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(54) Title: PEPTIDE-IONIC LIQUID CONJUGATE FOR THE PREVENTION AND/OR TREATMENT OF SKIN DISORDERS

(57) Abstract: The present application relates for a peptide-ionic liquid conjugate for the prevention and/or treatment of skin disorders. The peptide- ionic liquid conjugate comprises a cosmeceutical peptide comprising up to 10 amino acids and at least one ionic liquid comprising saturated or unsaturated hydrocarbon chain substituents from C1 to C18. The peptide-ionic liquid conjugates herein disclosed are suitable against skin disorders, such as, e.g., melanoma, non-melanoma skin cancers, diabetic foot ulcers, venous leg ulcers, pressure ulcers, acne, candidiasis, cellulitis, dermatophytoses, erysipelas, folliculitis, impetigo, psoriasis, rosacea, or eczema (atopic dermatitis), since they present potent activity against either antibiotic-susceptible strains or multidrug resistant clinical isolates of both Gram-positive and Gram-negative, a bactericidal type of action, anti-inflammatory and immunomodulatory effects, and collagenesis-inducing effects. The present application further discloses a topical composition comprising the peptide-ionic liquid conjugates and uses thereof.

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DESCRIPTION

"PEPTIDE-IONIC LIQUID CONJUGATE FOR THE PREVENTION AND/OR TREATMENT OF SKIN DISORDERS"

Technical field

This application relates to a peptide-ionic liquid (PIL) conjugate for the prevention and/or treatment of skin disorders, a topical composition comprising the same and uses thereof.

Background art

Ionic Liquids (ILs), though mostly known for their potential role as "green solvents", are becoming increasingly attractive as easily customizable and tunable organic salts for diverse specific purposes (task-specific ILs). There are infinite possibilities when combining organic cations with organic or inorganic anions, enabling production of ILs with diverse structural, physical and chemical properties [1] that can be adapted to the demands of areas as diverse as material sciences [2], biotechnology [3], or biomedicine [4]. Moreover, by making use of bioactive ions, ILs displaying relevant biological activities [5] can be produced, for instance, as anticancer [6], antimalarial [7], and antimicrobial agents [8]. ILs showing broad-spectrum activity against both Gram-negative and Gram-positive bacteria and antibiofilm activity have been reported [9]; as such, ILs are emerging as appealing alternatives to counteract antimicrobial resistance, while the world is running out of effective antibiotics, especially against Gram-negative bacteria. Furthermore, many ILs have gained attention as dermal permeation enhancers [10], making them particularly attractive for topical applications. ILs presenting both antimicrobial activity and dermal permeation enhancement can be quite helpful to treat skin disorders including infected injuries, as recently demonstrated in an *in vivo* biofilm-infected wound assay, where an IL was able to kill 95% of the bacteria [11].

The prevalence of diabetes, peripheral vascular disease, or conditions forcing patients to be bedridden, among others, is increasing alongside with life expectancy, and is often associated with problems like diabetic foot ulcers (DFU) and other skin disorders, such as complicated skin and soft tissue infections (cSSTI) [12]. Despite

cSSTI are mostly caused by multidrug resistant (MDR) bacteria from the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter spp.*) group, a high prevalence of fungi in chronic wounds has also been reported, and linked to both healing time, and formation of mixed biofilms with bacteria; *Cladosporidium spp.* and *Candida spp.*, mainly *C. albicans* and *C. parapsilosis*, were described as the most prevalent fungi in cSSTI [13,14]. The burden of cSSTI increased during the COVID-19 pandemic due to reluctance in exposing elderly patients to healthcare facilities [15] and is exacerbated by both the (i) dissemination of MDR pathogens, and (ii) installation of polymicrobial biofilms that are refractory to both current antibiotics and the immune system, and delay or impair healing.

In the specific case of cSSTI, treatment requires debridement or incision and drainage, complemented with antibiotic therapy. The guidelines for cSSTI treatment recommend the administration of systemic antibiotics that are effective against methicillin-resistant *Staphylococcus aureus* (MRSA) strains, which are among MDR pathogens that prevail in health care facilities, followed by an antibiotic therapy program based on the culture assessments for a definitive treatment [16]. When this fails, amputation emerges as the last resource to avoid life-threatening sepsis.

Resistance to the available antibiotics is rapidly increasing and currently widespread to many different species of Gram-positive and Gram-negative bacteria. Likewise, fungal infections are becoming more difficult to treat due to the development and spread of resistance among pathogenic fungi.

Peptide-based antimicrobials, like the cyclic lipopeptide daptomycin, offer clinicians an alternative to tackle MDR bacteria in hospital settings, but daptomycin is exclusively active against Gram-positive species, including MRSA (17). However, most cSSTI are of polymicrobial nature, also involving Gram-negative bacteria and fungi, meaning that new options are urgently needed to cope with the emerging post-antibiotic era (18). Fungal colonization of non-healing wounds has been traditionally neglected (14), but Kalan et al. have highlighted the importance of identifying not only bacterial, but also fungal

species involved in cSSTI (14), namely, *Cladosporidium spp*. and *Candida spp*., including *C. albicans* and *C. parapsilosis* (19).

The healing process in skin disorders is often delayed or even impaired due to age- and/or disease-related pathophysiological traits, such as high oxidative stress, neurophaty, angiopathy, poor tissue oxygenation, chronic inflammation, and poor immune response; this scenario is further aggravated by installation of microbial pathogens that promptly form biofilms in the wound bed, and which are refractory to the action of most current antibiotics (20). In such cases, effective treatment of skin lesions must, most of the times, both quell infection and promote fast and correct healing. In this regard, collagen, as a structural protein from the extracellular matrix (ECM), plays an important role in key steps of wound healing and closure (21). Because collagen is an endogenous protein, it is regarded as a desirable component for the development of biocompatible and biodegradable wound dressings/biomaterials (22). Along with collagen itself, collagen-derived/inspired peptides, such as cryptic collagen peptides (23), have been also considered as potential promoters of cell migration and proliferation, capable of inducing fibroblasts to produce new collagen, and consequently promoting faster wound healing (24). This motivated the development of the so-called cosmeceutical peptides (CP), and derivatives thereof, for topical application in the treatment of skin disorders, acting by promoting faster skin regeneration. CP are small peptide fragments that result from proteolysis of ECM macromolecules like collagen or elastin, with diverse potential biomedical applications, including in cosmetics (25, 26). For instance, "pentapeptide-4" (or PP4, with amino acid sequence KTTKS) is a widely studied CP that derives from type I human collagen and is the smallest peptide sequence known to retain a potent ability to stimulate ECM (collagen and fibronectin) production (27, 28). The *N*-palmitoylated form of KTTKS, known as "palmitoyl pentapeptide-4" or Matrixyl®, is used in the cosmetics industry due to its ability to cause a skin-rejuvenation/anti-wrinkle effect, probably associated to a collagenesis-inducing action (29, 30).

Noteworthy, as previously mentioned, a sensible choice of peptide and ionic liquid building blocks to be conjugated enables the development of a range of products tailored for their intended specific topical

use, covering from severe non-healing skin ulcerations (diabetic foot ulcers, pressure ulcers, venous leg ulcers, and alike) to usually milder bacterial or fungal skin infections (acne, atopic dermatitis, candidiasis, cellulitis, dermatophytoses, erysipelas, folliculitis, impetigo, rosacea, and others), but also post-surgical wounds, common injuries or burns. Moreover, considering that peptide-based antimicrobials have been widely associated to other activities such as immunomodulatory, antioxidant, anti-aging and anti-tumoral effects, our products will likely find application also in the topical treatment of auto-immune/inflammatory, aging and cancer skin disorders, such as psoriasis, atopic dermatitis, cutaneous lupus erythematosus, impetigo, skin aging, and melanoma, either in absence or presence of secondary infection.

The potential of PP4 to promote skin regeneration in the context of skin lesions has recently attracted attention. Thus, given that this CP is devoid of antimicrobial action, when the KTTKS sequence is conjugated to an antimicrobial peptide (AMP), this results in a chimeric peptide displaying (i) antibacterial activity against reference and MDR bacteria from clinical isolates; (ii) antibiofilm action; and (iii) a collagenesis-inducing effect comparable to that of Matrixyl® (32).

Further *N*-terminal modification of the aforementioned chimeric peptide with an imidazolium-based ionic liquid afforded an equally potent antimicrobial construct with increased stability toward enzyme-mediated modification (31). Indeed, IL are becoming quite attractive for biomedical applications, given their unique physicochemical characteristics, low cost, and high structural diversity that enables the synthesis of a wide panoply of different IL which can be easily tuned to meet specific requirements, including broad spectrum activity against bacteria (4) and fungi (33). Recently, alkylimidazolium-based IL have been proposed as an alternative antibacterial treatment for cSSTI focusing on Gram-positive pathogens (34). In addition, several IL have been found to improve the skin permeation of drugs (10,35,36,37), including ceftazidime, an antibiotic possessing poor water solubility and low skin permeation (11).

Document "Clicking" an Ionic Liquid to a Potent Antimicrobial Peptide: On the Route towards Improved Stability (31) describes an analogue of the peptide 3.1-PP4 (composed of the antimicrobial peptide 3.1 and the cosmeceutical pentapeptide-4) conjugated with the ionic liquid methyl imidazolium at the *N*-terminus (MeIm-3.1-PP4) and its biological application as antibacterial, antibiofilm and antifungal agent. This conjugate retained the unmodified parent chimeric peptide's activity (3.1-PP4) against multidrug-resistant clinical isolates of Gram-negative bacteria, and antibiofilm action on a resistant clinical isolate of *Klebsiella pneumoniae*, while exhibiting much improved stability towards tyrosinase-mediated modifications.

Document "Disclosure of a Promising Lead to Tackle Complicated Skin and Skin Structure Infections: Antimicrobial and Antibiofilm Actions of Peptide PP4-3.1" (38) describes an analogue of the peptide PP4-3.1 (composed of the cosmeceutical pentapeptide-4 and the antimicrobial peptide 3.1) conjugated with the ionic liquid methyl imidazolium at the *N*-terminus (MeIm-PP4-3.1) and its biological application as antibacterial, antibiofilm and antifungal agent. Although the conjugated peptide presented activity in all the biological assays, the unmodified parent chimeric peptide (PP4-3.1) stood out for its potent activity against Gram-positive and Gram-negative bacteria, including against MDR clinical isolates, and against three clinically relevant species of *Candida* fungi, with an overall performance superior to that of MeIm-PP4-3.1.

Both documents describe peptide analogues with antibacterial, antibiofilm and antifungal activity *in vitro*, exhibiting *N*-terminal modification of chimeric peptides combining a cosmeceutical and an antimicrobial sequence. Nonetheless, these documents do not describe peptide analogues comprising a quaternary imidazolium moiety with long hydrocarbon chain substituents and do not provide any demonstration of a significant collagen biosynthesis-inducing activity of the peptide derivatives therein reported. Also, the peptide analogue sequences reported in the two documents comprise long and highly cationic host defence peptide sequences, given their well-known direct antimicrobial action. Yet, many host defence peptides lack the fast and potent skin regeneration ability that can be afforded by cosmeceutical peptides, and most are too long (with over 10 amino acids) for an efficient

dermal and transdermal delivery (39) and for cost-effective production at industrial scale. Moreover, host defence peptide-resistant strains of the most prevalent bacterial pathogen in infected skin lesions, *Staphylococcus aureus*, have been reported to possess an increased membrane surface charge, which lowers the efficiency of the initial peptide-bacterial electrostatic interaction that precedes the peptide's bactericidal action by bacterial membrane destabilization (40); this means that the formerly developed constructs based on host defence peptides might trigger selection of resistant microbial strains. In turn, the new antimicrobial constructs derive from small CP and not from highly cationic host defence peptides, hence are less likely to induce resistance.

Gomes et al. in 'The Emerging Role of Ionic Liquid-Based Approaches for Enhanced Skin Permeation of Bioactive Molecules: A Snapshot of the Past Couple of Year', Int. J. Mol. Sci., 2021, 22, 11991, discloses a review of ionic liquids as dermal permeation enhancers, but it does not disclose technical information related to the present invention.

Bergfeld et al. in 'Safety Assessment of Tripeptide-1, Hexapeptide-12, their Metal Salts and Fatty Acyl Derivatives, and Palmitoyl Tetrapeptide-7 as Used in Cosmetics', Cosmetic Ingredient Review, 2014, is a study in the safety of using peptides in cosmetics but does not address antimicrobial action of the peptides or discloses technical information related to the present invention.

Gomes et al. in '"Clicking" an Ionic Liquid to a Potent Antimicrobial Peptide: On the Route towards Improved Stability', International Journal of Molecular Sciences, vol. 21, n.° 17, 2020, does not disclose a cosmeceutical peptide with up to 10 amino acids, it is a 16-aminoacid antimicrobial peptide that comprises both antimicrobial and cosmeceutical motifs within its sequence. The present invention differs from this document because the ionic liquid is covalently attached to the cosmeceutical peptide, which is devoid of antimicrobial action *per se*, without resorting to antimicrobial peptide motifs.

The present invention refers only to small (up to 10 amino acids) CP already amply used (and patented) as active ingredients in cosmetics,

for cosmetic applications, but in no case as topical antimicrobials, given their lack of intrinsic antimicrobial action. Relevantly, the features of PIL conjugates to which the present invention refers are not simply a mere sum of the intrinsic properties of each of the two individual building blocks, as simply mixing them (instead of linking them through chemical conjugation) delivers mixtures with bacteriostatic rather than bactericidal action. In addition, the chemical modifications of peptide analogues from the two documents mentioned above (31, 38) are only carried out at their *N*-termini, while the PIL conjugates in the present invention have one or multiple IL building blocks attached to either the *N*-terminus or amino acid side chains (e.g., azido-lysine side chains) or both. Insertion on amino acid side chains for chemical conjugation of IL to peptides has never been reported so far.

Summary

The present invention related to a peptide-ionic liquid conjugate for the prevention and/or treatment of skin disorders, wherein the peptide-ionic liquid conjugate comprises a cosmeceutical peptide comprising up to 10 amino acids and at least one ionic liquid comprising saturated or unsaturated hydrocarbon chain substituents from C1 to C18.

In one embodiment the cosmeceutical peptide is selected from a cosmeceutical peptide selected from any of the sequences SEQ. ID NO 1 to 20.

In one embodiment the at least one ionic liquid is selected from a pyridinium, an imidazolium, a phosphonium, or a cholinium ionic liquid.

In one embodiment the conjugation between the cosmeceutical peptide and at least one ionic liquid occurs on the *N*-terminus of an amino acid of the cosmeceutical peptide.

In one embodiment the conjugation between the cosmeceutical peptide and at least one ionic liquid occurs on a side chain of an amino acid of the cosmeceutical peptide.

In one embodiment the conjugation between the cosmeceutical peptide and the ionic liquids occurs on the *N*-terminus of an amino acid and on a side chain of an amino acid of the cosmeceutical peptide.

The present application also relates to a topical composition comprising at least one peptide-ionic liquid conjugate type.

In one embodiment the topical composition further comprises nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers including liposomes or polymeric nanoparticles, and/or at least one hydrogel including polysaccharide-based hydrogels or poly(lactic-co-glycolic acid)-based hydrogels.

In one embodiment the topical composition is for use in the prevention and/or treatment of skin disorders.

In one embodiment the skin disorders are severe non-healing skin ulcerations such as diabetic foot ulcers, pressure ulcers, venous leg ulcers; milder bacterial or fungal skin infections such as acne, atopic dermatitis, candidiasis, cellulitis, dermatophytoses, erysipelas, folliculitis, impetigo, rosacea; auto-immune/inflammatory disorders, aging and cancer skin disorders, such as melanoma, non-melanoma skin cancers, psoriasis, cutaneous lupus erythematosus, impetigo, skin aging, and melanoma, either in absence or presence of secondary infection, treatment of post-surgical wounds, common injuries or burns.

General description

This application relates to a peptide-ionic liquid (PIL) conjugate suitable for the prevention and/or treatment of skin disorders.

The peptide-ionic liquid conjugate described herein is a novel type of prospective Active Pharmaceutical Ingredient (API) suitable for topical compositions to prevent and/or treat disorders of the skin.

The present invention relates to the conjugate of a cosmeceutical peptide (CP) comprising up to 10 amino acids, with at least one ionic liquid, forming a PIL conjugate.

As an example, the direct coupling of alkylimidazolium-based IL to the non-antimicrobial pentapeptide-4 (PP4) with sequence SEQ. ID 1 (a cosmeceutical peptide with up to 10 amino acids: KTTKS) was studied to understand if it would afford a new type of peptide-based construct displaying collagenesis-inducing and antimicrobial action, despite not harboring an antimicrobial peptide (AMP) motif. To this end, three different alkylimidazole-based ILs were chemically modified to introduce the alkyne moiety required for subsequent coupling to different azide derivatives of PP4. Seven different IL-PP4 conjugates were produced, and their antibacterial, antifungal, and collagenesis-inducing properties studied, as herein reported and discussed.

These conjugates present potent activity against either antibiotic-susceptible strains or multidrug resistant clinical isolates of both Gram-positive and Gram-negative bacterial species belonging to the so-called "ESKAPE" group of pathogens. Noteworthy, their antibacterial activity is preserved in simulated wound fluid, which anticipates an effective action in the setting of a real wound bed. Moreover, their collagenesis-inducing effects *in vitro* are comparable to or stronger than those of Matrixyl®. Altogether, IL-PP4 exert a triple antibacterial, antifungal, and collagenesis-inducing action *in vitro*. These findings provide solid grounds to advance IL-PP4 conjugates as promising leads for future development of topical prevention and/or treatment for skin disorders.

Further studies are envisaged to incorporate PIL conjugates into suitable nanoformulations, to reduce toxicity, improve resistance to proteolytic degradation, and retain the active pharmaceutical ingredient at the intended site of action (wound bed, lesion area).

The major advantages of the PIL conjugates of the present invention are:

1. the fast skin regeneration ability and safety for use on human skin conveyed by the CP, which are already used in cosmetics for advanced skin care;

2. the small size of the CP (up to 10 amino acids), favouring their cost-effective production and skin permeation ability, without the need for using non-trivial/expensive dermal and transdermal delivery

enhancement methods (e.g., electroporation, iontophoresis, sonophoresis, microneedles, etc.);

3. the fact that CP are already produced at industrial scale according to current good manufacture practices (cGMP);

4. the widely reported antimicrobial and skin permeation ability of IL, namely but not limited to, imidazolium and pyridinium-based; for instance, cetylpyridinium chloride is an antiseptic IL used in mouthwashes, toothpastes, and throat/nasal sprays that combines microbicidal action with dermal permeation ability (41);

5. the possibility of using a fast and straightforward chemoselective conjugation between the CP and the IL building blocks by click chemistry approaches, namely but not limited to, copper(I)-catalysed azide-alkyne coupling (CuAAC);

6. the likelihood of delivering new products that could be fine-tuned not only to reflect the sum of the properties of each of its building blocks, but also to display new properties (e.g., immunomodulatory), hence expanding its prospective range of therapeutic (and cosmetic) applications beyond management of skin infections and wound healing (i.e., topical use on autoimmune skin disorders like psoriasis or eczema).

Brief description of drawings

For easier understanding of this application, figures are attached in the annex that represent the preferred forms of implementation which nevertheless are not intended to limit the technique disclosed herein.

Figure 1 - Route to the target IL-PP4 conjugates. (**A**) Synthesis of the alkyne derivatives of the IL: (i) 1.1 eq of C_{16}Im, C_{14}Im or MeIm and 1.0 eq of propargyl bromide (80% in toluene), 40 °C, 24 h. (**B**) Synthesis of the azide derivatives of KTTKS and their coupling to the alkynyl-IL *via* CuAAC: (ii) 5 eq of Fmoc-protected amino acid, 10 eq of *N*-ethyl-*N*,*N*-diisopropylamine (DIEA) and 5 eq of *O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HBTU) in *N*,*N*-dimethylformamide (DMF), 1 h, room temperature (r.t.); (iii) 20% piperidine in DMF, 15 min, r.t.; (iv) 5 eq of azido acetic, 10 eq of DIEA, 5 eq of HBTU, 1 h, r.t.; (v) 10 eq of DIEA, 10 eq of 2,6-lutidine, 1 eq of copper(I) bromide, 1 eq of sodium *L*-ascorbate and 1

eq of either Pr-MeIm, Pr-C_{16}Im, or Pr-C_{14}Im, in DMF:acetonitrile (ACN) (3:1 *v/v*), 24 h, r.t.; (vi) trifluoroacetic acid (TFA)/ triisopropylsilane (TIS) distilled water (95:25:2.5 *v/v/v*).

Figure 2 - Structure of IL-PP4 conjugates and corresponding molecular weight (in Da). The amino acids are represented in single letter code as defined by the IUPAC-IUBMB guidelines on nomenclature and symbolism for amino acids and peptides; exception is made to the amino acid residues whose side chain was coupled to ionic liquids *via* click chemistry, in which case the full modified structure is shown.

Figure 3 - Collagen synthesis by Human Dermal Fibroblasts (HDF), in presence of C_{16}-KTTKS-OH (Matrixyl®), KTTK(C_{16}Im)S and C_{16}Im-KTTKS at 5 μM, using the Sircol™ Kit. Data are presented as mean±SEM (3 independent experiments in triplicates) expressed in collagen amount (% of control); * $p<0.05$, ** $p<0.01$.

Description of embodiments

Now, preferred embodiments of the present application will be described in detail with reference to the annexed drawings. However, they are not intended to limit the scope of this application.

The present invention relates to a peptide-ionic liquid conjugate of a cosmeceutical peptide (CP) comprising up to 10 amino acids and at least one ionic liquid, forming an PIL conjugate.

In one embodiment, the cosmeceutical peptide is selected from, but not limited to, PP4 (SEQ. ID 1), KVK, PKEK (SEQ. ID 2), GEKG (SEQ. ID 3), GHK, copper tripeptide-1, manganese tripeptide-1, palmitoyl tripeptide-1, tripeptide-5, palmitoyl tripeptide-5, tetrapeptide-3 (SEQ. ID 4), pentapeptide-3 (SEQ. ID 5), pentapeptide-18 (SEQ. ID 6), hexapeptide-11 (SEQ. ID 7), acetyl hexapeptide-3 (SEQ. ID 8), hexapeptide-10 (SEQ. ID 9), hexapeptide-12 (SEQ. ID 10), palmitoyl hexapeptide-12, acetyl octapeptide-1 (SEQ. ID 11), SA1-III (SEQ. ID 12), lipospondin.

In one embodiment the at least one ionic liquid is selected from, but not limited to, any ionic liquid comprising a saturated or unsaturated hydrocarbon chain substituent from C_1 to C_{18}. In one embodiment the

ionic liquid is selected from diverse ionic liquid families, namely but not limited to, a pyridinium (e.g., cetylpyridinium), an imidazolium (e.g., 1-alkyl-3-methylimidazolium), a phosphonium (e.g. trihexyltetradecylphosphonium), or an cholinium ionic liquid.

The conjugation between the cosmeceutical peptide and at least one ionic liquid occurs on the *N*-terminus of an amino acid and/or on a side chain of a specific amino acid in the peptide sequence, namely but not limited to, lysine, arginine, and histidine side chains.

In one embodiment, the PIL-conjugates are for use in the treatment and/or prevention of skin disorders, from severe non-healing skin ulcerations such as diabetic foot ulcers, pressure ulcers, venous leg ulcers, and alike, to usually milder bacterial or fungal skin infections such as acne, atopic dermatitis, candidiasis, cellulitis, dermatophytoses, erysipelas, folliculitis, impetigo, rosacea, and others, but also auto-immune/inflammatory disorders, aging and cancer skin disorders, such as melanoma, non-melanoma skin cancers, psoriasis, cutaneous lupus erythematosus, impetigo, skin aging, and melanoma, either in absence or presence of secondary infection. It may also find application in the treatment of post-surgical wounds, common injuries or burns.

The present application also relates to a topical composition comprising at least one type of PIL conjugate.

In one embodiment, the topical composition comprising the PIL-conjugate is for use in the treatment and/or prevention of skin disorders, from severe non-healing skin ulcerations such as diabetic foot ulcers, pressure ulcers, venous leg ulcers, and alike, to usually milder bacterial or fungal skin infections such as acne, atopic dermatitis, candidiasis, cellulitis, dermatophytoses, erysipelas, folliculitis, impetigo, rosacea, and others, but also auto-immune/inflammatory disorders, aging and cancer skin disorders, such as melanoma, non-melanoma skin cancers, psoriasis, cutaneous lupus erythematosus, impetigo, skin aging, and melanoma, either in absence or presence of secondary infection. It may also find application in the treatment of post-surgical wounds, common injuries or burns.

In one embodiment, the topical composition further comprises nanoparticles, including, but not limited to, solid lipid nanoparticles, nanostructured lipid carriers (liposomes or polymeric nanoparticles) and/or at least one hydrogel including, but not limited to, polysaccharide-based hydrogels or poly(lactic-co-glycolic acid)-based hydrogels.

It has been previously demonstrated that it is possible to produce a dual-action antimicrobial and collagenesis-inducing chimeric peptide, by combining the amino acid sequence of an AMP to that of the non-antimicrobial well-known cosmeceutical peptide PP4 (32). It was further shown that such potent antimicrobial activity was preserved when coupling an imidazolium IL to the *N*-terminus of the chimeric peptide *via* the CuAAC "click" approach, which conferred the peptide resistance to enzyme-mediated modification (31).

Based on these findings, the studies disclosed in the present application were to investigate if the dual antimicrobial and collagenesis-inducing activity was preserved by removing the AMP sequence and coupling at least one IL directly to the amino acid sequence of the cosmeceutical peptide PP4.

The findings disclosed in the present application strongly indicate that the direct conjugation between an IL and other cosmeceutical peptides comprising up to 10 amino acids will have the same properties observed in the examples herein disclosed.

In the experimental examples ahead the PP4 peptide will be mentioned by its amino acid sequence, i.e., KKTKS.

The antibacterial activity of the new constructs herein presented, IL-KKTKS, was assessed against reference bacterial strains and results obtained (shown in the Examples section) allowed to advance a couple of structure-activity relationships (SAR) on the (i) IL insertion site, as the covalent graft was at either the *N*-terminus or the Lys1/Lys4 side chains of the KTTKS sequence, and (ii) length of the alkyl substituent in the imidazole ring, which was varied between one (methyl or Me), fourteen (tetradecyl or C14), and sixteen (hexadecyl or C16) carbons. Hence, antibacterial activity (i) increased with the

length of the alkyl substituents in the IL moiety [KTTK(C14Im)S versus KTTK(C16Im)S] and (ii) is depleted in all conjugates bearing the methyl-substituted imidazolium IL, regardless of other structural features.

Moreover, MIC values were also determined for the parent building blocks KTTKS and [C16 M1Im][Br], as well as for their noncovalent equimolar mixture, indicated as KTTKS:[C16 M1Im][Br] (1:1), so that the importance of covalent conjugation could be assessed. The noncovalent mixture KTTKS:[C16 M1Im][Br] (1:1) presented MIC values similar to those of [C16 M1Im][Br] alone, confirming that the IL building block is the main responsible for the activity observed for the mixture, as expected.

Interestingly, when comparing the MIC values of the noncovalent mixture KTTKS:[C16 M1Im][Br] (1:1) with those of the covalent conjugates KTTK(C16Im)S and C16Im-KTTKS, it is apparent that covalent conjugation is clearly beneficial for activity against Gram-negative bacteria, but not so much against Gram-positive bacteria. Still, whereas the noncovalent mixture is bacteriostatic for Gram-positive species at MIC values, the covalent conjugates are bactericidal at these concentrations. These results indicate that the antibacterial activity of the covalent conjugates KTTK(C16Im)S and C16Im-KTTKS is not only modulated by the IL building block, but also by its conjugation to the peptide. Hence, biophysical studies will be performed to further explore the mechanism(s) of action of IL-KTTKS conjugates. The conjugates KTTK(C16Im)S and C16Im-KTTKS also showed a potent activity against MDR clinical isolates of Gram-positive and Gram-negative bacteria, being more active than the reference antibiotic ciprofloxacin.

This is a relevant finding, considering that the three clinical isolates tested refer to bacterial species belonging to the "ESKAPE" group of pathogens which encompasses life-threatening nosocomial pathogens, namely, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp*. The ability of "ESKAPE" pathogens to escape the action of currently available antibiotics is one of the

major healthcare threats of our times, especially for Gram-negative bacteria, against which the world is running out of effective options.

Additionally, the conjugates showed to retain (C16Im-KTTKS) or slightly decrease (KTTK(C16Im)S) the antibacterial activity against *S. aureus* in simulated wound fluid (SWF). These preliminary observations using SWF, which mimics the wound exudate, are relevant as they are indicative that the IL-peptide conjugates, especially C16Im-KTTKS, can be more stable in the wound environment as compared to analogues where the peptide is not protected at the *N*-terminus.

The IL-KTTKS conjugates herein described have also shown interesting antifungal properties, especially against *C. parapsilosis*. This is one of the most common non-*C. albicans* species of *Candida*, which are regarded as important nosocomial pathogens of concern as they were reported to be involved in cases of sepsis and cSSTI. In this regard, the antifungal activity of the conjugates was assessed, and MIC values observed were as low as 2.4 and 2.7 µM against *C. parapsilosis* and 4.7 and 5.7 µM against *C. albicans*.

Still, as observed in antibacterial activity assays, the non-covalent mixture and the parent IL are more potent than the IL-KTTKS conjugates against *Candida spp.*, which indicates that the antifungal activity of the covalent conjugates is not only modulated by the IL building block, but also by their conjugation to the peptide (devoid of antifungal activity). Therefore, further biophysical studies will be performed to shine some light into possible mechanism(s) of action of IL-KTTKS conjugates against *Candida* species.

The evaluation of the cytotoxicity of the IL-KTTKS conjugates is obviously important on its own to check for selectivity, but also due to the toxicity effects often associated to IL, depending on, e.g., cation alkyl chain length or specific ions used.

The parent IL [C16 M1Im][Br] and its covalent equimolar mixture with the peptide, KTTKS:[C16 M1Im][Br] (1:1), were significantly toxic against the human cell lines tested. Therefore, covalent conjugation of the IL to the peptide confers on one hand, antimicrobial activity

to an otherwise peptide building block devoid of such activity and, on the other, reduced cytotoxicity as compared to the parent IL.

The conjugates C16Im-KTTKS and KTTK(C16Im)S were further assessed for their ability to induce collagen production by human dermal fibroblasts *in vitro*. The conjugates showed to be comparable to the reference cosmeceutical Matrixyl® and more active than the control. No significant difference between both conjugates were observed, indicating that changing the side chain of Lys4 did not affect the peptide's collagenesis-inducing behavior.

Altogether, the findings on the present examples are unprecedented as well as remarkable, as they provide confirmation by advancing a couple of PIL conjugates, C16Im-KTTKS and KTTK(C16Im)S, that possess antibacterial, antifungal, and collagenesis-inducing activity *in vitro*, the latter being actually comparable to that of the cosmeceutical ingredient Matrixyl® based on the KTTKS peptide. Further, the site of insertion of the IL does not significantly affect the overall *in vitro* properties of the conjugates [C16Im- KTTKS versus KTTK(C16Im)S], although *N*-terminal conjugation seems to better preserve the conjugates' antibacterial action in SWF and to improve the collagen synthesis by human dermal fibroblasts.

Further studies are envisaged to incorporate PIL into nanoformulations and/or hydrogels, which will reduce toxicity, improve resistance to proteolytic degradation, and retain the active pharmaceutical ingredient at the intended site of action (wound bed, lesion area). Moreover, since the IL-KKTKS conjugates will be applied in the treatment of cSSTI, which are mainly polymicrobial infections, the antimicrobial activity of IL-KTTKS on polymicrobial cultures will be further investigated. This will enable selection of best IL-KTTKS based formulations to advance for *in vivo* studies.

Considering that KTTKS and other small cosmeceutical peptides are already produced at industrial scale as ingredients for cosmetic products, these findings unveil the value of IL-CP conjugates as a promising start for future development of cost-effective topical formulations for the prevention and/or treatment of skin disorders, from mild to severe ones like cSSTI.

Examples:

1. Synthesis of the target conjugates

The route towards the target IL-KTTKS conjugates started by the synthesis of the alkyne-modified imidazolium IL (Figure 1 - A). 1-methyl-imidazole (Me-IM), 1-tetradecyl-imidazole (C14-Im) and 1-hexadecylimidazole (C16-Im) were reacted with propargyl bromide according to Hu *et al.* (Figure 1 - A) (42), to afford the three target imidazolium ILs, propargyl-MeIm (Pr-MeIm), propargyl-C_{14}Im (Pr-C_{14}Im) and propargyl-C_{16}Im (Pr-C_{16}Im). The structures of these ILs were confirmed by ^{1}H-NMR, ^{13}C-NMR, and ESI-IT MS.

In parallel, conveniently modified derivatives of PP4 (amino acid sequence KTTKS) were produced by Solid Phase Peptide Synthesis (SPPS), to afford diverse final IL-KTTKS conjugates (Figure 2) that differed in the: (a) propargyl-imidazolium building blocks used, (b) insertion site of the latter (*N*-terminus, side chain of either or of both lysine residues), and (c) length of the spacer between the imidazolium moiety and the peptide's *N*-terminus. To this end, the PP4 sequence was first assembled, according to steps ii and iii in Figure 1 - B, and conveniently protected lysine (Fmoc-Lys(Boc)-OH) or azido-lysine (Fmoc-Lys(N_3)-OH) building blocks were inserted in the respective positions of the sequence, according to the desired site for the subsequent introduction of the imidazolium moiety *via* "click" copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC). To produce the peptides modified at the *N*-terminus, the sequence bearing two natural Lys residues was assembled and further elongated through coupling of azido acetic acid (step iv, Figure 1 - B) yielding a 2- carbon (ethyl) spacer between the *N*-terminal lysine and the imidazolium moiety to be incorporated *via* CuAAC. This click reaction was next performed on-resin on all precursor azido-peptides, using the desired propargyl-imidazolium IL (step v, Figure 1 - B) and CuAAC conditions previously reported by us (31). After acidolytic cleavage (step vi, Figure 1 - B) and purification of the crude conjugates thus obtained by reverse-phase preparative high performance liquid chromatography (RP-HPLC), all the resulting IL-KTTKS conjugates were isolated in high purity (>95%), and their expected molecular weights confirmed by ESI-IT-MS.

In addition to the target conjugates, the reference cosmeceutical peptide Matrixyl® (C_{16}-KTTKS-*OH*), its *C*-terminal carboxamide analogue (C_{16}-KTTKS-*NH_2*), and the native PP4 (KTTKS) were also assembled by SPPS, following procedures recently reported (32). For the palmitoylated peptides, after the full amino acid sequence of PP4 was assembled, palmitic acid (C_{16}) was coupled. Then, acidolytic cleavage from the solid support delivered the crude peptides that were purified by RP-HPLC. The final peptides were obtained in high purity and their molecular weights confirmed by ESI-IT MS.

2. Antibacterial activity *in vitro*

The antimicrobial activity of the IL-KTTKS conjugates was assessed *in vitro* against reference bacterial strains (American Type Culture Collection, ATCC). The minimal inhibitory concentration (MIC) was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (43) against Gram-positive (*S. aureus*, *E. faecalis*) and Gram-negative (*E. coli*, *P. aeruginosa*) bacteria. The MIC values obtained are shown in Table 1. Notably, the reference peptides C_{16}-KTTKS-*NH_2* and C_{16}-KTTKS-*OH* were respectively soluble in water and dimethyl sulfoxide (DMSO), but both precipitated when diluted in cation-adjusted Mueller-Hinton broth (MHB2), the culture medium recommended by the CLSI guidelines, which hampered the determination of the MIC values for these reference peptides. Data in Table 1 show, as expected, that the peptide KTTKS alone is devoid of significant antibacterial activity, and that MIC values for the [C_{16} M1Im][Br] IL are in agreement with those previously reported (44). Interestingly, all conjugates bearing methyl imidazolium (MeIm) units were inactive against the tested bacterial species, even at the highest concentrations used, regardless the number or position of the MeIm moieties in the overall structure. In turn, replacing the methyl substituent in the imidazolium ring by either a tetradecyl (C_{14}) or a hexadecyl (C_{16}) group, led to an improvement in the antibacterial activity, delivering MIC values from 6.45 to 52.6 µg/mL, hence adding antimicrobial activity to the parent KTTKS peptide. Given that KTTK(C_{16}Im)S and C_{16}Im-KTTKS showed the strongest antibacterial activities, and reflect two different conjugation positions, both these peptides were further investigated by determining their MIC against *S. epidermidis*, *S. pyogenes* (both Gram-positive) and *K. pneumoniae* (Gram-negative), chosen due to their abundance in the skin

(*S. epidermidis*) (45), relevance to cSSTI (*S. pyogenes*) (46-48), and relation to the so-called "ESKAPE" pathogens (*K. pneumoniae*) (49). The noncovalent mixture of the parent peptide KKTKS and the [C_{16} M1Im][Br] ionic liquid, presented MIC values comparable to those of [C_{16} M1Im][Br] alone.

Table 1: MIC values (n=3) in µM (in µg/mL) of the IL-KTTKS conjugates against Gram-negative and Gram-positive bacteria (ATCC reference strains).

	MIC in µM (in µg/mL)						
Peptide	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. aureus ATCC* 29213	*E. faecalis* ATCC 29212	*K. pneumonia ATCC* 138830	*S. epidermidis* ATCC 14990	*S. pyogenes* ATCC 19615
K(MeIm)TTKS	> 1030.1 (731.2)				ND[a]	ND[a]	ND[a]
KTTK(MeIm)S	> 954.2 (677.3)				ND[a]	ND[a]	ND[a]
K(MeIm)TTK(MeIm)S	> 1245.5 (1067.4)				ND[a]	ND[a]	ND[a]
KTTK(C_{14}Im)S	29.5 (26.3)	58.9 (52.6)[b]	29.5 (26.3)	58.9 (52.6)[b]	ND[a]	ND[a]	ND[a]
KTTK(C_{16}Im)S	7.0 (6.45)	32.5 (29.9)	14.0 (12.9)	32.5 (29.9)	53.8 (49.5)	5.4 (5.0)	10.9 (10.0)
MeIm-KTTKS	> 825.9 (633.4)				ND[a]	ND[a]	ND[a]
C_{16}Im-KTTKS	14.3 (14.0)	28.7 (28.0)	14.3 (14.0)	28.7 (28.0)	27.4 (26.8)	9.5 (9.3)	18.9 (18.5)
KTTKS	>1820[e]				ND[a]	ND[a]	ND[a]
[C_{16} M1Im][Br]	60	>240	0.94[d]	0.94[c]	60	ND[a]	ND[a]
KTTKS:[C_{16} M1Im][Br] (1:1)	60	>240	0.94[d]	0.18[c]	60	ND[a]	ND[a]
Ciprofloxacin	0.012 (0.00	0.18 (0.06)	1.5 (0.5)	0.38 (0.125	0.75 (0.25)	0.75 (0.25)	6.04 (2.0)

	4)			)			
[a] *Not Determined;* [b] *the MBC was 2× the MIC;* [c] *MBC = 15 µM;* [d] *MBC = 30 µM; in all other cases, the MBC was equal to the MIC;* [e] value from ref (32)							

The antibacterial activities of the best couple of conjugates, i.e., C16Im-KTTKS and KTTK(C16Im)S, and of the reference antibiotic ciprofloxacin, were further assessed against MDR clinical isolates of *K. pneumoniae* (KP010), S. aureus (SA007), and *P. aeruginosa* (PA004). MIC values thus obtained are displayed in Table 2 and show that both conjugates preserve their antibacterial activity observed against susceptible ATCC bacterial strains. Relevantly, the conjugates were clearly more active than the reference antibiotic ciprofloxacin against the MDR isolates; for instance, the MIC value obtained for C16Im-KTTKS against SA007 is nearly 10-fold higher than that of ciprofloxacin.

Table 2: MIC values (n=3) in µM (in µg/mL) for C16-Im-KTTKS and KTTK(C16Im)S against MDR clinical isolates of Gram-positive and Gram-negative bacteria

MDR	**Peptide**		**Ciprofloxacin**
	C_{16}Im-KTTKS	KTTK(C_{16}Im)S	
KP010	37.9 (37.0)	21.7 (20.0)	48.0 (16.0)
PA004	18.9 (18.5)		96.0 (32.0)
SA007	18.9 (18.5)[a]		193.0 (64.0)
[a] *The MBC was 2× the MIC; In all other cases the MBC was equal to the MIC*			

The antibacterial activity of C_{16}Im-KTTKS and KTTK(C_{16}Im)S was also assessed in SWF (50) against *S. aureus* (ATCC 29213), to check if it was preserved in this medium. MIC values were obtained in both SWF and MHB media in three independent experiments run in triplicates (Table 3), and indicated that the antibacterial activity in SWF was the same as that in MHB for C_{16}Im-KTTKS, and decreased for KTTK(C_{16}Im)S displaying a MIC twice as low.

Table 3: MIC [MBC] values (n=3) in µg/mL for C16Im-KTTKS and KTTK(C16Im)S against *S. aureus* (ATCC 29213) in MHB and SWF.

Peptide	MIC in µg/mL [MBC]	
	MHB	SWF
C_{16}Im-KTTKS	16-32 [32-64]	16-32 [32 - >64]
KTTK(C_{16}Im)S	32 [64-128]	64-128 [128 - >128]

3. Antifungal activity *in vitro*

The antifungal activity of the best couple of IL-KTTKS conjugates, their parent building blocks, and respective noncovalent 1:1 mixture, were all assessed against three species of *Candida*, namely, *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), and *Candida parapsilosis* (ATCC 22019). The MIC values were determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) protocol (51-53) and are shown on Table 4. Both conjugates, KTTK(C_{16}Im)S and C_{16}Im-KTTKS, were equally active against all *Candida* spp., with MIC values ranging from 2.4 to 5.4 µM. Both peptides were twice more active against *C. parapsilosis* than against the other two *Candida* species. Relevantly, the noncovalent mixture KTTKS:[C_{16} M1Im][Br] (1:1) showed a potent activity against all *Candida* spp., being equipotent to the parent IL alone, and both seven times more active than the reference antifungal drug fluconazole.

Table 4. MIC values (n=2) in µM (in µg/mL) for the best performing conjugates, their parent building blocks, and respective 1:1 noncovalent mixture on ATCC *Candida* spp.

Peptide	MIC in µM (in µg/mL)		
	C. albicans ATCC 90028	*C. glabrata* ATCC 90030	*C. parapsilosis* ATCC 22019
KTTK(C_{16}m)S	5.4 (5.0)	5.4 (5.0)	2.7 (2.5)
C_{16}Im-KTTKS	4.7 (4.6)	4.7 (4.6)	2.4 (2.3)
KTTKS	>60	>60	>60
[C_{16}M1Im][Br]	0.93	0.93	0.93
KTTKS:[C_{16} M1Im][Br] (1:1)	0.93	0.93	0.93
Fluconazole	1.6 (0.5)	26 (8)	6.5 (2)

4. Toxicity to HFF-1 and HaCaT cells

The cytotoxicity of KTTK(C_{16}Im)S and C_{16}Im-KTTKS conjugates was assessed on human foreskin fibroblasts (HFF-1) and human immortalized keratinocytes (HaCaT). The results shown in Table 5, are expressed as the conjugate concentration causing a 50% cell growth inhibition (IC_{50}). As expected from previous reports (54), both the parent peptide sequence KTTKS and the derived reference cosmeceutical Matrixyl® (C_{16}-KTTKS-*OH*) did not show relevant toxicity against the cell lines tested, at up to 100 μM. In turn, the parent IL [C_{16} M1Im][Br] and its covalent equimolar mixture with PP4, KTTKS:[C_{16} M1Im][Br] (1:1), were significantly toxic. Interestingly, covalent conjugation of the peptide to the IL resulted in an intermediate situation, as conjugates were more toxic than the peptide alone, but clearly less toxic than the IL alone or than its noncovalent mixture with the peptide.

5. Collagen production *in vitro*

The point of conjugating antimicrobial ILs to a collagenesis-inducing peptide was to afford a simple construct able to exert a dual antimicrobial and skin rebuilding action. Therefore, the two best IL-KTTKS conjugates were further tested for their ability to promote collagen production by human dermal fibroblasts (HDF) *in vitro*. This was assessed using the Sircol® kit assay, whereby the amount of newly formed collagen in the ECM that is deposited in the microwell-plated cell cultures is solubilized in an acidic medium and next quantified through a collagen standard curve according to the Sircol® kit assay procedure (55). Assays were conducted in different conditions for comparison, namely, in the presence of the reference cosmeceutical Matrixyl® (positive control – C_{16}-KTTKS-*OH*), of the test conjugates KTTK(C_{16}Im)S and C_{16}Im-KTTKS, and in the absence of any peptide (negative control). Data presented in Figure 3 show that both conjugates induce HDF cells to produce more collagen, as compared to the negative control. No significant difference was observed between both conjugates or between KTTK(C_{16}Im)S conjugate and reference Matrixyl®, demonstrating that the ability of Matrixyl® to induce collagenesis is not affected by the introduction of the imidazolium IL at the Lys side chain of the peptide sequence.

This description is of course not in any way restricted to the forms of implementation presented herein and any person with an average knowledge of the area can provide many possibilities for modification

thereof without departing from the general idea as defined by the claims. The preferred forms of implementation described above can obviously be combined with each other. The following claims further define the preferred forms of implementation.

Sequence listing:

SEQ ID NO 1 = PP4

SEQ ID NO 2 = PKEK

SEQ ID NO 3 = GEKG

SEQ ID NO 4 = tetrapeptide-3

SEQ ID NO 5 = pentapeptide-3

SEQ ID NO 6 = pentapeptide-18

SEQ ID NO 7 = hexapeptide-11

SEQ ID NO 8 = acetyl hexapeptide-3

SEQ ID NO 9 = hexapeptide-10

SEQ ID NO 10 = hexapeptide-12

SEQ ID NO 11 = acetyl octapeptide-1

SEQ ID NO 12 = SA1-III

SEQ ID NO 13 = KVK

SEQ ID NO 14 = GHK

SEQ ID NO 15 = copper tripeptide-1 = Cu(II)-GHK

SEQ ID NO 16 = manganese tripeptide-1 = Mn(II)-GHK

SEQ ID NO 17 = palmitoyl tripeptide-1 = C16-GHK (palmitoyl from palmitic acid, CH3-(CH2)14-COOH)

SEQ ID NO 18 = palmitoyl tripeptide-5 = C16-KVK

SEQ ID NO 19 = palmitoyl hexapeptide-12 = C16-VGVAPG

SEQ ID NO 20 = lipospondin = elaidyl-KFK (elaidyl from elaidic acid, $HOOC-(CH_2)_7-CH=CH-(CH_2)_7-CH3$)

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CLAIMS

1. A peptide-ionic liquid conjugate for the prevention and/or treatment of skin disorders, wherein the peptide-ionic liquid conjugate comprises a cosmeceutical peptide comprising up to 10 amino acids and at least one ionic liquid comprising saturated or unsaturated hydrocarbon chain substituents from C1 to C18.

2. A peptide-ionic liquid conjugate according to the previous claim, wherein the cosmeceutical peptide is selected from any of the sequences SEQ. ID NO 1 to 20.

3. A peptide-ionic liquid conjugate according to any of the previous claims, wherein the at least one ionic liquid is selected from a pyridinium, an imidazolium, a phosphonium, or a cholinium ionic liquid.

4. A peptide-ionic liquid conjugate according to any of the previous claims, wherein the conjugation between the cosmeceutical peptide and at least one ionic liquid occurs on the *N*-terminus of an amino acid of the cosmeceutical peptide.

5. A peptide-ionic liquid conjugate according to any of the claims 1 to 3, wherein the conjugation between the cosmeceutical peptide and at least one ionic liquid occurs on a side chain of an amino acid of the cosmeceutical peptide.

6. A peptide-ionic liquid conjugate according to any of the claims 1 to 3, wherein the conjugation between the cosmeceutical peptide and the ionic liquids occurs on the *N*-terminus of an amino acid and on a side chain of an amino acid of the cosmeceutical peptide.

7. A peptide-ionic liquid conjugate according to any of the previous claims, wherein the skin disorders are severe non-healing skin ulcerations such as diabetic foot ulcers, pressure ulcers, venous leg ulcers; milder bacterial or fungal skin infections such as acne, atopic dermatitis, candidiasis, cellulitis, dermatophytoses, erysipelas, folliculitis, impetigo, rosacea; auto-immune/inflammatory disorders, aging and cancer skin disorders, such as melanoma, non-

melanoma skin cancers, psoriasis, cutaneous lupus erythematosus, impetigo, skin aging, and melanoma, either in absence or presence of secondary infection, treatment of post-surgical wounds, common injuries or burns.

8. Topical composition comprising at least one peptide-ionic liquid conjugate type described in any of the previous claims.

9. Topical composition according to the previous claim, wherein it further comprises nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers including liposomes or polymeric nanoparticles, and/or at least one hydrogel including polysaccharide-based hydrogels or poly(lactic-co-glycolic acid)-based hydrogels.

10. Topical composition according to any of the claims 8 to 9, comprising the peptide-ionic liquid conjugate described in any of the claims 1 to 6 for use in the prevention and/or treatment of skin disorders.

11. Topical composition according to claim 10 wherein the skin disorders are severe non-healing skin ulcerations such as diabetic foot ulcers, pressure ulcers, venous leg ulcers; milder bacterial or fungal skin infections such as acne, atopic dermatitis, candidiasis, cellulitis, dermatophytoses, erysipelas, folliculitis, impetigo, rosacea; auto-immune/inflammatory disorders, aging and cancer skin disorders, such as melanoma, non-melanoma skin cancers, psoriasis, cutaneous lupus erythematosus, impetigo, skin aging, and melanoma, either in absence or presence of secondary infection, treatment of post-surgical wounds, common injuries or burns.

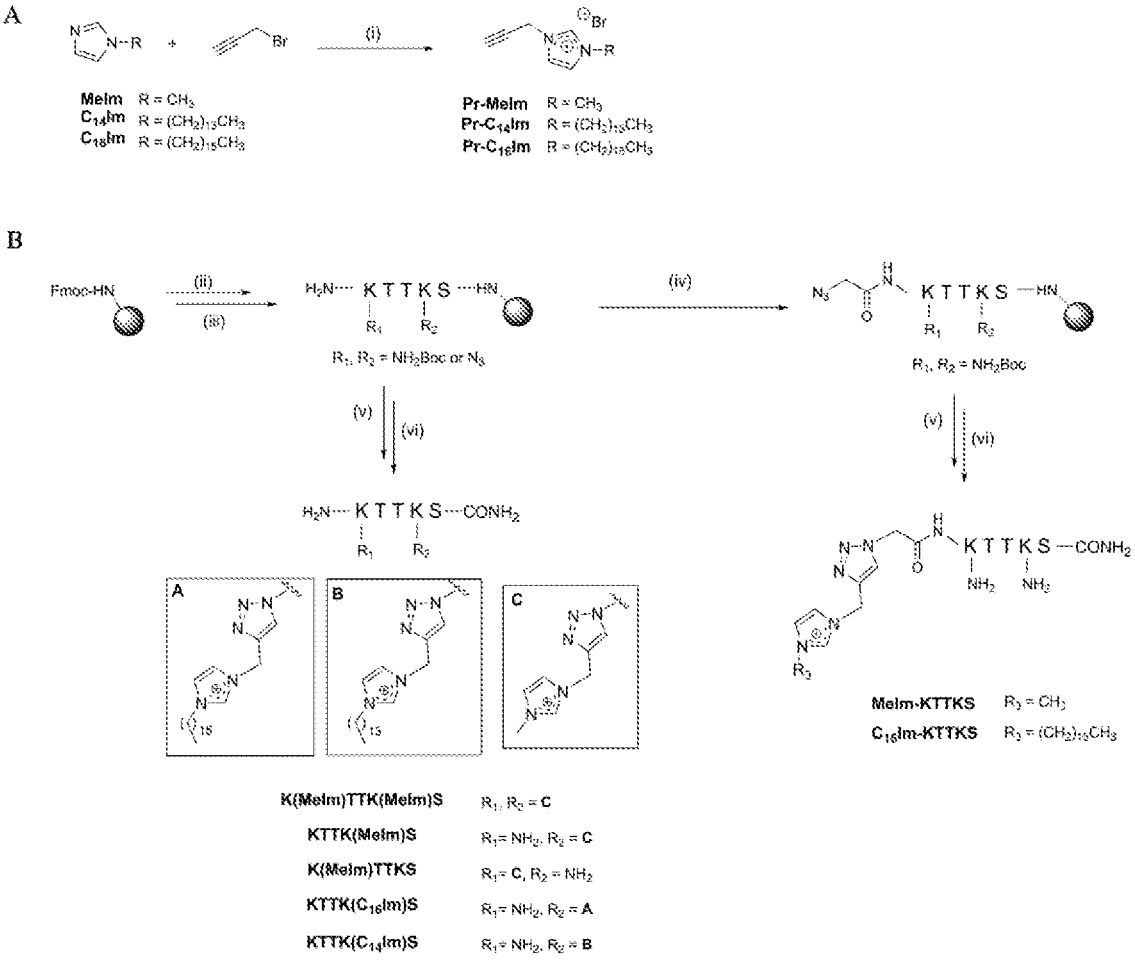

Figure 1

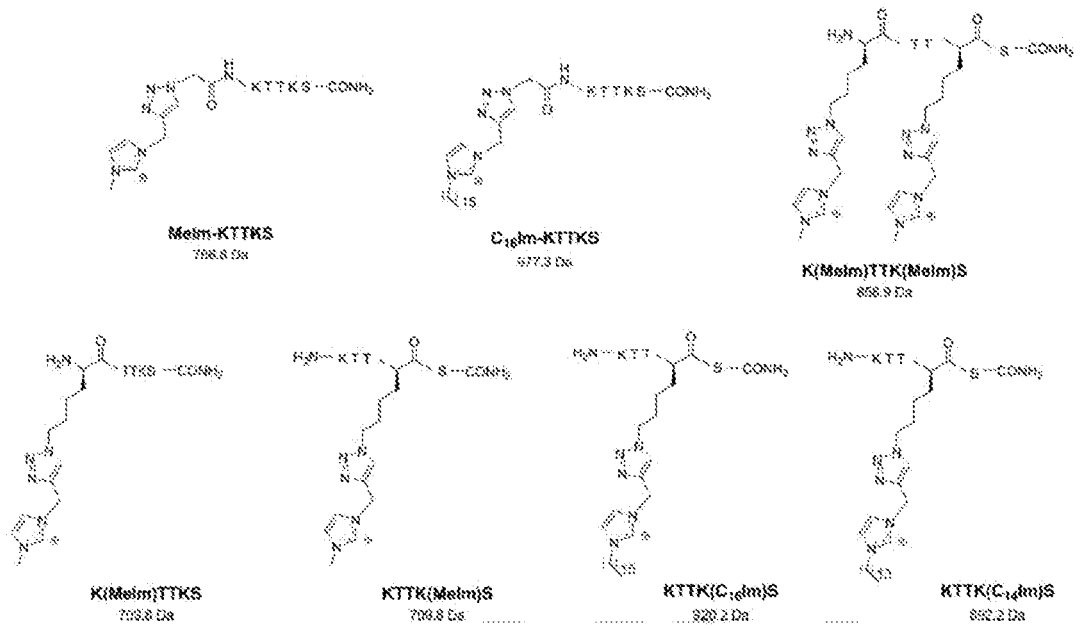

Figure 2

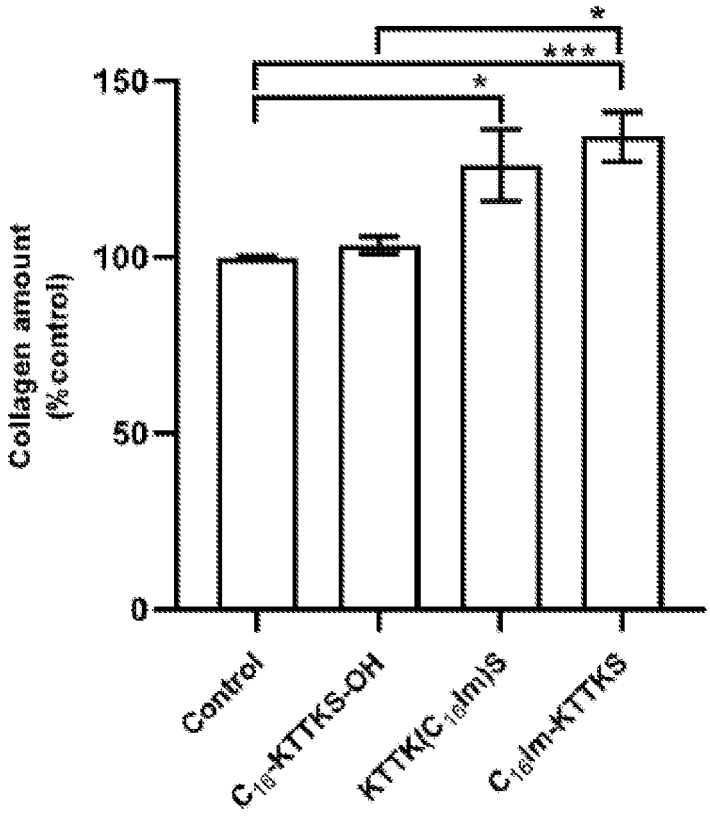
*
***
*
150
100
50
0
Collagen amount
(% control)
Control
C16-KTTKS-OH
KTTK(C16dm)S
C16dm-KTTKS

Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/056516

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/54 A61K8/64 A61P17/00 A61P17/02 A61P17/06
A61P17/10 A61P31/04 A61P31/10 A61P35/00 A61Q19/00
A61K38/00

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

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, CHEM ABS Data, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; 26 August 2020 (2020-08-26), GOMES ANA ET AL: ""Clicking" an Ionic Liquid to a Potent Antimicrobial Peptide: On the Route towards Improved Stability.", XP002810219, Database accession no. NLM32859111 cited in the application abstract -/--	1-11

[x] Further documents are listed in the continuation of Box C.
[ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"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
"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 October 2023	17/10/2023

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	Strenkowska, Malwina

2

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& GOMES ANA ET AL: ""Clicking" an Ionic Liquid to a Potent Antimicrobial Peptide: On the Route towards Improved Stability.", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 26 AUG 2020, vol. 21, no. 17, 26 August 2020 (2020-08-26), XP002810231, ISSN: 1422-0067 abstract scheme 1 page 2, paragraph 2 - page 4, paragraph 2	
A	WEISHI MIAO ET AL: "Ionic-Liquid-Supported Peptide Synthesis Demonstrated by the Synthesis of Leu5-enkephalin", 15 April 2005 (2005-04-15), THE JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY, PAGE(S) 3251 - 3255, XP002409192, ISSN: 0022-3263 abstract figure 1 schemes 1-3	1-11
A	DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; 17 December 2014 (2014-12-17), REINHARDT A ET AL: "Novel imidazolium salt--peptide conjugates and their antimicrobial activity.", XP002810232, Database accession no. NLM25428117 abstract -& REINHARDT A ET AL: "Novel imidazolium salt--peptide conjugates and their antimicrobial activity.", BIOCONJUGATE CHEMISTRY 17 DEC 2014, vol. 25, no. 12, 17 December 2014 (2014-12-17), pages 2166-2174, XP002810233, ISSN: 1520-4812 abstract figure 1; table 1	1-11

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PENDLETON JACK NORMAN ET AL: "The antimicrobial potential of ionic liquids: A source of chemical diversity for infection and biofilm control", INTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS, vol. 46, no. 2, 30 March 2015 (2015-03-30), pages 131-139, XP055778138, AMSTERDAM, NL ISSN: 0924-8579, DOI: 10.1016/j.ijantimicag.2015.02.016 cited in the application the whole document -----	1-11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2023/056516

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments: