Porcine Integumentary System Models:
Part 1-Dermal Toxicology

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Introduction
Swine have been used extensively in dermal research because of the similarities of the integument to humans. In the field of toxicology the skin has been used for acute and repeat dose dermal toxicology, dermal absorption, phototoxicity, allergic contact dermatitis and photosensitisation studies. Both miniature and domestic breeds have been used for these types of studies, however, miniature breeds may be more advantageous because of their size at sexual maturity. The Sinclair, Yucatan and Hanford minipigs may all be utilized in some aspect of dermal toxicology. Using them allows the investigator to conduct experiments in mature rather than pediatric animals with a consistent size and health status as compared to farm pigs. Models are done both in vivo as well as in vitro with skin membranes and grafts. Reviews of the use of swine in these studies have been published. 1-8

Information on porcine models involving the integumentary system will be published on this website in two parts. The first part will summarize models related to dermal toxicology and Pt. 2 will discuss skin closure and wound healing models.

Anatomy and Physiology
As with all animal models there are both similarities and differences between the pig and humans. Macroscopically, the pig is a relatively hairless animal with a fixed skin that is tightly attached to the subcutaneous tissues. The cutaneous blood supply and sequence of events in wound healing are also similar to that in humans. Miniature breeds can either have pigmented or non-pigmented skin. For example, the Sinclair and Yucatan breeds may be procured in a spotted variety in which both types of skin are available on the same animal. 7, 11, 12

Swine and humans have similar body surface areas which makes them more comparable than smaller animals, such as rodents. Body surface area can be calculated as per the formulas below: 1

Human — (height[cm] X weight[kg]) / 3600
Miniature pig — 0.121 BWkg 0.575 = m²
or
(70 X BW0.75)/1000 = m²

However, the skin of the pig is thicker and somewhat less vascular overall than human skin. The thickness of the skin is especially pronounced in sexually mature animals on the dorsal surface of the neck and back and in some breeds such as the Yucatan. The thinnest skin is located on the ventral abdomen and pinnae. Epidermal and dermal measurements have been made in domestic and miniature breeds and are comparable between animals of a similar age. 5, 7, 8

Humans have been described as having an
epidermis of 70 μm (50-120 μm) with a stratum corneum of 10 μm and a dermis of 2.28 mm. The stratum corneum was 20 μm with 15 layers and the epidermis ranged from 40-100 μm depending upon the location. Juvenile pigs (1.5-3 mo) have a stratum corneum of 10 μm, an epidermis of 50-65 μm, and a dermis of 1.15-1.56 mm. Normal pig skin has been described both microscopically (Figure 1) and ultrastructurally. The pH of the skin is 6-7 in swine and approximately 5 in humans. The epidermis was described as being 70-140 μm and composed of the following layers from outside to inside: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale. The epidermis ends at the epidermal-dermal junction. The cellular turnover rate in the skin is approximately 28-30 days, which is similar to humans. The stratum corneum contains approximately 15 layers of keratinized stratified squamous epithelium which is continuously desquamated. Beneath it is the thin translucent stratum lucidum with keratinized cells devoid of nuclei. This layer contains protein bound phospholipids and eleidin, especially in thick, hairless skin. The stratum granulosum is the next layer and consists of cells containing keratohyalin and lamellar granules which release lipid by exocytosis into the intercellular space.

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Figure 1. Histologic section of pig skin from the abdomen. H&E staining 10X

Hydrolytic enzymes interact with the lipids which results in an intercellular lipid matrix. The lipid matrix between keratinized cells is both the primary barrier and the pathway for penetration of topical drugs. The physical properties of this matrix contribute to the similarities in transdermal penetrance to humans. The stratum spinosum is immediately below this layer. The stratum basale consists of columnar or cuboidal cells with the dual function of attaching to the dermis and producing new epidermal cells. There are approximately four viable cell layers. Non-
keratinocytes in the epidermis are melanocytes, Merkel cells and Langerhans cells\(^4, 9, 10\).

The epidermal-dermal junction provides the basement membrane to the epidermal cells and the connection to the dermis. There are eight separate antigenic epitopes in this structure. Six of these cross react with those of humans: laminin, type IV collagen, fibronectin, GB3, BP and EBA. However, L3d and 19-DEJ-1 do not. This structure is ultrastructurally similar to humans and invaginates into the dermis with finger-like projections (epidermal pegs and dermal papilla). Besides its adherence and maintenance functions, this structure also is a selective barrier for restriction and transport of molecules, wound healing and immunological function\(^4,9,10\).

The dermis (corium) is composed of dense irregular connective tissue containing elastic, collagen and reticular fibers in amorphous ground substance. This layer contains numerous cellular and adnexal structures. Cells commonly found are fibroblasts, mast cells, plasma cells, macrophages, chromatophores and fat cells. Blood vessels, lymphatics and nerves are predominantly located in this layer. Glands include sweat glands, sebaceous glands, and hair follicles with their associated arrector pili muscles\(^4,9,10\).

There are approximately 60-75 capillary loops/mm\(^2\) in human and pig skin and approximately .7 m of blood vessel length/cm\(^2\).\(^10\) Blood flow depends upon the region and is highest in the ventral abdomen at approximately 18 ml/min/100 g and lowest in the dorsum and buttocks at approximately 3 ml/min/100g. The capillaries are more involved with body temperature regulation than in humans but otherwise perform the same essential functions of nutrient transport, removal of waste products and blood pressure regulation\(^5, 9, 10\).

Hair follicles are arranged in a triad and contain an intrafollicular muscle in addition to the arrector pilimuscles which contribute to the erection and rotation of hair shafts. There are approximately 11 hair follicles/cm\(^2\) in pigs and humans. The hair follicle is also a potential source of transdermal penetrance of test substances\(^5, 10\).

Sebaceous glands as well as apocrine and eccrine sweat glands have a difference in function, number and location from humans. In pigs, apocrine rather than eccrine sweat glands are extensive but do not contribute to sweating or thermoregulatory functions significantly. Eccrine sweat glands are limited to the snout and carpal glands. Consequently, pigs thermoregulate by blood flow modulation and either finding shade or wallowing in mud. The
secretions on the skin may contribute to preventing fluid loss but do not have a sweating function similar to humans. There is also a mental gland on the ventral chin which is a mass of apocrine and sebaceous glands with tactile hairs.4, 5, 9, 10

The hypodermis or subcutis is a layer of loose connective tissue which produces fascia and elastic fibers connecting the skin to muscle. The subcutaneous fat (panniculus adiposus) is located in this region. In boars, this layer becomes transversed with collagen fibers which provide a protective shield on the dorsolateral aspects of the animal.9, 10

In summary the similarities between pigs and humans are a sparse hair coat, relative thick epidermis, epidermal turnover kinetics, lipid composition, carbohydrate biochemistry, enzyme histochemistry, lipid biophysical properties and arrangement of dermal collagen and elastic fibers. The differences are the interfollicular muscle, the apocrine versus eccrine sweat glands, the thicker stratum corneum, the basement membrane epitopes and Cytochrome P-450 biotransformation isoenzymes.4, 5

**Models**

Four basic types of in vivo models of dermal toxicity have been suggested.4, 6 They are transdermal absorption, phototoxicity, chemical vesication, and allergic contact dermatitis.

**Transdermal Absorption:** Penetration of the skin by topical agents may be due to intercellular, pore, skin breaks, and/or interfollicular pathways. Absorption may be variable depending upon the temperature, the humidity, the skin condition, the surface area of the application, the location on the skin, and whether the area is covered. The properties of the agent and its vehicle are also important. In general, the pig is accepted as an appropriate model for topical agent testing and skin penetrance is second only to macaques in its similarity to humans for both lipophilic and hydrophilic drugs. Human permeability is higher than pigs for most compounds tested. The pig ear has been used as a predictive model because it is relatively thin and highly vascularized but transdermal absorption has been performed on the ventral abdomen and dorsum as well. In the case of the caudal ventral abdomen there is the opportunity to study absorption inareas of direct cutaneous blood supply in the region of the last nipples versus musculocutaneous blood supply cranial to that region.1-13

Fluxes of drugs in μg/(cm2h) have proved to be similar between pigs and humans in vitro.8 Drug metabolism of xenobiotics does take place in the skin by phase 1 and phase 2 enzymes. The
Cytochrome P-450 isoenzymes are located in the keratinocytes of the basal layer and the hair follicles. Transdermal drug delivery also includes the technique of iontophoresis, which involves the use of electrical current to enhance penetration of drugs that ordinarily would not be permeable. Using this technique charged drugs are transported after applying an opposing electrical field.

Skin can be prepared by washing with gentle detergent and adhesive tape stripping to reduce the thickness of the stratum corneum for these studies.

**Phototoxicity:** Sunburn can be induced in swine by exposure to ultraviolet light, predominately UVB, using wavelengths of 280-320 nm delivered using 8 watt bulbs. Swine develop erythema, microvesicles, edema, dermal inflammation and sunburn cells. Sunburn cells develop eosinophic cytoplasm with pyknotic nuclei. This model can be useful in studying skin protectants and effects of the depleted ozone layer.

**Chemical Vesication:** The pig has been described as a model of chemically induced vesication. Caustic chemicals can induce blisters and epidermal vesicles in humans and a similar sequence of events has been induced in swine with mustard agent (HD). Pigs developed blisters, epidermal vesicles, edema and microvesicles. The epidermal-dermal junction was involved immunologically in the sequence of events. The pig may be a valid model to test the pathogenesis and treatment of chemical agents which cause vesication in humans.

**Contact Allergic Dermatitis:** Allergic contact dermatitis is a T cell mediated event which can be induced in swine. Many of the clinical and pathological events which occur in humans occur in minipigs and a study with 2,4 dinitrofluorobenzene was used to demonstrate these effects. The similarities include epidermal acanthosis, spongiosis, intracellular edema, exocytosis and intradermal abscess formation. Immunohistochemical characterization was performed and there were many similarities to humans and a few differences. Pigs have some differences in subsets of T cells and have extrathymic CD4+ and CD8+. Using the thin skin on the pinnae, medial aspect of the thigh or ventral abdomen may validate the pig as an appropriate model of allergic contact dermatitis.

**Summary**
Swine as a model in toxicity testing of pharmaceuticals and other chemicals is now being well accepted by Japan, EU and USA regulatory agencies. Swine are specifically mentioned as a potential non-rodent species in the guidelines of Japan and Canada and would generally be considered superior to dogs and rabbits as a dermal model. The OECD 409
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guideline lists pig and minipig as optional species. However, evidence should be provided that it is a suitable species in order to overcome residual regulatory resistance. Increases in the amount of background information on this species will continue to demonstrate their usefulness in toxicology and specifically as a dermal model.5, 14, 15

References
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