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SKIN CARE PRODUCTS AND EVALUATION OF FEW SKIN PARAMETERS: IS THE FUTURE ALREADY HERE?

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Synopsis

In recent years several sophisticated non-invasive methods for the evaluation of skin physiology and pathology have been developed. The interest increasingly attracted by cosmetics has prompted several studies on such methods, all aiming at assessing the effects of various skin care products on different properties of the skin. The Authors describe the latest and most significant techniques introduced in the study of some important cutaneous parameters.

Riassunto

In anni recenti sono stati sviluppati diversi sofisticati metodi non invasivi per la valutazione della fisiologia e della patologia della pelle. Il crescente interesse destato dai cosmetici ha stimolato vari studi su questi metodi, tutti tendenti alla valutazione degli effetti sulle proprietà della pelle di diversi prodotti. Gli Autori descrivono le ultime e più significative tecniche introdotte nello studio di alcuni importanti parametri dello strato corneo.

INTRODUCTION

In recent years several sophisticated non-invasive methods for the evaluation of skin physiology and pathology have been developed. The interest increasingly attracted by cosmetics has prompted several studies on such methods, all aiming at assessing the effects of various skin care products on different properties of the skin. Skin topography, for instance, has been measured on skin replicas by profilometry; friction with a newly-developed friction instrument; capacitance with a computerised apparatus - the 3C System; the barrier function in normal conditions with an Evaporimeter to assess transepidermal water loss (TEWL), and in pathological conditions by the application of an irritant followed by the measurement of the irritative reaction (1,2).

Some of these techniques were initially employed to characterise the differences between dry atopic and normal skin. More recently, however, researchers have begun to accept the idea that skin care products do not merely form an inert epicutaneous layer, but penetrate and influence the structure and function of the skin. For instance, a scrub cream removes the outermost part of the stratum corneum (SC) resulting in smoother skin. The application of moisturises modifies the skin's frictional response. Since the new friction instrument has given results comparable to those of skin friction can predict the degree of penetration of moisturisers (1). Moisturisers augment skin hydration, supplying water directly from their water phase. Skin hydration is also increased by a higher degree of occlusion, as measured by lower TEWL values. In dry skin, roughness parameters are higher and topographical peaks lower; TEWL is higher, indicating impaired barrier function. Friction and capacitance levels are lower and correlate significantly with each other, whereas TEWL does not appear to correlate with either of these parameters. Moisturisers can also alter the diffusional resistance of SC and reduce the skin's susceptibility to the surfactant sodium lauryl sulphate (SLS). Lipids in moisturiers can influence already developed SLS-induced irritatio (1).

In the light of these data, it is interesting to describe some of the latest and most advanced techniques introduced in the study of some important cutaneous parameters.

CORNEOSURFAMETRY

The stratum corneum is the outermost layer of the epidermis. The flattened keratinocytes of the granular layer lose visible evidence of the keratohyalin granules and, as they die, lose their nuclei: these dead keratinocytes form the SC (Fig. 1).

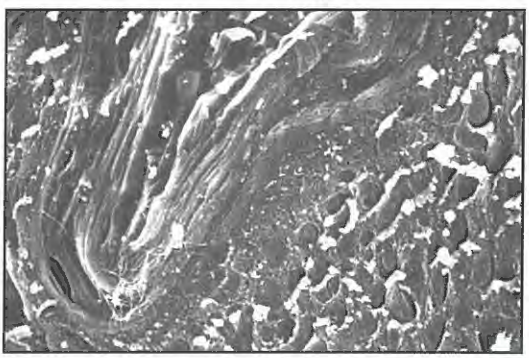

Fig. 1 Epidermis is the outermost part of the skin, note the stratum corneum layer (SEM x 906).

Corneosurfametry allows to study SC samples by measuring the variations in staining of samples obtained in different skin (3), however, it presents less interindividual variability than in-vivo testing, and allows for better discrimination among mild products. More exhaustive morphological information about surfactant induced loosening of corneocytes may be increased by testing surfactants on human skin equivalent. Results are similar to those provided by specimens used for corneosurfametry. The corneosurfametric prediction of surfactant irritancy seems to correlate well with in-vivo testing and in-vivo and in-vitro evaluation on human skin equivalent (3).

SKIN EQUIVALENT

Nonetheless, the legal procedure for evaluating the toxicity of household, cosmetic, chemical and pharmaceutical products is still the irritancy Draize

test on rabbits. Several irritation tests are currently being developed as alternatives to in-vivo animal testing. An interesting in-vitro model system is 24 equivalent dermis (ED) consisting in a chitosan-cross-linked collagen glycosaminoglycan matrix populated by foreskin fibroblasts (Fig.2) (4).

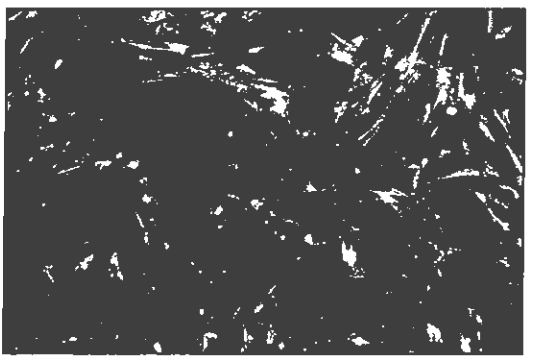

Fig. 2 Scanning electron micrograph of skin fibroblasts (SEM x 500).

Three main parameters of toxicity - MTT (dimethylthiazol diphenyltetrazolium bromide) reduction, lactate dehydrogenase and interleukin-6 can be used to determine the usefulness and the predictive value of this system compared with methods employing chemical products (cadmium chloride, lauryl sulphate, benzalkonium chloride). Preliminary results confirm the efficacy of ED as an in-vitro model for the prediction of cutaneous and ocular toxicity.
This and other in-vitro models are all the more significant in view of the European Union's directive banning the utilisation of animal models to test the safety of cosmetic products as of 1st January 1998. Devising effective alternative models is therefore an issue of outstanding interest to cosmetological research (5).

CONFOCAL LASER SCANNING

Confocal laser scanning microscopy allows the direct visualisation in unfixed material of diffusion pathways and of the cellular distribution of fluorescent markers following topical applications. Optically sectioned tissue specimens are analysed for the changes occurring in the distribution patterns of topically applied compounds depending on vehicle penetration time and depth, without the interference of the chemical alterations induced by most of the usual fixation techniques. With confocal laser scanning the permeability properties of in-vitro-reconstructed epidermis can be compared with those of the native or aged counterpart. The epidermis is reconstructed by culturing human adult keratinocytes at the air-liquid interface either on fibroblast populated collagen or on de-epidermised dermis. A fluorescent probe, Nile red, is applied in association with one of three different vehicles - polyethylene glycol with a molecule mass of 400 da, propylene glycol and dimethyl sulphoxide - which perturb the SC barrier function to different extents (6). These methods allow a better evaluation of permeability in different structural conditions.

FREEZE FRACTURE AND SMALL-ANGLE X-RAY SCATTERING

The interactions between three liposomal formulations and human SC can be visualised by freeze-fracture electron microscopy. Human SC is immersed for 48 h in liposome suspensions that can be prepared from commercially available phospholipid mixtures. The main difference between formulations may be the hydrophilicity of the headgroups. This technique investigates the composition dependence of the interactions between these vesicles and human SC. Different types of interaction can be observed: adsorption of the liposomes onto the outer surface of SC, and/or ultrastructural alterations in the deeper layers of SC caused by mixing of the liposomal constituents and SC lipids (7). The electron microscopical observations are verified with small-angle X-ray scattering. It is possible that liposomes composed of phospholipids containing relatively small hydrophilic headgroups showed a marked interaction with the skin lipids of human SC in vitro (7). These results are very promising for future applications of small-angle X-ray scattering in cosmetology, which is expacted to provide

essential information on the molecular structure and organisation of intercellular lipids of SC.

MAGNETIC RESONANCE

To gain a more datailed knowledge of the precise mechanism underlying SC elasticity, the molecular dynamics of chemical residues within the keratin fibres of human plantar SC can be investigated in various conditions by polarisation-image angle spinning 13C nuclear magnetic resonance (NMR) (8). In a recent study (8), the intensities of NMR spectra responsible for amide carbonyl, C alpha methine, and side-chain aliphatic carbons in the intact SC decreased markedly with increasing water content of up to 30% in dry SC, and remained consant over 30%. Lipid extraction of intact SC with acetone-ether (1:1) did not induce significant changes in the NMR spectrum, whereas additional treatment with water, which released natural moisturising factors (mainly amino acids), caused SC to lose elasticity (8). Elasticity recovered after treatment with basic and natural, but not acidic, amino acids. With the latter treatment, the movement of amino acid molecules was significantly disturbed, suggesting a strong interaction with keratin fibres. Parallel studies of the complex alastic modulus of a pig SC sheet with a rheovibron have also demonstrated that removal of natural moisturising factors reduce SC elasticity. This effect is also reversed by the application of basic and neutral, but not acidic, amino acids. These findings suggest that the structural keratin proteins, mainly consisting of 10 nm filaments, acquire elasticity with the help of hydrated natural moisturising factors via the reduction of intermolecular bindings, probably through nonhelical regions between keratin fibres (8).
The future seems indeed to be already here.

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