THE CINCO DE MAYO STUDY

Temporal Changes in the Facial Skin Microbiome - A One-Year Longitudinal Study in Normal Healthy Men and Women

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Amway Corporation, Ada, Michigan
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WORLD CLASS BRANDS - Four Business Categories

BEAUTY / PERSONAL CARE

- ARTISTRY™
- SATINIQUE™

ENERGY SPORT

- XS™

NUTRITION

- TRUVIVITY™
- NUTRILITE™
- bodykey™

HOME

- Amway Queen® Cookware
- Atmosphere™
- eSpring™
PRESENTATION OUTLINE

- Background and Objectives
- Study Design and Methods
- Findings
- Conclusion
BACKGROUND -

RESIDENT MICROFLORA

<table>
<thead>
<tr>
<th>Predominant Microflora</th>
<th>Skin Physiology</th>
<th>Microbial Diversity and Stability</th>
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<tbody>
<tr>
<td><strong>Phylum</strong></td>
<td><strong>Genus</strong></td>
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<tr>
<td>Actinobacteria</td>
<td><em>Propionibacteria</em></td>
<td>Sebaceous</td>
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<tr>
<td>Actinobacteria</td>
<td><em>Corynebacteria</em></td>
<td>Moist</td>
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<tr>
<td>Firmicutes</td>
<td><em>Staphylococci</em></td>
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<tr>
<td>Proteobacteria</td>
<td><em>Pseudomonas</em></td>
<td>Dry</td>
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</tbody>
</table>

Depending on the skin conditions (sebaceous, moist or dry) - Different bacterial species harbor different parts of a human body
Skin Commensals act as Immunomodulators

- **Educate Prime Adaptive Immunity** –
  - Tune local cytokine production
  - Educate adaptive immunity
  - Influence regulatory T-cells

- **Enhance host innate immunity** –
  - Increase AMP production
  - Reduce inflammation after injury
  - Strengthen epidermal barrier

- **Directly inhibit pathogen growth** –
  - Competition - Occupy space and nutrients
  - Inhibition - Produce AMPs
  - Antagonism - Prevent *S. aureus* biofilm formation

BACKGROUND

Despite the skin’s exposure to the external environment,

• Microbial communities are largely stable over time

Variability in community composition among healthy individuals exceeds the variability within individuals over time:

• “Inter-individual” variability is higher than “Intra-Individual”
MICROBIOME STUDY OBJECTIVES

1. Measure the “Stability of Skin Microbiome” over several years in a large population of healthy adults.

2. Measure the association of “Skin Microbiome Diversity and Composition” with facial skin condition using biophysical and imaging methods.

3. Determine if the skin microbiome composition co-varies with important signs of skin health and beauty to develop microbiome-based treatment interventions.

Goal – Target Scientifically Sound Technology in Amway Products
Cinco de Mayo Cohort

• ~150 Amway employee volunteers
• Skin measurements annually in May since 2014
• Apples vs apples: Same staff, instruments, etc..
• Added skin microbiome in 2017 and 2018

Note: Most women used make-up between two sampling points for microbiome
CINCO DE MAYO – LONGITUDINAL STUDY

Conducted in 2017 and 2018 -
• On 150 Normal Healthy Men and Women

Measured -
• Biophysical Skin Properties -
  - Barrier Function, Skin Surface pH and Elasticity,
• Visible Facial Features -
  - Wrinkles, Hyperpigmentation, Porphyrins, Changes in Skin Health
• Microbiome -
  - Changes in Skin Microbiome Composition and Diversity

The study is designed to collect data over a 10-year period to identify the visual signs of skin aging, and wrinkles and shift in natural skin microflora composition.
2017 AND 2018 COHORTS

Longitudinal Study Subjects

May, 2017
n=155
90% Caucasian
Age 26-61

May, 2018
n=150
90% Caucasian
Age 27-62

90% at Age = 30s - 50s

2017 Age Distribution

Decade
20s 30s 40s 50s 60s
Number of Subjects
0 10 20 30 40 50 60

n=88
n=67
n=86
n=64
STUDY FLOW

Individual Consent → Microbiome → pH → Face Cleansing

Height/Weight → Elasticity → Imaging → Questionnaire Equilibrate 20’

Barrier Function → Exit

Microbiome Samples Requirement
Dirty Face –
• No face washing
• No Make Up
Facial Swabbing

Skin Microbiome
- Forehead
- Left+Right Cheeks
- Buffer-wetted Sterile Swabs
- 16S Amplicon Sequencing → V4 and V1-V3 Regions

Facial Features
- Left/Right Cheeks and Forehead
- Standardized Imaging and Image Analysis
- Canfield Scientific VISIA CR and VAESTRO

Region of Interest (ROI) – Exact Same
FACIAL MEASUREMENTS

Skin Feature Detection and Quantitation

- Wrinkles
- Visible Spots
- Brown Spots
- Red Features
- Porphyrrins
- Pores

Standard 1 for Wrinkles and Pores –
• Multiple Lighting Modalities

Ghost Repositioning for 2017 and 2018 image analysis –
• Locked image location for each subject to get the image at the same location each year
Skin Elasticity
Left Cheek
Suction Method
C&K Cutometer
N=3 per site.

Stratum Corneum Barrier Function
Left Cheek and Forehead
Transepidermal Water Loss
Delfin Vapometer
N=3 per site.

Surface pH (2018 only)
Left Cheek
pH meter - (Mettler Toledo flat probe)
N=3 per site.
Facial Microbiome Composition and Diversity
DATA - FACIAL COMMUNITY COMPOSITION

Cheeks and Forehead Microbiome Composition using **V4** and **V1-V3** Primers

Relatively moderate differentiation between the cheek and forehead microbiomes.

No difference between V4 vs. V1-V3 Primers

2017 vs. 2018 V4 vs. V1-V3 Composition

Insignificant differentiation between 2017 and 2018.
Alpha diversity – Diversity within a single microbial ecosystem –

Shannon Index –
• No significant change in alpha diversity over the one-year (slightly lower in 2018)

Similar results were noted with V4 Primer.
Within Individual – Microbiome diversity remains stable over time from 2017 and 2018

• In general, subjects with high (or low) diversity in 2017 maintained their high (or low) diversity over the one-year.

Within Individual - Temporal Diversity is Stable
CHANGE IN MICROBIOME DIVERSITY

Change in Cheek Diversity (●) = Diversity in 2017 – Diversity in 2018

- Most subject’s showed no change in diversity from 2017 to 2018.
- A few subjects showed significant increase or decrease in diversity from 2017 to 2018.

Subjects rank-ordered based on change in cheek diversity (●)
COMMUNITY DISSIMILARITY

Beta Diversity - Variability

Microbiome dissimilarity between subjects is greater than the dissimilarity within a subject from 2017 to 2018.

- High inter-individual dissimilarity
- High temporal stability

Inter-individual Community Dissimilarity > Intra-Individual

Distance: 0 = Identical Communities  1 = Dissimilar Communities
Facial Microbiome and Skin Parameters
MICROBIOME DIVERSITY vs. TEWL

Subjects with better stratum corneum barrier function (lower TEWL) tend to show higher diversity, however, the relationship is very weak.

- Shaded areas: with 95% confidence limits.

Microbiome Diversity is weakly correlated with trans-epidermal water loss
Forehead - Certain demographic and skin parameters were correlated with specific OTUs.

Strongest correlation was between forehead porphyrins and *Propionibacterium*.
STABILITY OF SKIN MICROBES - CHEEKS

Cheeks- Stability of associations between skin parameters and microbes

Cheeks - 2017

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<tr>
<th>Parameter</th>
<th>bmi</th>
<th>color_evenness</th>
<th>gender</th>
<th>mean_b_yellowness</th>
<th>mean_l_lightness</th>
<th>pore_area_fraction</th>
<th>porphyrin_area_fraction</th>
<th>r7_elasticity</th>
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Cheeks - 2018

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<th>gender</th>
<th>mean_a_redness</th>
<th>mean_b_yellowness</th>
<th>mean_l_lightness</th>
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</table>
Good co-relation between *C. acnes* abundance and cheek porphyrins.

Abundance of *C. acnes* seems to be necessary for having porphyrins.

Subject #: 205

Let’s look at this subject with high porphyrins and high *C. acnes*.

*C. acnes* Abundance - Strongly Correlated with Porphyrins
Subject #205 –
Despite very high porphyrins, visually skin showed clear complexion, healthy looking, evenness of color tone and no acne.

Imaging Analysis showed very high porphyrin fluorescence redness
Another person (subject #155) with High *C. acnes* and High porphyrins

Image Analysis Results
- Visually some redness
- Very high porphyrins fluorescence
- Moderate redness and red blotchiness.
Although high *C. acnes* seems to be necessary for having porphyrins -

Some subjects showed high *C. acnes* and low porphyrins level.

Subject #004 - with high *C. acnes* and very low porphyrins

Image Analysis
- Clear complexion
- A few Porphyrins
- No Inflammation
Subjects with low porphyrins in 2017 showed low porphyrins in 2018 and visa versa.

Let’s look at this subject #003 with moderately high porphyrins in 2017 but very low porphyrins in 2018.
Visually -
  • Improved Skin Health in 2018 as compared to 2017

Porphyrin Analysis Results – Decrease in Porphyrins Abundance
  • 2017 - 0.026 Porphyrin Area Fraction
  • 2018 - 0.004 Porphyrin Area Fraction

Skin Redness Analysis
  • A marked reduction in red blotchy skin on the cheeks
PORPHYRINS AND C. ACNES ANALYSIS

A 1:1 post-study interview with this subject revealed no obvious explanation for the dramatic decrease in porphyrins or C. acnes.
Corynebacterium kroppenstedtii (CK)

Age Dependent
In 2015, we characterized the skin microbiomes of 500 subjects in a cross-sectional study (‘ArtPrize Study’), where we found two mutually exclusive and age-dependent *Corynebacterium* OTUs.
AGE DEPENDENT CORYNEBACTERIUM OTUS

Cheek and the Forehead – (2017 and in 2018 using V4 Primer)

With age, there is a shift in abundance of Corynebacteria spp.

- Increase in *Corynebacterium kroppenstedtii*
- Decrease in *Corynebacterium sp.* (unclassified)

Similar results were noted with V1-3 Primer.
Corynebacterium kroppenstedtii and Corynebacterium unclassified are usually “Co-exclusive” in their abundance.
C. KROPPENSTEDTII - TEMPORAL STABILITY

*Corynebacterium kroppenstedtii* -
High intra-individual and temporal stability.....

If relative abundance of *C. kroppenstedtii* was low in 2017, it remained low in 2018 and visa versa.
Based on *C. kroppenstedii* abundance, the subjects were grouped into:

- **< 0.1 relative abundance** – Low *C. Kropp*.
- **≥ 0.1 relative abundance** – High *C. Kropp*.

The skin parameters (age, BMI, Redness etc.) of these two groups were compared using a t-test.
## C. KROPPENSTEDTII - ABUNDANCE

The group with higher *Corynebacterium kroppenstidii* (>0.1) had significantly higher Skin Redness (2.5a*)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low C. kropp. (&lt;0.1)</th>
<th>High C. kropp. (&gt;0.1)</th>
<th>P-value</th>
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<td>Age</td>
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<tr>
<td>BMI</td>
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<td>Red Features</td>
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<td>a*</td>
<td>23.1</td>
<td>25.6</td>
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<tr>
<td>L*</td>
<td>64.7</td>
<td>63.1</td>
<td>0.001</td>
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</table>

Increase in *Corynebacterium kroppenstedtii* - Increase in Skin Redness
In “Rosacea Patients” as compared to controls, the cheek and nose data showed that the relative abundance was significantly:

- **Higher** for *Corynebacterium kroppenstedtii* (4.35; p< 0.005) – Nose
- **Higher** for *Finegoldia magna* (+4.44 log; p<0.005) – Cheek
- **Lower** for *Propionibacterium acnes* (-3.48 log; p<0.005) - Cheek

**Rosacea Patients:**

*C. kroppenstedtii and F. magna* may have important roles in rosacea pathophysiology
Shift in skin microbiota of Western European women across aging

R. Jugé et al, 23 May 2018. Journal of Applied Microbiology - R&D department, SILAB, Brive-la-Gaillarde, France

Concluded: At the genus level, older skin exhibited: **A significant increase in Corynebacterium and a decrease in Propionibacterium relative abundance.**

Cinco De Mayo Study -
- With increase in age- Increase in *C. kroppenstedii*.
- With increase in *C. kroppenstedii* abundance of - Increase in Skin Redness

Rosacea Patients (Literature) –
- Higher abundance of *C. kroppenstedii* and *Finegoldia magna*
- Lower *P. acnes*

**Food for thoughts:** Is Rosacea a result of dysbiosis i.e. – high abundance of *C. kroppenstedii* and *F. magna* and low *P. acnes*?
SUMMARY

Results observed in two separate cohorts, multiple skin sites, two different primers (V4 and V1-V3) and over 1 year period.

1. Microbiome diversity and composition is relatively STABLE (over 1 year in this study cohort).

2. Microbiome diversity and composition are significantly associated with important parameters of skin health (e.g., redness, porphyrins).

3. *Corynebacterium kroppenstedtii* is:
   - Associated with age and skin redness parameters.
   - Mutually exclusive with other *Corynebacterium spp. (unclassified)*.
THANK YOU!

Skin Microbiome Team:
Greg Hillebrand PhD,
Brandon Iker, PhD

Amway Clinical Team
Pedro Dimitri, PhD
Bill Mohn, PhD