Healthy skin has healthy microbiota: how can we help?

Harald van der Hoeven, Dr Heiko Prade - CLR Chemisches Laboratorium Dr. Kurt Richter, Germany

'Microbiome skin care' has established itself in the market place. Where it began with niche brands, now mainstream brands have jumped on the bandwagon as well. What do we know about the skin microbiome? What is the skin microbiome?

Knowledge is lacking on the exact composition of the skin microbiota and the exact mechanisms with which it interacts with the skin. Virtually every day new species of microorganisms are discovered on the skin. Some species are associated with skin diseases. A well-known example is *Staphylococcus aureus* (*S aureus*) which plays an important negative role in the pathophysiology of atopic dermatitis. *Propionibacterium acnes*, now called *Cutibacterium acnes* (*C acnes*), is another species which is associated with a skin disease, in this case acne. *Staphylococcus epidermidis* is another well-known species, considered as one of the 'good' microbes on the skin. Many other species live on and in the skin, though, and is *S epidermidis* really just 'good'?

Are 'good' microbes good?

'The dose makes the poison'. This does not just go for 'molecules', but also for microorganisms. No species can be considered 100% harmless. *S epidermidis*, commonly recognized as 'good', is only good at a low enough dose and on intact skin. It can be considered to be a so-called 'pathobiont'. On compromised skin it can trigger severe adverse effects. The application of *S epidermidis* on sterilized skin leads to severe disruption of the quality of skin and barrier function. On the other hand, one of the predominant microbes living on oily skin, *Cutibacterium acnes*, is a commensal skin bacterium which is not always necessarily a pathogen. It can safely be concluded that there is no such thing as 'good' and 'bad' bacteria. We should rather consider a 'healthy' or 'unhealthy' mix of microorganisms. Healthy, non-diseased skin, shows a 'healthy' ('not unhealthy', at least partly 'mutualistic') mix, whereas with many skin diseases it is clear that the mix of microbes is unhealthy (at least partly pathogenic).

'Healthy' skin microbiota

What does 'healthy' skin microbiota mean? The following possible interpretations exist:
A: Many skin resident microbes interact with the skin. If this interaction has beneficial outcomes for the skin, the skin microbiota might be considered to be 'healthy'. The nature of this interaction is of great interest to the cosmetic industry. One thing is clear; the interaction between the skin microbiota and the skin cells is of biochemical nature. Molecules produced by the microbes interact with skin cells, for instance the differentiating keratinocytes. These cells react which leads to benefits for the skin and, simultaneously, the keratinocytes produce molecules which are beneficial for the 'healthy' microbes.
B: For non-diseased, 'healthy' skin it can be hypothesized that the skin microbiota is inherently 'healthy'. The microbiota lives in symbiosis with the skin and in homeostasis within itself. The hundreds of different microbial species making up the healthy skin microbiota reside on the skin in a heterogeneous, but stable equilibrium. The ability to keep this balance, its resilience, is a measure for microbial 'health'. After disruption of the balance the healthy microbiota returns to its original balance more quickly than when the microbiota is 'unhealthy'.

Going forward

In order for a cosmetic product to have a balancing, supporting benefit for the 'healthy' skin microbiota, first, the complexity of the topic needs to be appreciated. Analyzing single species, like *S epidermidis*, is only sufficient in the context of a larger analysis including the overall microbial composition. Added to that, most skin resident microbes cannot be grown on culture. This adds to the complexity of the topic.

Many unknowns still, but important knowledge is available: a 'healthy' mix of skin microbiota has important positive implications for the physical barrier function of the skin. It also positively influences the skin's immune capacity (immune competence). It is opportune using this knowledge in defining potentially good active ingredients which can support the claims 'balance' and 'support' the skin microbiota. Focus should be on the microbial environment.

Environment dictates microbial composition

Importantly, the skin provides important components for the microbial environment. Molecules which regulate skin pH, for instance and antimicrobial peptides. There are many more such important molecules

produced during the epidermal differentiation process. The interaction with the 'healthy' microbes will influence the differentiating keratinocytes in such a way that these produce ingredients which make the environment for these microbes beneficial. The skin 'returns the favor'.

The upper half of the stratum corneum is called the stratum disjunctum. It is in the lowest parts of the stratum disjunctum where the skin microbes reside and multiply. From this point of view, the lower stratum disjunctum can be considered to be the 'nursery' for the skin microbiota. After formation the microbes become attached to the corneocytes and, with them, they move upwards to the surface of the skin. There they are shed from the skin together with the corneocytes or washed away, killed or rubbed off, etc. Anything which happens at the skin surface is compensated for by the microbial renewal processes where 'new' microbes ('neo-microbiome') reach the skin relatively quickly.

An active ingredient which promotes the improvement of the epidermal differentiation process should be beneficial for the skin microbiota. It should promote a beneficial environment in the 'nursery' and therefore support and help balance the skin microbiota.

CLR developed ProRenew Complex CLR™ (PRC), a lysate of probiotic *Lactococcus lactis*, which was already shown to improve epidermal differentiation and was investigated for its ability to support, balance and protect the skin microbiota.

Designing the clinical study

The overall composition of the skin microbiota can be analyzed. The composition of the skin microbiota can be described by 2 main parameters: richness and diversity. Richness describes the number of microbial species on the skin. Diversity describes the evenness of the distribution of these species.

For the efficacy study with PRC an area of the skin was analyzed which shows both high richness and high diversity. This ground state allows for the valid analysis of the activity of the active ingredient as both parameters are reduced significantly after disturbance of the microbial composition under experimental conditions. After challenging the skin by tape stripping (described below), the kinetic of the re-establishment of the original microbial balance was determined.

The study was performed on the inner forearm. The composition/balance of the skin microbiota was challenged by tape stripping. Tape stripping allowed for a standardized challenge to the skin microbiota, where other approaches might be less relevant for performing this study. E.g. washing with harsh cleanser, which is difficult to standardize and use for performing an efficacy study. Like the use of a harsh cleanser, tape stripping severely alters the composition of the skin microbiota. In all cases the microbiota will try to regain its original composition from within the skin, essentially 'renewing' itself. Therefore tape stripping is representative of all other ways of challenging the skin microbiota and the results of the study can be deemed relevant for the cosmetic industry.

Table 1: Study design.

Area	Treatment
A	Placebo
B	1% Fructooligosaccharides
C	3% PRC
D	Positive control (untreated, undamaged)

Performing the study

The study was designed as blinded intra-individual comparison of the effect of 2 different formulations and a corresponding formulation without active ingredient ('placebo') on different, randomized locations. 17 study participants were included in the study. On day 0 tape-stripping was performed on 3 areas (A, B, C) and one area was not tape-stripped (D). Areas A, B and C were then treated with test products, as shown in Table 1 and the volunteers continued applying test products twice daily for the subsequent 7 days.

Volunteers applied the formulations bi-daily in the morning and evening at selected fields. Analysis of the microbial community took place before tape stripping and 2 and 7 days after tape stripping.

Method of microbial analysis

Traditional methods to identify and characterize microbes require growth of isolated microbes on culture plates. Most microbial species on the skin, however, do not grow readily in routine laboratory culture conditions or as monocultures. Next-generation sequencing technologies allow comprehensive and detailed sequence-based analysis of microbial communities. The 16S ribosomal RNA (rRNA) gene is conserved among the bacteria occupying the human body with specific genetic regions that can be used for taxonomic classification. This makes the 16S rRNA gene a molecular signature to identify members of bacterial communities.

Skin microbes are collected by noninvasive swabs. After lysis of the microbial cells, the microbial DNA is isolated. After this, the DNA is selectively PCR-amplified (Polymerase Chain Reaction) using primers targeting the 16S rRNA gene. The next step is sequencing the amplified PCR products to determine the DNA sequence in a high throughput manner. After DNA sequences are obtained, bioinformatic tools are used for basic sequence processing, taxonomic assignment, diversity analyses, and community comparisons.

Results

Composition: richness and diversity

Tape stripping led to significant changes in the composition of the skin microbiota. Both diversity and richness were clearly reduced. After 2 and 7 days after tape stripping the microbial analysis showed interesting results on both species richness and diversity:

The treatment of the skin with the formulation containing PRC lead to an accelerated recovery of species richness, whereas the treatment of the same formulation containing Fructooligosaccharides was not able to outperform the corresponding placebo. The benefit of the use of PRC was already clearly visible after 2 days of application. See Figure 1: richness on area D was set at 100%.

After 7 days, the application of PRC lead

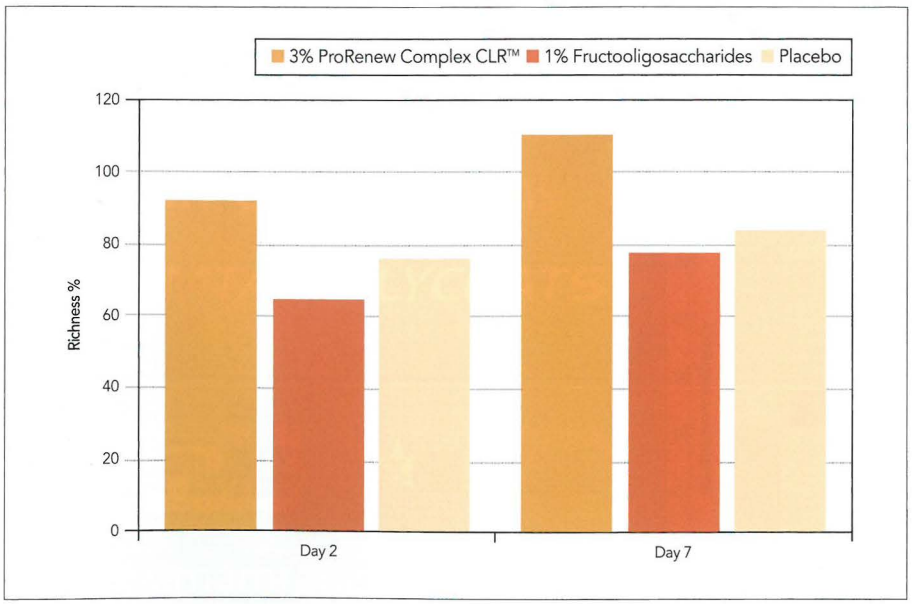


Figure 1: Recovery of microbial richness (%).

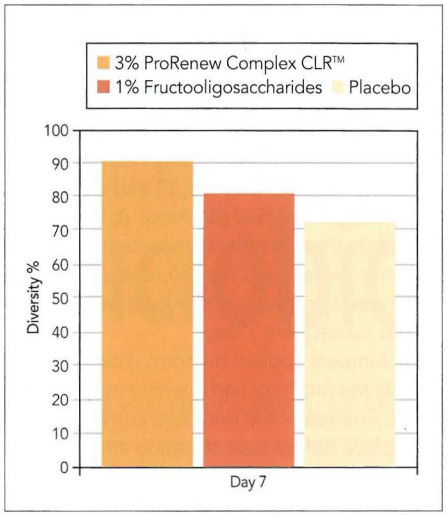


Figure 2: Recovery of microbial diversity (%) at day 7.

to an improved recovery of species diversity. PRC clearly outperformed the same formulation containing fructooligosaccharides and placebo. See Figure 2: diversity on area D is set at 100%.

From the results obtained on the above parameters it can be concluded that the topical application of PRC promotes the skin's ability to reestablish its microbiota after severe disruption. It clearly outperformed prebiotic fructooligosaccharides, underlining the importance of influencing the epidermal processes which favor the skin health. Healthy skin has a healthy microbiome and this could be convincingly shown in these experiments. From these studies it can also be concluded that prebiotic approaches using fructooligosaccharides do not suffice.

Species level

The approach with 16S rRNA gene sequencing as performed in this study allows for the analysis of single microbial species with extremely high resolution. Therefore, additional analyses on the species level were performed. In 2018 a scientific publication of Byrd et al. described the most abundant microbial species on the inner forearm, the area of the skin which was used for the study with PRC (Byrd AL et al., The Human Skin Microbiome, Nature Reviews Microbiology, 2018 Mar;16(3):143-155. doi: 10.1038/nrmicro.2017.157). With this knowledge it was possible to gather valid information on the activity of PRC on relatively abundant microbial species.

After tape stripping and dramatically altering the composition of the skin microbiota, for 7 of the most abundant microbial species it could be shown that the use of PRC leads to an acceleration of the recovery of the original abundance of these species. Here too, PRC clearly outperforms prebiotic fructooligosaccharides. See Figure 3: abundance of microbial species on area D is set at 0%.

Conclusion

The environment, the local habitat, dictates the microbial composition. For skin, the habitat is for the larger part made available by skin, the differentiating keratinocytes. As PRC is strongly able to positively influence the quality of epidermal differentiation it promotes a healthy microbiota on and in the skin. This was convincingly proven in the described studies. The studies were performed with valid technologies where both the overall composition and single abundant species were analyzed. On all parameters it was shown that PRC outperforms both the corresponding placebo and the same formulation containing prebiotic fructooligosaccharides.

Contrary to many prebiotics, probiotic lysates like PRC contain components which are known ligands for toll like receptors (TLR). Some of these molecules are situated in the membranes of (probiotic) bacterial cells. e.g. TLR2 is activated by bacterial lipoproteins, TLR4 is activated by lipopolysaccharides (LPS), peptidoglycan recognition protein (PGRP) is activated by peptidoglycan (PGN). The downstream signaling pathways used by these receptors can contribute in maintaining the proliferation and differentiation status of skin cells as well as in strengthening the immune system. Several recent reports have suggested that the functional outcomes of TLR and PGRP signaling have positive effects even on the antimicrobial defense system.

The use of prebiotic molecules in the cosmetic industry is a common phenomenon, but the results of these studies show that positively influencing epidermal differentiation is a clearly more powerful tool in balancing, supporting and protecting the skin microbiota. Interestingly, PRC, being a lysate of probiotic *Lactococcus lactis*, a postbiotic, interacts with the differentiating keratinocytes much in the same way as the healthy skin microbiota does. PRC 'co-operates' with the skin microbiota, which makes it an essential component in overall skin health and quality. PC

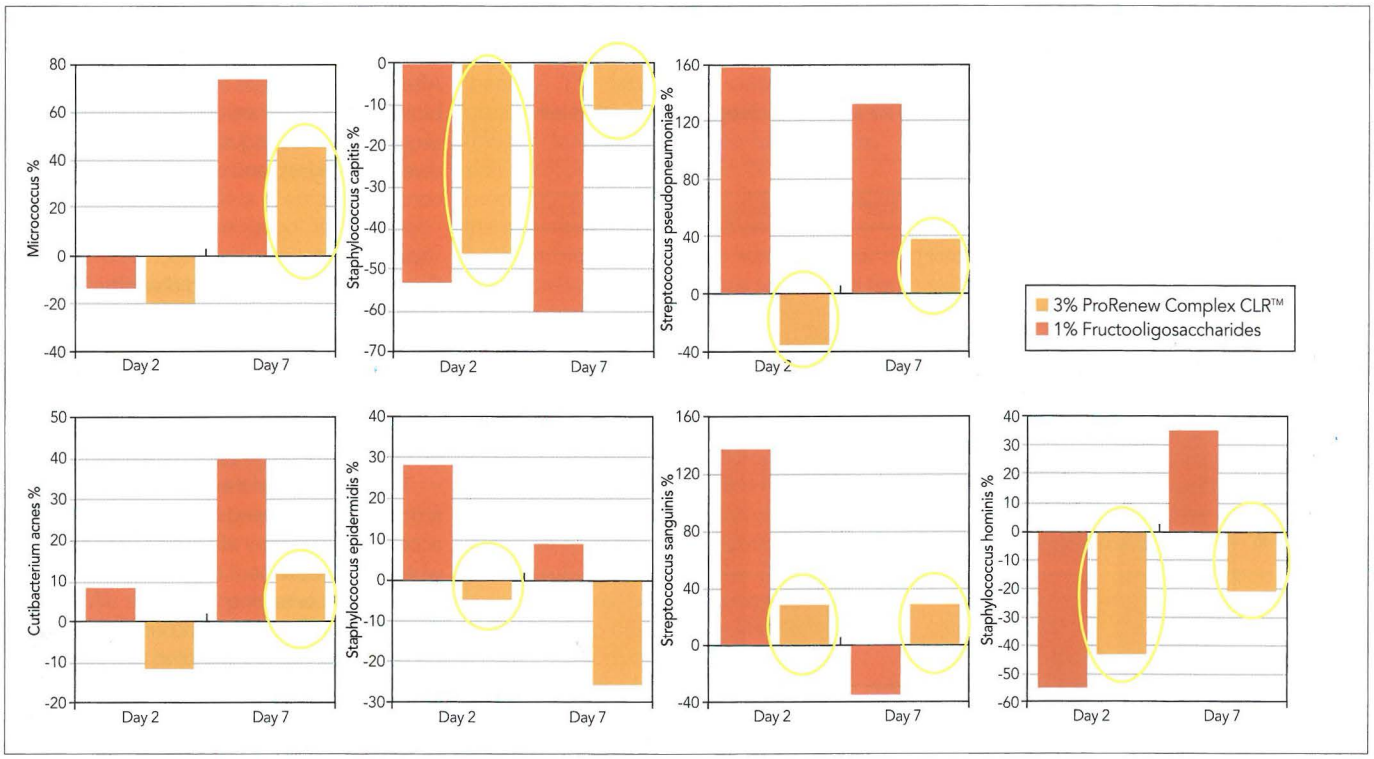
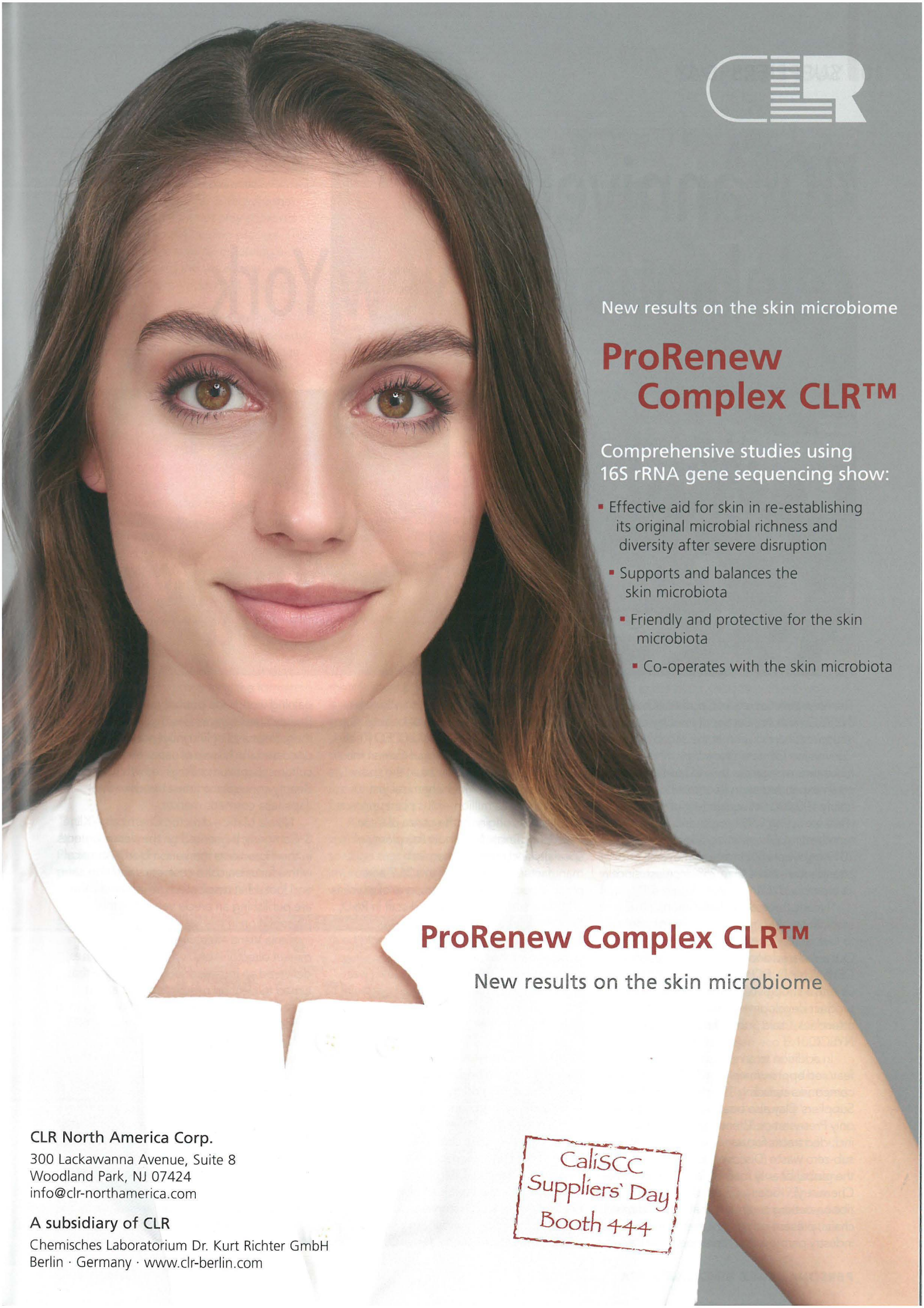


Figure 3: Difference with positive control (%).


New results on the skin microbiome
ProRenew Complex CLR™
Comprehensive studies using 16S rRNA gene sequencing show:
▪ Effective aid for skin in re-establishing its original microbial richness and diversity after severe disruption
▪ Supports and balances the skin microbiota
▪ Friendly and protective for the skin microbiota
▪ Co-operates with the skin microbiota
ProRenew Complex CLR™
New results on the skin microbiome
CLR North America Corp.
300 Lackawanna Avenue, Suite 8
Woodland Park, NJ 07424
info@clr-northamerica.com
A subsidiary of CLR
Chemisches Laboratorium Dr. Kurt Richter GmbH
Berlin · Germany · www.clr-berlin.com
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