The microbiome miracle – developing true microbiome-friendly formulations

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Introduction:

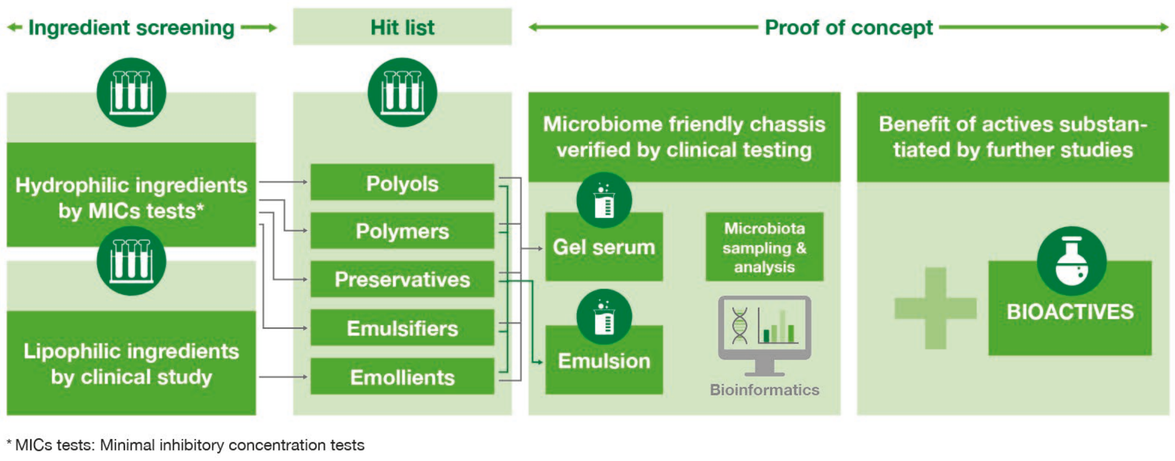
The skin's microbiome is revealing itself to be essential for the skin's health. The definitions vary currently, yet the definition of the microbiome is expanding to include not only the genomes of microorganisms but also the "theatre of activity", i.e. metabolites, interactions with other microorganisms and the host, etc. [1, 2]. As the skin is also the primary focus of many cosmetic applications, the effects of cosmetics on the microbiota of the skin are increasingly being explored. Currently much focus is being given to individual bioactives that can modulate the microbial composition of the skin. Bioactives are rarely applied directly to the skin – they are normally incorporated into formulations.

In this study, we took on the challenge to explore the effects of the "galenics", i.e. the non-bioactive components of the formulation. As the composition of a healthy skin microbiota varies between individuals, body location and environment effect [3; 4; 5], the main intent was to identify ingredients that do not disrupt the complex microbial community found on healthy skin, i.e. being "microbiome-friendly".

Materials & Methods:

Screening of ingredients

Due to the complexity of full microbiome studies, in a first step, many typical "galenic" ingredients [e.g. emulsifiers (n=10), polymers (n=6), polyols and preservatives (n=10)] were screened using minimal inhibitory concentration (MIC) tests. The effects of substances on the growth of two beneficial microorganisms *Staphylococcus epidermidis* and *Cutibacterium acnes*, as well as the commensal *Corynebacterium minutissimum* were evaluated. Three emollients were subjected to full microbiome testing with 16S rDNA-based analyses on human volunteers.



* MICs tests: Minimal inhibitory concentration tests

Formulation development and microbiome testing

The results of the screening were used to identify the most suitable ingredients and to develop formulations which were then subjected to human volunteers testing (four-week study; 20 volunteers, application twice daily) using 16S rDNA sequencing techniques. In addition, the "must haves" of skin care products, namely the moisturizing effects (assessed via corneometry), effects on skin barrier function (measured using trans-epidermal water loss), and consumer acceptance (via questionnaires) were also evaluated. Stability testing was conducted at -20°C, 4°C, 29°C, 40°C, 50°C for 3 months except for testing at -20°C and 50°C which were conducted for 1 month only. Challenge testing was conducted according to EN ISO 11930. Additional formulations containing bioactives were also developed, and formulation and microbiological stability tested.

Results & Discussion:

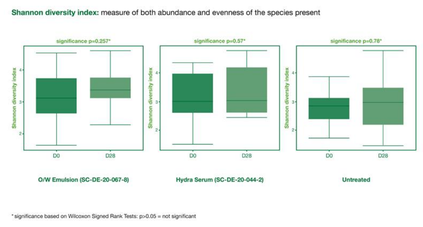
MIC results varied substantially depending on the ingredient type and composition tested. In particular, emulsifiers and preservatives had a negative effect on microbial growth. The three emollients tested neat *in vivo* did not perturb the skins microbiome.

INCI	Function	MIC (% active matter)	INCI	Function	MIC (% active matter)
Cetearyl Alcohol, Lecithin, Sodium Cetearyl Sulfate Olus Oil [EU]	Emulsifier	3.0	**Dipropylheptyl Carbonate**	Emollient	100
Laureth-7 Citrate	Emulsifier/surfactant	0.5	**Caprylyl Caprylate/Caprate**	Emollient	100
Polyglyceryl-2 Dipolyhydroxystearate	Emulsifier	1.0	**Dicaprylyl Carbonate**	Emollient	100
Xanthan Gum	Polymer/thickener	2.0	**Butylene Glycol**	Polyol	10.0
Glucomannan	Polymer/thickener	1.0	**Glycerin**	Humectant	5.0
PEG/PPG-120/10 Trimethylolpropane Trioleate (and) Laureth-2	Polymer/thickener	3.0	**Preservative 1, 2, 3**	Preservatives	0.25, 0.25, 1.00

Following four weeks of twice daily use of the two formulations by human volunteers, analyses of the alpha diversity (the mean species diversity in sites or habitats at a local scale) of the skin microbiota, as evidenced by Shannon alpha diversity index data, indicated no significant changes to the microbiome when compared to day 0. These results demonstrate that the chassis are truly microbiome-friendly, not just *in vitro* but also *in vivo*. Interestingly, even the untreated skin showed substantial variations over time, even within one individual.

Phase	Ingredients	INCI	% by weight	Function
A	Glycerin	Glycerin	5.00	Humectant
	1,3-Butanediol	Butylene Glycol	10.00	Humectant
	Rheocare® XGN	Xanthan Gum	2.00	Stabilizer
B	Water, demin.	Aqua	63.95	
	Sodium Benzoate	Sodium Benzoate	0.25	Preservative
	Dermosoft 1388 eco (Evonik)	Glycerin, Aqua, Sodium Levulinate, Sodium Anisate	1.00	Auxiliary
C	Dehymuls® PGPH	Polyglyceryl-2 Dipolyhydroxystearate	1.00	Emulsifier (W/O)
	Cetiol® 4 All	Dipropylheptyl Carbonate	3.00	Emollient
	Cetiol® CC	Dicaprylyl Carbonate	3.00	Emollient
	Cetiol® RLF	Caprylyl Caprylate/ Caprate	10.00	Emollient
	Plantapon® LC 7	Laureth-7 Citrate	0.50	Surfactant
D	Citric Acid (50% solution)	Citric Acid	0.30	pH Adjustment
Specifications				
pH value (23°C)			4.9	
Viscosity (Brookfield, RVT, spindle TE, Helipath; 4 rpm; 23°C)			62500 mPa s	

Phase	Ingredients	INCI	% by weight	Function
A	Plantaquat® NC	Cetearyl Alcohol, Lecithin, Sodium Cetearyl Sulfate, Olus Oil [EU], Cetearyl Alcohol, Lecithin, Sodium Cetearyl Sulfate, Vegetable Oil [CTFA]	3.00	Consistency agent
	Cutina® HVG	Hydrogenated Vegetable Glycerides	2.50	Consistency agent
	Cetiol® RLF	Caprylyl Caprylate/ Caprate	6.50	Emollient
	Cetiol® CC	Dicaprylyl Carbonate	4.00	Emollient
B	Water, demin.	Aqua	66.35	
	1,3-Butanediol	Butylene Glycol	10.00	Humectant
	Sodium Benzoate	Sodium Benzoate	0.25	Preservative
C	Glycerin	Glycerin	5.00	Humectant
	Rheocare® XGN	Xanthan Gum	1.00	Rheology modifier
D	Dermosoft 1388 eco (Evonik)	Glycerin, Aqua, Sodium Levulinate, Sodium Anisate	1.00	Auxiliary
	Citric Acid (20% solution)	Citric Acid	0.40	pH Adjustment
Specifications				
pH value (23°C)			4.9	
Viscosity (Brookfield, RVT, spindle TE, Helipath; 4 rpm; 23°C)			75000 mPa s	



An improvement in skin hydration and skin barrier function (moisture: +12.6%/12.9%, transepidermal water loss (TEWL): -4.1%/-11,3%), as well as good consumer acceptance (scale 1-10: 7.5/7.4) was also observed for the serum/cream, respectively. Following the addition of selected bioactives, the formulations passed stability and challenge testing.

Conclusions:

- An individual's healthy skin microbiome is almost as unique to an individual as are their fingerprints, and should therefore not be disrupted to any great degree.
- The chassis developed in this study were subjected to full microbiome studies on human volunteers with healthy skin to substantiate being "microbiome friendly" while also fulfilling the needs consumers expect from their skin care products (e.g. moisturization; good consumer acceptance).
- These chassis can then be used to incorporate specific bioactives that target defined skin conditions.

This study shows that by careful selection of ingredients and formulation know-how, truly microbiome-friendly skin care chassis can be developed.

Acknowledgments:

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