Review

Microfabrication-derived DDS: From batch to individual production

Kanji Takada*

Department of Pharmacokinetics, Kyoto Pharmaceutical University, Kyoto, Japan.

ABSTRACT: As a result of recent advances in microfabrication technology (MFT), microparticles including microcapsules and microspheres can be prepared individually and the disadvantages of the conventional microparticles produced by batch production, *i.e.* (i) low loading efficiency, (ii) large size variation, and (iii) initial burst release, have been remedied. In addition, all conventional microparticles have the same structure, a spherical shape, so they have only one function, sustained release. Three-layer microcapsules (TLMCs) have been designed to address these issues. TLMCs consist of a surface layer, a drug carrying layer, and a basement layer. TLMCs have sustained release as well as adhesiveness and targeting functions. TLMCs are prepared using ink-jet printer nozzle technology. The obtained TLMCs are used for the oral delivery of peptide/protein drugs and long-term sustained-release injection preparation. In addition, self-dissolving micropiles (SDMPs) can be individually produced by MFT as a percutaneous preparation. MFT allows biopharmaceutical drugs like insulin, erythropoietin, and growth hormone to be absorbed through the skin. Thus, advances in MFT have accelerated the development of pharmaceutical technology.

Keywords: Microfabrication, DDS, Three-layer microcapsules, Micropiles, Individual production

1. Introduction

Many scientists are interested in nanotechnology, and governments are supporting scientific research on nanotechnologies. In the field of pharmaceutics, nanotechnology is an attractive technology, and research on nanocarriers like liposomes and nanospheres has been widely performed (*1-3*). Nanospheres are a form of nanoparticles. However, nanospheres differ from nanocapsules as shown in Figure 1. As microcapsules, nanocapsules have a capsule structure. Preparing nanocapsules is very difficult and they are 1-2 orders smaller in size. Therefore, nanocapsules are not popular in pharmaceutics. Nanoparticles are fabricated by the same method as used for microparticles, *i.e.* they are produced by a batch system as shown in the figure. Batch systems have a long history; conventional methods of preparing microcapsules and microspheres are classified into two categories: dispersion of preformed polymers and polymerization of monomers. In addition, dispersion is further classified into three methods: (i) emulsion solvent extraction/evaporation, (ii) phase separation (coacervation), and (iii) spray-drying. Scientists have developed many modified methods to remedy the disadvantages of the three methods, *i.e.* low drug-loading efficiency and wide variation in particle size. Many scientists have struggled for three decades to resolve the problem of low drug-loading efficiency. In addition, conventional microparticles have only one function, controlled-release, due to their spherical shape. However, the batch production method has failed to provide clues to resolving these problems. With the advance of microfabrication technology, in contrast, microparticles including microcapsules and microspheres can be prepared individually. When microfabrication technology is used to produce microcapsules, microcapsules can be produced individually. In such instances, a high drug loading efficiency (100% theoretically) can be attained with microparticles of almost the same shape and size. Therefore, pharmaceutical technology is enjoying a renaissance. This review studies advances in microfabrication technology in pharmaceutics and describes the outcomes of microfabrication technology resulting from research by the author.

2. Oral preparation

The main purpose of oral microparticles, *i.e.* microcapsules and microspheres, is for oral

**Correspondence to:* Dr. Kanji Takada, Department of Pharmaceutics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan;
e-mail: takada@mb.kyoto-phu.ac.jp

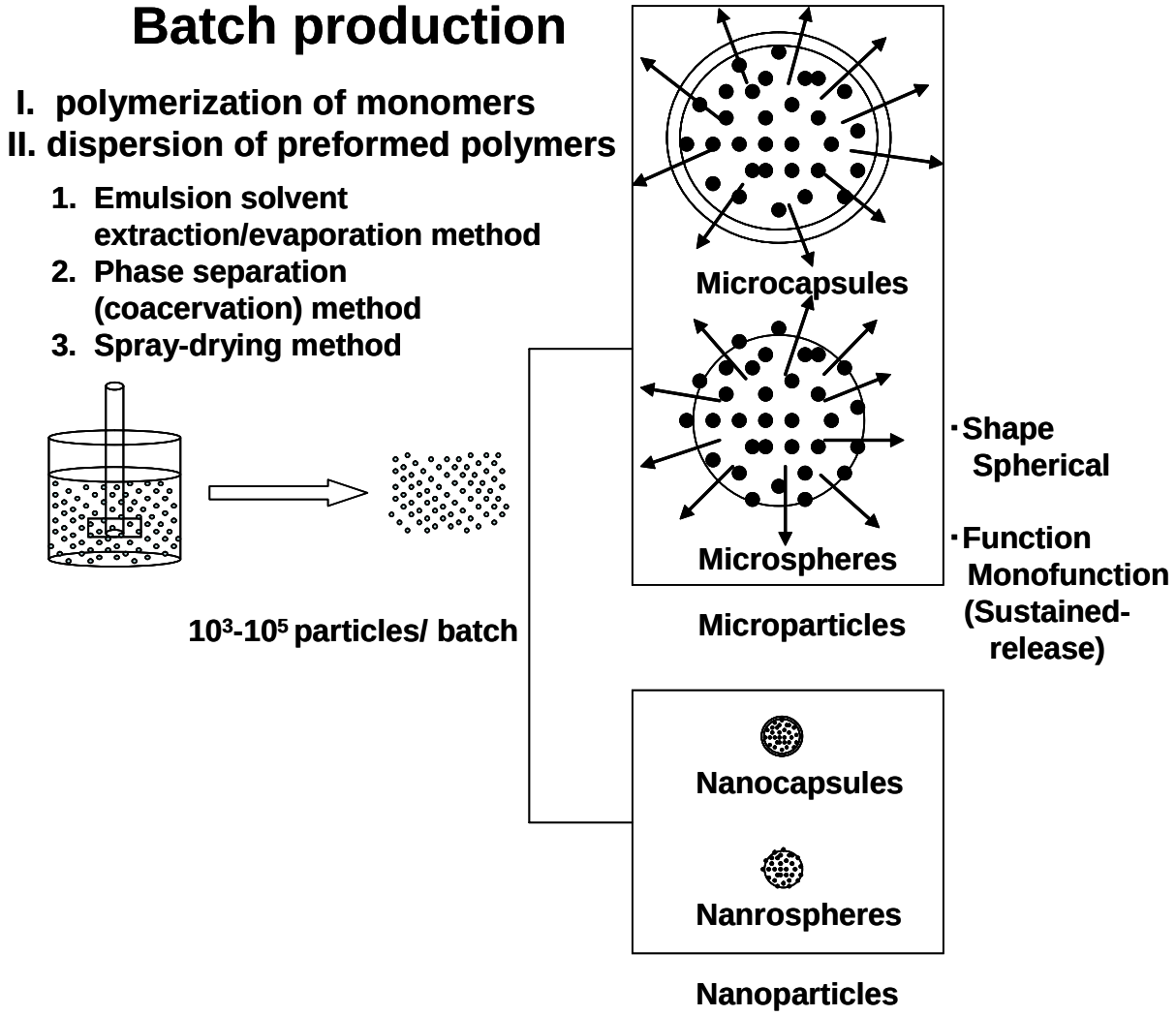

Figure 1. Conventional micro- and nano-particles prepared by batch production method.

sustained-release preparation. Both chemical and natural polymers are used as a wall-forming material. Cellulose and cellulose derivatives such as hydroxypropylmethyl cellulose (HPMC), ethyl cellulose (EC), cellulose acetate (CA), cellulose acetate trimellitate (CAT), cellulose acetate butyrate (CAB), cellulose acetate phthalate, cellulose acetate propionate, hydroxypropylmethyl cellulose phthalate (HPMCP), hydroxypropylmethyl cellulose acetate succinate (HPMCAS), carboxymethyl cellulose (CMC), methyl cellulose (MC), sodium cellulose sulfate, and sodium carboxymethyl cellulose are used as chemical materials. Natural polymers that are used as a wall-forming material include chitosan, gelatin, and alginate.

The polymerization method has not been used for oral pharmaceutical preparations because of the safety problem of the polymers obtained. In pharmaceuticals, the safety problem is crucial. When the polymerization method is used, polymerized substances with different degrees of polymerization are formed. If safety studies are performed with each polymerized substance, the production cost of the microparticles will increase tremendously. Therefore, the dispersion method is generally used.

Using these polymers as wall-forming materials, microcapsules and microspheres were prepared by both of the methods mentioned above. However, each method has both advantages and disadvantages.

The coacervation method can be performed under room temperature. However, a coacervating agent is needed. Therefore, the coacervation method often suffers from residual solvents and coacervating agents detected in the final microcapsules. In addition, each polymer requires its own coacervating agent, so there is no universal rule for the combination of a wall-forming polymer material and coacervating agent.

The solvent extraction/evaporation method has been widely used in pharmaceutics to prepare microcapsules. This requires the evaporation of the solvent by increasing the temperature during the preparation process. Therefore, the possibility of degradation increases when drugs that are susceptible to heating,

like biopharmaceuticals including peptide/protein drugs, are used as the active pharmaceutical ingredients (API). In addition, the microcapsules obtained have a substantial variation in size. The review by Freitas *et al*. provides useful information on microencapsulation by the solvent evaporation/extraction method (*4*).

The spray-drying method is simple and microspheres are easily obtained. This method cannot produce authentic microcapsules. However, microspheres can be converted to microcapsules by modifying the surface of the microspheres during the formation process. The disadvantage of this method is the difficulty in limiting the size of microspheres. Li *et al*. adequately described the conventional large-scale production of these microparticles by spray-drying method (*5*).

Table 1 summarizes the research on microparticles, indicating the core substance, API, wall-forming material, and method of preparation. These microparticles were prepared with either of the aforementioned methods or a modified form of one of those methods. In all cases, the obtained microparticles are spherical and have only one function, sustained-release of the formulated API.

In the last two decades, research focused on the wall-forming material, and natural polymers like sodium alginate were introduced in microcapsules and microspheres. Reports mentioned, for example, diclofenac sodium microspheres prepared by emulsification (*46*), L-lactate dehydrogenase microparticles prepared by spray-drying (*47*), and indomethacin microspheres prepared by precipitation (*48*). Egg albumin microspheres containing nitrofurantoin were prepared by phase separation (*49*). In addition, chitosan was used as a wall-forming material and ketoprofen was used as the core drug for preparation by emulsification/solvent evaporation (*50*). In addition, melatonin was loaded onto chitosan microcapsules by ionotropic gelation (*51*). Chitosan microspheres and nanoparticles were applied to insulin (*52,53*). The review by Kas provides useful information on microparticles made of chitosan (*54*). The purpose of those microparticles was to provide oral sustained-release preparations. On the other hand, mucoadhesive chitosan microspheres were prepared by spray-drying, and the interaction between the obtained microspheres and rat small intestinal mucosal tissue was investigated (*55*). In addition, chitosan microspheres and

Table 1. Microcapsules and microspheres as oral sustained-release preparation

Drug	Wall-forming material		Method	Reference
Acetylsalicylic acid Eudragit	RS	MS	solvent evaporation	(*6,7*)
	CMEC	MC	neutralization reaction	(*8*)
	Eudragit RS	MS	solvent partition	(*9*)
Allopurinol	EC	MC	solvent evaporation	(*10*)
Bacampicillin	Eudragit E	MS	solvent evaporation	(*11*)
Bitolterol	EC	MC	phase separation	(*12*)
Chlorothiazide	whey protein	MC	cross-link	(*13*)
Dexamethasone	Eudragit S100	NS	spray-dry	(*14*)
Diclofenac Na	CAB, PVA	MS	solvent evaporation	(*15*)
	CMC	MS	crosslink	(*16*)
Fenoterol	EC	MS	solvent evaporation	(*17*)
5-fluorouracil	EC	MS	solvent evaporation	(*18*)
Furosemide	EC	MS	spherical crystallization	(*19*)
Ibuprofen	EC	MS	solvent evaporation	(*20*)
	CAB	MS	solvent evaporation	(*21*)
	PHBV	MC	solvent evaporation	(*22*)
	Eudragit RS	MS	emulsion solvent diffusion	(*23*)
Indomethacin	EC, Eudragit RL	MS	solvent evaporation	(*24,25*)
	Polyesters	NS	spray-dry	(*26*)
Isosorbide dinitrate	EC/HPC	MC	oil-in-oil emulsion evaporation	(*27*)
Ketoprofen	CAT, EC	MS	spray dry	(*28*)
	CAB, HPMCP	MS	spray-dry	(*29*)
	Eudragit RS	MS	coacervation/spray-dry	(*30*)
Nifedipine	cetearyl alcohol/poloxamer	MP	hot air coating	(*31*)
Nitrofurantoin	CMC	MC	coacervation	(*32,33*)
Pantoprazole	Eudragit S100/HPMC	MP	spray-dry	(*34*)
Piroxicam	Hyaluronate	MS	spray-dry	(*35*)
Propranolol HCl	CAB	MC	emulsion non-solvent addition method	(*36*)
Quercetin	PMMA	MC	solvent evaporation	(*37*)
Sulfamethoxazole	CAP	MC	spray-dry	(*38*)
Theophylline	HPMC	MO	spray-drying	(*39,40*)
	CMC-Na, HPMC	MC	spray-drying	(*41*)
	EC	MC	emulsion non-solvent addition	(*42*)
	Polyglycerol esters of fatty acids	MS	spray-chilling	(*43*)
	Eudragit RL, Eudragit RS	MC	phase separation	(*44*)
Verapamil HCl	CA, cellulose acetate propionate, CAB	MS	emulsion-solvent evaporation	(*45*)

CA, cellulose acetate; CAB, cellulose acetate butyrate; CAP, cellulose acetate phthalate; CAT, cellulose acetate trimellitate; CMC, carboxymethylcellulose; CMEC, carboxymethylethylcellulose; EC, ethylcellulose; HPC, hydroxypropylcellulose; HPMC, hydroxypropylmethylcellulose; HPMCP, hydroxypropylmethylcellulose phthalate; MC, microcapsules; MP, microparticles; MS, microspheres; NS, nanospheres; PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PMMA, polymethyl methacrylate; PVA, poly(vinyl alcohol).

nanoparticles were prepared for the colon delivery of prednisolone (*56*) and oral delivery of protein (*57*). Pargaonkar *et al*. (*58*) used a new method, electrostatic layer-by-layer (LbL) self-assembling, to make core-shell structures for encapsulation of dexamethasone microcrystals with a polyelectrolyte shell. The LbL self-assembly process was used to encapsulate dexamethasone particles with up to five double layers formed by alternating the adsorption of positively charged poly(dimethyldiallyl ammonium chloride), negatively charged sodium poly(styrenesulfonate), and, depending on the pH, positively or negatively charged gelatins of type A (acid pretreated/porcine gelatin) or type B (alkali processed/bovine gelatin) onto the surface of the negatively charged dexamethasone particles. Direct surface modification of dexamethasone microcrystals *via* the LbL process produced monodispersed suspensions with diffusion-controlled sustained drug release *via* the polyelectrolyte multilayer shell. Although many studies have been performed with oral microparticle preparations, a high drug loading efficiency independent on the method of preparation was not attained. All of the methods of preparing these microparticles are batch production methods.

Since the primary goal of oral microparticles, *i.e.*, providing an oral sustained-release preparation, has been attained, scientists are now working to develop an oral delivery system for peptide/protein drugs with microparticle technology. Peptide/protein drugs undergo hydrolysis before being absorbed by the gastrointestinal (GI) tract. Microparticles are a solid preparation and can protect peptide/protein drugs from attack by hydrolytic enzymes. Cui *et al*. prepared insulin loaded copoly(lactic/glycolic) acid (PLGA)-hydroxypropylmethyl cellulose (HP55) nanoparticles as an oral DDS (*59*). The nanoparticles were prepared by diffusion of a modified emulsion solvent in water, and their physicochemical characteristics, drug release *in vitro*, and hypoglycemic effects in diabetic rats were evaluated. The particle sizes of the PLGA nanoparticles (PNP) and PLGA-HP55 nanoparticles (PHNP) were 150-169 nm, and the drug loading rates were 50.3-65.4%. The initial burst release of insulin from the nanoparticles in simulated gastric fluid over 1 h was 50.5-19.8%. In diabetic rats, the relative bioavailability (BA) of insulin from PNP and PHNP was, in comparison to subcutaneous (s.c.) injection (1.0 IU/kg) of insulin, 3.68-6.27%. Ye *et al*. (*60*) prepared chitosan and sodium alginate microcapsules containing insulin by LbL self-assembly.

In contrast, the current author designed three-layer microcapsules (TLMCs). TLMCs were prepared individually. Figure 2 shows the basic method of preparing TLMCs by discharge as is widely used in printing technology, where it is known as the ink-jet method. As each TLMC is prepared individually, the obtained microcapsules have far less variation in shape and size than conventional microcapsules. In addition, TLMCs do not have only one function, *i.e*. controlled release but other functions like targeting and adhesiveness. TLMCs were used in an oral preparation as a gastrointestinal (GI) mucoadhesive patch system known as GI-MAPS. GI-MAPS was designed to surmount the two hurdles for oral peptide/protein preparations, *i.e*. hydrolytic degradation by digestive enzymes and poor membrane permeability of peptide/protein drugs due to their 3D structures. As many conventional oral drug delivery systems (DDS) including absorption enhancers, emulsions, liposomes, and micro- and nano-capsules, protein unfolding technology, protein conjugates, and colon delivery technology have been examined to develop oral peptide/protein drugs. However, trials of all of these drugs have all faced the hurdle of a low BA because the dilution and spread of an absorption enhancer in the GI tract reduces the effectiveness of the absorption enhancer on peptide/protein drugs. GI-MAPS is designed to solve these problems. GI-MAPS consists of three layers: (i) a water-insoluble basement membrane, (ii) a drug-carrying layer, and (iii) a pH sensitive bioadhesive surface membrane. After oral administration, the surface layer dissolves at the targeted intestinal site and adheres to the small intestinal wall, where a closed space is created at the target site of the GI mucosa by adherence to the mucosal membrane. As a result, both drug and absorption enhancer coexist in the closed space and a high drug concentration gradient is formed between the system and enterocytes, contributing to the enhanced absorption of peptide/protein drugs because peptide/protein drugs are absorbed by a passive diffusion mechanism. As a result, the absorption enhancer is used to full advantage.

Microfabrication technology has been developed to prepare micron-sized GI-MAPS with a diameter of 500-1,000 μm. Figure 2 shows a manufacturing process using this method; a large-scale GI-MAPS-producing machine was developed in 2008. This machine has three to four nozzles that are modified to discharge a polymer solution prepared with an organic solvent. Three kinds of solutions are discharged in series; for example, an enteric polymer solution is first discharged onto the surface of a glass plate and then a drug solution with an absorption enhancer and adhesive polymer is discharged onto the dried enteric polymer layer with a smaller diameter of the drug layer than that of the first enteric layer. Finally, a water-insoluble polymer solution is discharged onto the drug layer with a diameter larger than that of drug layer. Figure 2 also shows the GI-MAPS obtained by this method. TLMCs are made individually *via* this method. A previous review by the current author provides useful information on the biopharmaceutical evaluation of GI-MAPS (*61*).

The advantages of TLMCs are: (i) high drug loading

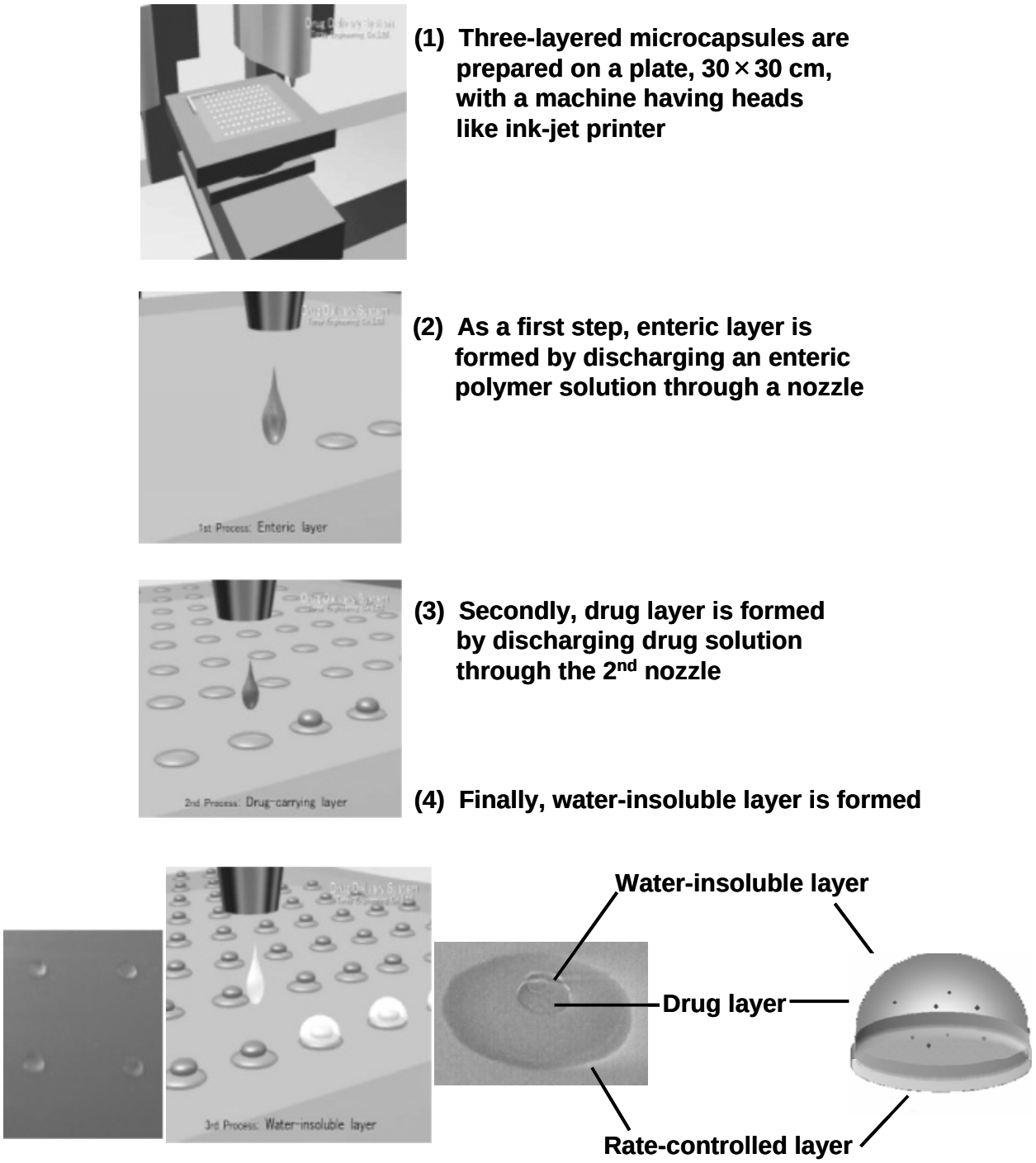

Figure 2. Three-layered microcapsules (TLMC) for oral delivery of drugs.

efficiency, *i.e.*, theoretically 100%, (ii) little size variation for the microcapsules obtained, and (iii) the obtained microcapsules have not only sustained-release but also other functions like site selectivity, targeting, and adhesiveness. The areas for use of TLMCs as an oral DDS are: (i) oral peptide/protein delivery, (ii) sustained-release preparation, and (iii) colon delivery of drugs. With oral delivery of peptide/protein drugs, their BAs improved dramatically, reaching 23% for G-CSF in dogs (*62*) and 12.3% for erythropoietin (EPO) in rats (*63*).

3. Injection preparation

Microparticles for injection preparation are also prepared by the methods described in the previous section. They are: (i) emulsion solvent extraction/evaporation, (ii) phase separation (coacervation), and (iii) spray-drying. The most clinically significant microparticle preparation is leuprolide acetate microspheres (Lupron^{TM}), created by Takeda Chemical Industries and registered with the FDA in 1989. PLGA microspheres containing leuprolide acetate

were prepared by an emulsion solvent evaporation method. A W/O emulsion was first prepared with an inner water phase containing leuprolide acetate and gelatin and an oil phase containing PLGA plus additives such as glyceryl monocaprate and D-lactide in dichloromethane. Then, a W/O/W emulsion was formed with a cooled aqueous 0.1% polyvinyl alcohol solution. Hardened microspheres were obtained by evaporating dichloromethane (*64*). Other researchers used phase separation to prepare polylactic acid (PLA) microspheres and PLGA microspheres. However, they did not succeed as fast as the Takeda group did. In such instances, a great deal of heptane was needed as an organic solvent (*65*). However, completely removing heptane proved difficult because some heptane remained as a contaminant inside microspheres. In addition, the great deal of heptane required to prepare microspheres resulted in difficulties with large-scale production of microspheres. The two water phases were prevented from mixing by increasing the viscosity of the W/O emulsion. As a result, an extremely high loading rate of leuprolide acetate was obtained. However, an initial burst release of leuprolide after injection into experimental animals occurred because the microspheres used did not have a microcapsule structure (*66*). During the initial period of development, monomers of lactic acid and glycolic acid connected by ester bonds were believed to undergo ester hydrolysis, resulting in the release of leuprolide (*67*). However, later research revealed that the basic amino acid residue of leuprolide acetate interacted with the terminal carboxyl group of PLGA inside the microspheres. This structure is very stable and leuprolide acetate was not easily released even when the dissolution medium, water, entered the microspheres. Therefore, leuprolide was released from the microspheres in a sustained manner (*68*). PLGA microspheres released leuprolide over a period of 1 to 3 months, alleviating the pain caused by daily injections and improving patient compliance (*69*). Thus, leuproleide acetate microspheres were an epoch-making preparation in the history of microparticle technology. A review by Andersen *et al*. provides useful information on the biological fate of PLA and PLGA (*70*).

Many reports have previously been published on both microcapsules and microspheres, including nanospheres, for use as injection preparations. Table 2 summarizes the reports on micro and nanoparticles as injection preparations.

As shown in this table, solvent extraction/evaporation is the most popular method for preparing PLA and PLGA microspheres. The review by O'Donnell *et al*. (*134*) provides useful information on the advantages of this method. The review by Soppimath *et al*. (*135*) also focuses on the preparation of microcapsules and microspheres by polymerization.

Microchannel emulsification was introduced to decrease the variation in size of gelatin microspheres (*136*). In such instances, the core material solution/suspension must continuously flow into the wall-forming material solution. A new trend offered by microtechnology in mechanics has allowed a continuous low flow rate, resulting in the decreased size of the obtained microcapsules with decreased variation in size (*137,138*). The basic concept of producing microcapsules is the same as that used in an encapsulator provided by Inotech AG (Switzerland) with a large size nozzle. Recent advances in microfabrication technology have allowed smaller double nozzles. PLGA in dichloromethane solution, 5 w/v%, was discharged through the outer nozzle as a wall-forming material and core material was discharged through the inner nozzle. The discharged particles were collected in a 1 w/v% polyvinyl alcohol solution. Then, microcapsules were obtained by the solvent/evaporation method. By changing the nozzle size, microcapsule size was controlled from 45 to 500 μm with less variation in size. Yeo *et al*. (*139*) described a new method for making reservoir-type microcapsules by an interfacial phase separation principle using ink-jet nozzles. Two ink-jet nozzles that comprise a dual microdispenser system continuously produce two streams of liquid droplets and are aligned so that droplets from one stream collide with droplets from the other stream. After a pair of droplets collides, the polymer droplet spreads over the aqueous droplet to cover the surface of the aqueous droplet. Mass transfer between the two liquids, *i.e.*, solvent exchange, results in the formation of a polymer membrane on the surface of the aqueous droplet. The formation of the polymer membrane depends largely on the favorable spread of the polymer solution on the aqueous droplets and fast solvent exchange and requires judicious selection of the organic solvent. Simple and fast screening methods were developed for selection of a proper solvent. Screening procedures identified ethyl acetate as one of the most desirable solvents. Ethyl acetate and the dual microdispenser system were used to form microcapsules that were subsequently examined by microscopic methods to demonstrate their unique geometry. Details are available in the review by Freitas *et al*. (*140*).

Thus microencapsulation technology has made advances in the past few decades. However, the microcapsules and microspheres obtained with either method have spherical shape. For an oral sustained-release preparation, initial burst release is not a critical factor because the rate of BA of the drug after oral administration is low. However, initial burst release is a critical factor for a sustained-release injection preparation where the rate of BA of a drug is fast after sc injection of microparticles. The conventional microparticles described above cannot solve the initial burst release of the core drug. On the other hand, TLMCs as designed by the current author do not

Table 2. Microcapsules and microspheres as sustained-release injection preparations

Drug	Wall-forming material		Method	Reference
Acetaminophen	PLA	MC	solvent evaporation	(*71*)
Amphotericin-B	albumin	NS	pH-coacervation	(*72*)
Ascorbic acid	pea protein	MP	spray dry	(*73*)
Bovine serum albumin	PLGA	NP	solvent evaporation	(*74*)
Bupivacaine	PLA	MS	solvent evaporation	(*75*)
Camptothecin	PCL	MS	solvent evaporation	(*76*)
Captopril	PLG	MS	solvent evaporation	(*77*)
Cisplatin	PLA	MS	solvent evaporation	(*78*)
	Chitosan	MS	emulsion-chemical crosslink	(*79*)
Ciprofloxacin	BSA	MS	spray dry	(*80*)
Cyclosporine A	PLGA	MS	solvent evaporation	(*81*)
	PLGA, PLA	MP, NP	solvent evaporation	(*82*)
Cytosine arabinoside	PGA/PGA derivative	NP	interfacial deposition	(*83*)
Dexamethasone	PLGA	MP	solid-oil-oil-oil	(*84*)
Diclofenac	PCL	NS	spray-dry	(*85*)
Enkephalin	PGA	MC	solvent evaporation	(*86*)
Ethyliopanoate	poly(benzyl L-glutamate)	MS	solvent evaporation	(*87*)
Finasteride	PPCM	MS	solvent evaporation	(*88*)
5-fluorouracil (5-FU)	PLA	MS	solvent evaporation	(*89*)
	poly(ortho ester)	MC	solvent evaporation	(*90*)
	poly(methylidene malonate)	MS	emulsion/extraction	(*91*)
	chitosan coated PLA	MS	solvent evaporation	(*92*)
	poly(methylidene malonate)	MS	emulsion/extraction	(*93*)
5-FU, indomethacin	PLGA	NS	emulsification solvent diffusion	(*94*)
Ganciclovir	albumin	NP	coacervation	(*95*)
	PLGA (Intraocular)	MS	solvent evaporation	(*96*)
G-CSF	PLGA	NP	emulsion/solvent diffusion	(*97*)
Gemcitabine	polycyanoacrylate	NS, NC	nanoprecipitation	(*98*)
Gentamicin	coralline hydroxyapatite	MS	dispersion polymerization	(*99*)
	BSA	MS	spray dry	(*100*)
	PLA/PLGA	MS	spray dry	(*101*)
human growth hormone	PLGA	MS	atomizer freezedry	(*102*)
	PLGA	MC	solvent evaporation	(*103*)
	dex-HEMA	MS	emulsion/polymerization	(*104*)
Glycine homopeptides	PLA	MS	solvent evaporation	(*105*)
Griseofulvin	PLA	MS	solvent evaporation	(*106*)
Heparin	gelatin	MC	coacervation	(*107*)
Indomethacin	polyesters	NS	spray-dry	(*108*)
Insulin	PLGA/agarose	MS	phase separation	(*109*)
	chitosan	MC	emulsion interfacial cross link	(*110,111*)
	PLGA	MS	solvent evaporation	(*112*)
Interferon	gelatin	MS	coacervation	(*113*)
	PLGA	MS	solvent evaporation	(*114*)
Levodopa	gelatin (Nasal)	MS	solvent evaporation	(*115*)
Methotrexate	gelatin	MS	azide coupling-grafting	(*116*)
Paclitaxel	poly(methylidene malonate	MS	solvent evaporation	(*117*)
	PLGA	NP	spray dry	(*118*)
Pentamidine	PLGA	MC	solvent evaporation	(*119,120*)
Peptides	PLGA	MS	multiple emulsion	(*121*)
	HSA/alginate	MS	emulsion transacylation	(*122*)
Prednisone	star oligo/poly(DL-lactide)	MS	ultrasonic-dispersion	(*123*)
Protein	gelatin	NS	coacervation	(*124,125*)
Ribonuclease, lysozyme	PLA	MP	supercritical carbon dioxide	(*126*)
Rifampicin	PLA	MS	solvent evaporation	(*127*)
Steroids	albumin	MS	solvent evaporation	(*128*)
Streptomycin	albumin and gelatin	MS	coacervation	(*129*)
Testosterone	PLA	MS	solvent evaporation	(*130*)
Tetanus toxoid	Poloxamer/PLGA	MS	solvent extraction	(*131*)
Timolol	PLG	MS	solvent evaporation	(*132*)
Vancomycin	PCL	MP	solvent evaporation	(*133*)

BSA, bovine serum albumin; CA, cellulose acetate; CAB, cellulose acetate butyrate; CAP, cellulose acetate phthalate; CAT, cellulose acetate trimellitate; CMEC, carboxymethylethylcellulose; dex-HEMA, hydroxyethyl methacrylated dextran; HPMCP, hydroxypropylmethylcellulose phthalate; HSA, human serum albumin; MP, microparticles; NC, nanocapsules; NS, nanospheres; NP, nanoparticles; PCL, poly(epsilon-caprolactone); PGA, poly(glycerol adipate); PLA, polylactic acid; PLG, polyglycolic acid; PLGA, copoly(lactic/glycolic) acid; Poly(THPMA), poly(2-tetrahydropranyl methacrylate); PPCM, poly(propylene carbonate maleate).

have an initial burst release of the encapsulated drug. TLMCs are unique and also applicable to a sustained-release sc injection preparation. In accordance with the preparation of TLMCs as GI-MAPS, as was described in the previous section, FITC-dextran was encapsulated into TLMCs and a proof-of-concept (POC) experiment was performed in which poly-ε-caprolactone (PCL) was used as the wall-forming material. The capsule size was less than 1,000 μm. A rate-control layer with a thickness of approximately 10 μm was first formed by discharging a PCL solution containing different amounts of plasticizer including a surfactant. Second,

FITC-dextran was discharged. Finally, the PCL solution was discharged and a water-insoluble basement layer was formed. The *in vitro* release experiment showed long term sustained-release characteristics as shown in Figure 3, and an initial burst release of FITC-dextran was not observed. TLMCs are also applicable to a wide variety of peptide/protein drugs. Therefore, TLMCs containing leuprolide acetate were prepared and sustained-release characteristics were ascertained from the serum leuprolide concentration *vs*. time profile for more than 10 days after sc administration of the TLMC preparation in rats. The advantages of TLMCs are: (i) a high drug loading efficiency, theoretically 100%, (ii) no initial burst release, and (iii) little variation in particle size. A GI-MAPS-producing machine can also be used to prepare TLMCs, although its nozzle size must be decreased.

4. Percutaneous preparation

Thanks to advances in biotechnology, several important biopharmaceuticals such as insulin, erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF), growth hormone (GH), and interferon (IFN) have been developed (*141*). The demand for the delivery of macromolecular biopharmaceuticals like peptide/protein drugs is increasing (*142*). Although the most preferable form of dosage is an oral preparation, the BAs of these drugs are almost 10-23% even when strong absorption enhancers are formulated into GI-MAPS. As a result, no oral preparation of these drugs has entered the pharmaceutical market. Even today, these drugs are administered by iv and/or sc injections. Percutaneous administration is an attractive alternative for the delivery of these drugs because of its many advantages: (i) no or less degradation by hydrolytic enzymes than in the GI tract, (ii) no first-pass effects of the liver associated with oral delivery, (iii) no or less pain than sc injection, (iv) convenience of administration over iv injection, (v) a better and continuously controlled-delivery rate than oral and sc sustained-release preparations, and (vi) easy removal when side-effects appear. Despite their many potential advantages, transdermal drug delivery systems (TDDS) are severely limited by the poor permeability of drugs through the human skin, *i.e*. most drugs do not permeate through the skin at therapeutically relevant rates. Many DDSs have been examined in order to increase the rate of drug permeation through the skin, including chemical enhancers and physical methods. Among them, chemical enhancers have contributed most to the development of TDDS. Table 3 shows TDDS products launched on the American market prior to 2007. The permeability of small molecules through the skin can be enhanced by chemical enhancers (*143*). However, their use is limited because they trigger skin irritation or cause other safety concerns. Iontophoresis, electroporation, and ultrasound have been studied as methods of enhancing physical absorption (*144*). Iontophoresis uses an electric field to drive ionized molecules across the skin by electrophoresis and nonionized molecules by electroosmosis. Despite concerns about skin irritation, iontophoresis may be useful in delivering some peptides and small proteins (*145*). As shown in Table 3, a TDDS with lidocaine by iontophoresis was launched on the American market in 2004. Electroporation and ultrasound also provided temporary enhancement of skin permeability of both small drugs and macromolecules (*146,147*).

However, recent advances in microfabrication technology have allowed preparation of microneedles, which may represent a novel TDDS. Since their first description by Henry *et al*. in 1998 (*148*), microfabrication techniques for the production of silicon, metal, glass, and polymer microneedle arrays with micrometer dimensions have been reported (*149-152*). The microneedles are either solid or hollow and posses a geometrical shape. A microneedle TDDS is roughly defined by a micron-sized needle preparation through and by which a drug is percutaneously administered. Microneedle TDDSs are classified as follows: (i) extremely small needles through which a drug solution can be injected into the skin, (ii) metallic

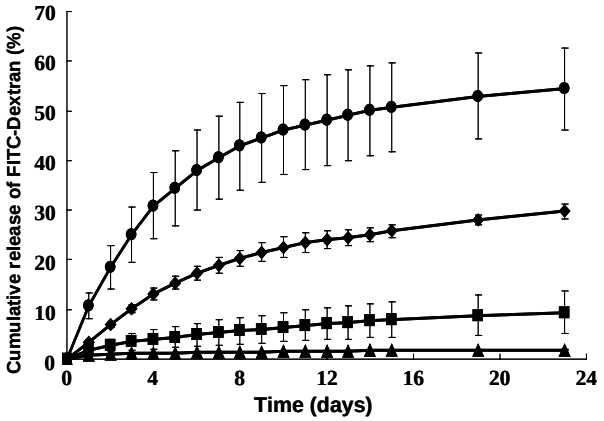

Figure 3. *In vitro* release profiles of FITC-Dextran from three-layered microcapsules (TLMC) made of poly-ε-caprolactone. Release rate was controlled by the addition of plasticizer, PEG 400, (●) 30%, (♦) 25%, (■) 20% and (▲) 5%. Each point shows the mean ± SE of 3-4 experiments.

Table 3. Transdermal DDS products in USA

1980	scopolamine patch
1981	nitroglycerin patch
1983	clonidine patch
1985	estradiol patch
1991	fentanyl patch
1992	estradiol/norethindrone patch
1993	nicotine patch
1994	testosterone patch
1999	lidocine patch
2002	norelgestromin/ethynyl estradiol patch
2003	oxybutynin patch
2004	lidocaine iontophoresis
2005	selegilene patch
2006	methylphenidate patch
2007	rotigitine patch, rivastigmine patch

and/or silastic microneedles onto which a surface drug is coated, and (iii) metallic and/or silastic microneedles by which conduits known as micropores are made on the skin and a drug solution is applied once the microneedles are removed. The physiology of the skin must be understood in order to fully appreciate the function of microneedles.

Human skin consists of three layers, *i.e*. stratum corneum (SC), epidermis, and dermis. The SC is the outer layer of the skin with a thickness of 10-15 μm and is dead tissue. The SC is a strong primary barrier against exogenous compounds including drugs. The second barrier is the viable epidermis (50-100 μm), which contains tissue-like living cells. However, there are no blood vessels in the epidermis. Deeper still, there are blood capillaries in the dermis, which accounts for the bulk of skin volume and contains living cells in the form of nerves. When microneedle arrays are inserted into the skin, conduits are created for the penetration of a drug across the SC. Once a drug penetrates the SC, it can diffuse rapidly through the deeper tissue and permeate the underlying capillaries for systemic absorption. As microneedles do not penetrate to the dermis, where the nerve system exists, pain does not result. Based on this understanding of the skin anatomy, microneedles were designed to penetrate the SC without stimulating the pain receptors found in deeper tissue (*153*).

Silicon microneedles are produced with a standard microelectromechanical system, *i.e*. microfabrication techniques. Chabri *et al*. (*154*) prepared arrays of microconduits for direct and controlled access of molecules across the SC; when inserted into the skin, the arrays enabled drugs to diffuse into the underlying viable epidermis and dermis. Although microneedle arrays originally utilized solid projections for delivery of materials, microfabricated microneedle arrays combined with fluidic microchannels for transdermal extraction of extracellular fluid and blood have also been investigated. Chabri *et al*. prepared microneedles using a modified form of the BOSCH deep reactive ion etching process, which consists of a combination of an isotropic etch and BOSCH reaction. The microfabrication of microneedles involves the use of tools developed by the microelectronic industry to make integrated circuits. Although these tools offer the potential for mass production of microneedles, production is often highly specialized and includes complex multi-step processes (*155*,*156*). For example, 450-μm-thick silicon wafers were spun-coated with photoresist and baked pre-exposure. The wafers were then exposed with the test mask and developed. The wafers were baked postexposure; the thickness of the resist obtained was approximately 8 μm. A standard lithographic mask bearing the appropriate dot array pattern was used during UV exposure to produce a photoresist etch mask. The surface was subsequently etched using a reactive blend of fluorinated and oxygen gases, with those regions directly underlying the photoresist mask being resistant to the etching process. The waters were loaded and subjected to an SF_6 etch to provide an isotropic etch profile. Subsequently, ASETM etch was used to define the length of the microneedle. Finally, the resist was removed in oxygen plasma. Thus, the method of Chabri *et al*. falls under technology used in the field of semiconductors.

In addition to silicon-based microneedles, metallic microneedles were also proposed. They are classified into two categories, hollow microneedles (*149*,*151*) and a microneedle array made of stainless steel (*157*) and titanium (*158*). Silastic and metallic microarrays are used in two ways. One is the application of a drug solution to the skin after physical conduits are made by inserting a metallic and/or silastic microarray. The second way is to use a microarray with the drug coating its surface. After the insertion of the microarray into the skin, the drug is dissolved and absorbed into the skin. Hollow microneedles have also been developed in which a drug solution is injected into the skin through hollow microneedles. As is readily apparent, these hollow microneedles are quite distinct from pharmaceuticals. Furthermore, silicon microneedle arrays are fragile, the use of silicon is relatively expensive, and silicon has yet to be proven to be a biocompatible material. Therefore, these microneedles fall under the category of medical devices.

After Prausnitz *et al*. showed that the absorption of a protein antigen, ovalbumin, was extensively increased by microneedle technology (*158*), the absorption-enhancing effects of microneedle arrays on the following drugs have been reported: (i) small compounds with a MW of less than 1 kDa like diclofenac (*159*), methyl nicotinate (*160*), and bischloroethyl nitrosourea (*161*), (ii) intermediate compounds (MW between 1 and 10 kDa) like FITC-Dextran (*162*), desmopressin (*163*), and insulin (*149*,*151*,*157*,*159*), and (iii) macromolecules (MW larger than 10 kDa) like FITC-Dextran (*162*), bovine serum albumin (*164*), ovalbumin (*158*), antisense oligonucleotides (*165*), plasmid DNA (*166*), and nanospheres (*167*).

Another area of study has been self-dissolving micropiles (SDMPs). Miyano *et al*. (*168*) proposed SDMPs made of maltose for the percutaneous application of dye for tattoos and cosmetics. In their system, maltose was used as a base to make SDMPs. To make maltose SDMPs, maltose was melted by heating it to its melting point, 103°C, and SDMPs were made by introducing maltose into a metallic mold. As a high temperature is needed to make SDMPs, insulin may easily degrade and lose its pharmacological activity. In addition, maltose is a disaccharide, so it causes difficulties in obtaining SDMPs with a hard, steep top because under high humidity in particular it absorbs

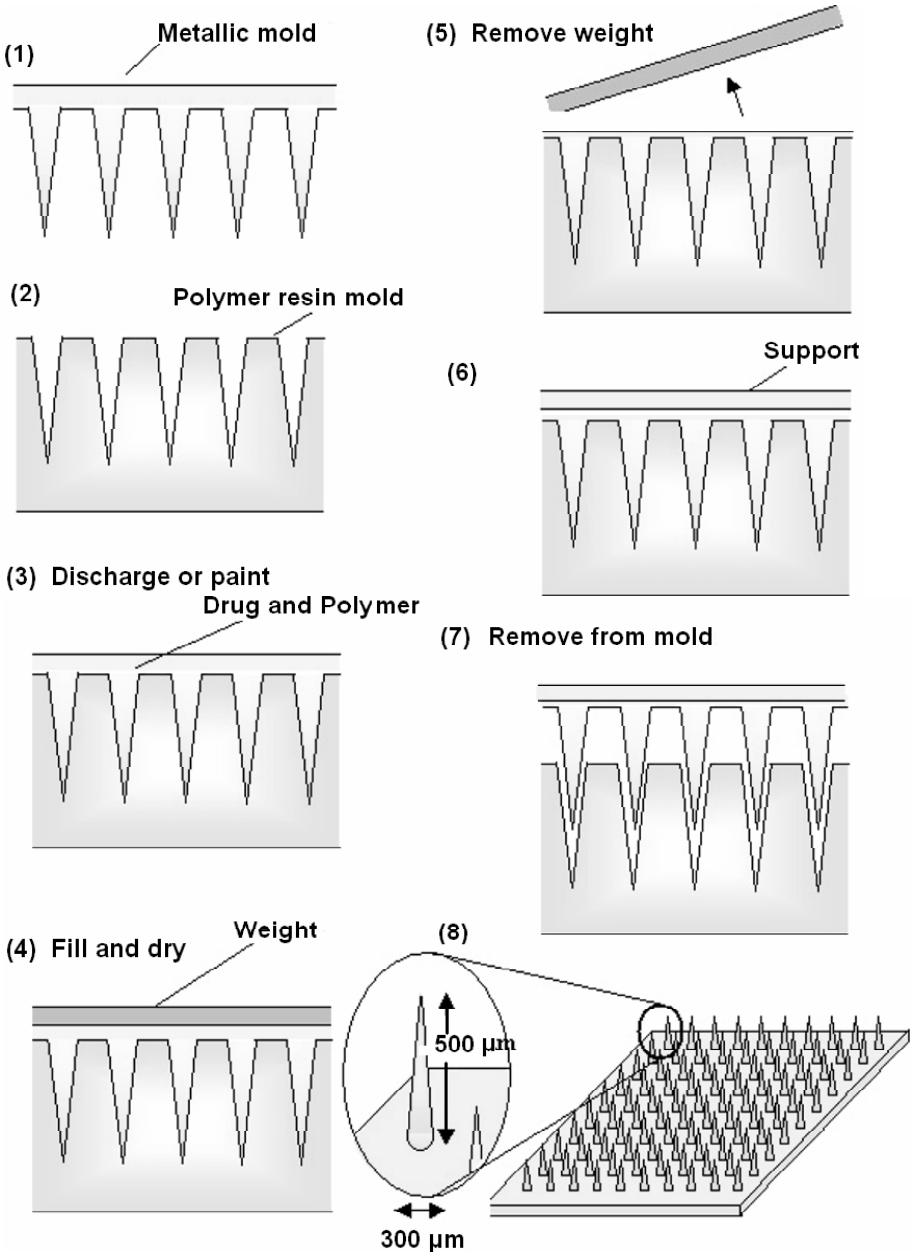

Figure 4. Fabrication process of self-dissolving micropile (SDMP) array.

water from the air; the top of micropile then bends, resulting in difficulty inserting the micropile into the skin.

To overcome these pitfalls, SDMPs made of water-soluble thread-forming polymer were developed. Polysaccharides like chondroitin sulfate, dextran, and hyaluronic acid, proteins like albumin, and synthetic polymers like polyvinyl alcohol were used as a water-soluble thread-forming polymer. A drug solution or drug powder was added to a dense solution of a polymer or combination of polymers. SDMPs were originally formed by withdrawing the top of the micropile tip after the drug and polymer were mixed under low or room temperature. However, as shown in Figure 4, microfabrication technology allows SDMPs to be made individually by means of a mold with micron-sized pores, for example, 500 µm in length and 300 µm in diameter, in the opening base. Their size can be changed from 500 µm to 100 µm in length and from 500 µm to 100 µm in diameter. The method of preparing SDMPs is simple in comparison to preparation by microelectromechanical technology. Namely, a mixture of polymer and drug solution is dispensed into a mold made of polymer resin and dried under pressure. After they fully dry, SDMPs are removed from the mold. A pressing system is useful in accelerating the polymer and drug mixture's insertion into the mold and drying. Metallic microneedles can be formed with MEMS, for example, to make a polymer resin mold. In research by the current author, 100 microneedles with a length of 500 µm and base diameter of 300 µm were formed in a 1.0-cm^2 area on a base plate. A polymer resin mold with 100 microwells was obtained wth these master micropiles. A mixture of polymer and drug was obtained by kneading water-soluble thread-forming polymer, chondroitin sulfate, and a small amount of

Figure 5. Micromissile capsules, 100 capsules, are formed on 1.0 cm^2 patch.

insulin solution; the resultant mixture was introduced into the wells of the polymer resin mold. Finally, the mold was covered with a stainless steel plate and stored in a cold room to dry overnight. The next day, the plate was removed and the hardened mixture was detaching onto supporting material to obtain SDMPs. Figure 5 shows SDMPs containing insulin and Evans blue (EB) obtained by this method. EB was used to stain SDMPs because SDMPs made of chondroitin sulfate and insulin are transparent. The mold can be changed to make SDMPs of different sizes, *e.g.* 200 μm in length and 100 μm in diameter. SDMPs can easily be prepared under low or room temperature. Therefore, SDMPs can be used with drugs that are sensitive to high temperature such as peptide/protein drugs, *i.e.* insulin, EPO, and GH. As water-insoluble drugs can be formulated into SDMN as suspensions, SDMPs can also be used with genetic materials, *i.e.* oligonucleotide delivery, and vaccines including both protein and DNA vaccines. As the shape of the obtained SDMPs is similar to that of a missile, this TDDS is often known as a "micromissile capsule". POC studies on the percutaneous absorption of peptide/protein drugs were performed using SDMPs and showed that high BAs were obtained in mice, rats, and dogs, specifically yielding 90% for insulin (*169*), 80% for EPO (*170*), 88% for IFN, and 95% for human GH. After insulin SDMPs were applied to the skin, the base polymer started to dissolve and the drug was immediately released and then absorbed into systemic circulation. In a dog experiment, the plasma glucose level decreased after insulin SDMPs were applied to the skin at 1.0 IU/kg. The same degree of hypoglycemic effect was observed after sc injection of an insulin solution in the same dogs at the same dose.

5. Conclusion

Thanks to advances in microfabrication technology, novel microparticles such as three-layer microcapsules (TLMC) and self-dissolving micropiles (SDMP) can be prepared on a large scale. As these microparticles are prepared individually, the loading efficiency of a drug is theoretically 100% and a low variation in microparticle size can be attained. These microparticles overcome the disadvantages of conventional microparticles and also have multiple functions. These microparticles have led to a renaissance in pharmaceutical technology.

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