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"NATURAL COSMETOLOGY": INNOVATIVE APPROACHES TO AMELIORATE THE SKIN BARRIERS

G. Biagini, F. Gabbanelli, F. Giantomassi, L. Virgili and M. Mattioli-Belmonte
Istituto di Morfologia Umana Normale, University of Ancona, Italy.

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Summary

Over the last few years, gelatin and chitosan have been widely used in cosmetology as active materials to maintain and/or improve skin-barrier functions. Their different structural and bio-functional characteristics appear to suggest different conditions of elective application.
Chitosan seems mainly suitable to maintain a high level of skin hydration with a parallel improvement in tissue homeostasis preventing water evaporation at the cutis-environment surface. By contrast, gelatin seems to be more suitable to stimulate poorly reactive skin microenvironments by virtue of its more prolonged in situ bio-presence compared with chitosan.
The association of chitosan and gelatin could offer considerable advantages as their main "stimulation" attitudes evolve through different bio-temporal pathways.

Riassunto

I nostri studi sono rivolti alla progettazione di molecole cosmetologiche che possano creare per le generazioni future una miglior presa sui sistemi ecologici di *life support*. Nel corso di ultimi anni, la gelatina e il chitosano sono stati ampiamente usati in cosmetologia quali materiali attivi per mantenere e/o migliorare le funzioni di barriera della pelle.
Le loro differenti caratteristiche biofunzionali e strutturali sembrano suggerire differenti condizioni di applicazione elettiva. Il chitosano sembra soprattutto adatto a mantenere un elevato livello di idratazione della pelle con un parallelo miglioramento dell'omeostasi tessutale, impedendo l'evaporazione dell'acqua a livello dell'interfaccia cute-ambiente.
La gelatina, grazie alla sua prolungata bio-presenza in situ rispetto al chitosano, sembra essere più adatta a stimolare i microambienti cutanei scarsamente reattivi. Pertanto l'associazione di chitosano-gelatina può offrire considerevoli vantaggi.

The Cosmetological Industry is continually synthesising new chemicals, the regulation of which requires assessment of their potential danger. Risk assessment is nowadays considered essential to taking these decisions on a scientifically sound basis; developing natural, biological cosmetical products, avoiding industrial chemical alternatives, seems a select key-route. We shall therefore briefly consider some natural molecular substances products aimed at improving skin barrier permeability and at addressing other dermal-cosmetological occurences.

The skin carriers out a number of functions; among which that of barrier is a major one. Intact healthy skin prevents fluids from flowing out transepidermally and germs and foreign matter from penetrating. Basically, the barrier function carried out by the horny layer, which develops via keratinocyte differentiation into horny layers and the intercellular accumulation of lipids. Thus, skin permeability depends largely on how lipids are arranged in the cells of the outer layers. Though quite similar to other biological membranes , these lipidic lamellas partly differ from them in lipid content and structure.

Among molecules other than lipids, polysaccharides as chitin and its derivatives are known as enhancer of pericellular and intracellular pathways, affecting higher functions and free plasma membrane surfaces. Aminoacids such as L-Arginine (poly L-arg) are also enhancer of transepithelial absorption. The barrier function thus seems to involve both lipid organisation and protein structures.

In the skin, cross-talks between arginine and glycine are becoming more and more evident. It is conceivable that nitric oxide (NO), which is generated in vivo through conversion of L-argininine to citrulline by NO synthase (NOS), may represent a cellular switch that changes the epithelial phenotype from stationary to locomotive. Glycine also seems to be deeply involved in the modulation of skin micro-environmental metabolism. The presence of glycine - which is structurally diffuse in tissues – within peptide RDG, which represents an important binding site between extracellular environment and cells, seems to be involved in cell proliferation, differentiation and migration processes. The soluble RGD module also stimulates collagenase production. Exogenous glycine may therefore have a strengthening role of the glycine present in tissue stromal RGD motifs, and its attachment to artificial substrates seems to promote cell adhesion (Figs 1-2).

***Fig. 1** Cell culture on Glycine Substrata*

***Fig. 2** Sem Micrograph of cell adhesion on Glycine substrate*

Work performed by our group seems to indicate that the presence of glycine bound to substrates such as gelatin promotes the adhesion and proliferation of keratinocytes, and lends further support to the notion that the association of glycine and arginine may contribute to improve cutaneous trophism in the medical-cosmetological field.

Carbohydrates can also significantly affect protein structure as glycoproteins and therefore influence protein functions. Carbohydrates are also used as tags to sort proteins in the Golgi apparatus, by targeting them to specific compartments within the cell or directing them to the cell surface. It is known as after a skin lesion, a series of reparative events begin to take place that range from homeostasis to inflammation, neoangiogenesis and, ultimately, fibroblastic and epithelial proliferation. These natural repair processes may be hampered by several exogenous and endogenous factors like microbial flora and/or venous stasis. We are thus called upon to devise means that prevent and reduce the onset of these complications but also favour and where possible accelerate cellular and extracellular metabolism.

Our research has outlined the possibility of using bioactive compounds such as collagens, GAG, and fibronectin, which are naturally occurring extracellular-matrix compounds that interact with various cell–adhesion molecules called integrins to improve skin homeostasis.

In our experiments, we used a gelatin- based dressing in the form of stiff sheets to supply the repairing skin tissue with mechanical support. Gelatin - which derives from the denaturation of collagen is endowed with metabolical stimulation properties that favour the correct recovery of the skin barrier. The gelatin sheets were obtained by cross-linking with low – concentration glutaraldehyde (GTA) solutions. Excess GTA was rinsed off with several washes in glycine, which enhances the results on the repairing skin environment, as previously outlined (Fig. 3).

Fig. 3 Wound healing in the presence of Glycine device

In line with other recent works, we also observed that molecules such as chitosan seem to favour the early phases of skin repair, while gelatin appears to promote cell proliferation and the production of extracellular matrix, which are characteristic of typical phases of skin stable reconstitution.

Our data can thus contribute to design a dermocosmetical protocol based on the utilisation of different natural molecules (also in the presence of low-concentrations of a biocidal ones such as AgSD, which is used to control infective activities in burned and debilitated patients). In fact gelatin has been seen to strongly favour the deposition of collagene fibres rather than cell proliferation at the level of both stromal and vascular elements in different experimental models. Collagen and gelatin macromolecules entrap platelets in early fibrin clots, with PDGF release and neosynthesis of extracellular matrix.

In addiction polysaccharide such as hyaluronic acid, by virtue of their large hydrated molecular volume and its ability to form molecular matrix, can expand interfibrillar collagen spaces to allow cell movements. Signal molecules interacting with specific cell membrane binding sites

are involved in this intriguing regolatory controls of cell traffic and perhaps also cell differentiation during embryogenesis and regeneration.

In the light of these data, we studied the behaviour of epithelial cells (Keratinocytes NCTC 2544) cultured on gelatine microstructured with hyaluronic acid (Hyal) or sulfated hyaluronic acid (HyalS) to evaluate the possibility of applying these molecules to stimulate the methaholic processes of a soft tissue as cutis (Fig. 4).

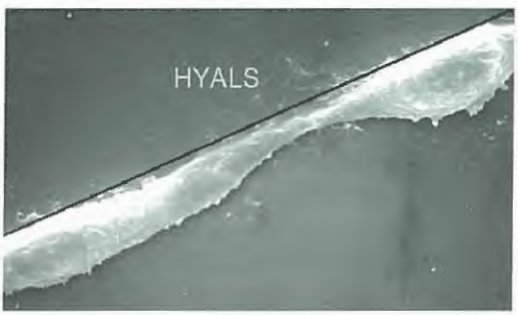

Fig. 4 Cell adhesion on hyals

Hyaluronic acid was deposited in the form of strip- like nanostructures on a homogeneous substrate, represented by gelatin sheets (Fig. 5).

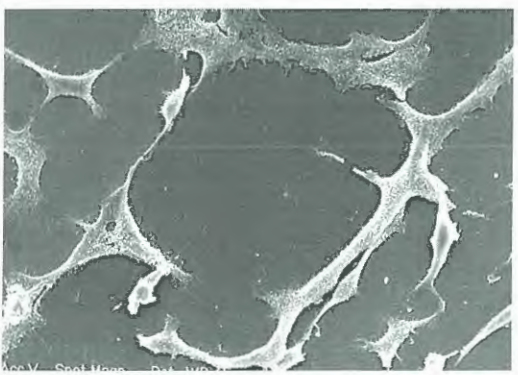

Fig. 5 Cell Behaviour on hyal deposed onto gelatin sheets

The quantitative- morphometric analysis of cell behaviour on this biomaterial coated with Hyals showed as a characterizing element that such behaviour depends partly on the nature of the substrate, and in large part on the topography of the strips of hyaluronic acid deposited on it. From a functional standpoint, as the cells in contact with the strips tended to arrange themselves parallel to them (which entails an active cellular locomotion phonomenon), it was observed that this occurred to a greater extent with 100 and 25 mm structures than with 50 and 10 mm ones. Indeed, in the larger structures (100 mm), which had wider interstrip spaces, cells were more numerous in these spaces, possibly because they were less sensitive to the ordered migratory stimulus provided by the structuring at 25mm (Fig 6).

Rather than a concept based on defined parameters, the "fragility" of the cutis is a situation that often obtains as a consequence of both increased life expectancy and environmental stress stimuli. It results in a series of borderline morphostructural and functional conditions that are of interest also to cosmetology. For these subjects halfway between the competence of the dermatologist and that of the "cutaneous hygiene expert", the utilisation of normal natural factors such as growth factors should be accepted also in the cosmetological field.

The develop of cosmetological products that for instance re-establish a good cutaneous vascularity and thus a better trophism should be undertaken and standardized. Because they are able to re- establish a good vascular network, factors such as VEGF associated with vehicles typical of cosmesis are to be considered as an operational time reality. The research towards health promotion and protection is redesigning our biomolecular knowledge.

Our group is studying the action of VEGF on skin explants: its use in vivo is not allowed at present, but in my opinion it is only a question of time and commitment.

In conclusion, we must address our attention to peculiar understanding "Humanity like all of nature's bounty is a product of evolution and a dipendence and an affinity for NATURE are undeniable components of the human community also for Dermal Cosmetological occurrences".

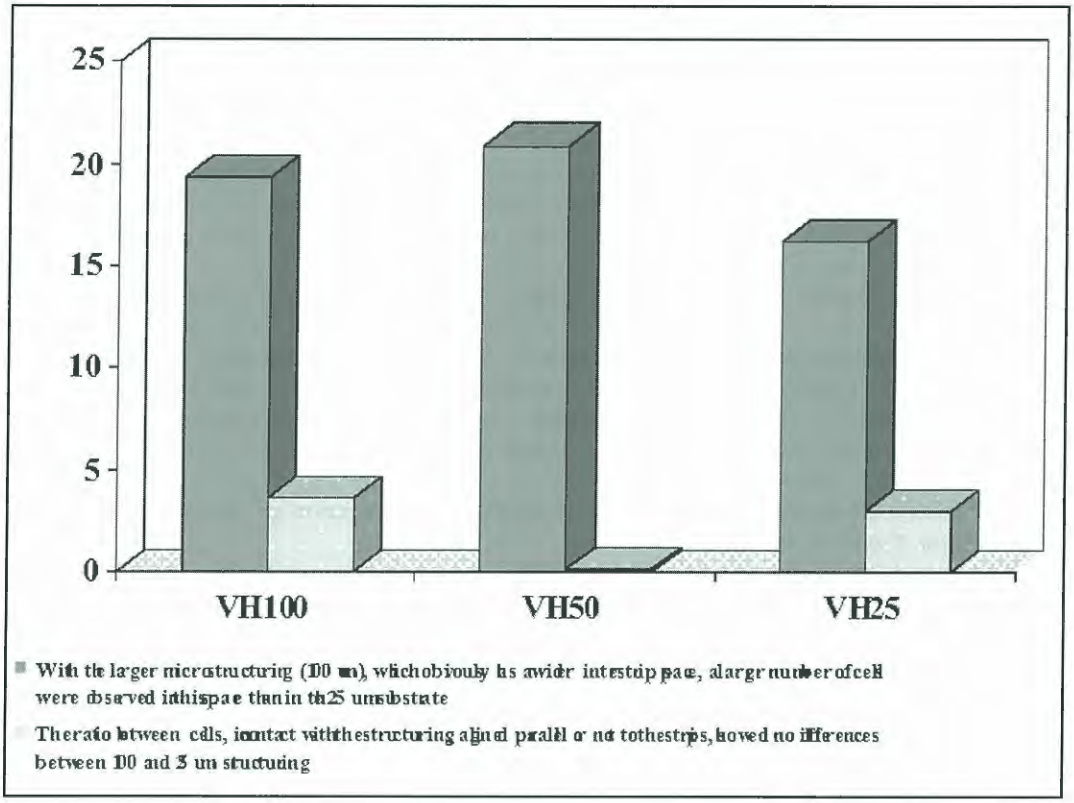

Fig. 6 Histogram of cell behaviour

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Author Address:

Graziella Biagini,
Istituto di Morfologia Umana Normale,
Facoltà di Medicina e Chirurgia,
Università Politecnica delle Marche
Via Tronto, 10/A
60020 ANCONA - Italy
Fax. 00390712206073