Demonstration of surfactant antagonism in the Open Source Reconstructed Epidermis (OS-REp) model

Roland Wedekind, Anja Fischer, Olaf Holtkötter, Karsten R. Mewes
Henkel AG & Co KGaA
E-mail contact: karsten.mewes@henkel.com

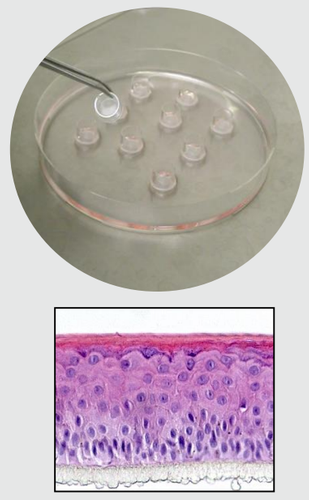
Introduction

Surfactants are main constituents of different consumer products, e.g. detergents or cosmetic cleansing products. However, surfactants show an intrinsic skin irritation potential which has to be considered when products are formulated. Human patch test studies, e.g. from Hall-Manning et al. (1998), have shown that the irritation potential of mixtures of surfactants is much lower than predicted by the summation of the irritation potentials of the single surfactants only. This behavior is known as surfactant antagonism.

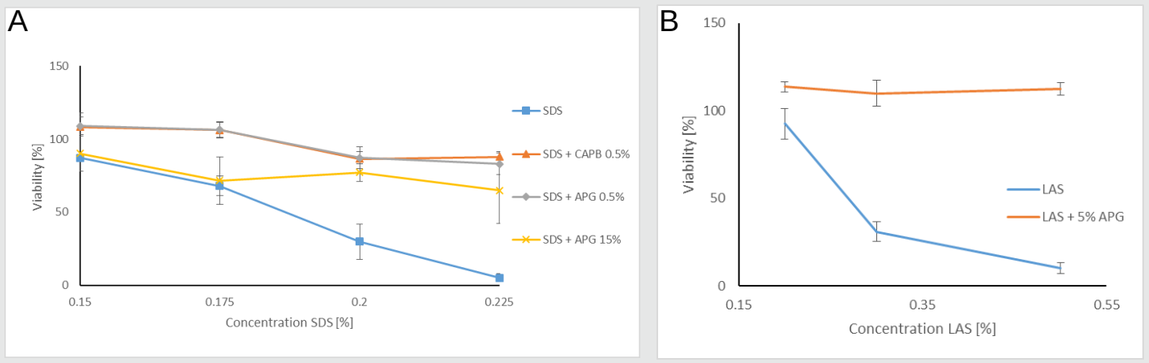
In this study, the irritation potential of binary mixtures of sodium dodecylsulfate (SDS), linear alkylbenzene sulfate (LAS), cocamidopropyl betaine (CAPB) and alkylpolyglucosid (APG) was compared with the effects elicited by the single compounds using the Open Source Reconstructed Epidermis (OS-REp) model. Apart from irritation effects that were assessed similar to OECD TG 439, the relevance of keratinocyte differentiation and the role of the model's barrier for surfactant antagonism was investigated.

OS-REp model

Primary human keratinocytes isolated from foreskin tissue ($3.15x10^5$) were seeded into inserts (∅ 12 mm) and cultured submersed for 24 h based on a publicly available SOP (Mewes et al., 2016). Then, cells were elevated to the air-liquid interface and cultured for additional 19 days. Tissue quality was assessed by histological staining, and applicability for skin irritation studies was evaluated by testing with the OECD TG 439 proficiency chemicals.



Surfactant antagonism in OS-REp models



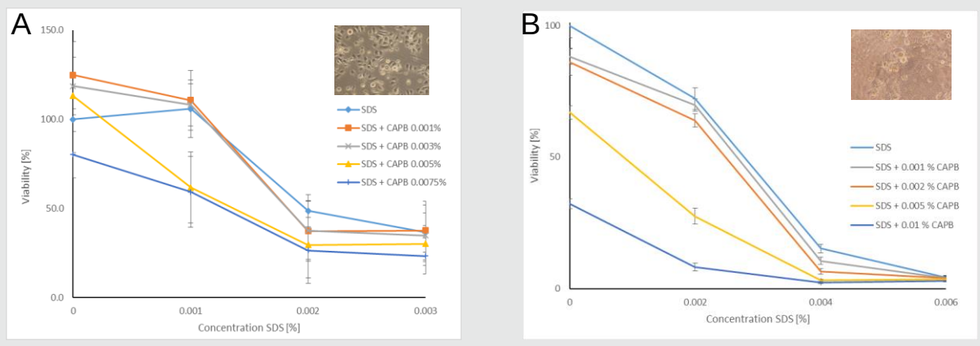
The concentration range at which the single surfactants decrease OS-REp tissue viability was determined with the MTT assay. Based on this approach, surfactants could be ranked with regard to their irritancy potential in the following order:

SDS > LAS >> APG > CAPB

(A) To assess surfactant antagonism, models were treated with SDS solutions of different concentrations alone or in combination with 0.5% CAPB, 0.5%, and 15% APG. After 35 min exposure and 42 h post incubation, viability was measured (n=3). Combination of SDS with either APG or CAPB resulted in higher tissue viability compared to SDS alone, corresponding to a reduced irritation potential of the surfactant mixtures.

(B) Corresponding results were attained by testing LAS alone and LAS in combination with 5% APG or 5% CAPB (not shown).

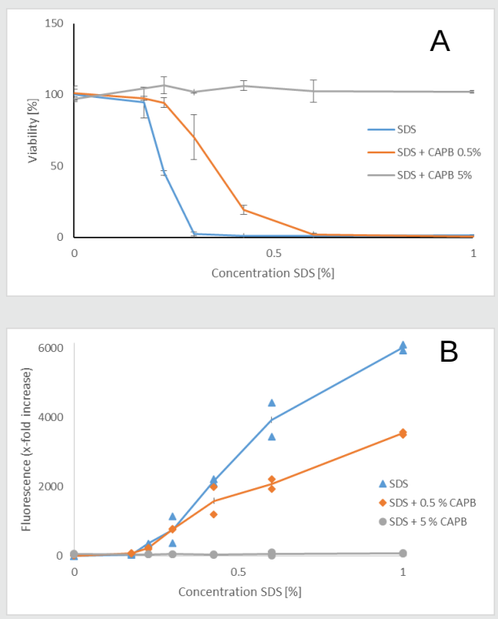
No antagonism in monolayer keratinocytes

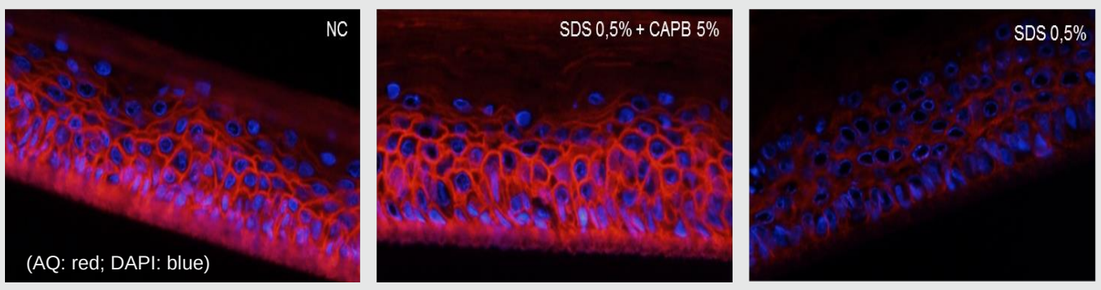


Keratinocytes were cultured in low calcium medium (A) for 24 h (sub-confluent culture) or (B) for additional 5 days after reaching confluence. Cultures were treated for 4 h with SDS alone or in combination with different concentrations of CAPB or APG (not shown), and viability was measured by MTT assay (n = 8). Combining SDS with APG or CAPB did not protect keratinocytes from viability loss, neither in the proliferating (A) nor in the differentiated state (B). Cell damage increased with increasing total surfactant concentrations.

Effects of surfactant mixtures on model integrity

Consequences for the OS-REp tissues after treatment with surfactants were analyzed by measuring fluorescein permeation (n=2) and immunofluorescence staining of aquaporin (AQ). Irritation effects of SDS, measured by loss of viability (A), came along with higher permeation of fluorescein due to impaired barrier function (B), and with loss of AQ staining due to cell damaging. These effects were ameliorated by combining SDS with CAPB.





Conclusion

- Epidermal equivalents like the OS-REp model offer a valuable *in vitro* alternative for the investigation of surfactant antagonism which previously was described primarily *in vivo*.
- Irritation effects of single surfactants, measured as loss of viability in OS-REp models, was ameliorated by combining surfactants, even though the total surfactant concentration increased.
- The absence of surfactant antagonism with monolayer keratinocytes indicates a pivotal role of the differentiated epidermis, including a functional barrier, as a prerequisite for this effect.
- In addition, reduced cell damage in the viable layers of the model correlates with reduced impairment of the model's barrier function.
- The model is a suitable tool to further investigate the mechanism of surfactant antagonism, e.g. the relationship between physico-chemical characteristics and the irritation potential of surfactant mixtures.
- Finally, analysis of surfactant antagonism in the OS-REp model might be applied for comparing the skin compatibility of surfactant-based products, e.g. cosmetic cleansers or hand dish washing products.

References

- Hall-Manning et al.: Skin irritation potential of mixed surfactant systems, Food Chem Toxicol. 36(3):233-8 (1998).
- Mewes et al.: Catch-up validation study of an in vitro skin irritation test method based on an open source reconstructed epidermis (Phase I), Toxicol in Vitro (2016), http://dx.doi.org/10.1016/j.tiv.2016.07.007

Henkel Excellence is our Passion

www.henkel.com