Recent Advances in the Replacement of Invasive Method of Protein Drug Delivery-A Review

A project submitted

By

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To

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Dedication

Dedicated to My Parents, Family and Respected Teachers

Certification Statement

This is to certify that this project titled "Recent Advances in the Replacement of Invasive Method of Protein Drug Delivery – A Review" is submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University. My work has been done under the supervision of Nishat Jahan, Lecturer, BRAC University. Here I used the information and evidences provided in different journal articles, websites and books.

Signed,

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Countersigned by the supervisor,

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Acknowledgement

Firstly I thank the Almighty for giving me the courage and strength to do this project work. Without the blessing of Allah this project work would have never been possible.

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Abstract

In today's era the necessity of non-invasive protein drug delivery is increasing day by day. Most of the global pharmaceutical companies are showing encouraging progress in alternative technologies rather than invasive delivery. Needle free delivery could aid improved safety and compliance to the diabetic patients, mass vaccination by increasing ease and speed of delivery, decreasing cost and reducing pain associated with protein drug delivery. This article include the development technologies in the last decade which brought limelight strategies that hold some promise to turn non-injectable way of protein drug delivery mainly insulin and vaccines from theory to reality. A rigorous research effort has been undertaken worldwide to replace the authentic invasive delivery. Several research has been carried out to focus on different routes. Oral delivery of protein drug through azometric group and nanotechnology provide desirable result. Aerosol technology for pulmonary route and micro needle, iontophoresis for transdermal route gives hope to the world health sector. Many work has also been carried out for rectal and ocular routes. This article summarizes all the recent work which have been done based on different routes to replace the invasive way of protein/peptide delivery.

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Acronyms

1. PPs : Peyer's Patches
2. FAE: Follicle Associated Epithelium
3. LCs : Langerhans Cells
4. TLR: Toll like Receptor
5. ASC: Anti-body Secreting Cell
6. PDMS: Poly Dimethyl Siloxane
7. CMIS: Common Mucosal Immune System
8. POELE: Polyoxyethylene-9-lauryl Ether
9. FDA: Food And Drug Administration
10. IIN: International Nonproprietary Name
11. GI: Gastrointestinal
12. UV: Ultraviolet
13. IgA: Immunoglobulin A
14. IgG: Immunoglobulin G
15. WHO: World Health Organization
16. IM: Intra Muscular
17. IV: Intravenous
18. MHIA: Monomeric Human Insulin Analogue
19. UCP: Ultraviolet Curable Polymer
20. CFSM: Conventional Film Shaking Method
21. MRA: Mannose Receptor Agonist
22. SLN: Solitary Lymphoid Nodules

1. Introduction

There are many techniques of drug delivery invented over the time. Non-invasive route of drug delivery is one of the most preferred ways of drug delivery. Non-invasive delivery means administration of drug that does not involve the making of relatively large incision of instrument into the patient which includes intramuscular (IM), intravenous (IV), subcutaneous route of administration and others (M, Al-Bataineh, Lweesy, & Fraiwan, 2012) . Non-invasive route of delivery is particularly important for therapies which are protein and gene based, for instance the delivery of insulin and vaccine where non-invasive routes are not fully invented (Shivanand, 2010). The most needed protein based drugs are now insulin and vaccine (M, Al-Bataineh, Lweesy, & Fwaiwan, 2012). It is fund from the research of WHO that from 366 million of people three quarter are in the risk with Type 2 diabetics worldwide. World Health Organization (WHO) expects the numbers will be double and rise up to over 500 million by 2030 (S, Chandra, & Sharma, 2006). The recent advances for replacing invasive route of insulin and vaccine delivery include: oral, transdermal, nasal, pulmonary and ocular and will be discussed in this review. This will provide a foundation for future development work on the area of invasive drug delivery (M, Al-Bataineh, Lweesy, & Fwaiwan, 2012).

1. Rationale

There are many valid reasons to replace invasive method of protein based drug delivery such as:

- Patient compliance
- Needle stick Injury
- Disposal problem
- Need experienced person to administer
- Cold-chain

2.1 Patient compliance: The traditional protein drug delivery is dependent on needle based delivery. The needles can penetrate up to epidermis to dermis where

nerve vessels are present and causes damage of nerve tissue (Morcol, Nagappan, Nerenbaum, Mitchell, & Bell, 2004). As a consequence, the insertion of needle into skin is perceived as painful and there arises a necessity to replace the invasive method. Beside this, sometimes inflammation also occurs at the site of administration (Morcol, Nagappan, Nerenbaum, Mitchell, & Bell, 2004).

2.2 Needle stick injury and contamination: Needle stick injuries are basically inflammation or wounds which occur due to needles that accidentally puncture the skin. In case of insulin delivery needle stick injuries are hazardous because of hypodermic syringes. These injuries can occur at any time with the diabetic patient (Niclas & Roxhed, 2007). Another life-saving medication is vaccine which acts as preventive agent against many life threatening diseases such as polio, cholera, influenza etc. Accidental punctures by contaminated needles can inject hazardous fluids into the body through the skin. There is a high risk of contamination for injection of hazardous drugs (Niclas & Roxhed, 2007). The risk of these injections getting in contact with infectious fluids, especially blood, is by far the greatest concern. Even small amounts of infectious fluid can spread certain diseases effectively (Finkel, Chark, & Cebeddu, 2009).

2.3 Disposal problem: Insulin therapy is needle based and most of the time disposal of needle creates problem like transmission of diseases. Proper disposal procedure is absent in many places so there is a high risk of transmission of blood-borne pathogens (Canadian Center for Occupational Health and Safety, 2004).

2.4 Need experienced person to administer: Insulin have to be administered to a proper depth with an accurate force and for this a trained person is required (Morcol, Nagappan, Nerenbaum, Mitchell, & Bell, 2004).Sometimes experienced person may not be available leading to patient anxiety which can be avoided by micro-needle patches that can be easily self-administered (Morcol, Nagappan, Nerenbaum, Mitchell, & Bell, 2004).

2.5 Cold chain: Many protein drugs especially some vaccine must be maintained within specific temperature ranges from (2 to 8 ℃) to maintain potency. For example some vaccine such as oral polio vaccine, in some cases cold chain or a temperature-controlled supply is a problem. An unbroken cold chain is the composition of several activities such as storage, distribution in a given temperature range for vaccine (Gawkrodger, 2002).

2. Different Noninvasive routes and their application in protein based drug delivery

There are several classes of drugs that should be administered invasively for getting the desired effect. As mentioned earlier, the drugs are mainly protein based product, for instance, insulin and vaccine. Some of the routes of delivery that are being investigated for replacing the invasive route of administration of these drugs are as follows:

- Oral Route
- Transdermal Route
- Pulmonary Route
- Nasal Route
- Rectal Route
- Ocular Route

3.1 Oral Route

This is the oldest route which has been used for conventional and novel drug delivery. The two main reasons for considering this route as the highly preferred one are the ease of administration and the high acceptability and comfort of the patients (Chase, Garg, & Md).

From the very beginning of the invention of exogenous insulin therapy, oral route is the most desired because it is pain free and also it has minimal chance of contamination or infection (Finkel, Chark, & Cebeddu, 2009). Another important benefit of oral insulin therapy is that before entering the systemic circulation, it reaches to the liver by portal

circulation. This route is similar to the physiological pathway of insulin secretion which decreases the glucose secretion and prevent hyperglycemic effect (Finkel, Chark, & Cebeddu, 2009).

Nevertheless, the oral administration is a challenge because of degradation of insulin by the acidic pH of the stomach and the presence of digestive enzyme/micro flora in the intestine (Morcol, Nagappan, Nerenbaum, Mitchell, & Bell, 2004). There are two apparent difficulties in oral administration of insulin; inactivation of insulin by digestive enzyme and another is lack of information about the mechanism of long chain amino acids (Morcol, Nagappan, Nerenbaum, Mitchell, & Bell, 2004).

However, new techniques for oral delivery are being investigated like coating by polymer of azometric group, nanotechnology and polymeric system targeted delivery system which have given promising results (Saffran, Kumar, Savariar, Burnham, Williams, & Neckers, 2001).

3.1.1 Polymer of azometric group

Peptide drugs can be protected from the degradation of digestive enzyme by coating the drug with an impermeable polymer film. In this technique, the drug is coated with polymer cross linked with azometric group forming impervious film that protect the drug from digestive enzymes (Saffran, Kumar, Savariar, Burnham, Williams, & Neckers, 2001). When the drug enters into large intestine the intestinal micro flora only reduce the azo bonds and breakdown the cross link, thus degrading the polymer only and the unaltered drug releases into the lumen of the colon for absorption and give therapeutic effect (Saffran, Kumar, Savariar, Burnham, Williams, & Neckers, 2001).

3.1.2 Nanotechnology

Nanotechnology is another latest way of oral administration of insulin and other peptide drugs. Nano particles is used as a career system for drug delivery (Sarmento & Pansky, 2007). The range of nanoparticles is about 100 nanometers and those are highly stable and the feasible incorporation of many hydrophilic and hydrophobic substance can release the drug in controlled rate with enhanced bioavailability with a specificity (Sarmento & Pansky,

2007). Polymers that are used for nanotechnology are polyacrylic acid polymer polylactides, alginate, chitosan polymer etc (Sarmento & Pansky, 2007). Inhibitory action against proteolytic enzyme can obtain by the use of novel polymers. Novel polymer also provide reasonable mucoadhesivity (Lowman, Morishita, Nagai, & Mkajita, 1999). Mucoadhesivity refers to the adhesion between polymeric carriers and the mucosa and is exhibited by certain polymers, which became adhesive upon hydration (K.Nakamura, R.J.Murray,J.I Joseph, N.A.Peppas et al, 2003).The binding of hydrophilic polymers, such as polyacrylates, cellulose derivatives and chitosan derivatives to biological surface is based on hydrogen bonding and ionic interaction. In the last few years, a large number of mucoadhesive system have been developed, including hydrogel-composite-based system lipid-based nano carriers and thiolated polymer system (Foss, Goto, Morishita, & Peppas, 2003). The feasibility of systemic insulin delivery by oral route using graft copolymer networks of methacrylic acid-g-ethylene glycol has been studied. The smaller sized nanoparticle insulin loaded polymer showed a rapid burst-type insulin release and higher insulin absorption (Foss, Goto, Morishita, & Peppas, 2003).

Another important protein based life-saving preventive medication is vaccine. For vaccine administration the most beneficial route compared to other route is oral delivery (Garinot, et al., 2007).Vaccines that have been developed for oral administration include viral vaccines against polio and rotavirus, bacterial vaccines against typhoid fever and cholera and so on (Tacket, Pasetti, Edlman, Howard, & Steattifield, 2004) . The problem in oral administration of vaccine is the production of low immune response. The reason of low immune response was premature degradation of the antigen in the gastrointestinal (GI) tract (Garinot, et al., 2007) . This problem is being addressed through encapsulation of the vaccine in a particulate system. It has been proved that oral immunization by antigen-loaded micro particles produce mucosal IgA and systemic IgG antibodies response which provide complete immunization (Robinson, Chamberlain, Schofield, Wells, & Page, 1997). Along with that this system protect the antigen from the harsh environment of the GI tract. M cell proactively take the nano carriers which play a key role in immune response. Besides this biodegradable particles give the advantage of sustained release of antigen, thus increasing the contact time between antigen and immune cell (Information sheet observed rate of vaccine reactions polio vaccines, 2014). As a result a favorable environment is created which helps in

effective immune response. The peyer's patches (PPs) structure contains immune cell of the intestinal mucosa. Follicle Associated Epithelium (FAE) cell is composed of enterocytes and M cell. This FAE is responsible for the separation of mucosa associated lymphoid tissue from the lumen (Robinson, Chamberlain, Schofield, Wells, & Page, 1997). It has been considered that M cell is responsible for entrance for pathogen to invade the body. Particularly M cell is adopted to antigen by three ways. The first one is by favoring the antigen interaction with apical membrane (Garinot, et al., 2007). This process is optimized through antigen endocytosis. Second process is by shortening the facility of antigen access to the basolateral compartment. Third one is by quick and straightforward interaction with immune cell and antigen presenting cell at the basal side (Robinson, Chamberlain, Schofield, Wells, & Page, 1997).

Oral route is the most effective and convenient routes of drug delivery and many research has been done regarding insulin and vaccine delivery. Comparing both insulin and vaccine delivery, it has been shown that oral insulin provide medication like normal physiology. The mechanism of action of orally administered insulin is similar to the physiological secretion of insulin. So oral route could be an ideal way of noninvasive insulin delivery.

2.2. Transdermal Route

The skin is the largest organ of human body which covers the entire external surface of the human body. Skin is the main part which involves in the interaction with the environment. It serves as a physical protective barrier which provides protection of internal tissues from exposure to trauma and ultraviolet (UV) radiation (Gawkrodger, 2002) . It also regulates body temperature, prevents fluid loss and gives protection from toxins as well as from bacteria. Along with that, skin works to convey sensory information to the nervous system and provides immunologic information to the immune system (Niclas & Roxhed, 2007). Different layers of skin have mentioned in the figure 1.

The skin can be divided into three parts:

1. Superficial Epidermis
2. Hypodermis
3. Dermis

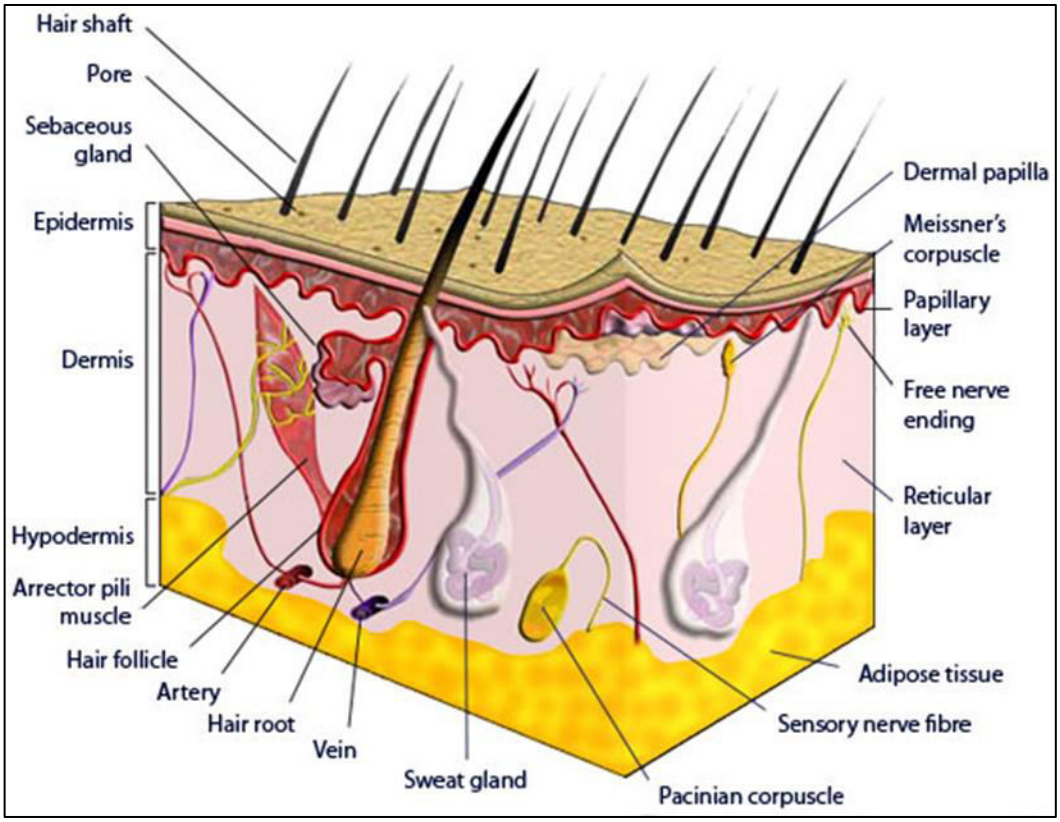

Figure: Cross-section of human skin (Virtual Medical Center, 2016)

3.2.1. Transdermal insulin delivery using piston-shaped PZT Transducer:

Transdermal delivery of insulin is a common route of drug delivery in case of the Type 1 diabetic patient (M, Al-Bataineh, Lweesy, & Fraiwan, 2012).Transdermal delivery of insulin by using piston-shaped transducer can improve the lifestyle of Type 1 diabetic patient. It is preferable over the traditional invasive painful subcutaneous insulin injection (M, Al-Bataineh, Lweesy, & Fwaiwan, 2012). It is proved by various studies that light weight compact cymbal transducer immediately decrease the blood glucose level. Piston-shaped ultrasound transducer operating in a frequency range 100-1000 kHz. The transducer housed by using silicone adhesive which includes a reservoir to hold insulin during *in vivo* delivery (M, Al-Bataineh, Lweesy, & Fraiwan, 2012). Since the mechanism of insulin delivery across the skin is not yet known, the various ranges of frequency of transducer have the capability to increase the permeability of the outer layer of the skin (Shivanand, 2010). Ultrasound energy of transducer facilitates the transportation of insulin across the condensed keratinocytes of stratum corneum

(Shivanand, 2010). Drug delivery across the skin is enhanced in low frequency due to the production of micro bubbles in the layer which allows water channel to be produced within the lipid bilayers. Convective, thermal effect, cavitation are the possible mechanism of transdermal insulin delivery through ultrasound transducer (M, Al-Bataineh, Lweesy, & Fraiwan, 2012).

Advantages of piston-shaped PZT transducer:

- Piston-shaped transducer delivers insulin in non-invasive manner across the skin which is pain free.
- Compared to other transducer method piston-shaped transducer is cheaper.
- Piston-shaped transducer is smaller in size and portable.
- Piston-shaped transducer have the capability to fabricate with predefined thickness for explicit frequency driving condition (M, Al-Bataineh, Lweesy, & Fraiwan, 2012).

3.2.2. Microneedles

Microneedle is one of the advanced ways of drug delivery. Micro needle is similar with traditional needle but these needles are fabricated on a micron scale which normally ranges from 1-100 microns in length and 1 micron in diameter (Dean, Fuller, & Osorio, 2003). Microneedle is considered a novel way of insulin delivery. Here the needles are so small that they can only penetrate the stratum corneum (Resik & Tejeda, 2010). The delivery of the medication from the device can be in the form of diffusion or through convection process. In convection method a force is applied to backing of the reservoir (Maaden, et al., 2014).This also proved that micro needle is compatible in local environment and in terms of toxicity it is also safe (Maaden, et al., 2014).

Types of Micro needles: Based on fabrication method microneedles can be classified as follows:

Solid micro needle

Solid micro needles are used for the purpose of pretreatment. Solid micro-needle create for the pore formation on the skin. Sharp micro needles penetrate the skin in order to make holes through which the drug can transport (Prausnitz, et al., 2008).After transportation they can give either systemic or local effect. The drugs can be applied over the skin surface through the pores using a drug loaded patch (Prausnitz, et al., 2008).

Hollow micro needle

Hollow microneedles are used for infusion of liquid drug for diffusion through needle. Through this microneedle we can administer medication on eye (Prausnitz, Park, & Kim, 2012).

Silicon micro needle

The process of fabrication of silicon micro needle depends on two points. One is the needle material and another is the geometry. Short silicon microneedle is prepared based on reactive ion etching with a chromium mask which is coupled with isotropic etching plasma etcher by silicon drying process (Prausnitz, Park, & Kim, 2012) .As an additional approach microneedles have been fabricated to serve as neural probes by dicing a silicon substrate to create a grid pattern of deep grooves (Prausnitz, Park, & Kim, 2012).

Metal Micro-needles

To produce metal microneedle three-dimensional laser ablation, laser cutting, wet etching, and metal electroplating method have been used. Several rows of solid metal microneedle are fabricated directly (Prausnitz, et al., 2008). Whereas two dimensional arrays of micro needles have been cut through stainless steel and titanium metal sheets. These sheets are bent at 90 degree angle (Prausnitz, et al., 2008).

Polymer micro-needle

Polymer micro needles have been made by photolithography under which optically curable polymer are also used (Prausnitz, Park, & Kim, 2012).These polymer then act a master structures for replication through molding. The (UCP) ultraviolet curable polymer SU-8 have been utilized extensively to fabricate the microneedles. The

mechanical strength of UV curable polymer is weaker than silicon or metal microneedles (Prausnitz, Park, & Kim, 2012).

Ceramic Micro-needles

Micro molding and sintering techniques are used to fabricate the ceramic microneedles (Shivanand, 2010).Solid ceramic microneedles were prepared from micro molding of alumina slurry using PDMS micro-needle mold and ceramic sintering (Shivanand, 2010).

Micro-needle rollers

The micro-needle roller was introduced to enhance skin restoration, facilitate collagen and increase permeability for drug application (Prausnitz, et al., 2008). It has been made by fabrication of planar micro-needle arrays and then applying them into the surface of cylindrical rollers (Prausnitz, et al., 2008).

Insulin is one of the protein drugs which by far have received the most attention to be administered by micro needles. Through different studies it is found that solid micro needle is an effective way of insulin delivery (Niclas & Roxhed, 2007). In studies solid microneedles were pressed into diabetic rat skin as a pretreatment. The application of topical insulin solution triggers the insulin delivery and reduction in blood glucose level (Niclas & Roxhed, 2007).

Dissolving microneedle encapsulating insulin has also been studied extensively in diabetic mice, dogs and in rats (Prausnitz, Park, & Kim, 2012). This approach also shows how to enable stable encapsulation of insulin and effective insulin delivery to reduce blood glucose level (Prausnitz, Park, & Kim, 2012).

Hollow micro needle design have also been developed for effective insulin delivery. Hollow microneedles have progressed into human studies of insulin delivery to type 1 diabetic subjects.

Advantages of micro-needle delivery:

- Less painful route
- It has increased pharmacokinetics effect almost two-fold.

- Avoid first-pass metabolism (Prausnitz, Park, & Kim, 2012).

Micro fabrication techniques provide a feasible alternative using microneedle. Microneedle can only penetrate a shallow part of skin <300 micrometer which offer the possibility to deliver vaccine into epidermis or dermis (Sebastiaan, et al., 2014). This area has the high possibility of immune response. Among the microneedles the hollow microneedle is attractive. These microneedles provide control and reformulate administration of the vaccine (Sebastiaan, et al., 2014).

Powder jet device also provides the needle free administration of vaccine into the epidermis layer of the skin. The recent research has been provided enough proof that transdermal route is the most effective way of protein drug delivery. The abundance and superficial location of LCs and their potential antigen presenting cell activity make the transdermal route an attractive choice for vaccine delivery (Dean, Fuller, & Osorio, 2003).

3.2.3. Iontophoresis

In recent years, iontophoresis is one of the most advanced technologies that have been developed which overcome all the obstacles of transdermal protein drug delivery (Borkute, Sivaprasad, & Panchagnula, 2004). From the beginning of transdermal insulin delivery one of the problems was low skin permeability. Iontophoresis is an advanced technique which enhance the transdermal delivery of the compound through the skin with the application of small electric current (O, Pillai, S.D. Borkute,et al, 2003). Iontophoresis works with the mechanism of electro migration and electro-osmosis, resulting in the increase of the permeation of the compound. It also gives the option of a programmed drug delivery techniques that facilitates the transport of the compound across the skin (Nair, Pillai, & Panchagnula, 2004). This technique provides several advantages, for instance, non-invasive, continuous, as well as programmed complex dosing. Iontophoresis in combination with absorption enhancer and ultrasound transducer has been tested which reduces the possible side effect (O, Pillai, S.D. Borkute,et al, 2003). It has been reported that transdermal insulin delivery through pulse-current iontophoresis, results the pulse permeation which correlates positively (Borkute, Sivaprasad, & Panchagnula, 2004).It has been found in research that administration of (MHIA) monomeric human insulin analogue (B9 Asp, B27 Glu) by iontopherosis through skin produce marked decrease in plasma glucose levels in the diabetic

rats (Nair, Pillai, & Panchagnula, 2004).Permeation of large peptide drug such as insulin has been achieved using combination strategy involving absorption enhancer with transdermal iontophoresis (Borkute, Sivaprasad, & Panchagnula, 2004).The major challenges in this field are the development of portable, cost-effective and suitable semisolid formulations that are compatible with skin as well as the device (O, Pillai, S.D. Borkute,et al, 2003).

3.3. Pulmonary Route:

In recent years, the need of peptide and protein drugs is increasing day by day. The recent research discovered many routes of non-invasive drug delivery and pulmonary route of drug delivery is one them (Finkel, Chark, & Cebeddu, 2009).Pulmonary route provides several advantages such as large absorptive area which is essential for effective systemic delivery. Another benefit of pulmonary route is extensive vasculature with easily permeable membrane. Furthermore, it has been proved that lung has low extracellular and intracellular enzyme activity.

One of the advantages of pulmonary route is that it is the oldest route which has been used for conventional and novel drug delivery (Huang & Wang, 2006).To administer protein/peptide drug suitable drug carrier is required. These carriers can be either liquid, solid or gaseous (Finkel, Chark, & Cebeddu, 2009). For the delivery of insulin proper incorporation of liposomes acts on drug release pattern. Here liposomes work for sustained delivery of drug which is very important for the chronic diseases such as diabetes (Finkel, Chark, & Cebeddu, 2009). Along with that liposomes mediated pulmonary drug delivery process has other advantages like liposome can increase drug retention time and can reduce extra pulmonary side effects which improve efficacy (Huang, Wang, 2006). It has been reported that the effect of formulation of mixed (dipalmitoylposphatidyl choline: cholesterol, 7:2) results in enhancement of the pulmonary bioavailability of insulin .Recently many company launched inhaled insulin for clinical trial as an INN drug (Hung,Wang, 2006).

In case of inhalation, insulin encapsulation of drug into liposomes need to be performed. Liposomal insulin can be prepared by two methods. The first one is (CFSM) conventional film shaking method (Hung,Wang, 2006).In this method, liquid film is formed at the bottom of the rotary vacuum evaporator where aqueous citric buffer containing insulin hydrate the

film. Through hydration and mechanical shaking multicellular vesicles can be formed (Hung,Wang, 2006). Another method of insulin delivery through liposomes is membrane destabilizing/detergent dialyzing method (Hung,Wang, 2006).

Pulmonary route has some unique parameters which makes high chance of immune response. Basically it is known that respiratory tract is the portal of entry of pathogens which causes pulmonary diseases (Tonnis, Kersten, Frijlink, Hinrichs, Boer, & Amorij, 2012) .The first vaccine was administered through pulmonary route in 1950. Currently there are two types of models under development (Tonnis, Kersten, Frijlink, Hinrichs, Boer, & Amorij, 2012).The first one is for the specific area of the lung like: trachea, bronchus, and bronchiole and for the alveoli in dosage form of dry powder (Tonnis, Kersten, Frijlink, Hinrichs, Boer, & Amorij, 2012).

Second form of vaccine delivery is the induction of immune cells by use of adjuvant delivery system. Here nanoparticles, liposomes or immune potentiates TLR (Toll like Receptor) mannose receptor agonist (MRA) or chemokine are used (F, Tinnis, kersten et al, 2012).

Both the insulin and vaccine delivery by pulmonary route is on the pre-clinical stage giving desirable result and there is a high chance that invasive route of peptide drug delivery can be replaced by pulmonary route.

3.4 Nasal Delivery:

Nasal mucosa has porous and thin endothelial basal membrane. This endothelial basal membrane has rapid blood flow as well as a vast absorption area about 150 cm (Yu, Zhao, Wu, & Zhang, 2004). Due to these characteristics, nasal route offer many advantages for drug delivery especially for insulin. Better effect is obtained when insulin is delivered in chitosan solution through nasal route (Yu, Zhao, Wu, & Zhang, 2004).Chitosan is a mucopolysaccharide obtained by DE acetylation of chitin in crustaceans such as crabs and shrimps. Chitosan is non-toxic and due to its biocompatibility it has been used as a pharmaceutical excipient (Yu, Zhao, Wu, & Zhang, 2004). This is proved that chitosan can enhance the absorption of poorly absorbable protein and peptide drugs (RD & Hill, 2002). It is found that there are two main effects of chitosan on nasal delivery of insulin. First one is the mucoadhesive property of chitosan that can reduce the clearance rate of drug from nasal

cavity (RD & Hill, 2002). As a result the contact time of chitosan delivery in the nasal epithelium is increased. The second effect is the interaction with positively charged amino group of chitosan with negatively charged sialic acid residues in mucus. As a result it causes the transient opening of the tight junction allowing large hydrophilic compound to be transported across the epithelium (Yu, Zhao, Wu, & Zhang, 2004).

Chitosan is an absorption enhancer so that there is a possibility that chitosan with other absorption enhancer can have synergistic effect (Yu, Zhao, Wu, & Zhang, 2004). It is found that increasing concentration of chitosan up to 1.5% (w/v) can increase the permeability of insulin across the nasal mucosa. Insulin formulation with chitosan solution is prepared by deionized water. These show higher relative pharmacological bioavailability (Yu, Zhao, Wu, & Zhang, 2004) . Chitosan solution's concentration, osmolality, and absorptivity have a significant effect on nasal delivery. Administration of insulin with chitosan solution is a possible way of insulin delivery on nasal route (RD & Hill, 2002).

Advantages of Nasal Route:

- Rapid absorption of the drug.
- It has vast absorption area about 150 cm.

Nasal vaccine delivery has emerged as a most beneficial route as an alternative of invasive route. Nasal route provides several advantages. This route is one of the most accessible routes which is highly vascularized (Huang, et al., 2004).Nasally administered vaccine including toxoid may also penetrate brain by olfactory region (Huang, et al., 2004). This nose to brain procedure may provide effective for certain vaccines. Beside this it can be provide a path for direct targeting organ drug in the case of neurological disease. (Partidos, CD et al, 2000). Often this process can create several adverse effect also. To solve this problem many adjuvant techniques are being investigated including liposomes, chitosan, microspheres and bacteria-derived particles (Partidos, CD et al, 2000). Human and other mammals have lymphatic tissue which enable to induce mucosal immune response in the airways. The upper airway structure also composed of palatine tonsils, lymph epithelial cell which stimulates the immune response (Partidos, CD et al, 2000). Recently it has also been identified that the nasal mucosa enriched with such specialized cells enhances local immune

response. One of the important benefits of nasal vaccine delivery is it provides response very rapidly, only few hours. This property of nasal delivery may work as a life-saver in the time of epidemics. For example the dry powder influenza vaccination through nasal route get significant IgA response (Partidos, CD et al, 2000).

Here nasal route can be an effective alternative for vaccine delivery. In many studies it has been proved that nasal vaccination give effective result without any significant side effect.

3.5. Rectal Route

Rectal route is also involved in the absorption of drug through gastrointestinal tract like oral route (RD & Hill, 2002). However rectal route bypasses the more proximal areas that are actively involved in digestion. As a result the drug is being unaffected by the diet, gastric or intestinal motility (RD & Hill, 2002).

Efforts have been made to administer insulin by rectal route in the form of suppository. From the anatomy of rectum we know that it has some Porto-systemic anastomoses in rectal vessels (Kantele, et al., 1998). The main responsibility of these vessels is to connect the portal system to the systemic circulation and allowing the absorbed drug to directly enter the systemic circulation (Kantele, et al., 1998). It is proved that insulin creates an adhesive interaction between the delivery system and the rectal mucosa. This interaction increases the residence time at the absorption sites (Kantele, et al., 1998). Nevertheless insulin administered through these route appears to provide high bioavailability and efficacy. The process for the improvement of the formulation is underway to increase the bioavailability (RD & Hill, 2002). The addition of absorption enhancers along with improved formulation can open up the mucoasdhesivity, hence result in improved rectal delivery (RD & Hill, 2002).

Mucosal delivery of any vaccine is the main target of current vaccine development. Rectal delivery of antigen is an alternative of gastrointestinal route which so far has not been explored (Kantele, et al., 1998). This is known that rectal mucosa is enriched of lymphoepithelial structures analogous to peyer's patches (Kantele, et al., 1998). Peyer's patches is the appendix with a small (SLN) solitary lymphoid nodules. These are follicles of

lymphoid tissue covered with a specialized epithelium containing M cell (Brayden, Jepson, & Baird, 2005). The several mucosal surface of the body is connected to each other by circulating lymphocytes which is recognized by the concept of common mucosal immune system (CMIS). So immune response in one mucosal site can lead to immune response in another, anatomically remote effector site (Kantele, et al., 1998). By This concept of mucosal immune response is done by transient appearance of anti-body secreting cell (ASC) in the peripheral blood and the immune response is found in distinct part of mucosal surface (Kantele, et al., 1998).It has been found that both mucosal and systemic immune response achieved against *Salmonella typhi* by rectal administration of antigen (Kantele, et al., 1998).

Rectal route has the same mechanism of absorption of drug like oral route. Rectal mucosa has the property like oral mucosa as well as these route avoid the first-pass metabolism.

That is why drug is being unaffected by diet and intestinal motility and will have better possibility for insulin delivery.

3.6. Ocular Route

Many scientists and research group have reported that systemic drug absorption can be possible via ocular route (Chiou, 1994). The efficient systemic absorption can be regarded as non-invasive way of protein drug delivery (Chiou, 1994). Eye tissue provides many advantages for instance, it is easier to administer rather than injection with fast absorption. Eye tissues are also less sensitive than other tissue to development of immunological reactions (Y.C.Lee, 2002). Along with that these route also bypasses the gastrointestinal and liver effect which are the main reason of low oral bioavailability of protein drug (Koevary, 2003). Recent research study has shown that conjunctival route of entry has important role for the penetration of drugs into the anterior segment (Yamamoto, Luo, & Dodda, 1989) . Besides it has been proved that a high molecular weight peptide like insulin can accumulate in the retina and optic nerve after topical application (Bartlett, Henson, & Atchison, 1999) . By supporting the contention topically applied drugs can reach the posterior segment which gives therapeutic effect (Y.C.Lee, 2002). Since the development of ophthalmic product has always been hindered by many obstructions such as local irritation, drainage, blinking and tearing and so on, to solve those problem several absorption enhancer have been evaluated for the delivery of insulin via ocular route (Bartlett, Henson, &

Atchison, 1999). Polyoxyethylene-9-lauryl ether (POELE), sodium glycholate, sodium taurocholate and sodium deoxycholates provide effective result. Commercially available Gelfoam®, an absorbable sponge is an ocular insert in the form of matrix system (Chiou, 1994) . This device gives a mentionable result for both *in vivo* and *in vitro* studies. The *in vivo* result have shown substantial improvement in insulin activity with desired therapeutic level. Gelfoam® ocular device have been developed as insulin carriers for systemic delivery of insulin (Y.C.Lee, 2002). This is must that all ocular device must be compatible with the iris, corneal, and conjunctival tissues. Ultimately, all the preclinical studies suggest the feasibility of delivering of insulin systemically via ocular route. However, the application of this approach still require further studies to be clinically useful (Koevary, 2003).

Table 1: Advantages and disadvantages of several route on insulin delivery:

Route	Advantages	Disadvantages	References
Oral: This is the oldest route which has been used for conventional and for novel drug delivery.	1. Ease of administration and patient comfort 2. Painless route of administration 3. No chance of contamination or infection 4. The mechanism of action of orally administered insulin is similar to the physiological secretion of insulin.	1. Inactivation of insulin by digestive enzyme 2. Degradation by acidic pH of the stomach 3. Lack of information about long chain amino acid.	(Kantele, et al., 1998) (Shivanand, 2010)

Pulmonary route: The recent research discovered many route of drug delivery and pulmonary route is one of them. Here the drug is administered through lung.	1. Large absorption area 2. Pulmonary route is extensively vascularized with easily permeable membrane. 3. Lung has low extracellular and intracellular enzyme activity	1. Dose estimation and dose reproducibility very difficult. 2. Need knowledge about device and administration protocols 3. Standardized technical information is also necessary.	(Hung,Wang, 2006)
Nasal Route: Nasal route is one of the most accessible route which is highly vascularized	1. Rapid blood flow 2. Vast absorption area almost 150 cm	1. Upper airway infection may increase the variability as may the extent of sensory irritation of nasal mucosa	(Yu, Zhao, Wu, & Zhang, 2004)
Transdermal Route: This is the most effective way of protein drug delivery. The abundance and	1. Insulin delivery by piston shaped across the skin which is pain free. 2. It has increased pharmacokinetics effect almost two fold	1. Chance of infection 2. Need trained personnel for administering by micro needle or transducer	(M, Al-Bataineh, Lweesy, & Fraiwan, Noninvasive Transdermal Insulin Delivery Using Pistion-shaped PZT Transducer:In vivo Rabbits Evaluation, 2012)

superficial location of LCs and their potential antigen presenting cell activity make the transdermal route attractive way for protein drug delivery.	3. Avoid first pass metabolism		(Morcol, Nagappan, Nerenbaum, Mitchell, & Bell, 2004)
Rectal Route: Rectal route has the same mechanism of absorption of drug like oral route. Rectal mucosa has the property like oral mucosa.	1. Avoid the first pass metabolism 2. The drug is being unaffected by diet and intestinal motility 3. Painless, safe way of administration	1. Long term administration can cause adverse reaction.	(Kantele, et al., 1998)
Ocular Route: Ocular route is one of	1. Easier way of administration than injectable.	1. Side effect found after long term administration	(Chiou, 1994)

route which has most efficient systemic absorption can be regarded as non-invasive way of protein drug delivery	2. Systemic absorption through ocular route is as fast as via injection	(3 month)	

Table 2: Advantages and disadvantages of different Non-invasive Vaccine Delivery:

Route	Advantages	Disadvantages	References
Oral	1. Ease and speed of delivery. 2. No risk of occupational or patient blood borne pathogen transmission 3. Has a good result with the use of oral polio vaccine.	1. Vaccine-associated paralytic poliomyelitis 2. Affected by gastrointestinal enzymes and acid.	(Garinot, et al., 2007) (Robinson, Chamberlain, Schofield, Wells, & Page, 1997)
Pulmonary	1. Ease of delivery 2. No pain	1. This delivery process needs more data to avoid unwanted transmission between subjects.	(Tonnis, Kersten, Frijlink, Hinrichs, Boer, & Amorij, 2012) (F, Tinnis, kersten et al, 2012)

		For example live attenuated measles vaccine.	
Nasal	1. No cold chain reaction with edible vaccine. 2. Low cost and ease of delivery 3. No risk of occupational and patient oriented blood-borne pathogen transmission. 4. Both systemic and mucosal immune response 5. Suitable for mass vaccination. 6. Faster onset and strong immune response.	1. Bell's palsy reaction found with nasal inactivated influenza (with LT adjuvant) 2. Narrow nasal entrance. 3. Complex geometry with narrow passages 4. Mucociliary clearance and nasal cycle. 5. Reformulation of vaccine required 6. Influences of nasal inflammation and obstruction.	(Yu, Zhao, Wu, & Zhang, 2004)

Table 3: Recent Marketed and Developed Formulation for Non-invasive Insulin Delivery:

Route	Trade name	Company/Developer and Researcher	Status	References
Oral	Eligen	Emisphere Technology,Inc	Phase II	(AR Sircar, 2002)
	Macurlin	Cortecs International / Provalis PLC	Phase II	(Emisphere, 2016)
	AI-401	AutoImmune, Inc/ Eli Lilly	Phase II	(Phoenix, 2016)
Nasal	Nasulin	Bentley Biotechnology Corp	Phase II	(Bentley, 2016)
		Vectura,Ltd/		(Vectura, 2016)
	QDose	MicroDose Technologies Inc	Phase I	
	ChiSys	West Pharmaceuticals Services	Phase I	(Edgar, 2016)
Pulmonary	Exubera	Nectar	Market available	(Pfizer, 2016)
	AERxiDMS	Therapeutics, Inc/Pfizer/Sanofi-Aventis SA	Phase III	(T. Qattrin,A.Belanger, et al, 2004)
		Adadigm Corp./Novo Nordisk A/S		(2016)

	HIIP	Alkermes.Inc/Eli Lilly		(Alkemers, 2016) (Lilly Corporation, 2016)
	Technosphere	PDC/Mannkind crop	Phase III	(Mankind, 2016)
	Aerodose	AeroGen,Inc/Nektar Therapeutics,Inc	Phase III	(Aerogen, 2016)
	Spiros	Dura Pharmaceuticals,Inc/Elan Corp	Phase IIIa	(Prnewswire, 2016)
	Insulin Inhaler	Kos Pharmaceuticals, Inc./Abbott Laboratories	Phase II	(Abbtt, 2016)
	MicroDose DPI	MicroDose Technologies, Inc/Novartis		(Microdose technology, 2016)
	ProMaxx	Epic Therapeutics, Inc/ Baxter Healthcare Corp	Phase IIa	(Alteatherapeutics, 2016)

			Phase I	
Transdermal	PassPort Transfersulin U-strip Macroflux	Altea Therapeutics IDEA AG Encapsulation System, Inc. Alza Corp	Phase I Phase I Phase II	(Alteatherapeutics, 2016) (Enesys, 2016) (Enesys, 2016) (Alza, 2016)

Table 4: Recent Marketed and Developed Formulation for Non-invasive Vaccine Delivery:

Routes	**Trade Names**	**Lead Product Candidates**	**Status**	**References**
Transdermal	Easy Vax™ Topical Patch	Influenza Traveler's Diarrhea	Phase II	(Iomai Corporation, 2016)
	Soluvia™	Trivalent inactivated seasonal influenza vaccine through micro needle	Phase III	(Vaxin, 2016) (J. Beran,A, Ambrozaitis et al, 2007)
	Macroflux®	Micro needle array	Phase III	(Macroflux,

		for influenza		2016)
Nasal	FluMist™	Live cold-adapted trivalent influenza via a prefilled single-use device.	Phase III	(S.A.Harper, K.Fukuda, N.J.Cox, C.B.Bridges, 2003)
Pulmonary	PREVNAR ®13	Pneumococcal 13-valent conjugate vaccine for pneumonia via nebulization	Marketed	(Pfizer, 2016)

4. Conclusion

Non-invasive delivery is desirable for many reasons including improved safety, better compliance, decreased pain (which is mainly important for children), easier and faster delivery, and likely reduced costs compared with invasive route of delivery. Needle phobia and stress encouraged scientist to investigate and exploit all promising routes for peptide drug delivery ranging from insulin to antigen delivery. Many approaches have been used to study certain strategy for different route for instance oral, pulmonary, transdermal, rectal and ocular. It has been found that some route provide more effective result for insulin whereas some for vaccine. From the beginning of the innovation oral route seems to be the most attractive way of drug delivery. In recent years, the development of innovative oral insulin delivery carriers that improve oral insulin absorption has shown some promising light on new horizon of oral insulin therapy. The main challenge of oral insulin delivery is the degradation of insulin by proteolytic enzyme of intestine. Using nanotechnology and polymer of azometric group the degradation of insulin can be prevented. Vaccine delivery through oral route has been already provided enough evidence that these route can be an alternative of invasive delivery. Comparing oral route for insulin and vaccine delivery, it has been found that oral route is more preferable for insulin delivery rather than vaccine. Oral insulin delivery works as the same way of physiological secretion of insulin. Another route that has been studied which showed remarkable result of both insulin and vaccine delivery is transdermal route. Through transdermal route many techniques and technology has been studied which gives satisfying result, although more clinical and post marketed surveillance is need. Ultrasound transducer, iontophoresis, micro needle technology reduce the human suffering. Microneedle technology can be an attractive alternative of invasive route for both insulin and vaccine delivery. Microneedle in the form of patch have been observed as a potential carrier for the delivery of peptide/protein drugs. Various research reports confirmed that microneedles can be the promising carrier for enhancing the permeation deep into the systemic circulation and providing painless, effective result for insulin delivery. Although micro needles have not yet been used clinically in immunization, several companies, such as Biovalv Technologies, Inc. which makes

Micro-Trans™ are currently working on developing the technology and are seeking FDA approval for their product. On the basis of the recent research it can be declared that transdermal route can be the best alternative of invasive method. In addition to other route pulmonary route can be an alternative of invasive delivery. Significant progress has been reported in recent years in the delivery of insulin using pulmonary route rather than vaccine. Pulmonary insulin Exubera® got market approval which will be in the market within few years. In recent research it has been shown that nasal route can also be preferred for insulin and vaccine delivery. Severally nasally delivered vaccine have been developed. One such vaccine is the live attenuated cold-adapted trivalent influenza vaccine FliMists™ which is administered via a prefilled single-use device that sprays vaccine into nostrils and was licensed by FDA in 2003 .The pharmacokinetic profile of intranasal insulin is similar to that achieved with intravenous injection. This route bears a close resemblance to the pulsatile pattern of endogenous insulin secretion during meal time. Another painless way of drug delivery is the rectal route. The efficacy and bioavailability of rectally delivered drug remained low compared with those delivered by invasive route. The irreproducible bioavailability and special storage condition required are also great obstacles for protein drug delivery by rectal route. In addition to other routes ocular route has great possibility to become effective way of non-invasive protein delivery. Still this route is not fully advantageous. . Non- invasive route of drug delivery is one of the most core demand in the health sector now-a-days. There are many possible ways of replacing invasive route which will resulting the improved life style.

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