Human Wound Healing Analysis using 2D-TOF-SIMS

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Background

- The skin is an important barrier to protect the body against microorganisms, abrasion, ultraviolet light, and water loss.¹

- **Chronic wounds** fail to heal after ~4 weeks.
  - Venous leg ulcer, arterial ulcer, diabetic foot ulcer, pressure sores

- **Chronic wounds** of the skin affect an estimated 8 million people in the US at a cost of $28 billion.²

- **Challenges** in research:
  - Complexity of wound healing processes.³
  - Translational limitations of animal and skin equivalent models.⁴,⁵

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Goals

- **Characterize** acute skin wounds using healthy and **newly-formed human epidermis** using Mass Spectrometry Imaging.
  - **Identify biomarkers** for wound healing.
- **Improve understanding** of role of **lipids** in acute skin wounds for treatment of non-healing chronic wounds.
- **Use a relevant ex-vivo model** for skin repair.
Ex-vivo model and Experimental Design

Dr. Ivan Jozic

Tissue collection from abdominoplasty

8mm biopsy punch followed by 3mm medial punch

Tissue grown on DMEM+FBS media

H&E staining of parallel tissue slice

Freeze-drying

Cryosectioning

TOF-SIMS analysis
Ex-vivo model and Experimental Design

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Tissue grown on DMEM+FBS media

24-96 hrs
Targets for Wound Closure

- **Caveolin-1**, a scaffolding protein, slows wound closure when over-expressed.¹

- In healthy wounded skin, Cav-1 is downregulated at the epithelial tongue.

- Cav-1 is dependent on cholesterol for incorporation into membranes.²,³

- Cholesterol removal limits Cav-1 expression and speeds wound closure.

- Cholesterol sulfate is a precursor to Cholesterol.⁴

(Jozic et al., in revision)

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TOF-SIMS

◊ Time-of-Flight Secondary Ion Mass Spectrometry
◊ Secondary Ions are extracted and analyzed in a TOF analyzer
TOF-SIMS

- Time-of-Flight Secondary Ion Mass Spectrometry
  - Secondary Ions are extracted and analyzed in a TOF analyzer
  - Mass Spectra are collected across a surface
  - Label-free technique and allows for retrospective analysis

- TOF detector
- 25 keV Bi$_3^+$ Primary ion beam
- Sample chamber

TOF-SIMS V
ION-TOF
Unwounded Human Skin Composition

Positive polarity: Cholesterol, Diacyglycerides, Triacylglycerides, Ceramides, and Phosphatidylcholines
Stratum Corneum is comprised of Cholesterol, Ceramides, and long chain fatty acids\textsuperscript{1,2}

2. J. van Smeden et al., *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 2014, 1841, 70-79.
Unwounded Human Skin Composition

Negative polarity: Cholesterol sulfate, Sphingomyelin, Triacylglycerides, and Phosphoinositol
Unwounded Human Skin Composition

Negative polarity: Cholesterol sulfate, Sphingomyelin, Triacylglycerides, and Phosphoinositol

Cholesterol sulfate
Long chain Fatty acids
Sphingomyelin

Sum of: 339.27 u, 353.30 u, 367.33 u, 381.32 u, 395.35 u, 409.36 u, 423.37 u
Sum of: 168.07 u, 616.48 u
Sum of: 465.30 u, 1406.03 u

MC: 23; TC: 1.483e+004
MC: 40; TC: 3.501e+004
MC: 24; TC: 6.346e+004
Imaging of Re-epithelialized Human Skin

48hr re-epithelization

Unwounded Skin

Re-epithelialized Tongue

SM  FA  Chol sulf  PI

0 100
Imaging of Re-epithelialized Human Skin

- Stratified epidermis
- Homogeneous lipid signal
Imaging of Re-epithelialized Human Skin

- Less stratified epidermis
- Diminished lipid signal
Imaging of Re-epithelialized Human Skin

96hr re-epithelialized tissue section

- Diminishing cholesterol sulfate across the epithelial tongue

Wound edge

Re-epithelialization

μm

0 100 200 300

300 200 100 0

SM FA Chol sulf PI

100 0
Imaging of Re-epithelialized Human Skin

96hr re-epithelialized tissue section

- Diminishing cholesterol sulfate across the epithelial tongue

![Image of tissue section with annotation](image)

- Re-epithelialization
- Cholesterol sulfate signal
- Wound edge

Legend:
- SM
- FA
- Chol sulf
- PI

Values:
- SM: 100
- FA: 0
Semi-quantitation of Cholesterol sulfate

Normalized Secondary Ion Yield

Wound recovery time

Unwounded
Re-epithelialized

$C_{27}H_{45}SO_4^-$

465.3 $m/z$

$1.5 \times 10^{-4}$

$1.1 \times 10^{-4}$

$7.4 \times 10^{-5}$

$3.7 \times 10^{-5}$

$0.0$

n=4

U

R

96hr
Semi-quantitation of Cholesterol sulfate

Unwounded
Re-epithelized

C_{27}H_{45}SO_{4}^{-}
465.3 \text{ m/z}

Normalized Secondary Ion Yield

Wound recovery time

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<tr>
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Semi-quantitation of Cholesterol sulfate

- **Diminished cholesterol sulfate quantity is characteristic** of the healing epithelial tongue
Conclusions

- TOF-SIMS imaging is an ideal method for studying lipid distribution and quantity

- Cholesterol sulfate and other lipids may be related to the rate of wound closure and expression of proteins

- Ex-vivo human tissue models will help understand wound healing and develop therapies for non-healing chronic wounds
Future Steps

- **Stain** skin tissue post-TOF-SIMS analysis
  - Parallel slices are not exact
  - Use Cav-1 antibody stain to compare with cholesterol sulfate distribution
- Relate lipid composition to rate of re-epitheliazation
Thank you for your attention!
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Group Members

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