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Review

# Animal models for cutaneous vaccine delivery

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ABSTRACT

Main challenges in skin vaccination are overcoming the stratum corneum (SC) barrier and targeting the antigen presenting cells (APC) in the epidermis and the dermis. For this purpose many delivery techniques are being developed. *In vivo* immunogenicity and safety studies in animals are mandatory before moving to clinical trials. However, the results obtained in animals may or may not be predictive for humans. Knowledge about differences and similarities in skin architecture and immunology within a species and between species is crucial.

In this review, we discuss variables, including skin morphology, skin barrier function, mechanical properties, site of application and immunology, which should be taken into account when designing animal studies for vaccination via the skin in order to support the translation to clinical trial outcomes.



**Contents**



## 1. Introduction

The skin is the largest organ in the body and protects against environmental challenges, such as pathogens, injuries and UV radiation. It harbors many immune cells, making it an attractive site for vaccination. The smallpox vaccine, the first vaccine used and also the most successful, was delivered in the skin, using bifurcated needles. Nowadays only three other vaccines are on the market for intradermal delivery: the bacillus Calmette-Guerin (BCG) and rabies vaccine are both administered in the skin using hypodermic needles and since 2009, an influenza vaccine was reformulated for use in a novel microinjection system (BD Soluvia™). This system enables medical personnel to inject the vaccine intradermally on a more reliable and consistent manner.

Regulations require that immunogenicity and safety of vaccines is first determined in animal models before entering clinical trials. The results of animal studies are difficult to extrapolate to the human situation. This is especially true for cutaneous vaccines. For this reason the risk of failure of clinical studies is high.

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An example is the development of a cutaneous vaccine patch against travelers diarrhea. The vaccine contains heat-labile enterotoxin (LT) from Enterotoxinogenic *Escherichia coli* (ETEC), an important causative agent of travelers diarrhea. Preclinical studies in mice immunized with LT applied on intact skin demonstrated very high anti-LT titers and full protection against lethal systemic challenge with the toxin (Beignon et al., 2001; Glenn et al., 1998). In a first clinical trial (Glenn et al., 2000) on intact skin, IgG titers were induced but responses were not as high as in preclinical trials. In following clinical trials (Behrens et al., 2014; Steffen et al., 2013), the investigators applied an abrasion step before vaccine application to reduce the skin barrier function. Although the delivery of LT in the skin was demonstrated, antibody titers remained much lower than in mice studies and the antibody titers were not protective.

Knowledge about differences in skin architecture and skin immunology between different species and within species is crucial in order to understand the limitations of skin immunization in experimental animals with respect to the situation in humans. For cutaneous vaccination, the difference in epidermis (especially stratum corneum) and dermis thickness is an additional challenge in the translation of animals to human. In this review, we discuss variables between animal species used to test cutaneous vaccines, some of which can be controlled by the investigator and which should be considered in the translation of animal-clinical studies of vaccines delivered via the skin.

The skin is composed of three layers: the epidermis, the dermis and the subcutis. The viable epidermis is composed of mainly keratinocytes (90%). The other 10% of the cells are represented by Langerhans Cells (LC), melanocytes and mast cells. Keratinocytes differentiate in the epidermis and during the differentiation, the cells move to the upper layer of the viable epidermis. Terminally differentiated keratinocytes are called corneocytes (dead cells), which form the surface layer of the skin, the stratum corneum (SC). This SC forms the main barrier for water loss from the body (inside-out diffusion) and for invading pathogens from the environment (outside-in diffusion). The dermis is located underneath the epidermis and is composed of dense fibroelastic connective tissue with lymph vessels, nerves, sebaceous glands, sweat glands and hair follicles. The cells present in the dermis are fibroblasts, dermal dendritic cells (dDC), mast cells, monocytes and macrophages. The subcutis is the deepest layer of the skin and is composed of mainly fat tissue.

Cutaneous vaccination delivers the vaccine either onto the SC, or deeper in the skin, in the epidermis or the dermis. In literature, there is a variety in definitions for vaccination into or onto the skin. In this review, we will use the terms "topical vaccination", "intradermal vaccination" and "cutaneous vaccination" (Table 1).

With topical application, the vaccine is applied onto intact skin or pre-treated skin. The vaccine formulation diffuses through (a part of) the SC, into the epidermis and the dermis. With intradermal delivery, the SC diffusion is circumvented by for example needle injections, liquid or solid jet injections, or microneedle insertion. With intradermal vaccination, the vaccine is delivered directly into the epidermis or the dermis. Cutaneous vaccination will be used to assign both topical and intradermal application.

## 2. Topical application

### 2.1. Stratum corneum as the main barrier

The main challenge of topical applications is the passage through the SC in a consistent way such that a substantial part of the antigen dose is delivered to the immune cells. The SC is composed of corneocytes (dead keratinocytes) and intercellular lipids, arranged in a brick and mortar structure, composed of corneocytes and lipids, respectively. This structure forms a barrier against invading pathogens. Topically applied vaccine formulations have to penetrate through this SC in order to reach the immune cells in the epidermis and the dermis. The thicker the SC, the longer the diffusion pathway for penetrating substances. Body sites with thinner SC would therefore be more appropriate for topical application. The thickness of skin (epidermis + dermis) is subject of many studies. However, data specifically on SC thickness is limited. SC thickness in animals is measured by using microscopic techniques on biopsy samples prepared by conventional formalin-paraffin processing or by cryo techniques. Formalin-paraffin processing distorts the skin morphology caused by swelling and shrinking and often results in a basket-wave appearance of the SC. This artefact makes thickness quantification a difficult task. Cryo-preparation techniques cause minimal structural changes of the sample. Boundaries between viable epidermis and SC in biopsy samples might be difficult to determine resulting in less accurate measurements. Monteiro-Riviere et al. compared the SC and viable epidermis thickness at 5 body sites in 8 animal species, by paraffin sections and frozen sections (Monteiro-Riviere et al., 1990). A selection of their data is presented in Table 2. The two methods showed significantly different results with smaller SC thickness with paraffin sections as compared to frozen sections. With the frozen section method, for some body sites relatively thick SC was found, sometimes even comparable to the viable epidermis thicknesses (SC of ear of rats is 8.49 μm; viable epidermis is 8.8 μm). This was confirmed by Bronaugh et al. (1983) and Sato et al. (1991). In these sections, the boundaries between viable epidermis and SC in biopsy samples might be difficult to define which may cause the large differences between the two methods when

**Table 1**
Definition of terminology used in this review.

| | Delivery site of vaccine | Delivery techniques |
|---|---|---|
| Topical application | Onto intact SC or partly removed or altered SC | Skin patches, sometimes in combination with pre-treatment of the skin by:<br>– Emery paper<br>– Tape stripping<br>– Electroporation<br>– Ultrasound<br>– Hydration, permeation enhancers<br>– Microneedle pre-treatment |
| Intradermal application | In epidermis or dermis | – Microneedles (coated, hollow, dissolving)<br>– Fluid Jet injections<br>– Powder jet injections<br>– Needle and syringe injections<br>– Invasive electroporation<br>– Tattooing |
| Cutaneous application | Onto SC or in epidermis or dermis | |