, the transmembrane pressure, and the dynamic interfacial tension. Abrahamse *et al.* (2001) carried out CFD (Computational Fluid Dynamics) simulation of the droplet formation process from a circular membrane pore.

Several research groups attempted to improve low throughput of an emulsion due to some restricted process conditions in membrane emulsification. Suzuki *et al.* (1996, 1998) proposed a high-throughput membrane emulsification using coarse emulsions as the continuous phase, which can achieve maximum dispersed phase flux up to 10 $m^3/(m^2\ h)$. Katoh *et al.* (1996) reported that a hydrophilic membrane pre-treated by immersion in the oil phase allowed the high-throughput production of a W/O emulsion one hundred times the dispersed phase flux in membrane emulsification using a hydrophobic membrane.

The preceded features in membrane emulsification have attracted various industrial fields that produce emulsions and emulsion-based products. The W/O membrane emulsification process using a hydrophilic SPG membrane pre-treated by immersion in the oil phase was commercialized to produce a very low fat spread (Katoh *et al.*, 1996). Mine *et al.* (1996) prepared monodisperse O/W, W/O, and W/O/W emulsions stabilized by egg yolk phospholipids. Stable W/O/W emulsions for arterial injection chemotherapy were prepared by double membrane emulsification (Higashi *et al.*, 1995, 1999). Polymeric microcapsules and microspheres for drug delivery vehicles have been prepared using either O/W or W/O membrane emulsification (Muramatsu and Kondo, 1995; Shiga *et al.*, 1996; Muramatsu and Nakauchi, 1998; Ma *et al.*, 1999, You *et al.*, 2001). In chemical field, a number of functional polymer microcapsules and microspheres with coefficients of variation of about 10% have been successfully prepared (Nakashima *et al.*, 1994; Omi, 1994; Omi *et al.*, 1995, 1999; Hatate *et al.*, 1995, 1997; Yoshizawa *et al.*, 1996a,b; Kage *et al.*, 1997a,b).

Microchannel (MC) emulsification

Kawakatsu *et al.* (1997) recently proposed microchannel (MC) emulsification to form monodisperse emulsion droplets using MC arrays precisely fabricated on a single-crystal silicon microchip. The silicon microchip including MC arrays, called a silicon MC plate, was originally developed as a capillary vessel model for blood rheology measurements and analysis (Kikuchi *et al.*, 1992, 1994). MC emulsification can form emulsion droplets by forcing the dispersed phase into the continuous phase through the uniformly sized channels (Fig. 1.5). Monodisperse emulsion droplets with diameters of 10 to 100 μm and coefficients of variation below 5% have been successfully formed (Kawakatsu *et al.*, 1999; Kobayashi *et al.* 1999; Sugiura *et al.*, 2002a). A silicon cross-flow MC plate allowed the continuous preparation of monodisperse emulsions (Kawakatsu *et al.*, 1999, 2000). An earlier study on the surfactant effect in MC emulsification verified that the channel surface must be sufficiently wetted by the continuous phase fluid in order to stably form monodisperse emulsion droplets (Tong *et al.*, 2000). Hydrophobic MCs pre-treated by silane coupler reagents were used to prepare monodisperse W/O emulsion droplets (Kawakatsu *et al.*, 2001a; Sugiura *et al.*, 2001a). Stability characteristics of monodisperse emulsions prepared by MC emulsification were studied (Liu *et al.*, 2001).

The MC emulsification process does not require any turbulent mixing (Kawakatsu *et al.*, 1997). Furthermore, MC emulsification has super energy efficiency exceeding 50% (Sugiura et al., 2001b), whereas high-pressure homogenizer has low energy efficiency less than 0.1% (Walstra, 1983). Optical observations of the MC emulsification process enabled by a microscope video system (Kikuchi *et al.*, 1992) provide useful clarification of the droplet formation behavior. Sugiura *et al.* (2001b) observed the droplet formation process from the channels using a microscope high-speed camera system and proposed interfacial tension-driven droplet formation mechanism from the channels with an elongated section. The model for predicting droplet diameter in MC emulsification was also proposed, based on the droplet formation mechanism and on experimental observation (Sugiura *et al.*, 2002b).

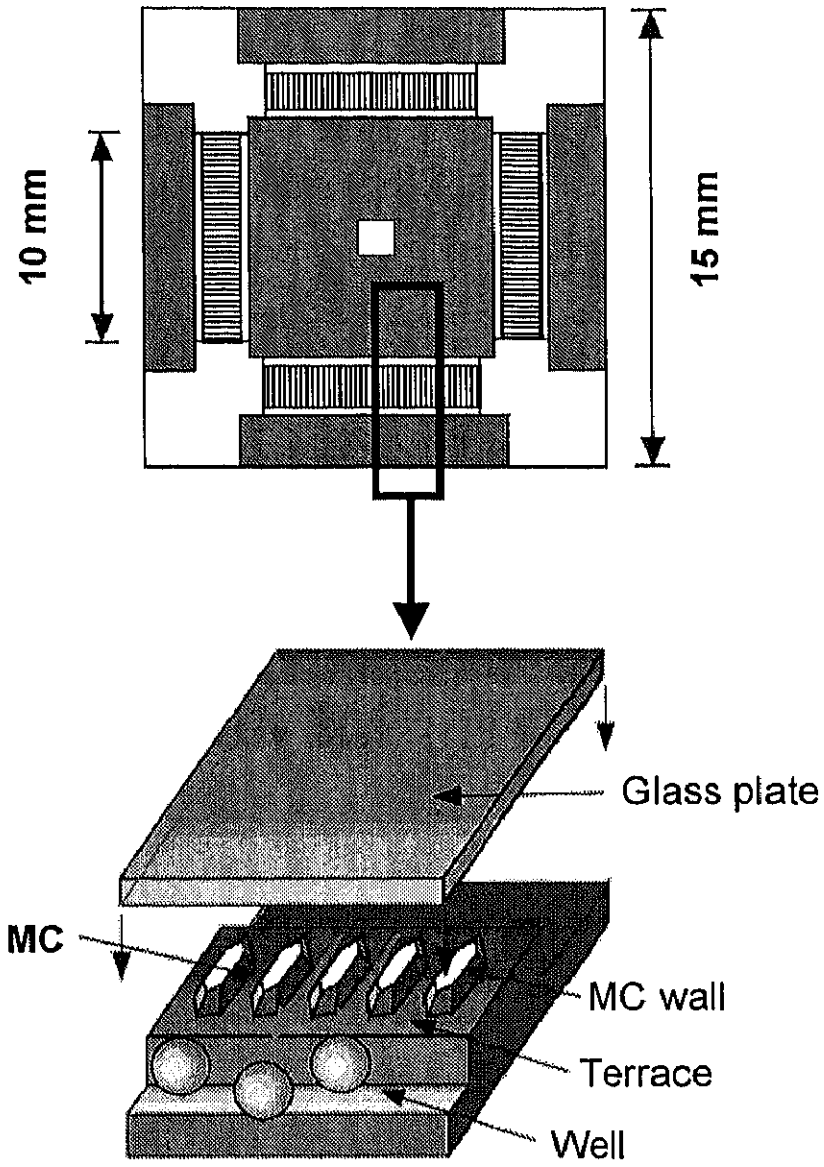


Fig. 1.5 Illustration of a microchannel (MC) emulsification process.

MC emulsification has been received a great interest in various industrial fields that produce emulsions and emulsion-based products. Potential applications of MC emulsification include monodisperse phospholipid emulsions (Tong *et al.*, 2002), multiple (W/O/W) emulsions (Kawakatsu *et al.*, 2001b), solid lipid microparticles (Sugiura *et al.*, 2000), and polymeric microspheres (Sugiura *et al.*, 2001c, 2002c) and microcapsules.

1.4 The Objectives of This Thesis

MC emulsification devices have a great potential for production of monodisperse emulsions due to excellent uniformity of the microfabricated channels (Kawakatsu *et al.*, 1997, 1999). For practical application of MC emulsification, it is required to extend the droplet size range of resultant emulsions and to improve throughput of emulsion droplets. The first primary purpose of this thesis was to develop small silicon MCs for the preparation of monodisperse emulsions with micron-scale droplets (**Chapter 2**). They include many potential applications in food, cosmetics, pharmaceutical and chemical industries. The preparation characteristics of emulsion with micron-scale droplets composed of biocompatible ingredients were also studied using an optimized small MC (**Chapter 2**). The preparation technique of micron-scale lipid microspheres with narrow size distributions by MC emulsification and subsequent solvent evaporation was proposed (**Chapter 3**). A micro-visualization system of MC emulsification allowed a detailed analysis of the droplet formation behavior (Kawakatsu *et al.*, 1997). The droplet formation behavior from a polycarbonate membrane under different operating conditions and surfactants was analyzed with a newly developed micro-visualization system of cross-flow membrane emulsification (**Chapter 4**). The second primary purpose of this thesis was to develop a novel microchannel emulsification device for high throughput of monodisperse emulsion droplets, which consists of a silicon array of uniform through-holes, named a straight-through MC (**Chapter 5**). Moreover, the effects of the device parameters and surfactant type on the straight-through MC emulsification behavior, on the resultant droplet size and size distribution were investigated (**Chapters 5 and 6**).