Chapter 18
Transcutaneous Immunization

Teerawan Rattanapak, Camilla Foged, and Sarah Hook

18.1 Introduction

Vaccination is regarded as the most cost-effective approach for controlling infectious disease (Coudeville et al. 2005). Currently, the main routes of delivery for vaccines are via either the oral or parenteral routes. Delivery by injection has many drawbacks; for example, vaccinators require injection training and there is a risk of needle-borne diseases associated with improper disposal of needles (Miller and Pisani 1999; Aylward et al. 1995). As a consequence, needle-free immunization has been investigated and developed for the safety of the vaccinator, patient and community. Additionally, it is likely that compliance will be improved by decreasing or eliminating injection site pain (Brown et al. 2006). The non-invasive vaccination routes include oral, buccal, nasal, pulmonary, vaginal and topical routes. In this chapter vaccination via the skin, transcutaneous immunization will be reviewed.

18.2 The Skin

The skin provides the first barrier of protection against the invasion of pathogens into the body. The skin is composed of two main layers, the dermis and the epidermis, which are separated by the epidermal-dermal junction (Fig. 18.1). The dermis

T. Rattanapak • S. Hook (✉)
School of Pharmacy, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand
e-mail: ratte848@student.otago.ac.nz; sarah.hook@otago.ac.nz

C. Foged
Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen Ø, Denmark
e-mail: camilla.foged@sund.ku.dk

C. Foged et al. (eds.), *Subunit Vaccine Delivery*, Advances in Delivery Science and Technology, DOI 10.1007/978-1-4939-1417-3_18

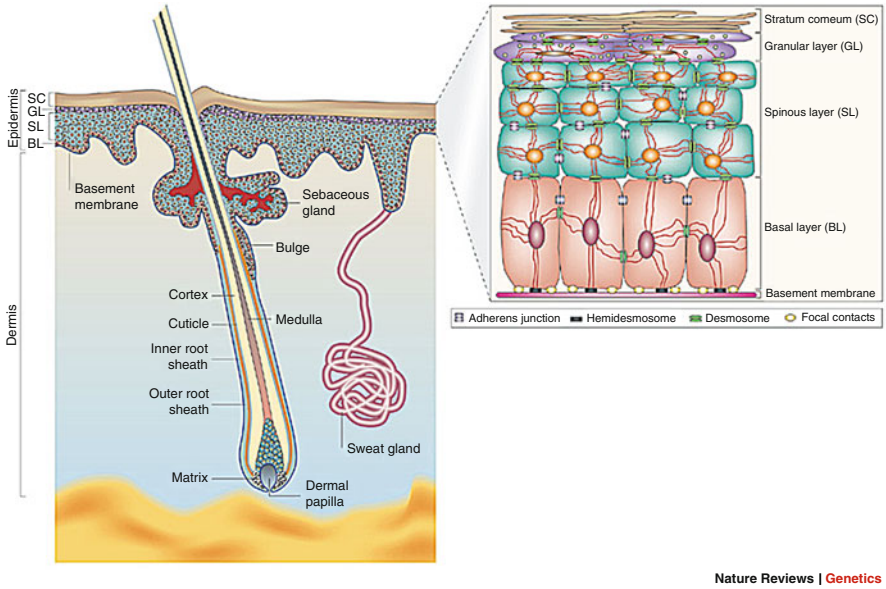

Fig. 18.1 The structure of human skin. The epidermis and dermis are separated by the basement membrane. The epidermis (*inset*) is composed of the stratum basale, the stratum spinosum, the stratum granulosum and the stratum corneum. Figure taken with permission from Fuchs and Raghavan (2002)

is made up of connective tissue, collagen, glycosaminoglycans and elastin. The dermis is a highly vascularized layer and provides the avascular upper layer, the epidermis, with nutrients. The epidermis is the most superficial layer of the skin consisting of keratinocytes and has a thickness of approximately 50–200 μm depending on the body region (Lambert and Laurent 2008).

The epidermis is divided into four different layers (Fig. 18.1). The stratum basale is a single layer of columnar basal cells which remain attached to the basement membrane. The cells begin to flatten and elongate in the stratum spinosum and the cells have lost their nuclei in the stratum granulosum. The stratum granulosum produces and organizes keratin proteins and water-proofing lipids. The stratum corneum (SC) is primarily composed of corneocytes (~90 %), which are flattened, dead, keratin-filled cells. These cells are surrounded by a cell envelope consisting of an inner layer of cross-linked proteins (cornified envelope proteins) and an outer layer of covalently bound lipid envelope (Menon 2002; Bouwstra et al. 2003; Proksch and Jensen 2008).

The SC resembles a brick wall. The corneocytes serve as the bricks and extracellular lipids as the mortar (Michaels et al. 1975). The densely packed and highly conformationally ordered arrangement of the SC results in low diffusion of drugs into skin. Thus, diffusion into the SC can be described as the rate limiting step and the main obstacle to transdermal delivery (Barry 2001).

18.3 Immune Surveillance in the Skin

Different skin layers contain different types of immune cell. $CD8^+$ T-cells and Langerhans cells (LCs) are found in the epidermis (Krueger and Stingl 1989), while the dermis contains various immune cells including macrophages, mast cells, dermal dendritic cells (DDCs), $CD4^+$ T-cells, γδ T-cells and natural killer T (NK T) cells (Nestle et al. 2009). The two key antigen-presenting cell (APC) subsets in the skin are the LCs and DDCs. These skin APCs possess the ability to take up and process antigen, migrate to draining lymph nodes and to present processed antigen to naïve T-cells (Glenn et al. 2003).

18.3.1 Langerhans Cells

LCs were first discovered by Paul Langerhans in 1868. LCs reside in the epidermis, where approximately 1,000 LCs are present per mm^2 (Flacher et al. 2010) of skin, equating to about 3–5 % of the total epidermal cells (Merad et al. 2008). LCs are surrounded by keratinocytes and the dendrites branching out from the LCs extend between individual keratinocytes (Pearton et al. 2010). Once activated, LCs disengage from the surrounding keratinocytes and migrate across the epidermal/dermal junction to the local draining lymph node (Dearman et al. 2004). LCs can be identified by their unique physical characteristics (presence of many dendrites), their location, the presence of Birbeck granules and high levels of expression of the C-type lectin langerin (CD207) (Valladeau et al. 2000).

LCs have been speculated to be the first APCs involved in capturing antigens delivered by transcutaneous immunization (TCI) due to their location in the epidermis. Kubo and colleagues (2009) found that LCs can extend their dendrites through tight junctions (TJ) and take up antigens via the dendrite tip. Romani et al. (2010) have postulated that the role of LCs in TCI will be dependent on several factors, such as the vaccination area, the amount of vaccine applied and the type of antigen and adjuvant used. For example, LCs were found to express toll-like receptor (TLR) 2, TLR4, and TLR9 but lack TLR7 (Mitsui et al. 2004). Hence, the type of adjuvant used in TCI should be taken into consideration when designing vaccine formulations to activate LCs. In addition, the site of vaccination has been shown to impact on LC activation. Wang et al. (2008) found LC activation was observed at the flank area but was absent in the ear. They suggested this was due to the SC in the flank area being much thicker than in the ear resulting in the vaccine accumulating in the upper skin layer leading to more opportunities for LCs to take up vaccine. More recently there have been conflicting reports on the role of LCs in stimulating effector immune responses and they have been reported to have an immunoregulatory function. In mice specifically depleted of LCs, contact hypersensitivity (CHS) responses were significantly augmented (Bobr et al. 2010). However in mice deficient in $CD207^+$ DDCs there was no difference in the CHS response (Honda et al. 2010). It can thus be concluded that LCs suppressed antigen-specific CHS responses (Bobr et al. 2010).

18.3.2 Dermal Dendritic Cells

The role of DDCs in TCI has been less studied due to their location in the dermis and the idea that therefore antigen uptake by DDCs would occur only rarely. However, recent evidence suggests that DDCs play a vital role in antigen-specific immune responses in the skin. Bursch et al. (2007b) found that LCs were not activated after epicutaneous immunization with a combination of peptide vaccine and adjuvant whereas DDCs migrated and accumulated in the dermis beneath the immunized area. In addition, surface expression of maturation makers was increased and DDCs migrated to draining lymph nodes stimulating T-cell proliferation.

DDCs reside in the dermis and are mostly found adjacent to the epidermal-dermis junction. Some DDCs cluster around hair follicles which has been suggested to facilitate contact with antigens that penetrate via hair follicles (Bursch et al. 2007a). Skin DDCs can be categorized into two subsets based on the expression of CD207. The main population of DCs in the dermis are the $CD207^-$ DDCs (82.1 %) (Henri et al. 2010). Although LCs and $CD207^+$ DDCs both express CD207 and are possibly derived from the same monocyte precursor, they do not have the same function (Ginhoux et al. 2006). Several studies have shown that efficient cross-presentation (Igyártó Botond et al. 2011) and activation of $CD8^+$ T-cells requires priming by $CD207^+$ DDCs (Elnekave et al. 2010; Henri et al. 2010; Stoecklinger et al. 2011). Stoecklinger et al. (2011) reported that following gene gun immunization with plasmid DNA $CD207^+$ DDCs were critical for the activation and functional differentiation of $CD8^+$ T-cells, but not for $CD4^+$ T-cell activation. In addition, the function of $CD207^+$ DDCs was specifically influenced by the nature of the antigen with protein vaccines being unable to stimulate protective immune responses. In the same study, they also reported that $CD207^-$ DDCs biased towards $CD4^+$ T-cell stimulation.

18.4 Immune Modulators for Transcutaneous Immunization

Most transcutaneous vaccines use proteins or peptide antigens and an issue with these are that they are either poorly immunogenic or non-immunogenic. Therefore, potent substances known as adjuvants are required to be delivered with the antigens to improve the immune response. Adjuvants enhance the immune response to vaccine antigens by several different means. For example, adjuvants are capable of increasing the immunogenicity of weak antigens and also of improving the speed and duration of the resulting immune response (Singh and O'Hagan 2003). Additionally, the utilization of adjuvants might decrease in the amount of antigen required to induce immunity, thus reducing costs and helping to overcome antigen competition in combination vaccines (O'Hagan et al. 2001).

Adjuvants play a critical role in TCI. The most common adjuvants used for TCI are cholera toxin (CT) and heat-labile enterotoxin (LT) (O'Hagan et al. 2001; Glenn et al. 1999). Numerous studies have demonstrated that these mucosal adjuvants can

enhance immune responses without toxicity after topical application (Scharton-Kersten et al. 1999; Chen et al. 2002; Eyles et al. 2004; Skountzou et al. 2006). Recently, bacterial lipopolysaccharide (LPS) has become an attractive adjuvant for TCI. According to Kahlon and Dutz (2003), LPS and its derivatives can activate TLR4 expressed by LCs and DCs. Additionally, Quil A (QA) has been incorporated into TCI formulations to enhance skin penetration and immune responses (Madsen et al. 2009). Combining adjuvants that act through different pathways can be used to further optimize immune responses (Garçon et al. 2007).

18.5 Transcutaneous Delivery Strategies

There are three possible pathways for compounds to penetrate into skin; the intracellular, the intercellular and the appendageal routes. The intracellular pathway is where the compound penetrates through the cells deeper into skin. The compounds that preferentially take this route are small hydrophilic molecules (Sznitowska et al. 1998). The intercellular pathway is where the compounds can penetrate into the skin through the extracellular lipids, fatty acids and cellular fluids, located between cells. Most of the compounds that preferentially use this pathway are lipophilic. The last pathway is the appendageal pathway which utilizes the sweat glands and hair follicles (Bolzinger et al. 2012). This route is of interest for nanoparticle delivery into the skin as the appendages can also act as a depot for particles from which drug can be slowly released (Liu et al. 2011; Morgen et al. 2011; Patzelt et al. 2011). Despite drug delivery via hair follicles being an effective delivery route, it cannot be a major route due to the fact that the number of pores in skin is only 0.1 % of the entire surface (Otberg et al. 2004). For larger molecules such as peptide and proteins, transcutaneous delivery is a challenge as even if minimal CD4 and CD8 peptides are used, they are still in excess of 500 Da and will therefore not be able to penetrate into the skin according to the "500 Dalton rule" which states that molecules with a molecular weight above 500 Da cannot cross the skin barrier (Bos and Meinardi 2000). Moreover, peptides and proteins are mostly hydrophilic compounds and according to Fick's law of diffusion (equation shown below) penetration of these large hydrophilic molecules without utilization of a skin penetration enhancer is not possible.

$$J = \frac{DK\Delta c}{h}$$

where J is the flux per unit area and per unit time, D is the diffusion coefficient, K is the skin-vehicle partition coefficient, Δc is the concentration difference across the skin and h is the length of the diffusion path.

The major obstacle for TCI is therefore penetration of the vaccine antigen (peptide, protein and DNA) through the densely packed and highly conformationally ordered corneocytes of the SC. As a result, diffusion through the SC can be

described as the rate limiting step for TCI (Michaels et al. 1975). Several approaches have been investigated to enhance skin penetration. These include both chemical and physical methods that, in general, work by temporarily reducing or disrupting the skin barrier and/or by providing a mechanism for actively driving the vaccine into the skin. These methods do not need to be utilized in isolation and there may be advantages or synergies to using combined approaches, for example Rattanapak et al. reported that using a physical penetration enhancer (microneedles) in combination with a lipid-based colloidal system (cubosomes) improved vaccine retention in the skin (Rattanapak et al. 2013).

18.5.1 Chemical Penetration Enhancers

The main mechanism for enhanced penetration by chemical enhancers is through the removal of the barrier provided by the SC. This occurs through a disordering of the intercellular lipid structure of the SC and through interactions with keratin. In addition, chemical enhancers increase the partitioning of drugs resulting in an increased diffusion rate (Hadgraft and Walters 1992; Parhi et al. 2012). The most commonly used penetration enhancers are alcohols (Morimoto et al. 2002), propylene glycol (Díez-Sales et al. 2005) and surfactants such as polysorbate (Akhtar 2011).

Ethanol is widely used as a solvent because it can increase the solubility of active ingredients in formulations. Ethanol is also well known as a potent skin penetration enhancer. Many studies have shown that ethanol can significantly increase drug permeation through the skin (Obata et al. 1993; Morimoto et al. 2002; Kobayashi et al. 1994). Ethanol enhances skin permeation and penetration by decreasing skin polarity (Kobayashi et al. 1994) and solubilizing the lipid components of the SC (Kai et al. 1990). Due to the concentration-dependent effect of ethanol on skin permeation enhancement, ethanol has been described by Heard et al. (2006) as having a so-called "pull" or "drag" effect.

Propylene glycol (PG) is regularly used in the cosmetics industry as a penetration enhancer. The skin penetration enhancement is due to hydrogen bonding with keratin (Takeuchi et al. 1992) and interactions with the polar head groups of the lipid bilayers (Bouwstra et al. 1991). Consequently, the structure of the SC is disordered and drug penetration into the skin is increased. Díez-Sales et al. (2005) reported the enhancing effect of PG on acyclovir penetration through human epidermis. Adding 50 % PG to a carbopol gel formulation increased drug permeation as compared to the unmodified gel (Díez-Sales et al. 2005).

Surfactants can be anionic, cationic or non-ionic. Cationic surfactants have the most potential to enhance skin penetration due to electrostatic interactions with negatively charged fatty acids in the SC (Lampe et al. 1983). However, the efficiency of the surfactant action is directly proportional to the amount of skin irritation induced. Thus, non-ionic surfactants are extensively incorporated into topical formulations due to their non-toxic properties. A mechanism for skin penetration enhancement by non-ionic surfactants proposed by Nokhodchi et al. (2003) is that

the surfactant molecules may fluidize SC intercellular lipids and also bind to the keratin, leading to disordering of the densely packed SC. Tween 80 is a commonly used non-ionic surfactant in topical formulations. The structure of tween 80 with its ethylene oxide and long hydrocarbon chain is relevant to the surfactant role. The lipophilic part modifies the intercellular lipid lamellae in the SC and the hydrophilic part disrupts protein domains of the corneocytes (Shokri et al. 2001). Akhtar (2011) reported that tween 80 increased the permeation of ascorbic acid through a hairless rabbit skin with an enhancement ratio of 5.07 in relation to the control formulation.

18.5.2 Lipid-Based Colloidal Systems

One of the most controversial methods for enhancing drug penetration into skin is the utilization of lipid vesicles. Around 30 years ago, vesicles were introduced for topical drug delivery by Mezei and Gulasekharam (1980). These authors suggested that intact liposomes were able to penetrate into skin. This investigation brought about numerous studies on vesicles for skin delivery (Fang et al. 2008a; Deshmukh et al. 2008; Lopes et al. 2007).

18.5.2.1 Liposomes

Liposomes are spherical phospholipid vesicles (see Chap. 5). They self-assemble spontaneously into bilayered structures containing an inner aqueous cavity (Castro and Ferreira 2008). Liposomes can be classified into three categories according to vesicle size and the number of lipid bilayers (Torchilin 1996). Vesicles with sizes in the range of 500–5,000 nm with several lipid bilayers are categorized as multilamellar vesicles (MLVs). Large unilamellar vesicles (LUVs) are liposomes with a single lipid bilayer with sizes in the range of 200 to 800 nm. Vesicles with a size of about 100 nm and a single lipid bilayer are referred to as small unilamellar liposomes (SUVs). Multilamellar liposomes can be reduced to LUVs or SUVs by extrusion through stacks of filters.

Phospholipids are biocompatible and biodegradable and these properties make liposomes a safe system, able to be used in the pharmaceutical field (Cosco et al. 2008). Liposomes can prevent the degradation of antigens resulting in prolonged primary activation of T-cells in vivo (Combadiere and Mahe 2008). Many studies have investigated the ability of liposomes to act as an immunological adjuvant and delivery system for subunit vaccines (Davidsen et al. 2005; Brunel et al. 1999; Holten-Andersen et al. 2004).

The ability of liposomes to increase transdermal drug delivery (as compared with non-vesicle formulations such as aqueous solutions, hydro-gels and creams) has been proposed to be due to the ability of vesicular systems to enhance drug penetration (Betz et al. 2005), improve pharmacological properties (Sharma et al. 1994),

control drug release (Fang et al. 2004) and serve as a photoprotection system for drugs (Arsic and Vuleta 1999). The penetration enhancement mechanism of liposomes is thought to be through disruption of the stratum corneum. Liposomes remain on the exterior of the skin, mixing with and fluidizing skin lipids thus disordering and loosening the SC resulting in improved drug penetration (El Maghraby et al. 2008). Because of their rigid membranes liposomes do not appear to be able to utilize the intercellular mechanism of penetration leading to the development of elastic vesicles such as transfersomes and ethosomes to improve skin penetration.

18.5.2.2 Transfersomes

Transfersomes, a more recent class of modified liposomes, was first reported by Cevc and Blume (1992) and are variously described as deformable, highly deformable, elastic or ultra-flexible liposomes or vesicles (Benson 2006). They are claimed to improve in vitro transdermal delivery of a variety of drugs. The deformability possessed by transfersomes is the outcome of incorporation of an edge activator within the phospholipid bilayers and this improves elasticity by means of lipid bilayer destabilization (Dubey et al. 2007). Edge activators commonly used are single chain surfactants such as sodium cholate (Boinpally et al. 2003) and tween 80 (Akhtar 2011). Transfersomes are claimed to be able to squeeze through conduits one-tenth the diameter of the vesicles, allowing them to spontaneously penetrate the stratum corneum (Cevc 1996). Moreover, Cevc and Blume (1992) reported that the driving force for penetration into the skin was the osmotic gradient. The osmotic gradient is caused by the difference in water content between the relatively dehydrated skin surface (varying from 15 to 20 % water in the SC) and the hydrated viable epidermis (approximately 70 % water). Aqueous lipid colloidal dispersions applied to the skin are subject to evaporation and this provides the impetus for the lipid system to follow the natural water gradient across the epidermis. Therefore, assuming this proposed mechanism is correct, transfersomes should not be applied under occluded conditions since this would decrease the osmotic effect (Cevc et al. 2002). Interestingly, transfersomes have been found to enhance skin permeation under occlusive condition in vitro whereas the opposite trend was observed when transfersomes were applied in vivo. It was suggested that the difference between in vitro and in vivo occurred because simple diffusion of free drug was a major pathway for permeation in vitro while the osmotic effect of vesicles was the major pathway in vivo (Cevc et al. 2008).

Topical delivery of peptides and proteins by transfersomes has been extensively investigated (Mishra et al. 2006). Transfersomes have been reported to improve vaccine entrapment efficiency, skin retention and penetration across the SC as compared to traditional vesicles (Paul et al. 1998). More robust immune responses were induced by antigen-loaded transfersomes compared with those induced by antigen-loaded liposomes and vaccine solutions (Li et al. 2011). Mishra (2010) reported that hepatitis B surface antigen (HBsAg)-loaded transfersomes triggered improved

antigen-specific systemic and mucosal responses against HBsAg in vivo as compared to other formulations including a physical mixture of transfersomes and HBsAg, HBsAg solution and intramuscularly administered alum-adsorbed HBsAg.

18.5.2.3 Ethosomes

Ethosomes have also shown potential for TCI. The efficacy and safety of ethosomal formulations has been convincingly demonstrated as compared to other transcutaneous carriers such as gels (Ainbinder and Touitou 2005), patches (Touitou et al. 2001) and conventional liposomes (Fang et al. 2008b). Ethosomes were first developed by Touitou and colleagues (2000). They are vesicles composed of phospholipid hydrated in water with a high ethanol concentration (up to 45 %). Some of the physical characteristics of ethosomes are their softness, flexibility and deformability. An additional characteristic of ethosomes is their multilayered structure, which is expected to increase drug entrapment, resulting in improved therapeutic efficacy. Furthermore, ethosomes have a negatively charged surface, due to the presence of high amounts of ethanol, which is one factor implicated in their ability to increase the permeation of drugs through the skin (Verma and Pathak 2010). According to Ogiso et al. (2001), the penetration rate of melatonin entrapped in negatively charged liposomes across the skin was higher than that of positively charged liposomes.

Touitou et al. (2000) developed a model to describe how ethosomes facilitate penetration. They proposed that free ethanol disrupts the SC by interacting with the polar head group region of lipid molecules. This interaction with free ethanol causes the structure of the SC to become loosely disordered, increasing fluidity and membrane permeability. Then ethosomal vesicles, which are flexible and deformable, easily penetrate through the disordered SC into deeper layers of the skin. Free drug in the ethosomal system can also penetrate into the skin via the loosened SC. An additional proposed mechanism is the fusion of ethosomes with skin lipids, resulting in drug release from the vesicles. Dayan and Touitou (2000) reported that ethosomes significantly increased the depth of penetration of a fluorescent probe (D-289) into skin as compared to classic liposomes. Moreover, the transcutaneous delivery of ammonium glycyrrhizinate in ethosomes was able to improve the anti-inflammatory effect of this drug as compared to ethanolic or aqueous solutions (Paolino et al. 2005). The immune enhancing abilities of ethosomes have also been reported. Mishra et al. (2010) reported enhanced antigen uptake by human DCs incubated with HBsAg-loaded ethosomes and the subsequent triggering of an efficient Th1-type immune response. However, it must be noted that the presence of ethanol as a component of ethosomes increased cell apoptosis. As regards safety, organic solvents are not necessary for the production of ethosomes whereas liposomes or transfersomes require organic solvents for dissolving the lipid phase, which may be a problem if these formulations contain residual solvents.

18.5.2.4 Cubosomes

Cubosomes are colloidal dispersions of the bicontinuous cubic liquid crystalline phase (see Chap. 7) and they possess the same microstructure as the parent cubic phase. Cubosomes have a significantly larger surface area and a lower viscosity than the bulk cubic phase. The low aqueous solubility of cubic phase-forming lipids allows cubosomes to exist at almost any dilution level, as opposed to most liquid crystalline systems that convert into micelles at higher dilutions. Thus, cubosomes can be easily incorporated into product formulations.

Variable entrapment and release of active pharmaceutical ingredient (API) from cubosomes has been reported and it has been suggested that this is due to the size of the API and any interactions occurring between the API and the cubosomes. Boyd (Boyd 2003) reported that release from bulk cubic phases was driven by simple diffusion resulting in the burst release of a small lipophilic drug. However, drug release from cubosomes is possibly influenced by the molecular weight of the drug. Rizwan et al. (2009) reported high entrapment and retarded release of the model protein ovalbumin (MW ~45,000 Da) from cubosomes. These particles have also been reported to act as an effective vaccine delivery system with increased interferon (IFN)-γ production in animals vaccinated subcutaneously with cubosomes containing ovalbumin and QA as compared to control groups (Gordon et al. 2012).

Cubosomes have been utilized as transdermal drug carriers. The penetration of hinokitiol, a hair growth promotion agent, was increased upon formulation into cubosomes (Kwon and Kim 2010). It has been reported that the penetration enhancing effect of cubosomes is due to the lipids of the particles forming a mixture with the lipids of the SC, which is facilitated by their similar cubic phase structure (Norlen and Al-Amoudi 2004; Esposito et al. 2005). Bender et al. (2008) visualized skin penetration of a fluorescence hydrophilic model drug formulated in cubic phase monoolein using two-photon microscopy and found high fluorescence intensity in micro-fissures and in a three-dimensional network of thin threads in the skin.

18.5.3 Other Delivery Systems

In addition to the lipid-based delivery systems, polymer-based delivery systems and virus-like particles (VLPs) have been investigated for transdermal delivery, although with variable success. Encapsulation of antigen in negatively charged poly(lactic acid) (PLA) nanoparticles did not enhance antigen delivery when applied on intact skin (Mattheolabakis et al. 2010). The nanoparticles were detected in the duct of the hair follicles indicating that the nanoparticles can penetrate the skin barrier through the hair follicles. However, when combining the microneedle approach (see below) with antigen-loaded PLGA nanoparticles, Zaric et al. observed efficient antitumour and antiviral immune responses upon transcutaneous vaccination (Zaric et al. 2013). In contrast, smaller VLPs (40 nm) adjuvanted with CpG were able to induce antigen-specific immune responses in mice characterized by high levels of IFN-ɣ and IgG1

(Young et al. 2006). Mittal et al. delivered ovalbumin-containing negatively charged poly(lactide-co-glycolide) (PLGA) or positively charged chitosan-coated PLGA nanoparticles to APCs in hair follicles, without any disruption of the skin (Mittal et al. 2013). Both formulations improved the delivery efficiency of ovalbumin into the hair follicles on excised pig ears by a factor of 2–3 compared to an ovalbumin solution, but it remains to be investigated if this improved delivery results in enhanced immune responses.

Slutter et al. compared different vaccine delivery systems for intradermal administration and found that N-trimethyl chitosan (TMC) nanoparticles were more effective carriers than PLGA nanoparticles (Slutter et al. 2010), positively charged liposomes (Slütter et al. 2011) and chitosan nanoparticles (Slütter et al. 2009). Bal et al. applied TMC nanoparticles loaded with diphtheria toxoid on skin pre-treated with microneedles to overcome the skin barrier (Bal et al. 2010a). After 1 hour of application of the nanoparticles, there was no enhancement of the immune response compared to a diphtheria toxoid solution. However, the authors suggest that TMC nanoparticle diffusion might be an important limiting factor for potency in TCI since the nanoparticles were more efficient in potentiating the immune response than a diphtheria toxoid solution when utilizing longer application times (Bal et al. 2010b).

Co-encapsulation of additional immunopotentiators with the ovalbumin antigen into TMC nanoparticles further improved the immunogenicity of the vaccine, since after intradermal vaccination, ovalbumin-loaded TMC nanoparticles modified with CpG and LPS provoked higher IgG titres than plain ovalbumin-loaded TMC nanoparticles (Bal et al. 2012). The potential of TMC as adjuvant was further increased by conjugating the antigen to the polymer, thereby creating a smaller unit (Slütter et al. 2010). Bal et al. found that TMC-ovalbumin conjugates were more immunogenic than physical mixtures of TMC and ovalbumin and ovalbumin-loaded nanoparticles after transcutaneous administration, likely because they penetrate the skin more easily than nanoparticles and consequently are better delivered to DCs (Bal et al. 2011). Size, choice of immunopotentiator and the use of combination approaches incorporating physical disruption of the SC thus play an important role for transcutaneous immunization.

18.5.4 Microneedle Arrays

Microneedle (MCN) arrays are novel drug delivery devices for percutaneous administration of bioactives developed in the 1970s by Gerstel and Place (1976). MCNs are breakthrough systems facilitating transdermal delivery by transiently and physically disrupting the SC and creating micron-sized pores. MCNs are attractive delivery devices because they allow painless drug delivery (Kaushik et al. 2001). Although, the length of the needles can be up to 1,000 μm and are likely to penetrate into the superficial dermis where pain receptors are located, the micron-sizes of needles reduce the chances of encountering and stimulating nerves (Prausnitz 2004).

MCNs have great market potential due to their low manufacturing and product distribution costs and the fact that they are easy to use do not require vaccine-administration expertize (Birchall et al. 2011).

18.5.4.1 Designs and Modes of Action

MCNs disrupt the SC and allow drug to pass through the skin. MCNs generally have a pyramidal shape with a sharp or dull tip and can be manufactured in different ways from a variety of materials. They are divided into four general categories depending on their mode of action (Fig. 18.2).

Solid MCNs: "Poke and Patch"

The "poke and patch" approach is to utilize MCNs to create micro-channels and then apply vaccine patches or formulations to the skin. Drugs penetrate into the skin via simple diffusion (McAllister et al. 2003). Solid MCNs were first used to enhance calcein permeation (Henry et al. 1999). Multiple studies have since reported the use of MCNs to enhance skin permeability, including studies using solid MCNs to transport recombinant virus (Carey et al. 2011; Hirschberg et al. 2012) and protein (Kumar et al. 2011; Ding et al. 2011) vaccines into the skin. Needles can be prepared using a variety of materials. Silicon has been commonly used to prepare MCN arrays. However, the fabrication of microneedles from silicon requires expensive microfabrication procedures and silicon needles may break off in the skin due to the brittle nature of silicon. Nowadays solid MCNs are usually made from polymers such as polyvinyl acetate (Donnelly et al. 2011) and polyetherimide (You et al. 2010). Their mechanical strength reduces the risk of needle breakage in the skin (Park et al. 2005).

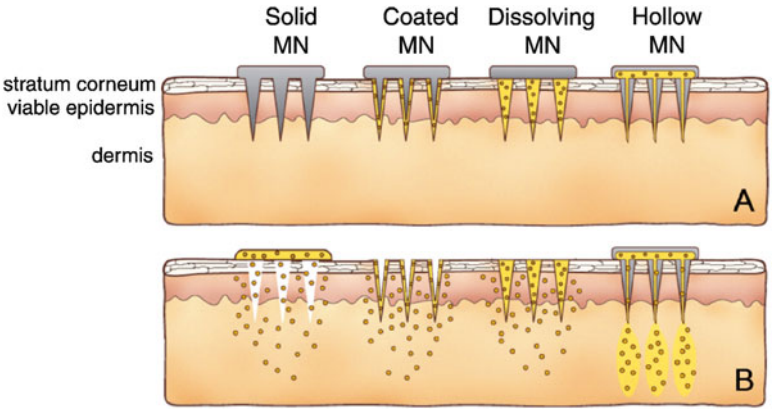

Fig. 18.2 Types of MCNs used for transdermal drug delivery. Adapted from Kim et al. (2012b)

Solid MCNs: “Coat and Poke”

The “coat and poke” approach is similar to the first approach except that the drug is not applied to the skin but is instead coated onto the needle surface. The solid-state vaccine on the surface of needle dissolves off in the skin following MCN insertion. Coated MCNs are an attractive approach as solid-state formulations are stable for longer periods of time as compared to liquid formulations (Kim et al. 2010). However, the amount of vaccine that can be coated onto the needles is limited. As a result, newer vaccine-coating processes have been developed in order to achieve increased vaccine coating. An example of this is an embossing process that fabricates groove-embedded MCNs (Han et al. 2009). An issue encountered with coated MCNs is loss of vaccine immunogenicity (Kim et al. 2011) and the use of stabilizers such as trehalose is essential to prevent this occurring (Kim et al. 2010).

Dissolving MCNs

Dissolving MCNs were developed due to environmental contamination issues arising upon improper disposal of used solid MCNs (Kim et al. 2012a). Dissolving MCNs are made from biodegradable materials such as polymers (Sullivan et al. 2008; Lee et al. 2008) and sugars (Lee et al. 2011; Martin et al. 2012) which dissolve upon exposure to intracellular fluids in the skin. Vaccines entrapped in the polymer matrix release into the skin after matrix degradation. Additionally, dissolving MCNs containing entrapped nanoparticles have been developed as complex controlled-release drug delivery devices (Kang et al. 2006).

Hollow MCNs

Hollow MCNs utilize the same mechanism of action as that used for traditional needle injection. Liquid vaccine formulations are transferred into the skin by active fluid flow or pressure-driven flow. Hollow MCNs are generally used with syringes and existing vaccine formulations but the injection rate through hollow MCNs is faster than subcutaneous injection (Burton et al. 2011). BD Soluvia™ and the MicronJet Needle (NanoPass) are examples of commercial hollow MCNs in the market.

18.5.5 Laserporation

Lasers have been used in medicine since the 1980s to remove or destroy tissue. Much work has focused on developing technologies that can be accurately targeted and have reduced heating and damage of surrounding tissue (Scheiblhofer et al. 2013). Ablative fractional laser (AFL) technologies are now available which can

generate a predefined pattern of micropores. A proposed advantage of AFL over other penetration enhancing technologies includes the degree of precision possible with laser technologies in the creation of the size and depth of microchannels which heal quickly to maintain skin integrity. Re-epithelialization of channels 71 μm wide and 40 μm deep was reported to occur within 24 h (Chen et al. 2012). The P.L.E.A.S.E.® (Precise Laser Epidermal System) technology uses a diode-pumped Er:YAG laser to painlessly create several hundred micropores with a typical diameter of 100–150 μm at a targeted depth sequentially in only a few seconds in an area with a diameter of approximately 3 cm (Yu et al. 2011). Studies using this technology have demonstrated the induction of both T- and B-cell responses that appear to be dependent upon antigen presentation by langerin negative DCs (Weiss et al. 2012). Interestingly laserporation has been reported to bias responses towards a Th2 phenotype; however this appears to be at least partially dependent upon the layer of skin targeted and could be modified through the inclusion of adjuvants into the vaccine (Weiss et al. 2012). As well as being used for TCI, laserporation can also be used to improve immune responses to vaccines delivered intramuscularly (Zeira et al. 2003) and intradermally (Zeira et al. 2007).

18.5.6 Other Emerging Technologies

Many different technologies are being investigated for the delivery of drugs into and through the skin. This includes the use of technologies such as electroporation, iontophoresis, sonoporation and jet injection (reviewed in Gratieri et al. 2013). Less work has been done utilizing these systems for vaccine delivery. However electroporation has been utilized for the delivery of DNA vaccines in to a variety of animal species including non-human primates. Laddy et al. compared immune responses to vaccination with an avian influenza DNA vaccine delivered either intramuscularly (i.m.) or intradermally (i.d.) using electroporation to macaques (Laddy et al. 2009). They found that while i.m. immunization induced superior antibody responses i.d. immunization provided better protection, suggesting the importance of cellular immunity in protection against this infection. Electroporation has also been used in combination with intradermal jet injection (whereby a CO_2-propelled needle-free device injects vaccine as a liquid stream into skin) in mice to deliver high doses of plasmid DNA (Hallengard et al. 2012).

18.6 Conclusions

The importance of being able to deliver vaccines without needles in a simple manner that does not require medical personnel or expensive or technical equipment should not be underestimated. While much of the research here is still in the early stages it is easy to imagine such vaccines being available in the future.

However research still needs to be done to develop formulations that efficiently activate the most relevant populations of APCs and induce the appropriate immune response. Such research will require multidisciplinary research teams including immunologists and pharmaceutical scientists.

References

Ainbinder D, Touitou E (2005) Testosterone ethosomes for enhanced transdermal delivery. Drug Deliv 12(5):297–303. doi:10.1080/10717540500176910

Akhtar N (2011) Penetration enhancing effect of polysorbate 20 and 80 on the in vitro percutaneous absorption of L-ascorbic acid. Trop J Pharm Res 10(3):281–288. doi:10.4314/tjpr.v10i3.1

Arsic I, Vuleta G (1999) Influence of liposomes on the stability of vitamin a incorporated in polyacrylate hydrogel. Int J Cosmet Sci 21(4):219–225. doi:10.1046/j.1467-2494.1999.181682.x

Aylward B, Lloyd J, Zaffran M, Mcnairscott R, Evans P (1995) Reducing the risk of unsafe injections in immunization programs—financial and operational implications of various injection technologies. Bull World Health Organ 73(4):531–540

Bal S, Ding Z, Kersten GA, Jiskoot W, Bouwstra J (2010a) Microneedle-based transcutaneous immunisation in mice with N-trimethyl chitosan adjuvanted diphtheria toxoid formulations. Pharm Res 27(9):1837–1847. doi:10.1007/s11095-010-0182-y

Bal SM, Ding Z, van Riet E, Jiskoot W, Bouwstra JA (2010b) Advances in transcutaneous vaccine delivery: do all ways lead to Rome? J Control Release 148(3):266-282; doi:http://dx.doi.org/10.1016/j.jconrel.2010.09.018

Bal SM, Slütter B, Jiskoot W, Bouwstra JA (2011) Small is beautiful: N-trimethyl chitosan–ovalbumin conjugates for microneedle-based transcutaneous immunisation. Vaccine 29(23):4025–4032. doi:10.1016/j.vaccine.2011.03.039

Bal SM, Slütter B, Verheul R, Bouwstra JA, Jiskoot W (2012) Adjuvanted, antigen loaded N-trimethyl chitosan nanoparticles for nasal and intradermal vaccination: adjuvant- and site-dependent immunogenicity in mice. Eur J Pharm Sci 45(4):475–481; doi:http://dx.doi.org/10.1016/j.ejps.2011.10.003

Barry BW (2001) Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 14(2):101–114

Bender J, Simonsson C, Smedh M, Engstrom S, Ericson MB (2008) Lipid cubic phases in topical drug delivery: visualization of skin distribution using two-photon microscopy. J Control Release 129(3):163–169. doi:10.1016/j.jconrel.2008.04.020

Benson HA (2006) Transfersomes for transdermal drug delivery. Expert Opin Drug Deliv 3(6):727–737. doi:10.1517/17425247.3.6.727

Betz G, Aeppli A, Menshutina N, Leuenberger H (2005) In vivo comparison of various liposome formulations for cosmetic application. Int J Pharm 296(1–2):44–54. doi:10.1016/j.ijpharm.2005.02.032

Birchall J, Clemo R, Anstey A, John D (2011) Microneedles in clinical practice–an exploratory study into the opinions of healthcare professionals and the public. Pharm Res 28(1):95–106. doi:10.1007/s11095-010-0101-2

Bobr A, Olvera-Gomez I, Igyarto BZ, Haley KM, Hogquist KA, Kaplan DH (2010) Acute ablation of Langerhans cells enhances skin immune responses. J Immunol 185(8):4724–4728. doi:10.4049/jimmunol.1001802

Boinpally RR, Zhou SL, Poondru S, Devraj G, Jasti BR (2003) Lecithin vesicles for topical delivery of diclofenac. Eur J Pharm Biopharm 56(3):389–392. doi:10.1016/S0939-6411(03)00143-7

Bolzinger M-A, Briançon S, Pelletier J, Chevalier Y (2012) Penetration of drugs through skin, a complex rate-controlling membrane. Curr Opin Coll Interface Sci 17(3):156–165. doi:10.1016/j.cocis.2012.02.001

Bos JD, Meinardi MMHM (2000) The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Exp Dermatol 9(3):165–169

Bouwstra JA, de Vries MA, Gooris GS, Bras W, Brussee J, Ponec M (1991) Thermodynamic and structural aspects of the skin barrier. J Control Release 15(3):209–219. doi:10.1016/0168-3659(91)90112-q

Bouwstra JA, Honeywell-Nguyen PL, Gooris GS, Ponec M (2003) Structure of the skin barrier and its modulation by vesicular formulations. Prog Lipid Res 42(1):1–36

Boyd BJ (2003) Characterisation of drug release from cubosomes using the pressure ultrafiltration method. Int J Pharm 260(2):239–247. doi:10.1016/S0378-5173(03)00262-X

Brown MB, Martin GP, Jones SA, Akomeah FK (2006) Dermal and transdermal drug delivery systems: current and future prospects. Drug Deliv 13(3):175–187. doi:10.1080/10717540500455975

Brunel F, Darbouret A, Ronco J (1999) Cationic lipid DC-Chol induces an improved and balanced immunity able to overcome the unresponsiveness to the hepatitis B vaccine. Vaccine 17(17):2192–2203. doi:10.1016/s0264-410x(98)00492-7

Bursch LS, Wang L, Igyarto B, Kissenpfennig A, Malissen B, Kaplan DH, Hogquist KA (2007a) Identification of a novel population of Langerin(+) dendritic cells. J Exp Med 204(13):3147–3156

Bursch LS, Wang L, Igyarto B, Kissenpfennig A, Malissen B, Kaplan DH, Hogquist KA (2007b) Identification of a novel population of Langerin+ dendritic cells. J Exp Med 204(13):3147–3156. doi:10.1084/jem.20071966

Burton SA, Ng CY, Simmers R, Moeckly C, Brandwein D, Gilbert T, Johnson N, Brown K, Alston T, Prochnow G, Siebenaler K, Hansen K (2011) Rapid intradermal delivery of liquid formulations using a hollow microstructured array. Pharm Res 28(1):31–40. doi:10.1007/s11095-010-0177-8

Carey JB, Pearson FE, Vrdoljak A, McGrath MG, Crean AM, Walsh PT, Doody T, O'Mahony C, Hill AVS, Moore AC (2011) Microneedle array design determines the induction of protective memory CD8(+) T cell responses induced by a recombinant live malaria vaccine in mice. PLoS One 6(7):e22442. doi:10.1371/journal.pone.0022442

Castro GA, Ferreira LAM (2008) Novel vesicular and particulate drug delivery systems for topical treatment of acne. Expert Opin Drug Deliv 5(6):665–679. doi:10.1517/17425240802167092

Cevc G (1996) Transfersomes, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit Rev Ther Drug Carrier Syst 13(3–4):257–388

Cevc G, Blume G (1992) Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim Biophys Acta 1104(1):226–232

Cevc G, Schatzlein A, Richardsen H (2002) Ultradeformable lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements. Biochim Biophy Acta 1564(1):21–30

Cevc G, Mazgareanu S, Rother M, Vierl U (2008) Occlusion effect on transcutaneous NSAID delivery from conventional and carrier-based formulations. Int J Pharm 359(1–2):190–197. doi:10.1016/j.ijpharm.2008.04.005

Chen D, Colditz IG, Glenn GM, Tsonis CG (2002) Effect of transcutaneous immunization with co-administered antigen and cholera toxin on systemic and mucosal antibody responses in sheep. Vet Immunol Immunopathol 86(3–4):177–182

Chen X, Shah D, Kositratna G, Manstein D, Anderson RR, Wu MX (2012) Facilitation of transcutaneous drug delivery and vaccine immunization by a safe laser technology. J Control Release 159(1):43–51; doi:http://dx.doi.org/10.1016/j.jconrel.2012.01.002

Combadiere B, Mahe B (2008) Particle-based vaccines for transcutaneous vaccination. Comp Immunol Microbiol Infect Dis 31(2–3):293–315. doi:10.1016/j.cimid.2007.07.015

Cosco D, Celia C, Cilurzo F, Trapasso E, Paolino D (2008) Colloidal carriers for the enhanced delivery through the skin. Expert Opin Drug Deliv 5(7):737–755. doi:10.1517/17425247.5.7.737

Coudeville L, Brunot A, Szucs TD, Dervaux B (2005) The economic value of childhood varicella vaccination in France and Germany. Value Health 8(3):209–222

Davidsen J, Rosenkrands I, Christensen D, Vangala A, Kirby D, Perrie Y, Agger EM, Andersen P (2005) Characterization of cationic liposomes based on dimethyldioctadecylammonium and synthetic cord factor from M. tuberculosis (trehalose 6,6′-dibehenate)—a novel adjuvant inducing both strong CMI and antibody responses. Biochim Biophy Acta 1718(1–2):22–31. doi:10.1016/j.bbamem.2005.10.011

Dayan N, Touitou E (2000) Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials 21(18):1879–1885. doi:10.1016/s0142-9612(00)00063-6

Dearman RJ, Bhushan M, Cumberbatch M, Kimber I, Griffiths CEM (2004) Measurement of cytokine expression and Langerhans cell migration in human skin following suction blister formation. Exp Dermatol 13(7):452–460

Deshmukh DD, Ravis WR, Betageri GV (2008) Improved delivery of cromolyn from oral proliposomal beads. Int J Pharm 358(1–2):128–136. doi:10.1016/j.ijpharm.2008.02.026

Díez-Sales O, Garrigues TM, Herráez JV, Belda R, Martín-Villodre A, Herráez M (2005) In vitro percutaneous penetration of acyclovir from solvent systems and Carbopol 971-P hydrogels: influence of propylene glycol. J Pharm Sci 94(5):1039–1047. doi:10.1002/jps.20317

Ding Z, Bal SM, Romeijn S, Kersten GFA, Jiskoot W, Bouwstra JA (2011) Transcutaneous immunization studies in mice using diphtheria toxoid-loaded vesicle formulations and a microneedle array. Pharm Res 28(1):145–158. doi:10.1007/s11095-010-0093-y

Donnelly RF, Majithiya R, Singh TRR, Morrow DIJ, Garland MJ, Demir YK, Migalska K, Ryan E, Gillen D, Scott CJ, Woolfson AD (2011) Design, optimization and characterisation of polymeric microneedle arrays prepared by a novel laser-based micromoulding technique. Pharm Res 28(1):41–57. doi:10.1007/s11095-010-0169-8

Dubey V, Mishra D, Nahar M, Jain NK (2007) Vesicles as tools for the modulation of skin permeability. Expert Opin Drug Deliv 4(6):579–593. doi:10.1517/17425247.4.6.579

El Maghraby GM, Barry BW, Williams AC (2008) Liposomes and skin: from drug delivery to model membranes. Eur J Pharm Sci 34(4–5):203–222. doi:10.1016/j.ejps.2008.05.002

Elnekave M, Furmanov K, Nudel I, Arizon M, Clausen BE, Hovav A-H (2010) Directly transfected Langerin+ dermal dendritic cells potentiate CD8+ T cell responses following intradermal plasmid DNA Immunization. J Immunol 185(6):3463–3471. doi:10.4049/jimmunol.1001825

Esposito E, Cortesi R, Drechsler M, Paccamiccio L, Mariani P, Contado C, Stellin E, Menegatti E, Bonina F, Puglia C (2005) Cubosome dispersions as delivery systems for percutaneous administration of indomethacin. Pharm Res 22(12):2163–2173. doi:10.1007/s11095-005-8176-x

Eyles JE, Elvin SJ, Westwood A, LeButt CS, Alpar HO, Somavarapu S, Williamson ED (2004) Immunisation against plague by transcutaneous and intradermal application of subunit antigens. Vaccine 22(31–32):4365–4373. doi:10.1016/j.vaccine.2004.02.049

Fang JY, Hung CF, Fang YP, Chan TF (2004) Transdermal iontophoresis of 5-fluorouracil combined with electroporation and laser treatment. Int J Pharm 270(1–2):241–249. doi:10.1016/j.ijpharm.2003.10.025

Fang JY, Fang CL, Liu CH, Su YH (2008a) Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). Eur J Pharm Biopharm 70(2):633–640. doi:10.1016/j.ejpb.2008.05.008

Fang YP, Tsai YH, Wu PC, Huang YB (2008b) Comparison of 5-aminolevulinic acid-encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. Int J Pharm 356(1–2):144–152. doi:10.1016/j.ijpharm.2008.01.020

Flacher V, Tripp CH, Stoitzner P, Haid B, Ebner S, Del Frari B, Koch F, Park CG, Steinman RM, Idoyaga J, Romani N (2010) Epidermal Langerhans cells rapidly capture and present antigens from C-type lectin-targeting antibodies deposited in the dermis. J Invest Dermatol 130(3):755–762. doi:10.1038/Jid.2009.343

Fuchs E, Raghavan S (2002) Getting under the skin of epidermal morphogenesis. Nat Rev Genet 3(3):199–209

Garçon N, Chomez P, Van Mechelen M (2007) GlaxoSmithKline adjuvant systems in vaccines: concepts, achievements and perspectives. Expert Rev Vaccines 6(5):723–739

Gerstel MSPA, Place, CA, Virgil A (1976) Drug delivery device. United States, Palo Alto, CA. Patent 3964482

Ginhoux F, Tacke F, Angeli V, Bogunovic M, Loubeau M, Dai X-M, Stanley ER, Randolph GJ, Merad M (2006) Langerhans cells arise from monocytes in vivo. Nat Immunol 7(3):265–273; doi:http://www.nature.com/ni/journal/v7/n3/suppinfo/ni1307_S1.html

Glenn GM, Scharton-Kersten T, Vassell R, Matyas GR, Alving CR (1999) Transcutaneous immunization with bacterial ADP-ribosylating exotoxins as antigens and adjuvants. Infect Immun 67(3):1100–1106

Glenn GM, Kenney RT, Ellingsworth LR, Frech SA, Hammond SA, Zoeteweij JP (2003) Transcutaneous immunization and immunostimulant strategies: capitalizing on the immunocompetence of the skin. Expert Rev Vaccines 2(2):253–267. doi:10.1586/14760584.2.2.253

Gordon S, Young K, Wilson R, Rizwan S, Kemp R, Rades T, Hook S (2012) Chitosan hydrogels containing liposomes and cubosomes as particulate sustained release vaccine delivery systems. J Liposome Res 22(3):193–204. doi:10.3109/08982104.2011.637502

Gratieri T, Alberti I, Lapteva M, Kalia YN (2013) Next generation intra- and transdermal therapeutic systems: using non- and minimally-invasive technologies to increase drug delivery into and across the skin. Eur J Pharm Sci 18;50(5):609–222; doi:http://dx.doi.org/10.1016/j.ejps.2013.03.019

Hadgraft J, Walters KA (1992) Skin penetration enhancers. In: Juninger HE (ed) Drug targeting and delivery. Ellis Horwood, Chichester, pp 169–177

Hallengard D, Brave A, Isaguliants M, Blomberg P, Enger J, Stout R, King A, Wahren B (2012) A combination of intradermal jet-injection and electroporation overcomes in vivo dose restriction of DNA vaccines. Genet Vaccines Ther 10(1):5–13. doi:10.1186/1479-0556-10-5

Han M, Kim DK, Kang SH, Yoon HR, Kim BY, Lee SS, Kim KD, Lee HG (2009) Improvement in antigen-delivery using fabrication of a grooves-embedded microneedle array. Sensors Actuators B Chem 137(1):274–280. doi:10.1016/j.snb.2008.11.017

Heard CM, Kung D, Thomas CP (2006) Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect. Int J Pharm 321(1–2):167–170. doi:10.1016/j.ijpharm.2006.05.018

Henri S, Poulin LF, Tamoutounour S, Ardouin L, Guilliams M, de Bovis B, Devilard E, Viret C, Azukizawa H, Kissenpfennig A, Malissen B (2010) CD207+ CD103+ dermal dendritic cells cross-present keratinocyte-derived antigens irrespective of the presence of Langerhans cells. J Exp Med 207(1):189–206. doi:10.1084/jem.20091964

Henry S, McAllister DV, Allen MG, Prausnitz MR (1999) Microfabricated microneedles: a novel approach to transdermal drug delivery. J Pharm Sci 88(9):948. doi:10.1021/js990783q

Hirschberg H, van Kuijk S, Loch J, Jiskoot W, Bouwstra J, Kersten G, Amorij J-P (2012) A combined approach of vesicle formulations and microneedle arrays for transcutaneous immunization against hepatitis B virus. Eur J Pharm Sci 46(1–2):1–7. doi:10.1016/j.ejps.2012.01.013

Holten-Andersen L, Doherty TM, Korsholm KS, Andersen P (2004) Combination of the cationic surfactant dimethyl dioctadecyl ammonium bromide and synthetic mycobacterial cord factor as an efficient adjuvant for tuberculosis subunit vaccines. Infect Immun 72(3):1608–1617. doi:10.1128/iai.72.3.1608-1617.2004

Honda T, Nakajima S, Egawa G, Ogasawara K, Malissen B, Miyachi Y, Kabashima K (2010) Compensatory role of Langerhans cells and langerin-positive dermal dendritic cells in the sensitization phase of murine contact hypersensitivity. J Allergy Clin Immunol 125(5):1154–1156. e1152. doi:10.1016/j.jaci.2009.12.005

Igyártó Botond Z, Haley K, Ortner D, Bobr A, Gerami-Nejad M, Edelson Brian T, Zurawski Sandra M, Malissen B, Zurawski G, Berman J, Kaplan Daniel H (2011) Skin-resident murine dendritic cell subsets promote distinct and opposing antigen-specific T helper cell responses. Immunity 35(2):260–272. doi:10.1016/j.immuni.2011.06.005

Kahlon R, Dutz JP (2003) Skin immune responses to peptide and protein antigen are TLR4 independent. Cell Immunol 226(2):116–123. doi:10.1016/j.cellimm.2003.11.007

Kai T, Mak VHW, Potts RO, Guy RH (1990) Mechanism of percutaneous penetration enhancement—effect of N-alkanols on the permeability barrier of hairless mouse skin. J Control Release 12(2):103–112

Kang EN, Wang HF, Kwon IK, Robinson J, Park K, Cheng JX (2006) In situ visualization of paclitaxel distribution and release by coherent anti-stokes Raman scattering microscopy. Anal Chem 78(23):8036–8043

Kaushik S, Hord AH, Denson DD, McAllister DV, Smitra S, Allen MG, Prausnitz MR (2001) Lack of pain associated with microfabricated microneedles. Anesth Analg 92(2):502–504

Kim YC, Quan FS, Compans RW, Kang SM, Prausnitz MR (2010) Formulation and coating of microneedles with inactivated influenza virus to improve vaccine stability and immunogenicity. J Control Release 142(2):187–195. doi:10.1016/j.jconrel.2009.10.013

Kim Y-C, Quan F-S, Compans R, Kang S-M, Prausnitz M (2011) Stability kinetics of influenza vaccine coated onto microneedles during drying and storage. Pharm Res 28(1):135–144. doi:10.1007/s11095-010-0134-6

Kim YC, Jarrahian C, Zehrung D, Mitragotri S, Prausnitz MR (2012a) Delivery systems for intradermal vaccination. Curr Top Microbiol Immunol 351:77–112. doi:10.1007/82_2011_123

Kim YC, Park JH, Prausnitz MR (2012b) Microneedles for drug and vaccine delivery. Adv Drug Deliv Rev. doi:10.1016/j.addr.2012.04.005

Kobayashi D, Matsuzawa T, Sugibayashi K, Morimoto Y, Kimura M (1994) Analysis of the combined effect of 1-menthol and ethanol as skin permeation enhancers based on a two-layer skin model. Pharm Res 11(1):96–103. doi:10.1023/a:1018953929457

Krueger GG, Stingl G (1989) Immunology inflammation of the skin—a 50-year perspective. J Invest Dermatol 92(4):S32–S51. doi:10.1111/1523-1747.Ep13074960

Kubo A, Nagao K, Yokouchi M, Sasaki H, Amagai M (2009) External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. J Exp Med 206(13): 2937–2946. doi:10.1084/jem.20091527

Kumar A, Li XR, Sandoval MA, Rodriguez BL, Sloat BR, Cui ZR (2011) Permeation of antigen protein-conjugated nanoparticles and live bacteria through microneedle-treated mouse skin. Int J Nanomedicine 6:1253–64. doi:10.2147/Ijn.S20413

Kwon TK, Kim J-C (2010) Preparation and in vitro skin permeation of cubosomes containing hinokitiol. J Dispers Sci Technol 31(7):1004–1009. doi:10.1080/01932690903224862

Laddy DJ, Yan J, Khan AS, Andersen H, Cohn A, Greenhouse J, Lewis M, Manischewitz J, King LR, Golding H, Draghia-Akli R, Weiner DB (2009) Electroporation of synthetic DNA antigens offers protection in nonhuman Primates challenged with highly pathogenic avian influenza virus. J Virol 83(9):4624–4630

Lambert PH, Laurent PE (2008) Intradermal vaccine delivery: will new delivery systems transform vaccine administration? Vaccine 26(26):3197–3208. doi:10.1016/j.vaccine.2008.03.095

Lampe MA, Burlingame AL, Whitney J, Williams ML, Brown BE, Roitman E, Elias PM (1983) Human stratum-corneum lipids—characterization and regional variations. J Lipid Res 24(2):120–130

Lee JW, Park J-H, Prausnitz MR (2008) Dissolving microneedles for transdermal drug delivery. Biomaterials 29(13):2113–2124. doi:10.1016/j.biomaterials.2007.12.048

Lee K, Lee CY, Jung H (2011) Dissolving microneedles for transdermal drug administration prepared by stepwise controlled drawing of maltose. Biomaterials 32(11):3134–3140. doi:10.1016/j.biomaterials.2011.01.014

Li N, Peng LH, Chen X, Nakagawa S, Gao JQ (2011) Effective transcutaneous immunization by antigen-loaded flexible liposome in vivo. Int J Nanomedicine 6:3241–3250

Liu X, Grice JE, Lademann J, Otberg N, Trauer S, Patzelt A, Roberts MS (2011) Hair follicles contribute significantly to penetration through human skin only at times soon after application as a solvent deposited solid in man. Br J Clin Pharmacol 72(5):768–774. doi:10.1111/j.1365-2125.2011.04022.x

Lopes LB, Speretta FFF, Vitoria M, Bentley LB (2007) Enhancement of skin penetration of vitamin K using monoolein-based liquid crystalline systems. Eur J Pharm Sci 32(3):209–215. doi:10.1016/j.ejps.2007.07.006

Madsen HB, Ifversen P, Madsen F, Brodin B, Hausser I, Nielsen HM (2009) In vitro cutaneous application of ISCOMs on human skin enhances delivery of hydrophobic model compounds through the stratum corneum. AAPS J 11(4):728–739. doi:10.1208/s12248-009-9149-5

Martin CJ, Allender CJ, Brain KR, Morrissey A, Birchall JC (2012) Low temperature fabrication of biodegradable sugar glass microneedles for transdermal drug delivery applications. J Control Release 158(1):93–101. doi:10.1016/j.jconrel.2011.10.024

Mattheolabakis G, Lagoumintzis G, Panagi Z, Papadimitriou E, Partidos CD, Avgoustakis K (2010) Transcutaneous delivery of a nanoencapsulated antigen: induction of immune responses. Int J Pharm 385(1–2):187–193; doi:http://dx.doi.org/10.1016/j.ijpharm.2009.10.033

McAllister DV, Wang PM, Davis SP, Park J-H, Canatella PJ, Allen MG, Prausnitz MR (2003) Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. Proc Natl Acad Sci 100(24):13755–13760. doi:10.1073/pnas.2331316100

Menon GK (2002) New insights into skin structure: scratching the surface. Adv Drug Deliv Rev 54:S3–S17

Merad M, Ginhoux F, Collin M (2008) Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nat Rev Immunol 8(12):935–947. doi:10.1038/nri2455

Mezei M, Gulasekharam V (1980) Liposomes—a selective drug delivery system for the topical route of administration. Life Sci 26(18):1473–1477

Michaels AS, Chandrasekaran SK, Shaw JE (1975) Drug permeation through human skin: theory and in vitro experimental measurement. Am Inst Chem Eng J 21(5):985–996. doi:10.1002/aic.690210522

Miller MA, Pisani E (1999) The cost of unsafe injections. Bull World Health Organ 77(10):808–811

Mishra D (2010) Evaluation of solid lipid nanoparticles as carriers for delivery of Hepatitis B surface antigen for vaccination using subcutaneous route. J Pharm Pharm Sci 13(4):495

Mishra D, Dubey V, Asthana A, Saraf DK, Jain NK (2006) Elastic liposomes mediated transcutaneous immunization against Hepatitis B. Vaccine 24(22):4847–4855. doi:10.1016/j.vaccine.2006.03.011

Mishra D, Mishra PK, Dabadghao S, Dubey V, Nahar M, Jain NK (2010) Comparative evaluation of hepatitis B surface antigen-loaded elastic liposomes and ethosomes for human dendritic cell uptake and immune response. Nanomedicine 6(1):110–118. doi:10.1016/j.nano.2009.04.003

Mitsui H, Watanabe T, Saeki H, Mori K, Fujita H, Tada Y, Asahina A, Nakamura K, Tamaki K (2004) Differential expression and function of Toll-like receptors in Langerhans cells: comparison with splenic dendritic cells. J Invest Dermatol 122(1):95–102

Mittal A, Raber AS, Schaefer UF, Weissmann S, Ebensen T, Schulze K, Guzmán CA, Lehr C-M, Hansen S (2013) Non-invasive delivery of nanoparticles to hair follicles: A perspective for transcutaneous immunization. Vaccine 31(34):3442–3451; doi:http://dx.doi.org/10.1016/j.vaccine.2012.12.048

Morgen M, Lu GW, Du D, Stehle R, Lembke F, Cervantes J, Ciotti S, Haskell R, Smithey D, Haley K, Fan C (2011) Targeted delivery of a poorly water-soluble compound to hair follicles using polymeric nanoparticle suspensions. Int J Pharm 416(1):314–322. doi:10.1016/j.ijpharm.2011.06.019

Morimoto Y, Wada Y, Seki T, Sugibayashi K (2002) In vitro skin permeation of morphine hydrochloride during the finite application of penetration-enhancing system containing water, ethanol and l-menthol. Biol Pharm Bull 25(1):134–136

Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ (2009) Skin immune sentinels in health and disease. Nat Rev Immunol 9(10):679–691

Nokhodchi A, Shokri J, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Barzegar-Jalali M (2003) The enhancement effect of surfactants on the penetration of lorazepam through rat skin. Int J Pharm 250(2):359–369. doi:10.1016/s0378-5173(02)00554-9

Norlen L, Al-Amoudi A (2004) stratum corneum keratin structure, function, and formation: the cubic rod-packing and membrane templating model. J Invest Dermatol 123(4):715–732; doi:http://www.nature.com/jid/journal/v123/n4/suppinfo/5602511s1.html

Obata Y, Takayama K, Maitani Y, Machida Y, Nagai T (1993) Effect of ethanol on skin permeation of nonionized and ionized diclofenac. Int J Pharm 89(3):191–198. doi:10.1016/0378-5173(93)90243-9

Ogiso T, Yamaguchi T, Iwaki M, Tanino T, Miyake Y (2001) Effect of positively and negatively charged liposomes on skin permeation of drugs. J Drug Target 9(1):49

O'Hagan DT, MacKichan ML, Singh M (2001) Recent developments in adjuvants for vaccines against infectious diseases. Biomol Eng 18(3):69–85

Otberg N, Richter H, Schaefer H, Blume-Peytavi U, Sterry W, Lademann J (2004) Variations of hair follicle size and distribution in different body sites. J Invest Dermatol 122(1):14–19. doi:10.1046/j.0022-202X.2003.22110.x

Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M (2005) Ethosomes for skin delivery of ammonium glycyrrhizinate: In vitro percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteers. J Control Release 106(1–2):99–110. doi:10.1016/j.jconrel.2005.04.007

Parhi R, Suresh P, Mondal S, Kumar PM (2012) Novel penetration enhancers for skin applications: a review. Curr Drug Deliv 9(2):219–230

Park JH, Allen MG, Prausnitz MR (2005) Biodegradable polymer microneedles: Fabrication, mechanics and transdermal drug delivery. J Control Release 104(1):51–66. doi:10.1016/j.jconrel.2005.02.002

Patzelt A, Richter H, Dähne L, Walden P, Wiesmüller K-H, Wank U, Sterry W, Lademann J (2011) Influence of the vehicle on the penetration of particles into hair follicles. Pharmaceutics 3(2):307–314

Paul A, Cevc G, Bachhawat BK (1998) Transdermal immunisation with an integral membrane component, gap junction protein, by means of ultradeformable drug carriers, transfersomes. Vaccine 16(2–3):188–195. doi:10.1016/s0264-410x(97)00185-0

Pearton M, Kang S-M, Song J-M, Anstey AV, Ivory M, Compans RW, Birchall JC (2010) Changes in human Langerhans cells following intradermal injection of influenza virus-like particle vaccines. PLoS One 5(8):e12410. doi:10.1371/journal.pone.0012410

Prausnitz MR (2004) Microneedles for transdermal drug delivery. Adv Drug Deliv Rev 56(5):581–587. doi:10.1016/j.addr.2003.10.023

Proksch E, Jensen J-M (2008) Skin as an organ of protection. In: Goldsmith LA, Katz SI, Gilchrest GA, Paller AS, Leffell DJ, Wolff K (eds) Fitzpatrick's dermatology in general medicine. McGraw-Hill, New York, pp 383–95

Rattanapak T, Birchall J, Young K, Ishii M, Meglinski I, Rades T, Hook S (2013) Transcutaneous immunization using microneedles and cubosomes: Mechanistic investigations using Optical Coherence Tomography and Two-Photon Microscopy. J Control Release 172(3):894–903; doi:http://dx.doi.org/10.1016/j.jconrel.2013.08.018

Rizwan SB, Hanley T, Boyd BJ, Rades T, Hook S (2009) Liquid crystalline systems of phytantriol and glyceryl monooleate containing a hydrophilic protein: characterisation, swelling and release kinetics. J Pharm Sci 98(11):4191–4204. doi:10.1002/jps.21724

Romani N, Clausen BE, Stoitzner P (2010) Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin. Immunol Rev 234(1):120–141. doi:10.1111/j.0105-2896.2009.00886.x

Scharton-Kersten T, Glenn GM, Vassell R, Yu JM, Walwender D, Alving CR (1999) Principles of transcutaneous immunization using cholera toxin as an adjuvant. Vaccine 17:S37–S43

Scheiblhofer S, Thalhamer J, Weiss R (2013) Laser microporation of the skin: prospects for painless application of protective and therapeutic vaccines. Expert Opin Drug Deliv 10(6):761–773. doi:10.1517/17425247.2013.773970

Sharma BB, Jain SK, Vyas SP (1994) Topical liposome system bearing local-anesthetic lignocaine—preparation and evaluation. J Microencapsul 11(3):279–286

Shokri J, Nokhodchi A, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Barzegar Jalali M (2001) The effect of surfactants on the skin penetration of diazepam. Int J Pharm 228(1–2):99–107. doi:10.1016/s0378-5173(01)00805-5

Singh M, O'Hagan DT (2003) Recent advances in veterinary vaccine adjuvants. Int J Parasitol 33(5–6):469–478. doi:10.1016/s0020-7519(03)00053-5

Skountzou I, Quan FS, Jacob J, Compans RW, Kang SM (2006) Transcutaneous immunization with inactivated influenza virus induces protective immune responses. Vaccine 24(35–36):6110–6119. doi:10.1016/j.vaccine.2006.05.014

Slütter B, Plapied L, Fievez V, Alonso Sande M, des Rieux A, Schneider Y-J, Van Riet E, Jiskoot W, Préat V (2009) Mechanistic study of the adjuvant effect of biodegradable nanoparticles in mucosal vaccination. J Control Release 138(2):113–121; doi:http://dx.doi.org/10.1016/j.jconrel.2009.05.011

Slutter B, Bal S, Keijzer C, Mallants R, Hagenaars N, Que I, Kaijzel E, van Eden W, Augustijns P, Lowik C, Bouwstra J, Broere F, Jiskoot W (2010) Nasal vaccination with N-trimethyl chitosan and PLGA based nanoparticles: Nanoparticle characteristics determine quality and strength of the antibody response in mice against the encapsulated antigen. Vaccine 28(38):6282–6291. doi:10.1016/j.vaccine.2010.06.121

Slütter B, Soema PC, Ding Z, Verheul R, Hennink W, Jiskoot W (2010) Conjugation of ovalbumin to trimethyl chitosan improves immunogenicity of the antigen. J Control Release 143(2):207–214; doi:http://dx.doi.org/10.1016/j.jconrel.2010.01.007

Slütter B, Bal SM, Ding Z, Jiskoot W, Bouwstra JA (2011) Adjuvant effect of cationic liposomes and CpG depends on administration route. J Control Release 154(2):123–130; doi:http://dx.doi.org/10.1016/j.jconrel.2011.02.007

Stoecklinger A, Eticha TD, Mesdaghi M, Kissenpfennig A, Malissen B, Thalhamer J, Hammerl P (2011) Langerin+ dermal dendritic cells are critical for CD8+ T cell activation and IgH γ-1 class switching in response to gene gun vaccines. J Immunol 186(3):1377–1383. doi:10.4049/jimmunol.1002557

Sullivan SP, Murthy N, Prausnitz MR (2008) Minimally invasive protein delivery with rapidly dissolving polymer microneedles. Adv Mater 20(5):933. doi:10.1002/adma.200701205

Sznitowska M, Janicki S, Williams AC (1998) Intracellular or intercellular localization of the polar pathway of penetration across stratum corneum. J Pharm Sci 87(9):1109–1114. doi:10.1021/js980018w

Takeuchi Y, Yasukawa H, Yamaoka Y, Kato Y, Morimoto Y, Fukumori Y, Fukuda T (1992) Effects of fatty acids, fatty amines and propylene glycol on rat stratum corneum lipids and proteins in vitro measured by Fourier transform infrared/attenuated total reflection (FT-IR/ATR) spectroscopy. Chem Pharm Bull (Tokyo) 40(7):1887–1892

Torchilin VP (1996) Liposomes as delivery agents for medical imaging. Mol Med Today 2(6):242–249. doi:10.1016/1357-4310(96)88805-8

Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M (2000) Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release 65(3):403–418

Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A, Levi-Schaffer F (2001) Intracellular delivery mediated by an ethosomal carrier. Biomaterials 22(22):3053–3059

Valladeau J, Ravel O, Dezutter-Dambuyant C, Moore K, Kleijmeer M, Liu Y, Duvert-Frances V, Vincent C, Schmitt D, Davoust J, Caux C, Lebecque S, Saeland S (2000) Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. Immunity 12(1):71–81

Verma P, Pathak K (2010) Therapeutic and cosmeceutical potential of ethosomes: an overview. J Adv Pharm Technol Res 1(3):274–282. doi:10.4103/0110-5558.72415

Wang L, Bursch LS, Kissenpfennig A, Malissen B, Jameson SC, Hogquist KA (2008) Langerin expressing cells promote skin immune responses under defined conditions. J Immunol 180(7):4722–4727

Weiss R, Hessenberger M, Kitzmüller S, Bach D, Weinberger EE, Krautgartner WD, Hauser-Kronberger C, Malissen B, Boehler C, Kalia YN, Thalhamer J, Scheiblhofer S (2012) Transcutaneous vaccination via laser microporation. J Control Release 162(2):391–399; doi:http://dx.doi.org/10.1016/j.jconrel.2012.06.031

You S-K, Noh Y-W, Park H-H, Han M, Lee SS, Shin S-C, Cho C-W (2010) Effect of applying modes of the polymer microneedle-roller on the permeation of l-ascorbic acid in rats. J Drug Target 18(1):15–20. doi:10.3109/10611860903115274

Young SL, Wilson M, Wilson S, Beagley KW, Ward V, Baird MA (2006) Transcutaneous vaccination with virus-like particles. Vaccine 24(26):5406–5412; doi:http://dx.doi.org/10.1016/j.vaccine.2006.03.052

Yu J, Kalaria DR, Kalia YN (2011) Erbium:YAG fractional laser ablation for the percutaneous delivery of intact functional therapeutic antibodies. J Control Release 156(1):53–59; doi:http://dx.doi.org/10.1016/j.jconrel.2011.07.024

Zaric M, Lyubomska O, Touzelet O, Poux C, Al-Zahrani S, Fay F, Wallace L, Terhorst D, Malissen B, Henri S, Power UF, Scott CJ, Donnelly RF, Kissenpfennig A (2013) Skin dendritic cell targeting via microneedle arrays laden with antigen encapsulated poly-D-L-lactide-co-glycolide nanoparticles induces efficient anti-tumour and anti-viral immune responses. ACS Nano. doi:10.1021/nn304235j

Zeira E, Manevitch A, Khatchatouriants A, Pappo O, Hyam E, Darash-Yahana M, Tavor E, Honigman A, Lewis A, Galun E (2003) Femtosecond infrared laser—an efficient and safe in vivo gene delivery system for prolonged expression. Mol Ther 8(2):342–350

Zeira E, Manevitch A, Manevitch Z, Kedar E, Gropp M, Daudi N, Barsuk R, Harati M, Yotvat H, Troilo PJ, Griffiths TG, Pacchione SJ, Roden DF, Niu Z, Nussbaum O, Zamir G, Papo O, Hemo I, Lewis A, Galun E (2007) Femtosecond laser: a new intradermal DNA delivery method for efficient, long-term gene expression and genetic immunization. FASEB J 21(13):3522–3533. doi:10.1096/fj.06-7528com