Cosmetic Preservatives and Skin Microbiota, what's new?

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In 2018, when skin microbiota was the personal care word of the year, and everyone seemed to be involved in this topic and related new discovers, we decided to investigate whether cosmetic preservatives, which are known and used for their antimicrobial efficacy, could have any impact on the microbial population inhabiting our skin. What we obtained, after an experimental trial involving several preservatives and boosters and twenty female volunteers, has been shared in an article on HPC Today journal (1).

Beside the results, what we realized thanks to this first experimental approach on preservatives and skin microbiota, are pros and cons of the method. We approached what we defined an *in-vitro* method: basically we isolated and identified some skin-resident bacteria (cheek and forehead) and then put them in contact with different substances (preservatives and boosters) through the Minimum Inhibitory Concentration (MIC) protocol. Doubtlessly, this approach has several advantages. First, it does not require excessive participation from the volunteer, indeed they are not requested to undergo any skin treatment and the sampling method (sterile soaked swab) is not invasive at all. Also, the protocol can be performed in the majority of microbiological labs, first because the techniques are quite easy and can be easily learned by basic operators, second because the required equipment is not sophisticated or difficult to use. Finally, another advantage of the *in-vitro* method, is that results are fast to obtain and easy to read and interpret. On the other hand, the *in-vitro* method is characterized by some limits, which result in the impossibility to use test results to exhaustively respond to the question "May preservatives alter skin microbiota composition?". Among the limits, one on all relates to microorganisms selection: the *in-vitro* method allows to work only on a selection of skin-resident microorganisms (those who are able to grow in the adopted cultivation conditions - e.g. temperature, O_2 presence, culture medium, ...), while it is not possible to extrapolate results for the whole skin microbiota. In a few words, even if the study was aimed at understanding if some substances could interfere with the skin microbiota (sampled from a defined skin area), actually it is only possible to analyse how a limited part of all the microorganisms inhabiting that skin area responds to the presence of substances. In addition to this, microorganisms behaviour and response to substances treatment would be probably different when *in-vivo* or *in-vitro* treated (details on this point are summarised in Table 1).

In lights of the above reported limits, an *in-vivo* method should be approached to answer the question whether cosmetic preservatives may or may not interfere with human skin microbiota. This method should be firstly based on volunteers prolonged treatment with products both including and not preservatives; secondly, microbiota sampling and metagenomics analysis should be performed to evaluate if changes in skin resident microorganism population occurred.

Before starting this new approach of experimental trials, we considered of interest to evaluate which is the current state of the art and knowledge on the topic, therefore we ran a bibliographic research on studies focusing on cosmetic preservatives and skin microbiota. This article is meant to resume up-to-date information on potential Personal Care preservatives interference with skin microbiota, it is also intended as a starting point for any future development in this field.

BIBLIOGRAPHIC RESEARCH

Among dozens and dozens of articles which are more or less titled "Cosmetics and skin microbiota" only few of them really focus on preservatives as ingredients and their potential interference with the skin microbiota. We selected six recent articles and summarized them in Table 2.

It is evident that most of the knowledge reported in these articles, derives from *in-vitro* test. Only Jeong and Kim (Article 1) approached an *in-vivo* study, whose protocol, unfortunately, is not deeply described in the article. All the other articles are based on the isolation of skin resident bacteria from volunteers, then followed by a microbiological *in-vitro* test (in some cases Minimun Inhibitory Concetratoin – MIC test, in some other cases Antibiotic susceptibility testing - AST like test).

Type of limit	Details
Skin microbiota representation	*Microbial isolation and lab cultivation limits tested species: only a limited part of all microorganisms inhabiting human skin are isolated through swab and will survive the selected laboratory cultivation techniques*
Artificial exposure to preservatives – type of contact	*According to the protocol, microorganisms come in direct contact with preservatives and boosters, which are usually contained in finished cosmetic formulations; Usually, preservatives and boosters are not applied directly on skin*
Artificial exposure to preservatives – time of contact	*In daily life, cosmetics remain on our skin for some hours of the day, whereas in the experiment the contact between microorganisms and substances lasts 24 hours*
Artificial exposure to preservatives – environment	*According to the protocol, microorganisms are isolated, cultivated and then putted in direct contact with test substances; in daily life, instead, they respond to external factors while living in their habitat (the skin)*
Artificial exposure to preservatives – community	*While inhabiting the skin, microorganisms are part of a community of living cells, instead during the in-vitro test, they are isolated and cultivated in single species microbial suspension/culture*

Table 1. List of *in-vitro* approach limits.

Ref	Article references	Investigated products	Experimental protocol details	Conclusions
1	*Effects of Cosmetics and Their Preservatives on the Growth and Composition of Human Skin Microbiota* Jin-Ju Jeong and Dong-Hyun Kim† (J. Soc. Cosmet. Sci. Korea Vol. 41, No. 2, June 2015, 127-134)	Metylparaben Ethylparaben Propylparaben 1,2-hexanediol Phenoxyethanol	1-MIC and MBC on purchased *P.a*, *E.c* and *S.a* 2- Isolation and cultivation of face skin bacteria (5 female volunteers) 3-DNA extraction, pyrosequencing and data analysis of skin microbiota 4-Evaluation of Preservatives effect on skin-resident bacteria 6-*In vivo* evaluation of Phenoxyethanol effect of human skin microbiota	*"... preservatives potently inhibited pathogens and skin-resident microbiota ... Based on these findings, preservative-containing cosmetics may disturb the microbiota and cause superinfection"*
2	*Effect of cosmetic chemical preservatives on resident flora isolated from healthy facial skin* Wang Q, Cui S, Zhou L, et al. (WILEY JCD Journal of Cosmetic Dermatology)	Methylisotiazolinone IPBC Ethylhexylglycerin Methylparaben Phenoxyethanol	1-MIC on purchased *S.aureus* and *E.coli* 2- before sample collection, volunteers undergo 24h pre-treatment (no washing/application topical medication) 3- Isolation and cultivation of skin bacteria (14 healthy adults) from face 4- DNA extraction and 16S RNA sequencing 5- Evaluation of Preservatives effect on skin-resident bacteria (MIC)	*"... The effect of common cosmetic preservatives on skin - resident bacteria in vitro can provide a reference for improving dosage of these ingredients in cosmetic formulations and preserve homeostasis of skin microorganisms"*
3	*Impact of superficial blends on skin micro biota* Chintha Lalitha, P.V.V. Prasada Rao (International Journal of Current Pharmaceutical Research)	Triclosan (*) Phenoxyethanol (*) Parabens (*) Talcum powder *RT Fairness cream *RT Deospray *RT (*) supposed to be tested *RT really testes	1 - Isolation and Identification of face skin bacteria 2 - Colony count on skin before and after application of cosmetics 3 – AST test: finished products vs skin isolated bacteria a)48 bacteria isolated from the skin, each one diluted and plated on agar b)50uL of each diluted cosmetic was spotted on sterile filter paper disc	*"... when the skin conditions are changed by the use of topical applications such as creams ... and other cosmetics, the chemicals that are present in the preservatives may alter in the population of skin biota"*
4	*Bacteriostatic efficacy of Germall BP on resident microorganisms in epidermis* CHEN Guan-wu, CAI Ying, TANG Bo, et al. (Journal of Environment and Health)	Germal BP (*Diazolidinyl urea + IPBC + Propanediol*)	1-Germall BP (diluted in water and added into cosmetics) 2-M.I.C. vs *Staphylococcus epidermidis* and *Propionibacterium acne* (4h-8h-24h)	*"... because of its bacteriostatic efficacy on resident microorganisms in epidermidis Germall BP shortening usage time of cosmetics is advised to minimize or even avoid this kind of adverse effects"*
5	*Bacteriostatic efficacy of Methylparaben on resident microorganisms in epidermis* Zhou Guang-bia	Methylparaben	1-Methylparaben (diluted in water and added into cosmetics) 2-M.I.C. vs *Staphylococcus epidermidis* and *Propionibacterium acne* (4h-8h-24h)	*"... Methylparaben presents a certain degree of bacteriostatic efficacy on resident microorganisms in epidermidis, but it would not inhibit and kill them significantly after short time contact (≤4h) within allowable Chinese limit (≤0.4%)*
6	*Antimicrobial Efficacy of Preservatives used in Skin Care Products on Skin Micro Biota* Chintha Lalitha, P.V.V. Prasada Rao (International Journal of Science and Research - IJSR)	Phenoxyethanol MethylParaben Propylpraben Sorbic acid Potassium sorbate Sodium benzoate Talcum powder (with Triclosan) Fairness cream (with Phenoxyethanol, Methyl and Propyl parabens) Deospray	1 – Isolation and cultivation of different bacteria from skin donors (20 volunteers) 2 – Biochemical identification of skin isolated bacteria 3 - Evaluation of finished product antimicrobial activity vs 10 skin isolated bacteria (AST test method) 4 – HPLC determination of preservatives in the finished products tested 5 - evaluation of MIC vs *P.aeruginosa*; *M.luteus* and *S.epidermidis* for all the cited preservatives	*"The study reveals that the six preservatives (Phenoxyethanol, Methyl paraben, Propyl paraben, Sorbic acid, Potassium sorbate and Sodium benzoate) shown antimicrobial activity with the three test organisms at various concentrations and time periods..."*

Table 2. Overview of articles investigating possible preservatives interference with skin microbiota.

Having a look on the type of products investigated, not only single molecules belonging to annex V of the European Cosmetic Regulation (Preservatives) have been chosen: Chen et al. on *Journal of Environment and Health* tested a commercial available preservative blend, and Lalitha and Rao in two different articles (ref. 3 and 6) also evaluated finished cosmetic products.

Among standard preservatives, the most investigated molecules are Methylparaben and Phenoxyethanol. Nevertheless, also on these two, it is not easy to draw a general and unanimous conclusion, answering the question "do they interfere with Skin microbiota?". In some cases, articles conclusions are even contradictory on the same molecule. Take the example of Phenoxyethanol: according to article 1 *"phenoxyethanol exhibited the most potent effect against S. aureus and P. aeruginosae and skin-resident bacteria"*, while in article 2 the conclusion is: *"Phenoxyethanol exerted the least inhibitory effect on the tested bacteria. Phenoxyethanol had the least impact on skin-resident bacteria at concentrations that could inhibit S. aureus and E. coli"*. It is obvious that only results deriving from reproducible test could be reasonably compared to draw unanimous conclusions; any type of result comparison deriving from different experimental methods would be risky and with no scientific value.

CONCLUSIONS

Doubtlessly, when speaking about skin microbiota cosmetics interference, preservatives are among the most interesting ingredients to be evaluated, because of their intrinsic antimicrobial efficacy. At the same time, it is not that easy (as highlighted by this article) to answer the question whether and to what extent different preservatives may have an impact on microbiota *eubiosis*. The difficulty lies in the experimental method to be used to evaluate cosmetic preservatives in regards to Microbiota. Why does it seem difficult? It would be recommendable, to evaluate the endpoint through *in-vivo* experiments, as described here above. Nevertheless, this type of approach is characterized by some limits: first, volunteers shouldn't apply the preservative, but finished formulations containing it (creams, emulsion, gel, ...) as it happens in real life. The point is that other ingredients of the finished formulation may also interfere with skin microbiota, affecting results. Second, through *in-vivo* testing, also intrinsic and extrinsic factors (diet, lifestyle, geographical location, pollution, ...) could affect results.

Our question mark on Preservatives and Skin Microbiota remains a key issue for the Personal Care field, and the cited articles are a good starting point to try to answer it. Much remains to be done, in order to be able to select double safety ingredients: safety for the formula, where we do not want pathogens, and safety for the skin, where we want to keep microbiota *eubiosis*.

Someone would sing *"Ain't No Mountain High Enough"* and that is true: despite all difficulties and unknown factors that could be encountered when testing skin microbiota, it is necessary to approach it and try to give preliminary answers that can be used as starting point for further studies. It is only step by step that all mountains can be climbed!

REFERENCES

1. Life on skin: Personal Care Preservative and Skin Microbiota, a preliminary study (2019) Pellegrino, Fracchia, Lincetti - H&PC Today - Household and Personal Care Today - vol. 14(2) March/April 2019.