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REVIEW ARTICLE

Transdermal Immunization: A Recent Tool for Immunization

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ABSTRACT:

Vaccination is one of the most powerful tools available in the ongoing battle against infectious agents. Virtually all recommended immunizations require parenteral administration, and many require a series of injections, therefore, new vaccine delivery methods, specifically alternatives to injections, are being sought. Transdermal immunization (TI) offers a new method for the delivery of vaccines, that relies on the application of antigen with adjuvant onto the outer layer of the skin and subsequent delivery to underlying Langerhans cells that serve as antigen-presenting cells. TI is a needle-free method of vaccine delivery that, will decrease the risk of needle-borne diseases, improve access to vaccination by eliminating the need for trained personnel and sterile equipment, and possibly provide a simple means for multivalent- or multiple boosting immunizations. This review presents various novel approaches for TI that is used alternative to parenteral immunizations.

KEYWORDS: Transdermal immunization, Langerhans cells, Skin, Vaccination.

INTRODUCTION:

Vaccination against infectious diseases is the major achievement of modern preventive medicine. The ultimate goal of vaccination is the stimulation of a specific immune response and the induction of a long-lasting immunologic memory to protect against subsequent disease[1-2]. Presently, most vaccines available are for intramuscular administration, which could be traumatic and their proper administration requires sterile technique, skilled and trained personnel. Thus, a needle free/non-invasive vaccination technique has become a global priority[3].

LIMITATIONS OF AVAILABLE VACCINE TECHNOLOGY:

One of the major impediments to ensuring vaccine efficacy and compliance is that of delivery. Presently all recommended immunizations require parenteral administration and many require a series of injections. Although, injectable vaccines are effective and widely used yet they may have number of drawbacks: [1, 4-5]

- Pain associated with injection
- Risk of accidental needle stick
- Spread of disease, especially in developing countries due to reusing needles
- Trauma, especially in infants
- Proper administration requires sterile technique, skill and trained personnel
- Expense in administration and disposal
- Stability and need of cold chain
- High cost

To make vaccine more economical and increasing compliance, vaccine should work after a single immunization using needle-free or non-invasive delivery systems. For optimal vaccination many factors can be considered such as: route of delivery, targeting of immune cell compartments and stimulation of humoral and cellular immunity[6-7].

There are several sites in which a vaccine can be administered including the muscle, mucosal surfaces and skin. Until the turn of century, the skin was thought to be impermeable. However, this view has changed and the progress achieved in this area clearly demonstrates that skin is a complex organ that may be one of the best sites for vaccination. Among the common routes of parenteral immunization, skin is the only site that shows potential as an immune organ.

TRANSDERMAL IMMUNIZATION (TI):

TI, a form of non-invasive vaccination involves the topical application of vaccine antigens to the skin that can elicit systemic antibody and T-cell responses. It is an innovative technique, having both practical and immunological merits requiring simple introduction of antigens to the host. The immunological implications of TI are potentially even more

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profound, as this technique appears to target highly accessible antigen presenting cells (APCs) in the skin that can be exploited for a variety of immune outcomes[8-9]. It could turn out to be a highly promising and advantageous route for vaccination with a variety of antigens to induce potent and functional immune responses. Thus, this new method may significantly impact both the delivery of vaccines and open a new avenue for manipulation of the immune response[10].

The feasibility of TI with protein-based antigen was first demonstrated by Glenn[4] *et al.* who used the term 'transcutaneous immunization (TCI). TCI was found to be aided by the use of adjuvant(s), such as cholera toxin (CT), heat-liable enterotoxin (ET) and their mutants which may induce potent immune responses when co-administered with antigen**.** Later on it was found that physical methods have limited success. Penetration enhancer reagents and topical adjuvants are not always desirable due to their toxicity. Thus, there is a need to develop new physical methods and topical adjuvants[11].

Advantages of TI [5]

- ❖ Target the antigen directly to the APCs of the skin
- ❖ Reduce the amount of antigens required for the immunization
- ❖ Sustained release
- ❖ Reduce the frequency of administration
- ❖ Patient compliance
- ❖ Self-administration is possible
- ❖ Eliminate accidental needle-stick
- ❖ Non-invasive zero order delivery
- ❖ Reduce the overall cost of immunization

Mechanism of TI:

The mechanisms involved in TI are yet to be fully characterized. The proposed hypothesis for the TI is the hydration of skin that allows superficial penetration of the stratum corneum (SC) by adjuvants. Throughout the epidermis, immune competent dendritic cells (DCs) called Langerhans cells (LCs), despite only composing 1% of the cell population, cover nearly 20% of the surface area through their horizontal orientation and long protrusions, which form a meshwork that allows them to uptake antigens that they encounter. LCs plays the chief role in the immune response to antigenic proteins in the skin. These cells phagocytose the antigen, and the adjuvants activate LCs, which then migrate to the draining lymph node where the antigen is presented to T-cells for the induction of an immune response (Fig. 1). During this process the LCs differentiate into DCs, which offer the antigen to naive CD^{4+} T-cells that have entered the lymph nodes through the high endothelial venules and finally initiate immune response[5, 8]. In addition to LCs, keratinocytes also play a role in the immunization process, which activated and synthesize a large number of cytokines involved in modulating the immune response and express intracellular adhesion molecules for various immune cells. In addition, the other two types of APCs in the skin (macrophages and lymphocytes) probably first require activation in order to present antigens and stimulate T-cells[12,13].

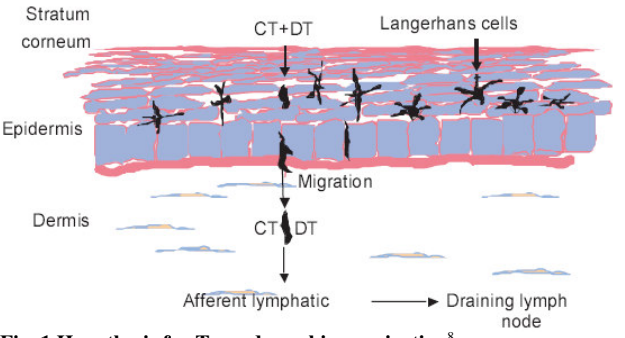

Fig. 1 Hypothesis for Transdermal immunization[8]

TCI protocols appear to be particularly promising by gaining access to skin resident APCs, which are highly efficient for the initiation of humoral and/or cellular immune responses. Hence, skin has been shown to represent a quite diverse organ for the manipulation of the immune system. Before the development of effective transdermal vaccination technique it is useful to review the structure and function of the skin.

NOVEL APPROACHES FOR TI:

Novel vaccine approaches comprised of various type of delivery systems and devices in order to deliver vaccines and target immune cell compartments of the skin. The various delivery devices consist of gene gun, jet injector, microneedles, microprojection arrays, electroporation, iontophoresis and sonophoresis etc. while delivery systems for effective vaccination are liposomes, modified liposomes, microcapsules, microparticles, nanoparticles, virus like particles etc.[7]. The ideal delivery systems/devices should be safe, non-toxic as well as non-immunogenic and also economical. It should be effective and reproducible for induction of proper immune responses against delivered antigen. Moreover such systems should be pharmaceutically acceptable, stable, biocompatible and amenable to patient.

Delivery Devices:

The skin is the most frequently used tissue for delivery devices due to the fact that it is easily accessible and monitored. Delivery device can be classified as instrument that uses brute force to deliver vaccine antigens through the SC into the skin. There are a wide range of delivery devices consisting of injection system, ballistics device as well as membrane de-stabilizers. Some important delivery devices are discussed below:

Gene gun:

The gene gun is essentially a miniature shotgun where gold beads act as small projectiles carrying DNA through the skin into and around cells. Since a gene gun delivers plasmids directly into cells, this circumvents degradation of DNA, increasing the efficiency of gene transfer[14]. Because of this direct introduction of plasmid DNA into cells immune responses are generated by picograms of plasmid DNA[15]. Some limitations of the gene gun are that it may be used only for DNA and it is very unlikely that it could be used to deliver proteins.

Jet injection:
Jet injection is a technique that uses air pressure to force liquid through the skin and around cells. One advantage with jet injectors is less tissue damage in comparison to conventional injection as the liquid follows the path of least resistance. This method has been used for intradermal, subcutaneous and intramuscular delivery of proteins as well as DNA[16,17].

Electroporation:
Electroporation is a method to transiently permeabilize a membrane by the application of a single or multiple short duration pulses. Electroporation *in vivo* is accomplished by placing naked DNA on the skin, followed by placement of calipers on the skin and pulsing the skin with electrical charges. It is a technique that has been used for several years to deliver DNA into cells *in vitro*; recently it has been used *in vivo* [18,19].

Iontophoration:
It is similar to electroporation, uses weaker electric fields for a longer period of time than the strong fields used in electroporation[20]. Iontophoresis is an electrically-assisted drug delivery technology that enables non-invasive, controlled administration of low molecular weight peptide therapeutics[21].

Sonophoration:
Sonophoration uses ultrasound waves to disorganize the SC lipids allowing the permeability of the skin to be increased. Several proteins such as insulin, IFN-γ and erythropoietin have been delivered through the skin in animal models. It is a relatively new technique that may be used for delivery of DNA and protein based vaccine antigens transdermally[22-23].

Photomechanical delivery:
This technique uses a laser pulse to transiently increase permeability of skin. High molecular weight dextrans were delivered through the skin making the delivery of proteins and DNA possible with this technique[24].

Microfabricated microneedles:
This technique has also been reported to overcome the barrier property of skin. These microneedles are approximately 150 µm long and create pathways across SC allowing transport of antigens. These are pointed projections fabricated into arrays that create conduits across the SC potentially allowing transport of DNA and proteins into the epidermis. Since these microneedles only penetrate through the SC and the viable epidermis, they do not reach nerve endings and are essentially painless[25,26].

Epidermal powder immunization (EPI):
This technology delivers antigens on 1.5 to 2.5 µm gold particles to the epidermis using a needle free powder delivery system[27]. The powder is delivered with the help of disposable or multiple dose system (powderject) that employs high velocity stream of helium into the skin. DNA or protein adsorbed on gold particles and formulated in the form of powder is filled in the cartridge and inserted into the delivery device. The pressure of helium gas controls depth of penetration of vaccine[28, 15].

Delivery Systems:
Various novel delivery systems have also been envisaged for safe and effective delivery of antigens thorough the skin. These delivery systems would provide controlled antigen release, immunoadjuvant properties and greater entrapment efficiency. The main delivery systems are discussed below:

Miroparticles/Nanoparticles:
Biodegradable micro/nanoparticles are suitable vehicles for delivery of the proteins and peptides to the APCs. These carriers offer numerous advantages like their potential ability for controlled release of encapsulated antigens and long lasting immune responses, efficient phagocytosis due to their particulate nature, and also capacity to induce T_H1 mediated cellular immune responses[29]. Various nanoparticles like gelatin, PLGA, chitosan, lipid microparticles have been used successfully for enhanced cellular uptake and induction of immune responses[30-32].

Liposomes:
Liposomes are colloidal particles containing concentric bimolecular layers and possess ability to encapsulate both polar and non-polar drugs. These lipid vesicles are usually made up of phospholipids and cholesterol. They can be prepared of various size, number of lamellae, structure and of different payloads. They are a versatile tool for delivery of large array of bioactives ranging from drugs to high molecular weight proteins and peptides. The amphipathic nature of liposomes may allow them to be used widely as a non-invasive delivery agent for vaccine antigens[33].

Four general mechanisms of modulation of skin permeability via liposomal carriers have been reported[34]. These include a) intact drug-laden vesicle penetration into the different strata of the skin; b) liposomes acting as penetration enhancers via their skin lipid-fluidizing property; c) direct carrier–skin drug exchange by 'collision complex transfer' between the drug intercalated in the liposomal bilayer and the surface phase of the SC; and d) liposome-mediated enhanced transdermal drug delivery via appendegal pathways.

However the results further obtained by various research groups about the effectiveness of liposomes for facilitating transdermal delivery were quite conflicting. The composition of the vesicles influences their physico-chemical characteristics such as size, charge, thermodynamic phase, lamellarity and bilayer elasticity. Thus, by modifying the structure and composition of liposomes we can have more versatile, safe and efficient tool for topical immunization. Addition of the surfactant provides flexible or ultradeformable property to the conventional liposomes (elastic liposomes) while addition of ethanol leads to enhanced skin penetration (ethosomes).

Elastic liposomes:

A novel class of modified liposome, containing an optimum amount of edge activator and providing it with a highly elastic nature, has been developed Transfersome® (http://IDEA AG-Science.htm). These carriers were first described by Cevc and Blume[35] (1992), and subsequently they have been the subject of numerous patents and literature reports.

Elastic liposomes (Transfersome®) are specially optimized, ultradeformable lipid supramolecular aggregates that have been claimed to penetrate and permeate the skin layers as intact vesicles to reach the systemic circulation. The elasticity possessed by these vesicles is the consequence of an edge activator (often a single-chain surfactant that enhances the deformability via lipid bilayer destabilization) incorporated within the phospholipid-based system. In most cases, the phospholipid and edge activator contents have been optimized to attain the desired deformable nature of the elastic liposomes, to increase elasticity and penetrability. These novel carriers are applied in the form of semi-dilute suspension, without occlusion and offer the efficient dermal and transcutaneous drug delivery of the high and low molecular weight substances[36-37].

Traditional liposomes are typically 100-400 nm in diameter and have a rigid structure. These are too large to fit within the intercellular lipid domains of the SC and to penetrate to the deeper layers of the epidermis. Due to the flexibility conferred on the vesicles by the surfactant molecules, elastic liposomes are claimed to be able to squeeze through channels one tenth of its diameter[38].

In the present context, a number of Transfersome® based products are at an advanced clinical trial stage, such as IDEA-033[39], which is expected to become the first truly effective topical analgesic for the effective management of osteoarthritis.

Salient features of elastic liposomes:

- Elastic liposomes can accommodate hydrophobic as well as hydrophilic moieties due to its infrastructure.
- Due to their deformable nature they can squeeze through narrow constriction without measurable loss and give better penetration of intact vesicles.
- They can act as a carrier for low as well as high molecular weight substances e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.
- They are made up of natural phospholipids hence are biocompatible and biodegradable.
- They have higher entrapment efficiency.
- They protect the encapsulated moieties from metabolic degradation.
- They act as depot hence release their contents slowly and gradually.
- They can be used for both systemic and topical delivery.
- They are easy to scale up.

Mechanism of modulation of skin permeability by elastic liposomes

Several studies investigated possible mechanisms by which elastic vesicles could improve skin delivery of drugs. Two mechanisms were proposed[40-42]. First, vesicles can act as drug carrier systems, whereby intact vesicles enter the SC carrying vesicle-bound drug molecules into the skin (mechanism 1). Second, vesicles can act as penetration enhancers, whereby vesicle bilayers enter the SC and subsequently modify the intercellular lipid lamellae. This will facilitate penetration of free drug molecules into and across the SC (mechanism 2).

The first mechanism was put forward by Cevc *et al.* for elastic liposomes[35,43,44]. They proposed that the driving force for the vesicles entering the skin is xerophobia (the tendency to avoid dry surroundings). The important difference between deformable liposomes and traditional liposomes is the high and stress-dependent adaptability of such deformable vesicles, which enables them alone to squeeze between the cells in the SC, despite the large average vesicle size. Thus, they can trespass the intact skin spontaneously, under the influence of the naturally occurring, *in vivo* transcutaneous hydration gradient, intact without permanent disintegration. Using confocal laser scanning microscopy (CLSM), Schatzlein and Cevc[45] further reported highly fluorescent interclusters and intercorneocyte pathways located within the intercellular lipid lamella of murine SC, which, according to them, could further act as virtual channels through which intact vesicles could penetrate.

A study by Verma[46] *et al*. utilising a CLSM technique, demonstrated that vesicular size greatly affects the transdermal potential of the prepared vesicular system. Their study indicated that larger vesicles (size $\geq$ 600 nm) were not able to deliver their contents into the deeper layers of the skin. These vesicles stay in/on the SC, and after drying they may form a layer of lipid, which may further strengthen the barrier property of the SC. Liposomes with a size $\leq$ 300 nm were able to deliver to some extent into the deeper layers of the skin. However, liposomes with size a $\leq$ 70 nm seemed to achieve the maximum depth. The exact mechanism of skin modulation by intact vesicle penetration is just a speculation that needs experimental corroboration.

When a suspension of Transfersome® vesicles is placed on the surface of the skin, the water evaporates from the skin surface and the vesicles start to dry out. Due to the strong hydrophilicity of major Transfersome® ingredients, the vesicles are attracted to the areas of higher water content in the narrow gaps between adjoining cells in the skin. The phenomenon, together with the vesicle's extreme ability to deform, enables each Transfersome® to temporarily open the pores through which water normally evaporates between the cells. This creates 20-30 nm wide pathways between the skin cells, two orders of magnitude wider than the original nanopores. Such newly activated inter-cellular passages can accommodate sufficiently deformable vesicles maintaining their integrity but changing their shape to fit the channel;

the calculated sequence in the above illustration highlights the process. Insufficiently deformable entities fail to pass through the channels. Along these said pathways in the horny layer, a Transfersome® reaches regions of high water content in the deeper skin layers. Subsequently, the vesicles that have crossed the skin barrier are distributed between the cells. Being too large to enter the blood vessels locally, a Transfersome® bypasses the cutaneous capillary bed and reaches the subcutaneous tissue. Ultimately, the vesicle may arrive into the systemic blood circulation *via* the fenestrated lymphatic system, which has openings (fenestrations) of sufficient width; most often, however, the vesicles applied locally are ultimately bio-processed and their building blocks are re-utilised in peripheral tissues below the application site[47].

Ethosomes:

Ethanolic liposomes or ethosomes are novel lipid based, non-invasive delivery carriers that enable biologically active agents to reach the deep skin layer and/or systemic circulation. These systems are mainly composed of phospholipids, a relatively high concentration of ethanol (20-50%) and water[37,42]. Previously it was generally thought that a high alcohol concentration lead to the destruction of lipid vesicular structure, owing to the interdigitating effect of alcohol on lipids. Later on Touitou[48] *et al*. demonstrated the coexistence of phospholipid vesicles with a high concentration of ethanol, leading to the formation of soft, malleable, highly fluid vesicles (ethosomes). They demonstrated the formation of vesicles using ^{31}P-NMR and paramagnetic ion NMR experiments.

Salient features of ethosomes

- Ethosomes provide a mode for passive non-invasive delivery.
- These carriers are suitable for hydrophilic, lipophilic molecules, peptides and other macromolecules.
- They can act as a carrier for low as well as high molecular weight substances e.g. analgesic, corticosteroids, sex hormone, insulin etc.
- They are biocompatible and biodegradable.
- Due to high ethanol content possess high entrapment efficiency.
- They possess high cell transfection efficiency.
- They may act as depot formulation hence sustained release is obtained.
- They can be applicable for topical as well as systemic delivery.
- Easy to scale up, as procedure is simple, does not involve lengthy procedure and unnecessary use of pharmaceutically unacceptable additives.

Mechanism of penetration of ethosomes

Ethanol is a well-known penetration enhancer and is commonly believed to act by affecting the intercellular region of the SC, thus enhancing permeation. This penetration enhancing effect of ethanol could be attributed to two factors: i) an increase in thermodynamic activity due to the evaporation of ethanol, known as 'push effect'; and ii) 'pull effect', in which the penetration of a drug molecule is increased due to a reduction by ethanol in the barrier property of the SC[49]. Ethanol encapsulated in lipid vesicles in the form of ethosomes provides fluidity to the ethosomal bilayers, and, when applied to the skin, it fluidizes the SC lipids and well demonstrated by differential scanning calorimetry[37].

In terms of the enhanced potential for the transdermal delivery of biologically active agents in ethosomal carriers, the exact mechanism of skin permeability modulation remains speculation. According to Touitou[50] *et al*. a synergistic mechanism between ethanol, vesicles and skin lipids exists, leading to an improved permeation profile. The proposed mechanism of ethosomal skin modulation lies in the interaction of ethanol with lipid molecules in the polar head group region, resulting in a reduction in the transition temperature (T_m) of SC lipids, thus enhancing their fluidity, leading to a disordered SC, which provides a potential site for soft, malleable ethosomes to penetrate more easily within the skin layers.

CONCLUSION:

TI is an innovative investigational technology, which promises to be an efficient and cost-effective means of immunizing patients. It not only allows ease of administration of vaccines, but may also elicit more potent immune responses than conventional needle injection given in equivalent doses due to the immunocompetence of epidermis along the skin border. Amongst various approaches for non-invasive immunization, vesicular approach is gaining wide acceptance for topical delivery. This is because they can act as carrier as well as exhibit adjuvant action, which help in boosting of the immune responses. The vesicular carriers including Elastic liposomes, Liposomes, Niosomes, Ethosomes etc. require no specially trained personnel and may avoid risk associated with needle prick. These carrier systems are intended to avoid the risks and complications associated with percutaneous delivery of vaccines (needles), to eliminate apprehension and pain associated with immunization, and to avoid the complications of reuse of disposable syringes and needles. It can be genuine approach by which discomfort related to all other routes such as cellular toxicity in case of intramuscular injection and degradation of antigen by oral route can be avoided.

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