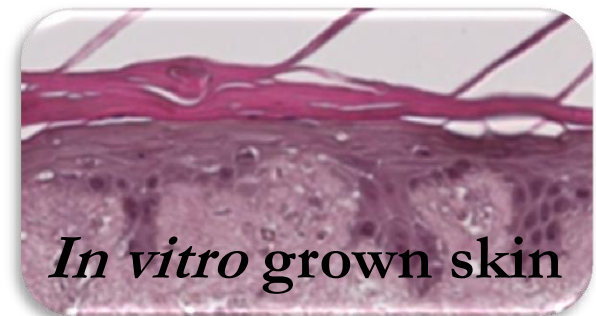




STATENS
SERUM
INSTITUT

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



1

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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

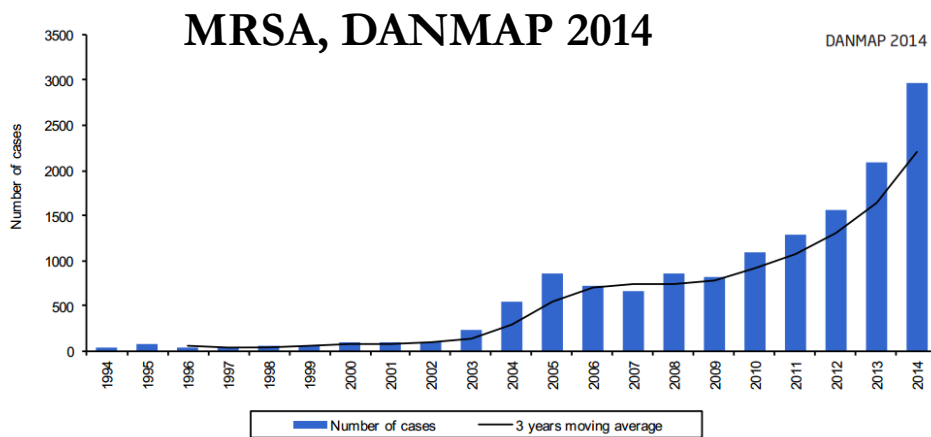
PROJECT START DATE: 1ST OF SEPTEMBER 2015

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

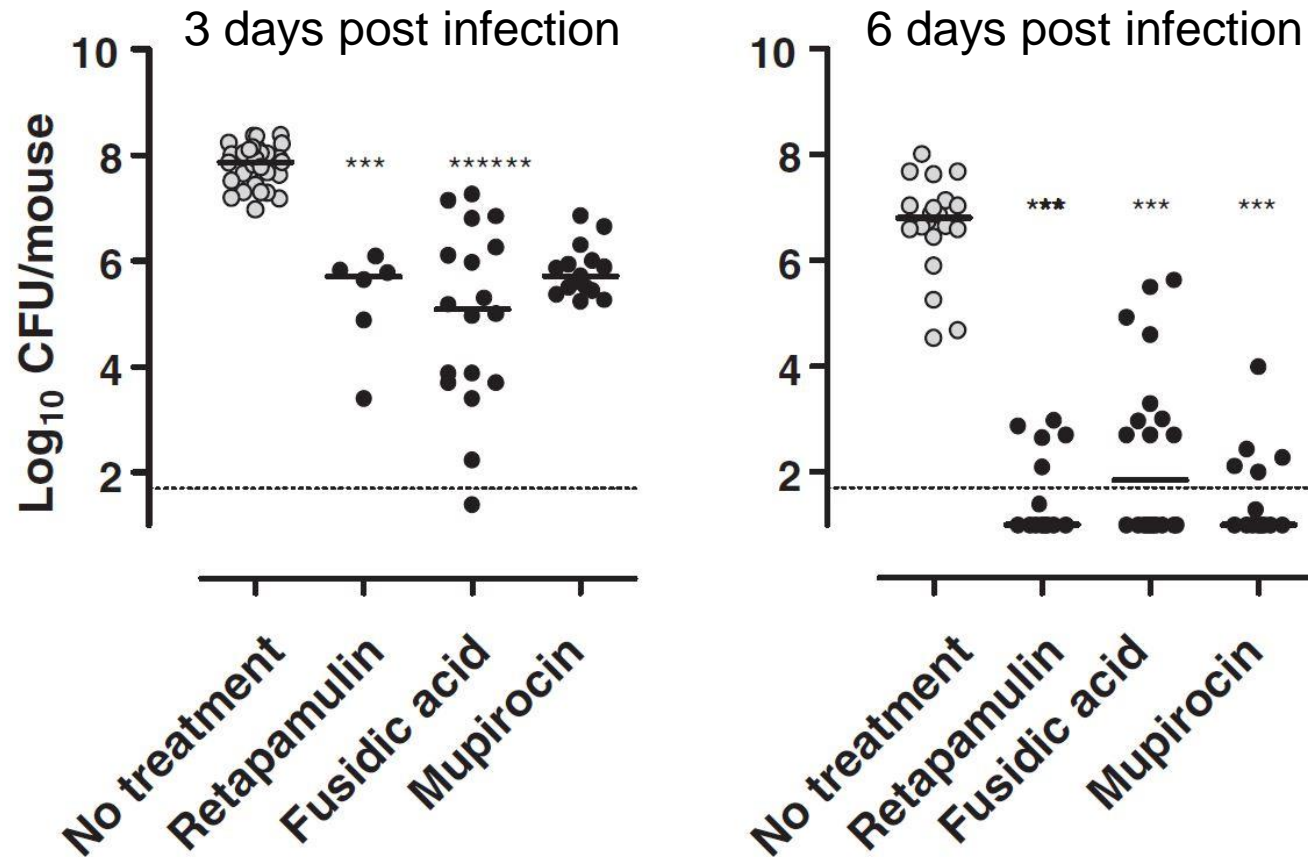


SKIN INFECTIONS

- Day 0: 10 μ l inoculum with MRSA ($\sim 10^7$ 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

TREATMENT OF SKIN INFECTION - MURINE MODEL



From CV Lundberg, N Frimodt-Møller, "Efficacy of topical and systemic antibiotic treatment of methicillin-resistant *Staphylococcus aureus* in a murine superficial skin wound infection model", International Journal of Antimicrobial Agents 42 (2013) 272– 275

ALTERNATIVES TO THE MURINE WOUND/SKIN INFECTION MODEL

THE TOOLS

RECONSTRUCTION OF HUMAN SKIN – THE SELF-ASSEMBLY APPROACH

Primary fibroblasts and
keratinocytes
(from abdominoplasty)

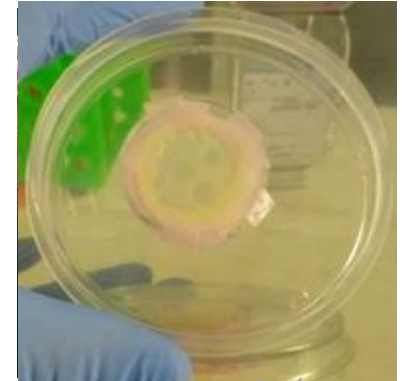


are expanded in cell
culture flasks

Freeze
isolated
cells
→



Two weeks
→

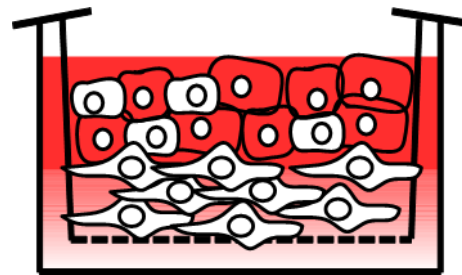


Two months
↓

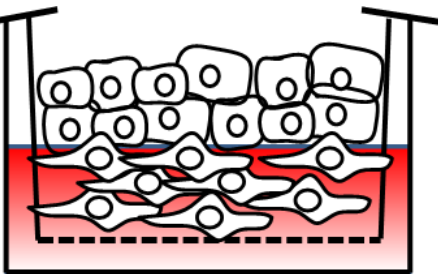


Stacked... and allowed to
develop into 3D

Two weeks
←



Two weeks
←

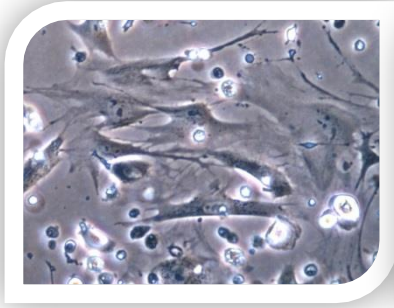


brought to air/liquid
interface

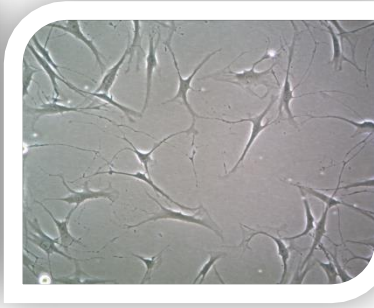
keratinocytes is added on
top

RECONSTRUCTED HUMAN SKIN

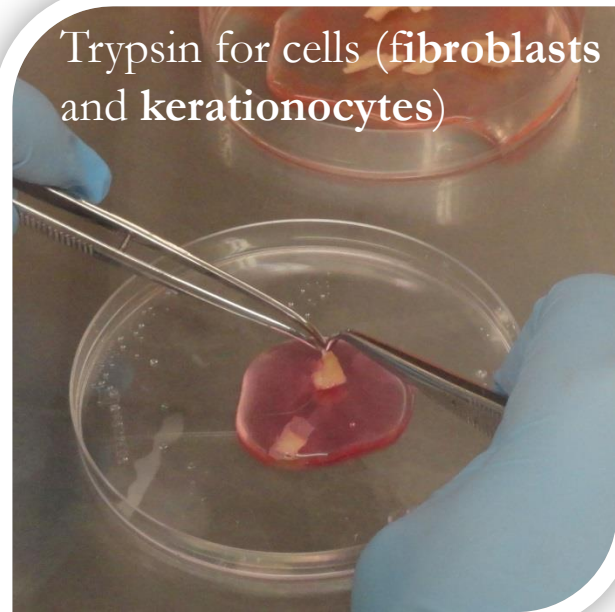
Keratinocytes



Fibroblasts



Trypsin for cells (**fibroblasts** and **keratinocytes**)



Human skin from abdominoplasty

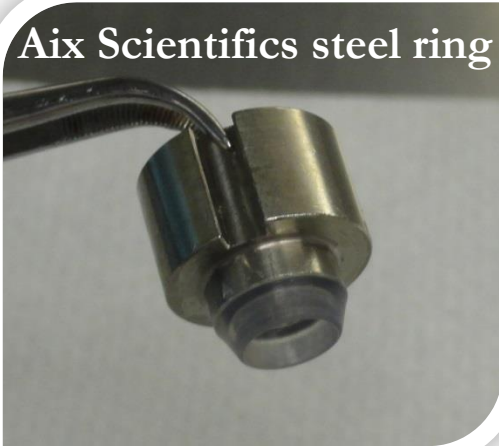


5 M NaCl for **dermis** preparation

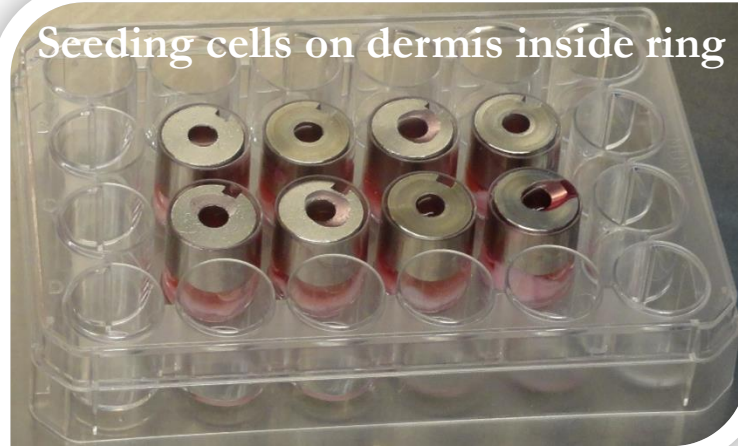


RECONSTRUCTED HUMAN SKIN

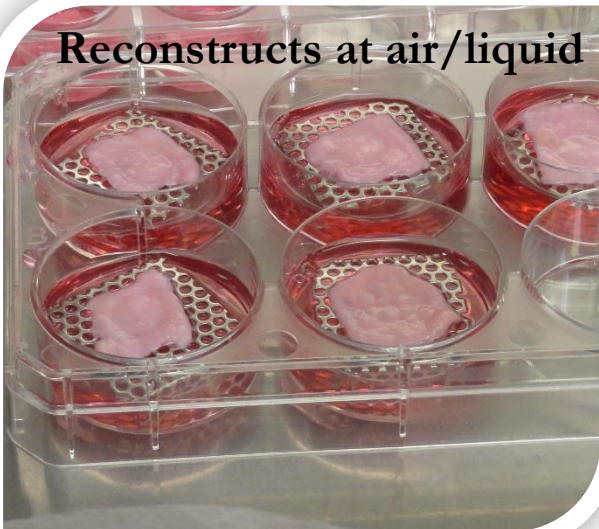
Aix Scientifics steel ring



Seeding cells on dermis inside ring



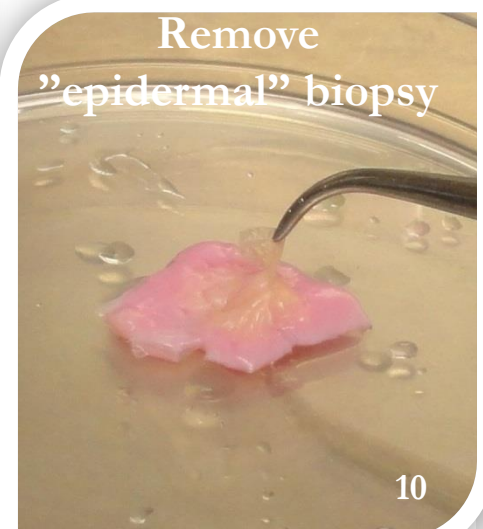
Reconstructs at air/liquid



Punch biopsy



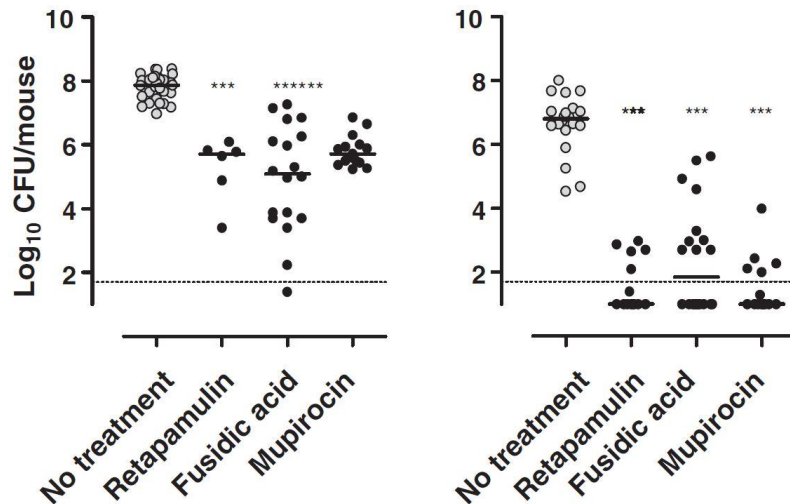
Remove
"epidermal" biopsy



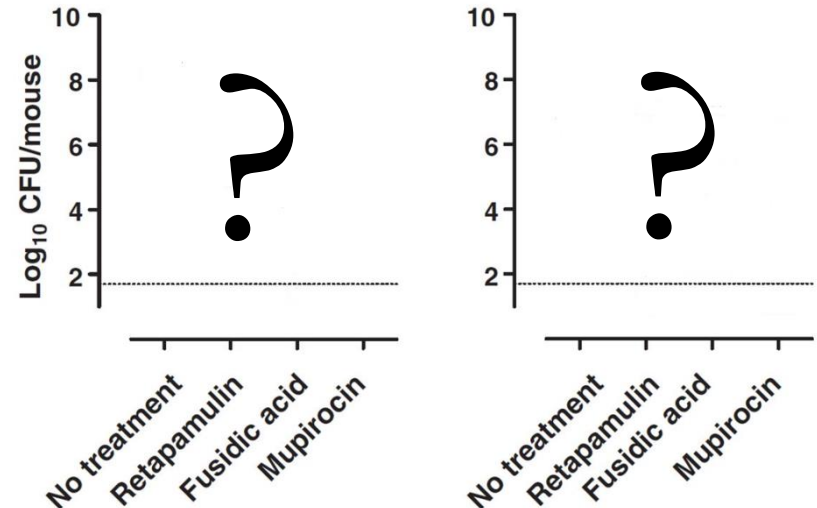
PROJECT PLAN

- 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

Murine skin infection



In vitro skin infection



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. dosis 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



www.animals.desktopnexus.com/wallpaper/1608764/

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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