Practical use of Göttingen Minipigs in Wound healing Studies

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Wound Healing Studies

Assessment of local tolerance & effect on the wound healing process

Type of Products

- Medical devices
- Pharmaceuticals
- Combination products

Regulatory guidelines

- No specific guideline addressing wound healing studies
- Local tolerance[1]
- Safety assessment[2,3]

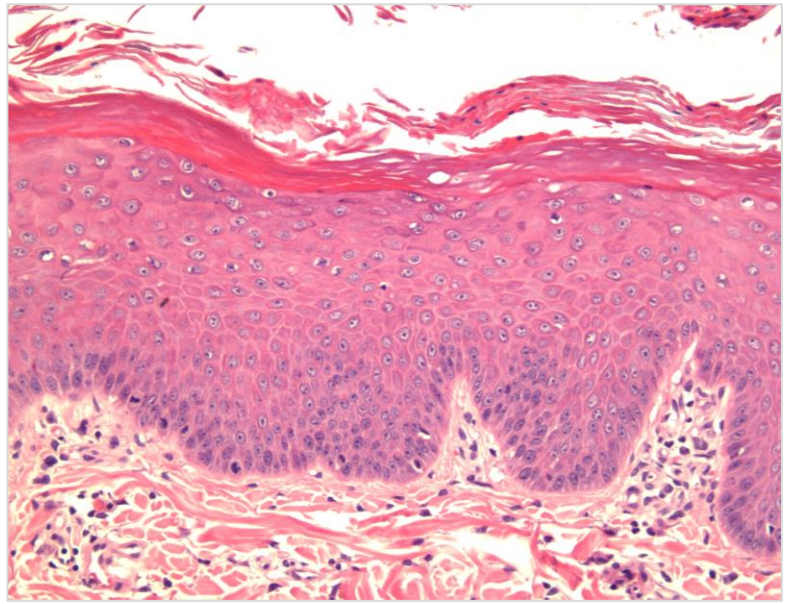
Wound Models

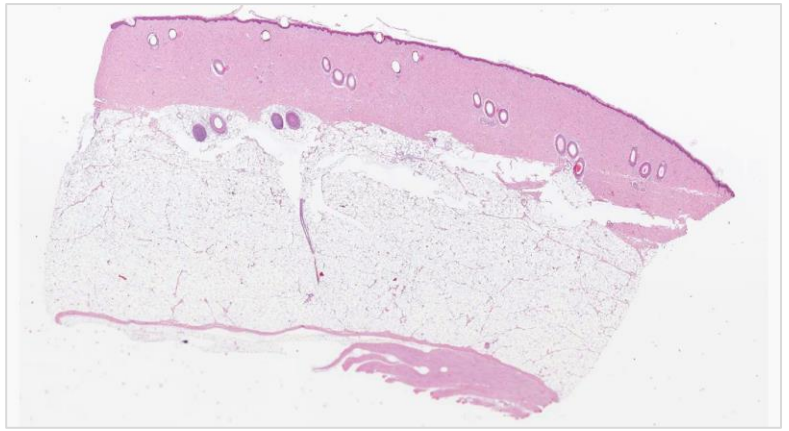
- Split-thickness wounds
- Full-thickness wounds
- Incisional wounds
- Burn wounds

[1] Guideline on non-clinical local tolerance testing of medicinal products. EMA/CHMP/SWP/2145/2000 Rev. 1, Corr. 1. 2015. EMA
[2] ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. EMA/CPMP/ICH/286/1995. 2009. EMA
[3] Guidance for Industry, M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. U.S FDA, January 2010

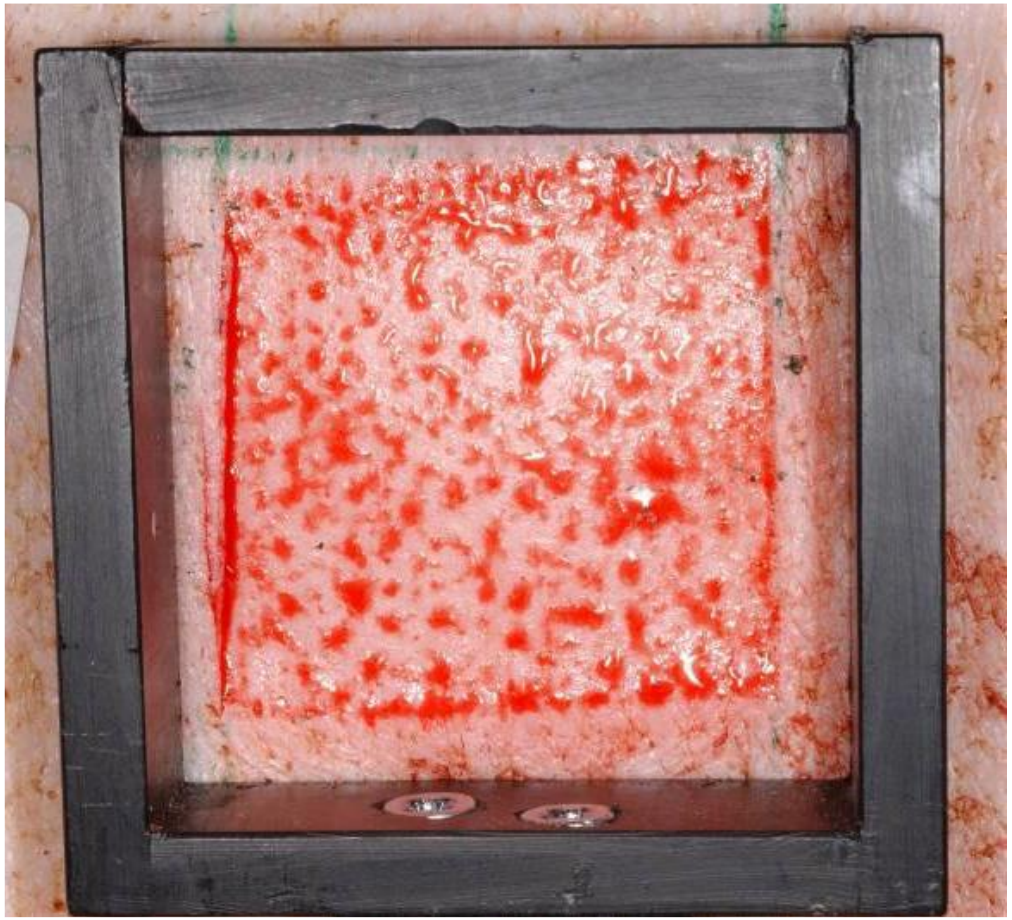
Split-thickness Wound Model

- Removal of epidermis and upper part of dermis
- Made by electrodermatome
- Square
- Healing by re-epithelialization
- Superficial lesion that will heal quickly by re-epithelialization
- Wounds re-epithelialized within approximately 7 days

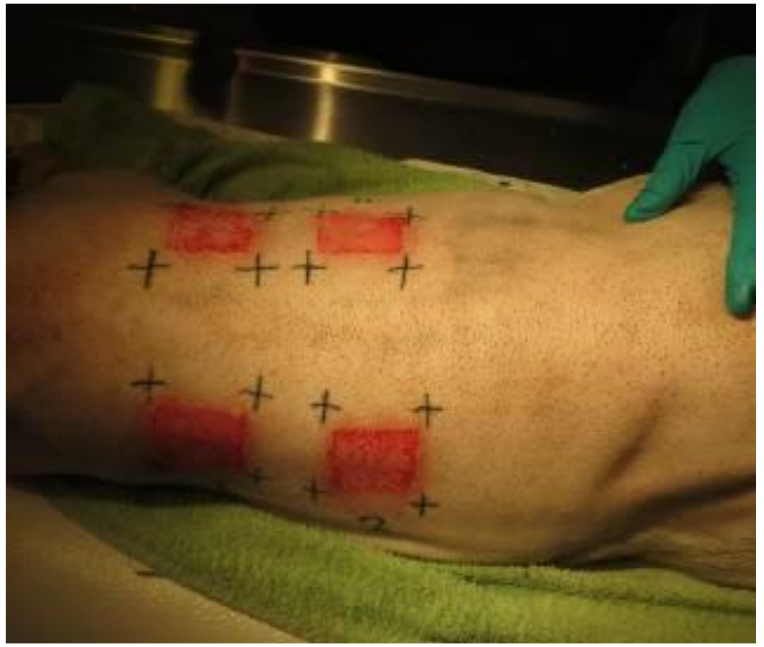




Split-thickness Wound Model



Split-thickness Wound Model



Split-thickness Wound Model

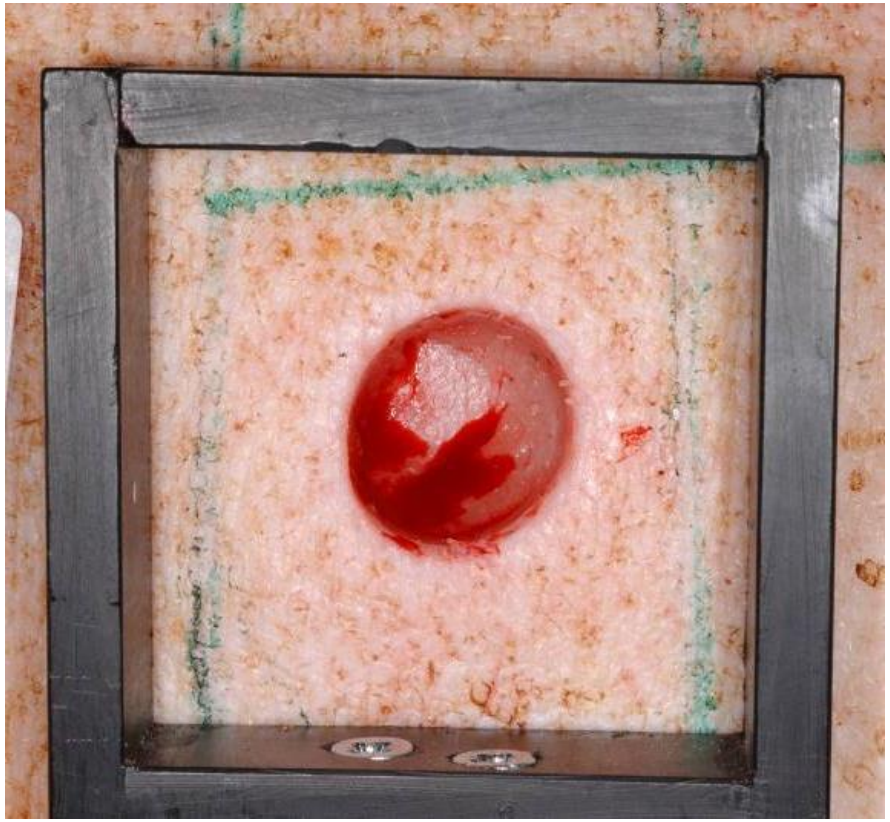
Day 3 after wounding

Day 7 after wounding

Full-thickness Wound Model

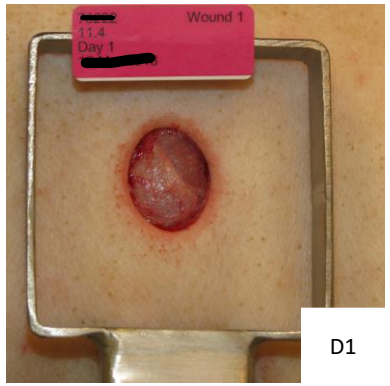
- Removal of epidermis, dermis and subcutis
- Made by circular knife or by use of scalpel
- Healing by granulation followed by re-epithelialization
- Wounds heal within approximately 3 weeks
- Alternatively, treatment can be continued via another relevant route of treatment, e.g. subcutaneously or dermal

Full-thickness Wound Model



Phases of Wound Healing

Wound healing process consists of the same elements as in humans (inflammation, contraction, proliferation, re-epithelialisation and re-modelling)



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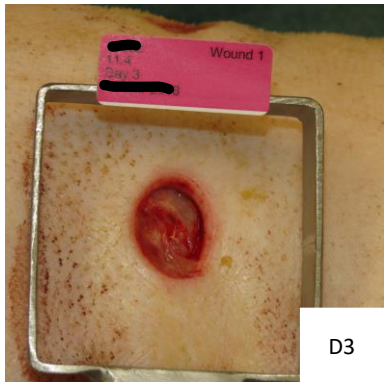

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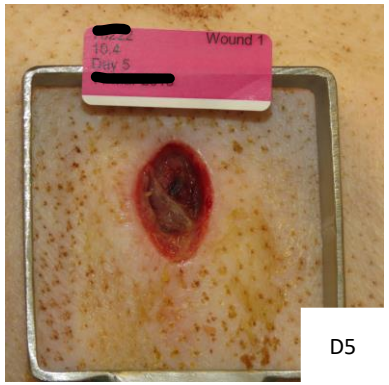

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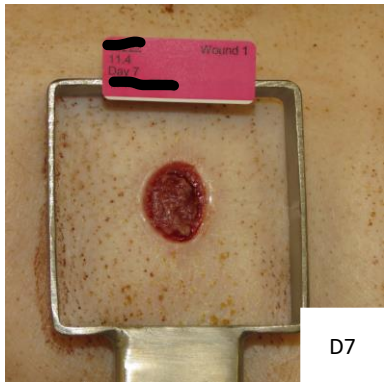

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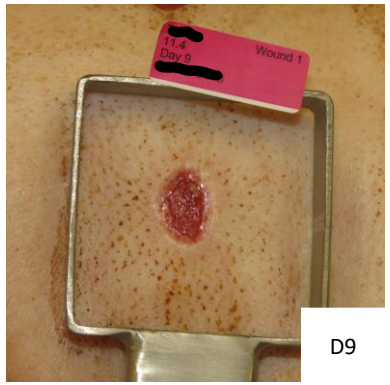

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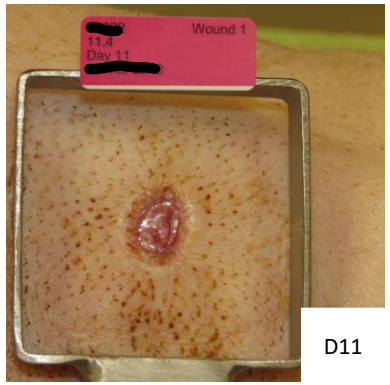

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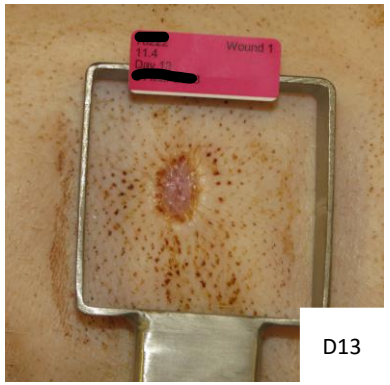

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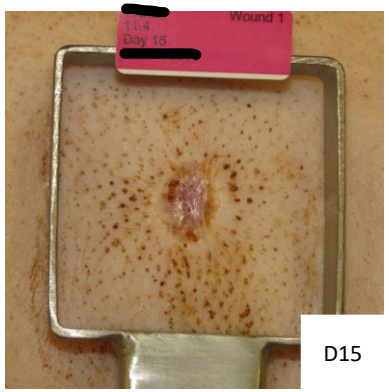

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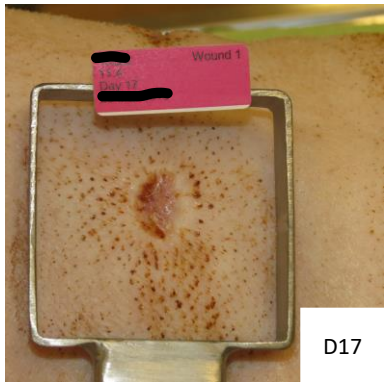

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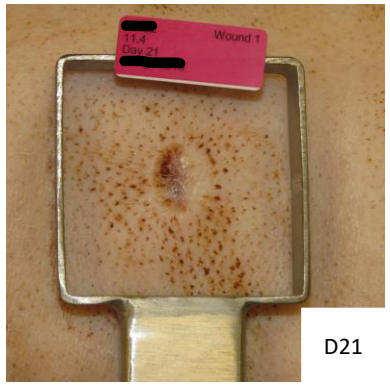


D21

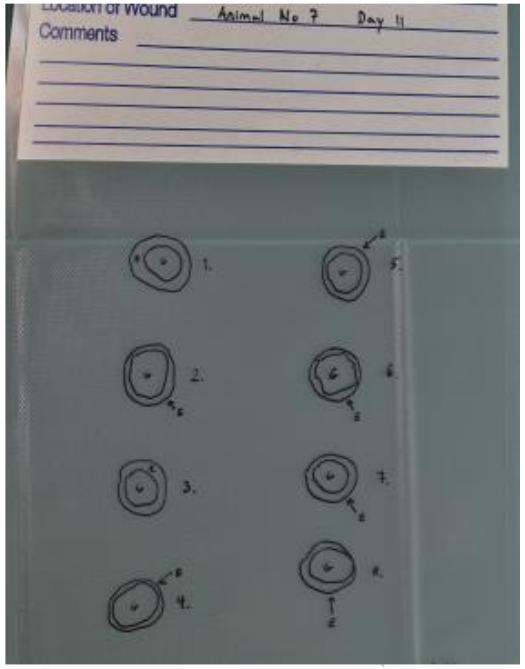
Macroscopic parameters evaluated

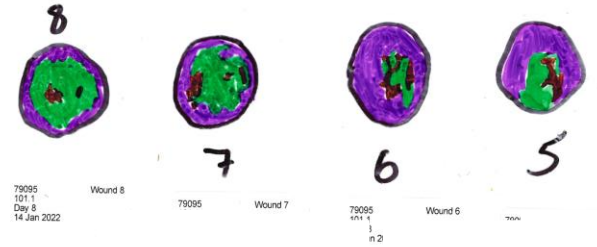
- Haemorrhage
- Wound secretion
- Inflammation
- Necrosis
- Granulation/hypergranulation
- Alternative parameters, adhesion of test material, re-epithelialization and discoloration from the test material

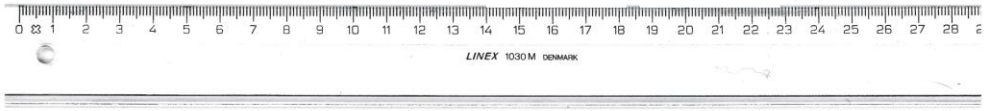
TREATMENT	AMOUNT OF EXUDATE					Total	p
	0	1	2	3	4		
1 (Placebo)	0	0	4	8	4	16	
2	0	1	4	9	2	16	
3	0	0	5	6	5	16	
4	0	0	1	6	9	16	
Total	0	1	14	29	20	64	

Planimetric Assessment

- 2-dimensional determination of wound areas
- Wound areas drawn on transparent sterile sheets or pictures can be taken
  - Total wound area
  - Area of granulation tissue
  - Area of re-epithelialized tissue
  - Area of necrotic tissue
- AI driven assessment of wound healing pictures using e.g. Visiopharm or other software

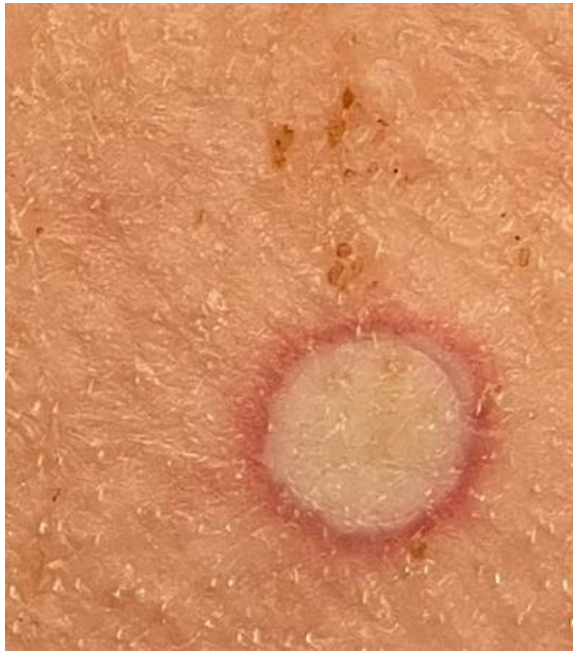




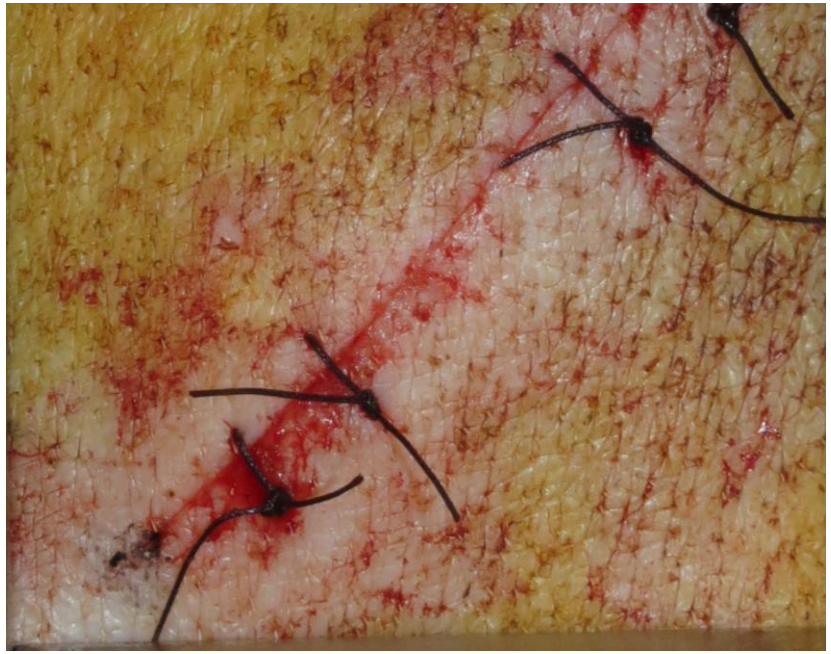


Other wound models

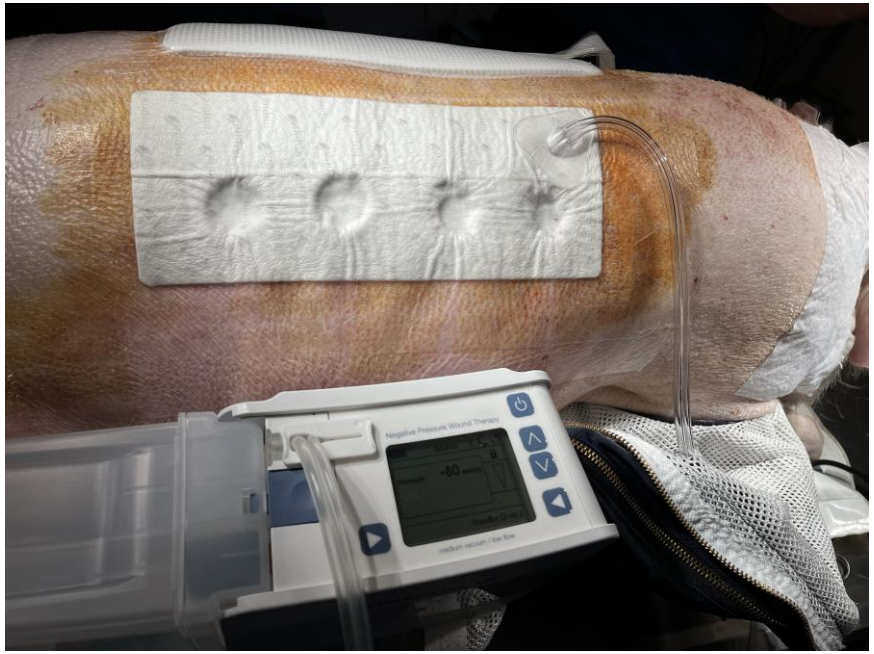
Burn wound



Incisional wound



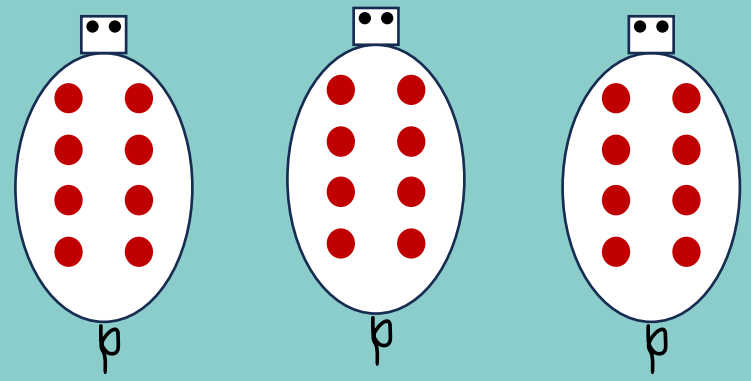
Negative pressure wound therapy



Considerations for wound healing studies

- Clinical program
- Test item
  - Amount, formulation type
- Local tolerance or systemic exposure
  - Number of animals
  - Number of wounds
  - TK assessment
- General tox study
  - Resources

Standard wound healing study



- 3 animals, 30 kg
- 8 wounds
- 4 treatments
- Each treatment represented on 6 wounds

Considerations for wound healing studies

Use of dressings

Primary dressing

- Direct contact with the wound surface
- Should be non-absorbent to allow the test item to stay in the wound
- Non-adherent

Secondary dressing

- Keep primary bandage in place
- Strong and sturdy
- Some absorptive capacity

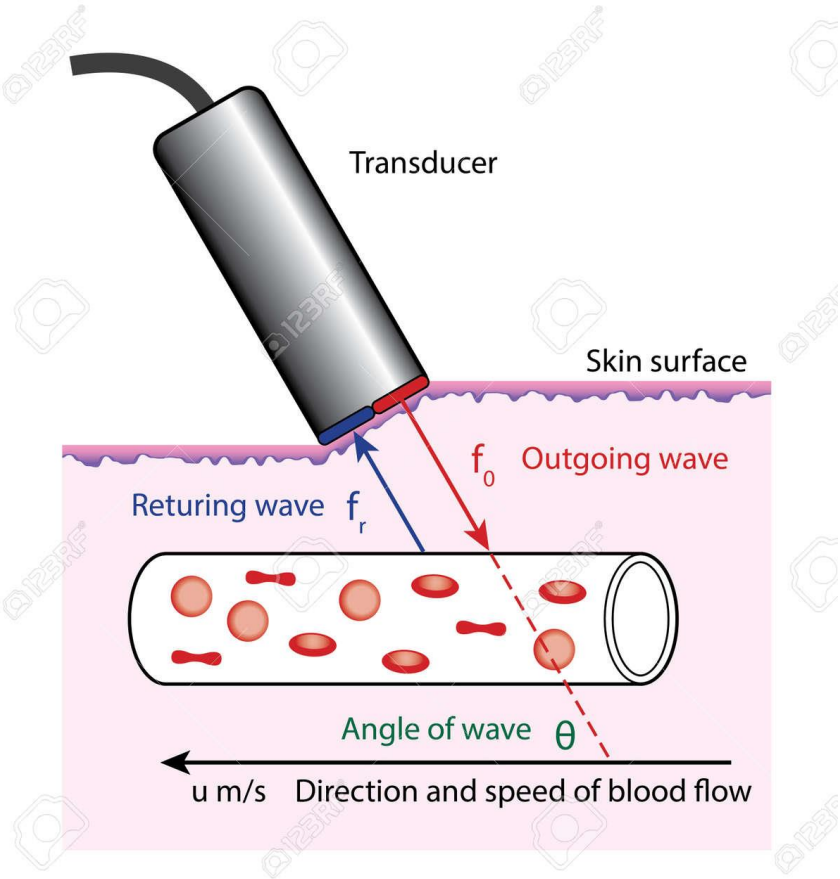
Considerations for wound healing studies

Additional measurements

- Tensile strength
- Laser doppler, skin blood flow

Animal welfare

- Anaesthesia
  - Frequency
  - Agents
- Pain management
  - NSAIDS and opioids



Thank you for the attention

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Practical use of Göttingen Minipigs in Wound Healing Studies

A pathologist's perspective

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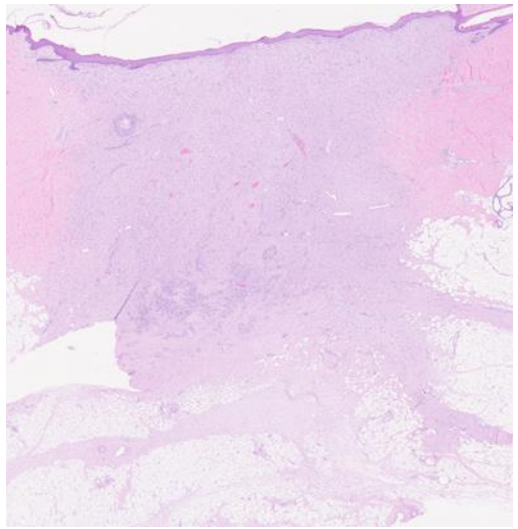


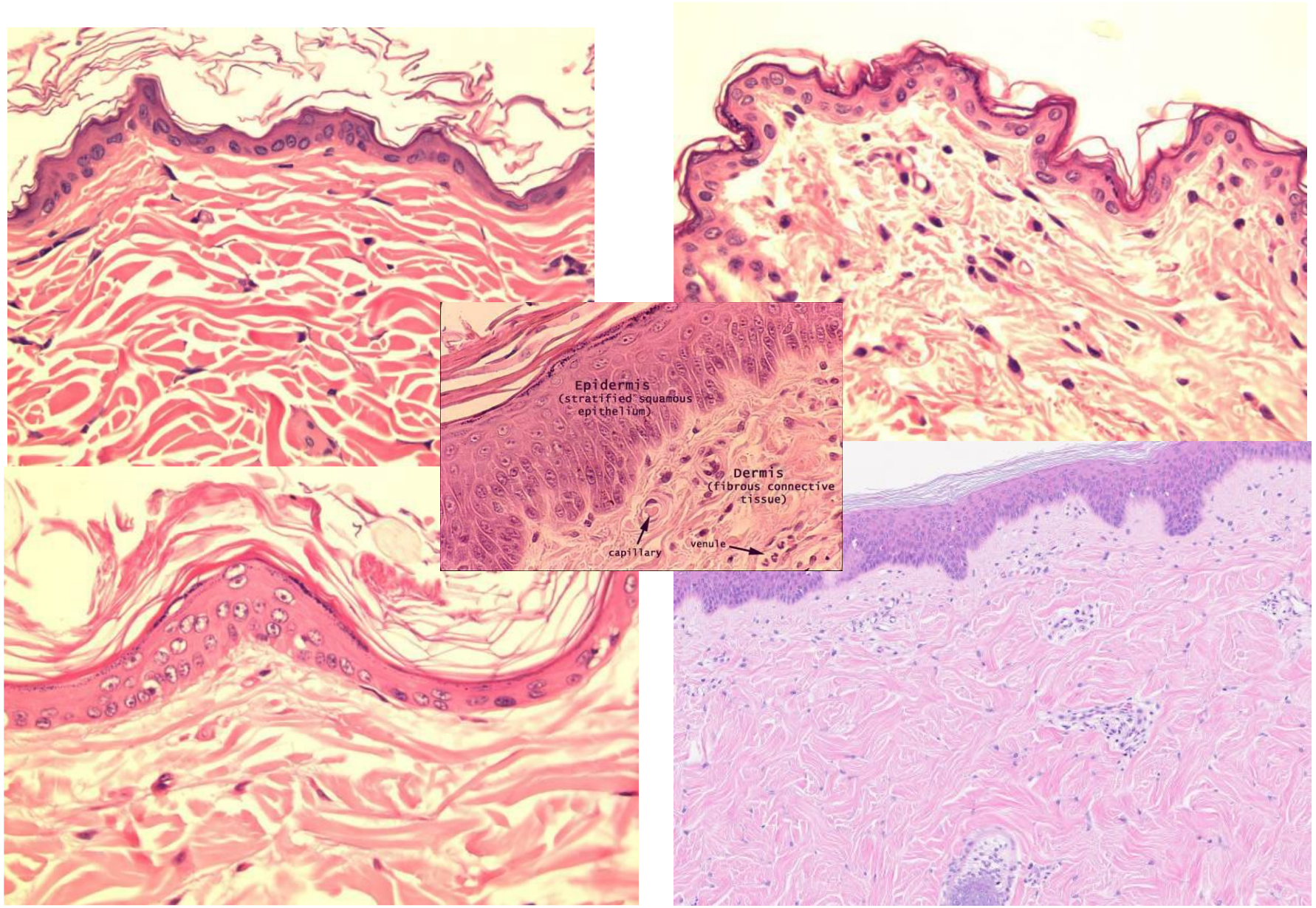
Histopathology in Preclinical Wound Healing Studies

- Histopathology is an important endpoint in preclinical wound healing studies
- It provides detailed information on the progression of the wound healing by characterizing the individual components of the healing wound
- The histopathology data provides a way to compare the effects of different test items and untreated control wounds on the wound healing parameters

Histopathology in Preclinical Wound Healing Studies

- The anatomical structure of the skin of the minipig supports the suitability of the minipig as a model of wound healing in human beings:
  - Attachment (firmly attached to the underlying structures)
  - Sparse hair cover
  - Thickness,70-140 μm in pig, 70-120 μm in human
  - The epidermis has an identical number of cell layers
  - Skin surface carved with fine intersecting lines
  - Rete ridges

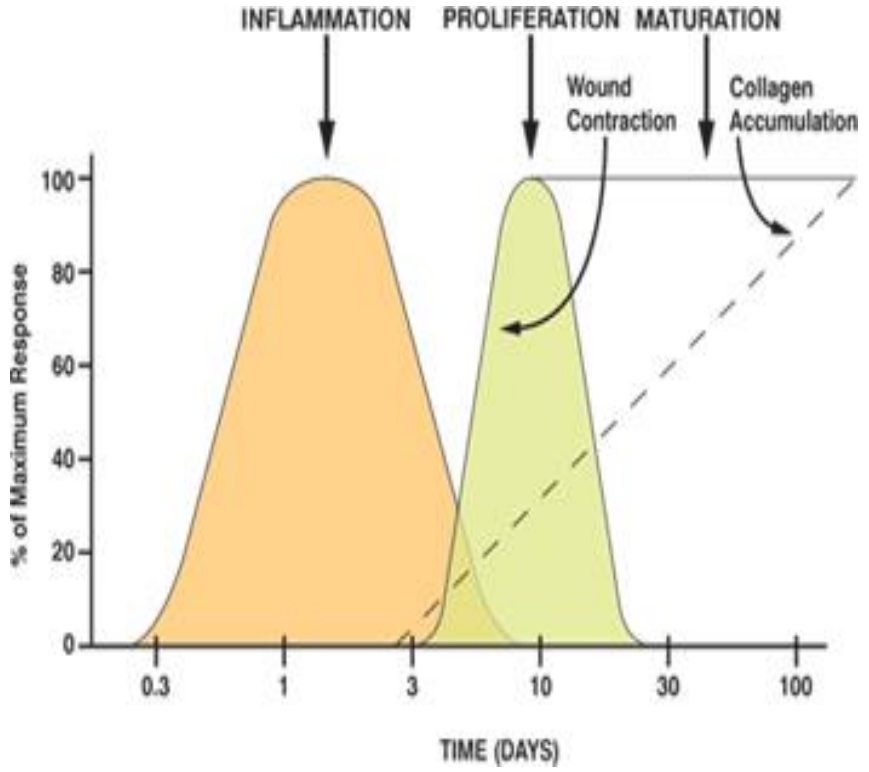
Epidermis of the rat, rabbit, dog, minipig and man



Brief recapitulation of the proces of wound healing

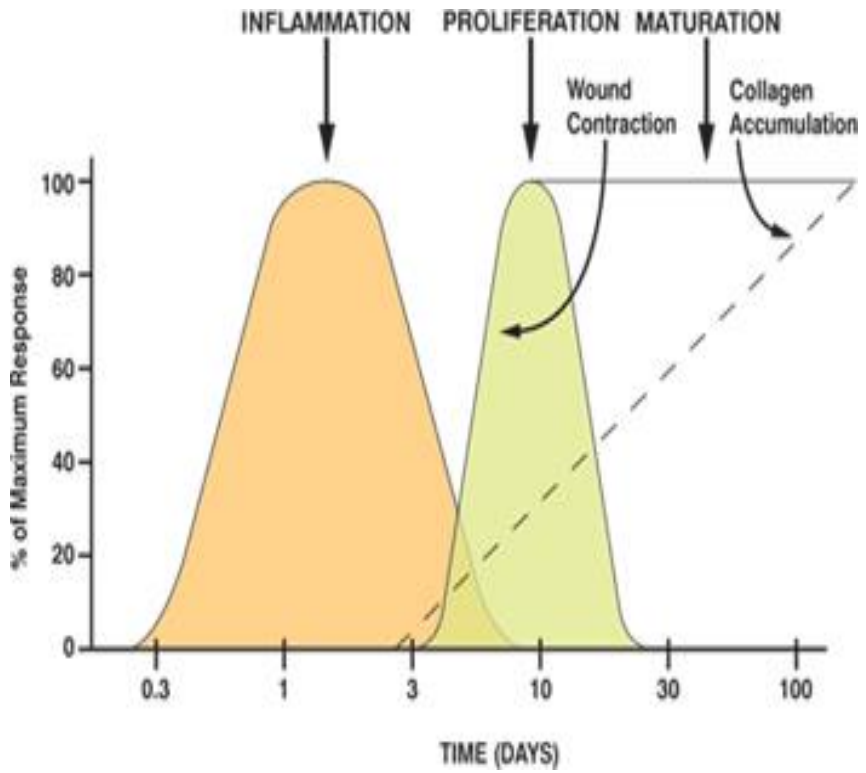
Wound healing:

- Epithelial regeneration and repair of the underlying structures (scarring, replacement of a highly specialised tissue with a less so)
- Three sequential, yet overlapping phases are part of the proces of wound healing:
  - The inflammatory (vascular) phase
  - The proliferation phase
  - The maturation phase



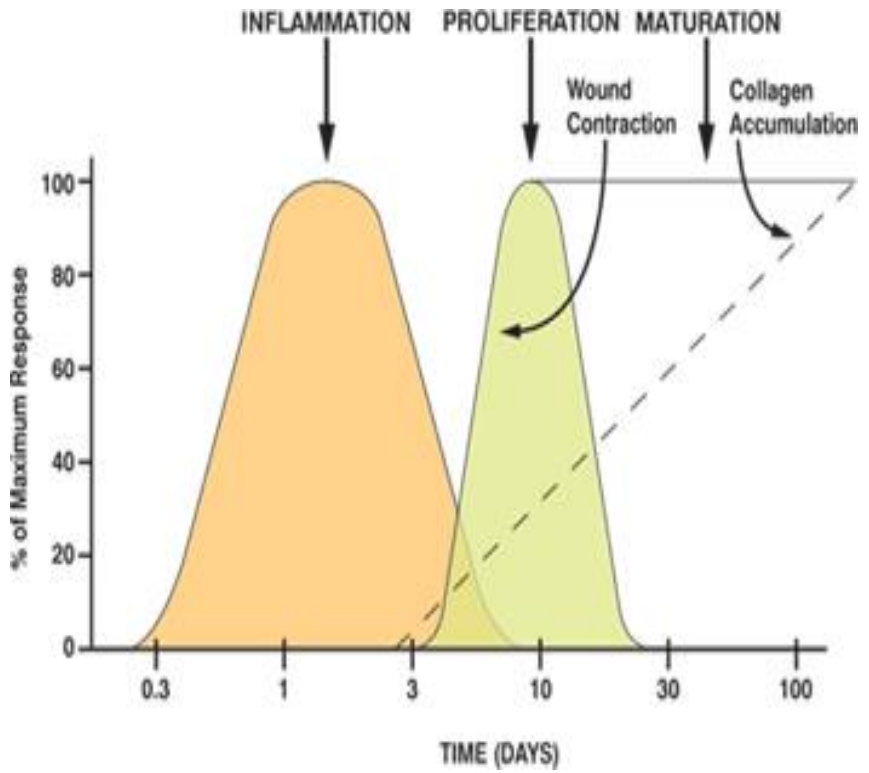
The inflammatory phase of wound healing

- Haemostasis =>dilation and increased permeability of blood vessels => leakage to the wounded area
- Neutrophils and macrophages are the predominant cells

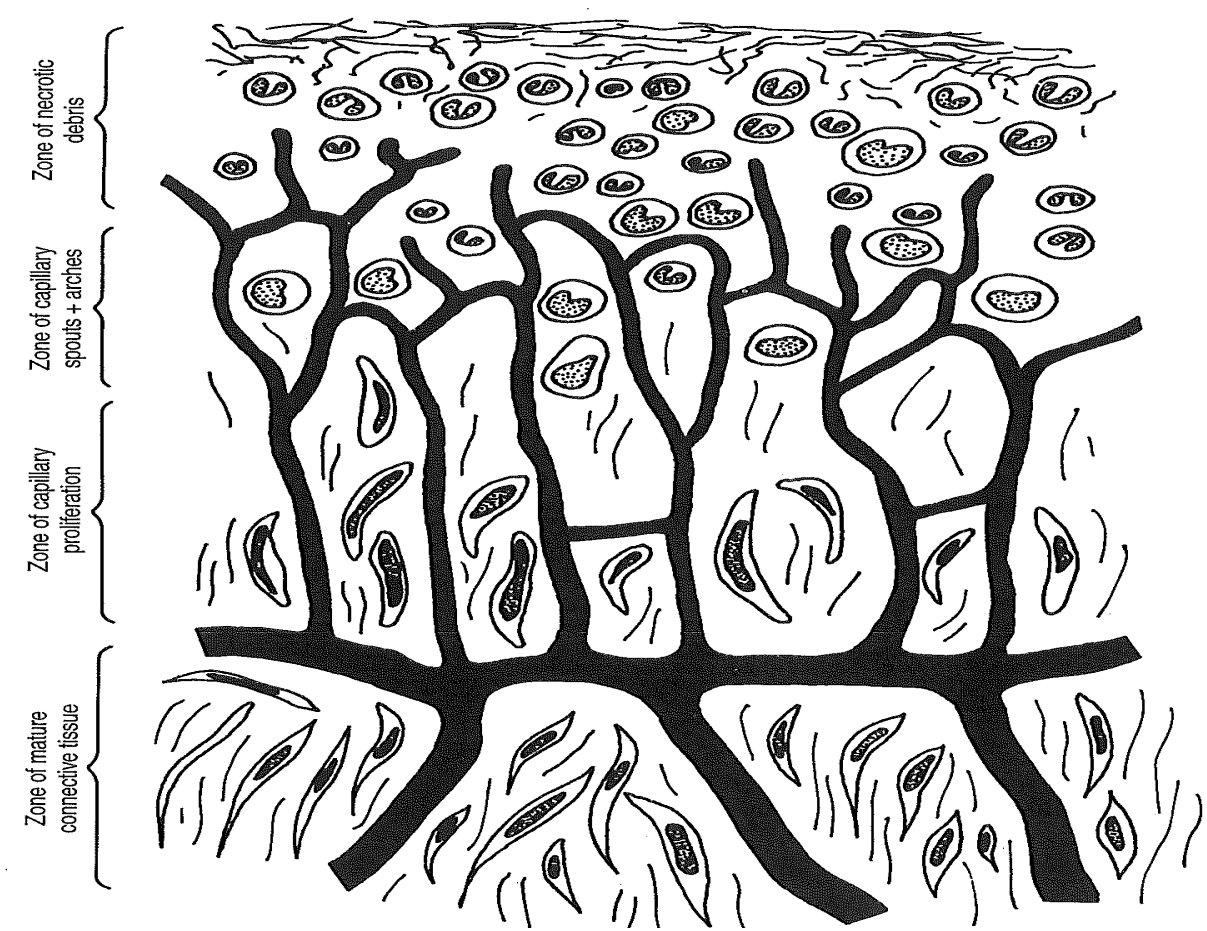


The proliferative phase of wound healing (1)

- The wound is ‘rebuilt’ with new granulation tissue, collagen and ECM, into which a new network of blood vessels develop (angiogenesis)
- Healthy granulation tissue is dependent upon the collagen secreting fibroblast receiving sufficient levels of oxygen and nutrients supplied by the blood vessels



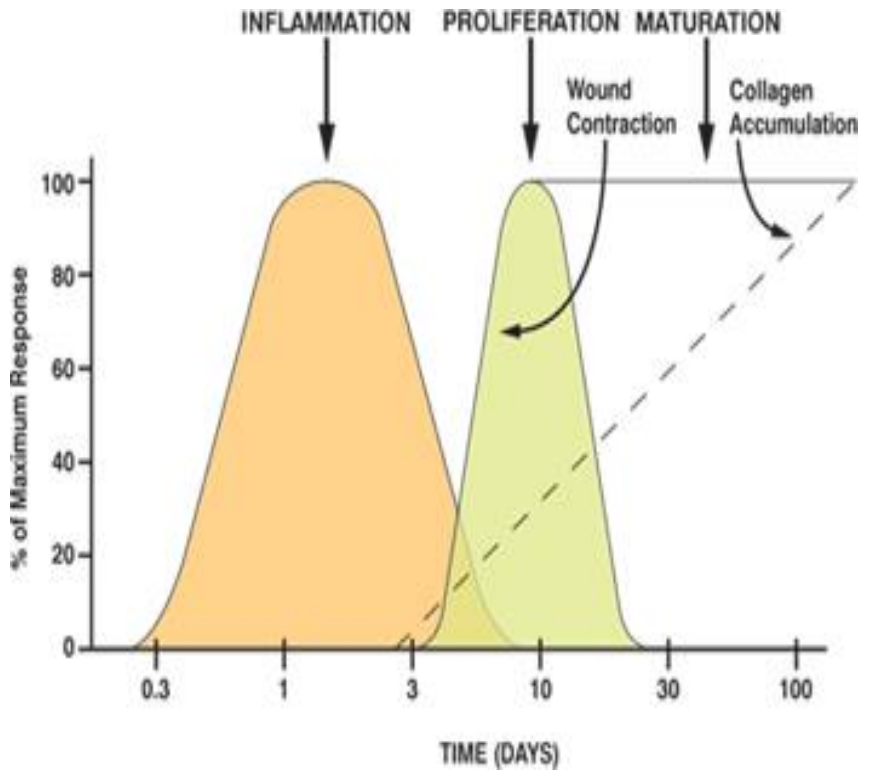
Schematic representation of granulation tissue



From Slauson and Copper: Mechanisms of Disease p. 231.

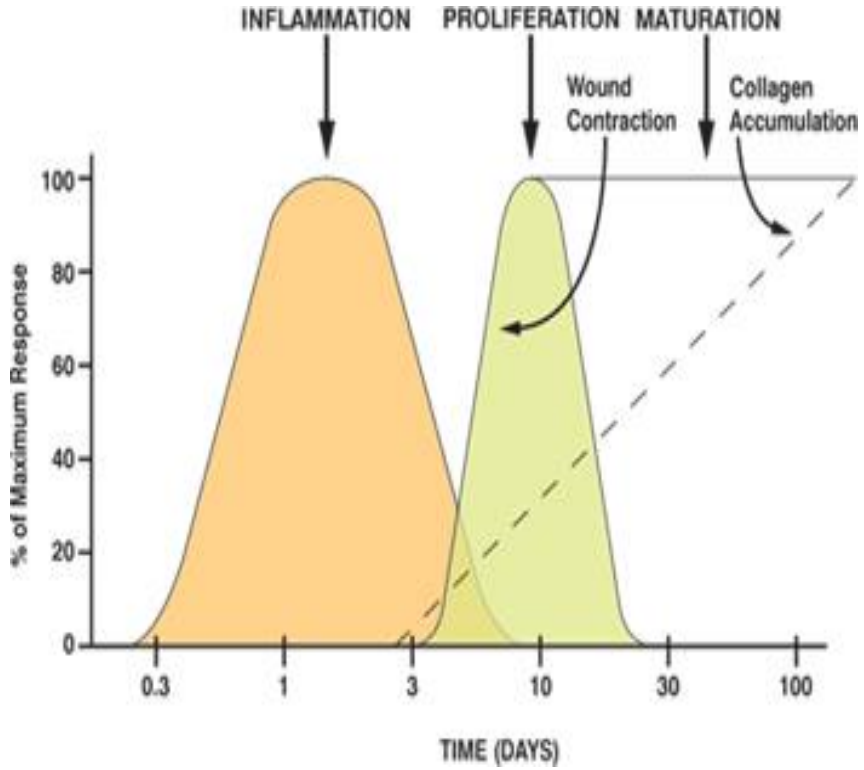
The proliferative phase of wound healing (2)

- Reepithelialisation occurs when the granulation tissue is on level with the surrounding healthy skin
- Proliferation and migration of epithelial cells occurs across the wound cavity
- Contact inhibition



The maturation phase of wound healing

- Occurs once the wound has closed
- Remodelling of collagen from type III to type I
- Cellular activity and the number of blood vessels decrease
- Myofibroblasts are active
- Apoptosis



Histopathology in Preclinical Wound Healing Studies

Factors affecting the process of wound healing

- Local factors: Mechanical factors, oedema, ischemia and necrosis, foreign bodies, low oxygen tension, excessive exudation, anatomical location

- Systemic factors: Inadequate perfusion, nutrition, metabolic disorders, inflammation, immunosuppression, connective tissue disorders

Histopathology in Preclinical Wound Healing Studies

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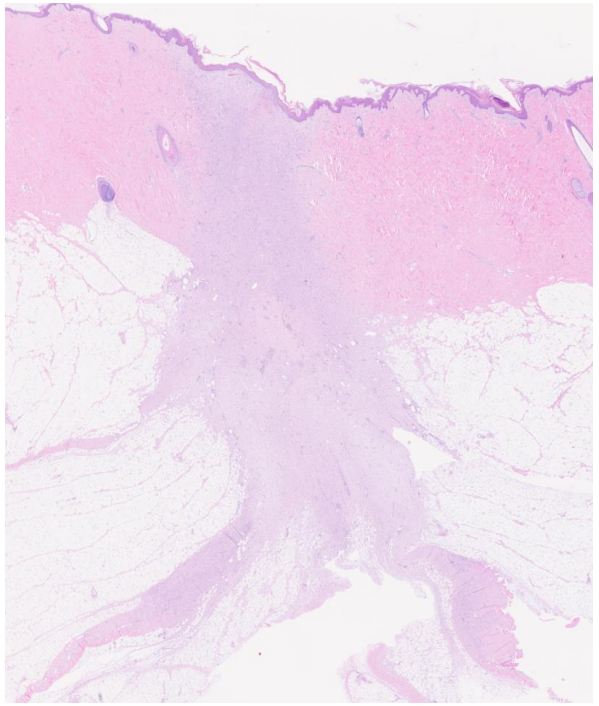
- **Regulatory aspects(1)**

  - No formal guidelines exist for wound healing studies *per se*
  - Some regulatory agencies ask to have the histopathological assessment done according to Annex E in “ISO 10993-6:2016 Biological evaluation of medical devices - Part 6: Tests for local effects after implantation”
  - In our point of view this poses serious challenges with the expectation of rigid application of pre-defined scoring systems that cannot accurately be applied for wounds and is insufficient to fully communicate microscopic pathology findings in a study



Histopathology in Preclinical Wound Healing Studies

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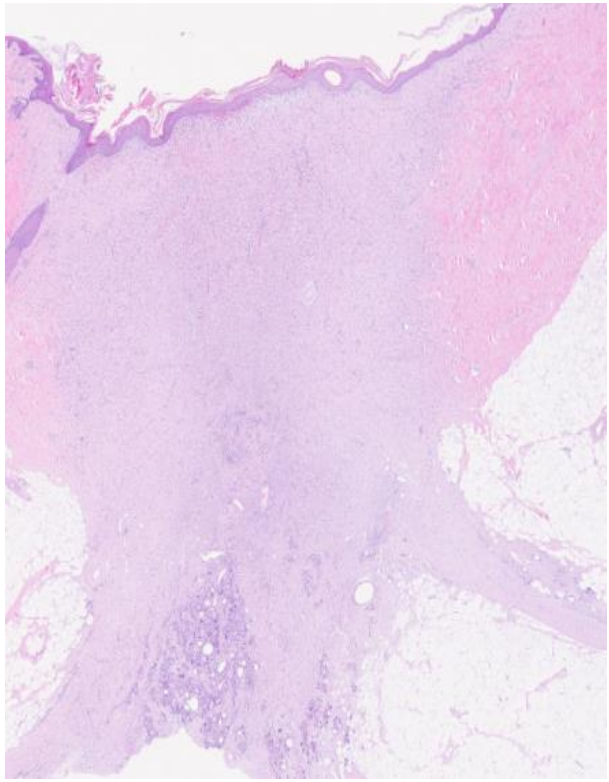
- **Regulatory aspects (2)**

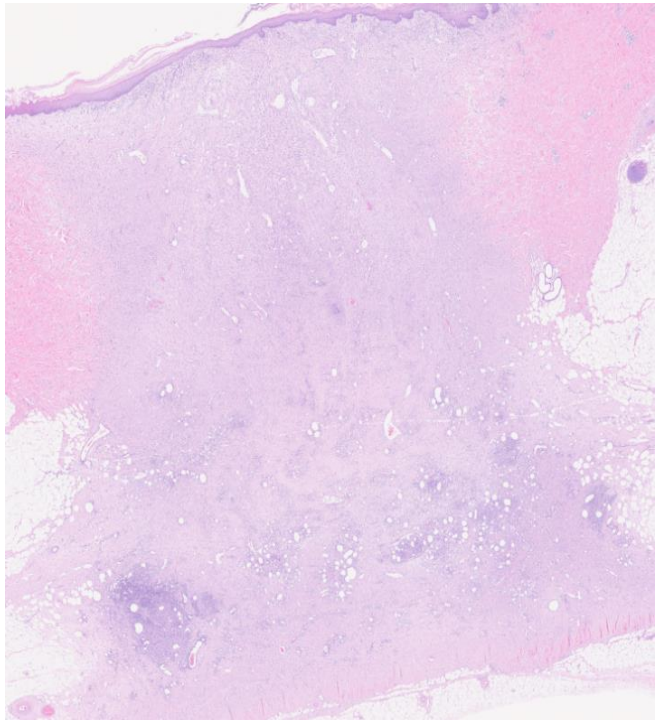
  - The purpose of pathology evaluation is to identify macroscopic and microscopic findings within a study and to interpret them in the context of the study design
  - It is not possible to predict/define ahead of time all possible microscopic findings to occur in a wound healing study
  - Well-established principles of microscopic evaluation as applied routinely in toxicologic pathology are relevant in wound healing studies
  - The observed changes do not always indicate that a tissue response is abnormal or undesirable and have to be put into context



Histopathology in Preclinical Wound Healing Studies

- **Regulatory aspects (3)**

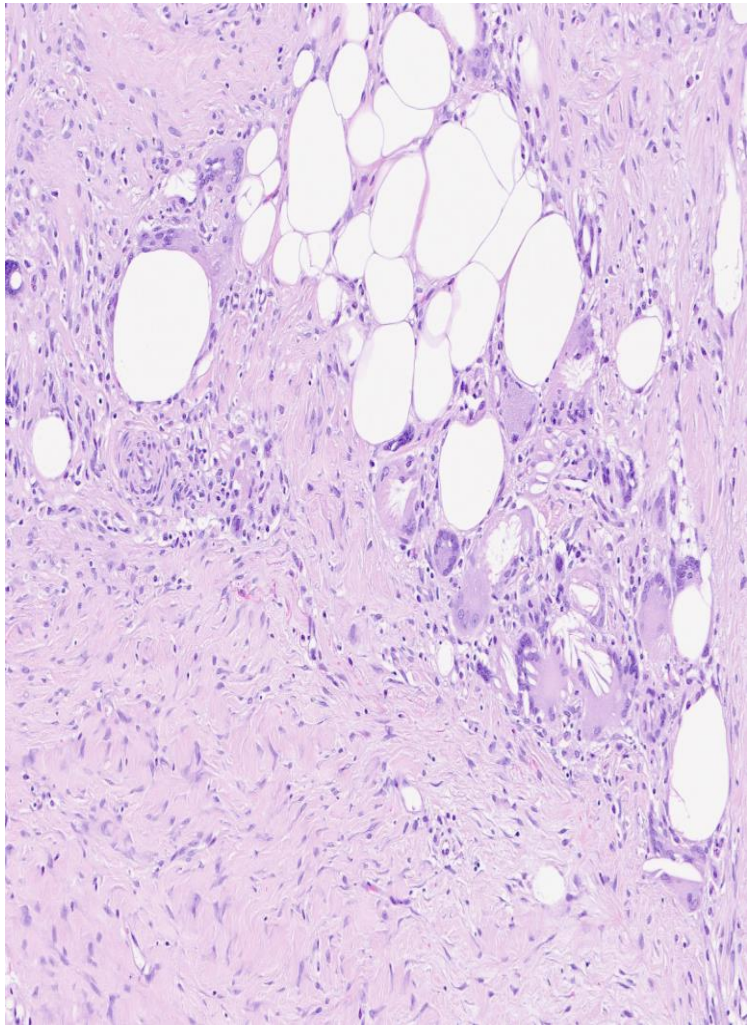
  - There should exist a flexibility for terminology and grading of the morphological findings
  - In ISO 10993-6:2016/Annex E, the basic suggested descriptors limited to specific cell types and fixed numerical grades are often not suited for robust descriptions needed to fully characterize the variety of local tissue responses in the wound healing studies
  - The scoring protocols presented in ISO 10993-6:2016/Annex E in general aims at generating a single composite value for a given wound or even wounds within a group/specific treatment. This provides an artificial value without any biological relevance
  - For wound healing studies a semi-quantitative scoring scheme rather than a purely numeric scheme is encouraged.



Histopathology in Preclinical Wound Healing Studies

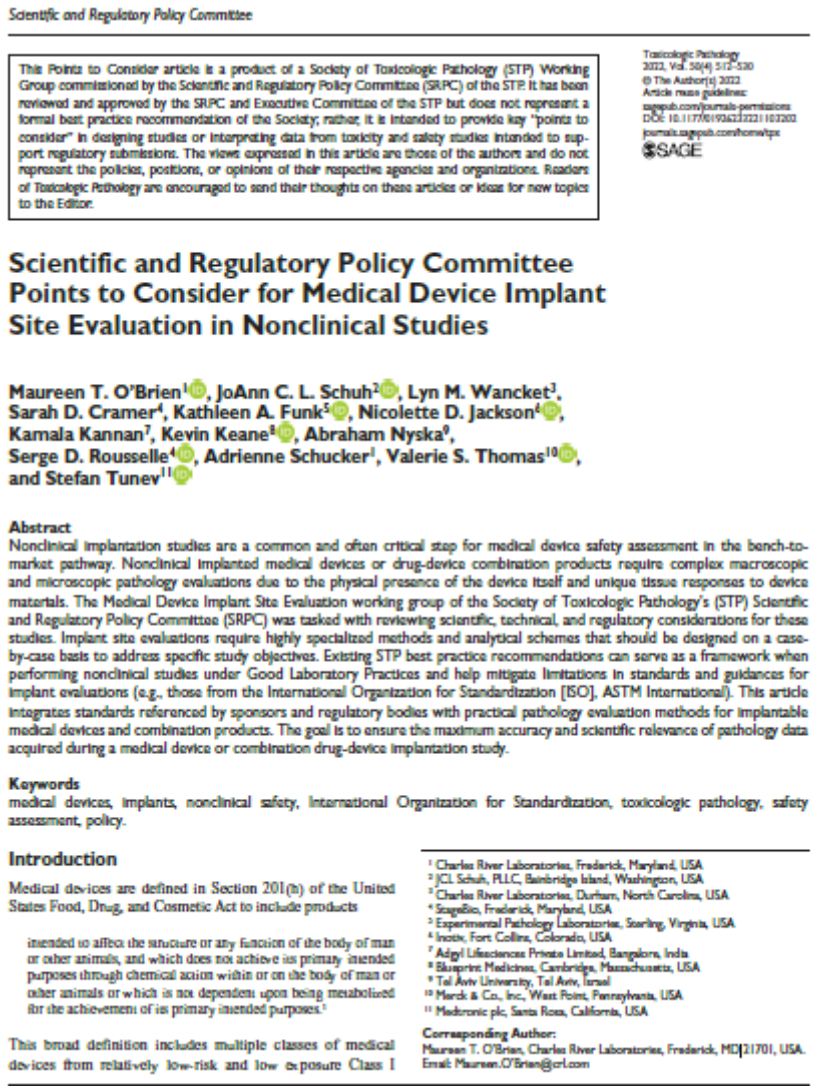
- **Regulatory aspects (4)**

  - Semi-quantitative grading criteria are typically applied by the study pathologist for each individual study due to the qualitative and context-based nature of the microscopic data
  - Trends in microscopic data should be based on incidence/severity tables for individual parameters rather than a combined nominal grade covering all parameters
  - Grading of microscopic findings is comparable within a given study but should not be compared from one study to the next



Histopathology in Preclinical Wound Healing Studies

- **Regulatory aspects (5)**
  - Efforts are being made to change the Annex E in “ISO 10993-6:2016 Biological evaluation of medical devices - Part 6: Tests for local effects after implantation”
  - We recommend not to use this standard for evaluation of wound healing studies – and to push back to the regulatory agencies when it is requested by providing the above listed arguments



Scientific and Regulatory Policy Committee

This Points to Consider article is a product of a Society of Toxicologic Pathology (STP) Working Group commissioned by the Scientific and Regulatory Policy Committee (SRPC) of the STP. It has been reviewed and approved by the SRPC and Executive Committee of the STP but does not represent a formal best practice recommendation of the Society; rather, it is intended to provide key "points to consider" in designing studies or interpreting data from toxicity and safety studies intended to support regulatory submissions. The views expressed in this article are those of the authors and do not represent the policies, positions, or opinions of their respective agencies and organizations. Readers of Toxicologic Pathology are encouraged to send their thoughts on these articles or ideas for new topics to the Editor.

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Scientific and Regulatory Policy Committee Points to Consider for Medical Device Implant Site Evaluation in Nonclinical Studies

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Abstract

Nonclinical implantation studies are a common and often critical step for medical device safety assessment in the bench-to-market pathway. Nonclinical implanted medical devices or drug-device combination products require complex macroscopic and microscopic pathology evaluations due to the physical presence of the device itself and unique tissue responses to device materials. The Medical Device Implant Site Evaluation working group of the Society of Toxicologic Pathology's (STP) Scientific and Regulatory Policy Committee (SRPC) was tasked with reviewing scientific, technical, and regulatory considerations for these studies. Implant site evaluations require highly specialized methods and analytical schemes that should be designed on a case-by-case basis to address specific study objectives. Existing STP best practice recommendations can serve as a framework when performing nonclinical studies under Good Laboratory Practices and help mitigate limitations in standards and guidances for implant evaluations (e.g., those from the International Organization for Standardization [ISO], ASTM International). This article integrates standards referenced by sponsors and regulatory bodies with practical pathology evaluation methods for implantable medical devices and combination products. The goal is to ensure the maximum accuracy and scientific relevance of pathology data acquired during a medical device or combination drug-device implantation study.

Keywords

medical devices, implants, nonclinical safety, International Organization for Standardization, toxicologic pathology, safety assessment, policy.

Introduction

Medical devices are defined in Section 201(h) of the United States Food, Drug, and Cosmetic Act to include products

> intended to affect the structure or any function of the body of man or other animals, and which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals or which is not dependent upon being metabolized for the achievement of its primary intended purposes.[1]

This broad definition includes multiple classes of medical devices from relatively low-risk and low exposure Class I

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[2] JCL Schuh, PLLC, Bainbridge Island, Washington, USA
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[6] Incite, Fort Collins, Colorado, USA
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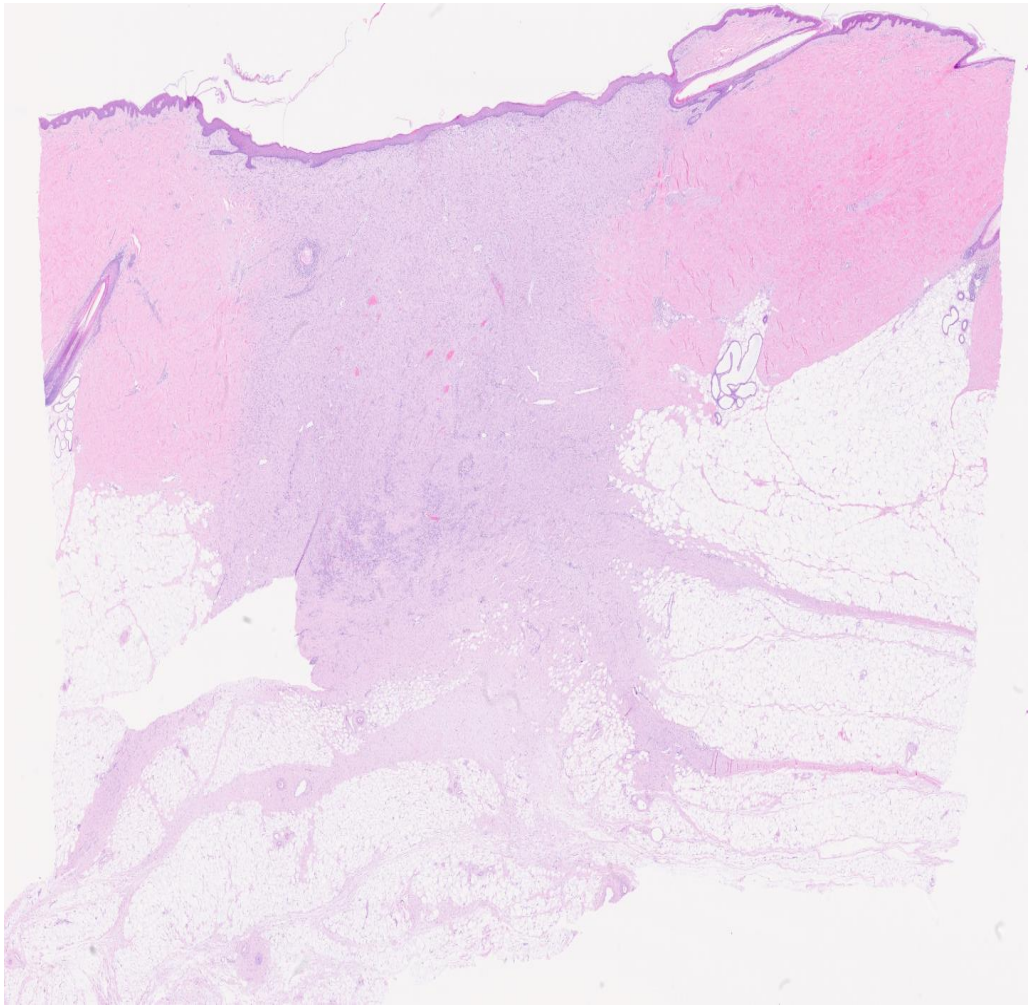
Histopathology in Preclinical Wound Healing Studies

- **Standard histopathological evaluation of wounds at Scantox A/S**

- A single section through the central part of the wound is examined
- Normal skin on both sides of the wound is included
- Care is taken to sample normal tissue subjacent to the wounds (dermis and/or subcutis)
- Standard H&E stain of FFPE samples is routinely employed
- On indication, special stains such as Masson Trichroma, Picrosirius Red or IHC for von Willebrand factor (angiogenesis) may be appropriate
- Pathology data is entered in the pathology module of Provantis using a semiquantitative 5 grade scoring scheme
- A detailed narrative report is prepared
- Illustrative photomicrographs may be warranted but it is recommended to allow the study pathologist the discretion to insert as many or as few images considered necessary to effectively communicate the pathology findings



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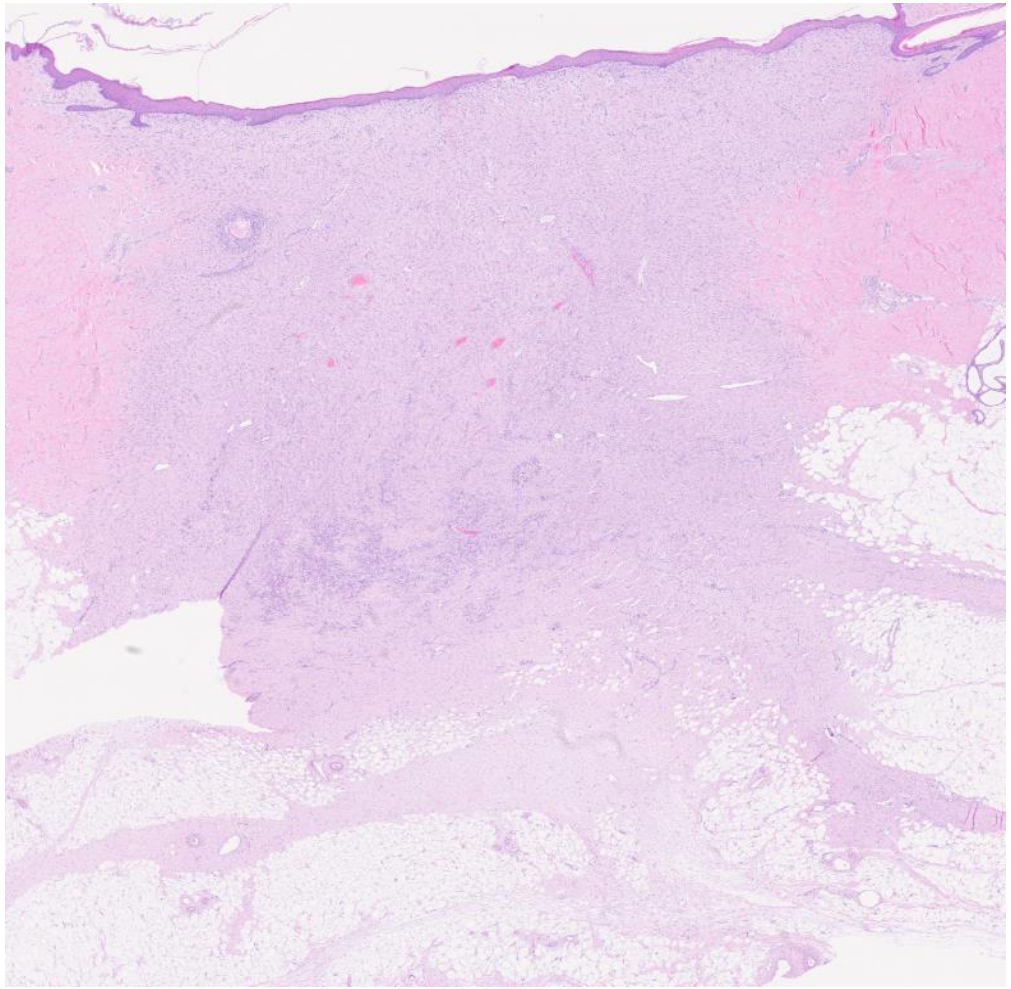
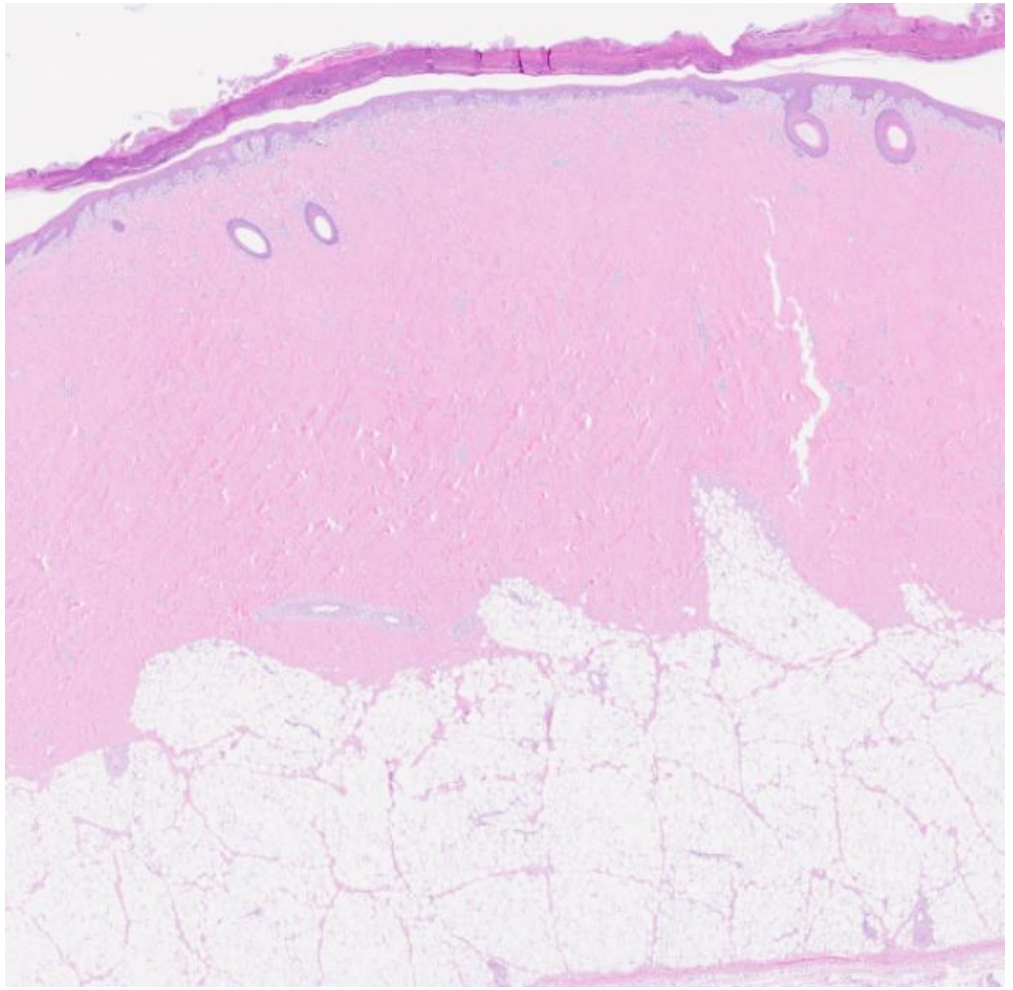
Full thickness wound day 21 post wounding



Split thickness wound day 8 post wounding



Histopathology in Preclinical Wound Healing Studies

- Common diagnosis used at Scantox include (H&E stain):
  - Reepithelialisation
  - Crust
  - Granulation tissue
  - Inflammation, superficial parts of the wound cavity (full thickness wounds only)
  - Inflammation, profound parts of the wound cavity (full thickness wounds only)
  - Hemorrhage
  - Mineralization
  - Infiltrate, giant cell
  - Vacuolation
  - Foreign body

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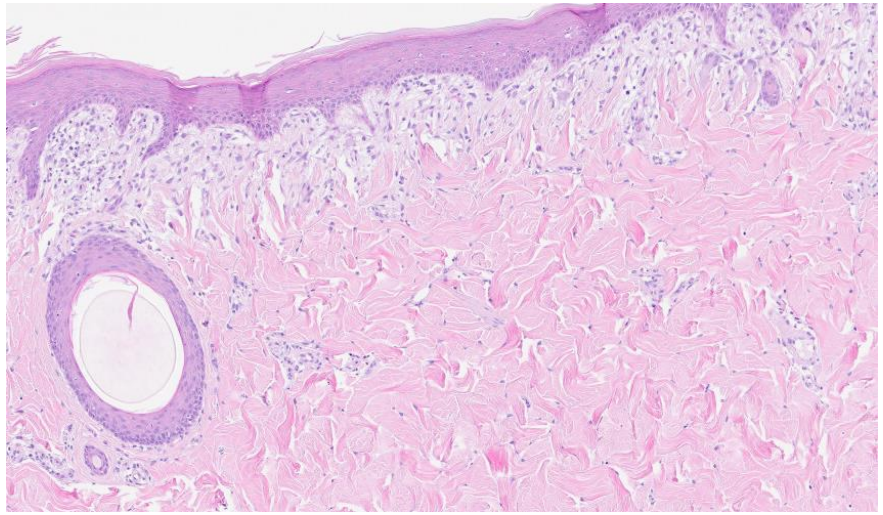

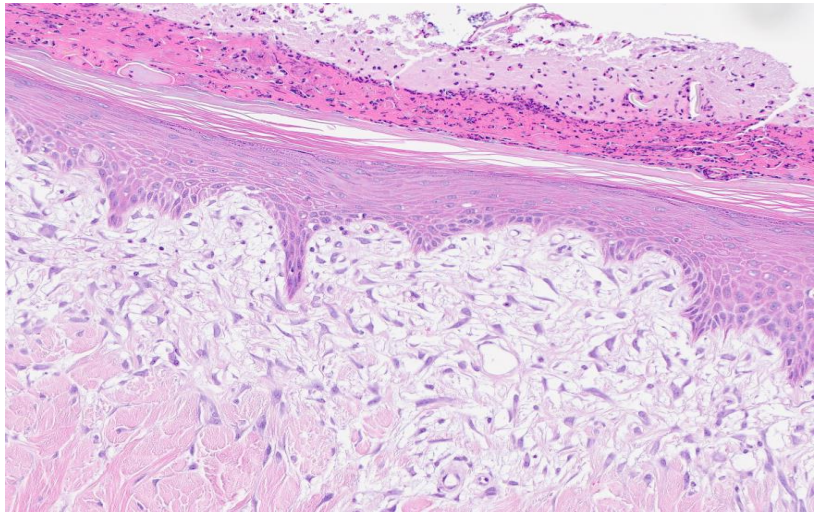

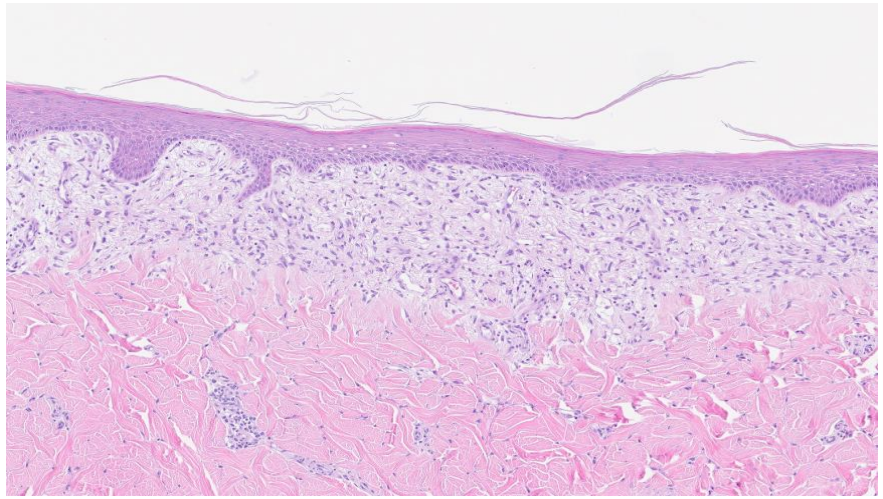

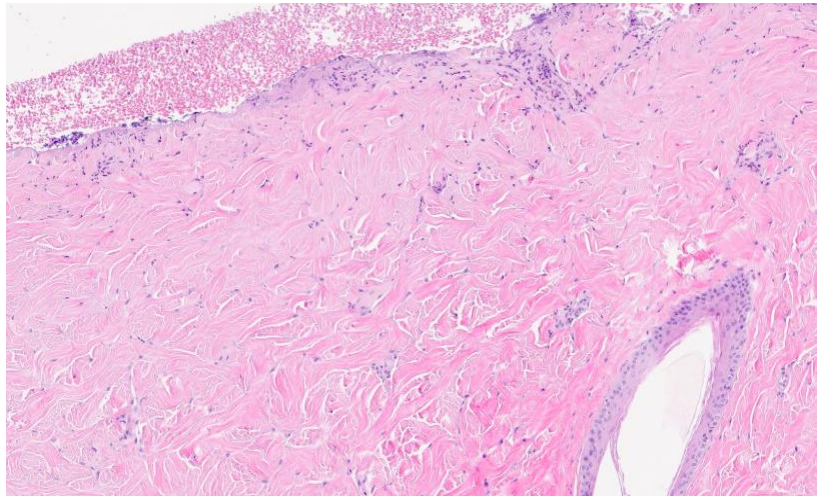

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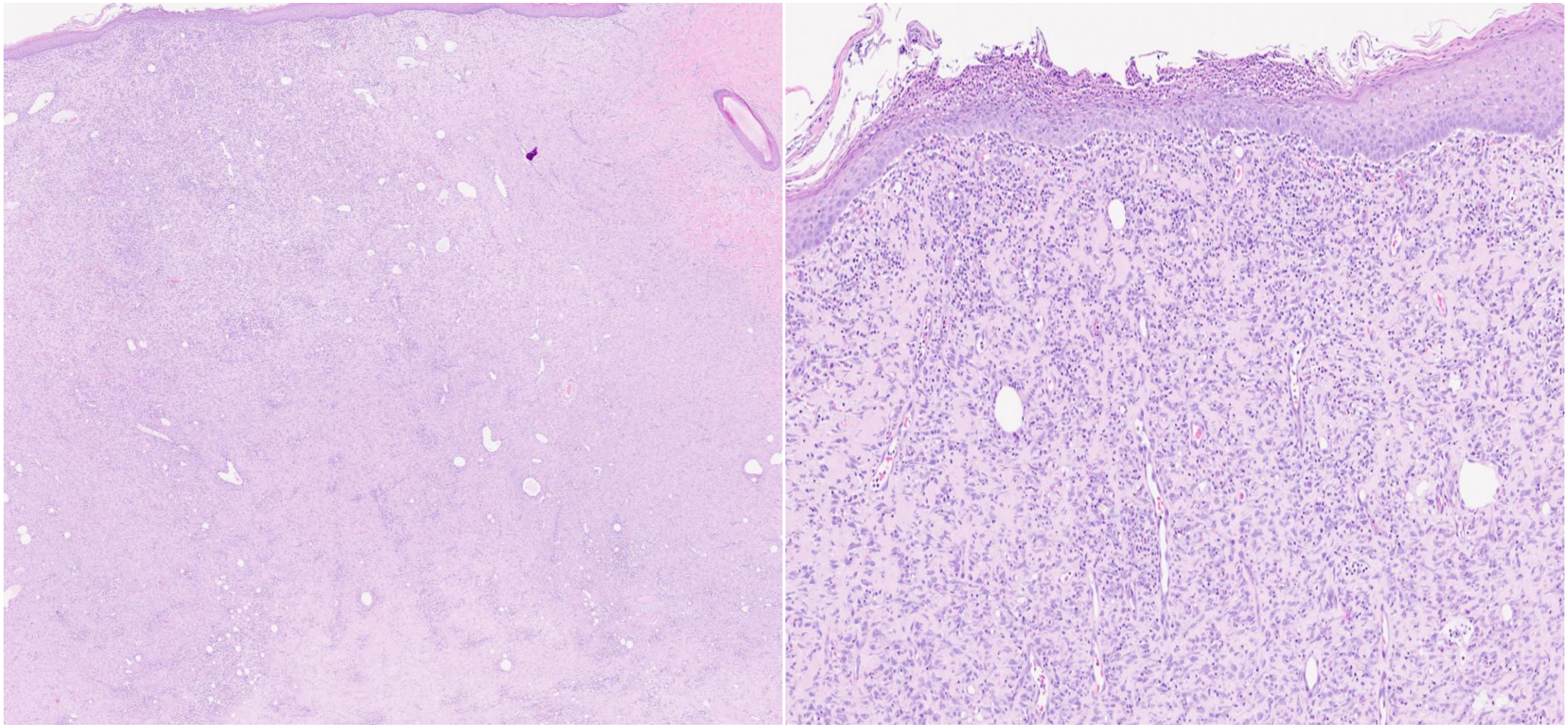

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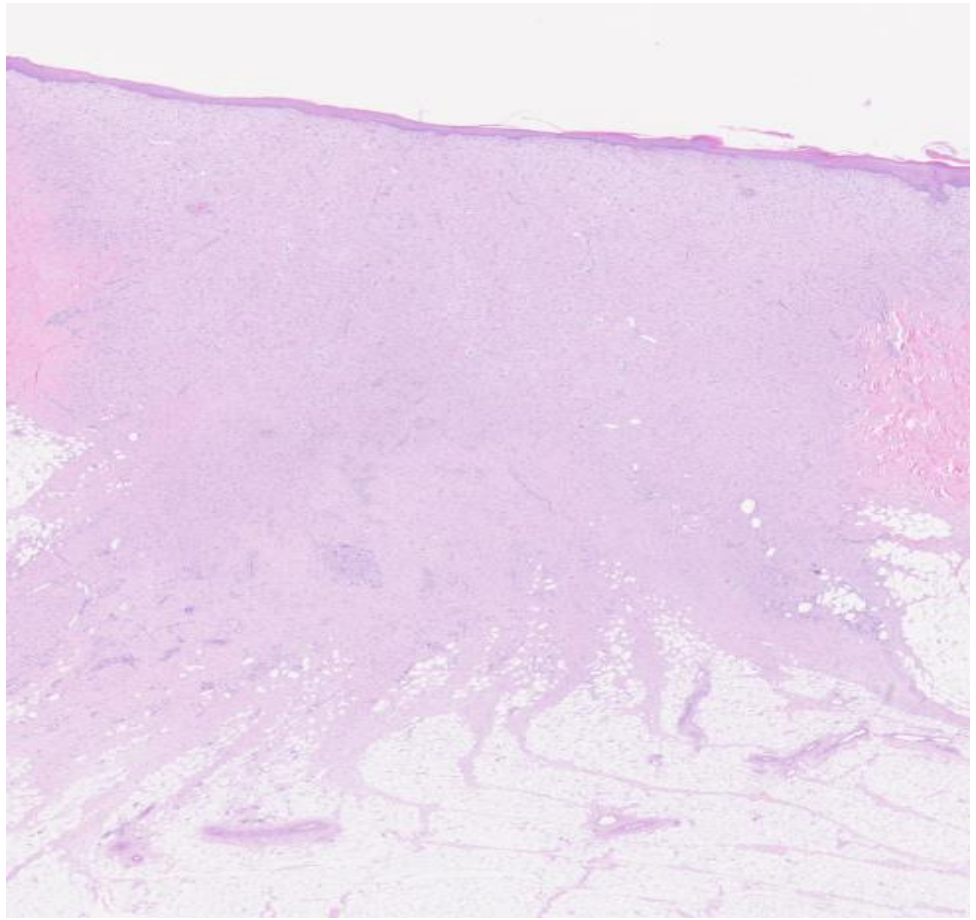

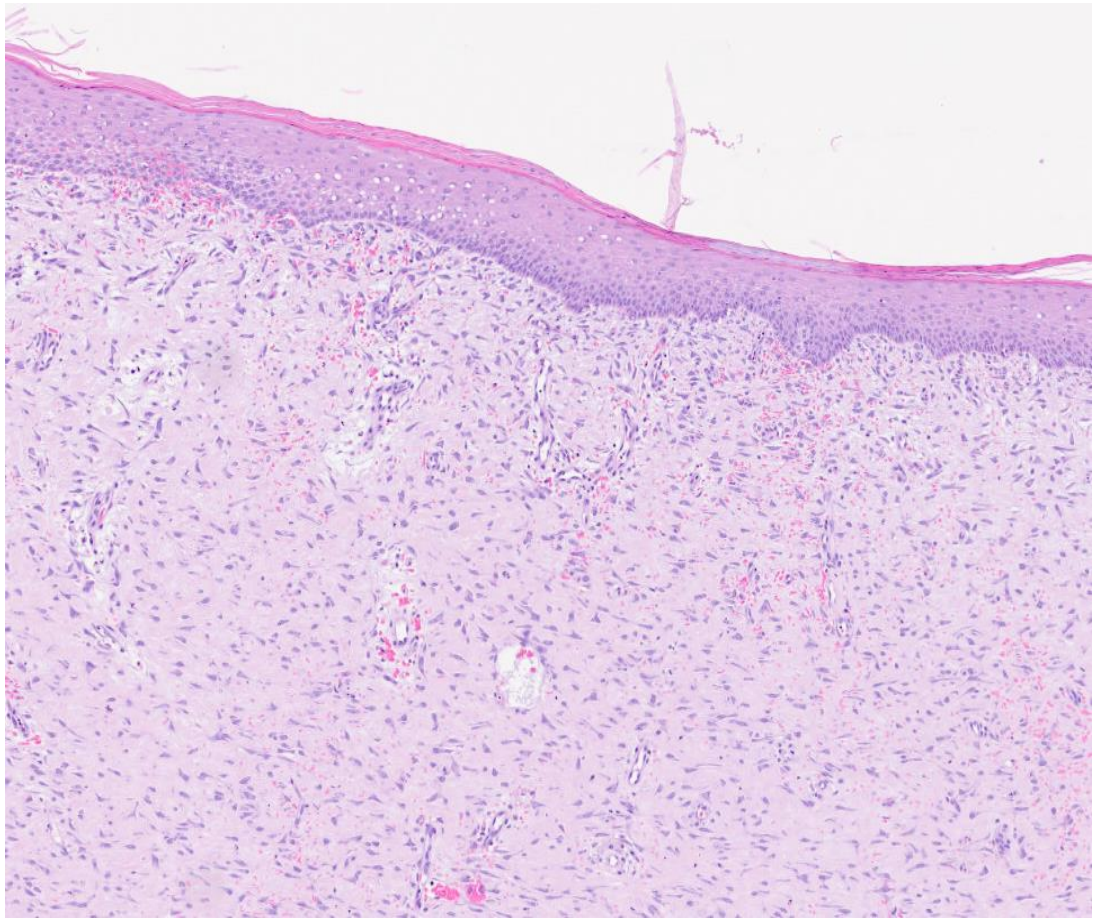

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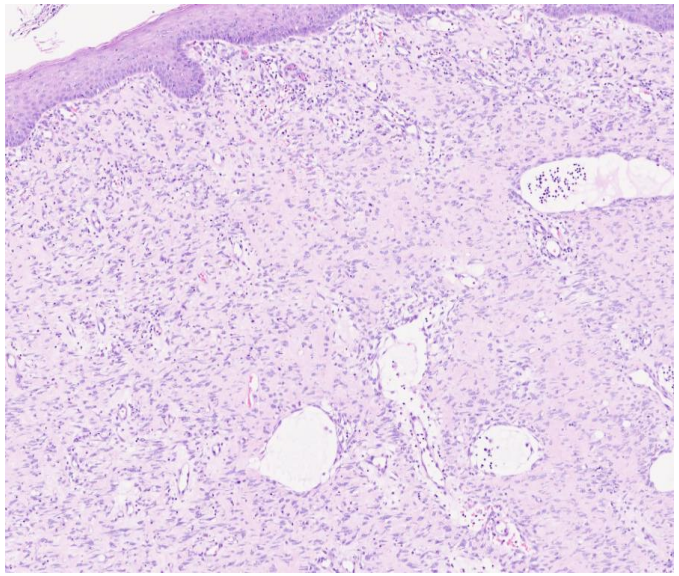
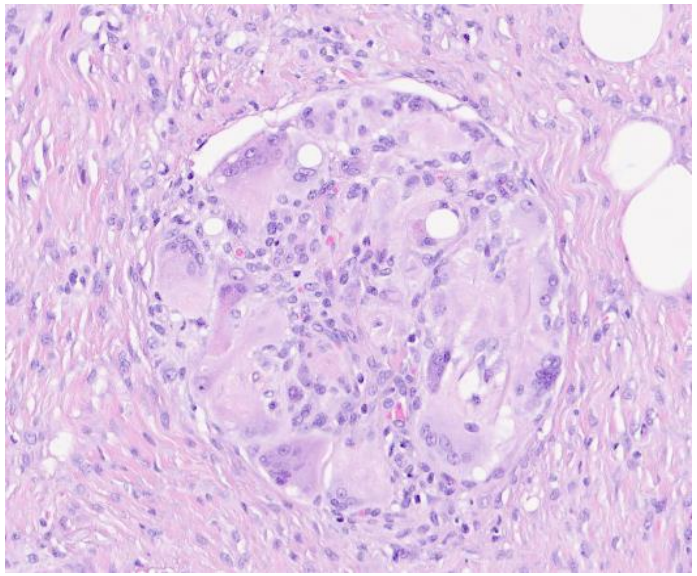
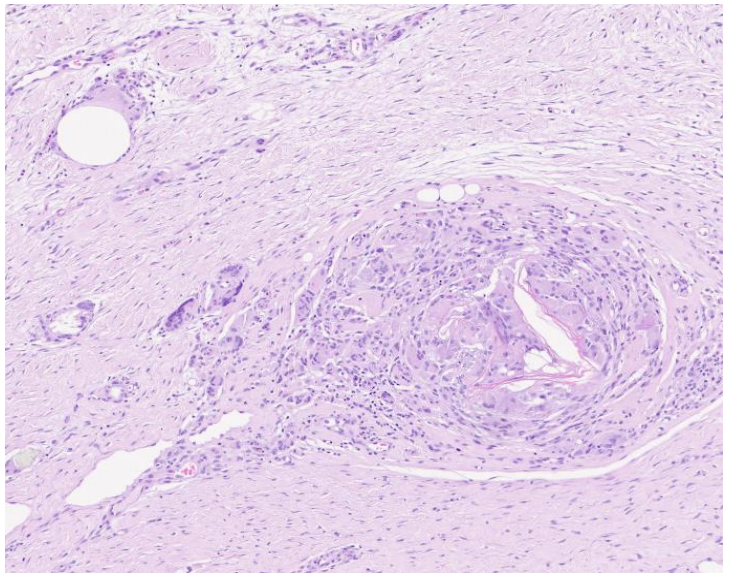
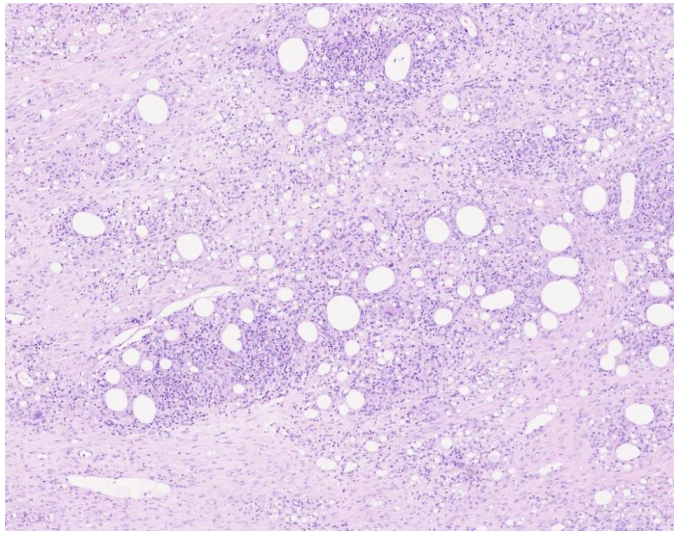
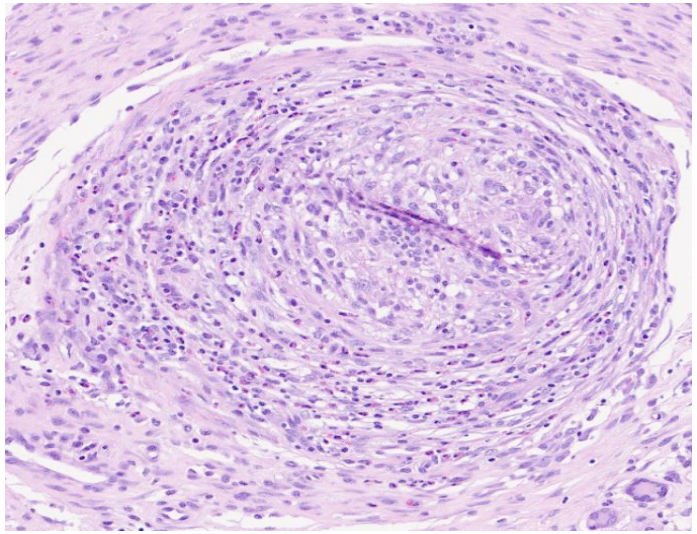
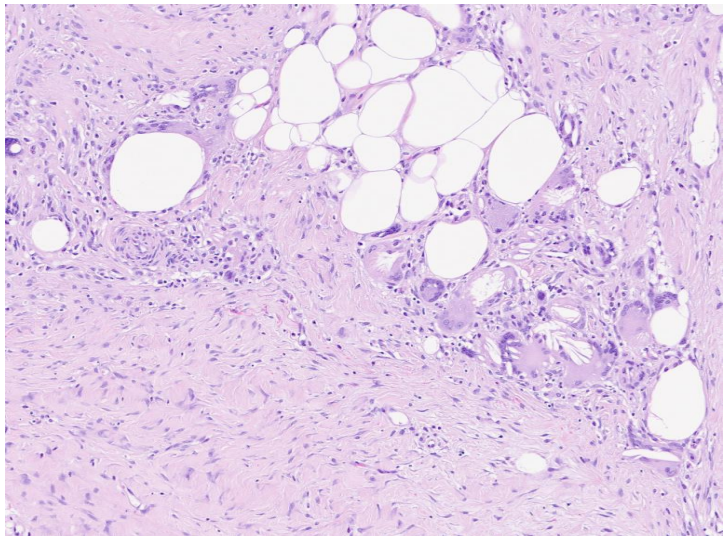

Thank you for your time☺

Any questions?