Preservation and Skin Microflora: A Conflict?
Understanding the Role of Skin Microflora in Cosmetic Formulation
Importance of the Skin Microbiome

Introduction

• Skin microbiome discovery is a revolutionary scientific breakthrough

• There are billions of bacteria, viruses, and parasites in our bodies
  • 10x more than human cells
  • Humanity has just been redefined
  • Billions of DNAs carried by organisms form an integral part of our bodies

• Science is elucidating new mechanisms through which these microorganisms affect the body

• Preservatives incorporated into our products may cause harm
  • Could natural antimicrobials uphold product integrity and maintain the health of the microbiome?
Outline

• The Importance of the Skin Microbiome
• The Importance of Preservatives
• The Role of HDAC
• Traditional Biocides
• Natural Antimicrobials
• Summary
Importance of the Skin Microbiome

Background

- The skin constantly exposed to the environment, making it an ideal location for the controlled growth of bacteria

- Microbial growth on the skin includes:
  - Resident and transient pathogenic bacteria
    (capable of invading and causing harm)
  - Commensal bacteria
    (protects against these pathogens)

- The skin is the body’s first line of defense
  - Protection against external pathogens such as bacteria, fungi, mold, and environmental stress

- Skin provides a unique ecosystem for microorganisms
Importance of the Skin Microbiome

Bacterial Symbiosis

• The perception of the skin as an ecosystem can advance our understanding of the skin and the skin microbiome

• Interdependence between the skin and the skin microbiome

• There is a delicate balance which can easily be disrupted
  • Leads to skin inflammatory events, stress, and skin aging

• Actions as simple as handwashing can upset this balance
  • Allow for transient microorganisms to flourish

• What effect does the application of personal care products to our skin therefore have on our skin microbiome?
  • Maintaining homeostasis of the microbiome may prevent skin disorders
Importance of the Skin Microbiome

Bacterial Symbiosis

• Natural microflora play a key role in our health
  • Beginning with the first microbial colonization shortly after birth

• Our skin – the stratum corneum, cellular layers, hair shaft, follicle, and skin glands all have associated microflora
  • Microflora coexists to prosper as a species\(^1\)

• Microbes defend against pathogenic invaders because their unwanted presence would force local flora to compete for the same resources

Importance of the Skin Microbiome

Bacterial Symbiosis

• Commensal microorganisms have evolved to defend their dwelling place
  • Big advantage

  • *Staphylococcus epidermidis* produces secretion that reduces inflammation and speeds wound healing
    • Also inhibits tissue colonization by *S. aureus*

• Other microorganisms perform a wider variety of functions
  • Overall immune system screening, tissue repair, wound healing, inflammation control

• Most people don’t realize the skin microbiota’s contribution to their well-being

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Importance of the Skin Microbiome

Bacterial Symbiosis

- Any disturbance of the microflora balance can be costly
  - Potential downside

- If the microbiome is altered or impaired, the health of the human host will likely suffer

- Dysbiosis has been associated with skin disorders
  - Atopic dermatitis, psoriasis, acne

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Importance of the Skin Microbiome

**Figure 1.** Survey of the Bacterial Communities on skin – Reveals several distinct skin microbiomes with fairly consistent patterns of microbial composition
Importance of Preservatives

Preservation and the Microbiome

- Product preservation is crucial to prevent microbial contamination in a product during its foreseeable life in use by the end consumer.

- The different microorganisms which have been found to grow in cosmetics are also resident commensal microorganisms found on our skin.
  - Traditional preservatives may destroy pathogenic & commensal bacteria.

- Protective microbiome should be considered.
  - Could unintentionally alter the skin’s natural defenses.

- This principle can help guide appropriate use of potential topical probiotics.
  - Promote the delicate balance of the microflora!
Importance of Preservatives

In an Ideal World...

- Cosmetics preservatives and biocides would prevent microbial growth within personal care products without affecting the skin’s natural microbiome.

- How can we evaluate the effect of an antimicrobial on the microbiome?
The Role of HDAC

**HDAC: Marker of Microflora Balance**

- We have tested and compared the selective activity of natural antimicrobials and traditional preservatives through the use of Histone Deacetylases (HDAC).

- HDAC are a class of enzymes expressed in skin cells.
  - HDAC maintains healthy skin by removing acetyl groups from histones, allowing histones to condense and organize DNA for easy replication.

- **HDAC serves as an innovative marker for microflora balance**
  - When the enzymes function properly, the microbial population of healthy skin remains intact.
  - Preserving skin’s integrity and natural barrier function.
The Role of HDAC

**HDAC: Marker of Microflora Balance**

- HDAC3 is most prominently expressed in N-TERT human keratinocyte cells
- HDAC3 expression is essential to maintain healthy skin
  - Regulates the relationship between commensal bacteria and cell function
- HDAC expression within multiple tissue systems such as the digestive tract and the skin is an essential factor in maintaining organ health and function
- When HDAC is altered or reduced, the skin’s commensal bacteria is no longer as effective against unwanted microbes
  - Leads to compromised immune system and reduced skin health

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The Role of HDAC

HDAC: Marker of Microflora Balance

- HDAC enzyme is sensitive to environmental and intrinsic factors
  - Including preservatives and biocides

- AMT investigated how antimicrobials affect HDAC activity
  - Indicates how the body regulates the relationship between the protective microflora and cell function to maintain healthy skin

- **We are focused on a solution which effectively eliminates unwanted microbes without negatively altering, and perhaps even promoting, the skin’s commensal microflora**
  - First step is to review and understand traditional antimicrobials
Traditional Biocides

Triclosan

• One of the most effective biocides on the market

• Over the last 20 years, its use has grown rapidly in personal care products⁶
  • Soap, cosmetics, toothpaste

• Also used in unexpected ways
  • Incorporated into handle rods of grocery carts to prevent bacterial growth

• Triclosan has some potentially harmful effects to skin health

⁶C Cooney, Triclosan Comes under Scrutiny, Environmental Health Perspectives, 118(6), A242 (2010).
Traditional Biocides

Triclosan

- Causes disruption of bacterial cell walls in nonspecific targets
- Results in disturbance of skin’s microflora balance
  - Pathogenic and commensal bacteria are killed
  - Skin left defenseless against new destructive microorganisms
- Can also cause dangerous antimicrobial resistance to vital medicines
  - Growing threat to overall healthcare
- Decreases HDAC expression in skin keratinocytes
  - Leads us to consider natural, effective alternatives
Natural Antimicrobials

The Natural Trend

- Natural products are often considered safer than synthetic ones
- Continual demand for natural, sustainable, ecologically friendly ingredients force formulators to learn more about alternatives
  - This includes antimicrobial agents
- Historically, cosmetic products made with natural or organic ingredients do not contain harmful ingredients that have been linked to cancer and other diseases
- Encourages the shift towards a safer, more natural and organic movement in the cosmetic and personal care industries
Some natural antimicrobials are equipped to kill pathogenic bacteria while maintaining a vigorous commensal microflora on the skin.

Other natural antimicrobials will not have a negative effect or inhibitory effect on HDAC.

The goal is to develop a new approach to preservation that will kill pathogenic bacteria and promote the delicate balance of the cutaneous microflora.

- Antimicrobial peptides may be the key!
Natural Antimicrobials

Antimicrobial Peptides

• Derived from the fermentation of lactic acid bacteria
  • Naturally acidify their environment and produce bacteriocins

• Promote a protective mechanism used by nature to achieve competitive advantage over other microorganisms
  • Providing antimicrobial protection by using same method that the commensal bacteria uses to protect itself
  • Allows for milder activity on skin while still restricting pathogenic microorganisms

• Via fermentation technology, these peptides are designed to provide:
  • Superior antimicrobial efficacy
  • Promotion of HDAC activity and commensal bacteria balance
Natural Antimicrobials

Antimicrobial Peptide Efficacy

- Minimum inhibitory concentration assays and challenge tests were conducted.

- Measures lowest concentration that will inhibit visible growth of a microorganism.

- Peptide MIC results demonstrate potential effectiveness at rates of 1-3%.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Minimum Inhibitory Concentration (ppm in final product)</th>
<th>Minimum Inhibitory Concentration (% in final product)</th>
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<td><em>E. coli</em></td>
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<td><em>S. aureus</em></td>
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<td>3.1</td>
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<td><em>P. aeruginosa</em></td>
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<td><em>A. niger</em></td>
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<td><em>Bacillus spp.</em></td>
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<td><em>Salmonella spp.</em></td>
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<td><em>Shigella spp.</em></td>
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*Figure 3. MIC Results*
### Natural Antimicrobials

#### Antimicrobial Peptide Efficacy

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<tr>
<th></th>
<th>S. aureus</th>
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*Figure 4. Challenge Test Results*
Natural Antimicrobials

HDAC Assay

- Screen each product for its effect on HDAC activity and microflora balance

- Used to determine histone deacetylase activity in cell-based or biochemical formats, providing accurate and efficient inhibitor profiling\(^7\)

- Bioluminescence-based detection so the light output or luminescence correlates to the amount of HDAC activity

- Less HDAC inhibition = higher light output

\(^7\)Promega HDAC-Glo™ I/II Assay and Screening System Technical Manuel, INSTRUCTIONS FOR USE OF PRODUCTS G6420, G6421, G6422, G6430, G6431, G6550, G6560 AND G6570.
Natural Antimicrobials

HDAC Assay

• More HDAC inhibition yields a lower luminescence value
  • Denotes the most damaging antimicrobial

• Antimicrobial peptide showed best HDAC activity

<table>
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<th>Name</th>
<th>Concentration/Dilution</th>
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<td>Peptide</td>
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<tr>
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Figure 6. HDAC Assay Results
Natural Antimicrobials

HDAC Assay

• Antimicrobial peptide did not inhibit HDAC in comparison to traditional preservatives

• Antimicrobial peptide best maintained microflora balance and overall skin health

Figure 7. HDAC Assay Results
Summary

Skin Microflora and Preservation

• Relationship between skin and its microflora is complex

• New information about the role of the skin microbiome will likely provide a key for innovation in cosmetic and personal care product development
  • Specifically preservation

• There are effective natural alternatives to conventional preservation methods that only target microbes but not the underlying enzymes and cell functions they affect, such as HDAC
Summary

AMT’s Innovation

• HDAC was evaluated as a novel indicator for skin microflora balance

• Research proved that some traditional preservatives decrease HDAC expression
  • Potentially leading to a compromised immune system
  • Overall reduced skin health

• Peptide technology provides a solution to finding the ideal balance!
  • Promotes the ultimate goal of eliminating pathogenic microbes while maintaining skin’s protective microflora balance
  • Supporting overall skin health
Selective Activity of Natural Antimicrobials:
Understanding the Role of Skin Microflora in Cosmetic Formulation