Altasciences, 562 State Road DD, Auxvasse, MO, 65231, United States
Altasciences.com | contact@altasciences.com

Why Use Miniature Swine in Dermal Research?

Madsen, T.[1]; Blair, E.[1]; Bouchard, G. F.[2]
[1]Altasciences, Columbia, USA; [2]Sinclair Bio Resources, Auxvasse, MO, USA

ABSTRACT

Swine have been used extensively in dermal research because of the similarities of the integument to humans. Miniature swine offer many distinct advantages for dermal research. Skin researchers require efficient animal models which are predictive of human responses. Pig skin is anatomically, physiologically, biochemically, and immunologically similar to human skin, and the skin is fixed skin like that of humans. Pig skin mirrors human skin in having a sparse haircoat; a relatively thick epidermis; similar epidermal turnover kinetics, lipid composition and carbohydrate biochemistry; lipid biophysical properties; and a similar arrangement of dermal collagen and elastic fibers. Porcine or miniature swine models offer significant advantages and have a record of predicting treatment modalities in humans better than other alternatives. Included uses are models for malignant melanoma, wound healing (including delayed diabetic model), phototoxicity, dermal toxicology, dermal pharmacokinetics/toxicokinetics (PK/TK), iontophoresis, dermal irritation, thermal injury, contact allergic dermatitis, depigmentation, and chemical vesication. These dermal study types will be briefly described, and aspects miniature swine studies will be discussed. Miniature swine models provide useful safety and efficacy data for novel cutaneous therapy product development. Miniature swine offer researchers unique tools in dermal research.

Dermal Studies in Miniature Swine

Mortensen et al. (1998) have reviewed the uses of miniature swine in dermal toxicology. Hanford, Sinclair S-1, Yucatan, and Micro-Yucatan miniature swine are utilized in some aspects of dermal toxicology testing and dermal research. Dermal study models and their applications are expanding. Below are selected model descriptions. (E² ERG system by Diagnosys, LLC with ColorDome stimulator)

Figure 1. Juvenile Sinclair S-1 miniature swine

Regressing Melanoma

Human melanoma is currently the fastest growing cancer in the USA and worldwide. Melanoma accounts for 5% of all skin cancers, but 71% of all skin cancer deaths. The risk for melanoma has steadily increased since 1935 when it was 1:1,500. Today one in 74 people are diagnosed with melanoma. A sub-lineage of Sinclair S-1 has naturally occurring spontaneously regressing malignant melanoma. At birth, 54% of Sinclair miniature swine have primary lesions, which increases to 85% at the age of one (Goldberg et al., 2004). Over 70% of primary lesions result in metastasis to regional lymph nodes. Tanaka et al. (2006) have developed a novel invisible near-infrared fluorescent method for *in vivo* Sentinel lymph node (SLN) mapping and resection using this model. Miniature swine offer insights into the genetics, immunology, and pathogenesis of malignant melanoma. (Sinclair miniature swine without melanoma are also available for other model uses.)

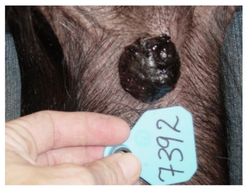

Figure 2. Large melanoma growth on ventral chest of Sinclair S-1 piglet

Dermal Toxicology

In light of the morphological and physiological similarities between human and porcine skin, miniature swine is the preferred species to evaluate the safety profile of dermally applied xenobiotics. In addition, miniature swine is an accepted second species for GLP toxicology/safety assessment (Jacobs, 2006). Swine as a model in toxicity testing of pharmaceuticals and other chemicals is now well-accepted by Japan, EU, Canada, and USA regulatory agencies. The OECD 409 Guideline lists pig and miniature pig as optional species for the second non-rodent species in toxicology testing. In most cases, swine should be selected as a primary species over dogs and rabbits as a dermal toxicology model. Young adult 3-month-old to 6-month-old Hanford miniature swine are most commonly used. Dermal maximum tolerated dose (MTD or dose escalation) studies are common in miniature swine, as the dermal surface area is adequate. Changes over time (during growth) have been studied to determine the potential effect upon the dose/unit surface area and dose/unit body weight. Dermal studies in miniature swine allow the evaluation of both local and systemic toxicity, and normal reference data is readily available.

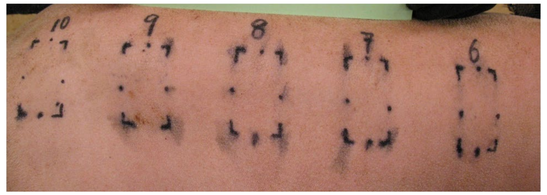

Figure 3. Demarcated dermal application areas

Wound Healing

Swine have been a standard for wound healing studies for many decades, including in the diabetic state (Velander et al., 2008). Because they have a fixed skin, their wound healing characteristics are similar to that of humans, with comparable elastic properties, and Sinclair, Yucatan, and Hanford miniature swine make good wound models. Porcine, small mammal, and *in vitro* models have been compared to determine which correlated most closely with human wound healing. The porcine *in vivo* methods were in agreement with human studies 78% of the time, while those of the other species were comparable only 60% of the time (Sullivan et al., 2001). Because of the size of miniature swine, multiple partial or full thickness wounds can be placed on the same animal, thus allowing the animal to also serve as its own control. Kerrigan et al. (1986) has published an account on various pig skin, myocutaneous, and fasciocutaneous flaps and grafts studied in cosmetic surgery.

Table 1. Dermatology and Wound Healing Models: Human vs. Porcine vs. Rodent

Parameter	Human	Porcine (Domestic)	Std. MS	Rat	Mouse
Skin thickness	50-120 µm (70 µm)	70-140 µm	70-140 µm	10-20 µm	Very thin (5-10 µm)
Epidermal thickness	Relatively thick 26 µm 2.67 layers	Relatively thick 52 µm 3.94 layers	Relatively thick: 50-65 µm 4-5 epithelial sub layers	21.7 µm 1.83 layers	13.3 µm 1.75 layers
Stratum corneum thickness	10-12.05 µm	12.28 µm	10-20 µm	5 µm	2.9 µm
Fixed skin	Yes	Yes	Yes	No	No
Hair coat	Sparse, 11 hairs/cm²	Sparse, 11 hairs/cm²	Sparse 'hairless', 11-25 hairs/cm²	Thick, 289 hairs/cm²	Thick, 658 hairs/cm²
Epithelial cellular turnover rate	28d	28d	28-30d	ua	ua
Skin blood flow rate, back (ml/min/100g)	3.12	3.0	3.0	9.6	20.6
pH of skin	5	6-7	6-7	ua	ua
Primary wound healing pattern	Re-epithelialization	Re-epithelialization	Re-epithelialization	Contraction	Contraction

Table 2. Miniature Swine Re-epithelialization Rates* by Microscopic and Macroscopic Observation

Strain MS	Status/Sex/ N Animals	Age/Wt	Type Dermal Wound	Post-Wounding Measurement Timepoint	Re-epithelialization Rate (mm/hr)	Reference
Yucatan	Normal Castrate/ male/2	1-2 yr, 54.3-57.5 kg	Full-thickness	d29	0.072**	Internal Data, SRC 2008
Yucatan	Diabetic Castrate/ Male/4	1-2 yr, 41.9-53.1 kg	Full-thickness	d29	0.064**	Internal Data, SRC 2008
Sinclair	Normal/ Female/5	9-10.8 yr Geriatric adult, 76.9-93.5 kg	Full-thickness	d32	0.048***	Internal Data, SRC 2008
Sinclair	Normal/ Female/5	0.5 yr Juvenile/Young Adult, 14.8-20.9 kg	Full-thickness	d19	0.109***	Internal Data, SRC 2008

*Rates per hour calculated from SRC research data; **microscopic observations; ***macroscopic observations

Phototoxicity

Morikawa et al. (1974) conducted experimental studies on the evaluation of laboratory animals, including miniature swine, for phototoxicity and photoallergy. Sambuco (1985) suggested the Yucatan species would make a good model for phototoxicity. Miniature swine react in an expected fashion to a combination of topical photoreactive 8-MOP and solar and UV irradiation (280 to 320 nm): they develop skin erythema, microvesicles, edema, dermal inflammation, and sunburn cells. The Hanford and Yucatan lineages have been validated by internal studies at Altasciences, and a peer-reviewed reference paper is currently in development. These models can be useful in studying skin protectants, solar irradiation damage, and the effects of the depleted ozone layer. Under the UVR test conditions, Hanford miniature swine are more sensitive to skin irradiation than Yucatan miniature swine.

Dermal PK/TK

Dermal absorption and percutaneous permeation of miniature swine skin has correlated well to human responses during both *in vitro* and *in vivo* studies. Absorption, distribution, metabolism and excretion (ADME) or distribution, metabolism, pharmacokinetics/toxicokinetics (DMPK)/(TK) studies are primarily conducted in the young adult Hanford miniature swine model. Radiolabel drug studies are a necessary component of these studies. Miniature swine are good models for the cytochrome P450 family (CYP3A), the main monooxygenase of drug biotransformation, and metabolism in the liver (Gad, 2008).

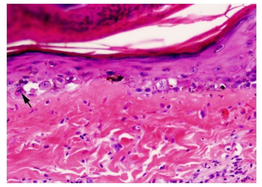

Figure 4. Swine 9836, Yucatan, Site 12. Severe multifocal epidermal apoptosis (dysplasia) with a sunburn cell (arrow), vacuolar degeneration, and serocellular crust on the epidermis (hematoxylin and eosin [H&E], 100x).

Table 3. Microscopic Pathology Mean Sum Scores (Erythema and Edema), Phase II

Animal ID/Duration	Control	Vehicle	0.0001% 8-MOP	0.001% 8-MOP	0.01% 8-MOP	0.1% 8-MOP
5727/Hanford/35 min	0	0	0	0	4	14
5727/Hanford/70 min	1	0	3	0	2	13
5659/Hanford/35 min	3	1	5	1	9	15
5659/Hanford/70 min	2	3	0	6	5	16
0181/Yucatan/35 min	0	0	1	0	2	6
0181/Yucatan/70 min	0	1	0	0	2	8
9944/Yucatan/35 min	3	3	1	0	4	11
9944/Yucatan/70 min	0	0	0	0	2	8

Transdermal Absorption

The miniature swine is an accepted model for determining topical agent skin penetrance for lipophylic and hydrophobic drugs. Human skin permeability is slightly higher than that of swine for most compounds tested on the dorsum. The swine ear is recommended as a predictive model, as the skin is relatively thin and highly vascularized. Transdermal absorption has been performed on the miniature swine dorsum and ventral abdomen. (The area of the last nipples is best for direct blood supply to skin.) Fluxes of drugs [µg/(cm²h)] across the skin have proven to be similar between swine and humans *in vitro*. Two novel dermal drug candidates were compared topically on the dorsal skin of young adult Hanford and Yucatan miniature swine as a TK study. The Hanford miniature swine had the most absorption through the skin for both drugs. In another PK study, topical absorption of a two-drug analgesia combination was studied from Hanford and Yucatan miniature swine dermal patches. Again, Hanford absorption was greater. The Hanford has the greater number of hair follicles, which may enhance absorption via the skin. Bronaugh et al. (1982) have shown that the hair density of human and pig skin is 11 follicles per cm², while that of rats, mice, and hairless mice is 289, 658, and 75 follicles per cm², respectively.

Iontophoresis

Electrical current enhances penetration of charged drugs that ordinarily would not be permeable to skin. Miniature swine have been successfully used as an animal model for these studies at Altasciences.

Dermal Irritation

Mannisto et al. (1984) studied the dermal toxicity (median erythema and irritation concentrations) of chemicals on 24 sites per miniature swine, which react to skin irritants with erythema and edema responses, as do humans (Draize endpoints). Studies on water-based cream, solvent-based, or alcohol-based drugs have been successfully conducted in miniature swine. Standardized skin scoring and training for grading of erythema responses have been established at Altasciences.

Burns

In response to thermal skin injury, miniature swine skin reacts grossly, biochemically, histologically, and immunologically like humans. Dorsal thermal wounds are created by applying an aluminum bar heated in a water bath to an anesthetized unconscious animal predosed with analgesia (pre-emptive analgesia). The animal is then recovered, placed on additional analgesia to control pain, and experimental treatments are administered to evaluate their effectiveness. The miniature swine recover very well under the proper analgesic regime. The adult Yucatan is our standard model, although other lineages should work equally as well.

Contact Allergic Dermatitis

T-cell-mediated allergic contact dermatitis (ACD) is inducible with 2,4-Dinitrofluorobenzene (2,4-DNFB). Vana and Meingassner (2000) reported that a frequently used model is ACD; however, this condition is not well-characterized in miniature swine. Erythema and cutaneous blood flow peaked at 24 hours. The major epidermal changes most pronounced at 48 hours were acanthosis, spongiosis, intracellular edema, exocytosis, and abscesses, mainly containing neutrophils and mononuclear cells (MNC). Perivascular infiltrates of MNC, as well as neutrophils and eosinophils, were the most significant dermal changes, with peak levels at 24 to 48 hours. In biopsies taken before the challenge, CD1+ dendritic cells were found in similar numbers and locations as major histocompatibility complex class II+ (MHCII+) cells in the epidermis. At this site, the maximum CD1+ cell decrease occurred at 24 hours, whereas the maximum increase in CD1+ stained cells was seen at 72 hours in the dermis. The dermal infiltrate (CD2+, CD5+, CD25+, and CD45+) was most dense at 48 hours. Between 8 and 48 hours more CD4+ were present than CD8+ cells, whereas at 72 hours CD4+ and CD8+ cells were similar in numbers. These findings closely resemble changes in human ACD. Miniature swine have some differences in T cells and have extrathymic CD4+ and CD8+ cells. Using the skin on the pinnae, medial thigh, or ventral abdomen is suggested. Therefore, DNFB-induced ACD in miniature swine is considered to be an appropriate animal model to study immunopathologic mechanisms and pharmacologic intervention.

Tacrolimus ointment, formulated for the treatment of atopic dermatitis, is the first in a class of topical immunomodulators. Tacrolimus was shown to be safe in a 52-week study with Yucatan miniature swine. No noteworthy macroscopic or microscopic changes (either dermal or systemic) related to the application of tacrolimus ointment (0.03% to 0.3% concentrations) were observed (Bekersky et al., 2001). Tacrolimus ointment was shown to be safe and effective in phase II and early phase III studies (Bekersky et al., 2001).

Depigmentation

Nair and Tramposch (1991) studied the Yucatan miniature swine as an *in vivo* model for screening skin depigmentation. Three depigmenting compounds (hydroquinone, 4-hydroxyanisole, and tert-butyl catechol, each at 5% concentration) were evaluated. The Yucatan miniature swine is an accepted *in vivo* model for screening for drug-induced depigmentation. The Yucatan can exhibit either hyperpigmentation or depigmentation at skin sites, depending upon the selective drug treatment. A chromometric measurement device is used to quantitatively assess pigmentation changes.

Chemical Vesication

Miniature swine have been used for medical chemical defense vesicant research for many years. Monteiro-Riviere and Riviere (1996) have reported that caustic chemicals can induce blisters, and epidermal vesicles in humans and a similar sequence of events have been induced in miniature swine with a mustard agent. Swine develop blisters, epidermal vesicles, edema, and microvesicles. The epidermal-dermal junction is involved immunologically in the pathogenesis. Miniature swine models offer tools for screening preventive skin protectants, treatment agents, and regimens for warfare agents and highly skin-caustic industrial chemicals.

DISCUSSIONS

Miniature swine offer many dermal models for testing and basic research. The anatomy and physiology of the cutaneous blood supply and wound healing characteristics have made the miniature swine a standard model for plastic surgical and wound healing studies (Chvapil & Chvapil, 1992; Kerrigan et al., 1986; Mertz et al., 1986). Besides the anatomic similarities, miniature swine are equivalent to primates for percutaneous absorption studies and have similar lipid biophysical properties, epidermal turnover kinetics, and carbohydrate metabolism in the skin (Montiero-Riviere & Riviere, 1996). They have fixed skin like humans and heal primarily by re-epithelialization rather than contraction. The ability to perform paired wound healing measurements reduces the effect of inter-animal variability and permits more robust paired statistical tests (Paddock, 1996). Unlike rodents, miniature swine possess sufficient body weight to tolerate prolonged topical administration of potent drugs (Nunoya et al., 2007). They can also serve as models of delayed wound healing associated with the diabetic state. Although dorsal pig skin does not contain eccrine sweat glands like human skin, the porcine model of partial-thickness skin wound healing has been validated and appears to successfully predict the effects of novel treatments in humans (Paddock, 1996; Montagna & Yun, 1964). Other valid dermal research applications exist, as well.

CONCLUSIONS

The miniature swine model is a predictive model for many dermal applications, including malignant melanoma, wound healing (including delayed diabetic model), phototoxicity, dermal toxicology, dermal PK/TK, transdermal absorption, iontophoresis, dermal irritation, burns, contact allergic dermatitis, depigmentation, and chemical vesication. Putative treatment drugs or modalities can be screened in these models, and basic biochemical assays for markers can be conducted.

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