Nonclinical Development of Products Intended for Treatment of Damaged Skin

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1 INTRODUCTION

For pharmaceutical products intended for dermal application, the minipig is an ideal animal model, as pig skin is well-known to resemble human skin with regards to characteristics such as thickness, attachment to underlying structures and presence of rete ridges.

Under normal conditions, the skin constitutes a relatively profound barrier to the external surroundings. However, in some human skin diseases, superficial abrasions and sores develop, potentially increasing systemic absorption of substances intended for topical skin use. This issue is not covered when using healthy animals with intact skin, in regulatory toxicity testing of products intended for dermal application.

Several parameters need to be taken into consideration, in the development of a standardized and reproducible model for repeated administration on damaged skin. There are different methods on how to abrade (scarifying, tape-stripping), but also the timing (pre-study, during treatment) and interval between abrasion need to be taken into consideration. Here we describe a standardized and reproducible minipig model of compromised or broken skin, which is used for regulatory toxicity testing of dermal products.

Table 1

Parameters evaluated and graded in a standard minipig wound healing study	
• Wound secretion	• Necrosis
• Haemorrhage	• Granulation
• Inflammation	• Re-epithelialization

2 MATERIALS AND METHODS

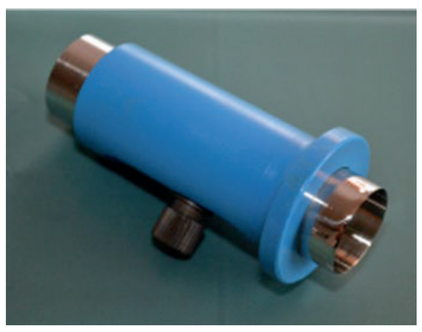
The Göttingen Minipig with full-thickness wounds is used for regulatory dermal toxicity studies, were sponsors would like to test their product on an animal model of compromised or broken skin.

The full-thickness wounds are created on the back of the animal, in the area between the scapula and the caudal costal curvature. The wounds are made using a specially designed circular knife, which removes epidermis, dermis and subcutis. Wound diameter is up to 20 mm and depending on the size of the minipigs, up to eight wounds can be made on each pig.

Dosing topically in the wound bed can be performed on a daily basis, depending on the intended human application scheme. The study design is build around a standard design for regulatory long-term general toxicity studies in non-rodents, containing all the elements required by various guidelines (clinical observations, ophthalmoscopy, electrocardiography, clinical pathology and pathology)

In addition, interference of the test substance on the wound healing process is evaluated (see table 1), and wound contraction, development of granulation tissue and the re-epithelialization process can be followed by planimetry recordings of the wound surface area.

Figure 1: Specially designed knife used to create the circular shaped full thickness wounds in minipigs



3 RESULTS AND DISCUSSION

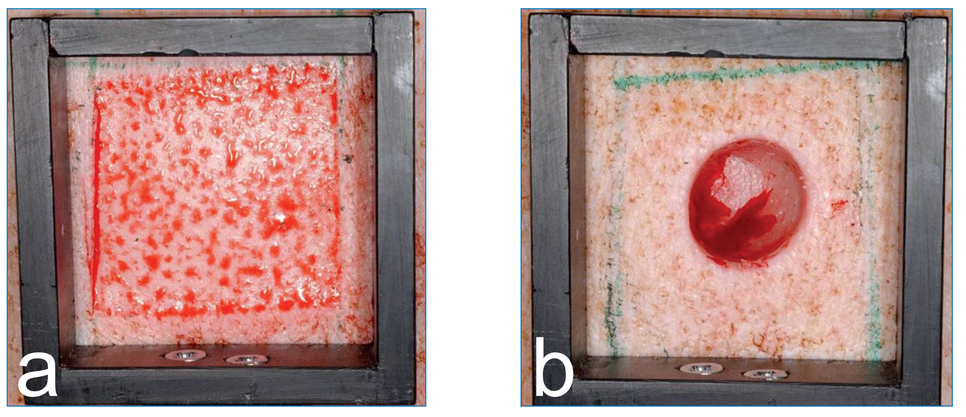
Full-thickness wounds, with removal of all three dermal layers (epidermis, dermis and subcutis), have been implemented in the study design, as these in contrast to superficial abrasions, can be inflicted in a very precise and reproducible way. Furthermore, whereas superficial abrasions tend to heal very quickly, the healing process of full-thickness wounds is protracted, enabling sufficient time for evaluation of local effects on the wound healing process.

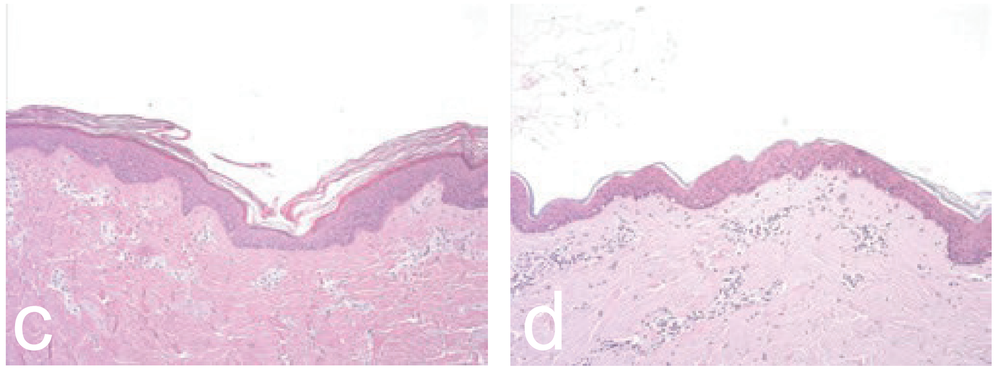
Repeated superficial abrasions may not be ethically acceptable, especially in animals that are showing clear reactions to treatment (erythema, oedema etc.). For legislative (Denmark) and animal welfare reasons, it is also not possible to establish new wounds on the animals upon complete wound closure, which would be necessary to evaluate the effects of test substances applied on abraded skin over longer periods.

In young, healthy animals, which are typically used in regulatory toxicity studies, full thickness wounds (20mm in diameter) will be completely re-epithelialized within a period of 21 days. Topical dosing in the wounds can therefore no longer be performed, and a different route of administration, for examples subcutaneous or a combination of subcutaneous and dermal dosing to ensure good systemic exposure to the Test Item, can be considered.

To prolong dosing by subcutaneous administration, it is imperative that the Test Item is provided as a formulation suitable for subcutaneous injection; i.e. it can be injected and it is not causing excessive local irritation. Moreover, one need to keep in mind that larger dose volumes cannot be applied, due to the tight adhesiveness of the skin to the underlying structures. Volumes of ≤ 0.3 ml/kg body weight are recommended.

Figure 2: **Skin abrasion methods applied in the minipig**.
a) Split-thickness wound, Day 1. Split-thickness wounds are made by removal of the epidermis and upper dermis with a dermatome. They heal by re-epithelialization in the course of around 7 days.
b) Full-thickness wound, Day 1. Full-thickness wounds are made using a circular knife, which removes epidermis, dermis and subcutis. Healing is by granulation followed by re-epithelialization.
c) Normal minipig skin x100.
d) Skin of a minipig after 20 tape strips x100. The superficial epidermis is removed by use of Scotch 3MTM Book Tape at 20 strips per occasion.





4 CONCLUSION

The combined wound healing and subcutaneous study in Göttingen Minipigs constitutes a valid and practically feasible method for evaluation of test compound, administration on abraded skin. Besides maximising systemic exposure of the test compound, the study design allows for evaluation of local effects on the wound healing process. This model therefore provides more useful safety documentation prior to human use of products intended for dermal application. Extending dosing by subcutaneous dosing as an alternative to re-scarifying is also considered a more ethically acceptable method, than re-scarifying animals with re-epithelialized full-thickness wounds or treatment related cutaneous reactions.