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(54) **Title**: ANTI-INFLAMMATORY EFFECT OF MICROFIBRILLATED CELLULOSE

(57) **Abstract**: The invention relates to use of microfibrillated cellulose as an anti-inflammatory agent for treatment of skin disorders.

Anti-inflammatory effect of microfibrillated cellulose

Field of the Invention

The present invention relates to the external use of a microfibrillated cellulose, particularly for the care and treatment of skin.

Background of the Invention

Cellulose is an organic compound, polysaccharide, consisting of a linear chain of several D-glucose units linked through β(1,4) glycosidic bonds. Many properties of cellulose depend on its chain length or degree of polymerization, the number of glucose units that make up one polymer molecule. Cellulose is mainly obtained from wood pulp and cotton but may be also secreted by some species of bacteria to form biofilms.

Microfibrillated cellulose (MFC) or nanocellulose is a material composed of cellulose fibrils, i.e. basic structural components of wood with a high length to diameter ratio (aspect ratio). The microfibrillated cellulose can be prepared from any cellulose containing material including wood (pulp fibres).

Generally, the cellulose fibrils are used in cosmetic compositions e.g. as a composite coating agent for hair, eyelashes or nails. Microfibrils are used, for example to improve the lengthening of the eyelashes. Microfibrillated cellulose may also be used as a binder or filler for solid dosage forms and as a bodying agent or a drug carrier in topical formulations or dermatological products. Additionally, cellulose fibrils may be used as a stabilizer for surfactant free oil-in-water emulsions.

European patent EP 820267 and the corresponding US patent 6001338 disclose the use of an aqueous solution or dispersion of a film-forming polymer and an aqueous suspension of natural cellulose microfibrils as a composite coating agent for hair, eyelashes, eyebrows and nails. The aim is to use cellulose microfibrils in cosmetics to improve the physical properties of cosmetic compositions.

Publication US 2002/192251 deals with cosmetic compositions, especially mascara, intended for human keratinous substances such as the skin, nails or keratinous fibers (eyelashes, eyebrows and hair) and containing a mixture of cellulose nanofibrils or microfibrils with second fiber and wax.

Up to now, in connection with human body, microfibrillated cellulose has only been proposed for enhancing properties of cosmetic formulations.

The skin is made up of layers of epidermis, dermis and subcutis. The epidermis is the outer layer of the skin and consists of sublayers: stratum corneum, granulosum, spinosum and basal layer. Keratinocytes are the main type of cells which make up the epidermis. The dermis is the middle layer of the skin and is held together by collagen made by fibroblasts. The subcutis or a subcutaneous layer is the deepest layer of the skin and consists of a network of collagen and fat cells.

There are a number of causes for skin inflammation (dermatitis), e.g. irritants such as some chemicals, overexposure to sun; infections; chronic or acute inflammatory conditions.

There are chronic and inflammatory skin conditions and disorders like atopic dermatitis, a type of eczema, for which there is currently no cure available. For controlling the symptoms, various treatments may be used, such as topical treatments focusing on reducing both dryness and inflammation of the skin, e.g. moisturizers. However, the moisturizers should not have any ingredients that may further irritate or aggravate the skin condition. Also topical corticosteroid products may be used. However, using steroids involves disadvantages, such as thinning of the skin. High potency steroids should also be avoided on the face or other areas where the skin is naturally thin. If there are also infections involved, antibiotics may be employed.

There remains a need for improved and efficient products comprising dermatological compositions with an anti-inflammation activity which allow local treatment of different chronic skin disorders, such as atopic dermatitis and psoriasis, while at same time avoiding the side effects and disadvantages of conventional treatments.

Summary of the Invention

It is an object to provide a use of a natural based ingredient, which has an anti-inflammatory effect, for topical care and/or treatment of skin inflammation conditions and disorders.

According to a first aspect of the present invention there is provided microfibrillated cellulose for use as an anti-inflammatory agent for the treatment of skin inflammation. Also a method of use of microfibrillated cellulose as an anti-inflammatory agent for the treatment of skin inflammation is provided.

According to a second aspect of the present invention there is provided microfibrillated cellulose for use in preventing inflammation induced keratinocyte de-differentiation. Also a method of use of microfibrillated cellulose in preventing inflammation induced keratinocyte de-differentiation is provided.

According to a third aspect of the present invention there is provided microfibrillated cellulose for use in the treatment of atopic dermatitis. Also a method of use of microfibrillated cellulose in the treatment of atopic dermatitis is provided.

According to a fourth aspect of the present invention there is provided microfibrillated cellulose for use in the treatment of psoriasis. Also a method of use of microfibrillated cellulose in the treatment of psoriasis is provided.

According to a fifth aspect of the present invention there is provided microfibrillated cellulose for use in the treatment of skin burns. Also a method of use of microfibrillated cellulose in the treatment of skin burns is provided.

Further embodiments of the invention are presented in the dependent claims.

The microfibrillated cellulose is included in an ointment, a serum, an aqueous gel, a foam, a cream, an emulsion of aqueous and fatty phase, a lotion, or a paste.

Description of the Drawings

In the following, the invention will be discussed with reference to accompanying figures, in which

Fig. 1 shows test results of anti-inflammatory effects of microfibrillated cellulose on the gene expression profile of normal human epidermal keratinocytes,

Fig.2 shows test results of the effects of microfibrillated cellulose on skin moisture and barrier function on the gene expression profile of normal human epidermal keratinocytes.

Detailed Description of the Invention

According to the present invention there is provided a method of use and use of a natural based ingredient for the treatment of keratinous material providing beneficial skin effects such as an activity against skin inflammation conditions. The ingredient may be multifunctional and provide also other beneficial effects.

According to the invention the natural based ingredient is microfibrillated cellulose (MFC). There are several widely used synonyms for microfibrillated cellulose. For example: nanofibrillated cellulose, nanocellulose, microfibrillar cellulose, nanofibrillar cellulose, cellulose nanofiber, nano-scale fibrillated cellulose, or cellulose microfibrils. Microfibrillated cellulose described in this application is not the same material as the so-called cellulose whiskers, which are also known as: cellulose nanowhiskers, cellulose nanocrystals, cellulose nanorods, rod-like cellulose microcrystals or cellulose nanowires. In some cases, similar terminology is used for both materials, for example by Kuthcarlapati et al. (Metals Materials and Processes 20(3):307-314, 2008) where the material investigated was called "cellulose nanofibers" although cellulose nanowhiskers were clearly referred to. Typically these materials do not have amorphous segments along the fibrillar structure as microfibrillar cellulose, which leads to a more rigid structure.

According to the invention, microfibrillated cellulose, MFC, is used as an anti-inflammatory agent for the treatment of skin disorders, such as skin

inflammation conditions. When the MFC is used as anti-inflammatory agent, it provides anti-inflammatory effects, i.e. calming and soothing effects, but it may also participate in other useful actions such as skin moisturizing. It may also be used for treatments of acute disorders to facilitate the symptoms of irritated skin and/or participate in or promote a healing process of the skin. The MFC may be used for example to help manage discomfort such as itching and rash of sun burns.

The microfibrillated cellulose, MFC, may be used as an active agent and/or as an ingredient in cosmetic and/or dermatological products. These products are preferably used for external and topical application onto keratinous materials such as the skin, the scalp, the lips and the eyelids.

The cosmetic and/or dermatological product is preferably semi-solid at room temperature and is easily absorbed into the outer layer of the skin, i.e. the stratum corneum.

The product comprising MFC may be in all the usual forms suitable for dermatological or cosmetic indication and for administration such as topical application, for example in the form of an ointment, a serum, an aqueous gel, a foam, a cream, an emulsion of aqueous and fatty phase, a lotion, or a paste. Additionally, MFC may be included in wound healing products, such as films, membranes or fabrics.

If necessary, the cosmetic or dermatological product may further include various components or ingredients which are compatible with the skin. The ingredients may be chosen according to the form of administration selected and may contain, but are not limited to, excipients and carriers such as gelling agents, stabilizers, surfactants, emulsifiers, thickeners, vitamins, oils, humectants, ultraviolet absorbers, preservatives, water, alcohol, colouring materials.

According to the invention MFC acts as an anti-inflammatory agent for the treatment of skin inflammation. The concentrations of micro fibrils (an active principle or dry matter of MFC), acting as an anti-inflammatory agent when incorporated in the product for the treatment of skin, may range from 0.00010 % to 1.3% or even 4%.

MFC may have different physical forms. It may be in the form of an aqueous dispersion, a film-like structure such as wet or dry membrane, non-woven or woven fabric.

In addition to its anti-inflammatory property, MFC may also provide a pleasant texture and act as a bodying agent (thickener) for the product. The thickener provides a suitable viscosity for the product. The suitable viscosity is important allowing the product to remain in place upon application to the skin. If necessary, the product may also include other thickeners in addition to MFC. MFC may also act as a stabilizer. It may also be used for emulsification of the product.

According to the invention, the cellulose microfibrils are extracted from a cellulose raw material source such as wood. The wood material can be obtained from softwood trees, such as spruce, pine, fir, larch, douglas-fir or hemlock, or from hardwood trees, such as birch, aspen, poplar, alder, eucalyptus or acacia, or from a mixture of softwoods and hardwoods. Alternatively, the micro fibrils may originate from a non-wood material such as cotton or bacteria. The non-wood material can be obtained from agricultural residues, grasses or other plant substances such as straw, leaves, bark, seeds, hulls, flowers, vegetables or fruits from cotton, corn, wheat, oat, rye, barley, rice, flax, hemp, manila hemp, sisal hemp, jute, ramie, kenaf, bagasse, bamboo or reed.

The term “microfibrillated cellulose, (MFC)” refers to a collection of isolated cellulose microfibrils or microfibril bundles derived from cellulose raw material. The cellulose fibrils may be isolated from wood based fibres through high-pressure, high temperature and high velocity impact homogenization. The homogenization process is used to delaminate or disintegrate the cell walls of the fibres and to liberate their sub-structural fibrils and micro fibrils. Enzymatic and/or mechanical pre-treatments of wood fibres may also be used. MFC is in native form, which has not undergone any chemical modification. The invention also encompasses chemical modifications of the native cellulose.

Cellulose microfibrils typically have a high aspect ratio: the length might exceed one micrometer while the number-average diameter is typically below 200 nm. The diameter of micro fibril bundles can also be larger but is generally less than

1 µm. The smallest micro fibrils are similar to the so called elementary fibrils, which are typically 2-20 nm in diameter. However, the present invention is not intended to be limited to these values. The dimensions of the fibrils or fibril bundles are dependent on the raw material and the disintegration method. The micro fibrillated cellulose may also contain some hemicelluloses; the amount may be dependent on the plant source.

The MFC is not cytotoxic to fibroblasts or to keratinocytes i.e. it does not cause mortality of the cells.

According to the invention, the MFC has an anti-inflammatory effect. The MFC has been found to efficiently reduce the inflammation of the skin. The microfibrillated cellulose limits the side-effects of inflammation in keratinocytes by preventing inflammation-induced keratinocyte dedifferentiation, for example in the case of an atopic dermatitis skin condition. MFC may also be effective for the treatment of other skin inflammation conditions such as psoriasis and skin burns. Skin burns may be a type of injury to flesh and may be caused e.g. by heat, chemicals, light, radiation, or friction.

Examples

The following examples serve to illustrate the invention, without, however, being limiting in nature.

Example 1.

Microfibrillated cellulose (MFC) was evaluated for its effects on skin using different skin biology-related *in vitro* models. The skin anti-inflammatory effects and moisture and barrier function were evaluated. For *in vitro* tests in normal human epidermal keratinocytes (NHEK), the test concentration of MFC was 0.01% (0.00016% active principle). Cell type of normal human epidermal keratinocytes (NHEK) in culture conditions of 37°C, 5% CO_2 was used. Culture medium of NHEK consists of keratinosyte-serum free medium (SFM) supplemented with epidermal growth factor (EGF) 0.25 ng/ml, pituitary extract (PE) 25 µg/ml and gentamycin 25 µg/ml. The test and the results are described in more detail in Examples 1.1 -1.2.

Example 1.1. NHEK Inflammation

The anti-inflammatory effects of the MFC compound (i.e. soothing, calming properties) were evaluated on the gene expression profile of normal human epidermal keratinocytes (NEHK) stimulated with an association of poly(I:C) and inflammatory cytokines. Inflammatory cytokines are natural small protein molecules involved in a variety of immunological and inflammatory phenomen. Effects of the compound were evaluated on gene expression profile using RT-qPCR technology on mRNA extracted from cell layers. Extracted mRNAs were analyzed on a dedicated PCR array containing 32 target genes, including 2 housekeeping genes, selected for their importance in skin inflammation and their involvement in atopic dermatitis disease.

Keratinocytes were seeded in a culture medium and cultured for 24 hours. The medium was then replaced by an assay medium. The assay medium consisted of a keratinocyte-serum free medium (SFM) supplemented with gentamycin 25 µg/ml. After an adaptation time, the medium was replaced by assay medium containing or not (in the case of control) the test compound of MFC or the reference of NFκB inhibitor III at 5 µM, and the cells were pre-incubated for 24 hours. The assay medium was then renewed and the cells were treated identically treated with test compound or the references in the presence or not (in the case of non-stimulated control) of the association of Poly(I:C) and the Th2 type cytokine mix and the cells were incubated for 24 hours. Three parallel experiments were performed.

At the end of the incubation, supernatants were discarded and the cell layers were washed using phosphate buffered saline (PBS), and covered with TRI-Reagent®. The plates were immediately frozen at -80 °C.

The test results are provided in Fig. 1. Atypical melting curves are indicated by the abbreviation nd. Down-regulated genes (arbitrary selection for inhibition: % less than 65) are indicated by a), and up-regulated genes (arbitrary selection for stimulation: % more than 150) are indicated by b).

Treatment of NEHK with the association of poly(I:C) and cytokines clearly induced an inflammatory profile and induced a characteristic gene expression modulation of many markers involved in atopic dermatitis pathology. The

treatment induced a strong up-regulation of all inflammatory markers analyzed in this PCR array, which was observed as the simultaneous increase of cytokine markers (IL1A, IL18, IFNB1, IL4R, TSLP), chemokine markers (CCL3, CCL5, CCL7, CCL20, CCL22, CCL27 and IL8) and innate immunity markers (TLR3, S100A7, S100A11, RNASE7). Inflammation of NHEK also resulted in a decreased expression of keratinocyte differentiation markers (FLG, IVL, KRT10, LASS6). The reference NFκB inhibitor III (5 μM) strongly inhibited the inflammation induced by the association of poly(I:C) and cytokines. Except for the keratinocyte differentiation markers, almost all the effects of pro-inflammatory association (increased expression of cytokine markers, chemokine markers and innate immunity markers) were reversed by the reference. These results were expected and validated the assay.

The main effects which can be expected from the test compound of MFC in this model will be [1)] an anti-inflammatory effect: decreased expression of cytokine; chemokine and innate immunity related markers, and [2)] a pro-differentiating effect. The pro-differentiating effect is expected since the inflammation induced in this model also results in keratinocyte dedifferentiation, which can be seen as a side-effect and which will in *in vivo* models affect skin's aspect and quality.

Based on the results shown in Fig. 1, the compound of micro fibrillated cellulose (MFC) was observed to reverse the effects of inflammation on keratinocyte differentiation even though it does not decrease the expression of inflammatory markers. All the differentiation markers analyzed (IVL, FLG, LOR, KRT10, LASS6) were observed to increase in the presence of MFC. Expression of several inflammatory markers, such as S100A7, CAMP, IFNA2, AFNB1, CCL20 and CCL22, were also found to increase. Expression of oxidative-stress related marker HMOX1 was also found to increase due to the presence of MFC.

MFC was shown as a compound limiting keratinocyte dedifferentiation in an inflammatory model mainly relevant for atopic dermatitis.

Based on the results it is found that MFC can be used to treat atopic dermatitis. In addition, it may be used for the treatment of other forms of skin inflammation e.g. psoriasis, skin burn etc.

Example 1.2. NHEK Differentiation

The effects on skin moisture and barrier function the compound of MFC was evaluated on the gene expression profile of normal human epidermal keratinocytes (NEHK) in basal conditions. Effects of the compound on keratinocytes were evaluated using RT-qPCR technology on mRNA extracted from cell layers. Extracted mRNAs were analyzed on a dedicated PCR array containing 64 target genes, including 3 housekeeping genes, selected for their importance in keratinocyte differentiation, growth and epidermal renewal.

Keratinocytes were seeded in culture medium and cultured for 48 hours. The medium was then replaced by an assay medium. The assay medium consisted of keratinocyte-serum free medium (SFM) supplemented with gentamycin 25 µg/ml. After an adaptation time, the medium was replaced by assay medium containing or not (in the case of control) the test compound or the reference of calcium chloride at 1,5 mM and the cells were incubated for 24 hours. Three parallel experiments were performed.

At the end of the incubation, supernatants were discarded and the cell layers were washed using phosphate buffered saline (PBS), and covered with TRI-Reagent®. The plates were immediately frozen at -80 °C.

The test results are provided in Fig. 2. Atypical melting curves are indicated by the abbreviation nd. Down-regulated genes (arbitrary selection for inhibition: % less than 65) are indicated by a), and up-regulated genes (arbitrary selection for stimulation: % more than 150) are indicated by b).

Based on the test results, MFC presented no pro-differentiating effect in basal conditions.

Claims:

1. Use of microfibrillated cellulose as an anti-inflammatory agent in skin conditions and disorders selected from eczema, psoriasis, irritated skin and inflammation induced keratinocyte de-differentiation.

2. The use according to claim 1, wherein the microfibrillated cellulose is included in an ointment, a serum, an aqueous gel, a foam, a cream, an emulsion of aqueous and fatty phase, a lotion, a paste, an aqueous dispersion, wet or dry membrane, non-woven or woven fabric.

3. The use according to claim 1, wherein the eczema is atopic dermatitis.

4. The use according to claim 1, wherein the microfibrillated cellulose is used in the prevention of inflammation induced keratinocyte de-differentiation.

5. A method of use of microfibrillated cellulose as an anti-inflammatory agent for the treatment of skin inflammation.

6. The method according to claim 5, wherein the microfibrillated cellulose is included in an ointment, a serum, an aqueous gel, a foam, a cream, an emulsion of aqueous and fatty phase, a lotion, or a paste.

7. A method of use of microfibrillated cellulose in preventing inflammation induced keratinocyte de-differentiation.

8. The method according to claim 7, wherein the microfibrillated cellulose is included in an ointment, a serum, an aqueous gel, a foam, a cream, an emulsion of aqueous and fatty phase, a lotion, or a paste.

9. A method of use of microfibrillated cellulose in the treatment of atopic dermatitis.

10. The method according to claim 9, wherein the microfibrillated cellulose is included in an ointment, a serum, an aqueous gel, a foam, a cream, an emulsion of aqueous and fatty phase, a lotion, or a paste.

11. A method of use of microfibrillated cellulose in the treatment of psoriasis.

12. The method according to claim 11, wherein the microfibrillated cellulose is included in an ointment, a serum, an aqueous gel, a foam, a cream, an emulsion of aqueous and fatty phase, a lotion, or a paste.

PCR array **H-Atopic dermatitis 32**		**Stimulated control**	**NFκB inhibitor III 5 µm**		**Microfibrillated cellulose 0.01%**	
Gene name	**abbrev**	**Cycles**	**Cycles**	**% control (Average HK)**	**Cycles**	**% control (Average HK)**
Average Housekeeping	Avg HK			100		100
Ribosomal protein L13a	RPL13A	19,92 19,97	19,82 19,76	83	20,33 20,54	88
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	18,78 18,74	18,29 18,19	107	18,99 18,97	105
Antimicrobial peptide, innate immunity						
Toll-like receptor 3	TLR3	27,08 26,64	27,88 27,91	**a)** 36	26,86 26,82	123
S100 calcium binding protein A7	S100A7	26,72 26,64	32,68 32,22	**a)** 1	25,47 25,64	**b)** 268
S100 calcium binding protein A11	S100A11	19,14 19,50	19,54 19,67	**a)** 61	19,52 19,64	102
Ribonuclease, RNase A family, 7	RNASE7	24,32 24,31	26,46 26,55	**a)** 16	24,13 24,08	142
Cathelicidin antimicrobial peptide	CAMP	30,46 30,72	31,25 31,10	**a)** 50	30,08 30,11	**b)** 172
Interleukin family members						
Thymic stromal lymphopoietin	TSLP	34,28 34,22	38,52 38,52	**a)** 4	35,01 34,72	81
Interleukin 1, alpha	IL1A	19,81 19,82	20,61 21,01	**a)** 38	19,58 19,78	135
Interleukin 18 (interferon-gamma-inducing factor)	IL18	31,30 30,82	33,35 33,70	**a)** 13	31,11 30,75	134
Interferon, alpha 2	IFNA2	36,41 36,49	35,68 35,70	127	34,54 33,94	**b)** 580
Interferon, beta 1, fibroblast	IFNB1	27,47 27,43	29,86 29,32	**a)** 17	27,13 27,16	**b)** 152
Interleukin 4 receptor	IL4R	32,77 33,20	35,80 35,50	**a)** 12	33,71 33,51	79

Fig. 1

PCR array **H-Atopic dermatitis 32**		**Stimulated control**	**NFκB inhibitor III 5 µm**		**Microfibrillated cellulose 0.01%**	
Gene name	**abbrev**	**Cycles**	**Cycles**	**% control (Average HK)**	**Cycles**	**% control (Average HK)**
Chemokines						
Interleukin 8	IL8	24,01 24,60	27,47 27,11	**a)** 9	25,32 25,21	**a)** 62
Chemokine (C-C motif) ligand 3	CCL3	19,94 20,14	23,32 23,72	**a)** 7	19,85 20,08	129
Chemokine (C-C motif) ligand 5	CCL5	17,23 17,00	17,30 17,40	**a)** 63	17,07 17,19	121
Chemokine (C-C motif) ligand 7	CCL7	32,90 32,99	34,80 34,80	**a)** 21	33,39 32,97	105
Chemokine (C-C motif) ligand 13	CCL13	nd nd	nd nd	nd	nd nd	nd
Chemokine (C-C motif) ligand 20	CCL20	23,04 22,84	26,51 26,63	**a)** 6	21,91 22,12	**b)** 233
Chemokine (C-C motif) ligand 17	CCL17	nd nd	nd nd	nd	nd nd	nd
Chemokine (C-C motif) ligand 11	CCL11	nd nd	nd nd	nd	nd nd	nd
Chemokine (C-C motif) ligand 22	CCL22	28,51 28,66	26,21 26,00	**b)** 418	28,17 28,16	**b)** 164
Chemokine (C-C motif) ligand 27	CCL27	24,81 24,91	28,31 28,09	**a)** 7	24,65 24,72	139
Differentiation markers						
Involucrin	IVL	30,07 30,09	31,76 31,22	**a)** 29	29,26 28,84	**b)** 253
Filaggrin	FLG	31,45 32,04	31,72 32,10	66	30,32 29,87	**b)** 382
Loricrin	LOR	34,34 34,51	33,98 34,09	98	33,47 33,47	**b)** 238
Keratin 10	KRT10	33,12 33,03	31,57 31,10	**b)** 253	32,23 32,43	**b)** 206
LAG1 homolog, ceramide synthase 6	LASS6	29,57 29,27	29,06 28,84	103	28,87 28,64	**b)** 194
Cell-cell interactions						
Corneodesmosin	CDSN	24,21 24,12	27,46 27,30	**a)** 8	24,35 24,02	122
Transcriptional regulation						
Retinoic acid receptor responder (tazarotene induced) 3	RARRES3	24,30 24,08	25,32 24,93	**a)** 39	24,25 24,51	108
B-cell CLL/lymphoma 3	BCL3	29,72 29,28	28,78 28,70	125	28,62 28,94	**b)** 201
Oxidative and cellular stress response						
Heme oxygenase (decycling) 1	HMOX1	27,09 27,58	29,30 29,34	**a)** 19	26,58 26,83	**b)** 188

Fig. 1 (continued)

PCR array **H-Keratinocyte 64**		**Control**	**$CaCl_2$ at 1.5 mM**		**Microfibrillated**	
Gene name	**abbrev**	**Cycles**	**Cycles**	**% control (Average HK)**	**Cycles**	**% control (Average HK)**
Average Housekeeping	Avg HK			100		100
Ribosomal protein L13a	RPL13A	19,22 19,26	18,57 18,62	110	19,64 19,66	75
Actin, beta	ACTB	18,08 18,21	17,59 17,63	102	18,36 18,17	91
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	18,22 18,21	17,86 17,74	94	18,54 18,56	79
Differentiation markers						
Calmodulin-like 5	CALML5	33,67 33,91	30,84 30,83	**b)**542	33,62 33,43	119
Cornulin	CRNN	33,32 33,41	33,48 33,71	**a)** 60	33,63 33,75	79
Filaggrin	FLG	29,04 28,86	26,56 27,02	**b)** 317	28,99 28,75	105
Involucrin	IVL	28,35 28,61	25,53 25,57	**b)** 532	28,34 28,28	111
Keratin 1	KRT1	29,77 29,87	27,47 27,49	**b)** 355	29,87 29,76	100
Keratin 10	KRT10	31,56 31,84	30,97 30,66	130	31,95 31,84	86
Keratin 2	KRT2	34,57 34,20	33,67 33,68	114	34,41 34,01	112
Keratin 6A NM_005554	KRT6A	20,78 20,79	19,55 19,21	**b)**187	21,03 21,57	71
Loricrin	LOR	34,96 35,69	34,46 35,57	91	34,32 34,63	**b)**174
Peptidyl arginine deiminase, type I	PADI1	33,63 33,61	29,75 30,11	**b)** 912	33,50 32,60	**b)** 154
Small proline-rich protein 1A	SPRR1A	23,87 23,82	19,89 20,09	**b)**1017	24,06 23,91	90
Small proline-rich protein 1B (comifin)	SPRR1B	22,52 22,13	18,78 18,81	**b)** 803	22,22 22,14	109
Small proline-rich protein 2A	SPRR2A	31,82 31,93	26,89 26,80	**b)**2291	31,78 31,81	105
Transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase)	TGM1	24,52 24,25	20,36 20,29	**b)**1165	24,46 24,48	93
Lipide synthesis						
Acyl-CoA synthetase short-chain family member 2	ACSS2	23,25 23,27	22,66 22,63	107	23,58 23,07	96
Fatty acid binding protein 5 (psoriasis-associated)	FABP5	24,18 24,17	23,29 23,27	130	24,60 24,64	73
Fatty acid synthase	FASN	25,27 25,48	24,81 24,89	101	25,51 25,46	92
Glucosidase, beta; acid (includes glucosylceramidase)	GBA	25,24 25,15	24,31 24,38	126	25,58 25,62	75
Sphingomyelin phosphodiesterase 1, acid lysosomal	SMPD1	32,96 33,28	33,27 33,09	67	34,34 33,94	**a)** 49
Serine palmitoyltransferase, long chain base subunit 1	SPTLC1	23,68 23,72	23,02 23,04	112	24,07 24,11	76
Sulfotransferase family, cytosolic, 2B, member 1	SULT2B1	27,91 27,93	23,47 23,74	**b)**1402	27,96 27,80	102
UDP-glucose ceramide glucosyltransferase	UGCG	25,00 24,71	23,76 23,69	**b)** 153	25,11 25,47	74

Fig. 2

PCR array **H-Keratinocyte 64**		**Control**	**$CaCl_2$ at 1.5 mM**		**Microfibrillated**	
Gene name	**abbrev**	**Cycles**	**Cycles**	**% control (Average HK)**	**Cycles**	**% control (Average HK)**
Proliferation marker						
Keratin 19	KRT19	25,96 26,07	23,63 23,65	**b)** 364	26,09 26,11	93
Desquamation						
Kallikrein-related peptidase 5	KLK5	23,21 23,06	21,17 21,34	**b)** 258	22,84 22,95	117
Kallikrein-related peptidase 7	KLK7	24,37 24,30	20,30 20,27	**b)** 1162	24,71 24,74	76
Dermo-epidermal junction						
Collagen, type IV, alpha 1	COL4A1	26,34 26,45	26,48 26,54	**a)** 65	26,67 26,71	81
Collagen, type VII, alpha 1	COL7A1	25,52 25,52	25,94 26,03	**a)** 51	25,47 25,53	101
Laminin, gamma 2	LAMC2	22,87 22,76	22,34 22,67	87	22,66 22,77	106
Growth factors / Mitotic factors						
Epidermal growth factor (beta-urogastrone)	EGF	34,75 34,51	33,69 33,95	123	34,91 34,34	101
Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	EGFR	22,30 22,30	22,37 22,35	67	22,68 22,58	79
Hyaluronan synthase 3	HAS3	31,21 30,66	31,44 31,00	**a)** 57	30,85 30,67	110
Heparin-binding EGF-like growth factor	HBEGF	25,92 26,03	26,01 26,00	69	26,36 26,25	79
Transforming growth factor, beta 1	TGFB1	26,64 26,54	26,47 26,36	79	26,44 26,88	96
Water, glycerol transport						
Aquaporin 3 (Gill blood group)	AQP3	22,77 22,93	21,49 21,48	**b)** 180	22,93 23,13	88
Apoptosis						
Caspase 14, apoptosis-related cysteine peptidase	CASP14	27,75 27,94	26,69 26,81	150	27,85 27,79	101
Inflammation						
Chemokine (C-X-C motif) ligand 5	CXCL5	32,05 32,23	31,77 31,92	86	32,66 33,08	**a)** 60
Interleukin 1, alpha	IL1A	22,84 22,75	22,17 22,31	103	23,08 22,91	86
Interleukin 1 receptor antagonist	IL1RN	23,04 23,05	20,79 20,81	**b)** 333	23,44 23,34	78
Interleukin 8	IL8	nd nd	nd nd	nd	nd nd	nd

Fig. 2 (continued)

PCR array **H-Keratinocyte 64**		**Control**	**$CaCl_2$ at 1.5 mM**		**Microfibrillated**	
Gene name	**abbrev**	**Cycles**	**Cycles**	**% control (Average HK)**	**Cycles**	**% control (Average HK)**
Antimicrobial peptide, innate immunity						
Cathelicidin antimicrobial peptide	CAMP	30,76 30,84	30,82 30,88	68	30,26 30,66	127
Defensin, beta 4	DEFB4	34,21 33,92	32,15 31,78	**b)** 302	33,12 33,74	**b)** 157
Peptidase inhibitor 3, skin-derived	PI3	26,61 26,69	22,59 22,63	**b)** 1154	25,97 26,58	131
Ribonuclease, RNase A family, 7	RNASE7	25,67 25,71	25,53 25,63	76	26,48 26,45	**a)** 58
S100 calcium binding protein A7	S100A7	31,58 31,73	29,08 29,52	**b)** 363	32,07 32,89	**a)** 58
cell-cell interactions, epidermal cohesion						
Corneodesmosin	CDSN	29,79 30,26	26,47 26,54	**b)** 794	30,63 30,48	68
Claudin 1	CLDN1	21,91 21,92	20,68 20,77	**b)** 160	21,90 22,04	96
Catenin (cadherin-associated protein), alpha 1, 102kDa	CTNNA1	26,05 26,16	25,29 25,62	111	25,97 26,01	107
Desmoglein 1	DSG1	29,79 29,87	26,95 26,89	**b)** 527	29,87 30,06	90
Desmoplakin	DSP	20,37 20,47	19,20 19,27	**b)** 159	20,83 20,71	78
Epiplakin 1	EPPK1	30,33 29,84	30,51 30,63	**a)** 49	31,09 31,33	**a)** 45
Envoplakin	EVPL	29,18 28,92	29,29 29,05	**a)** 65	30,28 30,17	**a)** 44
Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	ITGA2	25,79 25,67	25,11 25,05	110	26,15 26,12	75
Secreted protein, acidic, cysteine-rich (osteonectin)	SPARC	21,71 21,80	21,80 21,69	71	22,13 22,15	76
Dystonin	DST	20,21 20,40	21,01 21,17	**a)** 41	20,90 20,90	66
CD44 molecule (Indian blood group)	CD44	22,66 22,60	22,20 22,07	99	22,93 22,77	85
Syndecan 1	SDC1	21,79 21,68	20,48 20,51	**b)** 166	21,80 21,80	95
Extracellular matrix degradation, regeneration-related markers						
Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	MMP3	31,87 31,89	33,87 33,97	**a)** 17	32,03 32,61	75
Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	MMP9	27,94 28,15	27,48 27,30	110	28,34 28,19	85
Stratifin	SFN	21,68 21,34	21,33 21,02	88	21,92 21,97	73
Oxidative and cellular stress response						
Heme oxygenase (decycling) 1	HMOX1	30,45 30,43	28,70 28,86	**b)** 222	30,09 30,44	113
Heat shock 27kDa protein 1	HSPB1	21,29 21,28	21,16 21,27	74	22,00 21,97	**a)** 61

Fig. 2 (continued)

INTERNATIONAL SEARCH REPORT

International application No
PCT/FI2012/050129

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K8/73 A61K9/00 A61K9/16 A61K9/20 A61L15/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61L A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	US 2010/254961 A1 (NISHIO TOSHIHIKO [JP] ET AL) 7 October 2010 (2010-10-07) examples 3, 13, 16, 19, 20,22,24,25,29, 47-52, 77-82, 84,86	1-12
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2012	26/07/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kling, Isabelle

INTERNATIONAL SEARCH REPORT

International application No
PCT/FI2012/050129

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Form PCT/ISA/210 (continuation of second sheet) (April 2005)

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