

# **A Review On Transdermal Delivery of Vaccines**

A project submitted  
by

A.K.M. Salman Haque

ID: 12146013

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Inspiring Excellence

Dhaka, Bangladesh  
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*Dedicated to my Parents, who have sacrificed their worldly happiness in fulfilling my ones to their best and also to my beloved siblings and friends.*

Certification Statement

This is to certify that this project titled ‘A Review On Transdermal Delivery of Vaccines’ submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Ms. Najneen Ahmed, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

\_\_\_\_\_salman\_\_\_\_\_

Countersigned by the supervisor

\_\_\_\_\_Najneen\_\_\_\_\_

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## Abstract

For therapeutic responses against various diseases especially those are infectious in nature and also malignant such as cancer; vaccine induces cellular and humoral immune responses endowed with prophylactic measures by both structurally and physiologically, which provides front-line defense and opposes various environmental assaults to protect the skin which is an important part of the immune system. The delivery of water soluble compounds such as peptides, proteins and vaccines, transdermal route overcome the barrier properties of stratum corneum of the skin. The capillaries, lymphatic, blood and interstitial fluid within the dermis and epidermis where the dermis is the major site of fluid exchange in between the both the dermis and epidermis. So immunization through transdermal route is more effective to achieve therapeutic drug level besides sustained release of drug can also be achieved to have a prolonged action of drug delivery. In this review summarizes the different delivery strategies in order to improve the delivery of transdermal vaccines along with the carrier systems and the adjuvants which can be used to overcome these confronts and thereby enhance the transdermal vaccination or delivery. Dendritic cells which is an essential component of the immune system and induces a stronger immune response for the transdermal route of delivery by processing microbial antigens which migrate into lymphatic capillaries to lymph nodes. Immunogenic materials are being protected during the delivery and facilitate specific target oriented delivery system along with incorporation of various types of adjuvant materials. Moreover transdermal patches may also improve competitiveness in a large market segment of which known examples are nitroglycerin patches in the field of cardiovascular, it was favorably introduced for more than 30 years within the market of nitrate and latest introduction of rivastigmine patches in the indication of Alzheimer and dopamine patches Parkinson disease and restless leg syndrome which are acted as a agonist by the indication. (B. Boroojerdi; H.M. Wolff et al. 2010). Besides transdermal vaccination has developed vaccines like-hepatitis B (Deng et al, 2011), Foot and Mouth disease vaccines (Smartvet, 2012), Dendritic antitumor vaccines (Tendeelo et al, 2001) which is yet to developed. However, transdermal system supports lifecycle management of approved drugs by empowering the patients situation.

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## **List of Abbreviations**

DNA- Deoxyribonucleic acid

RNA-Ribonucleic Acid

TLR's- Toll-Like Receptors

mRNA- Messenger RNA

IgD-Immunoglobulin D

IgA-Immunoglobulin A

DCs-Dendritic cell

C-Complementary system

HIV-Human Immunodeficiency Virus

ADCC-Antibody Dependant cell Mediated Cytotoxicity

IFns-Interferons

CC-Chemokine Receptor

ELISA-Enzyme Linked Immunosorbent Assay

APC's-Antigen Presenting Cells

Hbs Ag-Hepatitis B Surface Antigen

Th- T Helper

HPV- Human Papilomma Virus.

LPS-Lipopolysaccharide

EMDA-Electromotive Drug Administration

rdAds-replication defective adenoviruses

**Chapter - 1**  
**INTRODUCTION**

## **1 Introduction**

To deliver drug successfully and efficiently, drug delivery has been an aim of researchers for ages. As the skin is one of the most sophisticated areas due to its ease of access and ubiquity, it is a widely used organ for drug delivery. Although parenteral route provides the quickest therapeutic action for any drug, it is not so patient convenient. As almost every person who has gone through pinching of needles has found this route disadvantageous. The most common limitations are:

- i) Pain and phobia due to needle
- ii) Various infectious diseases which can be transmitted through use and repeated use of the same needle (Walter & Wright, 2010).

A study has been made which results in 30% of adult suffer from needle phobia. (Andrew, Jeong, & Prausnitz, 2012). Moreover, significant populations are in unintentional injuries of the needle to both patient as well as the physician. It involves million of dollars- worth of additional medical treatment and raises transmitted infectious diseases ratevariably(Dahlan, Alapar, & Stickings, 2009)

Due to the hazardous needle practices, the world Health Organization estimated 1.3 million deaths were caused. In fact estimation for the developing countries says 50% of the injections are unsafe and there are 800,000cases reported of needle injuries by medical professions each year.(Miller and Pisani, 1999)

### **1.1 The Physiology and Anatomy of skin**

The skin which is one of the largest organs which is mainly responsible for sustaining homeostasis by regulation, protection of underlying tissues, temperature, retardation of water loss, housing sensory receptors, synthesizing certain chemicals, and excreting wastes along with it. The skin comprises of an outer epidermis and adermis, associated to underlying tissue along the subcutaneous layer (hypodermis). The epidermis is built up of

stratified squamous epithelium and dermal blood vessels. It comprises melanocytes and is well nurtured along with dermal blood vessels. The epidermis is significant because it helps against loss of water, microorganisms, mechanical injury and chemicals. The dermis ties the epidermis to elementary tissues. The dermis comprises of connective tissue along with collagen as well as elastic fibers between a gel-like ground substance. Now the dermal blood vessels convey nutrients to uppermost layers of skin and assist to govern temperature. The dermis also keeps nerve fibers, hair follicles, sensory fibers, sebaceous glands and sweat glands. The subcutaneous layer (hypodermis) is consists of loose connective tissue and insulating adipose tissue. It connects the skin to elementary organs and that contains the blood vessels that conveys to the skin. The auxiliary organs of the skin consists sweat glands, hair follicles, sebaceous glands and nails.

## **1.2 Immune system and its overview**

Immune system has been evolved in order to shield the host from pathogenic microbes which are themselves continually evolving. It generally helps the host to eradicate toxic and allergenic substances that penetrate through the mucosal surfaces. Central to the immune system's capability a response to invading pathogens, toxin or allergen is distinguished self from non self. Both the innate and adaptive mechanisms are being used by the host to detect and eliminate pathogenic microorganisms as well as self-nonsel self discrimination is included.

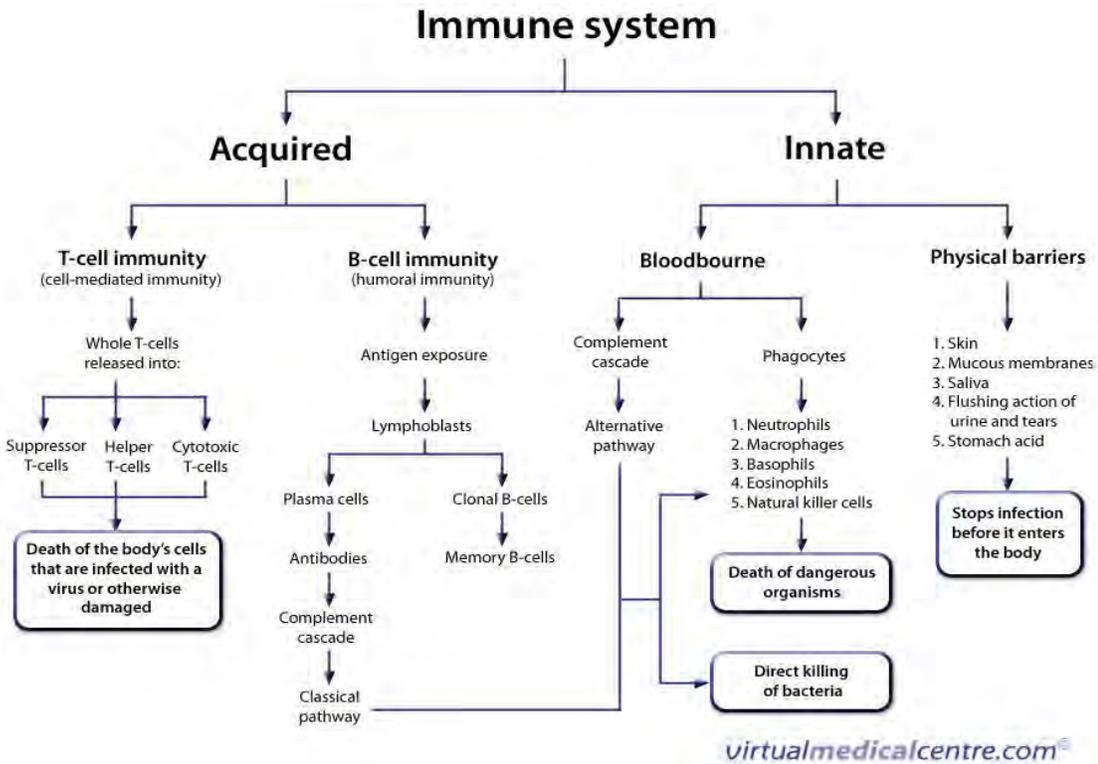
T-cells have been evolved to recognize foreign antigen along with self antigen as a molecular complex as a result self tolerance is maintained. Without having selftolerance, there is possibility of being attacked by autoimmune disease.

Now the innate response shows that the first line of host defense with adaptive response becomes for active several days following antigen specific T and B cells have endured clonal expansion. Synergistic activity of both the innate and adaptive response are necessary to have an enact and full immune response.

Innate immune response acts as physical barrier for the pathogens and contains epithelial cell layers having tight junction. Now the epithelium of the gastrointestinal, respiratory, and genitourinary tracts along with the epithelial cilia which sweeps away the mucus layer allowing it to be constantly refreshed after contamination due to inhalation or ingestion of particles. Boroojerdi, Wolf, Braun and Blom (2010) found that the complement proteins defenses and ficolins present in biological fluids or can be released from cells as they are activated. Cytoplasm proteins which have membrane bound receptors with that bind with molecular patterns that are stated on the surface of invading microbes.

Adaptive immune system maintains specificity of its target antigens where the antigen receptors expressed on the surfaces of T and B lymphocytes. Adaptive response is ordinarily encoded by genes which are assembled by somatic re arrangement to form T-cell receptors and immunoglobulin genes.

B-cell consists of approximately 15% peripheral blood leukocytes and produces immunoglobulin.



**Figure 1.1:** Overview of Immune system (Adapted from virtualmedicalcentre.com©)

Adapted from: <http://www.myvmc.com>

### 1.3Vaccine Terminology

A particular disease can be defined as a biological preparation which provides active acquired immunity assisted by Vaccine. A vaccine from killed or inactivated forms is typically comprises of a disease-causative microorganism that is often made of the microbe, its toxins or one of its surface proteins. It has been found out that the body's immune system is excited by this agent to identify it as threat, and destroy it, and maintains an account of it, and any such consecutive similar infection can be more easily identified by the immune system and destructed(Nir, Paz, & Sabo, 2003).

#### **1.4 Vaccination Terminology**

Administering of an antigenic material (vaccine) within a living mechanism, vaccination which can be defined by this process in which individual's immune system is to cause excitation, the clinical effect desired of an so that it can grow an adaptive immunity against the pathogen forming the vaccine. Diane, McAllister, Ping and Shawn (2003) has demonstrated that vaccination is the most ideal method of prevention for infectious diseases (V, wang, & Davis, 2003).

#### **1.5 Types of Vaccination:**

- I. Live, attenuated Vaccines
- II. Inactivated Vaccines
- III. Subunit Vaccines
- IV. Toxoid Vaccines
- V. Conjugate Vaccines
- VI. DNA Vaccines
- VII. Recombinant Vector Vaccines

##### **1.5.1 Live, Attenuated Vaccines**

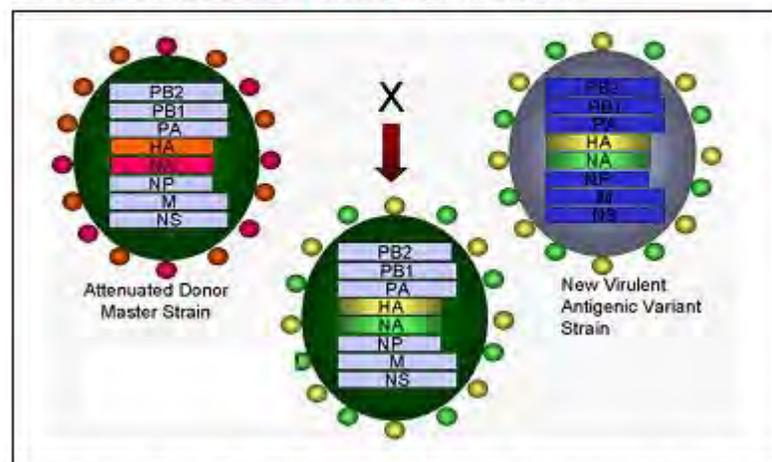
A living microbe which has been weakened in the lab so it cannot cause any disease includes live, attenuated vaccines. These vaccines are right “teachers” of the immune system, as live, attenuated vaccine is one of the closest things to a natural infection, strong antibody and cellular responses which often give lifelong immunity by only one or two doses are evoked by them.

In spite of the benefits of live, attenuated vaccines, there are some disadvantages. The organisms used in live, attenuated vaccines are indifferent as it is the nature of living things to change, or mutate. The distant possibility exists that an attenuated microbe in the vaccine

might revert to a virulent form which causes disease. Besides, not everyone can securely receive live, attenuated vaccines. By their own protection, people who have disabled or damaged immune systems because they have undergone chemotherapy or have HIV, for example and cannot be given live vaccines.

## Live Vaccines

- Also called **attenuated** vaccines



- Virus is still alive, but can't cause disease

**Figure 1.2:** Influenza Treatment: Drugs and Live attenuated vaccines.

Adapted from: <http://employees.csbsju.edu>

### 1.5.2 Inactivated Vaccines

The disease that affects microbe with, radiation, heat, or chemicals are the process of killing. Such vaccines are found to be safer and stable than live vaccines. The inactivated vaccines commonly do not require refrigeration and they can be readily stored and transported in a freeze-dried form that makes them available to people in developing countries.

But mostly they stimulate a weaker immune system response than do live vaccines. For this reason action has to be taken to go for several additional doses or booster shots, in order to maintain a person's immunity. It could be a drawback in areas where people don't have regular approach to health care and can't get booster shots on spell. (Inactivated vaccine,2013; Retrieved from: <http://www.vaccines.gov>)

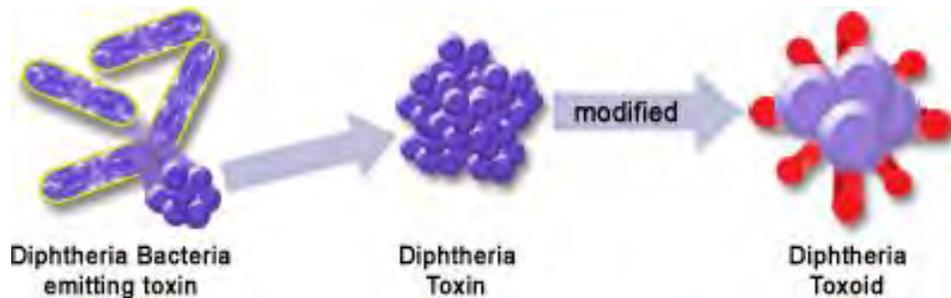
### **1.5.3 Subunit Vaccines**

Subunit vaccines contain only the antigens that best stimulate the immune system in place of the whole microbe. In few cases, these vaccines utilize epitopes which is the very particular segments of the antigen where T cells or antibodies identify and bind to. As subunit vaccines include only the requisite antigens and not entire of the other molecules which make up the microbe, the risks of adverse reactions to the vaccine are lower. (Subunit vaccines, 2013; Retrieved from: <http://www.vaccines.gov>.)

### **1.5.4Toxoid Vaccines**

A toxoid vaccine might be the response since bacteria that discharge toxins, or injurious chemicals .When a bacterial toxin becomes the main cause of illness and these vaccines are utilized. Inactivate toxins by compensating them with formalin which is a solution of formaldehyde and sterilized water which Scientists have found out. These kind of "detoxified" toxins, called toxoids, are secure for exercise in vaccines.

While the immune system acquires a vaccine including a harmless toxoid, it assimilates to fight with the natural toxin. The immune system generates antibodies which lock onto and obstruct the toxin. Vaccines opposed to diphtheria and tetanus are models of toxoid vaccines. (Toxoid vaccine,2013; Retrieved from: <http://www.vaccines.gov>.)



**Figure 1.3:** The Diphtheria and Tetanus portion of the inactivated Toxoid vaccines.

Adapted from: <http://www2.cdc.gov>

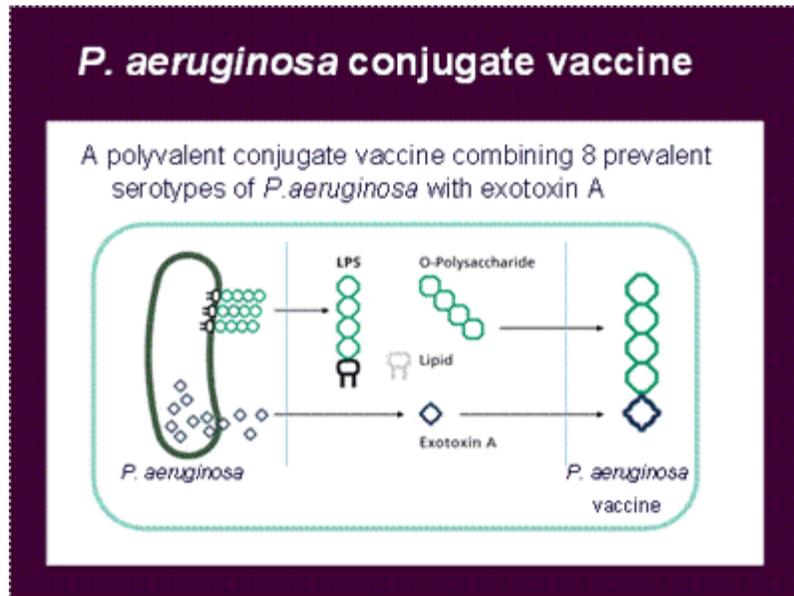
### 1.5.5 Conjugate Vaccines

When a bacterium retains an external coating of sugar molecules named polysaccharides, as several toxic bacteria do and researchers making a conjugate vaccine for it will attempt. The immature immune systems of younger children and infants can't identify or react to them on which Polysaccharide coatings conceal a bacterium's antigens. Conjugate vaccines, a unique type of subunit vaccine, get almost this problem.

T- Antigens or toxoids from a microbe that an infant's immune system can identify to the polysaccharides is connected by scientists while preparing a conjugate vaccine. The immature immune system responds to polysaccharide coatings and guard against the disease-causing bacterium is helped by the bond.

The vaccines which mainly shields against *Haemophilus influenzae* type B (Hib) which is a conjugate vaccine.

(Conjugate vaccine, 2013; Retrieved from: [http://www.vaccines.gov/more\\_info/types](http://www.vaccines.gov/more_info/types).)

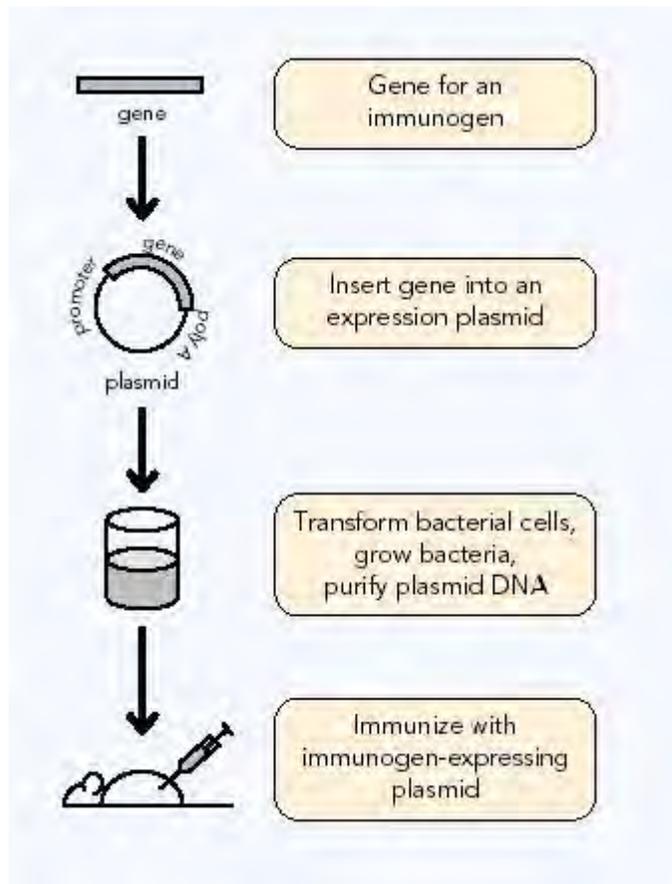


**Figure 1.4:** A possible anti*Pseudomonasaeruginosa*Conjugate vaccine.

(Adapted from:<http://www.cfww.org>)

### 1.5.6DNA Vaccines

It is still within the experimental stages, on which these vaccines reveal great promise and so many types which has been tested in to humans.Fresh technological plane immunization is taken by DNA vaccine. These vaccines allot with both the full organism as well as its parts and get right down to the fundamentals, the microbes and its genetic substantial. In specific, DNA vaccines exercise the genes which code for all those significant antigens.(DNA Vaccines,2013; Retrieved from: [http://www.vaccines.gov/more\\_info/types](http://www.vaccines.gov/more_info/types)).



**Figure 1.5: Study of DNA vaccine**

Adapted from: <http://www.cfww.org>

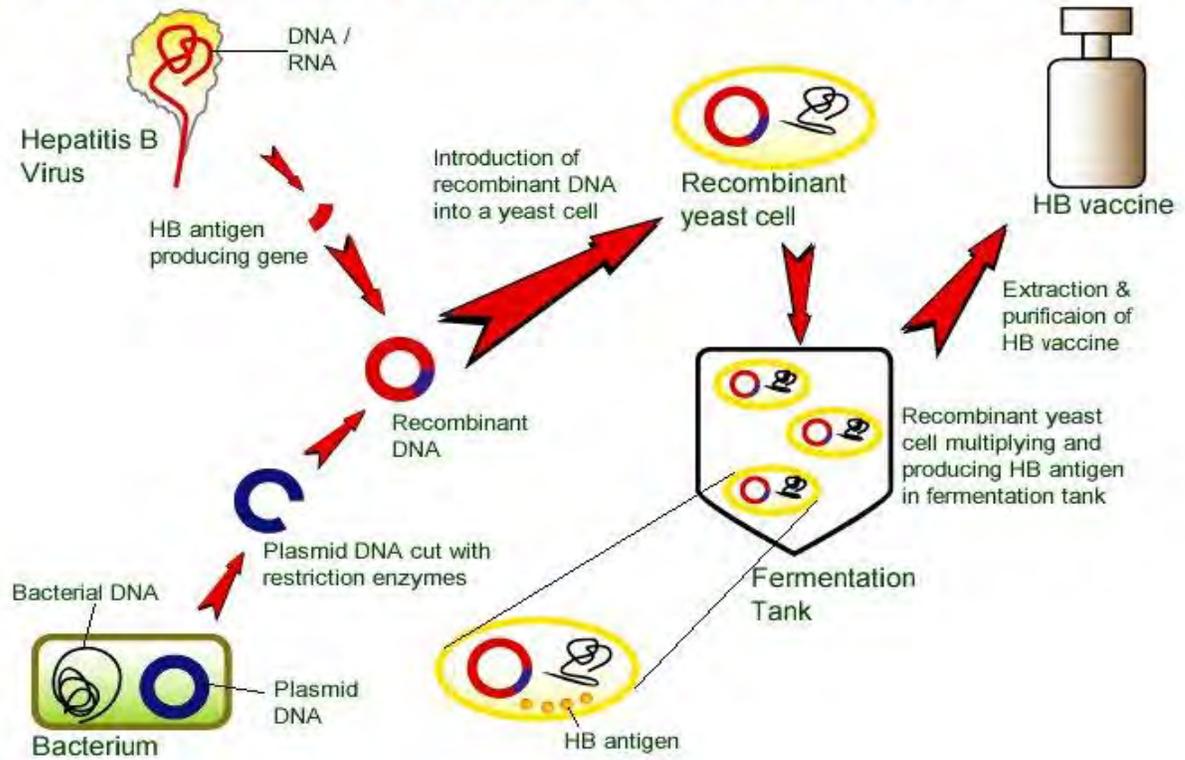
### **1.5.7 Recombinant Vector Vaccines**

Recombinant vector vaccines are basically experiential vaccines that are same to DNA vaccines, although they use an attenuated bacterium or virus in order to insert microbial DNA cells within the body. “Vector” relate to the virus or bacterium employed as the carrier.

Mostly in this kind, viruses lock on to cells and introduce their genetic material within them. No-win the laboratory, scientists have undertaken benefit of this method. They have find out in which way to take the spacious genomes of definite safe or attenuated viruses and embedded portions of the genetic stuff from another microbe into them. Then the carrier viruses transport that microbial DNA into cells. Well recombinant vector vaccines exactly copy a natural infection and therefore do a better job of exciting the immune system. Attenuated bacteria where utilization is made as vectors. The antigens of other microbes on its exterior, the injected genetic material drives the bacteria to show here. Inducing an immune response the effect of the safe bacterium imitates a harmful microbe.

Researchers are working on both viral-based and bacterial recombinant vector vaccines for measles, Rabies, HIV. (Recombinant vector vaccines, 2013; Retrieved from: [http://www.vaccines.gov/more\\_info/types](http://www.vaccines.gov/more_info/types)).

# Production of Recombinant HB Vaccine



**Figure 1.6:** Production of Recombinant HB Vaccine.

Adapted from: <https://www.ied.edu>

## **Chapter 2**

# **RESEARCH METHODOLOGY**

## **Research Methodology**

The summarization of the current status of transdermal delivery method of vaccination has been done in this article. The results were cross checked by searching Elsevier's "Science Direct", PubMed, SpringerLink. As well as other references and conference proceedings have also been included. Presently, there can be found a large benefit in expanding transdermal vaccinations are opposing many viral diseases as most of the pathogenic inclusion occurs through transdermal membranes. So, the effective vaccination measure can be taken via transdermal route to have a painless and desired efficacy of drug. However the development of transdermal vaccines, of infectious diseases or for oral immunotherapy-tolerance, requires effective antigen delivery along with adjuvant systems, even if for prevention. To provide effective adaptive immunity by the stimulation mostly opposing infections and tumor growth which Corresponds the system that should protect the vaccine against the physical elimination as well as enzymatic degradation, targeted inductive sites and suitably the innate immune system is generated which involves M cells. Better transdermal vaccine delivery system which is an indicative advancement has newly been built in the progression of it. Although it has been already found out that drugs or antigens delivered via gene gun, electric charge, ultrasound frequency are well recognized by the immune system which is innate and are powerful inducers of immune system provides anxiety free delivery system. Various types of DNA and RNA transdermal vaccines are on the way of enriched development. The objective of this review is to discuss several distinct advents to induce transdermal immunity into vaccines accentuating on transdermal tissue targeting and novel immunization routes with a particular coverage on the delivery system.

## **Chapter 3**

### **METHODS OF TRANSDERMAL VACCINATION AND DISCUSSION**

### **3.Classes of Transdermal drug delivery system**

There are various routes of drug administration. But the specific choice of delivery of drug mainly depends on diseases, physiological condition of the patient and the characteristics of the therapeutic compound. Lipophilicity is an important concept for the drug delivery within body with the molecular weight of  $< 500$  Da in order to get liberated round the skin. The conveyance of the drugs with varying lipophilicity have been reported to improve by dynamic iontophoresis, mechanical commotion and energy dependent techniques such as ultrasound and needle-less injection.

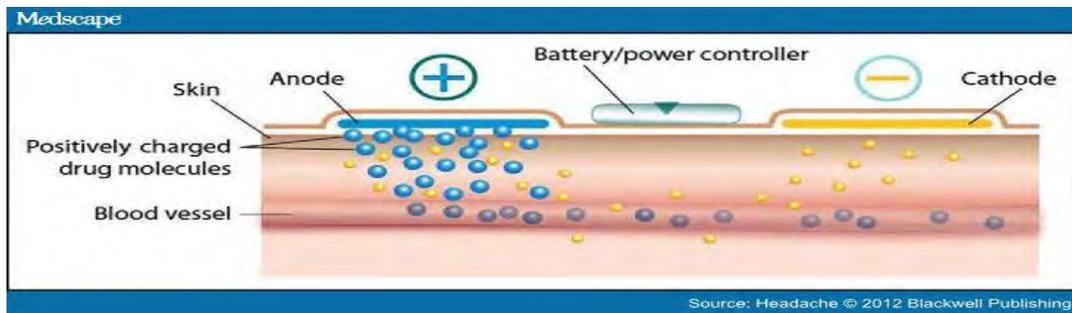
#### **3.1Iontophoresis**

This may be also called as an Electromotive Drug Administration (EMDA) basically a needle-free injection mechanism which uses electric charge to transfer drugs or chemicals throughout the skin. An ionophoretic system includes a positive electrode (anode), a negative electrode or cathode, a battery, microprocessor and drug storage. The drug particle containing by the active electrode compartment and the return electrode is kept near the skin which completes the circuit. At that time a phenomenon is termed as electro migration and it is the dominant electro transport mechanism. Now the skin which acts as a cation selective membrane below physiological conditions and it governs to the formation of a conventional solvent flow in the anode to cathode course and which is known as electro osmosis and it is the insignificant instrument for the electrically aided transfer of cations. Neutral molecules are also being helped in electro transport from anode (Agarwal & Dhawan, 2009)

The main usefulness of iontophoresis is that it is competent of leading the delivery kinetics. The extent and rate of the drug transfer is conditional on intensity and the duration profile of the flow entity applied. Now a days the challenge is being faced is that to provide a pre-filled iontophoretic patch system similar to the convectional transdermal patches This process have got the signal of United States Food and Drug Administration and this classifies iontophoresis as one of the best efficient physical improvement technique for the

betterment of drug permeation over the skin(Jain, 2008)theelectrical current will be different as the matrices are different.

This can be related to differences in viscosities, material electrical charge and porosities the migration of the drug under the influence of it(Gaur, Mishra, Purohit, & Dave, 2009) Delivery of high molecular weight compounds, like insulin has achieved through this technique in combination with chemical enhancers the larger peptides(Hansen, 2010)



**Figure 3.1:** Iontophoresis delivery

(adapted from Headache© 2012 Blackwell Publishing)

### **3.1.1 Hepatitis B Vaccination through Iontophoresis:**

Hepatitis B vaccines were investigated in order to assess the penetration of vaccine under passive diffusion and iontophoresis conditions. After removing the stratum corneum, the retention and permeation amount of hepatitis B vaccines increased. During administration of hepatitis B vaccine through the skin induction via Iontophoresis increases both of cumulative and retention time. Initial drug loading of 23 microg x mL(-1) and 46 microg x mL(-1) by application of iontophoresis significantly enhanced the permeation of hepatitis B vaccines ( $P < 0.05$ ) by 2.7-folds and 6.6-folds for the intact skin, and by 1.6-folds and 1.8-folds for the tape-stripped skin oresis ( $P < 0.05$ ) respectively. The amount of skin retention

was nearly the same as passive diffusion for 24 h both from intact skin after applying iontophoresis for 6 hour. and tape-stripped skin respectively. However, hepatitis B vaccines may be improved by iontophoresis, which can be potentially used in the field of transcutaneous immunization (Polat, Blanckstein, & Langer, 2010).

### **3.1.2 Appliances based on velocity**

Here Gene gun is the instrument used. They drive vaccines from a reservoir into the stratum corneum layer of the skin. Small molecules and proteins from powder and liquid formulations are delivered by it. (Dahlan, Alpar, & Stickings, 2010)

Compressed gas is generated at high velocity 100-200 m/s there is a nozzle having an orifice of diameter 50-360  $\mu\text{m}$  from where the jet is propelled. The benefits of using needle-free devices comprehend accidental needle sticks, concerns over disposal, decreased amount of costly and hazardous wastes are prevented. The interstitial liquid may splash back from the skin and contaminate the nozzle so; the risk of cross contamination is not eliminated completely since. For multi dose drug delivery to the same individual, however, the multi-use nozzle jet injectors are not used for mass vaccination nowadays. Sumavel® DosePro® needle-free delivery system (Zogenix, 2010) which was launched in 2010 for delivery of sumatriptan and the Biojector® 2000 (Bioject Medical Technologies Inc., USA) which has been passed by FDA for intramuscular injections. This device is in its clinical trial phase for delivery of DNA vaccines are some examples of partially disposable devices. (Timmerman, Hermanson, Hobart, Taidi, & Caspar, 2002)

The working of power jet injectors is akin to the liquid jet injectors since the power of jet injectors promote stronger immune responses because they transfer vaccines to the superficial layers of the skin (Prausnitz, Mikszta, Cornier, & Andrianov, 2009).

Found out that the clinical tests of Phase I have proved PowderJect XR-1 ; the efficacy of which delivering gold particles coated with DNA for hepatitis and influenza. (Soderholm, Tjelle, Kjekken, Frelin, & Hogland, 2002)

Ballistic impact with the tissue can be sustained with the gold micro particles, attained a great success. The drawbacks associated with this mode is that it may give pain, bruising, burning sensations, tingling, discoloration, hyper-pigmentation, mild erythema at the site of injection.

### **3.1.3 Sonophoresis:**

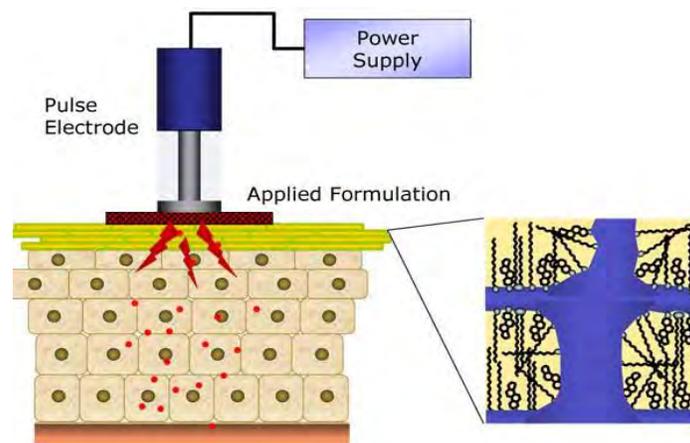
Sonophoresis is another developing technology for the delivery of vaccines. Ultrasound of frequency (20-100) khz or high frequency ultrasound like 90, 7-0.16) kHz are used(Jeong, Andrews, & Prausnitz, 2012)Sonophoresis increases skin permeability mainly for the drugs such as insulin and heparin. For various low and high molecular weight drug can be induced. As it is costly process so the expenditure for preparing it must be cheap for availability of the use in future. This is an united effects with electroporation and iontophoresis, its exercise in gene therapy, hormone replacement, cardiovascular disorders, cancer therapy.(Polat, Blankschtein, & Langer, 2010)sonoprep is the skin permeation device which applies frequency 55 khz for 30 seconds within the skin. When the desired level of permeability is obtained it stops automatically. The skin permeability is scaled by the stream moving through a return electrode. Although, stratumcorneum of the skin layer may get damaged due to breakdown of cavitations foams formed within the coupling medium (Polat et.al, 2010). Extensive survey has been made to find out commercially available transdermal drug delivery system and along with the drugs for which they are being used. Following lists of transdermal delivery and drugs has been represented:

Rabies vaccine (Blankschtein, Polat, & Langer, 2010)Influenza vaccine can be prepared by sonophoresis.

### **3.1.4 Electroporation**

Transdermal delivery has opened up a new possibility to introduce larger molecules such as peptide hormones and vaccines as well as minigenes and RNAi etc. via the application of electroporation through the transdermal route. Among different geometrical arrangements

these devices include both non-puncturing surface electrodes as well as puncturing electrodes. To maximize transport, uptake and minimizing pain different electroporation protocols have been developed. Sonic, vibrational and thermal treatments are used to enhance the transport.



**Figure 3.2:** Delivery of Vaccines by Electroporation.

Adapted from:<http://www.skin-care-forum.basf.com>

### 3.1.4.1 Vaccination through Electroporation

**A. Dc (Dendritic cell) based vaccines:** This type of vaccine is loaded with tumor antigens in which an approach has been made to prepare DC based tumor vaccine. Already in a study cytoplasm, which was expressed based on mRNA electroporation to efficiently expressed tumor antigens into DC which showed a improved transfection efficiency and induced a very low toxicity. So, electroporation mRNA encoding tumor antigens was a heavy technique to charge human dendritic cell with tumor antigen and can be served as future DC vaccine. (Tezel, Paliwal, & Shen, 2005)

- B. Foot and mouth vaccines:** Transdermal Vaccine projects of SmartVet's are aimed at expanding the use of its versatile remote delivery technologies into the field of vaccines. Current research efforts are in the field of infectious diseases particularly. Foot and Mouth Disease (FMD) on which the focus remains. Particularly the stratum corneum and underlying tight junctions, which the main challenge facing topical vaccines has been penetrating the protective barrier of the skin. The research showed that, this may facilitate antigen penetration via the intercellular pathway to the deeper layers of the skin deeper layers, which is uniformly accepted as an ideal target for antigen delivery are a highly immune competent zone. To generate strong immune responses to the topically applied antigens, VetCaps could further enhance the potential (Damme, Kafeja, Wielen, & Almagor, 2009)
- C. Influenza vaccine:** Use of Nanopatch allowed a 100-fold reduction in the required dose of Fluvax® influenza vaccine in which preclinical in vivo studies.. Vaxxas' Nanopatch™ vaccine delivery technology consists of a 1 cm<sup>2</sup> silicone array that carries about 20,000 vaccine-coated microprojections that painlessly perforate the outer layers of the skin when applied with the associated applicator device, and deliver the vaccine directly to key immune (Damme, Kafeja, Wielen, & Almagor, 2009)

### **3.2 Transdermal Nanoemulsion**

Recently researchers developed transdermal vaccines delivery improvement in the form of nano-size particulate, such as nanoemulsions, liposomes and nanoparticles. In vitro as well as in vivo absorption can be enhanced via this technique with good bioavailability. Now a model was selected which is named Artin-M, because the hydrophilic nature of both lectin and protein require same strategy for skin penetration (Amacker, Kammer, Rasi, Westerfield, & Moser, 2012)

So both the lectin and protein were incorporated into the oil phase of nanoemulsion as nanosize globules to improve the penetration of skin. This delivery is also appropriate for synergistic delivery of bovine serum albumin (BVA) which is a protein vaccine model.

### **3.3 Transdermal vaccination for immunization of Influenza via vaccine coated micro needle arrays:**

For simple administration fabrication of the micro scale needle utilizing minimal cost method for cheap mass output, for administration purpose a patch like format is made, perhaps by the patient to approach the resident dendritic cells themselves they inserted painlessly across the stratum corneum and into the skin's epidermis and dermis. It increases immunogenic responses but needs specialized hands. The procedure begins with the micro needle is pushed manually toward for a few minutes the skin is left in place meanwhile the vaccine coat, after which the micro needles are castaway disperses off within the skin. (Duffy, Weintraub, Vellozzi, & Destefano, 2010)

### **3.4 Methods and Materials for fabrication of Micro needles**

Fabrication of microneedles is done with stainless steels (Trinity Brand Industries, Atlanta). By laser cutting and Electro polishing are done to clean the edges and to make the tip sharp dissolved in a solution containing glycerol ortho-phosphoric acid (85%) and deionized water in a ratio of 6:3:1 by volume. The geometry of the final micro needle where the spacing was made between the needles was 1575 mm.700 mm long, measured 170 mm by 55 mm in cross section at the base and tapered to a tip with a 5 mm radius of curvature for each needle(Almagor, Cormier, Mikszta, & Andrianov, 2009)

#### **3.4.1 Micro dip coating**

It is another method Micro needles with a specially formulated coating solution described before were coated using a dip-coating process (Donnelly, Berry, & Ulmer, 2003) includes

(w/v) carboxymethylcellulose sodium salt (low viscosity, USP grade, Carbo-Mer, San Diego, CA), 0.5% (w/v) Lutrol F-68 NF (BASF, Mt. Olive, NJ), 15% (w/v) D-(+)-trehalosedihydrate contained by it and virus of 5 mg/ml inactivated A/ Aichi/2/68 (Aichi). The virus was concentrated by microfiltration using 300 kDa cutoff filters in order to reach high coating efficiency, (Vivaspin 500, Sartorius Stedim Biotech, Germany). Manually, the coating was done. To measure the amount of vaccine coated per row of microneedles, three rows from every batch of coated micro needles were drowned into 200 ml of PBS buffer per row for 5 min. By estimating the concentration of protein in the solution by BCA protein assay (Pierce Biotechnology, Thermo fisher Scientific, Rockford, IL) coupled with a standardization curve formed using accepted concentrations of Aichi virus (Ellen, Jet, & Wilson, 2008)

Discussions made on the development: Micro needle immunization technique provides better immunity as strong as that of Intramuscular injection. Studies showed that it provides more humoral and cellular immune response with single immunization.

As the study was performed within the mice bodies' solid metal inactivated influenza virus micro needle coated with conferred more than 99% protection regardless of lethal viral dispute and short-lived antigen-secreting cells in the lungs and spleens of immunized mice as well as anti influenza memory B cell responses are inducted. It gives almost similar immunization as that of regular IM route having same grade of useful antibodies at high or low concentrations of antigen.

### **3.5 Vaccination using cutaneous route applying micro needles coated with hepatitis C DNA vaccine**

The DNA vaccine that is much potent to produce vigorous humoral as well as cellular responses of immune. Characteristically it requires appreciably 5-10 mg of DNA producing a robust immune response in human. With the help of electroporation, potency can be increased to a great deal in humans and nonhuman who approximates three orders of magnitude in primates but the method is expensive than the micro needle delivery.

Microelectronics industry are there to fabricate micron-scale needles containing compounds that can be easily and painlessly delivered within the skin, applying techniques that are appropriate for economical massive production. Drug or vaccine introduced into the solid micro needle on the surface as a solid film following injection within the skin, interstitial fluid of the cutaneous rapidly discharges the film and liberates the drug or vaccine within the skin. To vaccinate mice with inactivated influenza virus and guinea pigs along with ovalbumin the method was already utilized (Pilar, Jiskoot, & Riet, 2008) (Booms, Stucker, & Gambichler, 2006). To vaccinate mice, plasmid DNA has been used (Booms, Stucker, & Gambichler, 2006). Other procedure using empty microneedle have been used to inoculate antigens within the skin of humans and animals a number of antigens where humoral responses were created (Osterhuis, 2007). Electroporation combines smallpox DNA vaccine with micro needle to persuade humoral responses in nonhuman primates (Stucker et al, 2006). Administration needs little or no training. It can also be prepared for self application, patch-like devices (Makidon, 2009). The hypothesis was examined utilizing a suitably developed vaccine of DNA encoded with nonstructural (NS) 3/4A protein hepatitis C virus which has previously been displayed to persuade powerful in vivo functional T-cell reactions in mice while transferred by intramuscular injection or gene gun, following by in vivo electroporation. The comparison has been made between

(1) Intramuscular DNA distribution by hypodermic injection, which is extensively used in animal studies but is basically useless in humans and

(2) Cutaneous approach using gene gun in humans that can be effective (Pathan, Seti, & Makidon, 2009)

### **3.5.1 Discussion based on result using cutaneous route**

The lines of micro needles were planned to permit steady penetration within the skin of mice into the highly pliant. After coating with vitamin B2 rows (5 Microneedles) of micro needles were found to be uniformly coated. Afterwards, new micro needles) were covered with DNA, along every micro needle line was established in order to be coated with  $1.6 \pm 0.2$  mg

of DNA. 700 mm was) the design made for the micro needle, with 2-3mm thickness. Swine skin is a better anatomy for skin of human model. Skin was pierced with it sulforhodamine-coated micro needles demonstrates that micro needles. Moreover, coated micro needles have been shown to have less or same 90% delivery efficiency.

Mice were vaccinated along with 8 mg DNA using microneedles and 4 mg DNA using gene gun in order to determine whether cutaneous immunization of mice with micro needles with plasmid encoded hepatitis C virus NS3/4A protein could evoke a lytic cellular immune response. Verily, DNA-coated which was immunized with micro needles inducted the generation of lytic Cytotoxic T- lymphocyte. Cell lyses was considerably greater following micro needle treatment contrasted with naive mice (P less than 0.05). A cellular immune response which shows that micro needles liberated coated DNA that primed, most likely in the skin. However the overall study showed that DNA plasmid which was expressed by the NS3/4A expression which can be liberated within the skin utilizing covered micro needles to evoke CTL's priming which were same doses at same to gene gun, which refers that the responses of immune which was liberated using micro needles may be enough for vaccine applications of DNA (Rims, Pinsky, & Osdol, 2008).

### **3.6 Topical immunization via Microporation**

This is an unique technique for painless and needle liberal transmission of adenovirus based vaccine.

An investigation was made for genetic immunization utilizing replication-defective adenoviruses (rdAds) refers to replication defective adenoviruses as prototype vaccines using microporation as a device (Walter, Wright, & Fuller, 2010).

RdAds(replication defective adenoviruses) makes them appealing vectors for genetic vaccination possesses number of properties that are:

- (1) They evoke potent cellular and humoral response.
- (2) 8kb of foreign DNA they can easily hold up to
- (3) Using basic molecular biology and tissue culture techniques they are readily manipulated and propagated.
- (4) Immunocompetent host have not been associated with the disease. (Walter et. al,2010)

For topical delivery of rdAds is one of the noble methods. Hairless mice exhibit a hair follicle which are immune-competent and frequency (approx. 75 follicles/cm<sup>2</sup>) closer to human skin (approx.10 follicles/cm<sup>2</sup>).Though there are difference in hair follicle 10 fold density but same level of gene expression is observed following hairless mice which is microporated(Skountzou, Quan, Vzorov, & Gangadhara, 2007).

AdLuc (adluciferase) was applied to both of the intact skin and microporated skin in order to determine whether microporation would develop the delivery of Ad vectors on to the skin. To measure luciferase gene expression then twenty-four hours later, the site of inoculation was eradicated and processed. The reproducibility of this technique where same levels of gene expression were observed in B6/129, BALB/c, and C57Bl/6 demonstrated to increase in luciferase activity which is compared to intact skin controls application of AdLuc in to microporated skin resulted in a 100–300-times. By applying vaccine to the intact skin-

It is observed that hairless mice are more responsive than the hairy mice.

For enhancing topical application of rdAd vectors in two settings:

- (1) Gene transfer to the skin

(2) Genetic immunization; discussions can be made that the effectiveness of the microporation technology (Osdol, Rims, & Pinsky, 2008)

### **3.7 Polysaccharide-based vaccines**

These are also introduced but lacked in immunogenicity conjugation of polysaccharide with a toxic antigen carrier renders activated T-cell Dependent antigens. As a result, the overall immunogenicity improves and the efficacy of the vaccine is enhanced. Including Prevnar this approach has led to the development of other polysaccharide the success of conjugate vaccines (Jonathan and Jeffrey, 2008) a quadri-valent meningococcal vaccine licensed in the U.S. in 2004.”(p. 36) a 7-valent pneumococcal conjugate vaccine approved in the U.S. in 2000, and Menactra (Sanofi- Pasteur). Some form of genetic engineering that we predict that the development of virtually all vaccines licensed from this point forward will involve. Allowing manipulation of genes prior to rescue or regeneration of infectious organisms in culture the entire viral genomes can now be cloned into bacterial or yeast vectors. The rapid custom design of organisms for using in vaccines which has been enabled these methods. The virus DNA can be modified or parts of it can be omitted out to yield safer DNA strand. Using plasmid DNA instead of using diverse organisms or strains has become more common now-a-days. Currently, Influenza vaccines are developed by mainly 2 methods involving random and molecular methods where desired vaccine strands are generated by trial and error and strictly controlling the production of the strands respectively. The molecular method is preferred as it gives the scope to eliminate several steps in the production process. Modified viruses are being used in cases of treatment of other viral diseases where they can cause immunogenic response towards the specified virus types. For instance, genetically modified adenoviruses are stripped off from the virulent genes and are used against other virus types such as HIV, Ebola and malaria virus. Scientists have found new ways to deal with viruses. Multiple proteins or vaccine cocktails can be introduced to treat multiple viruses in one host. Moreover, multiple types of vaccines made from the same type of virus with different type of proteins can show immunogenic property

against a number of viruses (Scarponi, Nassori, & Pavani, 2009) Instead of using live virus (Scarponi, Nasorri, & Pavani, 2009). Virus-like particles do not contain the necessary material to replicate (VLPs) are self-assembling constructs that express a viral antigen. Vaccines can be administered via different routes to greatly increase the effectiveness of the vaccines. Immunization action coalition has regulations to administer vaccines in the human body.

Among the delivery systems of the vaccines, for a long time transdermal drug delivery has been used. It provides advantages in certain aspects- controlled release of the drug, reduced first-pass effect, prolonged duration of the steady-state level of the drug.

### **3.8 Bird flu vaccine**

The studies that has been made so that expected vaccine strategies H5N1 can be identified as one of the most pathogenic constriction of the avian flu, the currently proposed technologies insufficient for rapid mass vaccination. H5N1 antigen within the antigen presenting cell (APC)-rich that is epidermal layer of the human skin in order to deliver the immunogen. The ability of the electrospinning method to organize a nanocomposite nonwoven mat which effectively encapsulates a peptide at derived from the H5-hemagglutinin (HA) and contains its immunoreactivity through the process now which the study examines. To assure immune reactivity after has exposed of HA peptide to the conditions encountered in developing electrospun nanocomposite mats and just to quantitate levels of the immunogen on silicon wafer mark has been propose to employ for transdermal delivery on which the assay that have employed opt immune blotting with a slot blot apparatus in this process has permitted. Incorporation of numerous epitopes by a preferred antigen which has rapid and efficient way to create the huge supplies of effective bird flu vaccines, in such case of its utilization and cost effectiveness which attempts the utilization of epitopes.

Influenza vaccination Pandemrix: GlaxoSmithKline in Europe is manufacturer of Pandemrix was specifically produced for pandemic 2009 H1N1 influenza which is oil in water emulsion with adjuvant ASO3

Pandemrix emulsion and suspension for influenza vaccine (H1N1)v contains split virion – inactivated and adjuvanted. As an excipient it contains 5 microgram of Thimerosal

( Glaxosmithkline UK,2013).

### **3.9 Ebola vaccine**

Ebola Vaccine Team which has developed an opportunity in order to discuss and address many important issues which are related to. To the broader aspects of global infectious disease prevention and control several key lessons, however, are generalized.

Firstly, potential and ingenious funding strategies are required to ensure vaccines move efficiently from discovery and research through clinical trials and licensure to manufacturing as well as delivery, when ongoing the recent market-driven approach for vaccine development is not adequate to guard impoverished populations from emergent infectious diseases of pandemic or epidemic.

Manufacturer's profitability for vaccine is not assured. Secondly, enhanced international coordination and transparency for Ebola epidemic illustrates the need of West Africa, and approaching international regulatory outcomes for licensure of new vaccines particularly regarding which are approval processes for doing clinical trials in developing countries. Besides this process to identify community trust needs in advance of a crisis situation, better readiness designs that need to be in place. Now the west Africa Ebola epidemic accents that need to further strengthen disease surveillance systems in the region and in other geographical areas within the continent and globally and finally. Several Ebola vaccines were in preclinical growth, owing to solid expenditures by many, the pharmaceutical industry, government agencies and private foundations at the onset of the Ebola epidemic in West Africa. But delivery of efficacious licensed products to be contrived areas has yet to

occur before work on product development platforms allowed relatively rapid progress to clinical trials for many vaccine candidates. On the feasibility and suitability of study plans to evaluate efficacy more quickly, validity assessment of these vaccines would have been possible if those had been evaluated for initial safety and immunogenicity in phase 1–2 studies before the onset of the outbreak, and if there had been better consensus prior to the epidemic (Moser, Amacker, Kammer, Rasi, Westerfield, & Zurbriggen, 2012). It is required to identify a developed method which emerges coronaviruses, such as severe acute respiratory syndrome coronavirus and middle Eastern respiratory syndrome coronavirus as well as other haemorrhagic fever viruses such as Marburg virus, Rift Valley fever virus, and Crimean-Congo haemorrhagic fever virus; emergent enteroviruses. Hendra virus and Nipah virus to ensure develop vaccines and other medical countermeasures for such pathogens that more robust research is initiated and supported (Amacker, Moser, Kammer, Rasi, & Westerfield, 2012).

Mainly, three nucleoproteins obtained from the Zaire strain of the Ebola virus which shows conserved domains, along with two glycoproteins that help in viral entry and mediated with Sudan/Gulu species which causative agent of hemorrhagic fever of Africa. To optimize expression in human cells, the Ebola virus GP inserts have been modified. Vaccine contains deletions in the transmembrane region of GP that were intended to eliminate potential cellular toxicity observed in the *in vitro* experiments using plasmids expressing the full-length wild-type GPs, the Ebola virus GP genes expressed by plasmid DNA constructs within cell (Westerfield et al. 2012).

### **3.10 Mechanisms of Virosome immunopotentiality**

Whether the epitopes of the antigen are set on the surface of the virosome (PeviPRO™) the type of the elicited immune response to virosome formulations is dependent on (Moser et al, 2007) or inside the virosome (PeviTER™) (Makidon, 2009). MHC II antigen presentation. PeviTER™ which are formulated antigens released *in vivo* not only a CD4+

and CD8+ positive response but are also capable to induce a strong cytotoxic T-cell response (CTL ), the antigen is degraded within endosomes of the cell and, thus, creates predominantly. Virosomal encapsulation the MHC I pathway as the antigen is generated in a natural way into the cytosol of the antigen presenting cell, that assures a proper presentation of the antigens through MHCI pathway.

Vaccines that are against hepatitis A (Epaxal<sup>®</sup>) and influenza (Inflexal<sup>®</sup>V)(Hartman, Kunzi, Herzog, & Lazar, 2009) have been validated the excellent characteristics of virosomes as an adjuvant as well as carrier system are now the registered. Together, over 45 countries, and more than 10 million patients have been immunized by these two vaccines are accepted in to date.

So, this new generation of vaccines gives additional advantages because the vaccines are effective even in immune-suppressed patients as well as in infants. Moreover, they have a high safety profile as enclosed viruses that do not replicate.

### **3.11 Polymers used on transdermal vaccine Delivery**

In a transdermal drug delivery system polymers are considered as the backbone where a fabricated multi layer polymeric laminates a drug reservoir or a drug polymer matrix and that is sandwiched between two polymeric layers. Outer layer prevents loss of a drug and the inner layer functions as an adhesive or rate controlling membrane.(Ramario & Diwan, 1998)

#### **3.11.1 Polymers used intransdermal patch**

The patches of transdermal vaccine may contain:

**A. Crosslinked polyethylene glycol network:** Patches containing protein can be delivered by PEG with isocyanate means of urethane allophanate bond to obtain polymers network which are capable of swelling matrix buffered saline or ethanol containing gels. (Bromberg, 1996)

- B. Acrylic acid matrices:** The patch is used with acrylic acid matrices along with plasticizers have been used to make drug polymer-matrix films for transdermal delivery. Eudragit RL PM, Eudragit S-100, Eudragit RS PM, and Eudragit E-100 (Costa, et al., 1997) u-dragit NE-40D (a copolymer of ethyl acrylate and methylmethacrylate), a non adhesive hydrophobic polymer, also has been used as a former matrix.
- C. Ethyl Cellulose and polyvinylpyrrolidone:** The patch of this delivery system contains EC and PVP matrix films with 30% dibutyl phthalate as a plasticizer. PVP acts as an antinucleating agent and ethyl cellulose enhances its release rate constants (Ramaro, 1998)
- D. Hydroxypropyl Methyl Cellulose:** In the design of patches propranolol hydrochloride is used. Adequate solubility of the drug can be obtained. (Guyot & Fawaz, 1998)
- E. Polyurethane:** The patch mostly used this type is polyether as polyurethane is prone to hydrolysis. Dirty or ugly scars can be removed by using polyurethane patches (Fitri, 2005)
- F. Acrylic polyisobutylene:** This is a silicone based adhesives are mostly used in the design of transdermal patches (Barnart & Pftiser, 1998). Selection of adhesives depends on number of factors which includes patch design, drug formulation and skin compatibility.

### 3.11.2. Polymers used in Needle

- A. Fabrication of Silicone microneedle:** Silicon and chromium deposition within the needle is made and lithographically patterned (20x20 arrays of 80 micrometer diameter dots with 150 micrometer center to center spacing). Microneedle fabrication is considered to be finished when the chromium masks become fully undercut and fell off the needle tips. (Wang, Park, & Allen, 2003)
- B. Polymer micromolds for solid needles:** were fabricated from silicone or polymer substrates. The needle is 8mm thick and applied vacuum at 90 degree for 1 hour to remove any air bubble (Park, Allen, & Wang, 2003)

- C. Fabrication of metal micro needles:** Fabricated by electrode position on to polymer or silicon micromolds sputter coated with Ti/Cu seed layer (Allen, Wang, & Park, 2003) On hollow micro needle the mold adjacent to the outer surface on which needle tip is covered with powdered coating tape to protect it from being electroplated (McAllister, 2000).
- D. Fabrication of Polymer microneedle:** This type of transdermal microneedle is made by polyglycolic acid, polylactic acid into the PDMS (Polydimethylsiloxane) at 120 degree to 250 degree celsius applying and peeling the molds off (McAllister, 2000).

## **Conclusion:**

In the formation and discovery of new medicinal agents, the former twenty five years have seen an outbreak. The prosperous implementations of many of these novel pharmaceuticals associated alterations in drug delivery systems have not only empowered, but have also allowed the formation of new medical treatments with prevailing drugs. One of the most important of these innovations the origination of transdermal delivery systems has become, which offered a number of benefits over the oral route. Significant impact this field has made on the administration of various pharmaceuticals; explore limitations of the current technology as well as discuss methods under research for overcoming those boundaries and the challenges beforehand, on this article, we tried to discuss. (Langer & Prausnitz, 2009). The word “transdermal” at [clinicaltrials.gov](http://clinicaltrials.gov) responded 456 entries on exploration of April 2011 (U.S National Institutes of Health 2011). For existing products that are available, the main focus of these tests is both novel products and novel delivery routes. These transdermal clinical trials involved the opportunity of some of which: Insulin patch, Sufentanil patch for pain of chronic cancer, cessation of smoking with a high dose of nicotine patch Varenicline for fast, metabolizers patch like available for post-menopausal women for depression in the elderly and cocaine addiction Estrogen and testosterone are Selegiline patch is used.

Though having some drawbacks, transdermal drug delivery gives lot of benefits competent of developing tolerant fitness and character connected with lifetime. Molecules which can be liberated through the skin, 1st and 2nd generation TDDS offer all these advantages though are limited in the scope. The capacity to transform TDDS in the close future and propose even more clinical benefits to the patient now Three Generations of Transdermal Delivery technologies that exists is doing advanced medical promotion to the technical field of medicine (Eady, Pop, & Maacgrath, 2008)

**Future work:**

This system at future will replace all sorts of anxiety and fear of needle injections in order to apply a soothing way to deliver drugs for a quick systemic circulation.. As well as research are taking places for the invention of new vaccines like 'Dendritic vaccine' which acts as an antitumor vaccine are on the way to develop in its full form has already been discussed on this review paper. The development of this delivery system is not static but on the way to create a dynamic revolution. More valid researches are going on for the development of this delivery system. And unusual death due to needle infection can be avoided by this method. By attaining such dimensions in the medical technology will diversify the knowledge, field of research, safe use of drug and innovation of new process as well as equipment.

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