A Review on Microneedle Mediated Transdermal Drug Delivery

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

Department of Pharmacy
Brac University
August 2019

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.
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Approval

The project titled "A Review on Microneedle Mediated Transdermal Drug Delivery" is submitted by Al-Sabbir (13346056) of Summer, 2013 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on August, 2019.

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Ethics Statement

This project does not involve in any human or animal trial.

Abstract

As a very good replacement of hypodermic needle micro needle can be used. Micro needles are very small needles as its name suggest. We all know that skin has three layers. Micro needles basically skip through the upper layer of the skin. Generally four types of micro needle are present. These are solid microneedle, hollow microneedle, coated microneedle and dissolvable microneedle. They can deliver drugs through different mechanisms. Many types of vaccine have successfully been coated in to micro needle. Among all some of their name can really be mentioned like influenza, insulin anthrax, hepatitis c. Finally it can be concluded that using micro needle has many benefits over the conventional hypodermic needles. The future aim of this technology is to coat more vaccine on micro needle and the delivery of drugs to the eyes by micro needle.

Keywords: Micro needle; Vaccine; Influenza; Anthrax; MVA; Dermal

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Dedicated to my parents

Acknowledgements

This research could not be completed without the following people to whom I am very much grateful. It is my absolute honor to acknowledge them. At first I will acknowledge my supervisor Dr. Md. Jasim Uddin, Assistant Professor, Department of Pharmacy, Brac University. He has provided a great support to complete my project by sharing his valuable knowledge. Next, I want to acknowledge Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy, Brac University. She has provided an enormous support and inspired me to complete my research. I am also thankful to Md. Samiul Alam Rajib, Senior Lecturer, Department of Pharmacy, Brac University for helping me to manage my references. And last but not the least I am thankful to all the lab associates and the research facility of Brac University those helped me to complete this project.

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List of Acronyms

ADN	Adeno virus
AFM	Atomic force microscopy
CD	Circular Dischroism
CLT	Cisplatin
CTL	Cisplatin T Lymphocyte
DNA	Deoxyribo Neucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
HI	Hemagglutination Inhibition
HPLC	High Performance Liquid Chromatography
IIV	Influenza virus A/pr/834
MEMS	Micro Electro Mechanical System
MVA	Modified Vaccinia Virus Ankara
POX	Poly (2-ethyl-2oxazoline)
PVA	PolyVenyl Alchohol
PVP	Polyvenyl Pyrrolidone
SOL	Polyvinyl Caprolactame- Polyvinyl Acetate-Polyethylene Glycol
USFDA	United States Food and Drug Administration

Chapter 1

Introduction

It is mcroneedle that has a size in microns and length up to 1mm and it helps basically to circumvent the upper layer of skin. The main purpose of micro needles is drug delivery dermally or transdermally. Transdemal drug delivery has provided an option to the injectable and oral administration of drug (Haj-Ahmad et al., 2015). The needles of the micro needles need to be strong enough to administer drugs (Lee, Lee, You, Lee, & Jung, 2013). Transdermal delivery of drugs means delivery of drugs through the skin and then they reach in the systemic circulation (Ling Teo, Shearwood, Ng, Lu, & Moochhala, 2005). In microneedle arrays a lot of micron scale like projections are attached to the solid support (Donnelly et al., 2014). Various types of materials are used to produce micro needle. Furthermore, since microneedle can be combined with an approach that is called lab-on-a-chip system by this system hollow microneedle can be used to diagnose diseases like diabetes. As skin is the largest organ it protects the body from different pathogens by stratum corneum that inhibits foreign particles to reach in the body. It is transdermal delivery that deals with the administration of drugs through the skin and after that they got distributed in systemic circulation (Ashraf et al., 2010). The skin has some more layers like dermis, epidermis and hypodermis conventional injection used to target the hypodermis for drug delivery but as it is painful so scientists made micro needles (Van Der Maaden et al., 2012). There are some problems in case of vaccination by using hypodermic needles, like for many of the viruses a booster dose is needed to maintain the effectiveness. Furthermore, these vaccines generally do not work well in case of patients of vitamin deficiency. Again, trained health care provider is needed for good vaccination that is rare in developing countries. Along with that in developing countries the infrastructure is not good and also the storage of

vaccine requires freezing that is costly. Also, needle stick injuries are really very common and it is really very painful. Similarly, these needles can leave biohazardous sharp wastages that requires disposal. So, due to these drawbacks scientists developed microneedle that can overcome many of the above shortcomings. There are some types of microneedles like solid microneedle, hollow microneedle and dissolving microneedle. In this paper I have tried to find out their advantages and disadvantages so that we can use the perfect microneedle for our coating purpose. Also, different microneedles deliver drugs in different ways like poke and patch, poke and release coat and poke and poke and flow. We also have tried to discuss about that. Again, we have discussed about the recent coating of drugs and biomolecules on microneedle. Among them we have discussed about influenza vaccine, proteins, peptides, hepatitis c vaccine, ebola vaccine, coating of anticancer agents, measles etc. As a result we have tried to see is microneedle is better than those conventional needles or syringes. We all know from our above discussion that if the vaccines can be coated on microneedle instead of hypodermic needles than can be really helpful as we can get some benefits like dose sparing , no needle injury , no needle phobia, no infection etc. On the other hand as microneedle gives us benefit over the conventional hypodermic needles, it can certainly increase the patient compliance. As it has been proven that microneedle is rarely associated with any kind of infection. Sometime pinpoint bleeding was noticed but it is ignorable. Thus in this research it was found that microneedle has increased patient compliance and Transdermal delivery has evolved as a very good option for delivering drugs through the skin (Escobar-Chávez et al., 2011).

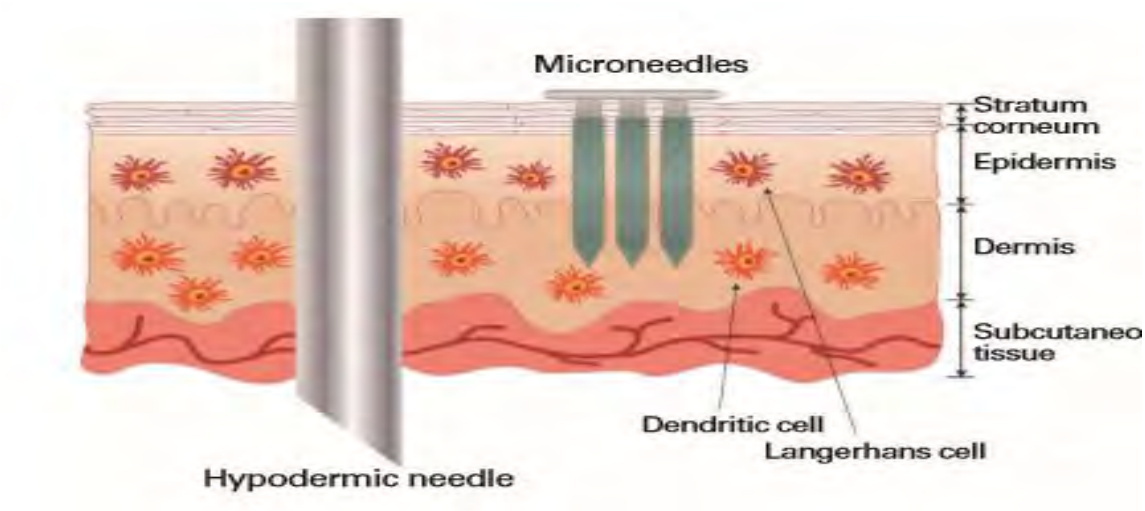

Figure 1: Difference between hypodermic needle and microneedle (Van Der Maaden et al., 2012).

1.1 Skin Structure

1.1.1 Stratum Corneum

Skin is the largest human organ and also works as a barrier. The skin is about one sixth of the total body weight and has a good surface area. There are three layers in skin. The name of the first layer is epidermis, second one is dermis, and the third one is subcutaneous layer. Among them subcutaneous is the outermost layer that is responsible for the protective mechanism (Duarah, Sharma, & Wen, 2019).

1.1.2 Epidermis

We all know that skin has three layers. They are dermis, epidermis and hypodermis. Among them epidermis is the outermost layer. It is composed of straum corneum the outermost layer, and then there are some other layers like stratum granulosum and stratum spinosum.

1.1.3 Dermis

Dermis layer is situated just underneath the epidermis layer and is much thicker than the epidermis. Dermis layer can contain some cells like dendritic cells and langerhans cells thatare present in a huge density in epidermis and dermis. Dermis and epidermis have many dendritic cells those also worked as antigen presenting cells (Ita, 2016). These cells can play a strong role in adaptive immunity by processing and presenting of antigen (Duarah, Sharma, & Wen, 2019).

1.1.4. Hypodermis

Hypodermis is the innermost layer just underneath the dermis layer. This layer has some complex capillary network. Hypodermis layer is very important for delivery by using the transdermal route systematically (Duarah, Sharma, & Wen, 2019). So we can say that the skin has three layers dermis epidermis and hypodermis. Among them epidermis is the outermost layer with physical barrier that prevents microorganisms from entering the body. Furthermore, the epidermis is subdivided into stratum corneum that is the outermost layer then the stratum granulosum and stratum spinosum at last. There are some other types of cells like melanocytes and langerhans cells (Mi& Hk, 2017). Among the deltoid suprascapular waist and thigh the deltoid, suprascapular and waist are the most appropriate body cites (Laurent et al., 2007).

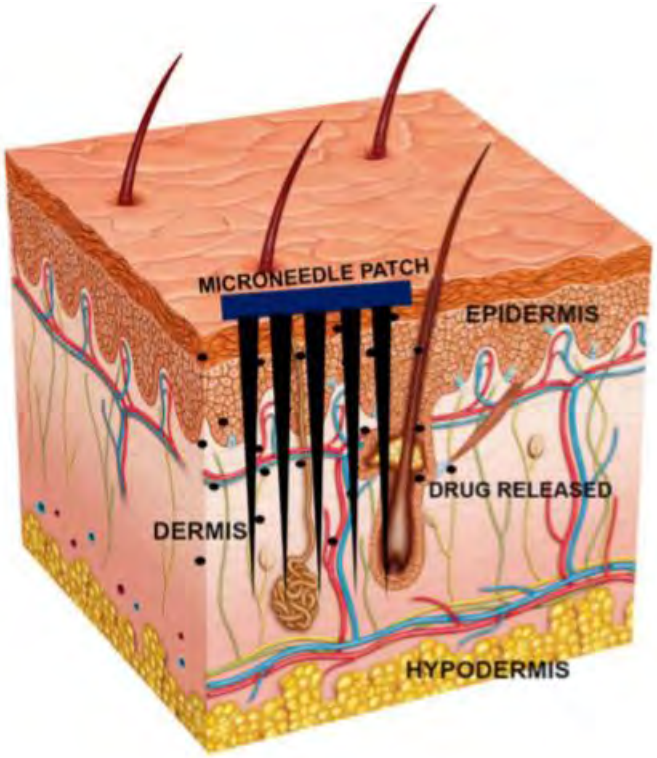

Figure 2: *Different layers of human skin (Duarah, Sharma, & Wen, 2019).*

1.2 Microneedle and its History

1921 is the year when the term “microneedle” was first introduced. The concept of microneedle was first used by two scientists. They used hollow and solid microneedle for this purpose. They also introduced the first drug coated microneedle. They also used it for significant evaluation and for proof of concept for that they used silicon microneedles prepared by micro fabrication technology for the delivery of calcein. They were also the first to report the in vivo evaluation of pain. The safety issues of microneedles were first evaluated by misztkaet all. Solid and hollow microneedles were used to deliver insulin albumin and latex beads for the first time and the result is reported. The first cosmetic microneedle based application is used in 2005. Microneedle roller was used at that time (Duarah et al., 2019).

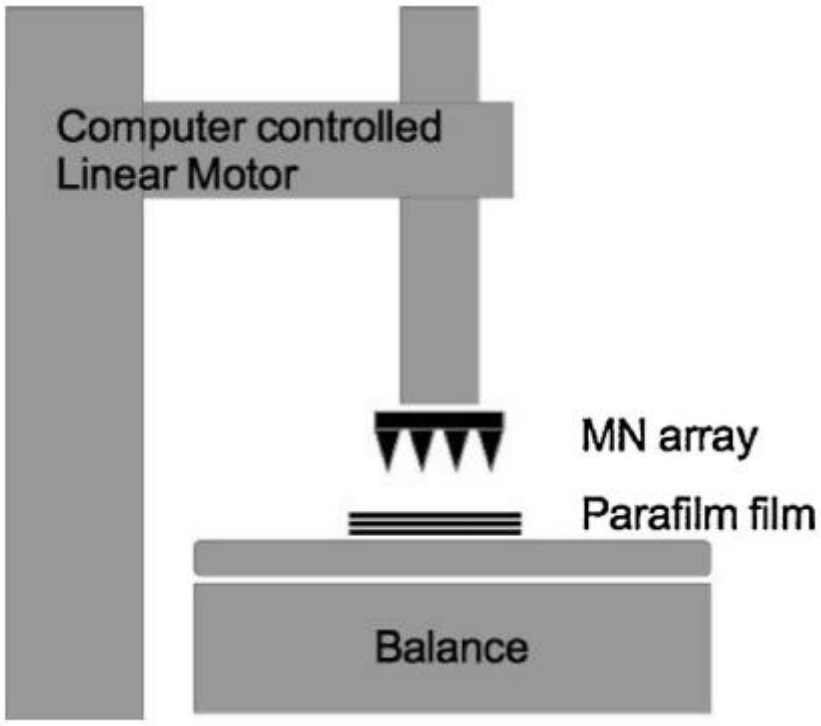

Figure 3: Penetration capability of microneedle (Lhernould et al., 2015).

1.3 Types of Microneedle

Microneedles have size in microns and have length of 1 mm. There are four types of microneedles they are solid micro needles, hollow micro needles, dissolving micro needles and coated microneedle.

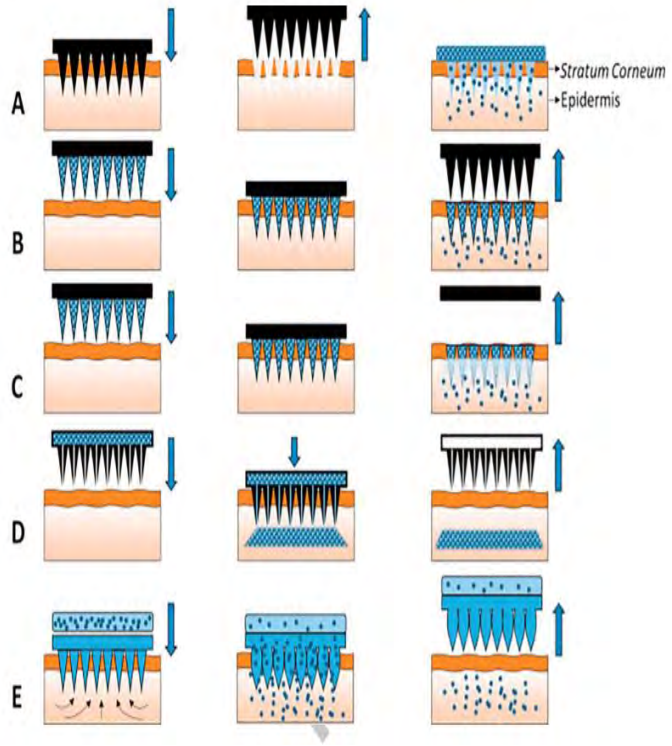

Figure 4: *Different types of microneedle and their drug delivery processes (Duarah et al., 2019).*

1.3.1 Solid Microneedle

Solid microneedle can deliver drugs by creating microchannels in the skin layers. Thus the permeability is increased. One important thing is that the skin need to be retract quickly just after the removal of the patch. MEMS technology is basically used for the fabrication process of microneedle devices. Fabrication process will vary in accordance with the needle material and geometry. The MEMS technology has some series of controlled operations and techniques which are sequential. Solid microneedles can deliver drugs in three different ways. These are

- Coat and poke approach
- Poke and release approach
- Poke and patch approach

Advantages

The advantages of using solid microneedles are like solid microneedle can pierce the skin easily so drug can be delivered in more easy manner (Bariya, Gohel, Mehta, & Sharma, 2012). Dissolvable/degradable and hollow microneedles can deliver drugs at a higher dose drug release can be controlled (Duarah et al., 2019).

Disadvantages

The disadvantages of solid microneedle are like there are some problems associated with solid microneedles in term of biocompatibility. The reuse of silicon microneedle and metal microneedle is generally not possible. Dissolvable and hollow microneedle can deliver drugs are efficiently than solid microneedle (Duarah et al., 2019). By using solid and coated microneedles we can just deliver drugs of lower and definite molecular weight but in hollow microneedles we can deliver a higher amount of drug (Duarah et al., 2019).

1.3.2 Hollow Microneedle

Hollow microneedle is also used to deliver drugs. Hollow microneedle can deliver drug more efficiently than solid microneedles those can only deliver drug of small molecules. MEMS technique is used just to fabricate the hollow microneedles. Bosch process is used to introduce hollow shell structures. For the tip sharpness we can use the wet etching process.

Advantages

The advantages of hollow microneedles are like the working process of hollow microneedle is Known as ‘poke and flow’ in this approach drug is just flown from the reservoir (Bariya et

al., 2012). By using solid and coated microneedles we can just deliver drugs of lower and definite molecular weight but in hollow microneedles we can deliver a higher amount of drug (Duarah et al., 2019). Hollow microneedles can deliver drugs at higher dose. By using hollow microneedles we can deliver drugs at a faster rate. The dose can be easily controlled by using hollow micro needles (Bariya et al., 2012).

Disadvantages

The disadvantages of using hollow microneedle are like hollow microneedles miss the opportunity of introducing dry formulations that can be done by solid microneedle. For hollow microneedle it is important to generate a constant flow rate so that the drugs can be delivered more efficiently without really affecting the needle strength.

1.3.3 Dissolving Microneedle

Dissolving microneedle will dissolve as soon as it comes in contact with skin and after that it will release its all encapsulated drug. The dissolving microneedles can be made from different types of polymers include polysaccharides. They release drug by poke and release method. The fabrication of dissolving microneedle can be done by various methods like hot embossing, ultrasonic welding etc.

Advantages

The advantages of using dissolving microneedle are like. Dissolvable microneedle can deliver drugs at a higher dose than solid microneedle (Bariya et al., 2012). Dissolvable microneedle can deliver some substances at a higher rate (Duarah et al., 2019). In case of dissolving microneedle there is very low chances of remaining any tips that is biohazardous (Duarah et al., 2019). Another advantage of dissolving microneedle is it is easy to use and also self-administrable (Duarah et al., 2019). The polymer microneedles actually dissolve

completely so no tips remain as the residue that is biohazardous (Y. C. Kim, Park, & Prausnitz, 2012).

Disadvantages

The disadvantages of using dissolvable microneedle are likelarge amount of drugs introduction is a limitation for dissolving micro needles (Bariya et al., 2012).The mechanical strength and stability is comparatively lower, Due to increased temperature substantial degradation can occur (Duarah et al., 2019). The microneedle arrays are difficult to storage and processing (Duarah et al., 2019).

1.3.4 Coated MNs

Coated MNs are coated with the drug-containing dispersion and are considered particularly attractive for rapid bolus delivery of high molecular weight molecules such as vaccines, proteins, peptides and DNA to the skin. Once inserted into the skin, the drug is rapidly released from the coating into the tissue. However, one serious limiting factor in attaining a relevant drug release profile is the infinitesimal surface area of the MN structures which leads to the limited extent of drug that can be successfully coated onto them. Besides, there are additional issues of concern such as consistency, uniformity, reproducibility and stability of the MN coating materials. In addition, precautions should be taken such that there is negligible deleterious drug loss from the MN surface during the coating process and also prior to insertion into the skin. Coated MNs have also been demonstrated for the efficacious and minimally-invasive delivery of therapeutic nucleic acids such as small interfering RNA (siRNA) etc. Coated MNs are also particularly attractive candidates for vaccine delivery to the skin, as antigens can be released in the skin to target the Langerhans cells in the epidermis or the dendritic cells in the dermis for a more effective immune response. To demonstrate this, ovalbumin, a model antigen, was coated onto microneedles and delivered to guinea pigs

to induce a pronounced immune response. Furthermore, as only small quantities of antigen are required to elicit an immune response, the restricted quantity of drug that can be coated onto MNs does not actually hamper their utilization in vaccine delivery. In a recent study, drug-coated poly (L-lactic acid) (PLLA) MN arrays were fabricated to induce rapid and painless local anaesthesia in the skin (Duarah et al., 2019).

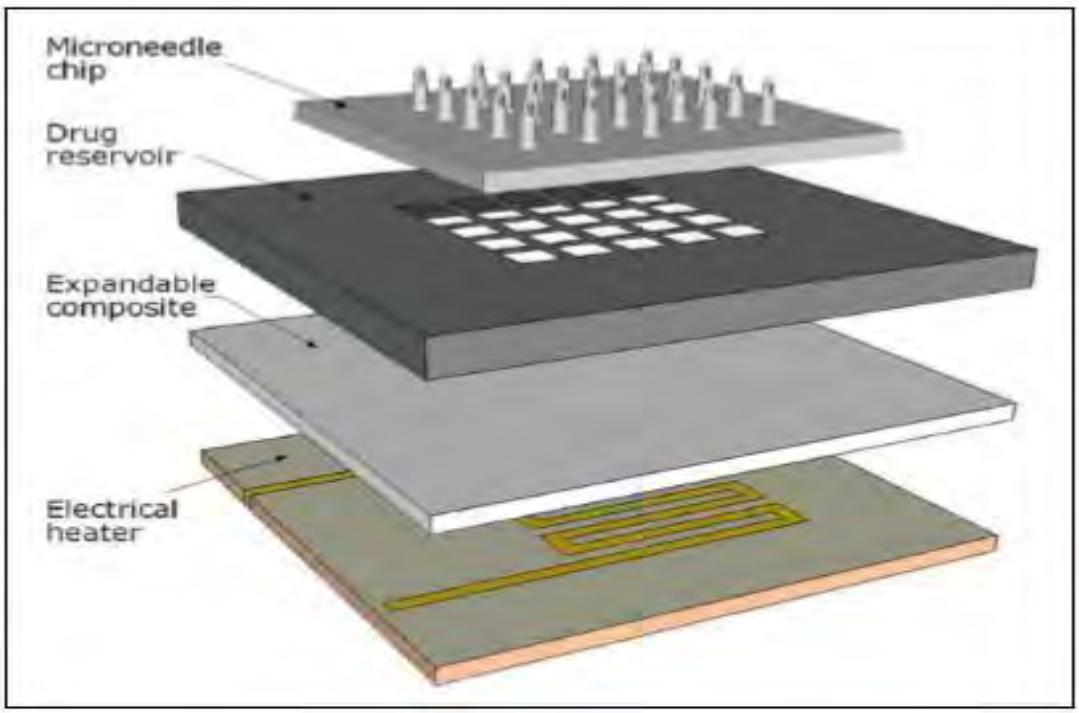

Figure 5: *An overview of MEMS technology (Hultström et al., 2014).*

1.4 Materials Used to Constitute MNs

As we mentioned earlier MNs can be categorized in three categories. First one is solid MNs. Second one is dissolvable MNs. Third one is hollow MNs and lastly coated MNs. The materials that need to be selected to produce microneedles based on some criteria. The first

criteria is that the microneedle that will deliver biomolecules need to be fabricated gently. Secondly, if the microneedle needs to disrupt the skin then sufficient mechanical strength is needed. For those microneedles that require controlled or rapid drug delivery. The materials need to be selected based on this idea. Some materials are used to fabricate microneedles most often. In these materials glass, silicon and materials glass, silicon and metals are used. Polymers are also used to make microneedles. Generally, solid microneedles are made of polymer those are biodegradable. There are some reasons to choose the polymers as materials for production of microneedle. The advantages those polymers have are firstly, the cost of this polymer is very low. Secondly, polymeric microneedles are not so brittle like metal microneedle. Thirdly, polymers are safer because the incident of accidental breakage of needles is very rare. Another type of material like polysaccharides is also used to make microneedles those are dissolvable. Carbohydrates can also be used to fabricate microneedles but they are rarely used because of some limitations.

1.5 Different Types of Release from Microneedle

1.5.1 Poke and Patch

Solid microneedle was first introduced the poke and patch approach. The poke and patch approach is also used by the dissolvable microneedle. In this method microneedles are first applied and then removed. Since a drug formulations that is reserved in a patch is introduced in the skin so the drug can e delivered. It is very important to remain the microspores should remain open at the time of drug application. A disadvantage of this is the skin irritation.

1.5.2 Poke and Release

The second approach of delivering drug from solid microneedles is the poke and release approach differs from the poke and patch approach is that the microneedle should remain in the skin until the drug is released.

1.5.3 Coat and Poke

By using solid microneedles coat and poke approach is used to increase the drugs uptake through the skin. Various types of drugs can be coated on to micronedle. The shortcoming of this approach is microneedles those are solid can be used to coat small amount of drug and the delivery efficiency is not very good. Last of all the challenge that need to be overcome is the relatively high loss of drugs.

1.5.4 Poke and Flow

Hollow microneedles deliver drugs via the “poke and flow” approach. An important benefit of hollow microneedles over solid microneedles is the possibility to facilitate force-driven fluid flow, thereby allowing faster rates of drug delivery. Furthermore, the dose of the desired drug in solution can be more easily controlled according to the need of the patient. This method of drug delivery can be achieved via passive diffusion through the bore of the microneedle. Other methods are also possible whereby the drug in solution is actively delivered through the bore of the microneedle. The latter requires a driving force through pressure (Van Der Maaden et al., 2012).

1.6 Fabrication of Microneedles

1.6.1 Fabrication of Solid MNs

Significant advances has been occurred in the design and microneedle fabrication (Chandrasekaran, Brazzle, & Frazier, 2003). Silicon MNs are the first MNs that are used. Now, different types of micro fabrication technology are used. MEMS technology is one of them. At present MEMS is used to fabricate MNs. Previously it was used to make micrometer or micro pumps. MEMS technology has many operational techniques. Previously micro fabrication technology was very cost effective. But now it has many problems. It has very complex processing steps and special handling is needed. However there are some basic steps for MEMS technology. Firstly the thin films of material are needed to be deposited. Secondly, photolithographic imaging is applied for a patterned mask. Thirdly, etching process of the films is done.

Photolithography imaging is for polymers those are optically curable so that master structures can be made. These master structures will help in future application. Photolithographic imaging is used to apply mask on top of the tip. Etching can be classified in some categories. First one is reactive ion etching. Second one is anisotropic etching. Third one is isotropic etching. Some time isotropic etching and anisotropic etching can be used in combined. There is another type of etching that is called acid etching. Acid etching is used so that the tips can be sharpened. Previously solid MNs were made of silicon but now solid MNs can be made of stainless steel. Also the other metals like titanium and palladium can be used to make solid MNs. Now very recently a technique is developed to make solid MNs. The name of this technique is magnetorheological drawing lithography. This method is very useful for the fabrication of solid MNs. It is so efficient that it is called the novel method for fabricating solid MNs. The mechanism of this technique is very simple. At first in an external magnetic

field a fluid is drawn. The fluid has certain properties like it is magnetorheological. The fluid is drawn from a substrate and is used to make a 3D microneedle. The whole process occurred under an external magnetic field as previously mentioned.

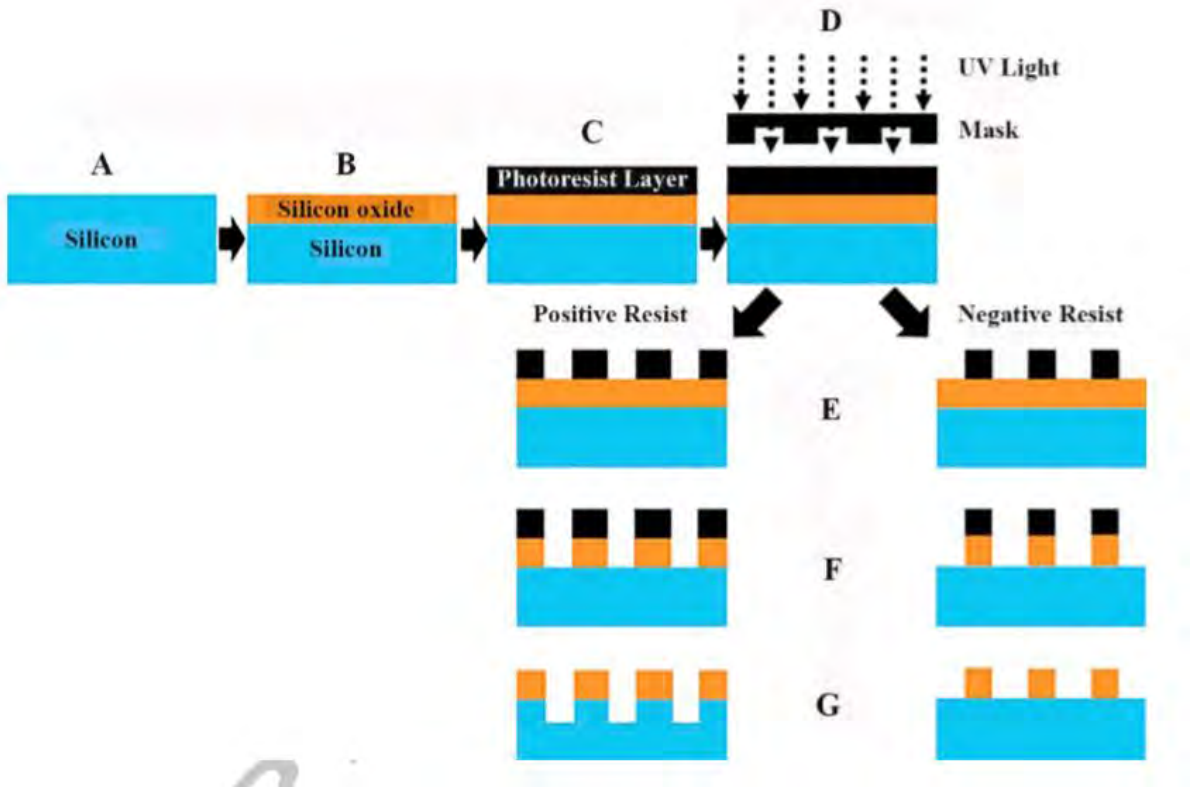

Figure 6: *Processes in fabricating solid MNs (Duarah et al., 2019).*

1.6.2 Fabrication of Hollow Microneedles

Hollow MNs are another type of microneedle. Like solid MNs hollow MNs can also be prepared by using MEMS technology. MEMS technique like laser micro matching is used to make hollow MNs. After that there is a process called bosch process. This process is used to make hollow shell structure that is dip. After that as mentioned previously the etching process is done. Generally there are both chemical and non chemical etching processes. Among the chemical etching process isotropic and wet etching is popular. This isotropic and wet etching is done so that a sharp tip can be gained. There is also a technique to gain sharp tips without

chemical etching. In this process a dicing saw is used to make the sharp tips. Moreover hollow MNs can be made from glass polymer and metal. There is a technique named micro pipette technique that is used to make glass hollow polymer MNs. There are different types of technique to make hollow MNs from polymer. At first the holes are made. After making the holes the tips need to be beveled. After this step the beveled tips need to be molded. For this a special technique is used. The name of this technique is milling. There is another way by which hollow polymeric MNs can be fabricated. In this technique the hollow polymeric MNs are made from the polymerization of liquid resin. In this technique a very specialized instrument is used. The name of this instrument is digital micro mirror stereolithography. Another technique can be use to fabricate hollow MNs. In this technique a process is followed. In this process the direct polymerization of two photons is done to make a hollow polymer MNs. This system is called a prototyping system that is very rapid. This rapid proto typing system is a laser based system. Another type of hollow MNs array are nickel HMNS arrays. This type of microneedle is prepared by a process. These processes are sequential. This hollow nickel microneedle arrays are generally made from the combination of electro less copper and nickel plating. The etching process is done by a chemical etching. The name of this chemical etching is copper wet chemical etching. In this study they presented a fabrication technique that they claimed to be the novel fabrication technology. In this technology it has been presented that this technology has a deep x ray exposure that is vertical. Then, a deep X-ray mask is used (Moon, Lee, Lee, & Kwon, 2005).

1.6.3 Fabrication of Dissolving Microneedles

There are several techniques for fabricating dissolving MNs. These techniques are mould based. Among these techniques are laser machining, hot embossing, microinjection molding and solvent casting. Among these techniques solvent casting is the most popular method. This technique is also very simple. In this technique at first the polymers are dissolved in

appropriate solvents. In the next step it is mould cavities that are used. These mold cavities are used for filling purpose. After that these are kept for drying. After drying the centrifugal forces are added. There are other types of MNs named tapered MNs. These tapered MNs are actually made by fabrication method. To fabricate tapered MNs at first a liquid preparation is made. In the next step the liquid preparation is drawn. Then the liquid preparation is solidified and the tapered MNs are made. The tapered MNs are very useful to coat different types of drugs. The drugs those can be coated in tapered MNs are dextrin, albumin etc. these materials are coated on tapered MNs. For this tapered MNs coating every needle of tapered MNs are fabricated by drawing a liquid preparation. The liquid preparation is drawn by using pipettes. Another technique is called ultrasonic welding. This technique is also used to fabricate hollow MNs. The mechanism of this technique is discussed below. At first the polymers are fused together. The fusion process of these polymers has a condition. The condition is that the polymers need to be fused without the help of any heating process. Actually this process has a benefit like a very less amount of damage is done to the materials that are encapsulated in the dissolving MNs.

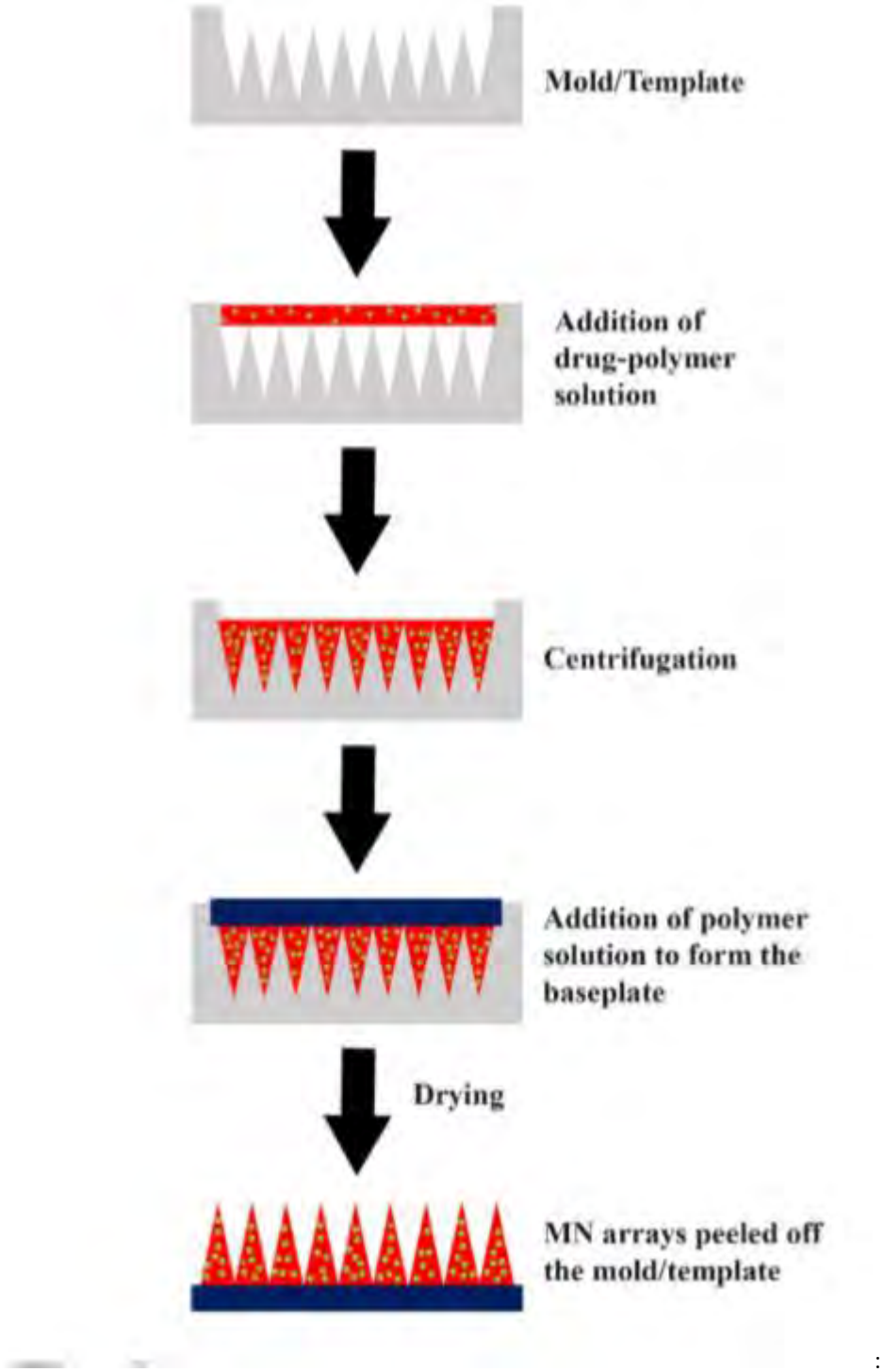

Figure 7: Process in fabricating dissolving MNs (Duarah et al., 2019).

1.7 Vaccine Delivery by Using Microneedle

Microneedles are used for the delivery of vaccine. The vaccines that are delivered through microneedle can be divided into three types. First one is the viral vaccine. Second one is the bacterial vaccine and third one is DNA vaccine.

1.7.1 Virus Vaccine

At present there are four types of viral vaccines those are delivered by using microneedle. They are

1.7.1.1 Live Attenuated Virus

This type of virus is the whole weakened forms of normal viruses. In this form of viruses they can skip the occurrence of diseases. They are also capable of maintaining their immunogenicity. These types of vaccines have some advantages. The immune response is very potent. They can induce this immune response in even a smaller dose. But there is a serious disadvantage of this type of viruses. The disadvantage is that they can come back to their original virulence form. So, that is a tremendous risk for this type of viruses. This generally happens to the patient those have a very weak immune system (Bariya et al., 2012).

1.7.1.2 Inactivated Virus

The vaccines that are made of the inactivated virus can also be delivered by using microneedle. This inactivated virus is mainly remaining in killed form. In addition to that they are well capable of maintaining immunogenicity and they can generate a well immune response. In case of influenza vaccination it has previously seen that at first their immune response is not so good. However, this immune response can be increased with the addition of an adjuvant (Bariya et al., 2012).

1.7.1.3 Subunit Vaccine

Subunit vaccine generally contains a very small portion of diseases containing virus. This small portion of diseases containing virus can act as an antigen (Bariya et al., 2012).

1.7.1.4 Virus like Particles

VLPs (virus like particles) are generally made of the envelope and capsid. The one characteristic of virus like particles is that they cannot make replica of themselves. Generally the viral portions are very active in case of VLPs this portion can create a strong B cell and T cell response (Bariya et al., 2012).

1.7.1.5 Bacterial Vaccines

There are a very good number of bacterial vaccines that can be delivered through microneedle. Generally in this type of vaccine a solution is used. This solution contains an epitope portion of bacterial polysaccharides. Moreover, this solution is intrinsic antigenic. There is a list of bacterial vaccine that can be formulated. These bacterial vaccines can be delivered through microneedle. This type of bacterial vaccines can give protection against the diseases that is fully bacteria created. The list of diseases is quite vast that can be treated by bacterial vaccine. This list includes anthrax, diphtheria, tetanus etc (Bariya et al., 2012).

1.7.1.6 DNA Vaccines

The DNA vaccines those are delivered through microneedle generally delivered in a formulation form. This formulation is coated by vectors of plasmid. These vectors can help to express specific proteins. After that these types of specific proteins give immune response. It has many advantages over conventional vaccine. First of all, it does not contain a whole virus. As a result it has a better safety profile. Secondly, the DNA vaccines can increase vaccine stability. Lastly, a large scale of production is possible (Bariya et al., 2012).

1.8 Successful Coating on Microneedle

1.8.1 Influenza Vaccine

In recent times three different types of influenza vaccines are administered for the preclinical use of microneedle technology. In this study we have witnessed some dose sparing in case of microneedle based drugs. The whole inactivated virus is given in i.d route and it shows 100 fold doses sparing than i.m injection. I.d trivalent human vaccine gives 10 fold doses sparing than i.m injection. In case of DNA plasmid vaccine we have noticed 5 fold doses sparing. And one thing is that in all the cases we have seen the antibody responses are same as the i.d delivery of vaccine (Alarcon, Hartley, Harvey, & Mikszta, 2007).

1.8.2 Insulin

By using the inkjet printing technology we can apply the insulin polymer layers on microneedle for the transdermal delivery of drug. This study showed that inkjet printing is an effective way for i.d delivery of insulin in solid state (Ross, Scoutaris, Lamprou, Mallinson, & Douroumis, 2015). They used a smart insulin patch. This smart patch is actually a synthetic glucose responsive device. In this device a hypoxia trigger is used that will regulate the insulin release. As a result it is very useful in controlling hyperglycemia and hypoglycemia in type 1 diabetes (Cegla, 2015).

1.8.3 Anthrax Vaccine

In this study an antigen of bacillus anthracis that is recombinent is tested in a rabbit and mice model. The result is like 90 percent of sero conversion in case of i.d delivery compared to 20 percent of i.m delivery. It has been seen that In case of rabbits, it is i.d delivery that gave very good protection against spores of anthrax. It is also seen that Combination vaccines help to reduce the overall number of injections that needed for each component that is administered

in a separate manner and usually give the disease protection in a same manner (Tammariello et al., 2008).

1.8.4 Hepatitis C Vaccine

DNA vaccine can be easily made and they can create strong antibody responses. Moreover, there is a common shortcoming of DNA vaccine when it is given intramuscularly is that low immunogenicity. However, in that case gene gun and electroporation for cutaneous immunization has potential but it requires different types of equipment and protocol. That is why there is a need for convenient delivery method for DNA vaccine that can create good cellular responses. Among the various approaches microneedle is also a good approach. This study a DNA plasmid expressingns3/4A can be given through the skin by using microneedle those are coated to elicit CTLs response that are specific for hepatitis C virus. In this study, a hypothesis that is microneedle that are DNA coated can produce a very good CTL cellular immune response that was tested. Furthermore, in this study it is cutaneous DNA that is delivered by using microneedle is compared to (1) by using hypodermic injection i.m DNA delivery, (2) delivery cutaneously by using gene gun. Since, Microneedle are very good delivery system, it has some reasons like it is painless, can be administered by minimally trained personnel etc. Though Gene gun-mediated delivery system has good potency but its clinical and logistical applicability is uncertain. The study also showed that if it is delivered via i.m route then the doses need to be increased by many folds to get the same protection. In addition to that the dose in case of hypodermic rote can be reduced by using adjuvants (Gill, Söderholm, Prausnitz, & Sällberg, 2010).

1.8.5 Delivery of Synthetic Peptide

It is biomolecules like proteins peptides etc are very important part in present pharmaceutical industry. It is preferable to introduce them orally but because of some problems like

enzymatic degradation and first pass metabolism the bioavailability in oral route is low. As a result hypodermic needle is used to administer them. As there are some problems associated with it like pain, infection and the requirement of trained personnel. So, a microneedle is designed that is dissolving microneedle where the drugs are encapsulated can deliver drugs without creating any pain and leaving no biohazardous sharps (Sullivan, Murthy, & Prausnitz, 2008).

1.8.6 Delivery of Dried Virus

Vaccine is very sensitive so continuous refrigeration is needed. Although the vast majority of vaccines have given through hypodermic needles it is very beneficial to coat them in microneedle. In this study the methods of coating thelive recombinant adenovirus and Modified Vaccinia virus Ankara (MVA) vectors into the solid microneedle arrays. The result is that coating of the live viruses and administration of it was successful and the virus delivery was also very successful and it was also comparable to the conventional use of drug delivery by using the syringes and needles (Vrdoljak et al., 2012).

1.8.7 Protein Delivery

It is biomolecules like proteins peptides etc are very important part in present pharmaceutical industry. It is preferable to introduce them orally but because of some problems like enzymatic degradation and first pass metabolism the bioavailabilty in oral route is low. As a result hypodermic needle is used to administer them. As there are some problems associated with it like pain, infection and the requirement of trained personnel. So, a microneedle is designed that is dissolving microneedle where the drugs are encapsulated can deliver drugs without creating any pain and leaving no biohazardous sharps (Sullivan et al., 2008).

1.8.8 Anticancer Agents

Three types of anticancer agents 5-fluororacil, curcumin and cisplatin has been coated by ink jet printing technology via transdermal route. Soluplus1 that was a hydrophilic graft copolymer was used as a carrier of drug and the coating solution was the drug polymer solutions. In this study it was found that inkjet printing technology was very beneficial for coating on metal microneedle and it is very effective in delivering the drug via transdermal route (Chowdhry et al., 2015). In this study they presented a sustained delivery of anti-PD-1 (aPD1) by using a microneedle patch. The microneedle is made of nanoparticles those are biocompatible and combined with hyaluronic acid. Now, aPD1 and glucose is encapsulated on them. In the next step that is actually converted to gluconic acid. They found that this type of microneedle shows a strong immune response and it can be combined with other strategies to get a great anti tumor efficacy (Sadeghifar, Gu, Ye, Wang, & Hochu, 2016).

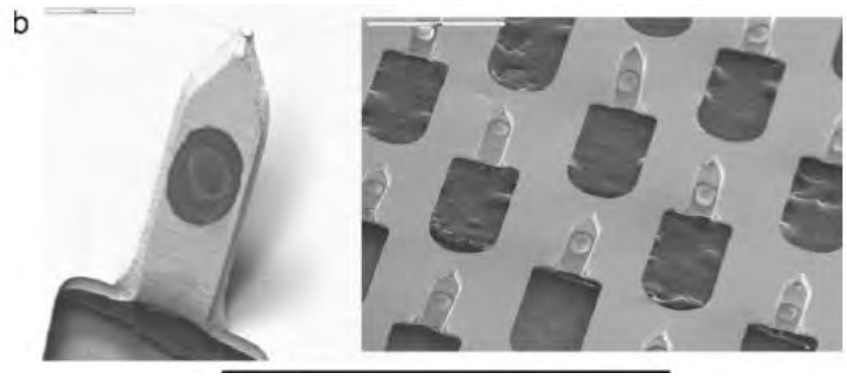

Figure 8: *Inkjet printing of MNs (Chowdhry et al., 2015).*

1.8.9 Measles

It is very beneficial for the vaccines like measles can be given by new delivery system like microneedle. As conventional subcutaneous injection can have some problem like needle stick injury, infection, pain etc, so it will be beneficial to use microneedle. In this study microneedle is used to deliver the measles vaccine so that the shortcomings of hypodermic needles can be overcome. In this study it was found that measles vaccine can successfully be

coated upon microneedle and it can produce same kind of antibody responses that is actually generated by subcutaneous injection (Collins, Ayers, Edens, Prausnitz, & Rota, 2012).

1.8.10 Ebola Vaccination

As we all know that there are some problems associated with hypodermic needle and subcutaneous injection like pain, needle stick injury, need of trained professionals etc are also applicable in case of measles vaccination. In this study measles vaccine is coated in a microneedle patch. It is found that microneedle based measles vaccination is a potential approach for future (Yang et al., 2017).

1.9 Drugs Given by Using Microneedle

In this study a rapidly dissolvable MN patch is made that can deliver exenatide very efficiently. It also can be useful for the delivery of other biotheraputics (Zhu et al., 2014). Desmoprossin is used in the treatement of enerusis in children. They can be delivered in different routes. But there bioavailabilty is very low. So this drug is delivered by using microneedle. This microneedle based delivery of desmoprossin has increased the bioavailability of desmoprossin (Cormier et al., 2004).

Mithotrixate is the drug that is inserted successfully. In this process biodegradable microneedle is used. By using microneedle delivered mithotrixate it has been seen that there is no evidence of toxicity. Moreover, there is not any inflammation also (Palakurthi, Correa, Augsburger, & Banerjee,2011).

1.10 Patient compliance

1.10.1 Pain

Pain is not safety concern and does not affect the patient compliance. In some initial studies we have seen that microneedle length from 50-200 mm and with 400 arrays of micro needles

and they are generally painless (Kaushik et al. 2001; Mikszta et al. 2002). Then again in some studies we have noticed that the pain is mainly associated with the length of microneedle. Again, tip angle, thickness, width do not have any correlation with pain. In all cases has found that microneedle creates less amount of pain than the hypodermic needle (Laurent et al. 2007). Microneedle with a length of a few hundred micrometers, only penetrates the superficial layers of the skin where the density of nerve receptors is low. As a consequence, insertion of microneedle into skin is perceived as painless (Shakeel et al., 2011). A table has been given below to see the relation between microneedle and pain

Table 1: *Relation between pain and microneedle (Duarah, Sharma, & Wen, 2019).*

MNS material /drug	Lengths of MNs(µm)	Comment
Silicon	150	painless
Stainless steel	200 and 400	painless
silicon	180 and 280	Lower pain
Stainless steel	620	One fourth pain of the hypodermic needle
Silicon	200	painless
Borosilicon glass	1	Less painful
Silicon/influenza vaccine	450	Swelling observed
Insulin / borosilicate glass	500,750,1mm and 4 mm	Painless if volume is more than 1mm then pain is caused

1.10.2 Infection

Because of the contaminated needles the risks of infection mainly occur. But in this case microneedle is considered as a safer option tan hypodermic needle. Most microorganisms generally found in the upper layers of the skin. So those microneedle arrays that contain

thousands of microneedle can be problematic. On the other hand, it is clinically proven that the infection caused by microneedle is minimal. In case of human studies it is found that no infection occurred due to microneedle is reported and no serious adverse effect occurred (Adams et al. 2005; Barker and Ryan 1995).

1.10.3 Bleeding

This is microneedle which have are needles those are very small in size and used for the delivery of drugs (Ma & Wu, 2017). First of all there is no vasculature in the upper layer of skin. The capillary those are superficialis generally placed above the dermis. So the microneedle whose length is 100 nm can cause bleeding. Generally in the experiments those were done to see whether MNs create bleeding or not showed that MNs generally are not associated with bleeding. However, in the study with 150 mm microneedle can produce pinpoint bleeding, and 600 mm microneedle can cause significant bleeding. But in case of human subjects bleeding can occur for microneedle that ranges from 500-100 mm length. However, for solid microneedle it is reported that 1.5 mm length can cause sometime bleeding or injection where hollow microneedle is used (Gill et al. 2008).

1.10.4 Skin Irritation

No noticeable skin irritation has occurred in case of using microneedle in human. However, in some experiments some localized red spots have seen but that generally is not a matter of concern. In a recent study it has been seen that by using 1.5 mm length microneedle can cause some sort of irritation but that generally passes away after some minutes (Laurent et al. 2007).

1.11 Application of Microneedle

The use of microneedle in the delivery of different agents is vast.Microneedle with different virus coated is in under development (Larrañeta, McCrudden,Courtenay,&Donnelly,2016).

1.11.1 Immunobiologicals

Generally the immunobiologicals are delivered via the skin. In this purpose generally the hypodermic needles were used. Before the use of micro needles immune biologics are generally delivered in intra muscular or subcutaneous route. As we all know using hypodermic needles have many drawbacks. The main problem is the pain that is created by the hypodermic needle. The problem of the hypodermic needle is needle phobia. On the other hand microneedles have various advantages. The first advantage is the lack of pain. Generally microneedle creates very less pain or no pain. Second advantage is that microneedles are self-administrable so no extra person is needed. The other advantage of microneedle is drugs can be rapidly delivered. Another advantage of microneedle is generally the conventional vaccines require cold conditions to store them and this liquid vaccines have a very short half-life. On the other hand the vaccines those are coated into microneedle also have the ability to cross the upper layer of skin that is stratum corneum and give clinical response. It has been seen in different studies that microneedle can create a better immune response at lower dose than the hypodermic needles.

1.11.2 Bioactive Macromolecules

Generally insulin, heparin and growth hormone is considered as bioactive materials. Generally the bioactive materials are delivered through the parental route. This parenteral route delivery has some limitations. As a result a method of delivering bioactive materials is needed that is noninvasive and that Can deliver bioactive macromolecules.

1.11.3 Drugs

Very few drugs have the ability to cross the skin barrier and show its action. Actually the main reason behind that is the lack of physicochemical properties that are needed to deliver dugs via the skin. The physicochemical properties are the hydrophilic lipophillic balance, the solubility and finally the molecular weight. All these challenges can be overcome by using microneedle.

1.11.4 Phlebotomy

Phelbetomy is the process by which blood samples can be withdrawn. This withdrawal blood sample can be used in the diagnosis process. A microneedle named hollow microneedle is used in this purpose. Diabetes is the proper example of phelbetomy. In diabetes the blood sample is collected and after that the glucose amount is measured. In phelbetomy using of microneedle is very helpful because microneedle can reach the depth that is required for the purpose of collecting the blood sample and it does not cause too much pain.

1.11.5 Additional Uses

Additionally microneedle can be used in cosmetics products delivery. The reason behind that is only a very limited portion of cosmetics can reach through the skin. Microneedle has also proven to be useful for many types of skin problems like scarring (Bs & Elbuluk, 2015).

1.12 Safety Issues in Microneedle

Most of the studies found that microneedle are very safe to use. However, the safety issues of microneedle are a great concern because it can create microchannels by which the drug is delivered. As micro needles disrupt the skin so the safety issues need to be monitored.

1.13 Commercial and Regulatory Status of Microneedle

At first microneedle were used to deliver drugs and many materials. Influenza is the first vaccine that is actually delivered by using microneedle. This influenza vaccine is first marketed in Australia, Canada and Europe.

Very recently USFDA has approved the coated microneedle for drug delivery. Although many of the pharmaceuticals are involved in making microneedle related products but only a few are marketed. However, a very strong specific guideline needs to be prepared for microneedle related products (Bs & Elbuluk, 2015).

1.14 Ocular Drug Delivery by Microneedle

Human eyes are very sensitive. If the intra ocular pressure is increased then the glaucoma is caused. This can cause blindness (Yadav, Rajpurohit, & Sharma, 2019)**.** The needles of the microneedle need to be strong enough to administer drugs in the eye (Lee et al., 2013). Microneedle is an emerging tissue for delivering drugs in the posterior segment of eye (Ashaben Patel, Kishore Cholkar, Vibhuti Agrahari, 2013). Microneedle has a significant potential in delivering drugs into the eye. Micronedle can penetrate but not through the sclera or cornea. As a result it is very safe to use (Khandan,Kahook,&Rao,2016).

Chapter 2

Methodology

I have been searched renowned journals, published research papers and different kinds of research databases like Pub med, Science Direct, Academic Search, and Web of Science etc. I have gone through all the articles those I have collected for writing a review on microneedle technology. This microneedle technology can create a great change in vaccination sectors. I have gone through around 100 articles and collected information from around 55 articles to know about this revolutionary technology and after that assembled them. Here I have summarized some of the methods that were used in different articles.

There are arrays of three microneedle exhibits utilized in this examination. The first is microneedle and they are collective and those have different needle lengths. Another type is made of steel and it is stainless, and finally the third one is microneedle arrays but they are hollow. Then the mice were anesthetized and the mice were pierced by the microneedle arrays. After that the in vivo assessment of the penetrating is estimated promptly by the estimation of the Trans epidermal water loss. At that point the mice were immunized by the dermal diphtheria and flu antibody. At that point antibodies those can neutralize the toxin of the diphtheria in mouse sera were surveyed by utilizing a Vero cell test. The antibody profiles were checked. After that the Hem agglutination inhibition (HI) examine was finished (Ding et al., 2009).

In this work the solid microneedle were made of the polylytic acid particles. These were biodegradable. A material named polyvinyl acid was given so that the concentration of the solution can be controlled. For this reason the solution that uses for coating sticks to the microneedle surface. It was Sucrose that given so that the vaccines remain unlikely to change. A drug used and it was a model drug (Y. Chen, Chen, Wang, Jin, & Guo, 2017).

Firstly, a process named dip coating is introduced to make polymer that is coated. The PLA microneedle was in the horizontal plate. The reservoir is filled with the coating solution. Secondly, the MNs those were made of polylytic acid were taken down by the use of a holder that is portable (Y. Chen et al., 2017).

Now, the MNs those are made of poly lytic acid are submerged in the coating solution. The solution of drug coated onto the surface of the needles. At last, the portable holder was moved up and the coated polymer MNs got removed from the horizontal plate. A dynometer was used to do the neanical property test of the solid microneedle. The coated polymer microneedle were inserted to evaluate the efficiency of the transdermal drug delivery. Just before the test the skin was pre treated in a well ventilated location, coated polymer MNs those were dried were vertically pressed and kept for 2 min. After that, the MNs were peeled off from the skin and the skin was observed by using a stereomicroscope. It is the drug loading and high drug delivery efficiency those were crucial factors to determine the practical application of the coated polymer MNs. As a result the drug delivery and drug loading efficiency was measured. After that the coating microneedle were fabricated. Then the in vivo drug delivery process was measured (Gill, Söderholm, Prausnitz, & Sällberg, 2010).

To begin with, the plasmid coated microneedle was designed so that they can be penetrated constantly. Then the fresh microneedles are coated with DNA. In addition to that the coated microneedle have very good delivery efficiency. A cellular immune response is created as a result of mconeedles released coated DNA. We have seen that the intramuscular injection without adjuvant cannot be so efficient. Again, this study was used to assess the potential of DNA coated microneedle to induce a CTL response and then compare it with gene gun and also with intramuscular routes. It is very important to note that mice those were immunized by microneedle actually receive much less DNA in comparison to the intramuscularly injected mice. Lastly, it has been seen that to get the same level of protection by the

intramuscular injection a higher dose is needed but it can be reduced to a large extent by adding an adjuvant (Bouwstra et al., 2017).

At first mice were collected. They were kept in a good condition, microneedle those were hollow were fabricated. A system that was controlled digitally was developed. The accuracy of this digitally controlled system was validated. A peptide containing liposomes was prepared. The particle size of that formulation was calculated. Some antigen specific responses were measured. Other responses were also determined and finally the analysis that can be done by using statistics were done (Alarcon et al., 2007).

At first the rats were collected and they were given anesthetics. An injection was given. Influenza virus was also given. After that a DNA but known as plasmid prepared. All the hair was removed from the injection site. The places where the injection will give were marked. Homogenization was done for different samples. The centrifugation was also done. The upper layers that are found from the centrifugation were assayed. The rats were injected by whole vaccine but it was inactivated. Process that was used for DNA immunization was done. The titters those were found at the last point their calculation was also done. At last the analysis that was performed by using statistics was done. At first the rats were anesthetized by intra peritoneal injection that contains ketamine, the influenza virus that was inactivated was prepared. After that the labeling of the whole virus is done. The molecules those were not bound were removed. Microneedle those are known as coating microneedle was made. After that the vaccination was done and antibody samples were collected. The samples those were taken from the lungs analysis of them were done. At last the analysis that were done by using statistics (Y. Ki.m et al., 2009).

Firstly, the measles vaccine was made. The stability tests were performed. Micro needles fabrication was performed. The study that was known as immunization study was performed.

The antibody titers were collected. At last the analysis that were done by using statistics. At first the live attenuated measles vaccine is prepared and then the vaccine stability tests are done. Here, microneedle that are made of stainless steel were fabricated for that at first the shape of the microneedle is defined and after that the microneedle need to etch in chemical bath. After that the immunization studies of measles vaccination is done on cotton rat Immunization. Neutralizing antibody measurement the standard plaque reduction neutralization assay is used to determine the Measles neutralizing antibody titers in serum samples that are obtained from the cotton rats. Lastly, all the statistical analysis is done (Collins et al., 2012).

At first the rabbits were collected. Then the sample which was rabbits was immunized. The blood sample needs to be taken. All the necessary injections were given. The concentration of the sample was taken. The titer that is used to neutralize the toxins is measured. All the analysis by using statistics was performed. To predict sample concentrations from the calibration curve a four parameter logistic curve was used. Then, titers of toxin neutralizing antibody were determined. T test was done to compare the antibody titers between the groups. The Values that were reported represent two-tailed P values. Last of all, logistic regression was used to determine the link between d56 anti-PA antibody levels and the survival was determined by the logistic regression (Mikszta et al., 2006).

In this study the microneedle arrays were used are 4 by 4structures and are conical and they have an internal conical cavity. In addition to that they are fabricated by using injection that is molding of a polymer that is biocompatible. The actual length of the needles is 900mm and the base diameter is 600 m. The tip diameter is 60m.There is an internal cavity. The hole is situated on the side so that the fluid is allowed to exit from the cavity. In the initial step of fabrication, a partially hollow microneedle is got. In a second step, post-processing is required to open the fluidic channels into the needle wall. Here, the microneedle array is

inserted through the eight membrane layers by the help of a linear motor. The MNs array is removed unfolded after insertion. Careful attention need to be paid to ensure that the membrane is effectively ruptured because the deformation of the skin can be caused due to the microneedle pressure. At first the insertion test is done in order to measure the precision of the force measurements. After that the microneedle array is projected against the Parafilm.Same kind of tests were done against an aluminium block (Lhernould et al., 2015).

The solid microneedle arrays were fabricated. The needles structures were made by cutting with the help of infrared laser. The needles were cut from the stainless steel sheets. The needles were bent manually. Then the needles were electropolished so that the debris can be removed. Polymers were dissolved. For disolvation, deionized water is used. The disolvation process was done prior to the addition of drug molecule. Uniform coating layers of metallic MNs arrays were produced. Then the three anticancer drugs were used. NanoploterII was used for the inkjet printing. Before that the skin is prepared. The main purpose of the skin preparation is the in vitro MNs testing. The skin is excised from pig's abdomen. After that the skin is modified to required thickness. HPLC analysis was done to measure the samples. Among the samples there were 5-FU and CRC. The CRC analysis was done. To do this CRC analysis the mobile phase that was selected was consisted of water, methanol, acetonitrile and acetic acid. Then the injection volume was measured. After that the absorbance was measured. HPLC analysis was done to measure drugs those were free. Finally for the CPT the atomic absorption spectroscopic analysis was done (Chowdhry et al., 2015).

Chapter 3

Discussions

In this study the rats were immunized by the whole inactivated influenza virus both in i.d route and also in i.m route. The vaccine is delivered by microneedle in i.d route and the vaccine is delivered by hypodermic needle in i.m route. Then, the immune response for the both route is measured. The titer does not reduce significantly in case of i.d route for various doses. On the other hand the titer reduces significantly where dose varies for i.m route. Here, in this study the antibody responses for H1N1, H3N2, and B strains of influenza is measured. The antibody responses are measured. At first the serum antibody response is measured by HAI and ELISA. In case i.d for H1N1 strain dropped less markedly than i.m injection for different doses. Again, immunization is done with high dose of vaccine against the H1N1 strain regardless of method of administration. Also the i.d route is proved to be better because no animals were immunized by the i.m injection while the number of immunized animals in case of i.d route is much higher. However, there is less difference between i.d and i.m route for both of the H3N2 and B strain. In ELISA it was found that dose sparring can occur if the vaccine is delivered in i.d route. To check the feasibility of using plasmid DNA the i.d injection has provide to be more convenient than the i.m injection. For, DNA vaccine dose sparring is apparent in case of i.d vaccine. Hence, we also found clear evidence of dose sparring in case of all of the virus strain (Alarcon et al., 2007).

To see whether the microneedle gives proper lytic cellular immune response or not mice were vaccinated. The mice were vaccinated by 8mg DNA through the microneedle and 4 mg DNA was delivered by gene gun. NS3-specific CTLs was delivered by microneedle to see that microneeedle that is coated with hepatitis c virus NS3/4A protein could give a lytic cellular immune response or not. The results of this study show that in case of microneedle the cell

lysis is significantly high. The cellular immune response in microneedle is comparable to the gene gun based immune response. Moreover, the group that was injected intramuscularly showed a very good CTLs response. Though the immune response is comparable but the instrumentation is complex in gene gun than the microneedle. The microneedle is very simple to use. On the other hand, tumor expansion was significantly repressed in mice those were treated with microneedle that is plasmid coated. Tumor enlargement was also repressed in comparison to other mice. Altogether, this outcome shows that microneedle vaccination introduces NS3-specific CTLs that are functional and eradicate an tumor expressing factor. Importantly, the mice that were immunized by microneedle got less DNA and the mice that were injected intra muscularly got more DNA. The microneedle based immunization was more effective.

This study was done because better immunization is needed in opposition to influenza particularly also with the other diseases. The main aim of this study was to see that the microneedle based immunization were more effective or not than the conventional hypodermic needle based immunization. The study shows us that microneedle based immunization is better than any other hypodermic based immunization. More specifically, this study showed that immunization by using microneedle could stimulate robust immune responses. It also can give full protection. Other options for immunization were also been reported. Interestingly, the immune response that we got from MNs immunization is compared to the immune response that we got from immunization. The result was different for MNs and immune response. This result can help in making new vaccines. Microneedle based immunization can give significant immune response at low doses. The immune response in case of immunization is dose related. In comparison to that in case of antibody response the result is different in case of mm than the i.m injection. Microneedle can give strong immune response even in a low dose. The MNs vaccination has some more advantages

than the immunization. First, MNs can introduce antigen to the skin mainly in the epidermis and also in the dermis layer those are upper. For that reason dose sparring is the main benefit of MNs. Another benefit is that MNs are really very simple to use. In addition to that these arrays are self-administrable. Another point is that MNs are painless. We have seen that delivery by microneedle needed a significant less antigen for inducing antibody responses in comparison to i.m injection. However, this effect that caused dose sparring vanished and the survival ratio of the animals is not so after aerosol spore challenge. The results of this study showed that the protection of i.d delivery is better than the protection that is caused by i.m injection for anthrax immunization. It also shows that i.d delivery is better than the i.m injection by using conventional delivery system. The study also recommends for clinical evaluation to use i.d drug delivery instead of i.m delivery for anthrax immunization (Mikszta et al., 2006).

The MNs needed the coating solution to be optimized. That will help in uniform coating and to avoid the formation of thick layers. The coating solution was optimized because three type of anticancer agents need to be coated. The three types of anti cancer agents were CRC, 5FU and CPT. For inkjet printing the concentration of the coating solution need to be higher. The reason behind that was to creating small particles and to avoid nozzle blockage. However, a washing step is required because continuous jetting can cause nozzle blockade. The jetting efficiency actually depends upon the size of nozzle, applied voltage and also pulse duration. For small MNs arrays the nozzle need to be kept at 45º angle between the nozzle axis and the MNS array plane. The distance between the nozzle and the surface of microneedle is very important because it can affect the quality of coating. If this can be maintained then the coating will appear smooth and uniform. In addition to that inkjet printing has some advantages like it can form thin layers instead of the thick one. The other advantage includes targeting of the microneedle surface with accuracy and also droplet printing. In this study the

anti-proliferative action of the 5FU, CRC and CPT is measured. The potentiality is higher for CRC followed by CPT and 5FU. The release pattern OF 5-FU is similar but rates are lower due to higher water solubility. SOL can be used as a drug carrier because it can cause increased solubility (Chowdhry et al., 2015).

To begin with there has been seen some advances in the stabilization process in live viral vectors that will help in the making of microneedle devices. The micro needles are coated with dried live viral vectors. It is microneedle that helps the vaccination programme to rich in its full potential. The actual goal of this study is to make microneedle that contain dried live viral vectors. Furthermore the spray coating is very much important for pharmaceutical sectors because of its scalability and simplicity. The focus of this study is whether spray coating can be used to coat the vaccines that are coating sensitive in the microneedle arrays. On the other hand there is also a secondary objective that is to know how the coating solution affects the viability of the vaccine that is coated in microneedle arrays. In this study it is found that we can use spraying as a better technique superior for formulation dropping microneedle arrays. Here, it is found that spraying is better for the coating for some reasons like the formulation remain very viscous round the microneedle. Another reason is that no materials will get deposited in the needle space. On the other hand by the use of the surfactant just below their critical concentration lipid enveloped virus can be made. Again, it has not very good effect on virus containing protein capsid. Another thing is the rate of spraying that need to be optimized. It is found that the rate of spraying has different impact on the coated ADV and MVA virus's viability. The reason behind that is both of the viruses have different susceptibilities in case of air liquid surfaces exposure. Another factor is the solution dehydration rate. It is found that ADV is more vulnerable in case of air interface. In this case the envelop of the MVA acts as a protective layer. Additionally, the rapid dehydration is occurred due to the slower rate of spraying. Furthermore, the substrate of the surface does not

seem to have any effect on the survival of ADV and MVA. It is also found that the delivery efficiency is very important to microneedle in comparison to the conventional needle. The array design can affect the drug loading efficiency (Vrdoljak et al., 2012).

First of all, the main purpose of this study is to see that the protein antigen that is delivered by microneedle can create immune response efficiently or not. For this reason the mice is immunized by using BSA coated MNs. The result is then compared with the injection that is given intramuscularly. The antibody responses by the i.m injection are minimal in contrast to that the antibody responses by MNs are much higher. The antibody responses are also different. The results of this study shows that delivery by using MNs can reduce IIV immunogenicity and a compensatory enhancement is seen in case of i.d delivery. The post challenge parameters are quite similar in both cases. The results of this study shows that target vaccine can be coated and delivered by using MNS quite efficiently. Moreover vaccination by using MNs has some advantages. Firstly, strong antibody responses can be induced. Secondly, it will give protection against high lethal dose challenge. Thirdly, the given protection is really very effective. MNs are very useful for intradermal vaccination. There are some reasons behind that. Firstly, we can coat vaccine in a dry state so no reconstitution is needed. Secondly, vaccine can be delivered into patches and it is very simple to use. Lastly, vaccine stability is increased. Now in the results of these studies shows that higher level of antibody responses are seen in case of i.d delivery than i.m injection for the delivery of BSA. The results also show that in i.m vaccination reactivity to internal protein e.g NP and m1 is seen. It is also seen that the antibody response to A/WSN strain for i.m vaccination is much higher than the i.d vaccination. The possible reason could be the increased reactivity to the protein those are internal (Gill et al., 2009).

The actual objective of this study was to check the ability of microneedle to see whether the microneedle can disrupt the barrier of the skin and also to determine the safety issue of

microneedle in case of irritation and pain sensation. For these purpose microneedle of different length diameter and shape is used. First of all we have seen a significant difference in case of microneedle lengths but having the same shape. In this study we found that the shape is a very important parameter for the disruption of the skin barrier. It is also mentioned in this study that microneedle are developed to reduce the pain and discomfort that is caused due to the conventional vaccination. To assess this in this study it is found that for maximum of the volunteer's microneedle did not cause pain or discomfort irrespective of the needle length or shape. Again it is found that pain can be subjective so here we found that two volunteers found the microneedle a little bit uncomfortable. On the other hand it has also been seen in this study that whether the microneedle causes any irritation or not in terms of length and shape. It has also been found that the level of irritation is not directly depending on the microneedle lengths. Again, it is also found that the microneedle shape has a clear relation with the irritation. However, in this study we have seen some differences in case of assembled microneedle and solid microneedle. It has been seen that the solid microneedle creates more irritation than the assembled microneedle. The sold microneedle can cause more skin disruption but this irritation can be good as it can be helpful for good immune response. The aim of this study was to obtain insight into the ability of microneedle to disrupt the (Bal, Caussin, Pavel, & Bouwstra, 2008).

According to the literature a dissolvable MNs has some advantages. Firstly, abroad range of drug can be given by this. Secondly, it is also applicable for the sustained release of drug. Now, there is some special fabrication technology for fabricating MNs. It needs to keep in mind that the dissolving microneedle needs to design in such a way that it can meet the regulatory aspect alongside with its own superiority than the other methods. Again, according to this experiment a manufacturing method will be useful if it meet certain criteria. Some of the examples of this criteria is it need to be applicable for specific material those are

dissolvable and the fabrication capability need to be reproducible. Now, many of these requirements can be solved by a technique named spin-casting technique. This technique has some advantages. Firstly, it is cost effective. Secondly, it can be used for a broad range of material. Thirdly, it can be processed in low temperature. However, the techniques that are used for the fabrication of master molds have some limitations like they are applicable for only some materials, e.g. silicon, silicon dioxide etc, also it is also mentioned that they are also applicable for only some fixed geometries fabrication. Now for these shortcomings mechanical micromilling is suggested as an alternative for fabricating master molds. There are some advantages of this technology like its capacity to create any micro scale geometry, low unit cost, shorter lead-time for fabrication and metals, most polymers, compositor ceramics can be processed through it so this study found micromilling as a very good alternative to photolithographic processes. A very useful advantage of it is like it has no post process method. Micromilling is very helpful in this purpose. Finally reproducibility of the dissolvable MNs are very important for the regulatory approval and clinical adaptation (Korkmaz et al., 2013).

First of all the quality of coating depends on some factors. Firstly it depends on the dispensary size. Secondly it also depends on the applied voltage. Lastly, the duration of pulse is a factor in which the coating quality depends. The microneedle those generally have smaller tips they cannot dispense so viscous solutions as it requires solution with less concentration. It has also been proved that the droplet size is just the linear function of the voltage. If the voltage increases the diameter of the droplets will also increase. However, it has some problems like it can cause splashing. 100v and 60µs is the best voltage and duration. As it was previously mentioned that the distance of the nozzle from the microneedle is very important so the microneedle need to be positioned at 45º relative to the dispenser. In this article it is also mentioned that one of the advantages of ink jet printing is like, low

material losses, the accuracy is high, reproducibility etc. However, the inkjet printing can apply solvent coatings. The toughness of the polymeric film can be investigated by AFM and also the uniformity. Generally, the studies showed that smooth polymeric surfaces can be produced by the ink jet printing but when the insulin was added the printed layers become rougher. However, the coating should be smooth enough. Finally, the AFM analysis is a good agreement for the smooth layers. CD analysis is a technique and it is spectroscopic that provides information about denaturation and also the transformation of helix of insulin. However, in this study it has been seen that in the CD evaluation there is no significant change in the helixes. In contrast, there is a significant change observed in the POX coatings. This can be done by the interaction of the insulin and polymers. This type of interaction can cause some kind of conformational changes. Franz cell diffusion can be used to determine which drug stabilizer will give the most rapid delivery. By using this insulin delivery can be estimated. According to this study it is SOL that has the highest release profile. In addition to that the GLN and SOL dissolve very quickly. For this reason the insulin can be delivered quickly. In comparison to GLN and SOL the POX have really a very poor release profile (Ross et al., 2015).

The cornea is responsible for almost 80 percent of the refractive power of the eye. Eye drops and topical drug formulation are some conventional drug delivery approaches for eye treatment. The tissue concentrations of eye generally depend on many factors. Epithelium barrier, tear turnover are the two main factors of them. The dose that is given in the eye can be quickly washed away by these factors. MNs for ophthalmic use can be classified into two types. First one is solid and other one is hollow microneedle. There are some polymers like PVA and PVP. These can be used in making polymeric MNs. These polymeric MNs have some advantages like they are transparent and easy to see. The second one is they are dissolvable rapidly. As a result the drug can be delivered at a faster rate. For polymeric MNs

the mechanical strength completely depends on the concentration of polymer. Based on this concentration the microneedle can be hard or soft. So this article recommends an optimum concentration of polymer that is 15 %w/v (Bhatnagar et al., 2018).

Besilifloxacin is an antibacterial agent. This article stated that the wastage of dose of besilifloxacin can be minimized by coating the drug in polymeric MNs. These MNs can rapidly dissolve and we can find besilifloxacin in a free drug form. Finally the literature concluded that MN arrays can be used to increase the concentration of drug in cornea. Sealed hollow microneedle have several advantages. There are some opening techniques for sealed hollow MNs. The microneedle membrane needs to be strong enough to endure the forces that were given during the insertion period. From the result of the study we found that gold membrane is relatively weak. There are some tests like burst tests that result shows membrane can endure high pressure. The result of insertion test shows that while the needles are about to penetrate the membranes break down. This means if the needle is not properly inserted then the membrane will be found intact and no delivery of drug will occur. The gold is used in this study as a membrane for the microneedle. Using gold can have some problem. Firstly, the biocompatibility is a major concern. Secondly it is used in different biomedical application (Roxhed, Griss, & Stemme, 2008).

Cosmetics, vaccines etc can be induced by other MNs instead of injection. This literature shows that the needle length of the micrineedles needs to be minimized because if the needle is larger in length then the needle can be deformed. In this study a new microneedle is developed that can be activated by NIR. This delivery system can deliver drug precisely and accurately thus it is comparable to the ideal drug delivery system. The study shows that the new delivery system can successfully suppress the tumor growth. It has also been seen that the recurrence of tumor is not occurred (M. C. Chen, Lin, & Ling, 2016).

In situ implant forming system remains as a solution under normal condition. It can be converted to gel if it is exposed to the stimulus that is external. There are some factors those are responsible for this conversion. They are dependent on temperature or in ph. The presence of ions, phase conversion, enzymatic and photo cross linking are also the factors those work as the external stimulus. According to this article poloxemers are used for the first time for drug delivery. Poloxemers are triblock copolymer in nature. In normal condition they are generally liquid but they can convert to gel just above the temperature that is needed for gelation. Gel formation actually completely depends on the concentration of poloxemer. They can be used in the eye treatment because they have some really good properties like solubility, opacity etc. the problem that is associated with rapid transition can be overcome by using two polymers of different molecular weights. However, one problem of intravitreal injection is the occurrence of pain. In this literature to overcome this pain a device named HMNS device is prescribed. This study presented the ability of HMNS device to localize the gel system. Now this literature shows a noninvasive method is found to apply implant forming gels. Sustain drug delivery also can be obtained by varying the needle depth penetration. This article claimed that more investigations are needed to know the effect of HMNS device. Finally, this article clai.ms that less sclera damage is done by HMNS device and it is less painful than the other intravitreal injection but further investigations are needed (McMillan, Thakur, Donnelly, Fallows, & Jones, 2013).

Chapter 4

Future directions

Vaccine delivery by using the conventional needles can cause problems like needle phobia, needle injury skin irritation etc. Now, after the invention of microneedle for vaccination these problems actually pass away. Studies have shown that microneedle is more superior to conventional needles in terms of dose sparing, immunogenicity etc. Some studies is going on based on the increasing vaccine stability and delivering of that vaccine to different body areas apart from skin (Shin, 2017).

1. Polymeric MN devices have opened a new era in MN technology. Polymeric MNs can solve many problems that occurred with silicon made solid MNs. Also it is nontoxic and biocompatible.
2. There are many pharmaceutical companies those are trying for the commercialization of MN technologies. Among these companies Zosano pharma, Coriunm 3M etc can be mentioned. If some problems can be addressed then this technology can be the pharmaceutical dosage forms and monitoring device in near future. Moreover, Zosano pharma are trying to deliver parathyroid hormone by using microneedle for the treatment of osteoporosis but this is in phase 3 trial now (Tuan-Mahmood et al., 2013).
3. One more thing is that though polymeric microneedle can solve many limitations associated in other types of microneedle, the therapeutic efficiency of polymeric microneedle is still not satisfactory. It will be very useful in future if the nano medicines containing drugs incorporated into functional nano particles. If it is done then this problem associated with polymer microneedle can be solved (Wang, Hu, & Xu, 2017).

4. In the future it is urgent to come up with some good adjuvant that will increase the immunogenicity. Moreover, there is a very good chance that the research community will give more chance to the microneedle product for clinical trial (Ita, 2016).

Conclusion

Microneedle is a very tiny and small size needle. Basically they are used to deliver drugs through dermal and transdermal route. Some of the examples can be given like hepatitis c vaccine, anthrax vaccine, insulin etc. In this literature the results of many articles were reviewed. A lot of research is going on related to the use of microneedle. Still many of the vaccines are yet to be coated. Especially the temperature related vaccines need to be coated. A lot of studies are ongoing for this purpose. Moreover a lot of researches are needed to deliver drugs to the eye by using microneedle. As we all know eyes are very sensitive and it is really pain full and risky to inject drugs on the eye. Finally, it needs to be said that microneedle can create a revolutionary change in the vaccination process.. Moreover, the needle spreading is increasing at an alarming rate. So the microneedle can be a very good preventive factor for needle spreading diseases.

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