# 3D Printed Microneedle for Vaccine Delivery: Prospect and Application

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelors of Pharmacy (Honors)

Department of Pharmacy
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September, 2020

## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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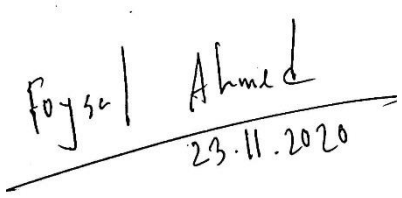


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# Approval

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## Ethics Statement

This study does not involve any animal or human trails.

## Abstract

Microneedle mediated delivery based research has garnered great interest in recent years. Microneedles (MNs) are designed to target the outermost skin barrier layer, the stratum corneum. A quick response can be observed due to disruption of stratum corneum by microneedles. For immunization, skin is an attractive administration site which might be an alternative for traditional intramuscular or subcutaneous vaccination. Vaccination using microneedles is especially appealing because it not only offers expected advantages but also enable vaccine targeting to the skin. To solve the traditional vaccine delivery problem, 3D printing might be an option because of allowing the rapid realization of customizable yet complicated microfluidic and microneedle features. The aim of 3D printing is the targeted release production and customized drug delivery system. The 3DP technique controls the thickness, shape, percentage fill, dose of the drug, and adjustment of dose as per patient need. In this review, we will discuss the probability of success in case of 3D-printed microneedle for vaccine delivery.

**Keywords:** Microneedle; 3D printing; Immunization; Vaccine

## Dedication

I want to dedicate this project to my respectable supervisor Dr. Md. Jasim Uddin, Assistant Professor in Department of Pharmacy, Brac University for his continuous guidance throughout my project.

## Acknowledgement

I would like to begin by thanking the Almighty who is the source of our strength and knowledge which have enabled me to complete this project with full diligence.

I would like to express my deepest gratitude and appreciation to my project supervisor, Dr. Md. Jasim Uddin (Assistant Professor, Department of Pharmacy, Brac University), whose expertise, ample time spent and consistent guidance in every step have helped me to accomplish this project efficiently. I would like to thank him for his great advice and patient behavior throughout this phase whenever I encountered difficulty.

I would also like to express my sincere gratitude to Dr. Eva Rahman Kabir, Professor and Chairperson, Department of Pharmacy, Brac University, who has been a constant source of inspiration for me.

Finally, I would also like to thank my parents for their support and words of encouragement which motivated me to work harder to overcome the difficulties.

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## List of Acronyms

| | |
|---|---|
| 3D | Three dimensional |
| MN | Microneedle |
| PDMS | Polydimethylsiloxane |
| AM | Additive manufacturing |
| DMN | Dissolving microneedle |
| CAD | Computer aided design |
| SLA | Stereolithography |

# Chapter 1

## Introduction

The creation of transdermal drug delivery system (TDDS) has been one of the most sophisticated and innovative approaches of drug deliveries (Z & C, 2014). The skin has a large surface area for drug application and the (trans)dermal route bypasses the first-pass effect of the liver (Van Der Maaden, Jiskoot, & Bouwstra, 2012). The skin, which is mainly composed of epidermis, dermis and hypodermis, is not only the largest organ of the body, but also the body's first barrier (Hao, Li, Zhou, Yang, & Qian, 2017). The viable epidermis and dermis contain many antigen presenting cells (APCs) such as Langerhans cells (LCs) and dermal dendritic cells (dDCs) (Leone, Mönkäre, Bouwstra, Kersten, & Ma, 2017). LCs and different types of DDCs in the epidermis and dermis can project their dendrites, capture antigens, be activated, traffic to the draining lymph nodes and activate T cells, either directly or through resident follicular DCs, to stimulate or regulate immune responses. DDCs and LCs preferentially induce humoral and cellular immunity, respectively (Hegde, Kaveri, & Bayry, 2011). The formidable barrier properties of the skin are stratum corneum (SC), also known as the horny layer, which is a part of epidermis. This uppermost layer of the skin consisting of corneocytes embedded in lipid-enriched matrix with a thickness of approximately 10-15 μm, is a key factor in regulating drug flux through the tissue (Mccrudden et al., 2013). Its multilayered wall-like structures are formed by terminally differentiated keratinocytes and multiple hydrophobic lipid bilayers of ceramides, cholesterol, cholesterol esters and fatty acids (D. J. Lim, Vines, Park, & Lee, 2018). The dermis is the middle layer of the skin, and includes papillary layer, the sub papillary layer, and the reticular layer, which are mostly made up of collagen and elastin (Hao et al., 2017). The SC is 10–15 μm thick with 15–20 corneocyte layers which is made up of corneocytes embedded in an intercellular lipid matrix (Ita, 2015). In the

deep dermal layer, there are most of the lymphatic vessels, and the collagen content of that layer is much greater than in the superficial layer. It has long been recognized as a highly immune reactive tissue containing an abundance of antigen-presenting cells and immunocompetent cells, especially within the epidermal and dermal skin layers (Hong et al., 2013). Microneedles are effectively taken and processed by Ag-presenting cells (APCs), which enhance cross-presentation through major histocompatibility complex class I (MHC I)-mediated cytotoxic (CD8+) T cell immune responses (R. J. Bose et al., 2019). For vaccine applications, the high density of antigen presenting cells (APCs) in skin microenvironments could be targeted directly, thereby multiplying the efficacy of drugs/vaccines/adjuvants and providing a dose-sparing effect, necessitating only a small fraction of the amount of drugs used in hypodermic delivery to obtain the same effect (Bediz, Korkmaz, Khilwani, & Donahue, 2013).

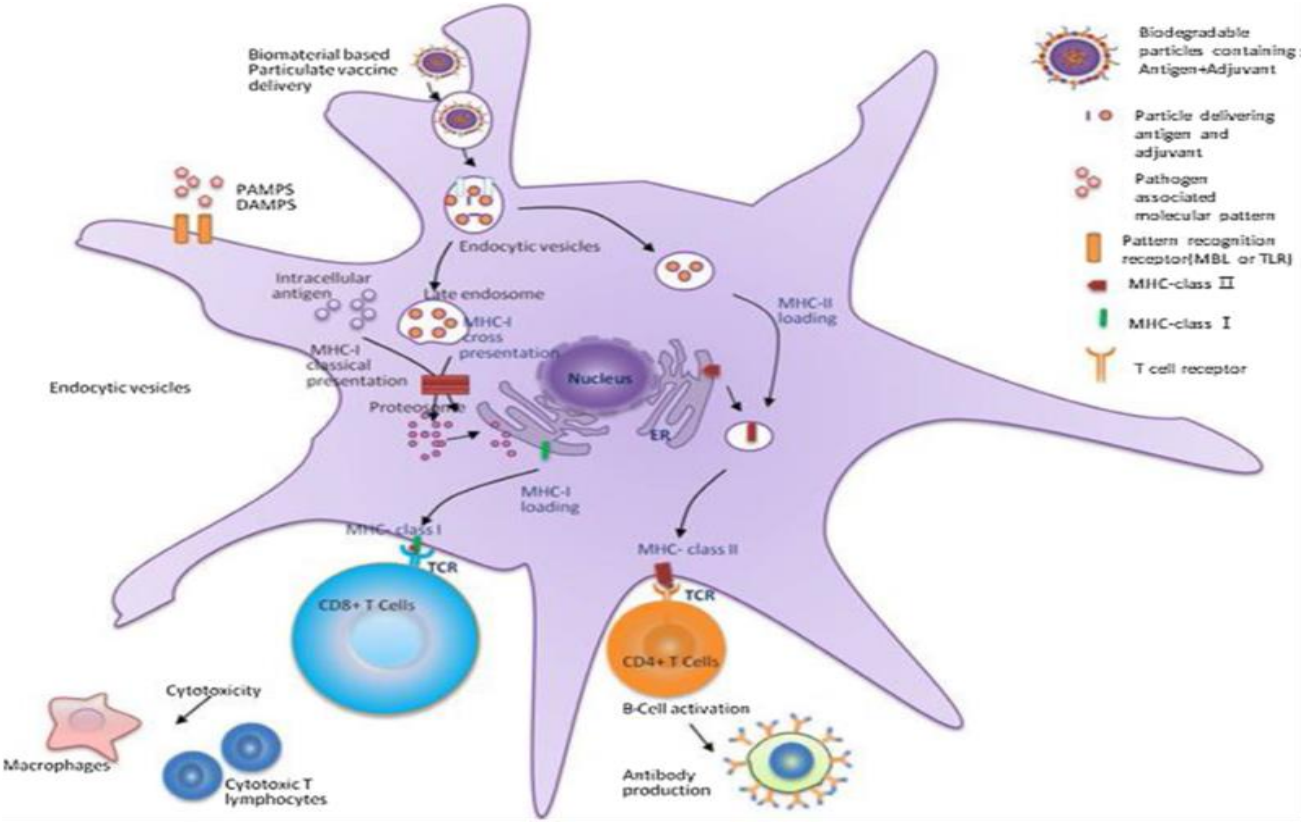


*Figure 1:Schematic illustration of MHC-1 mediated cytotoxic T cell immune system* (R. J. Bose et al., 2019)

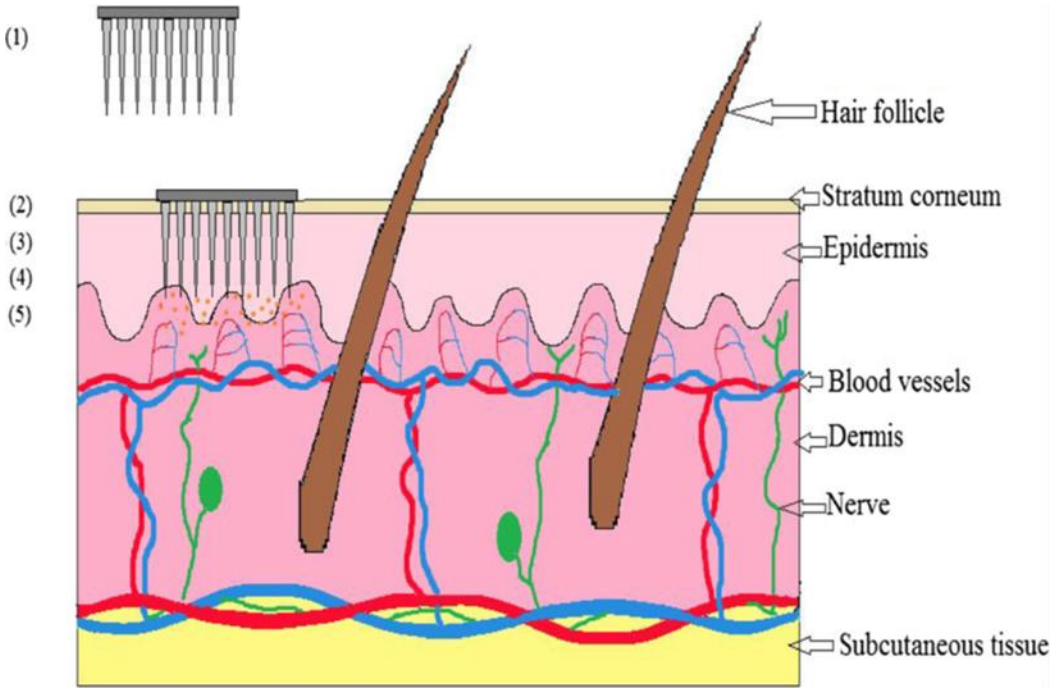


*Figure 2: Mechanism of drug delivery by microneedle device*

*(1) Microneedle device with drug solution; (2) Device inserted into the skin; (3) Temporary mechanical disruption of the skin; (4) Releasing the drug in the epidermis; (5) Transport of drug to the site of action* (Waghule et al., 2019a)

By definition, vaccines are pharmacological formulations that incorporate the disease-causing antigen which could innocuously induce an immune response when administered into a healthy human being, without causing the disease itself (Suh, Shin, & Kim, 2014). Immunization has become one of the most widespread and successful of all health interventions after the provision of safe drinking water. The reason for this is simple: the first immunization campaigns were directed at diseases that had very high mortality and morbidity in their communities (Doherty, Buchy, Standaert, Giaquinto, & Prado-Cohrs, 2016). To protect the body against future micro-organism encounter, vaccine therapy is most potential to stimulate the immune system. Usually the form of killed or weakened micro-organisms which are capable of causing diseases constituted in vaccine where its toxin or on of its surface proteins are used (Waghule et al., 2019a). While therapeutic drugs are regularly self-administered by patients, there is minimal precedent for self-vaccination. Advantageous self-vaccination may extend vaccination coverage and lessen administration costs (J. J. Norman et al., 2014). The

development of effective adjuvants and the use of alternative routes of vaccine administration, such as intradermal. Intradermal injection of influenza vaccine could be a highly desirable antigen-sparing strategy (Prausnitz, Avenue, & View, 2009). Most vaccines are given by intramuscular injection, even though the muscle is not a highly immunogenic organ (Abramo et al., 2012). Most bio therapeutics and vaccines are injected using a hypodermic needle. Injection provides a low-cost, rapid and direct way to deliver almost any type of molecule into the body (Abajo, 2007). Despite familiarity, widespread use and proven efficacy, the hypodermic needle is associated with accidental needle stick injury, spread of blood-borne infections, as well as phobias, pain and significant anxiety (Marshall, Sahm, & Moore, 2016). Due to pose risks of infection like poor sanitation, immunosuppressed patients and needle re usage in developing countries, hypodermic needles are invasive and irritating. Moreover, medically trained professional is required for the administration of hypodermic needles and it also produce medical waste (Economidou, Lamprou, & Douroumis, 2018). A major limitation of hypodermic needles is the pain and risk of infection from blood borne pathogens. Pain from needle insertion leads to distress and poor volunteer compliance and in extreme cases can produce needle phobia, which is characterized by fear, anxiety, and vasovagal reaction that can lead to fainting or sometimes even death (Gill, Denson, Burris, & Prausnitz, 2008). Another route is oral route which eliminates all the problems related to hypodermic needles. Although oral delivery has lots of merits in respect of patient compliance, painless and low cost, numerous drugs often suffer from poor absorption caused by drug degradation resulted from the first-pass metabolism in the gastrointestinal route and microenvironment change (Yang, Liu, Fu, & Song, 2019).Another way to deliver vaccine is using microneedle which pierce the skin to deliver vaccine. Immune responses resulting from dermal delivery are sometimes more analogous to parenteral delivery routes like intramuscular delivery, while delivery to the oral mucosa reliably results in immune responses analogous to other mucosal delivery route

(Creighton & Woodrow, 2019). In comparison to vaccine in microneedle patches where hem agglutination (HA) activity lost 40%-50% activity during or shortly after fabrication, liquid vaccine completely lost potency as determined by HA activity within 1-2 weeks outside of refrigeration. In case of vaccine in MN patches there is no significant additional loss within 3 months after 40-50% loss and also it is independent of temperature (Nicholas Dias, Yung Peng, 2017). However, there are several pitfalls in MN skin delivery, related to the manufacturing process and materials used for the development of MN (Pere et al., 2018).

The use of Additive manufacture, commonly known as 3D printing has brought the pharmaceutical industry a whole step closer to the era of personalized medicine. Even when given the same dose, there may be significant inter-individual differences in drug responses (Chen, Xu, Kwok, & Kang, 2020a). 3D printing is a technology that adds material to produce the part, and hence, it is also called additive manufacturing. Our notion of printing involves transferring ink to paper, line-by-line until the document is completed (Oropallo & Piegl, 2016). Three dimensional printing (3D printing) was used to fabricate novel oral drug delivery devices with specialized design configurations (Goyanes et al., 2015). 3DP incorporates a wide range of assembling procedures, which are altogether founded on computer-aided design (CAD), and controlled deposition of materials (layer-by-layer) to make freestyle geometries (Ahmed & Fazil, 2018). For the fabrication of patient specific drug delivery, 3D printing has infinite effectivity. Drug products with a drug dose prescribed can be directly translated for designing the drug formulation according to patient's age, gender, weight, surface area and other physiological parameters (Beg et al., 2020). 3D printing may facilitate easy customization of transdermal drug delivery systems to accommodate for factors that influence delivery such as differences in skin thickness and hydration (Moussi, Bukhamsin, Hidalgo, & Kosel, 2020). There are many potential uses for 3D printing in medicine, including ophthalmology, which could have a significant impact in changing the ways patients are treated for various conditions

in the future (Schubert, Van Langeveld, & Donoso, 2014). Although previous industrial revolutions focused on mass manufacturing, 3D printing may be a counterrevolution for previous revolution where the focus is only mass manufacturing. 3D printing is shifting towards smaller batch sizes or even unique, on-demand, personalized, yet reasonably priced products and medicine and pharmacy are two areas that would benefit considerably (Dumitrescu et al., 2018).

After penetrating the skin barrier, microneedle delivers the target drug where the therapeutic effects of microneedles originate from its 3D structural geometry (Lee & Jung, 2012). fabrication schemes that can simultaneously create and integrate complex millimeter/centimeter long microfluidic structures and micrometer-scale microneedle features are necessary (Yeung et al., 2019). 3D printing has evolved via the introduction of different technologies such as stereo lithography (SLA), fused deposition modeling (FDM), and two-photon polymerization (TPP). SLA has been used in various works to produce MNs for transdermal drug delivery, mainly to fabricate microneedles (MNs) (Moussi et al., 2020).

*Table 1:Comparison between topical cream, transdermal patch, hypodermic needle, and microneedle drug delivery systems* (Waghule et al., 2019b)

| | **Topical cream** | **Transdermal patch** | **Hypodermic needles** | **Microneedles** |
|---|---|---|---|---|
| Description | Emulsion/ emulgel/cream/ ointments | Adhesive patch to be placed on the skin | Fine, hollow tube having a sharp tip with small opening at the end | Micron size needles are aligned on the surface of a small patch |
| Onset of action | Slower | Slower | Faster | Faster |
| pain | Painless | Painless | Painful | Painless |

| | | | | |
|---|---|---|---|---|
| Bioavailability | Poor | Insufficient | Sufficient | Sufficient |
| Patient compliance | Less | Better | Less | Better |
| Self-administration | Possible | Possible | Not possible | Possible |
| Mechanism of drug delivery | Permeation through skin pores | Drug has to cross stratum corneum barrier, thus poor diffusion of large molecules | Drug placed directly in the dermis | Bypass stratum corneum and drug placed directly into epidermis or dermis hence enhanced permeability |

Transdermal drug and vaccine delivery by microneedle is an attractive means of delivery of therapeutics for numerous reasons over the conventional methods of drug delivery, including-

- Avoidance of gastrointestinal degradation (Moffatt, Wang, Raj Singh, & Donnelly, 2017)
- Elimination of potential issues related to needle-stick injuries and needle reuse (Bediz et al., 2013)
- Patient compliance can be improved by MN as patient with needle phobia will be more comfortable to apply the patch because of its painlessness (Ita, 2015)
- The MNs are small enough in length to avoid touching nerve endings of a patient, thereby, causing little or no pain (Cheung & Das, 2016)

- Transdermal vaccination has primarily been explored for its ability to generate equivalent antibody responses at lower doses, a phenomenon typically described as “dose-sparing (Levin, Kochba, Hung, & Kenney, 2015)
- Because they do not have direct contact with blood vessels, they dramatically diminish contamination risks (M. Sausse Lhernould, 2013)
- Possibility of self-administration, especially, in the rural region lacking qualified health professionals (Li, Zeng, Shan, & Tong, 2017)
- Have flexibility in material composition that permits smart drug delivery systems (Luzuriaga, Berry, Reagan, Smaldone, & Gassensmith, 2018)
- Good stability and Valuable source of intellectual property (Bora, Kumar, & Bansal, 2008)

The purpose of this study is to identify the future of vaccine delivery through 3D printed microneedle. Now only influenza vaccine is delivered through 3D printed hollow microneedle. A wide range of vaccine has been delivered to animals through different types of microneedle. Fabrication of microneedle through 3D printed is now very famous due its cheap cost and also computer aided design process. If 3D printed microneedle can be properly utilized for drug and vaccine delivery, a new era of drug delivery system will be opened.

# Chapter 2

## 3D printed MN for vaccine delivery

The application of microneedles for the delivery of Drugs such as vaccine and anticancer agent into dermal lymphatics is of great clinical benefits as the dimension of the needles permits the drug delivery system to access the rich immune network with ease in a painless fashion (Sabri et al., 2019). 3D printing (3DP) is a new manufacturing technique, which builds up solid objects by deposition of many thin layers (Goyanes et al., 2015). 3D printing is synonymous with "rapid prototyping", "solid free form fabrication", and "additive manufacturing" (J. Norman, Madurawe, Moore, Khan, & Khairuzzaman, 2017). 3D printing provides key advantages over traditional manufacturing approaches, including the ability to fabricate complex geometrical products, the ease of personalized pharmacotherapy for patients, low cost, personalized doses, production of patient specific devices, and fabrication with high tunability and complexity (M. Wu et al., 2020). Three dimensions are built by subsequent overprinting and when the first layer is deposited, the model is reduced by the thickness of the next layer (Goole & Amighi, 2016). Additive manufacturing has been known for its cost efficiency, owing to its potential for low-cost production of small quantities of personalized products. The cost of AM is also becoming increasingly competitive, especially for small production runs such as small-sized standard implants, prosthetics or personalized dosing tablets etc (S. H. Lim, Kathuria, Tan, & Kang, 2018). The spatial efficiency and new degrees of freedom achieved by the rendered 3D and multi-layered microfluidic architectures allows for the incorporation of a multitude of bioanalytical operations (Lin et al., 2019). The 3D printer's ability to allow users to produce objects on demand has proven useful in construction, automotive and aerospace manufacturing and biomedical applications (Luzuriaga et al., 2018). The utilization of devoted program with adaptability for visual customizing abilities has reformed 3D printing in the course of recent

decades, with an assortment of procedures created for the structure and customization of 3D geometries, transformation of an image into mathematical models, and resulting interpretation into the objects of an ideal size and shape with the assistance of equipment control systems (Beg et al., 2020).

3D printing technology where the layer-by-layer fabrication considered as a cornerstone may be thoroughly utilized in favor of the TDD in several aspects. Systems can be designed and printed featuring layers with various drug concentrations for gradually increasing or decreasing doses or for forming of priming doses; alternating layers with different drug contents, designed along the lines of the therapeutic process, are also a prospect (Economidou et al., 2018). 3D printing technology has originated from the layer by layer fabrication technology of three-dimensional (3D) structures directly from computer-aided design (CAD) drawing. 3D printing technology is a truly innovative and has emerged as a versatile technology stage (Shahrubudin, Lee, & Ramlan, 2019). 3D printing: is a process by which 3D solid objects of any shape or geometry can be created from a digital file. The creation is achieved by laying down successive layers of a specific material until the entire object is created. The difference between traditional manufacturing and 3D printing is that the 3d printer involves additive approach but most of the traditional manufacturing processes involve subtractive approach that includes a combination of grinding, bending, forging, molding, cutting, gluing, welding and assembling (Saxena & Kamran, 2016). Rapid manufacturing is a new method of manufacturing where companies are using 3D printers for short run custom manufacturing (Al-maliki & Al-maliki, 2015).

3DP is now used as a production tool or for rapid prototyping in many diverse fields, including the aerospace industry, architecture, Nano systems, fashion, and biomedical research, and it is destined to be the next industrial revolution changing the way many things are created, transported, and stored (Goyanes et al., 2015). Food is one of the sector where 3D printing is used. Shelter is another basic human necessity which can be an interesting application for 3D

printing. Bio-Organ printing. Dental implants, Skull and jaw implants are other sectors where 3D printing is used (Soliman, Feibus, & Baum, 2015).3D printing is also used for device covers, custom parts, artistic items, spare parts and visual aids (Mpofu, Mawere, & Mukosera, 2014).

## 2.1 Classification of microneedles

(I)Solid microneedle

(II)Coated microneedle

(III)Dissolving microneedle

(IV)Hollow microneedle

(V)Hydrogel forming microneedle

### 2.1.1 Solid microneedle

Solid microneedles for (trans)dermal drug delivery were first used in the "poke and patch" approach (Van Der Maaden et al., 2012). Solid microneedles can be used to create micron scale holes in the skin through which molecules can more easily transport (Prausnitz, 2004). Solid microneedles are designed to increase skin permeability by piercing the stratum corneum and exposing the underlying skin layers to the drugs that are later applied to the skin surface, or alternatively to the drugs that already coat the surface of the needles or are embedded in a biodegradable polymer that is the structural material of the needles (Mansoor, Hafeli, & Stoeber, 2012). Drugs are delivered by solid MN via passive diffusion by creating micro channels to increase skin permeability which is followed by the application of a drug-loaded patch on the channels. For the safety perspective, micro channels need to close soon after needle removal to prevent permeation of unwanted toxic substances or infection by pathogenic microorganisms (Ita, 2015). These needles were inserted into cells and nematodes to increase

molecular uptake and gene transfection. Shortly after this work was published, microneedles were developed for transdermal delivery applications, which have been shown to insert into skin and thereby deliver a variety of different compounds in vitro and in vivo (Prausnitz, 2004). Solid microneedles have also been used for gene therapy and vaccination studies. Disruption of the stratum corneum was shown to create holes in epidermal sheets that were large enough to allow for passage of pDNA material through the microneedle pores (Sivamani, Liepmann, & Maibach, 2007).

### 2.1.2 Coated microneedle

Microneedles which follow 'coat and poke' principle are coated microneedles (Nagarkar, Singh, Nguyen, & Jonnalagadda, 2020). Coated microneedles have two main functions. One is to pierce skin and the other is to deliver desired drugs applying on the surface of microneedle. Unfortunately, the maximum drug dose is less than 1 mg. This is the reason for limiting the development of coated microneedles (Yang et al., 2019). Microneedles which have a coat with drug solutions/dispersions are consisted by a base of solid microneedles. For coated microneedles several methods have been studied. The most common method is dip coating which is a little bit complicated for the need for precise control to ensure that the MNs are inserted exactly into the dipping solution (Nagarkar et al., 2020). For drug delivery via coated microneedles, the microneedle surface is first coated with a drug. Upon piercing of the skin, the drug coating is hydrated and detaches from the microneedle surface, resulting in delivery of the drug into the skin (van der Maaden et al., 2015). Microneedles have been coated with a broad range of drugs, such as hydrophilic and hydrophobic low-molecular-weight drugs, DNA, RNA, proteins, peptides, inactivated pathogens, and particles (Van Der Maaden et al., 2012).

### 2.1.3 Dissolving microneedle

Dissolving MNs pursue the mechanism of "poke-and-release" method to deliver drugs into the skin. This mechanism is quite different from "poke-and-patch" method where drugs are usually encapsulated within MNs and after being inserted into the skin, MNs remain on the skin and then the drug releasing is realized when MNs completely degrade or dissolve in the skin (He, Sun, Zhuang, Xu, & Liu, 2019). DMN have received substantial interest for intradermal vaccination due to their ease of use and possibility for self-administration (Rodgers, Courtenay, & Donnelly, 2018). Biodegradable polymers are used to fabricate dissolving microneedle and the drug is encapsulated into the polymer. After inserting microneedle in the skin dissolution occurs which is responsible for the releases of the drug (Waghule et al., 2019b). microneedles with model drug encapsulated not within the microneedle tips but only in the backing layer, which served as a controlled-release reservoir that delivered molecules by a combination of swelling the backing with interstitial fluid drawn out of the skin and molecule diffusion into the skin via channels formed by dissolved microneedles (Hong et al., 2013). One possibility to reduce the antigen loss during the micro molding is to use polymer/antigen solution only for the dMNs and to produce a back plate only from the matrix material or even from other material. The back plate material should possess higher viscosity than that of the needles to reduce the diffusion of the antigen from the dMNs during preparation and drying (Leone et al., 2017).

### 2.1.4 Hollow microneedle

Hollow microneedles deliver drugs by following the "poke and flow" approach. An important benefit of hollow microneedles over solid microneedles are the possibility to facilitate force-driven fluid flow, thereby allowing faster rates of drug delivery (Van Der Maaden et al., 2012).

Drugs can be delivered into the skin directly through the holes in hollow microneedles, which can provide amounts of fluids into the skin at different pressure-driven flow rates (Hao et al., 2017). It involves injecting the drug through the needle with a hollow bore. This approach is more reminiscent of an injection than a patch (Bora et al., 2008). The advantage of the hollow microneedles compared to dissolving or coated microneedles is that little time is required for modifying the dose, formulation or administration depth (Du et al., 2017). The height of the needles is 900µm with a 600µm base diameter and a 60µm tip diameter. The presence of an internal cavity results in a 80µm wall thickness (Marion Sausse Lhernould, Deleers, & Delchambre, 2015). Although hollow microneedles and hypodermic needles are of similar kinds, hollow microneedles are a better means of vaccination delivery than intramuscular injections (M. Sausse Lhernould, 2013). with an analysis of blood flow and optimal values of diameter of hollow and fluidic parameters the ability to demonstrate continuous blood extraction without coagulation is at hand (Le-Thanh, Tran-Minh, Le The, & Karlsen, 2014).

### 2.1.5 Hydrogel forming microneedle

Hydrogel forming microneedles are polymers with a three-dimensional structure that exhibit the ability to swell in water and keep significant amount of water within the structure (Hong et al., 2014). Hydrogel-forming MNs, contain no drug themselves, but swell in skin to allow diffusion of drug contained in an attached reservoir layer to the dermal microcirculation for systemic absorption (Migdadi et al., 2018). Such MN swell in skin to produce continuous, unlockable conduits from patch-type drug reservoirs to the dermal microcirculation, thus allowing prolonged transdermal drug administration (Donnelly et al., 2012). The hydrophilic structure is established by the polymers which ultimately makes it prepared for receiving a large amount of water into their three-dimensional polymeric process. After inserting those

polymers into the skin, the polymers swell because of the presence of the interstitial fluid in the skin which ultimately prompts to the formation of diffferent channels between the capillary circulation and the drug patch (Waghule et al., 2019b). One of the difference and advantages in comparison with regular dissolving polymer microneedles is that by using this drug delivery system, delivered doses of drugs and biomolecules are no longer limited to what can be loaded into the needles themselves (Ita, 2015).

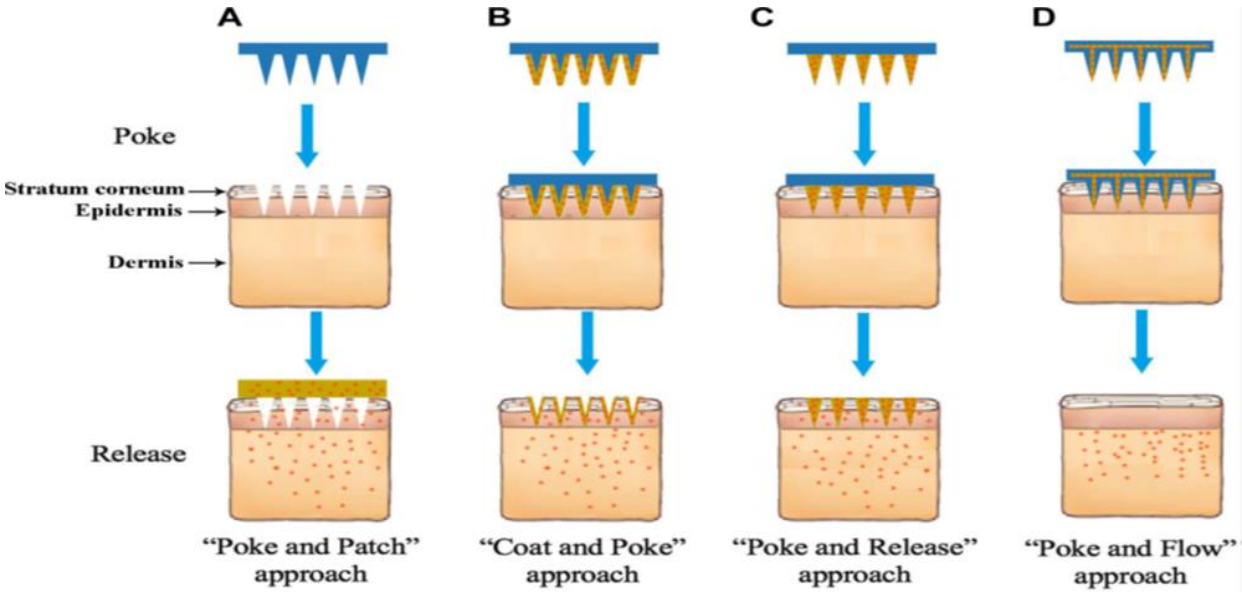


*Figure 3: Various microneedle drug delivery approaches.*

(A) Solid microneedles, for skin pretreatment to create micro channels, followed by the application of transdermal patch; (B) coated microneedles, for deposition of drug formulations into the skin, followed by removal of microneedles; (C) dissolving microneedles, incorporated into the substrate of microneedles, remaining in the skin and dissolving over time to release the drugs; and (D) hollow microneedles, for inserted into the skin and continuous infusion of drug through the created micro channels(He, Sun, Zhuang, Xu, Liu, et al., 2019)

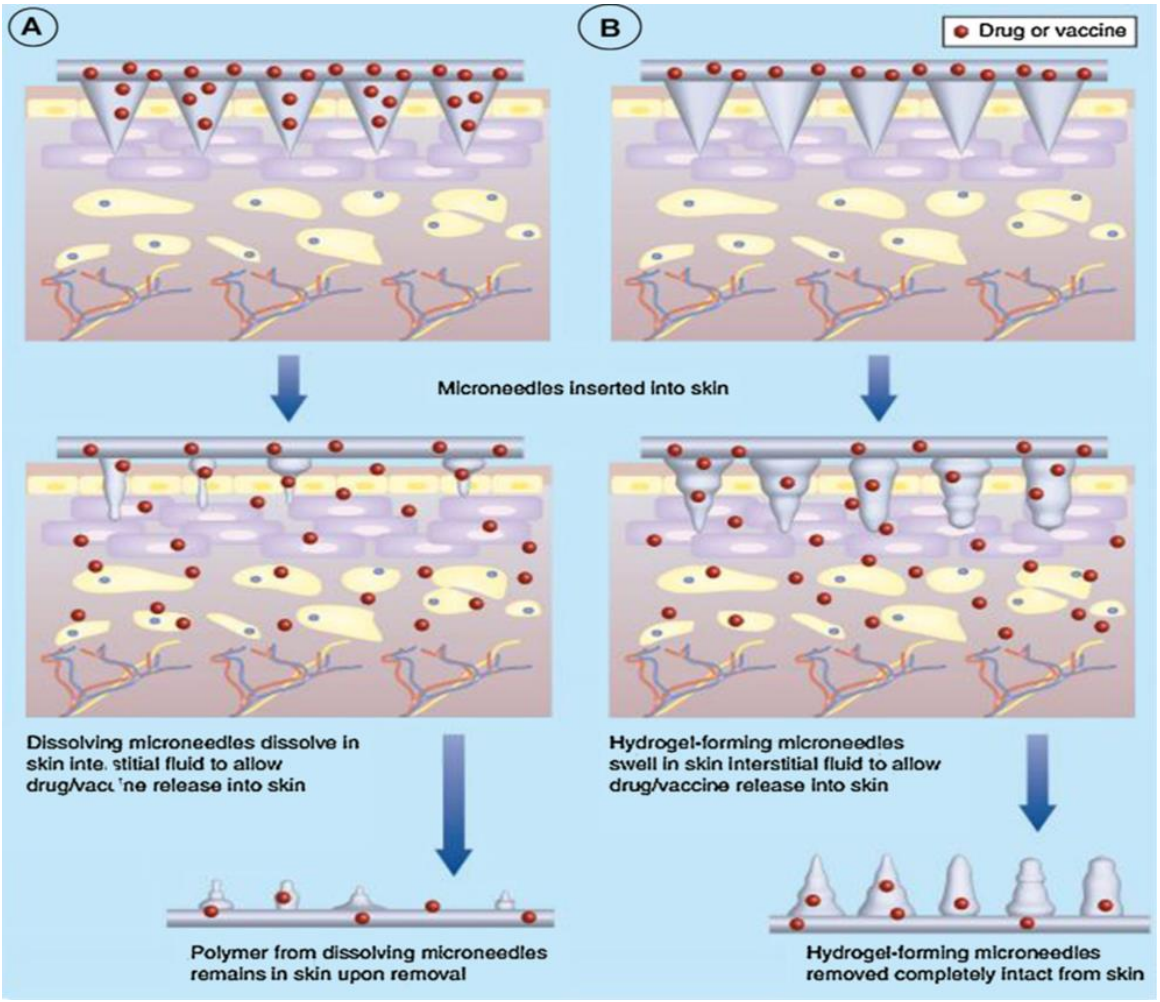


*Figure 4: Mechanisms of dissolving microneedles (A) and Hydrogel-forming microneedle (B)* (Ita, 2017)

A high-resolution 3D printing technique capable of producing complex 3D microstructures with sub micrometer solution, has also been used for fabrication of polymer microneedles, in terms of solid microneedles or hollow microneedles. Generally, these photo-polymerization-based 3D printing technologies can fabricate solid microneedles or hollow microneedles with high resolution and excellent mechanical properties (M. Wu et al., 2020). Among all those type of microneedles, 3D printing is already applicable for solid, coated and hollow microneedles drug and vaccine.

## 2.2 Dimensions of microneedles

Microneedles can be formed a number of sizes which depend on the type of microneedle and the material used to formulate. For the sufficiency to release the drug into the epidermis, the

needle length should be up to 1500 µm because of the epidermis thickness up to 1500µm. Mostly MNs are 150–1500 microns long, 50–250 microns wide, and have 1–25 microns tip thickness (Waghule et al., 2019a). When the needle length is increased from 500 to 1500 mm (constant needle number) and there is 10 times increase in the number of microneedles (at constant length 620 mm), the pain score was increased by 7- and 3-fold, respectively (Sharma, 2019). Microneedle length: 480, 700, 960, and 1450 mm; microneedle tip angle: 20, 55, and 90 degrees; microneedle width: 160, 245, and 465 mm; microneedle thickness: 30, 45, and 100 mm; and the number of microneedles: 5 and 50 (Gill et al., 2008).

The hollow microneedles arrays are fabricated with lumen diameter of 30 µm and height of 250 µm. The center-to-center the distance of the hollow microneedles array is 150 µm. The axis of lumen is fabricated with the distance of 10 µm to the axis of outside column (Shakeel et al., 2011).

# Chapter 3

# Development of 3D printed MN for vaccine delivery

## 3.1 Materials used for microneedle

### 3.1.1 Metal

#### 3.1.1.1 Silicon

Silicon has a structure like crystalline which is anisotropic in nature. Its features rely on the arrangement in the quartz lattice which displays several resilient moduli (50 to 180 GPa) (Waghule et al., 2019b). For microneedle devices silicon is most commonly used material and it has been explored for over two decades. Silicone MN arrays could be used as primary molds

In micromoulding ,to produce a primary molds silicon MN arrays could be used (Nagarkar et al., 2020). Nonetheless, silicon MNs are anything but difficult to sever during the procedure of insertion into the skin because of the fragile property. Hence, these MNs may remain underneath the skin after utilized and induce inflammation since silicon is not well established as biocompatible materials like a few polymers and metals (He, Sun, Zhuang, Xu, & Liu, 2019). The choice for silicon is driven by the refined production technologies developed for microelectronics which allow silicon to be shaped with microscopic precision into complex structures (Juster, van der Aar, & de Brouwer, 2019).

#### 3.1.1.2 Stainless steel, Titanium

Proper mechanical characteristics and proper biocompatibility are possessed by stainless steel and titanium. Usually Metals are that much strong which enable to avoid breaking which makes them more compatible materials different to silicon for microneedle production (Waghule et al., 2019a). They are used for MNs because it may not stay underneath the skin and will not induce inflammation (He, Sun, Zhuang, Xu, Liu, et al., 2019).

### 3.1.2 Inorganic materials

#### 3.1.2.1 Ceramic

Ceramic microneedles have been fabricated using ceramic micro molding and sintering. Solid ceramic microneedles were prepared by micro molding an alumina slurry using a Poly Di Methyl Siloxane (PDMS) microneedle mold and ceramic sintering (Kim, Park, & Prausnitz, 2012). To produce MN, alumina (Al2O3) is one of the most common type of material. Due to the porosity of alumina, it holds a defined volume of active. Gypsum and Brushite have also been used to fabricate MNs (Nagarkar et al., 2020).

### 3.1.2.2 Glass

To manufacture glass MNs, pulling of glass rods using pipette puller is used. Due to a kind of brittle material, same problems like silicon may occurred in glass MNs after penetrated into skin (He, Sun, Zhuang, Xu, & Liu, 2019). Although glass MNs are not used commercially, they maintain a very good potential for experimental purposes (Waghule et al., 2019b). Glass MN described in the literature are hollow in nature and have typically been used to bypass the stratum corneum and inject medicines (Larrañeta, Lutton, Woolfson, & Donnelly, 2016).

## 3.1.3 Synthetic polymers

Polymers are the most promising materials for MN fabrication. They may be versatile, biocompatible, readily available, cost-effective, and can have advanced properties, e.g., built-in controlled release mechanisms (Singh et al., 2019).

### 3.1.3.1 Biodegradable Polymer

Biodegradable synthetic polymers that are commonly used in biomedical applications include aliphatic polyesters such as poly (lactic acid) (PLA), PLGA, and PCL, as well as their copolymers, a diverse family of synthetic biodegradable thermoplastic polymers that have been investigated as potential adjuvants and vaccine carriers (R. J. Bose et al., 2019). In comparison to metal materials and inorganic materials, polymer materials are considered the most promising materials for microneedle fabrication. They can be used to prepare solid microneedles coated microneedles, dissolving microneedle and hollow microneedles (Hao et al., 2017). The polymers and polysaccharides are used to produce microneedles in easy and in large scale. This material presents advantages such as excellent biocompatibility, biodegradability, low toxicity, strength or toughness, rapid dissolution rate, low cost, a molecular weight below 40 kDa and are eliminate by renal excretion (Queiroz et al., 2020). Microneedles were fabricated by first making master structures using lithography-based

methods, then creating inverse molds of these master structures, and finally preparing replicate microneedles by melting biodegradable polymer formulations into the molds (Park, Allen, & Prausnitz, 2006).

### 3.1.3.2 Non-biodegradable Polymer

Polyvinyl acetate (PVA), Alginic acid, Gantrez AN-139, a copolymer of methylvinylether and maleic anhydride (PMVE/MA), Polyetherimide are used for microneedle fabrication (Bariya, Gohel, Mehta, & Sharma, 2012).

## 3.1.4 Natural polymers

Thermoplastic starch, Carboxymethylcellulose, Amylopectin, Dextran, galactose, chondroitin sulfate, Maltose are used for microneedle fabrication (Bariya et al., 2012).

For 3D printed microneedle, usually a number of polymers are used for this purposes. However, for micro molding process, PDMS are used mostly. PDMS (with the proportion of 10:1) was casted (to create a mold) on the silinized microneedles followed by degassing and restoring in an atmospheric oven. The silane layer makes a boundary between PDMS microneedles and PDMS mold, eliminating them from clinging to each other and facilitates their separation. The final PDMS mold was utilized to make microneedles from different polymers (Nejad, Sadeqi, Kiaee, & Sonkusale, 2018).

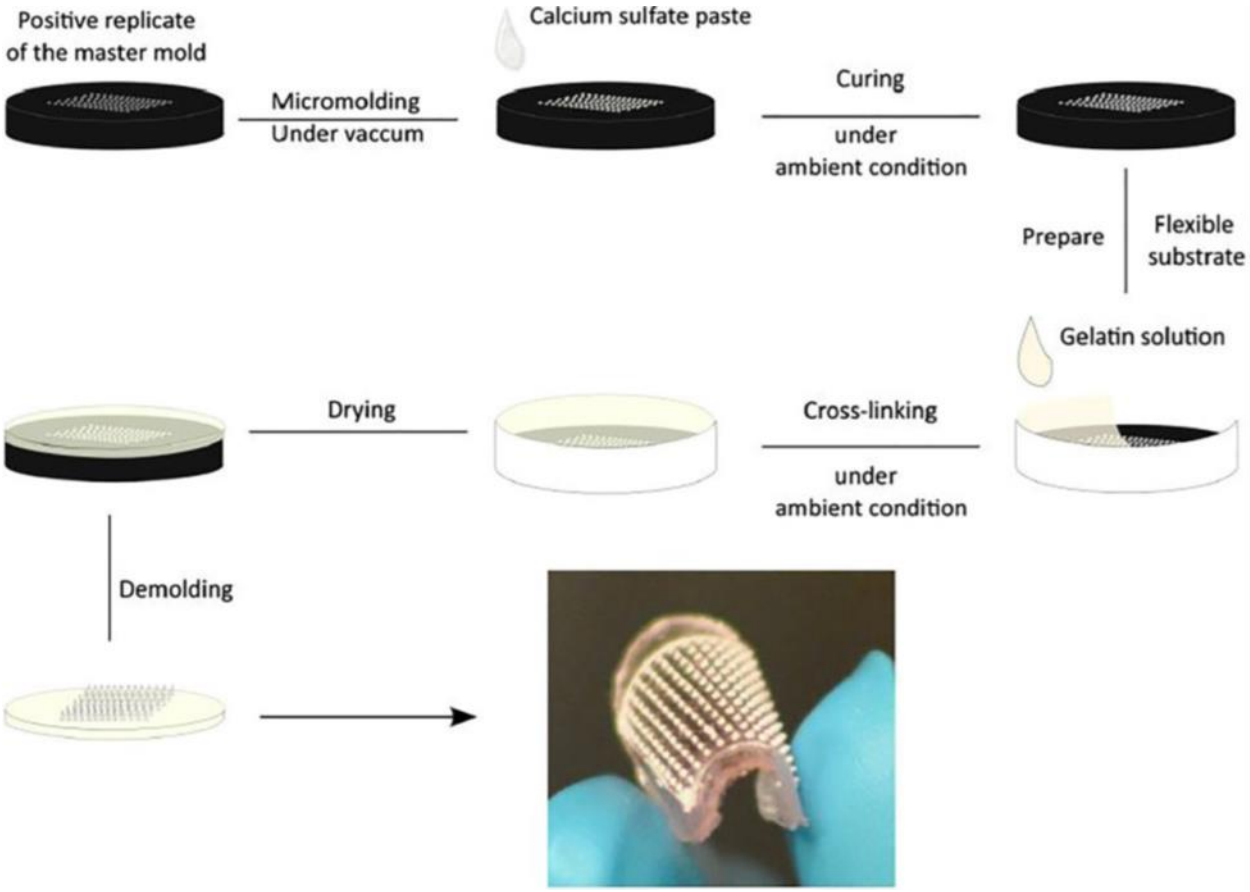


*Figure 5: Micro molding process* (He, Sun, Zhuang, Xu, Liu, et al., 2019)

## 3.2 3D printing fabrication technique

A number of 3D-printing techniques have been flourished for their appropriateness in drug delivery and biomedical uses. Some of the most important techniques include binder jet printing (BJP), fused deposition modeling (FDM), semi-solid extrusion (SSE), selective laser sintering (SLS), and stereo lithography (SLA) (Beg et al., 2020).

Among all these techniques, we will only focus on SLA because it is used to fabricate 3D printed microneedle.

### 3.2.1 Stereolithography (SLA) technique

Charles Hull developed SLA (patent 4575330 filed in 1984, awarded in 1986) (Beg et al., 2020). Stereolithographic 3D printing involves the curing of photosensitive material/s (photo-polymerization) to produce a 3D object (Alhnan et al., 2016). A laser beam is emitted in stereo

lithography and ‘draws’ lines of condensed polymer (Economidou et al., 2018). Stereo lithography (SLA) and Direct Light Processing (DLP) use photo-polymerization so as to frame solid parts, when layers of photosensitive material in which the API is disintegrated are cured, at that point solidified utilizing an UV laser or a light projector (Dumitrescu et al., 2018). This procedure is additionally named vat photo polymerization where a vat loaded with an exceptional resin in the form of a thick fluid is gone through a nozzle and quickly manifested to the light spectrum, whereas it is forever solidified to result in a solid object (Beg et al., 2020). SLA printers provide the opportunity to simultaneously and efficiently print submillimeter/ centimeter-scale components with larger build volumes (~20–30 cm maximum height), shorter print times (e.g., ~15 min for the equivalent 6mm tall spring), and substantially higher throughput than 2PP printers without any compromise in device performance. These operational advantages along with cheap cost and weight of commercially available SLA printers ultimately raise them durable for field deployment where the resource settings are low, where transdermal drug delivery devices can be rapidly prototyped and tailored toward an individual patient’s needs (Yeung et al., 2019).

There are two distinct configurations: the first one places the laser source underneath the hydrogel tank, with the stage that the part is being imprinted on, continually moving upwards; the next configuration has the structure stage inside the tank of gel or resin, continually moving downwards, sinking into the fluid as the laser cures each layer of material from over the tank (Dumitrescu et al., 2018). Stereo lithography uses UV light (or electron beam) to initiate a chain reaction on a layer of resin or monomer solution. The monomers (mainly acrylic or epoxy-based) are UV-active and instantly convert to polymer chains after activation (radicalization). After polymerization, a pattern inside the resin layer is solidified in order to hold the subsequent layers (Ngo, Kashani, Imbalzano, Nguyen, & Hui, 2018). A piston brings down the cured and shaped layer into the vat, permitting the process to rehearse and the other

layer of the part to be formed (Bhushan & Caspers, 2017). This printing process is based on solidification (curing) of successive layers of photosensitive liquid polymers (resins) through irradiation by a light source (e.g., UV laser). Through a laser beam or a digital light projector, the specific pattern defined by the CAD file (Zema, Melocchi, Maroni, & Gazzaniga, 2017). Advantages of this technology include production of high resolution objects at room temperature (Fina, Goyanes, Gaisford, & Basit, 2017). The primary limitation of this technique is the need for photo polymerizable raw materials, which are relatively uncommon in pharmaceutical manufacturing. Also, residual resin can represent a toxicology risk because the uncured Material is chemically distinct from the printed product and may Contain functional groups that are plausible structural alerts for Geno toxicity (J. Norman et al., 2017). The material of choice must be photosensitive. When the laser light shines onto the surface of the pool/bed of photosensitive, drug-loaded material, the material cures and solidifies. This method is extremely high resolution and considerably fast, but the nature of the pool of drug-loaded material has an inherent risk of cross contamination between the fabrications of different drug products (Lepowsky & Tasoglu, 2018).

## 3.3 Drug loading on microneedle

For applying drug solution on microneedle patches, dropping is one of the simple way. For a solution with high viscosity and surface tension, the solution will keep on the upper aspect of the microneedle surface after drying. Whatever the difference of viscosity is, when the surface tension of the drug solution is lowered, at that time all the drug solution will stay at the root area of the microneedle patches. However, for drug delivery into human body this situation may not be favorable. To retain drug solution on the microneedle surfaces, dipping is a better method. The microneedles would initially infiltrate into a drug solution pool and after that the microneedles were being pulled up. A donut ring would form in the retained drug solution on the microneedle surface. For a solitary needle, the volume of the donut ring, or the drug loading

amount relies on the initial capillary rise before the pulling activity and capillary number. On the capillary rise surface tension is the dominant parameter (Hsiao, Ye, Liu, & Wang, 2019).

### 3.4 Storage criteria for vaccine

Almost all vaccines are formulated in liquid form, which must be kept at refrigerated temperature to maintain vaccine quality. Due to this strict temperature requirement, vaccine management typically operates within a cold chain, which is a series of temperature controls during transport, storage and distribution of vaccine from the site of manufacturing to the final destination of delivery. However, even an established cold chain does not guarantee the quality of vaccine, as any accidental exposure to heat or unintentional freezing of vaccines during transport and storage can damage the vaccine (Chu et al., 2016).

# Chapter 4

## Different vaccines delivery through MN

Several vaccines are now delivered through different types of microneedles. However, the number of vaccine delivered to human through microneedle is still a few. Although this is new technology, a number of vaccines should be experimented widely for better purposes. The number of vaccines delivered through microneedles is given in table 2.

*Table 2: Disease targets for microneedle mediated vaccine delivery*(Marshall et al., 2016)

| **Model** | **Virus** | **Bacteria** | **Protozoa** |
|---|---|---|---|
| Mouse | Hepatitis B, Influenza, Human papilloma virus, west Nile virus, chikungunya, | Diphtheria, anthrax. Plague, tetanus | Malaria |

| | | | |
|---|---|---|---|
| | Rotavirus, Herpes simplex, Hepatitis C , HIV | | |
| Rat | Measles. Polio | | |
| Guinea pig | Influenza | Tuberculosis | |
| Rabbit | | Anthrax | |
| Pig | Hepatitis B | | |
| Macaque | Japanese encephalitis, Measles, Polio | | |
| Human | Influenza, Rabies, Polio | | |

## 4.1 Preferable route for vaccine delivery

Three routes are available for vaccine delivery.

(I) Oral route

(II) Transdermal route

(III) Dermal route

The transdermal route of drug administration combines the advantages of oral drug delivery such as convenience with the avoidance of presystemic metabolism observed with parenteral drug delivery (Ita, 2017). Delivery of large molecules, including proteins, peptides and vaccines, at the site of action is promising for the treatment of degenerative diseases. Because these molecules are larger in size, fragile, and poorly soluble, administration of these large molecules by traditional routes will be a bottleneck (Thuy et al., 2020). Vaccines are commonly administered as injections and they are useful because they stimulate specific immune

response, and induce long-lasting immunologic memory which is important for protection against subsequent infections. However, delivering vaccines as injections has several disadvantages such as pain, needle stick injuries, needle-phobia, and poor patient compliance (Ita, 2016). On the other hand, the small vertical length of microneedles allows to overcome main disadvantages of conventional methods of drug delivery (Longo, Strambini, Ventrelli, & Barillaro, 2014). Efforts that are being made to deliver the drug by microneedle array technique have placed them to the next level and eventually become recognized as a strong alternative to the hypodermic needles (Halder, Gupta, Kumari, Gupta, & Rai, 2020).

## 4.2 Polymers for vaccine delivery

Usually biodegradable polymers are used for preparing microneedles which are used for vaccine delivery. For micro molding technique, PDMS are used mostly. For other purposes, PLA, PGA, PLGA are used.

Laser ablation of acrylic substrate employing CO2 laser cutting machine in a COL model is shown to generate 3D conical molds following microneedle fabrication. The COL approach depends on the information that every carving line consequences in almost the similar depth per run for identical laser power and carving speed. When lines pass through, the carving depth at the cross-point is superior since this point is traversed many times, resulting in a sharp cone which become like the conical microneedle mold at this crossover point. By casting the PDMS on the mold, PDMS microneedles were fabricated. After that the PDMS microneedles were reused to fabricate a PDMS replica mold by using silanizing and PDMS casting (Nejad et al., 2018).

# Chapter 5

# Challenges to overcome

## 5.1 Limitations of 3D printed microneedle

i. Higher costs for large production runs relative to injection molding and other technologies (Berman, 2012)

ii. Accuracy of 3D printing is one of the limitation for microneedle (Gibson, Rosen, & Stucker, 2015)

iii. Complexity of part geometry, material used in the prototyping model, compatibility with 3D CAD models and other technical aspects still need in-depth study (Lemu, 2012)

iv. The limitations of rapid prototyping include cost and complexity, as well as the need for specialized equipment and consumables such as photoresist resins (Rengier et al., 2010)

v. Surface texture is generally too rough

vi. The effect of the use of 3D printing technology is will reduce the use of manufacturing labor so automatically will greatly affect the economy of countries that rely on a large number of low skill jobs (Shahrubudin et al., 2019)

## 5.2 Regulatory challenges

As a new manufacture technology, AM does not require special regulations. The existing regulatory framework may still be valid for AM pharmaceutical products. However, AM-enabled personalized medicine can become a challenge. As new regulations are needed to implement the clinical applications of AM pharmaceutical drug products, which can be made in pharmacy, doctor's office or at home (Chen, Xu, Kwok, & Kang, 2020b). The first 3D-printing product was approved by FDA in 2015 which generating some serious encouragement

within biopharmaceutical manufacturers to apply 3D-printing as one of the following-generation instrument for flourishing drug manufactures and biomedical instruments. Concerning the attribution of that similar quick advance in 3D printing, the FDA delivered in December 2017 Technical judgments for Additive Manufacturing of Medical instruments to 'give likely administrative experiences, the current thinking about the office and key chemistry, manufacturing and control (CMC) necessities for the endorsement of 3D-printed drug products and clinical devices (Beg et al., 2020). Approval of a sufficiently broad variety of thermoplastic polymers and liquid resins to be used in FDM and SLA, respectively, also constitutes a fundamental step that may strongly limit profitable exploitation of 3D printing in the manufacturing of medicines (Zema et al., 2017).

## 5.3 Challenges associated with 3D printed MN

SLS and SLA printers are capable of producing features smaller than 100 μm. However, these printers can be costly and most materials are not biocompatible. For instance, the photo-initiators required in the SLA printing process are toxic and are incompatible for transdermal drug delivery (Luzuriaga et al., 2018). The smooth surfaces, ultra-sharp tips and steep sidewalls of 3D printed microneedles structure processes are also challenging (O'Mahony et al., 2017).

# Chapter 6

# Conclusion

Vaccine development remains an important field in both research and pharma, whereby in addition to extending the spectrum of antigens for novel vaccines, developing improved administration strategies to ameliorate vaccine efficacy remains a challenge (Zaric, Ibarzo Yus, Kalcheva, & Klavinskis, 2017). Bringing the concept to drug therapy encountered a series of technical challenges from safety, drug delivery efficiency, to fabrication simplicity, due to which over a decade of research efforts have yet to achieve a practical transdermal 3D printed microneedle (F. Wu, Yang, Yuan, & Jin, 2012). The enhancement of the immune responses due to cutaneous vaccine delivery was particularly impressive for the influenza B vaccine strain (Vassilieva et al., 2015).

In comparison to subtractive manufacture, additive manufacturing has several benefits. Firstly, traditional subtractive method requires more amount of material than additive manufacture. Moreover, in subtractive manufacturing until the part geometry is achieved, material is removed from a block. On the other hand, in additive manufacturing the amount of material can be closely controlled since the part built in additive layers. Secondly, there is capability of producing parts or objects such as creating multi-material parts and biomedical objects including organ in additive system where traditional methods can't able to do (S. Bose, Vahabzadeh, & Bandyopadhyay, 2013). Thirdly, reducing time and cost of manufacturing are also an advantage for additive manufacturing. Although there are advantages. additive manufacturing does have some disadvantages. Additive manufacturing is fast and economical to produce in small orders but in case of large scale production of parts cost effective facility is not working due to extra time and poor part quality which is not applicable for traditional methods (Bhushan & Caspers, 2017).

3D printed microneedle is a recent technology. A number of microneedles now used 3D printed fabrication. Transdermal vaccine delivery through 3D printed microneedle create a new window for delivery system. Biodegradable polymers are used to fabricate microneedle through 3D printing and vaccines are loaded on that small tip. Since, MNs cannot reach dermis layer where the pain receptor is present, pain can't produce. Moreover, the pierce of skin sites easily removed within 1 to 2 hour. Vaccine delivery can be now more effective due to this process. Aside from several disadvantages, 3D printed microneedle might be an effective and efficient towards vaccine delivery because of its ability to produce a rapid painless action where the patient will be most influenced.

# Chapter 7

# Future direction

3DP is set to cause a digital revolution within healthcare. Owing to its simplicity, diversity and portability (Awad, Tren, Gaisford, & Basit, 2018). Despite making massive inroads into other manufacturing industries such as aerospace and automobile pharmaceutical 3D printing is still at its infancy (Alhnan et al., 2016). Delivery technology offers a means of accentuating or altering the desired immune responses from traditional vaccine formats (Beitelshees, Li, & Pfeifer, 2016). One can conclude that the 3D printing technology revolutionize and reshape the world as it is very exciting technology with huge potential also comprising the different technologies at one place, taking into account their economic benefits and social impact (Saxena & Kamran, 2016). A 3D printing stereolithographic technique was introduced for the fabrication of microneedle designs for transdermal delivery. SLA facilitated the printing of high quality MNs with various designs (Pere et al., 2018). Immunization via skin may target innate dendritic cell populations directly through lymphatics from proximal draining lymph nodes and simultaneously by activating the rich dendritic cell network that resides in skin (Sullivan et al., 2010).

However, for now solid, hollow and coated MN can be fabricated through 3D printing process and form that only hollow 3D printed MN is only available for commercialized which is not available for vaccine. For future, we need to focus on dissolving MN because of the pain produced from hollow microneedle. In addition, test should be done more to see efficacy of 3D printed microneedle for vaccine delivery. Finally, we also need to focus on how we can deliver a large amount of drug through 3D printed microneedle.

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