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NEW USE OF COMPOSITIONS
CONTAINING MOTHER OF PEARL

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NEW USE OF COMPOSITIONS CONTAINING MOTHER OF PEARL

This invention relates to a new use of mother of pearl for medicinal and/or
5 cosmetologic purposes.

The reference Chemical Abstracts Vol 124 n° 11. (1996) 1084 discloses a
preparation of mother of pearl associated to Keratine ; this preparation is obtained by
digesting with lactic acid, powdered mother of pearl, which destroys the mineral
10 components of the mother of pearl.

The reference US A 4.393.045 (E.G. Henderson and al.) has as a subject matter,
components of the mother of pearl having curative effects by applying the
preparation obtained having contacted the shell of a mollusc containing the nacreous
15 part, with a fruit containing citric acid. Only the organic part of the mother of pearl is
kept.

The reference EP A 0.393.267 (M.Kato and al.) discloses a bioactive substance
obtained by heating mother of pearl for several hours at very high temperature. It
20 seems that this heating activates a growth factor of vegetables und animals.

The reference WO A 95/30426 (Camprasse and al.) pertains to a product for
regenerating and for cutaneous healing obtained from a grinding of the nacreous
shell together with a lyophilisate of animal cells which have been treated by
25 enzymatic and thermic ways.

The reference DE A 3437184 (E.I Wan and al.) relates to an agent for treating burns,
based on a mixture of ground shells of mussels and snails. These shells have been
previously washed with water and dried.

The french patent FR A 2.715.568 (J.Virassamy) claims the use of powdered mother
of pearl for the bone reconstruction.

Mother of pearl is known since a longtime for its properties for regenerating the bones.

The applicant has stated that mother of pearl contained in its cristalline structure substances able to activate animal cells and mainly to allow the reparation of the shells of conchifers when they are damaged.

Some substances contained in the biomineral which is mother of pearl, are of proteic nature and will respond to the antibodies directed against human and rats collagen of type I and III. It exists then an analogy between the skin and the shell of conchifers. On the basis of this observation, the applicant has studied and verified that mother of pearl contains specific substances which could intervene in the proceedings of restructuration, of stimulation etc... of the skin.

The need to provide to the skin a stimulated or rejuvenated appearance, until yet utilizes cares which eliminate the superficial corneous layer in order to let appear the underlying cellular layers. It is thus that it will be given to the skin a rejuvenated appearance but with three consequences :

- the skin is slimmer due to a thinning of the epidermis, while the latter has a thickness which goes decreasing with the age.
- the number of the cells of the epidermis decreases, thus dipping into the capital of cell replication.
- the apoptosed or dead cells, will be eliminated from the superficial part of the epidermis, eliminating the protective film of stratum corneum and more particularly of stratum disjonctum, sensibilizing the skin to external aggressions.

Other methods of care call on some strictly visual aspect such as the layer of a lipophilic or siliconated film which makes tight proof the external stratas and thus avoid their dehydration. Finally the changes in the colour of the skin, insure a more warm (tanning) or juvenile (whitening) appearance, when often admixing solar protective (organic or mineral screens) or stimulants of natural or artificial tannings (dihydroxyacetone or DHA). These cares do not contributed in any manner to improve the native quality of the skin since they are in fact merely make-ups.

Therefore this invention trends to solve the technical problem until yet unsolved to present at the surface of the skin, a neoformed structure which endows it with a young and dynamic look without eliminating the cells.

- 5 The applicant studied the consequences of the use of mother of pearl on other types of cells such as fibroblasts, lymphocytes, keratinocytes and endothelial cells.

10 The observation of the cellular behaviours allowed to state the occurrence of novel properties, undisclosed until yet, and which allow to use mother of pearl for these new uses.

It has been found that powdered mother of pearl induces in an unexpected manner an increased synthesis of cytokeratines.

- 15 The structure present at the cutaneous surface has to be a substance which is natively produced by the epithelial cells such as the keratinocytes, which will contribute to the physiological thickening of epidermis.

20 The solution which has been retained, has been to choice not to scrap the skin to carry to the surface novel cell structures but to make them producing to the keratinocytes, neoformed substances -the cytokeratines- which are normally generated by these cells when they are young or active.

25 These proteic substances contribute in this way to the thickening of epidermis by improving the cytoplasmic quality of the keratinocytes as well as the corneocytes and consequently, to the passive resistance to the external aggressions. Moreover the rebuilding of the epithelial stratas, improve the mass of epidermis thus constituting a reserve of messengers and contributing to its active resistance to the external aggressions.

30 The most superficial part of the skin is constituted by unnucleated cells, the layers of which may be reflecting the shell of the bivalves.

They are then good grounds to think that the restauration proceeding of this structure (made of a matter without nuclear and genetic material such as it is the shell) may be mediated by the same signal of restauration. It remains however a difficult problem to solve as a result that the shell of the bivalves lie soaking in water and that the relargued substances from the shell on the occasion of a damage, have to respond to a very precise specificity of action.

From this fact the studies conducted by the applicant came up against unnumerous difficulties to make this activity expressed and to evidence it.

The analysis is of the proteins after usual grinding of the mother of pearl show that it may contain 1,6 mg proteinic substances per gramm. It has been stated that these substances do not show depending on the dilution- the same activity (alkaline phosphatase) on a culture of osteoblasts. Moreover it has been unexpectedly noted that when diluting the powdered mother of pearl with another powder essentially made of aragonite (mineral constituent of mother of pearl), the response of the cells to the presence of biocrystals of mother of pearl, will be expanded. Therefore the further described studies have been performed with a preparation made of aragonite and mother of pearl. According to the desired effect, it may be possible to use dilutions containing variable percentage of mother of pearl in the whole mixture.

The present invention more specifically relates to the use of a powder made of a mixture of mother of pearl and optionally aragonite, in a finely powdery form and admixed with suitable diluents to achieve pharmaceutical and/or cosmetological compositions, intended to improve the synthesis of new cytokeratinines.

The invention still relates to the use of a powder made of mother of pearl and optionally aragonite, with a view of its application in cell cultures medium in order to improve the synthesis of new cytokeratinines.

The very significant synthesis of new cytokeratinines insure the improvement of the qualities and appearance of the skin, and mainly allows to insure the proceeding of restauring the structure of the skin, to exert a protective effect on the skin and to give it an active resistance under external aggressive conditions, such as sun, cold,

protracted exposition to water etc..., to insure the improvement of the mechanical properties of the skin, to endow it with a young and dynamic look, thanks a neoformed structure at its surface.

5 The powder made of mother of pearl and aragonite is composed in such a manner that the mixture contains mother of pearl in a proportion ranging from 0.1 to 98 % in the whole mixture and preferably in a proportion ranging from 5 to 20 % in the whole mixture.

10 The studies herein after disclosed have been preferably performed with a composition containing 10 % of mother of pearl and 90 % aragonite.

The present invention also relates to pharmaceutical and/or cosmetological compositions insuring an improvement in the qualities and appearance of the skin
15 characterized in that they are constituted of mother of pearl, optionally admixed with aragonite, in admixture or compositions with an inert, non toxic carrier or diluent.

If mother of pearl allows to develop a dermo-protective effect, it is in the synthesis of constituent of stratum corneum that most of the alterations have been found. The
20 corneous layer being constituted by the condensation of cytokeratin, the applicant directed its analysis towards this material. The foreseeable consequences essentially are in the quality of the response of the cells to the stress of an aggression [synthesis of interleukin 1 (IL - 1)]. Moreover the skin is submitted to a significant photoluminescent activity to what it responds by the synthesis of a photoprotective agent, melanine. Accordingly it is observed a decrease in the synthesis of melanine
25 or a better distribution of that, in the keratinocytes.

Role played by mother of pearl in the synthesis of cytokeratin :

30 Cytokeratin distinguish themselves by their molecular weight and for most of them, it exists a specific antibody.

In order to study the whole phenomenon, it has been used an antibody directed against the whole of cytokeratin. Accordingly it is called as anti-pancytkeratin.

It has thus be achieved a cell culture of keratinocytes obtained from surgical removals of skin. The skin is treated by enzymatic digestion and incubated in a specific medium (Mac Koy's modified or of the KBM type - ie Keratinocytes Basal Medium). This medium does not contain any serum.

10^5 cells are incubated for 24 hours in the presence of 50 mg of mother of pearl per ml medium, free of fetal calf serum or similar proteic serum. The cells are rinsed, the supernatant is removed, the cells are separated from the mother of pearl, lysed then a « Western Blot » is performed on the cell fraction. The technique of « Western Blot » is an analytical proceeding based on the separation of the proteins as a function of their molecular weight by electrophoresis followed by an immunological detection . An antibody of a second layer grafted to an enzyme reveals the first antibody when contacted with a chromogenic substrate. In order to obtain results which could be interpreted, several dilutions are tested and the quantification of the substance to be dosed - herein the pancytokeratines - is performed by mere absorption spectrophotometry or by image analysis of the revelation plate. Under these conditions it has been evidenced that the basis hypothesis was verified and that mother of pearl was able to double the amount of cytokeratines synthetized by the keratinocytes. The results are studied taking account of the total proteic synthesis on several dilutions since on one part the number of cells is not exactly constant, and on the other part their activity is not necessarily coordonated.

Total proteins (in μ l)

tested volume in μ l	10	20	40
controls (without mother of pearl)	8	9,3	18,3
with mother of pearl	3	7,5	12

Signal cytokeratine

tested volume (in μ l)	10	20	40
controls (without mother of pearl)	62,8	70,9	178
mother of pearl	18,4	108	275

Signal cytokeratine (X 10) / total proteins

tested volume (in μl)	10	20	40
controls (without mother of pearl)	78	76	97
5 mother of pearl	61	130	230

Ratio mother of pearl / controls

tested volume in μl	10	20	40
ratio	0,78	1,89	2,36

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It is then noted that mother of pearl increases the synthesis of the pancytokeratines of a multiplication factor in the order of 2.

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Figure 1 is a graphic representation of the synthesis evolution of the pancytokeratines as a function of the tested volume. It has been shown therein that the ratio cytokeratine signal $10/\mu\text{g}$ of total proteins for the controls and for the preparations based on mother of pearl. It will be noted that analysis are more accurate and sensible when electrophoresis is performed with at least $20 \mu\text{l}$.

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When the cytokeratines synthesis improves the mechanical properties of the skin, it is possible to contemplate two types of response to the aggression : the responses to the aggressions of chemical or biological nature which gives a role to interleukin at first glance and the responses involving melanine.

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Role played by mother of pearl on the synthesis of interleukine 1 (IL - 1)

Figure 2 pictures the obtained results during the cell line culture HL 60 in the presence of mother of pearl.

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It has been noted a significant decrease of the secretion of IL - 1 when the cells are cultivated in the presence of mother of pearl.

Protective reactions involving melanine

The melanocytes culture (line of ECACC) line G 361 does in the presence of mother of pearl, modify only weakly the secretion of melanine

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				Mean
Controls	0,150	0,149	0,148	0,149

Mother of pearl 0,5 %	0,150	0,132	0,140	0,141
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Mother of pearl 1 %	0,139	0,137	0,143	0,141
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WHAT IS CLAIMED IS

1. Use of a powder made of mother of pearl and optionally aragonite, in view of achieving pharmaceutical and/or cosmetological compositions intended to improve the synthesis of new cytokeratinines.
2. Use of a powder made of mother of pearl and optionally aragonite, in view of its application in the cell culture media in order to improve the synthesis of new cytokeratinines.
3. Use of a powder made of mother of pearl and optionally aragonite according to claims 1 and 2, wherein the powder is composed in such a manner that the mixture contains mother of pearl in a proportion ranging from 0.1 to 98 % in the whole mixture.
4. Use of a powder made of mother of pearl and optionally aragonite according to claim 3, wherein the powder is so constituted that the mixture contains mother of pearl in a proportion ranging from 5 to 20 % in the whole mixture and preferably in a proportion of 10 % in the whole mixture.
5. Use of a powder made of mother of pearl and optionally aragonite according to claim 2, in a finely powdery form and admixed to suitable diluents, in view of its application in culture of keratinocytes.
6. Use of a powder made of mother of pearl and optionally aragonite, according to claim 1, in a finely powdery form, and admixed to suitable diluents, in view of the achievement of pharmaceutical compositions insuring an improvement of the qualities of the skin.
7. Use of a powder made of mother of pearl and optionally aragonite, according to claims 1 and 16, in view of the achievement of pharmaceutical compositions insuring restauration proceeding of the structure of the skin.

8. Use of a powder made of mother of pearl and optionally aragonite, according to claims 1 and 6, in view of the achievement of pharmaceutical compositions, fulfilling a protective role on the skin and endowing it with an active resistance under external aggressive conditions.

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9. Use of a powder made of mother of pearl and optionally aragonite, according to claim 1, in a finely powdering form, and admixed to suitable diluents, in view of the achievement of cosmetological compositions insuring an improvement in the appearance of the skin.

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10. Use of a powder made of mother of pearl and optionally aragonite, according to claims 1 and 9, in view of achieving cosmetological compositions insuring an improvement in the mechanical properties of the skin.

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11. Use of a powder made of mother of pearl and optionally aragonite, according to at least one of claims 1, 6 and 9, in view of the achievement of compositions endowing the skin with a young and dynamic look, thanks a neoformed structure on its surface.

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12. Pharmaceutical and/or cosmetological compositions insuring an improvement of the qualities and appearance of the skin, characterized in that they are made up of mother of pearl, optionally admixed with aragonite, in admixture or conjunction with an inert, non toxic, powdery carrier or diluent.