Development of an *in vitro* human skin model for evaluation of topical antimicrobial compounds

Native skin  
*In vitro* grown skin
PROJECT TITLE:
An alternative to animal experiments: Development of an in vitro human skin model for evaluation of topical antimicrobial compounds

RECEIVED THE DANISH 3R-CENTER RESEARCH PROJECT GRANT 2015

PROJECT START DATE: 1ST OF SEPTEMBER 2015
PROJECT BACKGROUND

- In Denmark at least 500 mice are used yearly for skin infection studies -> roughly corresponding to 25000 in the EU

- The purpose of the murine skin infection model is to evaluate new topical antimicrobials and effect of bacterial strains and mutants

- The murine skin infection model involves inducing a wound in the skin and infecting with bacteria -> severe distress for the mice

- Using an human in vitro skin model for infection studies may offer an alternative for skin infection studies
MRSA AND SKIN INFECTIONS

- *Staphylococcus aureus* is a leading cause of skin infections
- Increased prevalence of methicillin-resistant *S. aureus* (MRSA) in both hospital and community settings
- MRSA isolates are resistant to all available penicillins and most other β-lactams
- New treatments are urgently needed

MRSA, DANMAP 2014

Skindersoe *et al.*, accepted PLoS One
SKIN INFECTIONS

- Day 0: 10 µl inoculum with MRSA (~ $10^7$ CFU) spread on wound
- Day 1-6: Treatment with ointment containing antimicrobials or control
- Day 6: Euthanasia
  - infected skin area collected
  - homogenized in saline
  - CFU quantification agar plates w. polymyxin (5 g/L)

Part of the skin is collected in formalin and subjected to histology
ALTERNATIVES TO THE MURINE WOUND/SKIN INFECTION MODEL

THE TOOLS
Primary fibroblasts and keratinocytes (from abdominoplasty) are expanded in cell culture flasks then fibroblasts are seeded in six well plates with filter rings and ascorbic acid.

Two weeks
Two weeks
Two months

brought to air/liquid interface

keratinocytes is added on top

Stacked… and allowed to develop into 3D

Figures by MZS
Reconstructed Human Skin

Keratinocytes  Fibroblasts

Trypsin for cells (fibroblasts and keratinocytes)

Human skin from abdominoplasty

5 M NaCl for dermis preparation

Images by MZS
RECONSTRUCTED HUMAN SKIN

Aix Scientifcs steel ring

Seeding cells on dermis inside ring

Reconstructs at air/liquid

Punch biopsy

Remove "epidermal" biopsy

Images by MZS
PROJECT PLAN

- Infect and treat *in vitro* skin using the same procedures as for the murine skin infection model

- Perform CFU counts and perform histology to compare animal and *in vitro* results

Landberg, Frimodt-Møller, 2013
THE 3Rs AND THE *in vitro* WOUND/SKIN INFECTION MODEL

- **Replace:** it may be possible to completely replace the use of mice with *in vitro* skin for some research questions

- **Reduce:** it may be possible reduce the number of animals used by optimising e.g. dosage using *in vitro* skin

- **Refine:** histological findings from pre-experiments may enable identification of cytotoxic treatments/infections, thereby enabling lowering the dose causing less harm to the animals used
SUMMARY

- Skin infection and wound healing models are greatly needed

- Murine models not well suited due to interspecies differences (mice skin is not attached to muscle fascia, murine healing via contraction)

- Human skin grown *in vitro* may be used to investigate some questions related to skin infections and wound healing

- Research plan: compare *in vitro* skin infection data with existing data from the murine skin infection model
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