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2007

Philips L. (2007). Novel fabrication method for submicron hydrogel particles loaded with protein drug. Master's thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/36006>

<https://doi.org/10.32657/10356/36006>

A Novel Fabrication Method for Submicron Hydrogel Particles Loaded with Protein Drug

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A thesis submitted to the Nanyang Technological University
in fulfillment of the requirement for the degree of
Master of Engineering

2007

Acknowledgment

My greatest praise and thanksgiving is to The source of inspiration, motivation, creativity and life. This project was a hard and transforming lesson of life. My utmost appreciation is to my supervisors, Assoc Prof Chan Bee Eng and Prof Lam Yee Cheong, for your excellent standard, patient tutoring and continuous extra push at the back. This project will not be finished without you. I wish for productive and fruitful future years in your career.

To my friends and colleagues in BCPE and MEMS center, Dr Yan Yehai, Dr Chen Longqing, Dr Feng Jie, Dr He Bin, Ruoshan, JinYE, WenXiu, Zhang Jun, Murphy, Wee Koon, Andy, Wui Hean and others, your technical help, advises, smiles and presence are very greatly appreciated.

To all researchers out there, may your efforts be rewarded with the knowledge that we are doing what we can and contributing, however small, to the betterment of humankind.

Abstract

Bio-macromolecular drugs made from protein and DNA are being investigated as new and better cures for diseases such as cancer. However, they do not reach their target efficiently *in vivo* primarily because of damage by nuclease enzyme, scavenging by the immune system and difficult internalization by cells.

Encapsulation of these drugs into particulate drug delivery system having dimension in the micron and submicron range has been shown to increase their efficacy *in vitro*. Simultaneous demonstration of three important properties: submicron particle size, gentle particle fabrication condition and good drug encapsulation is rare. SCAMP, a novel particulate DDS fabrication method described in this project, is designed to accommodate these three properties.

SCAMP is molding of drug-containing particles inside (sub)micron cavities patterned on flexible polydimethylsiloxane. The particles were UV-cured from a precursor solution made of PEGDA, photoinitiator, water and Green Fluorescent Protein (model drug). Harvesting of the cured particles from the cavities was facilitated by a unique combination of stretch-release and freeze-peel techniques. SCAMP method was demonstrated at submicron particle size. GFP loading was demonstrated down to 2 μ m particle size. SCAMP particles can be made from high water content solutions with special water evaporation prevention step, which increases amount of drug loaded and provides a compliant environment for the drug. SCAMP can be developed further to include surface modification steps and to allow usage of other particle material and curing method.

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Chapter 1

Introduction

Drug delivery is an intensely researched subject and one particular interest is to increase efficacy of delivery of therapeutic protein and DNA. These water-soluble bio-macromolecular drugs are the first scope of this project because they are being researched for previously incurable diseases e.g. cancer and for better cure of other diseases.

Injection of these drugs into our body is largely ineffective because of drug denaturation inside our body, removal by white blood cells and also inefficient drug targeting to the intended site of action. Drug delivery system (DDS) has been proven routinely to increase drug efficacy in mice model and occasionally large primates and clinical trials are being held to test many proposed DDS, but mostly with chemical drugs. With protein/DNA drugs, preservation of their 3D conformation is important when combining them with a DDS.

One popularly reported DDS is submicron- and nano-sized drug-loaded particles. This form of DDS is the second scope of this project because its dimension facilitates

interaction with the body at the cellular and subcellular level. Also, it can travel through the circulatory system to potentially reach all parts of the body.

Current particle fabrication techniques rarely demonstrate the combination of three important requirements of a DDS: (a) preservation of fragile protein/DNA drug through the DDS fabrication processes, (b) submicron- or nano- size particles and (c) efficient drug loading into the particles. Top-down approaches (e.g. emulsification) rely on high-energy input and/or organic solvent to create sub-micron particles and these affect drug functionality. Bottom-up approaches (e.g. spontaneous self-assembly of block copolymers), rely on the materials ability to self-assemble from molecules to sub-micron particles. One common problem with these methods is inefficient drug loading. Simultaneous demonstration of these three requirements in a novel particle DDS fabrication method is the third scope of this project.

The objective of this project is to develop SCAMP (Stretch and Cavity Assisted Molding of Particles), a novel fabrication method for protein-loaded hydrogel particles. Figure 1.1 shows the steps of SCAMP.

A flexible, hydrophobic PDMS (polydimethyl siloxane) mold (1) was stretched and covered with particle precursor liquid (2). An example of its composition is 30%w (PEGDA+ 1%w I2959 photoinitiator) and 70%w (water + 8mg/ml Green Fluorescence Protein (GFP)). Atmosphere conditioning inside a chamber (2) was done to purge O₂, pressurize and humidify the atmosphere around the mold. The excess liquid on the mold surface was drained by 'discontinuous dewetting' (3) so that only the cavities were filled. The liquid droplets inside the cavities were cured with UV

irradiation (3). Outside the chamber, the stretching of the mold was released, resulting in the particles being partly ejected (4). Freeze-peel completely removed the particles from the mold surface (5).

Figure 1.1 Steps in SCAMP

Three sizes of blank PEGDA particles (10 μ m, 2 μ m and 500nm) were demonstrated in the initial development. GFP protein-containing hydrogel PEGDA particles were demonstrated subsequently in two sizes (10 μ m and 2 μ m). Complete harvesting (relocation of these particles from mold to an aqueous medium) were demonstrated. Fluorescence of GFP inside these particles was maintained and evaporation minimization of very small amount of water (10⁻¹³L) in order to keep an aqueous environment inside the particle was demonstrated.

Chapter 2

Literature Review

Sections 2.1 – 2.3 provide some brief introduction to the vast subject of drug delivery system (DDS). In section 2.1, gene and protein therapy is reviewed. In section 2.2, the requirements of an effective DNA/protein DDS particle are listed. In section 2.3, common DDS particle fabrication techniques are reviewed. These literature surveys help define the scopes of this project, which are:

1. The drugs of concern are DNA and protein (bio-macromolecular) drugs, which are relatively fragile and water-soluble.
2. The form of DDS of concern is micron and submicron particulate DDS.
3. The focus of the novel DDS of this project is on three aspects that are seldom demonstrated together: (a) submicron-size particles, (b) gentle fabrication conditions and (c) efficient drug loading.

In section 2.4, the supporting technologies used in SCAMP, our novel hydrogel particle fabrication technique, are discussed.

2.1 Gene and protein therapy

The pharmaceutical drugs today may be in the form of biomacromolecules such as protein and DNA. For curing disease, they work in at least two ways: by training the immune system to respond to pathogens and by treating directly the source of disease.

One example of the first type of treatment is vaccines. Vaccines have successfully diminished the incidence of smallpox and polio. Many other immunizations are commercially available, e.g. hepatitis, measles, rabies, and Bacillus of Calmette and Guerin (BCG), which are effective for a large percentage of recipients and have effective duration in terms of years. Their efficacy depends on training the immune system by introducing attenuated or mild variety of a specific pathogen. Our immune system adapts to the vaccines in a complex way, but one important aspect is recognition and memory of proteins (antigens) identified as belonging to the pathogen. As such, vaccines can be described as a form of protein therapy. One direction explored for vaccine development is delivery of DNA that expresses the antigens. This is hoped to reduce risks of direct introduction of live pathogens such as HIV.

Fig 2.1 shows the various methods to deliver DNA vaccines into a cell. Cells may be removed from the host, transfected in vitro, and then re-implanted. Alternatively, a virus or bacteria can be modified such that it is no longer virulent, may be unable to replicate, and contains a gene encoding the desired antigen (and sometimes other viral/bacterial vector proteins). The basic processes of DNA transcription and translation start in the nucleus of cells, and viruses are effective and efficient in

making their way there. Lastly, naked plasmid DNAs are simple rings of DNA, containing a gene encoding an antigen and a promoter/terminator sequence to make the gene expressed in mammalian cells [1], and plausibly the safest.

Figure 2.1 Various methods of gene delivery [1]

Studies so far indicate that plasmid DNA is tolerated in human [2]. It is exciting for its potential to induce various types of immune response: cytolytic T lymphocytes (CTL), T helper cells and antibodies [1]. It is also effective as treatment for single-gene defects, e.g. Adenosine Deaminase Deficiency, an autosomal recessive disease causing deficiency of ADA gene that codes for the enzyme Adenosine Deaminase, essential for the proper functioning of the human body's immune system. However, one of its drawback is transfection efficiency, as naked DNA has no effective means to deliver itself to the nucleus, nor is equipped to bypass numerous barriers encountered once injected inside the body [1,2,3]. A drug delivery system (DDS) that is safer than using virus and bacteria is urgently needed.

The second type of treatment is using drugs to treat the medical condition more directly. Proteins are the product of DNA expression and they run our biological

machinery. They are more direct compare to DNA as therapeutic agent, because they do not need the transcription step necessary for effective gene therapy. Also, the expression result of a gene is estimated to be 1-2 protein in bacteria, 3 in yeast, and 3-6 in human being since the expression is dependent not just on the DNA template itself but also on environment factors [4]. This makes protein therapy less complicated than DNA therapy, even more so if the disease is multi-gene.

Application of protein therapy includes external protein supply to compensate low protein levels due to missing or faulty gene(s), for example insulin for diabetic patients and hormone for post-menstrual women. Normal bodily functions can also be boosted by increasing relevant protein levels, e.g. for faster recovery of a wound site. Inhibition of specific proteins is also necessary sometimes, e.g. suppression of immunity system to prevent rejection of transplanted organ [5], killing of pathogens or cancer cells, or common painkillers for dental work. The effective treatment of cancer, a complex disease having genetic components, might be achieved through protein (and DNA) therapy.

It is reported that efficacy of protein therapy is often reduced in vivo because of rapid clearance by the scavenging system of our body, the Mononuclear Phagocyte System (MPS). Endothelial membrane bypassing and localization at intended site or organs is another delivery problem. Cell internalization at intended action site also needs to be facilitated, as cells do not take in proteins and DNAs indiscriminately. Also, compared to vaccine treatment in which the delivery target is relatively fixed (immune system), direct protein treatment amplifies the delivery issue because the variation of disease source and the complexity of each disease.

In summary, drugs of concern in this project are bio-macromolecular therapeutic protein and DNA, which are promising treatments that have been demonstrated in products such as vaccination, insulin and hormone treatments, and will continue to make greater impact in treating and curing diseases. There is a need for engineering of the delivery of the drug, rather than simply injecting these drugs into the body, because of the inefficacy of the latter approach due to in vivo conditions.

2.2 Requirements of a particulate DNA/protein drug delivery system (DDS)

An example of a form of DDS is particulate DDS of micron size and below (Fig 2.2). This form mainly addresses the delivery issue at cellular level because of its size. This form has the potential to be internalized by cells or simply be attached around the intended target and provide continuous drug release. This form can navigate through the circulatory system, potentially providing access to every cell in the body, because every cell is directly or indirectly sustained by the circulation system.

Effective cellular interaction alone does not make an effective delivery device. Examples of other important delivery issues on a larger scale are ways of intake (e.g. intravenous injection, oral intake, inhalation), place of entry (e.g. injection near stomach), localization technique (e.g. localized activation at the liver by heat or magnetic field) and the size and shape of DDS (e.g. tablet, needle, bulk hydrogel, stent). Issues at scale smaller than cell are drug-protein and drug-solvent interaction,

for example how the drug attaches efficiently to another membrane protein and elicit the desired response. However, this project limits its scope to (fabricating) particulate DDS only.

Figure 2.2 SEM image of ~10µm polymeric microparticles (made of dextran) [12]

The requirements of an effective protein drug particulate DDS are reviewed below.

1. **Particle size.**

Difference in size alone can determine a particulate DDS' final destination once injected into our body. Below 10 µm is necessary for macrophage cells uptake, while an optimum size of 2µm was observed in vitro [2]. Certain inflammatory vessels and tumor capillaries have fenestrations as large as 700nm [9]. Particles larger than 300 nm are largely captured by filtration in the red pulp of the spleen [9,19,20]. Smaller size particles (~100nm) avoid MPS detection better [21] by avoiding opsonization through minimal protein adsorption [22], are able to cross fenestration in epithelial linings and fine capillaries [9,10,22], and have prolonged circulation time [9].

Smaller size gives larger surface area, faster respond to stimuli and faster

equilibrium state [18]. Again, smaller particles were shown to be more effective in DNA transfection [23]. 100nm particles stimulate more uptake than micron-sized particles in certain cell lines [10]. It seems that submicron size, though not always demanded by applications, allow better specificity in targeting.

As a side note, a manufacturing technique that gives uniform size distribution is desirable because it offers more control over resulting particles' performances.

2. **Preservation of bio-drug function** through encapsulation up to the moment of release inside the body is important. Heat, mechanical stirring, organic solvent, pH, radiation and usage of excipients to minimize the effect are known to cause denaturing (list not exhaustive) [6,7].
3. **Biocompatibility.** The polymer encapsulating the bio-drug should not be toxic or immunogenic. This also concerns residue of materials used in processing.
4. **Biodegradability.** The polymeric carrier should be degradable into smaller fragments or molecules in physiological conditions. Though the carrier material must not be dissolvable in water, as it will fail as soon as it enters the body, biodegradability as a form of slow dissolution is desirable because it facilitates self-removal (prevents dangerous accumulation inside the body) and often helps produce the desirable controllable drug release (see 7th requirement).
5. **Isolation of drug.** Protecting drug from body's internal conditions is important because proteins are fragile and sensitive to many factors encountered inside the

body to varying degrees, and DNA can be degraded by nuclease enzyme and/or taken up by Mononuclear Phagocyte System (MPS). Reversely, protecting the body from the drug is also important because potent drugs might be toxic, even in small concentration, to sensitive part of the body.

6. **"Stealth" effect.** DDS with stealth ability are able to avoid clearance by MPS inside our body. It is typically associated with usage of poly(ethylene glycol) (PEG) or other hydrophilic molecules as coating material of the drug delivery particles, giving a steric-hindering outer layer capable of avoiding unwanted adsorption protein that is the catalyst for removal by macrophage cells. It seems that the PEG chains form flexible hydrated brush-like configuration (imagine rambutan fruit with dense, long waving hairs) that effectively discourage protein adsorption [8,9].

Other "stealth" conferring alternatives include (I) negatively-charged particle surface that prevents unselective attachment to negatively-charged cell membrane of macrophages, (II) usage of a "self" protein marker (CD47) present on the surface of red blood cells, which when removed, led to rapid clearance of the red blood cells by macrophages [9] and (III) high curvature (small size <100nm) of particles [14].

7. **Targeting / localization effect.** A drug typically works on a specific tissue or disease site and its biodistribution affects drug effectiveness. DDS localization can be effected by passive/active targeting to specific sites or cells, leading to attachment to cell membrane, uptake inducing, and escape from lysosomes.

Passive targeting takes advantage of bodily conditions and functions to localize the delivery system [9]. For example, through systemic injection, simple unmodified microparticles usually end up in macrophages, and if the drug's targets are macrophages, targeting is achieved [9]. By employing stealth effect, the carrier preferentially end up at lymph nodes or liver, lungs and spleens that have blood vessels with permeable walls [9]. Cancer cells also feed from blood vessels that are more permeable than normal cells, making them candidates for passive targeting with stealth particles [9]. Diseased regions might release different chemicals to its surrounding or become inflamed; these conditions can be used to localize the particles [9]. Particle uptake was observed to be dependent on concentration of particles outside and inside vascular smooth muscle cells and vascular endothelial cells, giving further incentive for localized targeting and maintenance of concentration [10].

Active targeting can be achieved by receptor-mediated attachment to targeted specific cells. For example, cancer cells express more folate receptors than normal cells, so they are preferential attachment sites for folate-tagged particles. The immune system's T cells can be targeted by antibodies, namely anti-CD3 and anti-CD5 [3].

After attachment, uptake needs to be induced so that the particles enter the cells. Sometimes this is automatic with attachment in certain cells, sometimes the right trigger for uptake inducing must be selected [12]. Besides uptake of particles, uptake of drugs released from particles attached to the outer side of cell membrane

is another form of internalization. Adhesion to mucus layer present at the eye, nasal, urinary and gastrointestinal tract is achievable, providing prolonged local concentration of drug [11].

Intracellularly, escape from lysosomes (cell organelle formed around the particles after internalization) must be facilitated. Fusogenic peptides derived from viruses, cyclodextrins and polyethyleneimine fused with the endosomal (matured lysosome) membrane and facilitated escape [13,12]. pH-sensitive liposomes (lipid bilayer vesicles) become fuse-competent in the acidic environment inside endosomes. Streptolysin-O can make pores exceeding 30nm in the membrane [13].

After escape from lysosome, specific cell compartments might need to be targeted, for example the nucleus to achieve effective DNA therapy. This however, is beyond the scope of this thesis.

8. **Controlled release.** The most common form is drug release resulting from biodegradation of particle material. Stimuli-induced release (by pH, heat, ionic concentration, or magnetic field) by means of special particle material can also be used. Tailoring of spatial drug release profiles becomes less relevant when DDS size approaches submicron, as release is accelerated in such high surface-to-volume system.

Figure 2.3 shows two conceptual designs of DDS that addresses the 7 requirements listed above. The first design [12] is a polymer-DNA complex with three surface modifications: a hydrophilic coating for stealth effect, homing device for active

targeting, and membrane fusion peptide to facilitate release once inside a target cell's endosome. The second design [15] has a similar concept, but uses liposome (lipid bilayer vesicles) as particulate material. Conventional liposomes are either neutral or negatively charged and can be modified by incorporating in the bilayer: (1) polymer coatings to sterically stabilize ('stealth') the liposome and obtain prolonged circulation times, (2) Immunoliposomes for antibody-mediated active targeting, and (3) cationic conjugates to impose a positive charge. Biodegradability, biocompatibility and drug loading are implied in the choice of encapsulation material (cationic polymer and liposomes).

Figure 2.3 Possible designs of a DDS
Polymeric carrier for DNA with surface components [12]
Drug-containing liposomes with associated surface molecules [15]

2.3 Particulate DDS fabrication techniques

DDS fabrication techniques can be classified as top-down approaches (making particles out of a larger starting material) and bottom-up approaches (composing particles from a smaller starting material). Examples of top-down approaches are emulsification, aerosolization and micromachining, while examples of bottom-up approaches include self-assembly of block copolymers and lipid bilayers.

We will briefly describe emulsification, aerosolization, micromachining, self-assembly and PRINT™ techniques of fabricating particulate DDS.

2.3.1 Emulsification

The basic technique of the family of emulsification techniques is oil-in-water emulsion method. Developments from this technique will also be described along with their advantages and disadvantages.

2.3.1.1 Oil-in-water emulsion

Illustrated in figure 2.4, the procedure starts with dissolving a suitable polymer (e.g. PLGA) and drug into an organic solvent (e.g. methylene chloride, dichloromethane, chloroform). Then this oil phase is mixed into an aqueous phase with dissolved surfactant (e.g. Polyvinyl alcohol (PVA), poloxamer, Tween) that preferentially orientates itself at the oil-water interface and serves to stabilize the emulsion. The mixture is homogenized, and if smaller particle size is desired, higher energy input and surfactant concentration are needed to keep the emulsion stable. Continuous

stirring for a few hours allow the organic solvent to dissolve out into the water and evaporates at the water-air interface. The remaining polymer solidifies to become solid hardened microparticles that can then be washed and filtered to remove residual surfactants and solvent. Solvent evaporation can be replaced with other methods, e.g. polymerization reaction for dissolved monomers by pH change, heating, or crosslinking.

Figure 2.4 Schematics of oil-in-water emulsion method

Scholes et al [19] reported a 90 nm, low polydispersity particles, using PLGA as polymer, PVA as surfactant, and optimization of the process parameters.

With o/w emulsion, water-soluble drug incorporation into the oil phase (which becomes the particle) is unacceptably low, as they are usually not soluble in organic solvent, and the existence of the continuous water phase further reduces drug entrapment. Reversing o/w to become water-in-oil (w/o) emulsion allows drug to be well trapped inside the water emulsion. Unfortunately, this also dictates the polymer

to be water soluble. An additional step to permanently harden the water soluble particles is required (e.g. crosslinking) so that they do not dissolve uncontrollably in vivo.

2.3.1.2 Water-in-oil-in-water double emulsion

This double emulsion method was developed by Ogawa et al (1988) as a solution for water-soluble drugs. The procedure (Fig 2.5) is very similar to the o/w emulsion, but with additional step early in the process: dissolving the drug in the first aqueous phase. This is followed by emulsification in the oil phase that contains dissolved polymer. This emulsion is quickly mixed into the second aqueous phase with surfactant dissolved, and the rest is the same. Proper ratio between phases, controlled mixing and enough surfactant is necessary to keep the water-oil-water interface stable.

Figure 2.5 Schematics of water-oil-in-water emulsion method

As with o/w emulsion, there are at least two problems regarding drug preservation. Firstly, the high-energy homogenization required to generate emulsion of (very) small droplets are potentially harmful to proteins and DNAs [18]. Secondly, solvent contact with fragile proteins and DNAs potentially denature and aggregate them, as they are preferentially adsorbed at the oil-water interface [6,7,18,24]. Also, large macromolecule surfactant like PVA is needed because of better drug entrapment and colloidal stabilizing property, but is not digestible and not easily washed after preparation [16,26].

2.3.1.3 Spontaneous emulsion/ solvent displacement/ nanoprecipitation

To circumvent high-energy emulsification necessary to reaching sub-micron size, spontaneous emulsification was developed by Niwa et al [27] since 1989. It is a modification of the basic o/w emulsion, as the main idea here is to use a co-solvent of both water and the polymer, with common examples including ethanol, acetone, and methanol. When the oil phase (polymer+drug+co-solvent) is added into the water phase, the ability of the organic solvent to dissolve in water causes self-emulsification, and particle size can reach 200nm or less [17,28] without high-speed homogenization. Another benefit is easier scaling-up of production since no homogenizer was used.

Avoiding usage of high-energy homogenization is an advantage, but hydrophilic drug loading suffers because water is the continuous phase [27], and organic solvent usage is also undesirable. Changing aqueous pH from 5.8 to 9.3 improves drug (procaine hydrochloride) loading and entrapment which might be due to decreased degree of ionization and hence lower solubility in the aqueous phase [28].

2.3.1.4 Solid-in-oil-in-water emulsion solvent evaporation

This technique modifies the o/w emulsion by solidifying and grinding the drug into very fine powders before being added into the oil phase, hence the name solid-in-oil-in-water emulsion. The idea is to reduce interaction with organic solvent and improvement of drug loading, as the drug is in solid form and is not soluble in organic solvent. Early attempt using Tetanus toxoid as model protein and relatively large particle size of 20-30 μ m shows that total antigenicity of the protein after release are still significantly reduced, but improves with dextran addition as protein stabilizer [24].

With γ -chymotrypsin as model protein, Castellanos et al [29] shows that PEG is a good excipient to reduce aggregation and protein structural perturbation, and when used as surfactant also, improves activity retention even more (reduction of less than 5%). Demonstration with another protein, horseradish peroxidase, also shows similar encouraging result [30].

The downside of this technique is that it is harder to make submicron particles, as it is limited by the technique used to make solid protein particles. At a more positive note, with IgG model protein, Wang et al shows that lyophilization and dehydration needed to atomize the protein does not significantly disturb the drug [31].

2.3.1.5 Water-in water formulation

Another modification of the emulsification technique is the water-in-water technique. One formulation [12] uses dextran, a water-soluble polymer in the first water phase,

and PEG as surfactant in the second water phase. This technique is possible because the first water phase containing dextran is very viscous, so it can be “emulsified” and quickly solidified in the second water phase before it has the chance to disperse and dissolve. Another example of this technique uses Chitosan as carrier material and PEO-TPP diblock copolymer as surfactant [21]. Using BSA as model protein, 80% loading efficiency and sustained release up to 1 week has been achieved.

This technique eliminates organic solvent interaction and PEG usage also gives additional stealth effect. The downside of this technique is again its dependence to high-energy emulsification.

2.3.1.6 Membrane emulsification [15]

The role of homogenizer is replaced by porous glass membrane [15]. The pressurized oil to be dispersed is passed through controlled pores of membrane and forms countless oil droplets on the inner surface of the membrane (Fig 2.6). The particles created were of ~5µm in size. Drug evaluation is not available.

Figure 2.6 (A) Schematic of the membrane emulsification apparatus [15]
(B) Principle of membrane emulsification in the case of preparing an O/W emulsion.

2.3.1.7 Section summary

Emulsification techniques is a top-down approach, creating small droplets from larger ones and stabilizing them by surfactant addition and droplet hardening (solvent evaporation or crosslinking). Submicron size and good drug loading are achievable. The problem with this large-to-small approach is that to break apart (emulsify) the material, high energy mixing or organic solvent (or both) are usually used, both of which are undesirable because of drug fragility and toxicity issues.

2.3.2 Aerosolization

While emulsification techniques form microdroplets inside another liquid phase, aerosolization forms the microdroplets in air/gas phase. This ‘atomization’ process of o/w emulsion is done with the help of a gas stream from a nozzle. Suspended in air, the droplets quickly dry and solidify to become solid-in-air (aerosol). To facilitate atomization and evaporation of solvent from the small droplets, heat can be supplied

Gander et al [32] uses this method to investigate 10 types of organic solvents to encapsulate Bovine Serum Albumin (BSA) with poly(lactic acid) (PLA). Notable findings are that water-miscible solvent causes more protein inactivation and that antigenicity test is still more reliable than simple checking M_w of protein. β -glucuronidase has been investigated by Burgess and Ponsart [33] showing that viscous dispersion is not suitable for spray drying, and that albumin-acacia ionic coacervate as excipient reduces activity loss to 20%. The pH of dispersion is low, around 4. Entrapment efficiency was shown to be excellent, around 99%.

Aerosolization depends on high temperature processing and organic solvent to form sub-micron particles. Though drug entrapment was regularly found to be superior to emulsion techniques, the harsh processing conditions are prohibitive for fragile drugs.

2.3.3 Micromachining

2.3.3.1 SiO₂ microwells

Micromachining technique like lithography, dry etching, and thin-film deposition techniques have been employed to machine empty wells out of a SiO₂ layer [41]. After filling them with therapeutic agent and closing the top opening, final etching of underlying substrate releases them into the medium to become microcapsules (Fig 2.7).

Figure 2.7 (A) Topview of micromachined SiO₂ microwells
(B) released SiO₂ microparticles [41]

Silane chemistry has been used to modify the microparticles surface with proteins for stealth and targeting purposes. Plug material can be tailored to achieve timed or stimuli-sensitive release. PMMA (poly-(methyl methacrylate), a biocompatible material, also a common resist material in photolithography application, has been demonstrated to be able to form microparticles too [41].

Drawbacks include limited material option due to compatibility issue with micromachining techniques, comparatively low rate of production, limitation of microparticle size due to optical lithography resolution limit, toxicity associated with etching process, and nonbiodegradability of material.

2.3.3.2 Hydrogel photolithography patterning

Patterned hydrogel by photolithography technique has also been also reported [42]. By applying UV-irradiation to crosslink a hydrogel precursor layer (instead of the usual photoresist) through a photolithography mask, the hydrogel layer can be patterned to uniform, small microparticles (Fig 2.8). Without adhesion-promoting steps to the underlying silicon substrate, swelling the patterned hydrogel in water might release the microparticles from the substrate.

Figure 2.8 Patterned hydrogel microparticles by UV-photolithography [42]

This technique provides more material choices as compared to silicon well micromachining [41] but possible limitations include protein agglomeration in the

precursor solution and activity loss after prolonged UV-irradiation, along with the low rate of production, size limitation and toxicity associated with photolithography techniques.

2.3.3.3 Section summary

In summary, micromachining techniques are a departure from the more conventional emulsification/atomization/self-assembly techniques and the associated problems are different. It has not been widely reported in literature, but what can be observed is that uniform size distribution is inherent; though submicron-size has not been reported. Drug exposure to harsh processing conditions more common to other techniques seems to be avoidable. Material selections are restricted by the nature of the technique. The first two reviewed techniques seem to have good water-soluble drug-loading efficiency. Depending on the technique, production rate might be relatively low, as the medium of particle production is a two-dimensional surface instead of three-dimensional volume in the case of emulsion, aerosolization, or self-assembly (next section).

2.3.4 Self-assembly

2.3.4.1 Liposomes [35 – 39]

Lipid molecules have the tendency to self-assemble into bilayers with hydrophilic head (round heads in Fig 2.9 A or B) facing outside to the aqueous medium and hydrophobic tails facing each other (double tails in Fig 2.9 A or B). Illustrations and electron micrographs are shown of different types of liposomes: (A) unilamellar vesicles (ULV) which contain a single internal aqueous compartment and typically

have a diameter of 0.02–0.5 μm ; (B) multilamellar vesicles (MLV) which contain multiple concentric internal aqueous compartments and typically have a diameter of 0.2–5 μm ; and (C) multivesicular liposomes (DepoFoam™ particles) which contain multiple non-concentric internal aqueous compartments and typically have a diameter of 1–100 μm [39].

Figure 2.9 Various structural configurations of liposomes

The aquatic core and lipid bilayer allow incorporation of both hydrophilic and hydrophobic drugs. The bilayer itself is easily disturbed and leaks out the drug; therefore it does not give acceptable controlled release. Polymer coating improves the integrity and stability of liposomes. It can also grant stealth effect, as liposome native surface properties interacts with various plasma components and Mononuclear Phagocyte System (MPS) leading to rapid clearance from the body.

2.3.4.2 Micelles

Figure 2.10 Illustration of micelles formed from block copolymer [40]

Block co-polymers can form micelles with core-corona structure with size ranging from 20-100 nm (Fig 2.10). One part of the block-copolymer is hydrophilic, while the other part of the block-copolymer is hydrophobic. In water, the hydrophobic part would aggregate together to form the core, while the hydrophilic part extends to the solvent and form the corona. Self-assembly is common, or when mediation is necessary, it is usually of low energy using dialysis or solvent evaporation, to increase drug loading in the core (Fig 2.11).

Self-assembly phenomenon has a Critical Micellar Concentration (CMC), below which the micelle structure dissociates and releases the drug content prematurely. When there is a possibility that concentration in vivo reaches below this value, further strengthening can be achieved by crosslinking the core. Material of choice for the corona would be PEG with excellent biocompatibility, water solubility and non-fouling property [18].

Figure 2.11 Drug loading to micelles by (a) dialysis and (b) o/w emulsion [40]

There is a trade-off between drug loading and minimization of organic solvent usage. The preferable solvent is water, and while it is possible to form micelles with it, preferential partition of drug outside the core (depot) will happen. To better trap the drug inside the core, organic solvent can be used, though undesirable for the drug and human consumption. Alternatively, the core part of block copolymer can be made cationic-anionic, resulting in enhanced drug trapping and stronger association compared to core-hydrophobic interaction [40].

A combination of cationic polymer and negatively-charged DNA forms a self-assembly aggregates too. Fig 2.12 shows schematic representations of lipid-coated polyplex formation. Plasmid DNA is condensed by adding the cationic polymer pDMAEMA (poly-(2-(dimethylamino)ethyl methacrylate)) to the DNA at a

DNA/pDMAEMA ratio of 1:3 (w/w). The formed cationic complexes are added to a solution containing the detergent octylglucoside (OG) and a total amount of 3 mmol of detergent-solubilized lipids. Slow removal of OG is done by adsorption to hydrophobic BioBeads, and that resulted in formation of lipid coats preferentially around positively charged complexes because of electrostatic interactions [12].

Figure 2.12 Formation of polyionic complexes coated with lipid bilayer [12]

2.3.4.3 Section summary

This family of technique takes the bottom-up approach, i.e. building particles from aggregation of smaller molecules. Submicron size and low energy processing are very common because of the self-assembly nature. Also, the simple processing condition allows easy scaling-up of production. However, for drug loading to be high, interaction of material with drug must be enhanced, since it is inefficient for water-soluble drugs to partition inside hydrophobic core of micelles or aqueous core of liposomes, when there is a bulk aqueous phase outside. One promising example would be the DNA-condensing cationic polymers with lipid bilayer outer coating [12]. Dependency on material's special properties is also high, which might make it difficult to design a material that also fulfills other DDS requirements such as biodegradability, biocompatibility, and having stealth effect.

2.3.5 PRINT™

PRINT™ refers to a recent particle fabrication technique by UV embossing on a non-wetting surface (Rolland et al, 2005) [59]. PRINT™ process is illustrated in Fig 2.13. In brief, because both the surfaces of the imprint mold and the substrate were fluorinated, they were non-wetting to the liquid and particles were formed by embossing a liquid droplet, trapping it inside the cavities, and hardening it by UV curing (or other means). This technique eliminates ‘scum layer’ that usually exist in ‘traditional imprint lithography’. In Fig 2.14, fluorescent confocal microscopy shows sample of 200nm and 500nm particles made with PRINT™.

PRINT™, as a top-down approach, is a unique approach to mold particles by embossing them. It is a relatively new technique (2005) which was published during the course of this project (2003-2006). It shares the same theme with this thesis that (a) pre-formed cavities avoid the need for agitation/solvent to create small particles and (b) top-down approaches suffer from poor drug preservation because of processing conditions. It is hoped that this approach is minimally disruptive to protein/DNA drugs but has good drug loading and submicron size, which are the strengths of top-down approaches. Though drug evaluation is not available, loading of various drugs in various materials is demonstrated, showing the versatility of this novel approach.

The authors of PRINT™, Rolland et al, made a common observation together with the writer after review of particulate DDS fabrication techniques, which is the rare simultaneous demonstration of small particle size, minimal disruption/denaturation of protein and high drug loading. Focusing the novel particulate DDS fabrication method

to these three factors became the third scope of this project. There are at least eight requirements of an effective particulate DDS (section 2.2) and those not included in these three factors were accounted for by material selection or making them plausible with further development beyond this thesis.

Figure 2.13 Illustration of the PRINT process compared to traditional imprint lithography in which the affinity of the liquid precursor for the surface results in a scum layer. In PRINT, the nonwetting nature of fluorinated materials and surfaces (shown in green) confines the liquid precursor inside the features of the mold, allowing for the generation of isolated particles [59]

Figure 2.14 Fluorescent confocal micrographs of PRINT results [59]
 (a) 200 nm trapezoidal PEG nanoparticles containing 24-mer oligonucleotides tagged with CY-3 fluorescent dye
 (b) 500 nm conical-shaped PEG particles containing avidin (68 000 Da) tagged with FITC fluorescent dye

2.4 Prior technologies used in SCAMP

A novel particulate DDS fabrication method termed SCAMP (Stretch and Cavity Assisted Molding of Particles) was developed and demonstrated. SCAMP can be described as a top-down approach that uses mold with (sub)micron cavities to break liquids into droplets and UV curing to make them solid, in contrast to emulsification techniques that have to rely on high-energy input and/or organic solvent. PEGDA is the DDS material of choice in this project. Submicron size was demonstrated. Drug loading was efficient because the drug cannot diffuse out of the solid cavities, therefore entrapping it together with the cured DDS material. The model pro-drug used in this project was Green Fluorescent Protein (GFP).

SCAMP starts by making an elastic PDMS mold with patterned (sub)micron cavities needed to fabricate DDS particles, followed by harvesting them out of the cavities. SCAMP utilizes available technologies and new techniques not reported before. Available technologies used are reviewed below.

2.4.1.1 Fabrication of flexible mold by micromachining and soft replication

Micromachining of submicron cavities is a commercially established technology that utilizes photolithography to cure or degrade specific areas of a resist layer spread on a substrate, typically a Si wafer. Uncured areas of the resist are washed off and the patterned resist serves as an etching mask that allows selective etching of the Si wafer.

Commercially available UV-line lithography system can resolve down to 350nm

feature size, while deep-UV line will extend the capability to 100 nm, a prediction for year 2007 [43]. Focused Ion Beam and Electron Beam Writing techniques are examples that allow even smaller dimension, but are typically not economically viable. The limit of micromachining techniques becomes the limit of our technique, as in SCAMP, cavity size determines particle size.

For SCAMP, cavities having 1:1 to 1:2 aspect ratio (height:width) is satisfactory. Available technologies can achieve very high aspect ratio features (17:1) with vertical sidewalls, now feasible with the latest Advanced Silicon Etch (ASE) technology from STS [44].

Usage of replication technique on the Silicon mastermold to create child molds gives at least three advantages: mass availability of the intended shape (as replication is quicker, easier, cheaper, and faithful to the original mold), preservation of master mold which is typically most challenging or tedious to make, and wider choices for final mold material, instead of being restricted to silicon or glass as conventional materials for most micromachining procedures. The last advantage is important for particle harvesting procedure, as shown in later section. Replication of microwells with minimum feature size of 10 μ m and aspect ratio of 1:1 has been reported by Chan et al [45]. Larger 72 μ m microchannels with aspect ratio of 5 were also replicated.

Poly(dimethylsiloxane) or PDMS as mold material has been shown to be compatible to the replication process [46]. Flexibility of the PDMS and low surface tension makes it a very robust as replication material which allows easy mechanical manipulation. Its low surface energy and chemical inertness prevents adhesion of polymer in contact

[43]. Very small water and ethylene glycol absorption [47] means it is unlikely to be affected by the normal replication and molding conditions. It is of low toxicity and its two-part base and curing agent do not present any toxicological hazard by normal industry handling.

2.4.1.2 Fabrication of particles utilizing discontinuous dewetting and UV-curing

The PDMS mold with patterned cavities was used to break bulk liquid into microdroplets inside its cavities. When the liquid droplets are made of photo-curable material, they could be hardened by UV irradiation to become particles.

Discontinuous dewetting [47] illustrated in Fig 2.15 was reported to be able to fill small microcavities made from PDMS with size ranging from $50\mu\text{m}$ to $2\mu\text{m}$ and with aspect ratio from 5:1 to 1:1. Before discontinuous dewetting starts, microcavities are covered overflowing by a layer of liquid. As Fig 2.15 shows, bulk liquid dewets the surface at an equilibrium receding contact angle (A). The dewetting movement of the liquid bulk can be achieved by slight tilting of the setup. On reaching the top edge of the well, the abrupt change in contact angle pins the drop at this edge (B). The drop hinges on the edge of the well as the bulk liquid recedes. As the liquid drains, it approaches its equilibrium contact angle at the top edge of the well, and the film at the lower edge of the well starts to thin (C). Liquid is left behind in the well when the thin film at the bottom lip of the well (marked *) ruptures (D). The droplets left inside the cavities is now isolated and can be formed into particles.

Important criteria for successful discontinuous dewetting are [47] (1) liquid must not swell PDMS, (2) liquid-on-PDMS receding contact angle is in the range of 16° - 81° -

wide material selection and (3) liquid must be of low viscosity ($< 500\text{cP}$).

Figure 2.15 Illustration of discontinuous dewetting process [47]

One mechanism for curing a liquid polymer to become solid is radical polymerization. It is a type of polymerization in which the reactive center of a polymer chain is a radical which is highly reactive because of its unpaired electrons. The polymerization can be divided into three stages: (1) initiation of radicals – creation of free radicals from (for example) a photosensitive molecule that can be excited by light (termed photoinitiator) (2) chain propagation – where the polymerization reaction of the reactive end group of a polymer chain regenerates the free radical to be used to polymerize another chain, thus propagating the polymerization, and (3) chain termination – where the free radicals are destroyed (or reacted with another molecule) and the polymerization reaction stops.

It was indicated that damage by UV irradiation to protein and DNA is possible through covalent binding by free-radical addition or Michael addition between lysine residues and acrylate groups existing in a diacrylated polyethylene glycol hydrogel matrix [21,50]. More specifically, the free radicals generated by photoinitiators are responsible for almost all DNA cleavage [56] as exposure to 365nm-UV radiation

only ($5\text{mW}/\text{cm}^2$ for 10 min) caused minimal change (5%) in DNA conformation. Radical scavenger can divert damage by free radicals from the drug [57]. The DDS encapsulating material itself has acrylate end groups for photopolymerization that are highly reactive to free-radicals. Relying on this does not seem to be enough though, observing that only a small fraction of the drug (3%) was recovered as the supercoiled DNA form (best transfection efficiency). Using a combination of protamine sulfate+vitamin C or HEPES buffer raised this value to 70%, besides increasing the total recovered DNA by a small amount [57].

Despite the damage potential of free radical UV photopolymerization, it was chosen as the mechanism of curing because it is relatively simple in equipment and operation so that it that facilitated development of SCAMP and is relatively unexplored for DDS application. The encouraging results of initial investigations would promote exploration of other hardening mechanism (e.g. collagen hardening) and also the many modification possibilities that come with using polymers in general.

2.4.2 PEGDA hydrogel as particle material

Hydrogel is a water-swollen insoluble polymer matrix with crosslinked structure [18,21,49] which is able to swell water in large quantities without dissolving. Poly(ethylene glycol) diacrylate (PEGDA) hydrogel was chosen based on the following facts.

1. Firstly, the material of choice must be compatible with the chosen manufacturing techniques. PEGDA is a UV-curable material, and was shown to be compatible to replication [45] and discontinuous dewetting process [47]. Photocrosslinking can be done under ambient or physiological conditions, with

minimal heat generation [48]. 16mWatt/cm^2 for 11sec is reported to be enough to cure PEGDA hydrogel precursor [46]. Lastly, being constructed from polymers, there is a wide modification possibility for the hydrogel material [49, 50].

2. Hydrogels are generally **biocompatible** [49,48], causing minimal inflammatory responses, thrombosis, and tissue damage. They also have high permeability for oxygen, nutrients, and other water-soluble metabolites [48]. It has been investigated in biomedical applications, e.g. tissue engineering, post-operative adhesion, drug delivery, and coatings for biosensors [48]. It is also potentially compatible with protein and DNA drugs [48].
3. Early reports [45] present PEGDA copolymer with poly(α -hydroxy acid) as **bioerodible** to become the harmless PEG chain and natural metabolites readily eliminated by the body.
4. PEG as surface-grafted material provides steric hindrance that functions as a barrier to protein adsorption that leads to opsonization by MPS inside the body (Section 2.2). Such configuration possibly allows the PEG to perform as surfactants too, maintaining colloidal stability and particle size in storage [16,17].
5. **Drug isolation.** Hubbell et al [53,54] reported that hydrogels made of PEG 2k, 4k, and 8k DA were all permeable only to myoglobin (22kDa). PEG 20k DA had larger cut-off point, allowing release of ovalbumin (45 kDa). Generally, reduced diffusion coefficient was observed with increasing PEGDA concentration and shorter length between crosslinks which usually corresponds to smaller mesh size.
6. **Controlled release** possibilities have also been investigated, including but not

limited to usage of hydrolytic monomer to make it biodegradable [45], copolymerization with proteolitically degradable peptides [48], stimuli-sensitive monomer to pH, temperature or ionic concentration changes that affects its swelling capability [51] and usage of UV light-sensitive monomers that experience photo-scission upon UV-irradiation, changing hydrogel mesh size [40-52].

7. **Water as solvent for PEGDA.** This is beneficial as the drugs of concern in this report are water-soluble and requires aqueous environment for proper functioning. It has been reported that usage of organic solvent can lead to denaturation of fragile drugs by preferential adsorption of protein to oil-water interface leading to aggregation which can be (partially) irreversible [6,7,12,10].

In summary, PEGDA was chosen for its biocompatibility, biodegradability, drug isolation, stealth effect and possibilities of controlled release.

2.4.3 Summary of novel manufacturing method

SCAMP is expected to encapsulate protein and DNA drugs with good efficiency in submicron particles, since both selected polymer and drug are water-soluble. The technique also minimizes contamination and has mild processing conditions to emphasize preservation of encapsulated protein/DNA by not exposing the pro-drug to extreme heat, mechanical agitation, strong radiation, strong solvents and potentially pathogenic and/or immunogenic excipient or surfactant found in other more popular manufacturing methods. Possible sources of damage to drugs are the radicals needed for photopolymerization and the low-intensity UV irradiation.

Chapter 3

Experimental procedure

The novel particle manufacturing method **SCAMP (Stretch and Cavity Assisted Molding of Particles)** summarized in Fig 1.1 is expanded in more detail in Fig 3.1. Steps 1's final result is an elastic PDMS mold with patterned cavities. Step 2 and 3 use this mold to fabricate particles. Step 4 and 5 harvest the particles.

Step 1a is the fabrication of an Si mastermold with (sub)micron-sized cavities using photolithography and dry plasma etching technique. **Step 1b** is the replication of the Si mastermold using polyurethane (PUR) diacrylate that gives the negative shape of the patterned cavities – short columns. **Step 1c** is a replication of the PUR replicate in step 2 using an elastic polydimethylsiloxane (PDMS) to reproduce the cavities of the Si mastermold. This PDMS replicate is used as mold to fabricate the particles. Prior to molding, preparation of the particle precursor liquid mixture of PEGDA polymer, GFP protein as model drug, water and I2959 photoinitiator was done (not shown).

Step 2a is stretching of the PDMS mold (cavities are elongated) with a stretcher (not shown) and covering of the mold with precursor liquid. However, air bubbles remain inside the cavities and prevent them from being filled. **Step 2b** is conditioning of the

atmosphere around the mold inside a chamber to (a) release the trapped bubbles and filling the cavities completely, (b) purge the O_2 in the air that inhibits curing and (c) pressurize (by Ar gas, 9 bar gage) and humidify (by wet tissue) the atmosphere inside the chamber to minimize water evaporation from the liquid precursor droplets. **Step 3a** is discontinuous dewetting that drains the precursor liquid by gravity (hence the tilting) and leaves only droplets inside each cavity. **Step 3b** is curing of these droplets to become particles by UV irradiation through a UV-transparent cover made from PMMA.

Step 4 is removal of the mold+stretcher from the chamber and release of the stretch, causing the cavities to be relaxed and the particles to partly ejected (half in and half out of the cavities). **Step 5** is complete detachment of the particles by encasing the particles in an ice layer that allows the PDMS mold to be peeled off the ice layer and be separated from the particles. The particles can then be released into an aqueous medium (not shown) for further experiments.

This chapter presents in detail the materials and equipments used in this project (section 3.1) and explains the details of PDMS mold fabrication (section 3.2), particle fabrication (section 3.3) and particle harvesting (section 3.4).

Three particle sizes (10 μ m, 2 μ m and 500nm) were investigated. To make the 10 μ m particles, PDMS molds with cavities measuring 10 μ m (length) x 10 μ m (width) with varying depths ranging from 3.1 μ m to 11 μ m were used. To make the 2 μ m particles, a PDMS mold with cavities measuring 2 μ m in diameter and 0.7 μ m in depth was used. For 500nm particles, a PDMS mold with cavities measuring 500nm (length) x 500nm

(width) x 200nm (depth) was used. 10 μ m and 2 μ m PDMS molds were replicated from polyurethane intermediate molds, which themselves were replicated from Si mastermolds made with photolithography and deep reactive ion etching (DRIE). The 500nm mold made by a proprietary technique of our group was supplied by Dr Chen Longqing.

Three particle precursor liquid formulations were used in the investigation:

1. “PEGDA” formulation consisting of 99/1 (w/w) PEGDA / Irgacure 2959.
2. “GFP-hydrogel” formulation consisting of 70/30 (w/w) GFP solution / PEGDA.
GFP solution concentration was 8mg / ml of DI water. PEGDA was as (1).
3. “Hydrogel” formulation consisting of 30/70 (w/w) DI water / PEGDA. PEGDA was as (1).

The first formulation was used for general debugging of SCAMP, the second formulation was used to demonstrate dug-loading capability of SCAMP and the third formulation was used to investigate water-evaporation from the cavities.

Figure 3.1 Detailed steps of SCAMP

3.1 Materials and equipments

3.1.1 Material and chemical

Si wafers, 100 mm-diameter <100> p-type/boron doped single-side-polish with average thickness of 455-575 μ m were purchased from Microserv (S) Pte Ltd. Hexamethyldisilazane (HMDS) adhesion promoter was purchased from Hoechst Industry Ltd. AZ-7220 photoresist and AZ-300MIF developer were purchased from Clariant Corporation (USA). Sulfuric acid (H₂SO₄), Hydrogen peroxide (H₂O₂), acetone and ethanol were purchased from JT Baker, USA. Trimethylolpropane trimethacrylate (TMPTA) ([H₂C=C(CH₃)CO₂-CH₂]₃CC₂CH₅) and Poly(ethylene glycole) diacrylate (PEGDA) (H₂C=CHCO(OCH₂CH₂)_nO₂CCH=CH₂) with average molecular weight of 700 were purchased from Aldrich Chem. Comp. Inc. (USA). Aliphatic Urethane Acrylate Resin (Ebecryl 270) and Acrylated Silicone (Ebecryl 350) were purchased from UCB Chemicals. Dipropylene Glycol Diacrylate with (SR508) was purchased from Sartomer Comp. Sylgard 184 silicone rubber kit was purchased from Dow Corning Corp, USA. 4-(2-Hydroxyethoxy)Phenyl-(2-Propyl)Ketone (Irgacure 2959) photoinitiator was purchased from Ciba Chemicals. Adhesion-promoted polyester film (Melinex) gage 500 was purchased from DuPont Teijin Films. Green Fluorescence Protein (2mg/ml) was provided by Xu Huibin who is a graduate student of Professor HS Yoon's group. All materials were used as received.

3.1.2 Equipment

The chrome photolithography mask is a dark field mask (Fig 3.2) with square/circle holes having 4 sizes, a quarter of circle for each dimension. The squares are 4x4 μ m,

6x6 μm , and 10x10 μm , and the circle is 2 μm in diameter. Wet bench with Teflon tools, temperature control and timer were supplied by Mediamac, Singapore. CT302 Spin dryer was supplied by Calitech. Delta 150 VPO HMDS coater with semi-automatic process control was supplied by SUSS Microtech Lithography GmbH. Cee 10CB spin coater with manual resist dispensing was supplied by Brewer Science, Inc. PMC 732 series digital hotplate was supplied by Dataplate. MA6 Mask aligner and UV exposure system was supplied by Karl SUSS.

Figure 3.2 Illustration of the dark field Cr photolithography mask used in this project. Each quarter contains different features. Only the top-left quarter containing 2 μm circular holes in regular 4 μm spacing and the bottom-right quarter containing 10 μm square holes in regular 20 μm spacing were used

Axiovert 200M microscope with digital image capturing software Axiovision 4.0 was supplied by Carl Zeiss Vision GmbH. STS Multiplex inductively coupled plasma system for Deep Reactive Ion Etching (DRIE) was supplied by STS. NT2000 Optical Profilometer (OP) with Vision operating software was supplied by Wyko. JSM-5600LV Scanning Electron Microscope (SEM) was supplied by JEOL. 97437 Oriel flood UV exposure source with Hg lamp (6 x 6 inch beam size) and uniform intensity of $8\text{mW}/\text{cm}^2$ measured at 365 nm was supplied by Spectra-Physics. PK 102 flood UV exposure source with Hg lamp having intensity of $96\text{mW}/\text{cm}^2$ (365 nm) was supplied by I&J Fisnar, USA. RZR 2021 mechanical agitator was supplied by Heidolph. BINDER vacuum oven was supplied by Fisher Scientific Pte. Ltd., USA. Direct-Q water ultrapurification system was supplied by MILLIPORE. 1-axis stretcher and pressure chamber with UV-window (poly (methyl methacrylate)) was self-designed and manufactured using standard milling and turning techniques in the workshop and CNC lab of school of MAE (NTU). Silicon master template mold with submicron features was provided by Dr. Chen Longqing (Fig 3.3).

Figure 3.3 Top-view illustration of the Si mastermold with 500nm features. The 500nm features are short square pillars with average height of 200nm and have regular $4\text{ }\mu\text{m}$ spacing

3.2 Fabrication of PDMS Rubber mold

PDMS molds with three feature sizes (10 μ m, 2 μ m and 500nm) were fabricated. 10 μ m PDMS molds have cavities measuring 10 μ m (length) x 10 μ m (width) with varying depths ranging from 3.1 μ m to 11 μ m. 2 μ m PDMS mold have cavities measuring 2 μ m in diameter and 0.7 μ m in depth. 500nm PDMS mold have cavities measuring 500nm (length) x 500nm (width) x 200nm (depth). The 10 μ m and 2 μ m PDMS molds were replicated from DRIE-etched Si mastermolds. Two replication steps to reproduce the cavities in elastic PDMS mold were illustrated as step 1a-1c in Fig 3.1 (pg 42). The 10 μ m and 2 μ m Si mastermolds (1a) had cavities patterned onto it (because the type of photolithography mask available was dark field), so two replication steps were needed to reproduce cavities in the PDMS mold. First replication gave short pillars made from PUR (1b) and the second replication gave cavities in PDMS (1c). Alternatively, the Si mastermold could start having the 1b profile (short pillars) as in the case of the 500nm Si mastermold (Fig 3.3), which was made by a proprietary technique of the group. In this case, the PUR replication step was unnecessary.

3.2.1 Si mastermold fabrication

The chrome mask used is shown in Figure 3.2 The MEMS center in the School of MAE (NTU) provided detailed procedures for the various machines needed.

New 100mm-diameter Si wafers were cleaned by dipping in piranha solution (H_2SO_4 : H_2O_2 = 15:1) at 120°C for 20 min to remove any organic contaminants. Spin-drying was then performed in stages: 1000 rpm for 2min, 1200rpm for 2min, and then 2000rpm for 2 min.

The resist coating process started with HMDS vapor coating (for resist adhesion promotion) for 20 sec in N₂ atmosphere at 100°C. Wafer was allowed to cool afterwards at room temperature for 1 min. AZ7220 resist was spin-coated at 3000rpm for 30s, with additional 5s before that to ramp up the speed. Resist was poured manually on the wafer before spin-coating. Afterwards, resist was prebaked on a hotplate for 90s at 100°C. Then the wafer was left on tissue papers for 60s to cool down to room temperature. Karl Suss MA6 mask aligner in contact mode was used to expose the resist with UV intensity of 8.9 mW/cm² at 365nm for 3.9s. Afterwards, postbaking on the hotplate was done at 110°C for 60s. Resist development was done manually by dipping and shaking the resist-coated wafer in AZ300MIF developer solution for 45s or until the patterned area stopped changing color. DI rinsing and spin-drying followed afterwards. Hardbake was conducted afterwards at 100°C for 3min. After cooling down, the wafer was stored in a wafer box until it was needed for DRIE etching.

Micrographs of the patterned resist were taken using an Axiovert 200M microscope from Carl Zeiss Vision. Dimension measurement of developed photoresist patterns were performed by the digital imaging software provided with the microscope.

The DRIE machine (STS) processes one 100mm-diameter wafer at a time. Etching process was controlled by setting parameters from the controlling software, as shown in Table 3.1a. The settings were recommended by the manufacturer. The etching process proceeded in cycles of etching and passivation that gives a ~90° vertical wall profile with sidewall undulations. SF₆ and O₂ were for the etching cycle, while C₄F₈

was used for the passivation cycle. The resulting cavity depth was determined by the total etching time.

Afterwards, the wafer was spun at 200 rpm and sprayed with acetone and IPA continuously for 60s each, to strip the bulk of resist on it. This was followed by dipping in 120°C piranha solution for 20 minutes, to give a clean surface. Spin-drying and vacuum evaporation dried the surface of the wafer.

The final step was to perform surface passivation using the same DRIE machine (STS) that etched the wafer. The parameters of passivation process are listed below in Table 3.1b. It was basically a prolonged version of the passivation cycle used in the etching process.

Parameters	Etching cycle	Passivation cycle
Sf ₆ flow	130 sccm	-
O ₂ flow	13 sccm	-
C ₄ F ₈ flow	-	100 sccm
Time/cycle	14 s	8 s
Coil power	800 W	800 W
Platen power	100 W	-
V peak-to-peak	35V	35V
V bias	0V	20V
Valve opening angle	70%	70%
Pressure	20mTorr	15mTorr

(a)

Parameters	Passivation
C ₄ F ₈ flow	100 sccm
Coil	300 W
V peak-to-peak	35 V
V bias	0
Valve opening angle	70%
Pressure	21 mTorr

(b)

Table 3.1 Machine settings for (a) etching process and (b) passivation process

Optical profilometer from Wyko was used to measure the etch depth. Micrographs were taken to measure the width of the cavities. SEM from JEOL provided top-view and cross-sectional images.

3.2.2 2nd generation replication: Polyurethane (PUR) diacrylate

The Si mastermold cut into approximately 2cm x 0.5cm piece was replicated with a UV-curable polyurethane prepolymer to get the opposite relief. This replicate needs to be replicated one more time using PDMS rubber to get the same relief as the Si mastermold.

The PUR precursor solution was made from 68 wt% EB270, 20 wt% SR508, 10 wt% TMPTA, 2% wt% EB350 and 0.3 wt% I651. Firstly, I651 (solid powder) was added to TMPTA and stirred until it dissolved. Afterwards, the other constituents were mixed followed by magnetic stirring and heating overnight (~8h) at 50°C to produce a uniform blend. The container must be covered with Al foil to protect the precursor from stray light that might cure it slowly.

The precursor was then spread on top of the Si master mold which was pasted on a glass plate acting as a backing. Degassing at 0.1 bar using a vacuum oven at room temperature was necessary to force the precursor into the cavities, as the precursor was viscous and does not automatically allow trapped air to be released from inside the cavities. During degassing, bubbles emerged to the air-precursor interface and burst. After 15 min, by visual confirmation, few or no bubble was left intact on the liquid-air interface and filling was considered complete.

To cure the precursor, it was necessary to isolate the precursor from O₂-containing atmosphere that can effectively stop free radical polymerization [45]. This was done by covering the precursor using adhesion-promoted polyester film (Melinex® film with gage 500). The polyester film was flexible and could be spread from one side of

the PUR precursor-covered Si mastermold to the other. This must be carefully done to prevent any air bubbles from being trapped beneath. Afterwards, a glass plate was put on top of the polyester film, and it was manually pressed as hard as possible. This caused extra PUR to flow out, resulting in a thinner PUR layer. This polyester film also served as substrate for the PUR replicate since the PUR layer itself was thin and had insufficient mechanical strength.

The whole setup was cured with UV-irradiation, with intensity of $\sim 90 \text{ mW/cm}^2$ at 365 nm for 7s using a PK 102 flood UV exposure source from I&J Fisnar, USA. Afterwards, the PUR-on-polyester film was peeled off the Si mastermold. Excess thick blocks of PUR usually present at the sides were removed by peeling it off the polyester film, leaving only the thin feature-containing replicated area.

OP and SEM were used to characterize the replication fidelity including the shape, size and wall profile of the resulting PUR replicate.

3.2.3 3rd generation replication: PDMS Rubber mold

To recreate the microfeatures on the Si master mold by replication from the negative 2nd generation PUR replicate, heat-curable Sylgard 184 elastomer kit from Dow Corning (USA) was used as the replicating material. The material will be referred to as PDMS rubber.

The two parts of Sylgard 184 - polymer and curing agent - were mixed in 10:1 w/w ratio and stirred with a mechanical stirrer at 500 rpm for 5min. Air trapped inside the viscous liquid was removed by degassing in 0.1 bar for 15 min at room temperature.

A spacer mold (Fig 3.4) was needed to achieve controlled and even thickness of the PDMS rubber replicate. The spacer mold was assembled from (Fig 3.4a) 2 glass plates (20cm x 20cm x3mm - larger area would need thicker glass to maintain sufficient plate stiffness), white Teflon spacers of 1mm thickness (this determines resulting rubber thickness), double-sided tape (not shown) and paper clips. The two glass plates were clipped together with the Teflon spacers between the plates and lining their edges (held in place by double-sided tape) as shown in Fig 3.4b.

PUR replicates were held onto the bottom glass plate by double-sided tape. Before top glass plate was put into place, liquid rubber precursor was spread on top of the PUR replicate, and degassed at 0.1 bar for 15min at room temperature to eliminate most bubbles that might still be present, minimizing potential defects in resulting PDMS mold. A layer of polyester film was used to carefully cover the rubber precursor before the top glass plate was placed over. It served two purposes: to minimize trapped bubbles because its flexibility provided better contact with the liquid PDMS precursors and to ease disassembly after curing. The fully assembled spacer mold was put inside an oven at 65°C for 12 hours to achieve full curing and high strength of the rubber. After curing and disassembly, the rubber mold was cut to suitable sizes.

Figure 3.4 PDMS rubber replication cast (20cm x 20cm x 5mm)
(a) Spacer mold parts (b) Fully assembled

Figure 3.5a shows the PUR replica and Figure 3.5b shows the PDMS replica both cut into rectangular strip. The grayish strip in the middle is where the pattern lies.

Figure 3.5 Photograph of (a) 2nd generation PUR layer on polyester substrate
(b) 3rd generation PDMS rubber

Defect-free PDMS mold is important to retain its ability to stretch excessively, as particle ejection effectiveness depends on the amount of strain (100% strain can give >99% ejection – results shown in chapter 4). Figure 3.6 illustrates the commonly found defects. Graph 3.1 shows effect of the various defects on the rubber's maximum strain ($\epsilon_{\max}$). The strain is given as a range (minimum and maximum) of $\epsilon_{\max}$ value where the PDMS mold broke. As can be seen, many of the defects cannot be ignored because the rubber cannot be stretched to 100% strain if there are defects.

This part was written to highlight the care that needs to be taken to achieve 100% strain with the PDMS mold. Classification of type 1-5 defects is largely qualitative and no systematic effort was made to induce the defects, nor is it practical to generate the same defects repeatedly e.g. to create the same size of bubbles or to create the same jagged profile of a deep scratch. The maximum strain measurements for type 1-5 defects were taken as they were observed to happen and are results of at least three measurements.

Figure 3.6 Illustration of the common defects of the PDMS replica. (a) Top view of rubber mold (b) side view of (a) (c) PUR replicate - sideview

Graph 3.1 Maximum and minimum strain needed to break PDMS rubber strip versus types of defect. Legend is according to figure 3.4

Bubbles defects (type 1 and 2) can be eliminated by cutting away the defective rubber to exclude them. Various precautionary measures (e.g. degassing and usage of flexible thin film cover) had been taken in the replication process to minimize occurrence of such bubbles. Edge nick (type 3) resulted from improper cutting of the rubber. It was

straightforward to prevent by using sharp blades and firm cutting strokes. Light scratches (type 4) and jagged scratches (type 5) usually cut across the width and cannot be cut away. Both are caused by rough, scratched, or dirty PUR replicate, and thus with care, can be prevented most of the time.

The PUR replicate (2nd replication) containing the micron features is a thin layer adhering to a larger, more robust polyester substrate. The polyester substrate is larger than the PUR layer to make it easy to handle and transport. This PUR layer has a thickness that makes the 3rd generation PDMS replica slightly thinner at the position where the PUR layer was (defect type 6 and 7)

When the size of the PDMS mold needed is small enough, it is possible to eliminate this difference altogether by using only the cavity-patterned area. In many of the experiments in this project, we usually used only a small PDMS mold with the size of 27mm x 3mm. But if thickness difference was unavoidable, the thickness variation was minimized because defect 6 (thickness difference less than 0.5mm) allowed more stretching than defect 7 (thickness difference 0.5-1mm).

OP and SEM were used to characterize the replication fidelity (shape, size and wall profile) of the PDMS replicate. Ultimate strain of the rubber is very important for subsequent particle manufacturing/harvesting process so it was investigated versus defects that were present.

3.3 Particle molding and ejection

Three particle sizes (10 μ m, 2 μ m and 500nm) were fabricated and investigated using the PDMS molds. The particle fabrication steps are illustrated by step 2-5 of Fig 3.1 (pg42). Briefly, the PDMS rubber mold was stretched and covered with particle precursor liquid. The mold and wet tissue paper were then placed inside a chamber and its surrounding atmosphere were conditioned by purging the O₂, pressurizing the atmosphere with Ar gas to 9 bar (gage), and humidifying the atmosphere (via the wet tissue). Droplets were formed inside each cavity by discontinuous dewetting, which were then cured using UV irradiation to give hardened, solid particles. After the mold was taken out of the chamber and the stretch was released, the particles were partly ejected out of the cavity. Freeze-peel completely removed the particle from the PDMS mold by encasing the particles in ice layer and separating the ice layer from the PDMS mold.

3.3.1 Preparation of particle precursor liquid

Three formulations were used in the investigation and they were:

1. "PEGDA" formulation consisting of 99/1 (w/w) PEGDA / Irgacure 2959.
2. "GFP-hydrogel" formulation consisting of 70/30 (w/w) GFP solution / PEGDA.
GFP solution concentration was 8mg / ml of distilled water. PEGDA was as (1).
3. "Hydrogel" formulation consisting of 30/70 (w/w) distilled water / PEGDA.
PEGDA was as (1).

The first formulation was used in initial investigations and debugging of SCAMP method, since it conserved GFP and does not introduce water evaporation factor. The second formulation was used to demonstrate drug loading of SCAMP down to 2 μ m particles and submicron particle size down to 500nm. The third formulation was used

for detailed investigation on water-evaporation prevention while reducing amount of water involved and conserving the GFP.

In the case of GFP-hydrogel formulation, GFP was dissolved in water first before being mixed with the polymer. Even with water in the mixture, GFP tended to aggregate when mixed with PEGDA and so mixture stirring with magnetic bar at low speed (10rpm) for 2 hours was done.

3.3.2 PDMS mold stretching

The stretch-release technique for particle ejection requires the rubber mold to be stretched and maintained in that state during cavity filling and UV-curing. To maintain the stretched condition, a simple prototype of '1-axis stretcher' was designed, fabricated and used in the experiment (illustrated in Fig 3.7). Detailed dimensioning is available in appendix A1. Subsequently this device will be referred to as 'stretcher'.

In Figure 3.7, parts pertaining to stretching of the mold are indicated in the legend. The gripper parts consist of a cylinder and a block. They gripped the mold at the grip points and were tightened with nuts and bolts. The mold strip was also wrapped around the cylinder in half circle to increase friction and made it possible for the mold strip to be gripped without tightening the nut-bolt pairs too much. This prevents large stress concentration that will cause premature failure of the PDMS mold when stretched. The stretching was effected by placing the spacer in between the gripping parts, and all parts were held together by the middle bar. The illustration shows the mold strip in the stretched position.

Fig 3.8a shows the exploded assembly view of the stretcher without the mold strip and the nut-bolt pair. Fig 3.8b is a photograph of the stretcher with the mold strip highlighted in white.

This method of stretching did not stretch the mold uniformly, as illustrated by the progressive narrowing of the mold strip from the side to the center. Usually the strain at the centre of the strip was controlled to be 100% (where the droplet was) which was observable under an optical microscope. To achieve this, the length of the unstretched mold strip was approximately 26mm. The stretcher was manually assembled and strain control was within $\pm 5\%$. Before stretching, the PDMS mold was cleaned by ultrasonication in acetone, ethanol, and DI water in that order, each for 15 minutes.

The patterned surface of the mold strip was the top surface (side view). The center area was covered by a precursor liquid droplet that was placed by pipetting it. The droplet was pinned onto the mold strip side edges to cover as large an area as possible. These are preparations (step 2a of Fig 3.1) before filling of the patterned cavities. The absorbent was placed on one side to help drain the droplet during discontinuous dewetting (next subsection).

3.3.3 Particle fabrication

Before the curing step, several issues need to be resolved to fabricate the particles successfully. Firstly, trapped air bubbles inside the cavities that prevented complete cavity filling with the precursor liquid need to be released. Secondly, the liquid covering the PDMS mold surface must be drained in such a way that the liquid remains only inside the cavities. This is the proper liquid shape to be cured to obtain particles. Thirdly, the atmosphere around the mold must be purged of O_2 that competes with radical polymerization reaction (the curing process) and prevents the liquid precursor to become solid particles. Fourthly, the water evaporation from the precursor liquid mixture must be slowed down, as in volume as small as the cavities,

water evaporates practically instantly. Step 2b and 3a of Fig 3.1 illustrate the solution to these problems and are explained in detail below.

A chamber was made for these purposes (Fig 3.9 – detailed dimensions in appendix A1). It has a compartment that fits the assembled stretcher. It has UV-transparent window (PMMA) on top to allow curing from externally-placed UV lamp. The double gasket design enables both vacuum and high-pressure operation. The vacuum gasket seals the chamber when the PMMA is sucked in during vacuum operation and the high-pressure gasket seals the chamber when the PMMA is pushed out during high-pressure operation (commercially available O-ring seals can also be used with alteration in the design).

After the stretcher with PDMS mold installed was placed inside the chamber, wet tissues were placed around the inside chamber wall before the chamber was closed. The tissue provided high surface area for the absorbed water to evaporate and humidify the atmosphere inside. To remove O₂/air, a cycle of vacuuming, backfilling with Ar gas to 2 bar (gage) and pause for 30s was repeated 3 times. This also degasses the air bubbles trapped inside the cavities (first issue). Afterwards, Ar pressure was increased to 9bar (gage). The chamber was then tilted to approximately 45° to allow the liquid to drain slowly to the direction of the absorbent material (Fig 3.5). UV lamp supplied by Spektra-Physics shone through the UV window for 1 sec with intensity of 16 mWatt/cm² (measured at 365 nm). The chamber was then depressurized and opened to remove the stretcher-mold assembly.

Figure 3.9 Pressure chamber

3.3.4 Particle harvesting

Harvesting of the particles was carried out in two steps: release of stretch to partially eject the particles out of the cavities and complete detachment from the mold by freeze-peel.

By detaching the mold from the stretcher, the cavities were relaxed into the original shape. This introduced dimensional difference with the particle that became the driving force to eject the particles out, though mostly the particles were half in, half out of the cavities (step 4 of Fig 3.1).

Another step, freeze-peel, was needed to completely detach the particles into a liquid medium e.g. water. The main idea of freeze-peeling was to trap the particles in another solid material, e.g. frozen water or PEGDA that allows them to be peeled off

the rubber mold. The freeze-peel material must be easily reversible between solid and liquid states such that releasing the particles is a simple matter of thawing the frozen material. Both water and PEGDA (average M_n 700 and melting point of 12-17°C) were used in this project.

In freeze-peel, to cover the surface of the PDMS mold (that has cured particles) with water, the PDMS mold strip was immersed fully in a container filled with DI water and degassed. Another relatively stiff material (e.g. a thick piece of polyester film or Si wafer) with surface area larger than the PDMS mold strip was immersed in the same container and brought into contact (slight pressure only) with the particle-covered surface. In this mated position, both materials were brought out of water and the excess water was drained/wiped. In between the two mated surfaces, there was a thin layer of water that was not very visible to the naked eye. In this setup everything was put into -30°C freezer for 15min together with a tweezers that was needed to separate the surfaces after freezing. If the tweezers was not of similar temperature, the heat transfer would melt the ice quickly. The setup should be put on an insulator when removed from the freezer and the surfaces should be separated immediately so that the ice did not melt first. Shearing action should be minimal when peeling, as it might weaken the ice' grip on the particles, causing the peeled material to not carry the particles with it. Successful freeze-peel results in the ice layer remaining on the other material together with the entrapped particles, which can then be scraped off with a cold blade for further purposes, for example to disperse the particles in an aqueous medium for in vitro experiment.

Sometimes the peeling did not result in complete separation of the ice layer and the PDMS mold, as shown in Fig 3.10. This micrograph of a PDMS mold strip has black/shaded parts covered with particles and brighter parts that have been stripped off any particles.

Figure 3.10 A PDMS mold strip showing incomplete freeze-peeling

It was not investigated why perfect peeling was not repeatable using this technique. Possible causes include ice temperature, surface properties, and improper peeling action. A spacer to increase the ice layer thickness guarantees perfect peeling. Thicker ice layer also made scraping of the ice layer easier.

Using PEGDA instead of water for the freeze-peel layer had some advantages. Incomplete peeling was seemingly less likely to happen and PEGDA's higher melting temperature (12-17°C) compared to water made it slower to melt after it was taken out of the freezer. The problem using PEGDA lies in extracting the particles from the liquid PEGDA, which is not as straightforward as using water since PEGDA does not evaporate easily in atmosphere conditions. In this project, water was almost exclusively used.

OP measurements were taken to characterize the filling and curing steps. SEM pictures were used to evaluate particle molding and ejection results. Fluorescence microscopy was used to observe GFP and GFP-loading inside the particles.

Chapter 4

Result and discussion

This chapter is divided into three sections. Section 4.1 presents the result of fabrication of elastic PDMS molds required for SCAMP (Stretch and Cavity Assisted Molding of Particle). Section 4.2 presents the result of making and harvesting the molded DDS particles with SCAMP. Further discussions are presented in section 4.3.

4.1 Fabrication of elastic PDMS mold with cavities

PDMS rubber mold that has patterned cavities is needed to fabricate the DDS particles using SCAMP technique. It was produced by a 2-step replication from a master Si mold (Fig 3.1 step 1).

In this project, three molds were used. The “10 μ m PDMS mold” has 10 μ m square cavities with various cavity depths (3.1 μ m - 11 μ m), the “2 μ m PDMS mold” has 2 μ m-diameter round cavities with 0.7 μ m cavity depth, and the “500nm PDMS mold” has 500nm square cavities with 0.2 μ m depth. 10 μ m particles produced from 10 μ m cavities are considered too large for cell-targeted drug delivery system, but they were helpful during preliminary debugging of various problems (e.g. particle harvesting and water evaporation) due to the relatively large size which allowed good observations using optical microscope. However, the 2 μ m and 500nm molds were used to make smaller more relevant particles to demonstrate the applicability of SCAMP.

Si mastermold was fabricated in-house instead of purchased because it provided an option to tailor its properties for investigation purposes. The fabrication techniques used were the same for 10 μ m and 2 μ m cavities, and the results were characterized, mainly using SEM. The 500nm mold was fabricated and donated by a colleague and was used as received. It was not further characterized because FESEM observation requires a part of the mold to be cut for gold coating. This was not conducted to preserve the original mold that was irreproducible by author.

For fabrication of 10 μ m and 2 μ m molds, the photolithography mask (Fig 3.2) used had different pattern sizes and shape at each quarter. One quarter had 10 μ m squares and another quarter had 2 μ m circles. Other quarters had different features but were not used.

Fig 4.1 shows the optical micrograph of resist-on-Si wafer after the photolithography process was completed. It shows well-defined $10.6 \pm 0.13 \mu\text{m}$ squares with rounded edges. The squares are the areas not covered by resist, and were etched down by the DRIE (Deep Reactive Ion Etching) process to give square cavities.

Figure 4.1
Optical micrograph of resist on Si wafer after lithography process

Figure 4.2 shows the SEM images of the DRIE Si mastermold, the PUR replicate and the PDMS replicate. The Si mastermold has cavities, the PUR replicate has short pillars and the PDMS replicate has cavities again. High-magnification SEM cross-section views of individual Si cavities, PUR short pillars and PDMS cavities were taken (Fig 4.3). The undulations of the sidewalls shown in Figure 4.3d were caused by the alternating passivation/etching steps of DRIE and have vertical periodicity of 0.2 μ m [44,55]. All pictures mentioned above show 10 μ m square cavities.

Figure 4.2 Low magnification SEM images of various stages of replication of 10µm mold
(a) Si master mold (b) PUR replicate (c) Silicone rubber replicate

Figure 4.3 High magnification SEM images of replication stages of 10µm mold
(a) Si master mold (b) PUR replicate (c) Silicone rubber replicate
(d) Close-up view of wall undulations

Variation of the cavity depth was desired because it translates to variation of particle thickness, a parameter that is useful in investigating the various particle parameters that will affect demolding. Two factors affect cavity depth. One factor is the DRIE etching time. Another factor is the spatial non-uniformity of DRIE etching process that caused different spots on the wafer to have different cavity depths. This spatial depth variation study was carried out using the 10µm mold only.

Table 4.1 summarizes the variation of cavity depth by changing the etching time from 1 min to 3 min and by measuring the depth at 5 different points across the wafer (Fig 4.4). The measurement value obtained from a “point” is actually an average of 5 spots

over an area of $200\mu\text{m} \times 200\mu\text{m}$ (illustrated in Fig 4.5). All measurements were taken by SEM pictures.

Dark gray: cavity area (4cm x 2.5cm)
Light gray: blank wafer surface

Figure 4.4 Illustration of different points of cavity depth measurement on the Si master mold (4in wafer). Point A, C, D, and E were taken 3mmx3mm from the edge of the cavity area (dark gray). Point B was taken at the center or the cavity area (dark gray).

Figure 4.5 Illustration of a "point" of measurement
Each measurement value is an average value of 5 spot over a $200\mu\text{m} \times 200\mu\text{m}$ area (10cavities x 10 cavities)

Table 4.1 The effect of etch depth time and location on etch depth. Average value and standard deviation are calculated according to the definition of a "point" in Fig 4.6

Etching time (mm:ss)	Point A (μm)	Point B (μm)	Point C (μm)	Point D (μm)	Point E (μm)
1:00	3.4 $\pm$ 0.10	<u>3.2</u> $\pm$ <u>0.06</u>	3.9 $\pm$ 0.05	3.6 $\pm$ 0.06	<u>3.7</u> $\pm$ <u>0.08</u>
1:20	<u>3.9</u> $\pm$ <u>0.07</u>	4.0 $\pm$ 0.08	4.5 $\pm$ 0.10	4.0 $\pm$ 0.10	<u>4.1</u> $\pm$ <u>0.10</u>
1:40	<u>5.5</u> $\pm$ <u>0.08</u>	5.6 $\pm$ 0.12	6.1 $\pm$ 0.15	5.9 $\pm$ 0.16	<u>6.7</u> $\pm$ <u>0.23</u>
2:00	6.5 $\pm$ 0.15	<u>6.4</u> $\pm$ <u>0.14</u>	7.2 $\pm$ 0.19	6.7 $\pm$ 0.23	<u>7.7</u> $\pm$ <u>0.26</u>
2:30	8.7 $\pm$ 0.13	<u>8.7</u> $\pm$ <u>0.21</u>	9.7 $\pm$ 0.24	9.5 $\pm$ 0.27	<u>10.5</u> $\pm$ <u>0.25</u>
3:00	10.1 $\pm$ 0.21	<u>10.0</u> $\pm$ <u>0.23</u>	11.2 $\pm$ 0.26	10.1 $\pm$ 0.29	<u>11.5</u> $\pm$ <u>0.14</u>

The trend of increasing cavity depth with increasing etch time is consistent at all 5 measurement spots with a calculated average etching average of $3.6\mu\text{m}/\text{min}$

The cavity depth more or less increases with increasing distance from wafer center. The points having minimum depth (single underlined) are spot A or B (Fig 4.4) which are nearer to wafer centre, while the points having maximum depth (double underlined) are spot E (Fig 4.4) which is furthest from the wafer centre. By subtracting the maximum point with the minimum point, dividing the result with the minimum point (thus obtaining percentage variation), and dividing that result with the distance between the points, a percentage of depth variation per distance can be obtained. The largest value of this is $6\%/\text{cm}$ for the 3min etching time. For preliminary investigations, a $5\text{mm} \times 5\text{mm}$ area was cut from this quarter of wafer, and this ensures a 3% or lower depth variation within this area, which is suitable for our project that did not require very high accuracy.

The width of the cavities also varied with different etching time and position, but is markedly less. For etching times of 1min and 3min, the width was measured to be $10.9 \pm 0.23\mu\text{m}$ and $11.0 \pm 0.21\mu\text{m}$ respectively (measurement at the center of the wafer – not displayed in table). Width variation with position is also small (not shown in table), measuring a minimum of $10.7 \pm 0.21\mu\text{m}$ and a maximum of $11.2 \pm 0.31\mu\text{m}$. Therefore, width variation with etching time and position is 5% or less which is considered suitable for this project.

For replication, one measure of quality is the amount of dimensional change. Table 4.2 summarizes the dimensional change after each replication step. Firstly, Si master

molds were fabricated and selected to have regular depth increment (approximately 1.5 μ m). Then the PUR replicates and PDMS replicates were fabricated and measured. All measurements were performed using optical profilometry.

Table 4.2 Depth measurements across three stages of replication, using Optical Profilometer. Average values and standard deviations are calculated according to the definition of a “point” in Fig 4.6

No	Si master mold cavity depth (μ m)	PUR replicate pillar height (μ m)	Rubber replicate cavity depth (μ m)
1.	3.3 $\pm$ 0.07	3.5 $\pm$ 0.10	3.1 $\pm$ 0.11
2.	4.7 $\pm$ 0.12	5.1 $\pm$ 0.17	4.4 $\pm$ 0.19
3.	6.3 $\pm$ 0.17	6.1 $\pm$ 0.21	5.8 $\pm$ 0.18
4.	7.8 $\pm$ 0.18	8.3 $\pm$ 0.22	7.5 $\pm$ 0.25
5.	9.3 $\pm$ 0.25	9.8 $\pm$ 0.31	9.2 $\pm$ 0.27
6.	10.9 $\pm$ 0.24	11.7 $\pm$ 0.35	11.1 $\pm$ 0.31

PUR replicates differ by less than 10% from Si master and PDMS replicates differ by less than 8% from Si master. The increase of PUR height compared to Si master was unexpected because replicates tend to shrink after the UV curing process to fabricate them. One possible reason is that OP might have slightly different accuracies for protruding and depressed structures, causing underestimation on the measurement of the Si master. Since this project did not require very high accuracy, this variation after replication was acceptable.

By applying the fabrication steps for 10 μ m cavities using 2 μ m photolithography mask, 2 μ m mold was obtained. The DRIE etching was set to give an etch depth of 0.7 μ m such that the aspect ratio (height:depth) achieved was approximately 1:3. The cavities were characterized using SEM and shown in Fig 4.6.

Figure 4.6 SEM images of SI mastermold having patterned 2μm cavities with 0.7μm depth
(a) low magnification, top view (b) high magnification, cross-sectional view

As mentioned before, characterization of 500nm Si mastermold was not carried out because of the interest to preserve the mold that was irreproducible by author (it was fabricated and donated by a colleague). By estimation from the colleague, the average depth of the 500nm cavities was approximately 200nm.

4.2 Particle molding

'GFP-hydrogel' (see definition at chap 3 pg 41) particles were fabricated with the 3 mold sizes with aspect ratio of approximately 1:3 (depth:width). To show the GFP encapsulation, images were taken with transmissive light microscopy and fluorescent microscopy (Fig 4.7 – 4.8). Clusters of 10 μ m and 2 μ m particles were shown to be fluorescent green in contrast to the dark background. The image of 500nm particles' clusters appeared to be thoroughly dark (not shown), possibly because the particles were too small to be detected by fluorescent microscopy. SEM image of 500nm particles' clusters are shown in Fig 4.9. The 500nm particles' clusters are less dense than the 10 μ m and 2 μ m particles' because the cavity density of the 500nm mold is less than the 10 μ m and 2 μ m molds. High magnification SEM images of individual particles are presented in Fig 4.10.

In Fig 4.7a & 4.8a, the dashed circle marks the area where particles are clearly separated from one another. The corresponding area under fluorescence microscope shows the particles also clearly separated, having the same level of brightness. However, some particles appear darker, dirty and unclear (marked by solid circles). The corresponding particles under fluorescence microscope (Fig 4.7b & 4.8b) are brighter compared to the surroundings. This indicates that the particles are stacking, not dirty, causing them to appear darker due to light obstruction under transmission light microscope, but brighter under fluorescence microscope because there are more GFP in the locality.

Figure 4.7 Transmissive optical micrographs of clusters of freeze-peeled 10µm "GFP- hydrogel" particles. Solid circles indicate single-stacking particles. Dashed circles indicate multi-stacking particles. Both pictures are of the same spot.
(a) transmissive light microscope (b) transmissive fluorescence microscope

Figure 4.8 Transmissive optical micrographs of clusters of freeze-peeled 2µm "GFP- hydrogel" particles. Solid circles indicate single-stacking particles. Dashed circles indicate multi-stacking particles. Both pictures are of the same spot.
(a) transmissive light microscope (b) transmissive fluorescence microscope

Figure 4.9 FESEM images of clusters of freeze-peeled 500nm “GFP- hydrogel” particles
(a) low magnification (b) high magnification

Fig 4.10 shows high-magnification SEM images of individual particles and gives an approximate measurement of their dimensions. The particles were elongated because they were cured inside stretched cavities. The 10 μ m particle actually measures about 21.3 μ m x 7.9 μ m x 2.1 μ m. Similarly, “2 μ m particle” measures 3.5 μ m x 1.3 μ m x 0.5 μ m (estimated thickness). The 2 μ m particle's shape is oval because the 2 μ m PDMS mold has circular cavities. 500nm particle measures 950nm x 380nm x 150nm (estimated thickness). All these particles were made with GFP-hydrogel solution.

Figure 4.10 SEM images of 10 μ m, 2 μ m, and 500nm “GFP- hydrogel” particles after stretch release, on PDMS mold

In the next four subsections, the importance of the fabrication steps in SCAMP: discontinuous dewetting, stretching, freeze-peel and atmosphere conditionings (O₂

exclusion, high pressure and high humidity) are discussed. These steps are illustrated in fig 3.1.

4.2.1 Effect of discontinuous dewetting

Discontinuous dewetting is the step performed after the PDMS mold was covered by the liquid particle precursor solution. A successful discontinuous dewetting results in liquid filling the cavities only, as opposed to (1) a thin layer of liquid still covering the entire mold surface, or (2) all liquid draining from the cavities and surface of the mold. The first situation would result in the particles joined together by a thin film after curing, while the second situation would leave no liquid to be cured.

At least three conditions need to be satisfied for successful discontinuous dewetting [47]. First, the mold surface needs to be hydrophobic because it promotes liquid draining, not leaving a continuous thin layer of liquid. Second, a minimum aspect ratio of 1:4 (height:width) is needed to retain the liquid inside the cavities and prevent total draining. Third, the viscosity of liquid must be less than 500 cP.

A convenient way to observe the success of the discontinuous dewetting process is to use Optical Profilometer. This is because under light microscope, it is not clear whether cavities are filled or empty due to the transparency of the uncured solution. Other characterization methods are less convenient or not suitable (SEM cannot image liquids and AFM are more complicated). Although measurements were not very accurate due to the presence of liquid and the measurement limit, OP was still useful because it was quick and because this project did not require a high degree of accuracy. OP was unable to characterize 500nm cavities though.

Fig 4.11 shows color depth profiles before filling (a) and after filling (b) of $10\mu\text{m}$ cavities with $3.1\mu\text{m}$ depth. The formulation used was “PEGDA”. Blue color means the lowest level and red color means the highest level, with other colors in between. Before filling (Fig 4.11a), the square cavities were empty (of a lower level) because they were blue in comparison to the red surrounding. After filling with discontinuous dewetting (Fig 4.11b), the cavities were filled with liquid because they were now red-greenish (indicating a convex surface) in comparison to the surrounding blue. The apparent discontinuity between the blue color and the green-red color is a good indication of a successful discontinuous dewetting, because if the whole surface was covered with liquid and not just the cavities, such discontinuous red-green squares would not be present.

Figure 4.11 OP depth profiles of filling and curing processed in $10\mu\text{m}$ cavities
 (a) before filling (b) after filling
 (Blue color - lowest level; red color - highest level)
 Formulation used was “PEGDA”

4.2.2 Effect of stretching

Various methods had been contemplated to collect the particles from inside the cavities after they were cured, and these include usage of sacrificial layer beneath the particles, sonication, suction, and adhesion layer on top of the particles to peel them off. It was decided to use stretch-release to eject particles because this method is simple, it requires no additional material (no additional contamination) and the process is gentle to the drugs.

Stretch-release ejection requires the cavities to be stretched before curing and released after curing. This will cause a dimensional difference between the cavities and the particles that becomes the driving force to eject the particles.

Pictures of empty relaxed cavities (Fig 4.12a) and stretched cavities (Fig 4.12b) were taken with light microscope. The cavities were stretched to 200% of original length (100% strain) and held in that position using an in-house manufactured stretcher. Particles formed using these two conditions of mold are shown in Fig 4.13 and 4.14, taken by SEM. The unstretched mold gave trapped particles (Fig 4.13). In contrast, application of stretch-release ejection gave elongated particles which were partly ejected out of the cavities (Fig 4.14). Particle shape elongation is parallel to the stretch direction, and one side perpendicular to the stretch direction was usually still attached to the cavity wall. These model particles shown here were made with “PEGDA” solution.

(a) (b)
Figure 4.12 Optical micrographs: top view of empty 10µm PDMS mold
(a) relaxed condition (b) pre-stretch applied and maintained

(a) (b)
Figure 4.13 SEM images of cured, non-prestretched PEGDA particles
in 10µm square cavities with 3.1 µm depth
(a) low magnification. top view (b) high magnification. cross-section view

Figure 4.14 SEM images of cured, pre-stretched PEGDA particles (stretch already released)
in 10µm square cavities with 3.1 µm depth

4.2.3 Effect of freeze-peel

The partially ejected particles still need further treatment to harvest them, i.e. to release them into an aqueous medium. Freeze-peeling is one way of accomplishing this. By trapping the particles in ice, the particles can be peeled off the mold and released completely to any aqueous medium, for further *in vitro* and *in vivo* experiments.

The effectiveness of freeze-peel in harvesting the particles is shown in Fig 4.15, where the mold was covered with particles before freeze-peel (Fig 4.15a) and was stripped clean of them after freeze-peel (Fig 4.15b). Both figures show the same spot as indicated by the distinctive blank area on the left. The mold used had square 10 μ m cavities which were 3 μ m deep. The model particles were made from “PEGDA” solution.

The images of clustering GFP-hydrogel shown earlier (Fig 4.7-4.8) were also taken after the freeze-peel process. The evidence that they were successfully harvested is that they were clustered on a microscopic glass slide (smooth and transparent), instead of being orderly arrayed on a square-patterned PDMS mold, as is the case after stretch-release. The ice block encasing the particles were left on the microscope slide to melt and afterwards the water evaporated, thus taking the micrograph became possible. In the same way the ice block can be melted in an aqueous medium to release the particles, which might be better suited for further experiments.

Figure 4.15 Optical micrographs of 10µm “PEGDA” particles removal by freeze-peeling
(a) after pre-stretch release: partly ejected particles
(b) after freeze-peel: no particles on the mold

4.2.4 Effect of atmosphere conditioning: O₂-free, high humidity and high pressure environment

By observation, macro-sized droplets of “PEGDA” liquid (2ml) could be UV-cured in open air, but there was a thin layer of uncured liquid on top of the cured macro-droplet. It is known that O₂ (in the air) inhibits free-radical polymerization initiated by the UV [50], so this was the cause of the uncured thin layer. In this project, the liquid droplet (inside the cavities, after discontinuous dewetting) was of (sub)micron size. The size seemed to aggravate the problem because diffusion of O₂ throughout the whole droplet was much faster. This in turn cause the (sub)micron precursor droplets to be incurable in open air.

Another observation is that macro-sized droplets of water (2ml) evaporate relatively slowly in open air. In comparison, (sub)micron size of the precursor droplets (in the case of solutions that contains water) will cause the water content to evaporate practically instantaneously. Without additional treatment, it is not possible to preserve the water inside the particles throughout the entire particle fabrication.

Based on the observations above, there are two purposes in conditioning the atmosphere around the liquid precursor. One is to exclude O₂ by excluding the air, because O₂ inhibits free-radical polymerization. Two is to prevent evaporation of water in the case of “GFP-hydrogel” solution.

The first conditioning (removal of O₂) was achieved by vacuuming the air out of the curing chamber and purging it with Ar gas 3 times. This should be done before the

UV-curing step. The vacuuming step can be combined with the one used to degass the cavities (see section 3.3).

The second conditioning is pressurization inside a pressure chamber through usage of high-pressure Ar and humidification through putting water-wetted tissues inside the chamber, both to prevent water evaporation. The difference between “GFP-hydrogel” particles cured with and without this conditioning is shown in Fig 4.16. The aggregated fluorescence GFP (tiny dots) inside the particles of Fig 4.16a is contrasted with the smooth, uniform distribution seen in Fig 4.16b. The aggregation is expected because GFP does not dissolve in PEGDA.

Noting that even without high-pressure and high-humidity, one, particles could still be formed, two, GFP was still trapped together with the cured PEGDA, and three, GFP was still fluorescent, further research is needed to determine the relevance of water-evaporation prevention to preserve bio-drug function in general. This step was introduced based on the assumption that any kind of aggregation is undesirable for bio-drugs and that GFP is a robust protein [56] that is not a good indication of SCAMP’s compliance for bio-drugs in general.

As a side note, the GFP aggregation of Fig 4.16a shows that the fluorescence does come from the GFP inside the particles, not from the particles' material (PEGDA).

Figure 4.16 Fluorescent micrographs of 10µm “GFP-hydrogel” particles
 (a) without and (b) with water evaporation prevention
 (a) aggregated GFP inside particle (b) smooth distribution of GFP

Another difference in the final result with and without the high-pressure and high-humidity conditioning is shown in Fig 4.17. The depressed shape of Fig 4.17a (without conditioning) is contrasted with the bulged shape of Fig 4.17b (with conditioning), indicating loss of mass (water) by evaporation without the conditioning. The depressed surface of Fig 4.17a is upside down; this is a common particle position after cross-sectioning the particles with a paper knife. Possibly the relatively gigantic blade caused rather large local disturbance when sectioning the particles and mold. These model 10µm particles were made with “hydrogel” solution.

To investigate the effectiveness of the conditioning to prevent water evaporation, a quantitative measurement of the particle's volume was also carried out. Specifically, SEM pictures (Appendix A2) of 60 “hydrogel” particles' cross-section, with (30 particles) and without (30 particles) atmosphere conditioning were taken, measured and statistically averaged for the final dimension comparison.

Fig 4.17 also illustrates how the particles' volume was estimated. First, the cross section area (height x width) was estimated. Certain height of the particles was estimated as rectangular in shape (H_2 – green rectangular). The rest of it (H_1 minus H_2) was estimated as triangles. The width (W) was measured from the widest part of the particle. Second, the length was measured from top view SEM pictures (Appendix A2). Volume can be obtained from cross section x length. Tabulated measurement data and volume calculation can be viewed in Appendix A2.

The volume difference is statistically significant, and it estimates that the particles cured with water evaporation prevention step contained 16.5 vol% of water. The water content was originally 30% wt. Though there might be some disparity in comparing volume to weight, it seems that the difference is large enough to conclude that evaporation prevention was not total. Noting that one, particle size more relevant for DDS lies at the submicron range, and that two, within submicron cavities, the water content available decreases in the order of 1 to 2 magnitudes compared to the 10 μ m particles measured above, the significant water retention demonstrated here is not a guarantee that it will be so in the submicron range. Therefore, more research will be needed in this matter.

Figure 4.17 Estimation of 10 μ m “hydrogel” particles’ cross-section area
 (a) depressed particle, water evaporation prevention not applied. The particle is upside down
 (b) bulging particle, water evaporation prevention applied

4.3 Discussion

4.3.1 On the mechanics of particle ejection

Two experiments were conducted to better understand the mechanics of ejection. The first experiment investigated the effect of strain and water swelling on the effectiveness of stretch-release to eject the particles. The second experiment set investigated the effect of particle thickness and water swelling.

A. Variation of strain and immersion time on ejection effectiveness

The effects of mold strain and swelling time on ejection effectiveness were investigated. 10 μm PDMS molds were used in this experiment. The particles in this experiment were made of “PEGDA” solution. The amount of strain applied before discontinuous dewetting was varied and they were: 0%, 25%, 50% and 100%. The particles were immersed in water after they were cured and before the stretch was released. The immersion time were also varied: no immersion, 15min (short time) and 7h (long time).

The objective of variation of strain was to give insight to the process of particle ejection. The objective of the extra immersion step was to investigate whether swelling of PEGDA particles before ejection can increase the effectiveness of stretch-release method to eject the particles. The two effects were summarized in table 4.3. For each condition, SEM images of arrays of 20x20 particles were analyzed. Examples of the SEM images are shown in Fig 4.18. They are images for 25% strain, with no immersion (0% effectiveness) and short immersion (50% effectiveness). Other SEM images can be viewed in appendix A3.

Table 4.3 Effectiveness of varying strain and immersion time (in water) to “PEGDA” particles ejection effectiveness

Strain	No immersion	Short immersion (15m)	Long immersion (7h)
0%	0%	0%	0%
25%	0%	50%	50%
50%	5-10%	80%	95%
100%	← >99% →

Figure 4.18 SEM images of ejection effectiveness of 10µm “PEGDA” particles with 25% strain
(a) without immersion in water
(b) with short 15min immersion in water before stretch was released

From table 4.3, without water swelling/immersion, increase in strain resulted in increase of ejection efficiency. For example, with 0% strain, all particles remained inside the cavities. The best result was achieved with 100% strain, as more than 99% particles were partly ejected. Instead of a linear progression (e.g. 25% strain = 25% particles ejected), the effectiveness change relatively abruptly from 50% strain (10% effectiveness) to 100% strain (99% effectiveness). This indicates that there is possibly a cut-off strain where above that amount, the phenomenon that causes particle ejection is triggered.

Based on the experiment results above and the occasional observation that particles were sometimes buckled inside the cavity (Fig 4.19), the ejection of particles is postulated to comprise the following sequential steps (Fig 4.20).

1. When stretch of the PDMS rubber is released, edges along the stretch direction break from the cavities, leaving edges perpendicular to the stress direction still adhering to the cavities
2. The particle buckles as the PDMS cavities return to the unstretched state
3. Typically, one end of the particles perpendicular to the strain direction breaks away from the cavities, allowing the particle to straighten, half-in and half-out of the cavity. This is a “partly ejected” particle.

Figure 4.19 SEM images of a “PEGDA” particle in buckled position
(a) Lower magnification (b) higher magnification

Figure 4.20 Illustration of the postulated process of particle ejection by stretch-release
Each part has a top view and a side view
(1) particle and cavity condition right after stretch is released
(2) when cavity is middle-way relaxed, the particle buckles to accommodate the shrinkage
(3) when cavity is fully relaxed, usually particle has 'snapped out' of the cavity and is straightened

A common observation of partly ejected particles is that one short side and one long side were attached to/touching the cavity wall. This is illustrated in Fig 4.20 and can be seen clearly in Fig 4.14 (pg 81). One possible explanation is as such. Adhesion force is present between each particle sides and their corresponding cavity wall before the stretch was released. Between the opposing sides (e.g. between the two short sides), the adhesion force was similar but not equal in value. Because of this, when the stretch was released, one side yielded first before the other. This left the particle adhering to/touching one short side and one long side.

Another observation is that although the length of particle is longer than the cavity (due to stretch-release), the width of the particle is shorter than the cavity (see illustration in Fig 4.20(3)). This is caused by the Poisson's effect and is explained more thoroughly in the next subsection 4.3.2.

The immersion step carried out in this experiment contributed significantly to the ejection effectiveness. For example, with 25% strain, 0% effectiveness without immersion changes to 50% effectiveness with immersion. One possible explanation is that swelling increases hydrogel size and lowers its modulus [49]. Both factors possibly contributed in making buckling easier, as buckling force decreases with a decrease in modulus of material and an increase in length. Another possible positive effect of immersion is that it might also reduce the adhesion strength of the particles to the cavity walls, which also facilitates buckling.

B. Variation of particle thickness and immersion time to ejection effectiveness

Ejection efficiency of PEGDA particles from cavities with different depths was evaluated. The cavities investigated were 10 μ m square with depths varying from 3.1 μ m to 11.1 μ m. The effect on water swelling (by 7h immersion) was also investigated. The particles in this experiment were made of “PEGDA” solution. The ejection efficiency was evaluated from SEM images of arrays of approximately 20x20 particles (appendix A4).

Table 4.4 summarizes the results. “Cavity depth” is the depth value of cavities used to mold the particles and “particle thickness” is the resulting particle thickness. Their difference is presented as “thickness reduction”. “No immersion” column gives percentage of partly ejected particles after stretch release. “Long immersion” gives percentage of (partly) ejected particles with additional step of prolonged water immersion (7h) before stretch release.

Table 4.4 Effectiveness of 100% pre-stretch and immersion time (in water) to eject “PEGDA” particles with different thicknesses

No.	Cavity depth (μ m)	Particle thickness (μ m)	Thickness Reduction	No immersion	Long immersion (7h)
1.	3.1 $\pm$ 0.11	2.4 $\pm$ 0.16	0.23	>99%	>99%
2.	4.4 $\pm$ 0.19	3.2 $\pm$ 0.25	0.27	0%	75%
3.	5.8 $\pm$ 0.18	4.3 $\pm$ 0.21	0.26	<10%	45%
4.	7.5 $\pm$ 0.25	5.6 $\pm$ 0.23	0.25	10%	50%
5.	9.2 $\pm$ 0.27	7.1 $\pm$ 0.24	0.23	10%	50%
6.	11.1 $\pm$ 0.31	8.9 $\pm$ 0.28	0.19	0.20%	20% (fully ejected)

“Thickness reduction” is the percentage reduction when comparing the original cavity depth and the molded particle. It is useful for comparison across the different cavity depths, and is a total of at least three effects. Firstly, there is a reduction of cavity

depth due to Poisson's effect when the cavity is stretched. Secondly, UV-curing of hydrogel precursor causes it to shrink and reduced the particle thickness. Thirdly, discontinuous dewetting leaves a convex liquid surface that rises above the rubber mold surface (see section 4.2.2). The height might have depended on the speed of discontinuous dewetting (the speed was uncontrolled) and mold surface properties. This factor increases the particle thickness.

Table 4.4 shows that thicker particles are less likely to be ejected successfully. For example, without any water immersion, the ejection efficiency changes abruptly from a >99% for 2.4 μm -thick particles to 0% for 3.2 μm -thick particles, i.e. from widespread ejection to total retention inside the cavities (see SEM images in Appendix A4). This again indicates a certain cut-off value that determines whether particles will be ejected or not. With additional water immersion, ejection efficiency improves for all thickness, with the general trend of thicker particles being harder to eject remains.

Interestingly, three particles thicker than 3.2 μm particle all have success rate of ~50% (with immersion), instead of linearly changing from a higher value to a lower value. To explain this, an observation was made on two rows of 5.6 μm -thick particles (Fig 4.21a). It can be seen that there is an alternation between ejected and stuck particles along the pre-tension direction. Fig 4.21b shows the common finding of severely deformed cavities. The cavity should have been square-shaped, but instead it looks like it is being pushed by neighboring stuck particles. This indicates that the particles are stiff compared to the rubber mold. Because of this, compression by the rubber was resisted, to the extent that the rubber was deformed instead by the PEGDA particles.

From Figure 4.21, we hypothesize that ejection might be neighbor-assisted. The particles were too thick to be ejected by stretch-release by themselves. But each particle was brought closer to its neighboring particle after the stretch was released, due to the return of PDMS mold to its original length. This might caused two neighboring particles to behave like one longer particle, lowering the force needed to eject them and making ejection possible. However, after one particle was ejected, its neighbors lose this effect and therefore could not be ejected themselves. This caused the alternating ejected-stuck row of particles.

Figure 4.21 SEM images of 5.6µm-thick 10µm “PEGDA” particles
(a) alternating stuck-ejected formation (b) severely deformed cavity

While the 4.3µm, 5.6µm and 7.1µm-thick particles appears to benefit substantially from the neighbor assisted-ejection (their ejection rate was ~50%), the thickest 8.9µm

particles have a smaller ejection rate of 20%. Probably, the 8.9 μ m particles were too thick to be affected by the neighbor-assisted method.

Observing the increased efficiency by water swelling and the alternating ejected-stuck phenomenon, it seems that buckling tendency is one plausible method to predict ejection efficiency, and it supports the proposed ejection process described before (Fig 4.20, pg 91).

The benefit of thicker particle compared to thinner particle for drug delivery itself has not been researched. In the event that it is shown to be beneficial, lengthening the particles is a more practical way to lower buckling force and increase ejection effectiveness. Also, eliminating the cavity wall undulations of sidewalls is likely to increase the ejection efficiency, because it will reduce adhesion (smaller contact area) and friction (smoother contact surface).

4.3.2 Ejection by 2-axes stretch

In our experiments, the PDMS mold was usually stretched to 100% strain before discontinuous dewetting to fill the cavities and the stretch was released after the particles were UV-cured, resulting in partially ejected particles. This stretch-release approach generates a dimensional difference between the rectangular cured particle and the relaxed square cavity to eject the particles which was induced with the release of the stretch. This dimensional difference (illustrated in Fig 4.22) is the difference of the length of the gray rectangle (particle (a)) and the dotted square (relaxed cavity (b)) in the y axis. For 100% strain, the release of stretch will cause a 50% dimensional difference.

Another stretch-release can be applied to the cavity perpendicular to the first. The shape of cavity after this 2nd stretching is illustrated in Fig 4.22 by the smooth dotted rectangle (c). The negative strain on the y-axis that happens when positive strain is applied to the x-axis can be calculated from the poisson's effect.

In following discussions, the stretch applied before cavity filling (the first, usual one) will be called pre-stretch, and the second stretch applied after the first one is released (i.e. after the particles are cured and ejected) will be called post-stretch.

Silicone rubber's Poisson ratio is very close to 0.5. Therefore, when a post-stretch of 100% strain is applied on the silicone rubber at the x-axis, a negative strain of 50% will be induced at the y-axis (and z-axis, not shown). This negative strain further reduce the dimensional difference between the particle and the cavity at the y-axis from 50% (result of pre-stretch) to 25% (Fig 4.22)

To achieve controlled and repeatable two-axes stretch, the mold must be cut into a square instead of the regularly used narrow strip, because it needs to be grippable on all four sides. For this purpose, at least two requirements need to be met. First, the increase in mold cross-section (from a narrow to a wide strip) requires an increase in the power needed to stretch the mold. Stretching by hand to 100% strain can provide power only up to a 1cm-wide rubber mold, so wider mold needs other sources of power. Second, the stretcher itself needs to be redesigned to accommodate the new power source and the increasing mold width. A wide mold, when unevenly gripped, will result in the mold slipping off or the mold breaking prematurely at one side before reaching 100% strain.

Figure 4.22 Combination of pre-stretch and post-stretch to increase dimension difference between particle and cavity
 (a) The size of particle, formed by curing in pre-stretched mold
 (b) Pre-tension released – cavity returns to square shape (double line)
 (c) Post-stretch applied – cavity deforms (dashed line)

These technical problems have yet to be overcome. This is the reason why the experiments on previous section were not conducted with 2-axes stretching. However, an improvised demonstration was carried out using a bigger prototype of the 1-axis stretcher that can grip a 4cm long x 2cm-wide PDMS mold. Pre-stretch (100% strain) along the 4cm-length was carried out normally by the stretcher, while the following post-stretch was carried out by (barely) gripping the rubber with fingers and stretching it along the 2cm-width. This achieved 75% strain but control was poor.

This improvised demonstration shows that 2-axes stretch can allow complete detachment of particles from cavity walls (Fig 4.23). This is in contrast to result of pre-stretch only where 1-sided attachment is common (Fig 4.14). However, the particles still remained adsorbed to the surface of the PDMS mold. This complete

detachment might be beneficial for surface modification on all the particle's surfaces (needed to reduce aggregation and *in vivo* particle internalization by white blood cells) or using the PDMS as template for cell growth and letting the cells internalize the particles (application in general cell culture and tissue engineering). Originally, two-axes stretching was designed for cases when pre-stretch was not enough to eject the particles, but it was easier to reduce the thickness of the particles and/or lengthen the particles to render them ejectable by pre-stretch only.

Figure 4.23 SEM image of full PEGDA particle detachment by pre- and post-stretch combination

Another interesting observation in the issue of particle ejection is trapped particles that could relocate onto the top surface of the PDMS mold without any pre- or post-stretch and freeze-peeling processes. These auto-ejected particles are shown in Fig 4.24 and the reason has not been found yet. Their occurrence was seemingly random,

rare and localized on a small area of the PDMS mold. If this phenomenon can be controlled and repeated, it might be possible to remove the pre-stretch step from the fabrication process.

Figure 4.24 SEM image of auto-ejected PEGDA particles

4.3.3 Comparison with PRINT method

The closest reported method of particle molding to SCAMP is PRINT [59] (reviewed in section 2.3.5) and we will compare them and highlight the potentially significant differences.

One comparison between them is on how particle liquid precursor is put inside cavities: SCAMP uses Discontinuous Dewetting (DD) and PRINT uses hydrophobic entrapment. PRINT seems to be better in speed of production, adaptability to

continuous production, and technique simplicity. SCAMP however, has a time period when the cured particles are inside the mold, leading to some possibilities:

- **Sandwich particles** - multiple layers of different material by stepwise layering and curing. Fig 4.25 show molded SCAMP particles made of GFP-hydrogel solution without the atmosphere conditioning to prevent water evaporation. The particle filled only less than half of cavity and was rather depressed. Because of the available space, another drug/delivery material can be placed on top on the cured layer. In this way, multiple layer particles are possible.

Figure 4.25 SEM images of GFP-hydrogel particle cured without water evaporation prevention and stretching

Such layering might enable particles comprising of different content in each layer that is not easily mixed into one homogeneous precursor solution. (As a side note, the lines visible at the side of the particles are not the result of layering; they are replicated from the cavity wall's undulations)

- **Single surface modification.** In SCAMP, when the particles were still trapped in the cavities (before stretch release), only the top surface was exposed. This period is an opportunity to selectively modify only one surface of the particles. In contrast, in PRINT the molded particles have their entire surfaces exposed except the bottom one.

Another comparison between PRINT and SCAMP is the harvesting technique. PRINT seems to excel because their harvesting with mechanical scraping seems very straightforward, while SCAMP needs stretch-release and freeze-peeling for complete harvesting. Still, SCAMP might have advantages in:

- **Extra-thin particles.** PRINT might face more difficulty in harvesting if particles were to be very thin. Harvesting with stretch-release (SCAMP) becomes more effective as particle becomes thinner.
- **All-surface modification.** SCAMP has a period where almost all surface area is exposed because of the position of the particles after ejection (Fig 4.26). PRINT seems to not have any period where all the particles are exposed, as the bottom surface were touching the substrate after curing, though full exposure can be done after the particles are dispersed in an aqueous medium. At situations when it is desirable for all-surface modification to happen before dispersion in aqueous medium, SCAMP has the advantage. Such situations may arise when particles need to be surface-treated to prevent aggregation in medium or that better control is possible and different treatments are faster when the particles are orderly arrayed on a surface instead of being dispersed in a solution.

Figure 4.26 SEM image of exposed 10um particles on PDMS mold

One last comparison between SCAMP and PRINT is on the completeness of particle harvesting. It was reported that PRINT particles was harvested by “simply gliding a doctor's blade across the flat substrate” and the result is shown in Fig 4.27a. In other words, the particles were scraped. Effective scraping is possible when the scraper mates (is completely in contact) with the substrate. Efficient scraping requires mating to happen throughout the length of the scraper such that in one glide everything is scraped.

An experiment similar to PRINT's scraping was performed by gliding a blade across PDMS covered with 2 μ m particles. The thick slightly irregular dark line in Fig 4.27b was where the blade stopped. Below it, the surface was still covered unevenly with particles. Above it, the surface was clean; no particles could be seen. It can be concluded that only if the substrate is flexible that efficient scraping is possible. This is because flexible substrate allows complete contact through the blade (scraper) length, allowing it to scrape the particles. A blade, however sharp, will have micro-irregularities and imperfect flatness throughout its length, such that it is not possible to scrape effectively and efficiently on a hard substrate e.g. glass slide.

Figure 4.27 Mechanical gathering (scraping) of particles
 (a) SEM image of 200 nm trapezoidal triacrylate particles (PRINT)
 (b) Micrograph of 2 μ m particles (SCAMP)

Because PFPE (material used in PRINT) is flexible, there is no doubt that gliding for collection was efficient on a sufficiently large scale. But since only collection/or gathering of particles were demonstrated, it is unclear whether it is also effective to lift the particles off the substrate, which is a good definition of complete harvesting. Freeze-peel (SCAMP) was shown to accomplish that.

By large magnification optical micrograph, it was evident that scraped particles (PRINT) stuck to one another when gathered and dispersing the particles in a solution might not alleviate the problem. SCAMP's particle, though not scraped, might also agglomerate after freeze-peeling. The difference is that SCAMP allows full-surface modification (e.g. attachment of long PEG chain or other surfactants) before freeze-peeling to potentially solve this problem, as compared to PRINT which has the underside unexposed and unavailable for surface modification.

In conclusion, PRINT and SCAMP are envisaged to be advantageous in specific applications. PRINT has demonstrated results in the 200nm range, with the advantages of simple and fast procedure, adaptability to many types of materials, proteins and nucleotides encapsulation, and of course, gentle fabrication process. SCAMP, through freeze-peel, has demonstrated effective transfer to aqueous medium. Also, SCAMP is a more direct method to produce sandwich particles and to modify one surface (top surface) of the particles. More careful studies are needed to demonstrate these.

Chapter 5

Conclusion & future work

SCAMP, a novel particle fabrication method, was demonstrated at submicron (“500nm”) particle size. The overall fabrication and harvesting processes were gentle and avoided most denaturing and contaminating sources. GFP loading was demonstrated down to “2 μ m” particle size. SCAMP particles were cured from high water content solutions with water evaporation prevention step. This increased amount of drug loaded and provided a compliant environment for the drug. Possible drugs damage sources are the free radicals, the UV irradiation and the freezing temperature involved. Future developments are suggested below.

5.1 Smoother cavity walls

To facilitate ejection of thicker particles, there are a few methods that can be employed to smoothen the sidewall undulations:

1. Optimization of the time ratio between the etch cycle and the passivation cycle in order to minimize undulations.
2. Chemical polishing by immersing the cavities into solution of $\text{HNO}_3 + \text{HF}$ (wet etching of Si). Kulkarni and Erk theorized that the undulation peaks will tend to etch faster, leaving less pronounced undulations [65]. Before this can

be done, the passivation layer must be removed first by O₂ bombardment (as with etching cycle but without SF₆ gas flow)

Alternatively, other methods for making submicron cavities can be attempted [66]: Si wet etching, e-beam lithography, focused ion beam lithography, and combination of Si etching and thermal oxidation, to name some.

5.2 Submicron particle

To fabricate DDS particles smaller than what was demonstrated in this project (we used 500nm square cavities), there are at least four potential issues.

Firstly, cavity size is the determining factor of particle size. Micromachining and replication techniques have been regularly demonstrated at 100nm level, so although obtaining the master mold with smaller features might be a little difficult and costly, cavity size should not be a big issue.

Secondly, filling of smaller cavities with precursor liquid might encounter new problems at smaller dimensions. For example, surface tension might not allow filling of smaller cavities irrespective of whether there are trapped air bubbles or not.

Thirdly, O₂ inhibition of free-radical polymerization might become more severe as dimensions become smaller. Better purging technique or different curing technique might be needed in the case the liquid precursor cannot be UV-cured because of this problem.

Fourthly, in this project, the 500nm particles made with GFP hydrogel solution appeared dark under confocal fluorescence microscope, while the 2 μ m particles appeared fluorescent green with prolonged exposure time when taking the digital photograph. This does not automatically conclude the absence of GFP inside the 500nm particle since another possible cause for the absence of fluorescence is that the sensitivity of the device was not enough. A feasible technique to evaluate drug content in submicron particles needs to be established first before evaluation of drug loading in submicron particles can be carried out. Also, GFP was observed to slightly aggregate in the GFP-hydrogel solution even with 2h of magnetic bar mixing. The aggregates were relatively small such that 2 μ m particles still appear to have smooth GFP distribution. But this might cause drug loading to be less efficient when particle size goes down to submicron.

5.3 Drug preservation

GFP was shown in this project to be fluorescent after encapsulation. But it is considered a robust protein [60]; therefore it is not a good indication that SCAMP is gentle enough to protein and DNA drugs in general. At least three possible denaturing/damaging factors need to be investigated further.

Firstly, Quick and Kristi [56-57] indicate that free radicals for UV-crosslinking purpose are damaging to DNA (and possibly protein). Their effect is reduced by the presence of crosslinkable polymer and radical scavengers. A non-damaging curing

technique can be a research topic to further develop SCAMP, since SCAMP does not depend on the free-radical UV-curing technique used in this project.

Secondly, cold denaturation of protein was described as a process that happens at a relatively low temperature, predicted and explained by thermodynamic of the protein [59,60]. As a specific high temperature denatures a protein, a specific low temperature can also denature it. This phenomenon usually occur at temperature below 0°C, made possible by the usage of very high pressure (>200 bar) to lower the freezing point of water. In SCAMP, usage of freeze-peel precludes lowering of water temperature down to, but not lower than, 0°C. Therefore it seems then that concern of this factor is minimal, but it is a factor to be investigated when other denaturing factors have already been researched.

Third, surface denaturation of protein has been demonstrated at interfaces of many sort (air, solid, solvent), indicating that proteins have the propensity to adsorb to such interfaces and unfold to exposes its hydrophobic regions. This in turn induces other proteins in the solution to do the same, thus forming aggregates.

Effective encapsulation of a drug inside a particle most probably requires interaction with many surfaces not encountered by proteins in vivo. Top-down approaches, for example, relies on partitioning by another solvent or material to create the submicron particles and effectively partition the drug inside. Bottom-up approaches are quite free from solvent or hydrophobic surfaces, but must rely on strong interaction of the polymer and drug to partition the drug effectively inside the formed particles. Therefore, by solid, solvent, polymer, or other material, the immediate presence of

other materials (surfaces) other than water is assumed to be unavoidable in fabricating a particulate DDS if effective drug encapsulation is required. A practical approach is to minimize the damage instead of eliminating usage of materials/surfaces.

In SCAMP, surface denaturation is possible by at least three ways: air-water surface during discontinuous dewetting step, ice-water surface during freeze-peel step and hydrophobic PDMS-hydrogel liquid precursor interaction before UV-curing.

Denaturation due to air-water surface was reported [61]. In SCAMP, when PEGDA droplets were formed inside cavities after discontinuous dewetting, there was a period of exposure to atmosphere for 30s – 1min before curing was done. SCAMP will benefit from usage of biocompatible surfactants to compete with the protein for adsorption at the surface, which was demonstrated to be effective [61].

Water freezing has been indicated to possibly affect the structure of protein as indicated by research on freeze drying [34]. Noting that freeze-drying is often a necessary protein post-production step for transportation and (paradoxically) preservation purpose, freezing is probably unavoidable at later stages anyway. Therefore, though freeze-peel step of SCAMP does not introduce a new problem, it will benefit from optimization recommended by researches on freeze drying.

SCAMP technique exposes the protein to mold cavity walls, which is hydrophobic. This hydrophobicity is beneficial during the discontinuous dewetting process, because it promotes clean and fast draining of liquid. But it has been indicated that hydrophobic surface of drug delivery device damages the insulin cargo [62]. One

plausible preventive strategy is to coat the inner surface with long-chain PEG that is known to exclude protein adsorption, such that proteins cannot interact with the hydrophobic surface and denature. This is the same strategy used to give stealth capability for in vivo applications [12]. A chemistry for this purpose is available [63]. Another possible strategy is usage of surfactant (e.g. Tween 80) to complete and displace the protein from the hydrophobic surface [64]

5.4 Efficacy evaluation of SCAMP particles

To evaluate efficacy of drug-loaded SCAMP particles compared to naked drug and drug loaded in particles made by other method, there are potentially two issues.

The first issue is to increase the particle production rate. The demonstrations in this project used relatively small molds with particle producing area of 1 cm x 0.5 cm. With 1 particle molded every 2 μ m spacing (for the 2 μ m and 500nm PDMS molds), this area gives approximately 12.5 million particles. Though such a small area gave a seemingly large amount of particles, better techniques to increase production rate will certainly make further experiments easier.

Fabricating a larger PDMS mold with cavities is one way to increase the production rate. Ensuring an even thickness and preventing defects from happening throughout a larger mold will be a challenge, but these things are important because it will affect the maximum strain of the mold. Furthermore, the stretcher must be redesigned to provide the larger grip area and the larger force required to stretch a bigger mold.

The second issue is to ensure sterility and non-contamination of the SCAMP particles, so that they won't negatively affect the efficacy test result.

5.5 Specific applications

Because formation of the particles in SCAMP utilized cavities, layer-by-layer molding might be possible to produce multi-layer particles. This possibility is discussed in section 4.3.3 and was not demonstrated in this project yet. One possible application for such multi-layer particles is combination of materials/drugs that are incompatible/immiscible with each other in the liquid state, using layer-by-layer curing of each material/drug to combine them into a single particle.

SCAMP has a period when the particles were partly ejected out of the cavities and were slightly adhering to the PDMS mold's surface. Their relatively stable position, regular arrangements and accessible surfaces provide an opportunity to modify their surface using materials relevant to DDS application e.g. targeted delivery and stealth.

The partly ejected particles adhering on the PDMS mold might also be applied for tissue engineering. The PDMS mold provides substrate for cell growth and can be patterned to control them. The particles on the substrate can provide controlled delivery of growth factors to the cell, possibly increasing the control on cell growth.

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Appendix A1
Engineering drawing of 1axis-stretcher parts: cylinder gripper
(All dimensions in mm)

Appendix A1
Engineering drawing of 1axis-stretcher parts: block gripper
(All dimensions in mm)

Appendix A1
Engineering drawing of 1axis-stretcher parts: middle bar
(All dimensions in mm)

Appendix A1
Engineering drawing of chamber parts: chamber body
(All dimensions in mm)

Appendix A1
Engineering drawing of chamber parts: acrylic window
(All dimensions in mm)

Appendix A1
Engineering drawing of chamber parts: top cover
(All dimensions in mm)

Appendix A2
10µm particles' SEM cross-section images made from 'hydrogel' solution with water evaporation prevention (10/30 images)

Appendix A2
10µm particles' SEM cross-section images made from 'hydrogel' solution with water evaporation prevention (10/30 images)

Appendix A2
10µm particles' SEM cross-section images made from 'hydrogel' solution with water evaporation prevention (10/30 images)

Appendix A2
10µm particles' SEM top-view images made from 'hydrogel' solution with water evaporation prevention, having a total of 30 particles (10/10 images)

Appendix A2
10µm particles' SEM cross-section images made from 'hydrogel' solution without water evaporation prevention (10/30 images)

Appendix A2
10µm particles' SEM cross-section images made from 'hydrogel' solution without water evaporation prevention (10/30 images)

Appendix A2
10µm particles' SEM cross-section images made from 'hydrogel' solution without water evaporation prevention (10/30 images)

Appendix A2
10µm particles’ SEM top-view images made from ‘hydrogel’ solution without water evaporation prevention, having a total of 30 particles (3/3 images)

Appendix A2

Tabulation of particles' dimension measurements from the SEM images

Particles made with evaporation prevention

No	H1 (um)	H2 (um)	W (um)	L (um)	Arbitrary Volume
1	4.2	2.6	6.4	30.5	
2	4.8	2.9	6.4	30.5	
3	3.4	2.5	6.2	30.5	
4	4.3	2.9	6.1	30.5	
5	3.5	2.6	6.7	30.5	
6	4.2	2.9	6.6	30.5	
7	5.0	3.2	6.0	30.5	
8	4.3	2.9	6.5	30.5	
9	3.5	2.6	5.9	30.5	
10	3.5	2.6	6.4	30.5	
11	3.8	2.9	6.9	30.5	
12	3.7	2.9	6.8	30.5	
13	3.8	2.8	6.9	30.5	
14	3.2	2.7	6.6	30.5	
15	3.8	2.8	6.9	30.5	
16	4.0	3.1	6.5	30.5	
17	4.1	2.9	6.9	30.5	
18	4.1	2.9	6.6	30.5	
19	3.6	2.9	6.9	30.5	
20	3.4	2.6	5.8	30.5	
21	4.1	2.9	6.7	30.5	
22	4.6	2.9	6.2	30.5	
23	4.2	2.6	6.6	30.5	
24	3.3	2.8	6.3	30.5	
25	3.3	2.8	6.4	30.5	
26	3.4	2.9	7.1	30.5	
27	3.9	2.9	7.0	30.5	
28	4.2	2.9	6.9	30.5	
29	4.2	2.2	6.4	30.5	
30	3.4	2.9	6.6	30.5	
Avg	3.9	2.8	6.6	30.5	681.7
Dev	0.5	0.2	0.3	0.0	65.7

Particles made without evaporation prevention

No	H1 (um)	H2 (um)	W (um)	L (um)	Arbitrary Volume
1	3.1	1.9	7.7	30.5	
2	3.3	2.1	8.2	30.5	
3	3.1	2.1	8.9	30.5	
4	2.8	1.7	7.9	30.5	
5	2.9	2.1	7.5	30.5	
6	3.1	2.0	8.8	30.5	
7	3.2	1.9	8.6	30.5	
8	3.2	2.1	7.6	30.5	
9	2.8	1.9	9.7	30.5	
10	2.8	1.8	10.3	30.5	
11	3.2	2.4	7.9	30.5	
12	2.9	1.8	8.1	30.5	
13	2.9	1.6	7.7	30.5	
14	3.1	2.0	7.5	30.5	
15	3.1	1.9	8.9	30.5	
16	2.9	1.8	8.9	30.5	
17	2.9	1.8	8.9	30.5	
18	3.0	1.3	8.9	30.5	
19	2.9	1.9	8.9	30.5	
20	2.9	1.9	8.9	30.5	
21	2.8	1.8	8.9	30.5	
22	3.1	2.3	8.9	30.5	
23	3.1	1.9	8.9	30.5	
24	2.8	1.7	8.9	30.5	
25	2.9	1.3	8.9	30.5	
26	2.8	1.8	8.9	30.5	
27	3.3	2.3	8.9	30.5	
28	2.9	1.9	8.9	30.5	
29	3.1	1.7	8.9	30.5	
30	2.9	1.7	8.9	30.5	
Avg	3.0	1.9	8.7	30.5	569.2
Dev	0.1	0.2	0.6	0.0	58.7

Average volume with evaporation prevention: V1, which is 681.7 (arbitrary volume)

Average volume without evaporation prevention: V2, which is 569.2 (arbitrary volume)

To determining how much (%vol) of the particles made with evaporation prevention are water, the formula $(V1-V2) / V2$ was used, resulting in the value 16.5%.

Appendix A3

SEM images of approx. 20x20 2.4 μ m-thick 10 μ m PEGDA particles with 25% horizontal pre-tension

- (a) after tension was released
- (b) immersed for 15min before tension was released
- (c) immersed for 7h before tension was released

Appendix A3

SEM images of approx. 20x20 2.4µm-thick 10µm PEGDA particles with 50% horizontal pre-tension

- (a) after tension was released
- (b) immersed for 15min before tension was released
- (c) immersed for 7h before tension was released

Appendix A4

SEM images of approx. 20x20 3.2µm-thick 10µm PEGDA particles with 100% vertical pre-tension

- (a) after tension was released
- (b) immersed for 7h before tension was released

Appendix A4

SEM images of approx. 20x20 4.3µm-thick 10µm PEGDA particles with 100% vertical pre-tension

- (a) after tension was released
- (b) immersed for 7h before tension was released

Appendix A4

SEM images of approx. 20x20 5.6µm-thick 10µm PEGDA particles with 100% vertical pre-tension

- (a) after tension was released
- (b) immersed for 7h before tension was released

Appendix A4

SEM images of approx. 20x20 7.1 μ m-thick 10 μ m PEGDA particles with 100% vertical pre-tension

- (a) after tension was released
- (b) immersed for 7h before tension was released

Appendix A4

SEM images of approx. 20x20 8.9 μ m-thick 10 μ m PEGDA particles with 100% vertical pre-tension

- (a) after tension was released
- (b) immersed for 7h before tension was released

Information About High Technology Silicone Materials

DOW CORNING

DESCRIPTION

SYLGARD® 184 silicone elastomer, base & curing agent, is supplied as a two-part kit comprised of liquid components. When the base and the curing agent are thoroughly mixed in a 10:1 weight ratio, the medium-viscosity liquid mixture has a consistency resembling SAE No. 40 motor oil. The liquid mixture will cure in thick or thin sections to a flexible, transparent elastomer ideally suited for electrical/electronic potting and encapsulating applications.

SYLGARD 184 silicone elastomer offers a flexible cure schedule from 25 to 150 C (77 to 302 F), without an exotherm. SYLGARD 184 silicone elastomer requires no post cure and can be placed in service immediately following the completion of the cure schedule at any operating temperature from -55 to 200 C (-67 to 392 F). See Table I for special features and benefits of this product.

USES

Because of its many special features, SYLGARD 184 silicone elastomer is ideal for a wide variety of electrical/electronic potting and encapsulating applications and provides environmental protection for:

- Equipment modules
- Relays, power supplies and magnetic amplifiers
- Transformers, coils and ferrite cores
- Connectors
- Fiber optic waveguide coatings
- Encapsulation of circuit boards

SYLGARD 184 silicone elastomer is especially useful in applications where clarity is desirable, including:

SYLGARD® 184 SILICONE ELASTOMER, BASE & CURING AGENT

Type	Two-part silicone elastomer
Color	Transparent
Physical Form	
as supplied	Medium-viscosity liquid
as cured	Flexible elastomer
Cure	25 to 150 C (77 to 302 F)
Special Properties	Transparent; reversion resistant; stable dielectric; high physical properties
Primary Uses	Potting and encapsulating
Benefits	Low toxicity; repairability

- Encapsulation of solar cells
- Encapsulation of opto-electronic displays

HOW TO USE

Mixing

SYLGARD 184 silicone elastomer is supplied in two parts, a lot-matched base and curing agent, mixed in a ratio of 10 parts base to one part curing agent, by weight.

For best curing results, glassware or tinned cans and glass or metal stirring implements should be used. Mix with a smooth action that will minimize the introduction of excess air.

Pot Life - Working Time

Cure reaction of SYLGARD 184 silicone elastomer, base & curing agent, begins with the mixing process. Initially, cure is evidenced by a gradual increase in viscosity, followed by gelation and conversion to a solid elastomer. Pot life is defined as the time required for viscosity to double following addition of curing agent to base. At 25 C (77 F), the pot life of SYLGARD 184 silicone

elastomer is 2 hours. Pot life of this product can be extended by refrigeration at 4 C (40 F); however, do not allow moisture from condensation to collect in the silicone elastomer.

SYLGARD 184 silicone elastomer will cure at 25 C (77 F), will become solid in 24 hours, and will reach full cure in 7 days. See "Curing."

Processing

Thoroughly mix SYLGARD 184 silicone elastomer, base & curing agent, in a ratio of 10 parts base to 1 part curing agent, by weight. Agitate gently to reduce the amount of air introduced. Allow mixture to set 30 minutes before pouring.

Since air bubbles are usually present following mixing, vacuum de-airing is recommended. De-air in a container with at least four times the liquid volume to allow for expansion of material. Air entrapped in the mixture can be removed by using a vacuum of 25 to 29 inches of mercury. Continue the vacuum until the liquid expands and settles to its original volume and bubbling subsides. This may take 15 minutes to 2 hours, depending on the

TYPICAL PROPERTIES

These values are not intended for use in preparing specifications.

As Supplied

CTM 0001A	Specific Gravity at 25 C (77 F) ¹	1.05
CTM 0050	Viscosity ² at 25 C (77 F), centipoise	5500

As Catalyzed³

CTM 0050	Viscosity at 25 C (77 F), centipoise	3900
CTM 0055	Pot Life ⁴ , minimum, hours	2

As Cured – Physical⁵

CTM 0176	Appearance	Transparent
CTM 0099	Durometer Hardness, Shore A	40
CTM 0137A	Tensile Strength, MPa (psi)	6.20 (900)
CTM 0137A	Elongation, percent	100
CTM 0159A	Tear Strength, die B, kN/m (ppi)	2.6 (15)
CTM 0022	Specific Gravity at 25 C (77 F)1.05	
CTM 0224	Thermal Conductivity, cal/cm ² -sec-(°C/cm)	3.5 x 10 ⁴
CTM 0585	Linear Coefficient of Thermal Expansion, cm/cm per °C from -55 to 150 C (-67 to 302 F)	3.0 x 10 ⁴
CTM 0585	Volume Coefficient of Thermal Expansion, cc/cc per °C from -55 to 150 C (-67 to 302 F)	9.6 x 10 ⁴
MIL-I-16923G	Thermal Shock Resistance, from -49 to 68 C (-55 to 155 F)	Passes 10 cycles
	Weight Loss, percent after 1,000 hrs at 150 C (302 F)	1.6
	after 1,000 hrs at 200 C (392 F)	3.2
ASTM D 570	Water Absorption, after 7 days immersion at 25 C (77 F), percent	0.10
ASTM D 746	Brittle Point, °C (°F)	-65 (-85)
CTM 0002	Refractive Index at 25 C (77 F)	1.430
	Radiation Resistance, cobalt 60 source at 200 megarads	Still usable
	at 500 megarads	Hard and brittle
UL 94	Flammability ⁶ Classification	94 V-1
UL	Temperature Rating, Mechanical, °C (°F)	130 (265)
	Electrical, °C (°F)	130 (265)

Electrical⁷

CTM 0114A	Dielectric Strength, volts/mil ⁸	450
CTM 0112	Dielectric Constant, at 60 Hz	2.7
	100 Hz	2.66
	100 kHz	2.65
CTM 0112	Dissipation Factor, at 60 Hz	0.001
	100 Hz	0.0009
	100 kHz	0.001
CTM 0249A	Volume Resistivity, ohm-cm	2.0 x 10 ¹⁵
CTM 0171	Arc Resistance, track, seconds	115

Electrical – after heat aging for 1000 hours at 200 C (392 F)

CTM 0114A	Dielectric Strength, volts/mil ⁸	600
CTM 0112	Dielectric Constant, at 60 Hz	2.7
	100 Hz	2.7
CTM 0249A	Volume Resistivity, ohm-cm	2.0 x 10 ¹⁴

¹In most cases, CTMs (Corporate Test Methods) correspond to ASTM standard tests. Copies of CTM procedures are available upon request.

²Brookfield Viscometer Model LVF, spindle #4 at 60 rpm.

³Mix ratio 10 parts base to 1 part curing agent, by weight.

⁴Time required to double catalyzed viscosity.

⁵Based on sample thickness of 125 mils and a minimum cure of 4 hours at 65 C (149 F).

⁶Tests, claims, representations and descriptions regarding flammability are based on standard small scale laboratory tests.

⁷Properties obtained on 1.58-mm-thick (0.062-inch) specimens, cured 4 hours at 65 C (149 F).

⁸Measured with 1.4-inch standard ASTM electrode, 500 volts per second rate of rise.

Specification Writers: Please contact Dow Corning Corporation, Midland, Michigan, before writing specifications on this product.

amount of air introduced during stirring.

Clean and degrease all application surfaces using a solvent to remove all mold release agent, processing oils and surface contaminant. Dry and remove all solvent before application.

For best adhesion the application substrate should be primed with SYLGARD® prime coat. Obtain a technical bulletin for proper application instructions. See "Bonding."

CAUTION: SYLGARD primer is flammable. Keep away from heat, sparks and open flame. Use only with adequate ventilation.

When pouring SYLGARD 184 silicone elastomer into the curing container, care should be taken to minimize air entrapment. When practical, pouring should be done under vacuum, particularly if the component being potted or encapsulated has many fine voids. If this technique cannot be used, the unit should be evacuated after SYLGARD 184 silicone elastomer has been poured.

After allowing time for the material mass to reach temperature, cure according to the cure time-temperatures listed in "Curing."

Repairability

In the manufacture of electrical/electronic devices it is often desirable to salvage or reclaim damaged or defective units. With most rigid types of potting and encapsulating materials, removal or entry is difficult or impossible without causing excessive damage to internal circuitry. SYLGARD 184 silicone elastomer can be selectively removed with relative ease, allowing repairs or changes to be completed and the area to be repotted with additional product. SYLGARD 184 silicone elastomer, base & curing agent, can be removed by cutting with a sharp blade or knife and tearing material away from the area to be repaired. Thin sections of the adhesive elastomer are best removed from substrates and circuitry by mechanical action such as scraping or rubbing; removal can be assisted by the application of isopropyl alcohol.

Before repotting a repaired device, if possible, roughen the exposed surface of the SYLGARD 184 silicone elastomer, base & curing agent, with an abrasive paper. This will enhance

adhesion and permit the repair material to become an integral matrix with the existing elastomer. Silicone prime coats are not recommended for adhering SYLGARD 184 silicone elastomer, base & curing agent, to itself in repotting applications.

Reversion Resistance

SYLGARD 184 silicone elastomer, base & curing agent, provides excellent reversion resistance even when exposed to temperatures in excess of 200 C (392 F) while under confinement or in deep section.

Temperature Stability

SYLGARD 184 silicone elastomer, base & curing agent, cures to a thermoset material that will not melt or appreciably soften even at elevated temperatures of 250 C (482 F). The material may harden or become brittle after prolonged exposure to these elevated temperatures. SYLGARD 184 silicone elastomer, base & curing agent, has a 130 C (265 F) UL yellow-card temperature index classification for both electrical and mechanical functional use.

Upon exposure to lower temperatures, SYLGARD 184 silicone elastomer, base & curing agent, does not approach a stiffening point until -55 C (-67 F). Overall, the cured elastomer will maintain its basic elastomeric flexibility over an extremely wide range of -55 to 200 C (-67 to 392 F), making it the ideal selection for applications that may experience high or low temperature cycling.

PROCESSING TECHNIQUES

Curing

SYLGARD 184 silicone elastomer, base & curing agent, can be satisfactorily cured either with exposure to air or completely sealed, and at temperatures ranging from 25 to 150 C (77 to 302 F). Curing time can be appreciably decreased by elevating the cure temperature. One of the following cure cycles is suggested:

- 25 C (77 F) for 24 hours
- 65 C (149 F) for 4 hours
- 100 C (212 F) for 1 hour
- 150 C (302 F) for 15 minutes

Large parts will require additional time in the oven to heat to the selected cure temperature. Satisfactory

sources of heat include circulating and non-circulating ovens, infrared heating lamps and heat guns.

Full mechanical strength will not be achieved for 7 days when SYLGARD 184 silicone elastomer, base & curing agent, is cured at 25 C (77 F). The majority of its physical strength, however, is present after 24 hours, although surface cure may not yet be complete.

Bonding

SYLGARD 184 silicone elastomer, base & curing agent, will not normally bond to clean, nonporous surfaces such as metal or glass. A primer coat is required to ensure adhesion to these surfaces. SYLGARD prime coat is recommended to obtain the best adhesion. The prime coat should be applied in a thin layer to clean, dry surfaces where adhesion is desired. It should be air dried 1 to 2 hours before the silicone elastomer is applied.

Lowering Viscosity

The viscosity of SYLGARD 184 elastomer, base & curing agent, can be lowered by the addition of 200[®] fluid, 50 cSt., from Dow Corning. Quantities of 10 percent or less will have little or no effect on the physical or electrical properties. Quantities of 10 percent or greater may decrease the physical strength and hardness but will have no effect on the electrical properties. At concentrations greater than 10 percent the fluid may possibly bleed from cured SYLGARD 184 silicone elastomer. The addition of thinning fluid does not change the mixing ratio of curing agent to base.

Varying the Hardness

Variations of up to 10 percent in concentration of curing agent in SYLGARD 184 silicone elastomer have little effect on cure time or on the physical properties of the final cured elastomer. Lowering the curing agent concentration by more than 10 percent will result in a softer and weaker elastomer; increasing the concentration by more than 10 percent will result in over-hardening of the cured elastomer and will tend to degrade the physical and thermal properties. Changes in the curing agent concentration will have little or no effect on the electrical properties, however.

Release Agents

When SYLGARD 184 silicone elastomer, base & curing agent, is cured in a mold, the mold should first be treated with a release agent to prevent sticking. Suitable release agents include DUPONOL^{®1} WAQ at a 5 percent concentration with isopropanol; DOW CORNING[®] 230 fluid at a 2 percent concentration with CHLOROTHENE^{®2} or similar chlorinated solvent; a mild liquid detergent at a 2 to 5 percent concentration with water; or petroleum jelly at a 5 percent solution in a chlorinated solvent.

LIMITATIONS

Inhibition of Cure

In the presence of inhibitors, cure of SYLGARD 184 silicone elastomer is poor. In the inhibited area (usually less than 0.020-inch thick) the silicone elastomer remains in a liquid or tacky state even though the cure schedule has been completed, and despite any subsequent attempts to convert it to a hard, dry, rubbery mass, this material will remain uncured. Extremely minute quantities of inhibitor may be sufficient to produce this effect. The most notable causes of inhibition include:

- Organotin and other organometallic compounds
- Silicone rubbers containing organotin catalyst
- Sulfur, polysulfides, polysulfones and other sulfur-containing materials
- Amines, urethanes, amine-containing materials and other nitrogen-containing materials
- Unsaturated hydrocarbon plasticizers

If a substrate or material is considered questionable in respect to potential cure inhibition, a small-scale compatibility test to ascertain suitability in the particular application is recommended. If inhibition is present it may sometimes be overcome by prebaking the unit at the highest tolerable temperature for approximately 1 to 4 hours to remove volatile chemicals. See bulletin No. 10-022, "How To

¹"Duponol" is a registered trademark of E. I. du Pont de Nemours & Company.

²"Chlorothene" is a registered trademark of The Dow Chemical Company.

Process SYLGARD® Brand Elastomers" for a listing of inhibition-causing materials.

Thermal Expansion

SYLGARD 184 silicone elastomer, base & curing agent, has a notable volume coefficient of thermal expansion ($9.6 \times 10^{-4} / ^\circ\text{C}$; see Typical Properties chart). The volume of the cured elastomer will increase or decrease approximately 9.6 percent for each 100 C (212 F) of temperature differential. When using SYLGARD 184 silicone elastomer, base & curing agent, at higher temperatures in applications that are highly confined or hermetically sealed, allowance should be made to accommodate volume expansion and prevent pressure build-up. Normal thermal expansion and contraction stresses may be minimized by selecting a cure temperature close to the midway point of the high and low extremes of the thermal cycle.

Temperature limits may be influenced by differences in the thermal expansion values between the silicone elastomer and the encapsulated or potted components, and also by their configurations. Therefore, thermal operating limits should be determined by testing before large scale use.

SAFETY REQUIREMENTS

Handling

SYLGARD 184 silicone elastomer, base & curing agent, does not contain volatile solvents. Special ventilation is not required in the normal use or storage of this product. Base and curing agent liquid components or their cured mixture do not present any significant toxicological hazard incidental to normal industrial handling. Minimal eye protection, such as standard safety glasses, should be adequate for normal industrial use. Direct eye contact can cause temporary eye discomfort; flush thoroughly with copious amounts of water should contact occur. See Material Safety Data Sheet for more detailed handling instructions.

TABLE I: SPECIAL FEATURES AND BENEFITS

Special Feature	Benefit
• Low toxicity	• No special precautions required for normal industrial handling
• No solvents or cure by-products; no exotherm during cure	• Requires no special venting; will not cause corrosion; low shrinkage during cure
• Cures to a transparent, flexible elastomer	• Provides stability and relief from mechanical shock; low transmission of vibration; visual inspection of components and easy repairability
• Environmental protection	• Low water absorption; good radiation resistance; little out-gassing in hard vacuum
• Excellent dielectric properties	• Maintains and protects existing electrical insulation requirements
• Stability over a wide temperature range; reversion resistant	• Maintains elastomeric flexibility and provides functional stability from -55 to 200 C (-67 to 392 F), even in confinement
• Flame resistant	• UL flammability classification of 94 V-1 and a temperature rating of 130 C (265 F)

Abnormal Exposures

CAUTION: The liquid curing agent component of SYLGARD 184 silicone elastomer may generate hydrogen gas if contaminated with strong acids, bases or catalytic oxidizing materials. If exposure is suspected, use appropriate caution to relieve hydrogen gas pressure. Keep away from sparks and flame, and supply adequate ventilation to reduce localized build-up of hydrogen gas.

Spills

Spills of the liquid base and curing agent components of SYLGARD 184 silicone elastomer can become extremely slippery. Sawdust or other absorbent material should be immediately applied to any liquid spill for temporary relief. The spill should be removed with high flash point mineral spirits or other suitable solvent.

SHIPPING LIMITATIONS

None.

STORAGE AND SHELF LIFE

When stored in original unopened containers at or below 32 C (90 F), SYLGARD 184 silicone elastomer, base & curing agent, has a shelf life of 24 months from date of manufacture.

PACKAGING

SYLGARD 184 silicone elastomer, base & curing agent, is shipped in kits that contain both the base and curing agent, liquid components in separate containers. Each kit contains the appropriate weight of curing agent for the amount of base. Complete kits are available in 1.1-, 8.8-, 44- and 495-lb (0.5-, 4-, 20- and 225-kg) quantities, net weight.

MSDS INFORMATION

ATTENTION: PRODUCT SAFETY INFORMATION REQUIRED FOR SAFE USE IS NOT INCLUDED. BEFORE HANDLING, READ PRODUCT AND MATERIAL SAFETY DATA SHEETS

AND CONTAINER LABELS FOR SAFE USE, PHYSICAL AND HEALTH HAZARD INFORMATION. THE MATERIAL SAFETY DATA SHEET IS AVAILABLE FROM YOUR DOW CORNING REPRESENTATIVE, OR DISTRIBUTOR, OR BY WRITING TO DOW CORNING CUSTOMER SERVICE, OR BY CALLING (517) 496-6000.

**WARRANTY INFORMATION—
PLEASE READ CAREFULLY**

Dow Corning believes that the information in this publication is an accurate description of the typical characteristics and/or uses of the product or products, but it is your responsibility to thoroughly test the product in your specific application to determine its performance, efficacy and safety.

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BL50528 50

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Printed in U.S.A. Form No. 10-042D-98