PREBIOTIC PROTECTION

Unlocking the skin microbiome code
The Noble task of Personal Care

The purpose of Personal Care

- To prevent skin/hair/nails/oral problems
- To preserve the beauty of the skin/hair/nails
- To maintain the health
- To improve the appearance

Not the purpose of Personal Care

- To repair the skin
- To cure infections (Acne)
- To change the skin’s biological activity
The classic skin barrier

Epidermis

- Dead keratinocyte
- Lamellar granules
- Langerhans cell
- Keratinocyte
- Melanin
- Melanocyte
- Merkel cell
- Basal lamina

Layers:
- Stratum corneum
- Granular cell layer
- Spinous layer
- Basal layer
The real skin barrier

- A layer of microorganisms: Stratum Microbiotum
- Epidermis

Diagram showing layers of skin with labels:
- Dead keratinocyte
- Lamellar granules
- Langerhans cell
- Keratinocyte
- Melanin
- Melanocyte
- Merkel cell
- Basal lamina
- Stratum Microbiotum
- Stratum corneum
- Granular cell layer
- Spinous layer
- Basal layer
Our Perception of Microorganisms

- Microorganisms are always associated with
  - Death
  - Sickness
  - Lack of hygiene
  - Bad smell
Our Perception of Microorganisms

Some microorganisms are beneficial

- Wine
- Beer
- Bread
- Camembert cheese
- Roquefort cheese
- Hyaluronic acid, DHA

What about the microorganisms on our skin?
It is generally assumed that microorganisms are or harmful, or beneficial
The Amount of Skin Microorganisms

- **Epidermis**
  - The microorganisms outnumber the skin cells
  - 10% human cells
  - 90% microorganisms (up to 1000 species)
The Amount of Skin Microorganisms

- Each person has his individual skin microbiota
- Each part of the body has a different population
- Even right and left hand are different
The delicate balance

The skin microbiota + skin cells are in balance (symbioses)
The skin nourishes the skin microbiota

1) Dead skin cells
2) Sweat (fatty acids)
3) Skin lipids
4) Sebum

No skin => no food => no growth/ no balance

However skin is not everywhere a party buffet, but mainly a desert.
The delicate balance

- Microorganisms provide
  - Personalized acid protection
  - Personalized antimicrobial peptides
  - Personalized genocide
  - Hygiene

No skin flora => no protection => immune system overloaded => Infections/Redness
Your personal mantle of bacteria

- 1000 musketeers: All for one, one for all
- The skin flora is competing for the same food
- Each person has his own personalized skin microbiota.
- The skin microorganisms are working in harmony together.
There are no 100% good and 100% bad bacteria!

Skin problems start with disturbing the skin microbiota balance:

1. Microorganism on the wrong place (in the skin)
   => C. xerosis in wound

1. Change in food (cosmetics) => one or more becomes dominant:
   => P. acnes, S. epidermidis, M. Furfur

3. Change in environment (UV, heat, wind, …)
   => one or more becomes dominant
Skin problems start with disturbing the skin microbiota balance:

4. Disinfectant, preservatives, antibiotics
   => the population changes

5. Aggressive products (shower gel, shampoo, depilation, AHA, …)
   => food disappears

6. Age
   => less skin cells, less sebum, less lipids => less food/changed food
Different stages of skin microbiota

- Stratum Microbiotumum in harmony - Balanced

- Bacteria Other
- Bacteroides
- Candida albicans
- Citrobacter
- Clostridium
- Corynebacterium Other
- Corynebacterium xerosis
- Escherichia coli
- Fusobacterium
- Kocuria kristinae
- Malassezia furfur
- Malassezia Other
- Moulds Other
- Peptostreptococcus
- Propionibacterium acnes
- Propionibacterium Other
- Staphylococcus aureus
- Staphylococcus epidermidis
- Staphylococcus hominis
- Staphylococcus Other
- Veillonella
Different stages of skin microbiota

Stratum Microbiotum disturbed – Out of Balance

- Bacteria Other
- Bacteroides
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✓ This is the beginning of an infection.
Different stages of skin microbiota

Stratum Microbiotum disturbed – Infection

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Empty
The Unfair Fight

- Homo sapiens exist 200,000 years on Earth
- Microorganisms exist 3,000,000,000 years on Earth

- Homo sapiens still has to learn how to survive
- Microorganisms knows how to survive
Microorganisms extremists

- Low pH (Shigella)
- High pH (H. halochloris)
- High pressure (deep in the ocean)
- Extreme Hot (up to 122°C)
- Extreme Coldness
- Extreme dry
- Extreme salt
- High concentration heavy metals
- No Oxygen
- Radioactive conditions
Bacteria live on a limited surface: the skin

- All species live together and work together
- They form a protective biofilm together
- They adapt => they change
- They *communicate*: quorum sensing
- They share food
- They control population in function of the available food.
- They never populate the whole surface
Microbial Politics

Population in balance

- The residual skin microbiota controls the growth of opportunistic organisms
Microbial Politics

- Disinfectant – Pollution – Chemical - UV
- Immediate effect

- The residual skin microbiota needs to adapt to the new environment
- Opportunistic organisms are less under control

Foreign/opportunistic microorganism
Beneficial skin microorganism
Microbial Politics

- Disinfectant – Pollution – Chemical- UV
- Possible result

- The residual skin microbiota didn’t adapt as well as the foreign/opportunistic organisms
- Skin problems start

Foreign/opportunistic microorganism
Beneficial skin microorganism
### Possible Demons for the Skin

<table>
<thead>
<tr>
<th>Bacteria/Species</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
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</table>
Occasional Demons for the Skin

Staphylococcus hominis

Staphylococcus epidermidis

Corynebacterium xerosis
Guardian Angels for the Skin

Kocuria kristanea
(Micrococcus kristinae)
Microbiological sport:
A matter of balance

**Experience**
- Make acquaintance with as many as possible micro organisms
  = training like athletes

**Prevention**
- Make sure no bacteria becomes dominant
- Use cosmetics that do not disturb the flora...so beware of preservatives
  = healthy food

**Fortifying**
- Use cosmetics that feed the protective microorganism. New!
Human interference

The aggressive approach

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>The balance is disturbed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The skin microbiota is not in control anymore</td>
</tr>
</tbody>
</table>
Human interference

The supporting approach

<table>
<thead>
<tr>
<th>Probiotic</th>
</tr>
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<tbody>
<tr>
<td>Traditional definition</td>
</tr>
<tr>
<td>A live microorganism with a beneficial influence on the gut flora. (Reid et al 2003)</td>
</tr>
<tr>
<td>Cosmetic definition</td>
</tr>
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<td>A live microorganism with a beneficial influence on the skin/oral/vaginal flora</td>
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<td>Foodstuff that can be fermented by the residual skin/oral/vaginal microbiota with a beneficial influence</td>
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</table>
Human interference

The supporting approach

| Synbiotic | The combination of prebiotic and probiotic |
Human interference
The supporting approach – Probiotic

- Well known from food (Lactobacillus, Bifidobacterium)
- In cosmetics several limitations

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Which micro-organism?</td>
<td>Each person is protected by a different skin microbiota. Each part of the skin is protected by a different skin microbiota.</td>
</tr>
<tr>
<td>2) How will the probiotic survive in the product?</td>
<td>Cosmetics are using preservatives</td>
</tr>
<tr>
<td>3) How to control the growth?</td>
<td>TVC &lt; 1000 cfu/g (SCCS guidelines)</td>
</tr>
<tr>
<td>5) How to adapt?</td>
<td>The skin is a harsh and dry environment. The first action a micro-organism needs to do is to connect to the skin. The probiotic has no experience to survive on the skin.</td>
</tr>
<tr>
<td>4) Limited effect</td>
<td>1 micro-organism is added to 1 million residual micro-organisms</td>
</tr>
</tbody>
</table>

Should we interfere with the composition of a well working skin microbiota community?
Prebiotics are present in mother milk

Prebiotics are chemically polysaccharides – polymer of mono-sugar (monomer)

The chemical bound between each mono-sugar requires a unique enzyme

Prebiotics are chemically resistant
Sugar is the energy source

- **Fructose** – F - monosaccharide
- **Glucose** – G - monosaccharide
- **Galactose** – G - monosaccharide
- **Mannose** – G - monosaccharide
Sugar is the energy source

The most famous disugar is sucrose

- Sucrose – GF – disaccharide

Enzymes such as amylase commonly present in microorganisms and mammals, digest these sugars.
Prebiotic sugars

**Inulin from Chicory root (Intibus chicorium)**
- The root contains 17% inulin
- Linear GFn n=2-60 (G=glucose, F=fructose)
- β (2-1) bound
- Only the inulinase enzyme can digest inulin
- Different qualities commercial available: Fn, GFn, GFn+Fn
Prebiotic sugars

Inulin from Agave pina (Agave tequilana)

- The pina contains 80% fructans
- Branched GFₙ n=3 - 60 (G=glucose, F=fructose)
- β (2-1) bound, β (2-6) bound, β (1-6) bound
- Mainly the inulinase enzyme can digest Agave fructans
Prebiotic balance

- Residual microflora can digest the prebiotic
  => Balancing effect in case of disturbance

- Foreign (pathogenic) microorganisms cannot digest the prebiotic
  => They lose the competition of food => they fade away
Demonstration of activity

- Inulin Fn n=10
- Mixture Inulin GFn + polyglucose
Cosmetic Prebiotic

Inulin $F_n \ n=10$

- High soluble fructose oligosaccharide
- $F_n, \ n=10$
Inulin Fn\textsubscript{n=10} - demonstration of activity

Fermentation (In-vitro)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>St aureus</td>
<td></td>
</tr>
<tr>
<td>St haemolyticus</td>
<td></td>
</tr>
<tr>
<td>P acnes</td>
<td></td>
</tr>
<tr>
<td>E coli</td>
<td></td>
</tr>
<tr>
<td>B bivius</td>
<td></td>
</tr>
<tr>
<td>B fragilis</td>
<td></td>
</tr>
<tr>
<td>B intermedius</td>
<td></td>
</tr>
<tr>
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</table>
P. acnes from an acne prone skin was incubated in anaerobic conditions for 24h in the presence of different concentrations of Inulin Fn n=10. The bacteria was quantified by DNA analysis through specific PCR conditions.

The initial population at T0 was $4,8 \times 10^5$. 

Population P.acnes after 24h
Inulin Fn n=10 reduces the speed of growth with 24%
St. Aureus was grown on a BHI medium containing 1% Inulin Fn n=10 during 8h. The growth was followed by Biorad and the slope of the growth curve was determined. The growth was compared to Glucose.
Inulin Fn \( n=10 \) - demonstration of activity
Oral care - Growth of Streptococcus Salivarius
St. Salivarus was grown on a BHI medium containing 1% Inulin Fn n=10 during 8h. The growth was followed by Biorad and the slope of the growth curve was determined. The growth was compared to Glucose and negative control (BHI).
Inulin Fn \(n=10\) - demonstration of activity
Stabilisation after a disturbance (Ex-vivo)

- Measurement with PCR
- 12 Skin explants with natural skin flora inoculated with extra \(10^7\) St. hominis
- 3 skin explants not treated (No treat)
- 3 skin explants treated with 50% ethanol (Placebo)
- 3 skin explants treated with 50% ethanol + 1% Inulin Fn \(n=10\)
Inulin F n \( n=10 \) - demonstration of activity
Stabilisation after a disturbance (In-vivo)

- A 40% solution of ethanol was used to disturb the skin microbiota
- The back of one hand was treated with a solution of 40% ethanol
- The back of the other hand was treated with a solution of 40% ethanol combined with 1% Inulin F n \( n=10 \)
- DNA was taken from the skin surface with DNA-swabs:
  - Before treatment
  - 5 min after ethanol-treatment
  - 4 hours after ethanol-treatment
- The complete skin microbiota was analysed through PCR (>900 micro organisms)
Inulin Fn $n=10$ - demonstration of activity
Stabilisation after a disturbance (In-vivo)

Results:
Hand treated with 40% ethanol

Pathogens

2.3%

Initial balance maintained

Unbalanced

Pathogens 2.3%
Hand treated with 40% ethanol

5min after disturbance

41.00% Initial balance maintained
59.00% Unbalanced

Pathogens 5.5% 139% MORE
Hand treated with 40% ethanol

4h after disturbance

Initial balance maintained: 66.80%
Unbalanced: 33.20%

Pathogens: 5.5%
95% MORE
Inulin Fn \( n=10 \) - demonstration of activity

Stabilisation after a disturbance (In-vivo)

Hand treated with 40% ethanol and 1% Inulin Fn \( n=10 \)

Before disturbance

Initial balance maintained

Unbalanced

Pathogens 1.4%
Inulin Fn \( n=10 \) - demonstration of activity
Stabilisation after a disturbance (In-vivo)

Hand treated with 40% ethanol and 1% Inulin Fn \( n=10 \)

5min after disturbance

- **Pathogens**
  - Initial balance maintained: 50% LESS
  - Unbalanced: 0.7%

98.60%
Hand treated with 40% ethanol and Inulin Fn n=10

4h after disturbance

7.80%

92.20%

Initial balance maintained

Unbalanced

Pathogens 1.0% 28% LESS

BACK TO THE INITIAL BALANCE
Conclusion:

- Without a prebiotic the skin microbiota recovered for 19%.
- Opportunistic micro organisms had the chance to become more dominant.
- With a prebiotic the skin microbiota recovered for 92.2%.
- Opportunistic micro organisms had no chance to become dominant.
Cosmetic Prebiotic

Inulin GFn n=10, Gn

- Inulin GFn n=<10 combined with Gn (n<10)

![Inulin](image1.png)
![Polyglucose](image2.png)

Inulin
Polyglucose
Demonstration of activity: Usertest Baby care
Clinical test performed at Leuven/Bonheiden
Baby cream containing 1.5% prebiotic combined with ZnO
Test persons: 15 babies < 30 months old, selected on prone to candida infections

Result:
No more infections were observed during the period using Prebiotic
GFn \textsuperscript{n=10}, Gn - demonstration of activity

Effect on Diabetic damaged skin

- Test performed by Sanofi
- Results published in Advances in Dermatology and Allergology (2016)
- Erythema: normalized
- Irritation: normalized
- Roughness: improved
- Dryness: improved
- TEWL: improved $<30\%$
- Skin pH: normalized
- Hydration improved $>20\%$
Dermatological evaluation of Feminine Wash with 1,5% prebiotic:

- All 50 patients have been tested with the test product during 2x2-4 weeks
- All patients are sensitive to C. Albicans infection
- No serious reaction with product containing Prebiotic
- 16 test persons reacted on product without Prebiotic

Result:
Product containing 1,5% Prebiotic is:
- suitable and safe for hypo allergenic claims
- suitable for sensitive patients
Claims General – Prebiotic

- Saves the skin
- Stay in control / controls the skin
- Protects the skin
- Works in harmony with your skin
- Preserves the beauty
- Essential protection whole your life
- Sustains the healthy look of the skin
- Secure the future of your skin
- Stabilizing
- Reinforce the delicate skin
- Vitalize (dry, dull, tired) Skin
Claims Inulin GFn \( n=10 \), Gn

- Happy baby skin
- Feel Fresh, Feel protected
Happy Feet / Sweet Feet

Reduces / Controls odor

Get back in control

Protect your teeth
Application Inulin GFn \(_{n=10}^n\), Gn

- Baby care
- Feminine care
- Diabetics
- Delicate skin (Cosmeceutical)
Application Inulin Fn \( n=10 \)

- Daily harmonization (Day Care)
- Balancing Body Care
- Elderly Cosmetics / Hospitals
- Deo Feet, Soaps
- Hygienic gels, washes
- Oral care (toothpaste, mouthwash)