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(54) Title: COSMETIC COMPOSITION WITH COMBINED FILLER AND SKIN REGENERATIVE EFFECT

FIG. 2



Δ T7 vs T0

(57) Abstract: The present invention, in one aspect, relates to a dermo-cosmetic composition having filler and regenerative effect on the skin, comprising the combination of a two-component dermal filler with a peptide component having biological activity on the skin.



**"Cosmetic composition with combined filler and skin regenerative effect"**

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DESCRIPTION

## 5 FIELD OF THE INVENTION

The present invention relates to a dermo-cosmetic composition with combined filling and regenerative effect on skin.

The present invention originates in the cosmetic and aesthetic fields.

In particular, the present invention relates to a dermo-cosmetic composition with  
10 filler and regenerative effect on the skin, comprising the combination of a two-components dermal filler with amino acid components having biological activity on the skin.

## PRIOR ART

Human skin aging is a multifactorial, complex and chronic biological process  
15 affecting the main skin components.

Even if the skin aging mechanism is not yet fully known, recent studies have shown that the skin tends to lose the molecules responsible for binding and retaining water molecules while a reduction in the number and functionality of fibroblasts and collagen occurs.

20 The exposure of the skin to external factors such as solar radiation, heat or cold temperatures contributes to accelerate the aging of the skin.

Skin aging especially in the face of an individual manifests with a thinning of the epidermis and a loss of elasticity and with the appearance of wrinkles, furrows, atrophy, relaxation and loss of skin elasticity.

25 In the cosmetic field, preparations containing substances with moisturizing, emollient and regenerating action are commonly used to improve the skin appearance and remedy to the signs of skin aging.

However, the cosmetic effects resulting from the topical applications of these cosmetic substances is not considered fully satisfactory by the users.

30 For this reason, certain techniques providing for the intradermal injection of dermo-cosmetic fillers have been developed in alternative to the topical application of cosmetic substances.

In aesthetic medicine, the dermal fillers are used to treat these signs of aging and to fill skin wrinkles or increase volumes.

Fillers are generally polymeric products injectable subcutaneously in the vicinity of wrinkles, skin furrows and in general in areas of the body where there is a sagging skin.

Once injected, they mainly carry out a mechanical action of filling the tissues, causing an action of stretching and relaxation of the skin which reduces the depth of the epidermal furrows.

The fillers available on the market can be divided into two main categories, those based on biodegradable substances and those non-biodegradable.

Non-biodegradable fillers are called permanent fillers because they are not absorbable and are generally encapsulated in fibrous tissues and are not subject to hydrolysis or phagocytosis. While exerting a prolonged action, however, permanent fillers have a higher risk of developing allergic and rejection reactions by the human body.

Typical examples of permanent fillers include liquid silicones, silicone particles in suspension, particles of acrylic hydrogel crosslinked with hyaluronic acid, calcium hydroxyapatite, polymethylmethacrylate microspheres dispersed in collagen and formulations of polyacrylamide gel.

Biodegradable fillers are called temporary because their effect wears off over time and their application needs to be renewed to maintain unchanged the filling effect. Biodegradable fillers are generally divided into two types, the intermediate duration ones and the long duration ones. The intermediate duration ones are absorbable after a few months of implantation and are made of materials subject to degradation by enzymatic hydrolysis, such as hyaluronic acid, and therefore they gradually lose the initial ability to fill and relax the tissues. Typically these polymers are biodegradable materials present in nature, with high biocompatibility and affinity with water.

However, these polymeric matrices, such as hyaluronic acid, are rapidly hydrolysed once implanted in a tissue, and the results obtained, although appreciable from an aesthetic point of view, do not last long.

In order to remedy these problems, the biodegradable polymer matrix have been

modified so as to obtain polymers in crosslinked form with longer duration of action.

At present, hyaluronic acids are available on the market which are treated with crosslinking agents so as to form covalent bonds which make the resulting polymer matrix less subject to enzymatic hydrolysis and enzymatic degradation, once injected into the tissues.

Because these crosslinked polymer matrices are less soluble and less prone to degradation, they behave as inert substances that play a mere tissue filling function, without making a contribution to improve the conditions of the tissues that have led to the formation or accelerated the formation of the wrinkle.

Some long duration of action fillers are also known which are made in synthetic polymer matrices. While having the advantage of being little biodegradable and thus having a long duration of action, these fillers are not free from drawbacks as they can cause allergic reactions or skin redness.

At present, there is still a demand for cosmetic products having an effective filling action combined with cosmetic properties.

One of the objects of the invention therefore is to provide a composition combining a filling effect with a biological effect on the skin.

A further object is to provide a dermocosmetic filler that, when injected in the skin, is almost free of side effects.

A further object of the invention is to provide an effective aesthetic or cosmetic method for the treatment of skin or photo-aging without using surgical treatments of the human body.

#### SUMMARY OF THE INVENTION

The inventors, in accordance with an aspect of the invention, found that the combination of a dermal filler component with a peptide component having biological activity provides a combined filling and regenerative effect on the skin.

In one aspect, the invention provides a composition combining the filling properties typical of dermal fillers with the eutrophic and regenerative characteristics of peptides having biological and antiaging activity.

Typically, the composition of the invention contains a two-component filler that fill in or plump body areas that have lost volume and smoothness combined with a

biologically active peptide component.

In accordance with a first aspect, the present invention provides a composition comprising a filler component in combination with a peptide having biological and antiaging activity on the skin, wherein the filler component comprises a hyaluronic acid and a type 1 recombinant collagen protein.

One of the two filler component is hyaluronic acid which may be in crosslinked or not crosslinked form.

The other filler component is a recombinant collagen, especially a Recombinant type 1 Collagen protein.

The cosmetic anti-aging components includes peptides having biological activity whose use improves the appearance of skin.

Typically, the peptides having biological properties are dipeptides preferably selected from AlaPro, ProGly, GlyPro and mixtures thereof. The composition of the invention may also contain additional amino acids or peptides.

The peptide having biological activity on the skin may be selected from AlaPro, ProGly, GlyPro and mixtures thereof.

Typically, the peptide having biological activity on the skin stimulate the fibroblast activity combining the eutrophic and anti-aging action with the mechanical function of the filler component.

The composition of the invention finds application in the cosmetic and aesthetic field and in aesthetic medicine as well.

Typically, the composition of the invention is a cosmetic composition designed to treat signs of skin aging and/or photo-aging in a human beings.

The composition may be used with the following indication of use: plump up thinning lips, enhance or fill in shallow areas on the face, decrease or remove the shadow or wrinkle under the eyes caused by the lower eyelid, fill in or soften the look of recessed scars, fill in or soften static wrinkles, especially on the lower face treat Static wrinkles which usually are a result of a loss of collagen and elasticity in the skin.

Typically, the composition is a cosmetic composition suitable to treat or ameliorate the aspect of wrinkles especially around the mouth, thin lips, and cheeks.

The composition may also be used on forehead wrinkles, scars, and other areas

that need extra volume for a smoother look.

The composition of the invention combines the mechanical function of a dermal filling with a cell regeneration activity and a delay in the skin aging process due to the sustained release of the peptides having biological activity to the tissues at the injection/implantation area.

Moreover, the combination of the fillers of the filling component increases the persistence time of the composition. For example, the composition exerts a combined filling and cosmetic effect lasting from 2 to 14 months, from 3 to 10 months, from 4 to 6 months.

Typically, the composition of the invention may be applied on the skin or administered by intradermal or subcutaneous injection or implantation.

According to an aspect, the present invention provides a cosmetic method for treating skin aging and/or photo aging comprising the injection of a cosmetically effective amount of a composition as defined herein.

According to certain embodiments, the cosmetic method of the invention stretches, reduces, mitigates wrinkles, facial furrows, reshape, or fill the volumes of the skin.

In accordance with another aspect, the present invention provides a method for ameliorating an aesthetic aspect of the skin and/or fill the volume of skin by applying on skin or injecting in the subcutaneous layer of skin an aesthetically effective amount of a composition according to anyone of the embodiments disclosed herein.

The present invention is described in details herein below by making reference to the enclosed figures.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Some features and advantages of the present invention will become apparent from the accompanying drawings, in which:

Figs. 1A and 1B are photographic images made at times before (T0) and after 7 days (T7) from the skin treatment of a facial area of a female individual by intradermal injection of a composition having the formulation of Example 1;

Fig. 2 shows a bluish area in 3D image elaboration of the facial area shown in previous Figs. 1A, 1B showing a reduction of nasolabial folds and an increase in the volume on and nearby the areas of treatment.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention originates from the finding that combining a dermal filler component with a biologically active peptide component, a synergistic cosmetic structural effect is achieved on the skin affected by skin aging, photo aging.

5 In particular, the invention originates from the finding that the subcutaneous or intradermal injection of a composition combining a dermal component with mechanical and/or filler properties with a biologically active peptide component provides an effective and prolonged antiaging action on the skin.

The prolongation of the antiaging action on the skin is due either to the extension  
10 of the filling effect and to the sustained release of the biologically active peptide component in the dermis where stimulates the production of collagen.

The application onto skin or the injection in the skin of the dermo-cosmetic composition increase the volume of the treated area determining the skin stretching and relaxation and a reduction of the depth of the epidermal  
15 corrugations.

The present invention provides either therapeutic or cosmetic uses of a composition as herein disclosed.

According to a first aspect thereof, the present invention therefore relates to a composition as defined in claim 1. Embodiments of the composition are defined in  
20 the appended dependent claims 2-7.

In another aspect, the use of a composition defined in claim 1 as dermal filler or to increase the volume of a soft tissue, especially skin, is disclosed herein.

Typically, for this use, the subcutaneous or intradermal injection of a cosmetically effective amount of the filler composition is provided.

25 A component of the composition is hyaluronic acid (HA), a naturally occurring non-sulfate linear polysaccharide comprising repeating disaccharide units of d-glucuronic acid and *N*-acetyl-d-glucosamine linked by  $\beta$ -1-3 and  $\beta$ -1-4 glycosidic bonds. Hyaluronic acid is classified in three groups depending on its molecular weights: high molecular weight (HMWHA) is greater than  $1 \times 10^6$  Da, low  
30 molecular weight (LMWHA) is from  $0.8$  to  $8 \times 10^5$  Da, and oligo-HA is equal to or less than  $6 \times 10^3$  Da.

Hyaluronic acid is a primary component of the extracellular matrix of human

connective tissues. It is also an important structural element in the skin.

The hyaluronic acid of the composition may be in free form or as a salt in the form of a physiologically acceptable salt such as sodium or potassium hyaluronate.

A suitable hyaluronic acid has a high molecular weight as defined above. For example, a suitable not-crosslinked hyaluronic acid may have a molecular weight greater than 1.000.000 Da, from 1.200.000, to 8.000.000 Da, of 1.500.000 to 3.500.000 Dalton.

Typically, the molecular weight of hyaluronic acid as described herein are weight average molecular weight MW, expressed in Dalton.

The hyaluronic acid molecular weight may be determined using standard methods, for example as described by Ueno et al., 1988, Chem Pharm Bull. 36, 4971-4975; Wyatt 1993, Anal Chim Acta 272: 1-40; Watt Technologies 1999 "Light scattering University Dawn Course Manual and "Dawn Eos Manual" Wyatt Technology Corp. Santa Barbara CA (USA).

In certain embodiments, the hyaluronic acid of the composition is at least partially cross-linked with a crosslinking agent. Suitable crosslinking agents include crosslinking agents based on dialdehydes and disulfides, PEG, diglycidyl ethers, bis-carbodiimides, bis-epoxides and mixtures thereof.

Examples of crosslinks include polyethylene glycol diglycidyl ether, polyethylene glycol epoxide, divinyl sulfone (DVS), pentaerythritol tetraglycidyl ether (PETGE), 1,4-butanediol diglycidyl ether (BDDE), 1,2-bis(2,3-epoxypropoxy)ethylene (EDGE), 1,2,7,8-diepoxyoctane (DEO), phenylene-bis-(ethyl)-carbodiimide, 1,6-hexane-ethylene bis-(ethylcarbodiimide), adipic dihydrazide (ADH), bis(sulfosuccinimidyl)suberate (BS), hexamethylenediamine (HMDA), 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane, BTDE or mixtures thereof.

The hyaluronic acid of the composition may be in the form of a gel which may be spread on the skin or administered by subcutaneous injection.

According to some embodiments, the hyaluronic acids of the invention have a crosslinking degree from 0.1 to 85%, from 1 to 50% to 20% in relation to the molecular weight of the polymer.

A component of the composition is a recombinant type 1 collagen especially recombinant type I collagen  $\alpha 1$  chain preferably produced by transgenic

silkworms. Preferably, said transgenic silkworms possess an expression cassette in its genome and expresses a recombinant protein such as recombinant type 1 collagen in its middle silk gland. The expression cassette comprises a polynucleotide having the SEQ ID NO: 1 or a part thereof as described on pages 5 13 and 14 of EP 1 811 027 B1 which typically is linked with a structural gene for the recombinant protein.

Typically, the recombinant type 1 collagen used herein may be obtained by the method disclosed in the European Patent EP 1 811 027 B1 especially in par. [0031], [0032] or claims 16 and 17, or in its priority application JP 2004301834 10 filed on 15 October 2004, especially as disclosed under par. 0040.

For example, the human recombinant type 1 collagen is obtained by a process for producing a recombinant protein comprising extracting the recombinant protein from cocoons of a transgenic silkworm which possesses (in its genome) an expression cassette for expressing the recombinant protein in silkworm middle silk 15 gland and secrete the recombinant protein in the sericin layer of silk filaments. A suitable polynucleotide comprises a polynucleotide corresponding to a promoter region of sericin 1 gene or sericin 2 gene which is functionally linked with a polynucleotide corresponding to baculovirus homologous regions especially having the nucleotide sequence of SEQ ID NO: 1 or a part thereof as reported 20 either in EP 1 811 027 or JP 2004301834.

According to some embodiments, the composition of the invention contains recombinant type 1 collagen, especially human recombinant type 1 collagen, from 0,0001 to 5%, from 0,001 to 1,000% by weight

Typically, the composition of the invention is a composition in a form suitable for 25 the application by subcutaneous or intradermal injection or by implantation in a body area, especially face, of a human being.

According to some embodiments, the composition of the invention contains from 0.01 to 80%, from 0.5 to 60%, from 1 to 50%, or from 10 to 40% by weight of HA.

According to some embodiments, the composition of the invention contains from 30 0.001 to 20%, from 0.01 to 10%, from 0,5 to 4%, by weight of recombinant type 1 collagen.

A further component of the composition is a dipeptides selected from AlaPro,

ProGly, GlyPro and mixtures thereof. The mixture of the three dipeptides is preferred to achieve a combined filler and regenerative effect on the skin.

Typically, the composition may also contains biologically active amino acid or peptide performing a function in skin care and selected from Alanine, Valine, Glycine, Proline, OH-Proline, Aspartic acid, Lisine, Glutamic Acid, Glutamine, Asparagine, Leucine and mixtures thereof. Preferably, the amino acids Alanine, Valine, Glycine, Proline, OH-Proline, lysine are L- isomers.

Advantageously, the amino acids or peptides have a biological activity on the skin, especially on skin collagen metabolism and improve the collagen synthesis rate.

The amino acids of the composition maintain the skin's hydration in physiological conditions, strength the immune system thus providing an overall healthy appearance to the skin. They amino acid also protect skin from free-radical damage and activates the physiological trophism of skin thus reducing the signs of ageing.

The above biologically active amino acids and/or peptides activate the fibroblasts and are active on the intracellular components of the skin thus improving the aesthetic aspect of the skin. This cosmetic effect on the skin is complementary to the main filling effects exerted by the filling components of the composition.

In accordance with an embodiment the composition combines cosmetically effective amounts of

- i) a filler component of hyaluronic acid and recombinant type 1 collagen with
- ii) biologically active peptides AlaPro, ProGly, GlyPro.

The composition may contain a physiologically acceptable excipient or carrier.

Typically, the physiologically acceptable carrier of the composition of the invention is an excipient, carrier or diluent suitable for intradermal or intracutaneous/intradermal administration or topical application. A suitable carrier may be water for the subcutaneous or intradermal.

Suitable carriers in liquid form include water, preferably ultrapure or for pharmaceutical use, isotonic saline solution (0.9%), aqueous solutions with phosphate buffer (PBS), Ringer's solutions, or aqueous solutions containing one or more of carbonate, citrate, acetate, glycine and mixtures thereof.

According to some embodiments, the composition of the invention contains one or

more pH regulating agents and/or buffer systems of the conventional type in the techniques of preparation of injectable pharmaceutical solutions.

For example, phosphate or tartrate buffers may be used to adjust the pH to physiologically compatible values.

- 5 In certain embodiments the composition contains a NaOH solution to have a pH from 4.5 to 8, from 6 to 7.6.

Typically, the composition of the invention in injectable form is a sterile and pyrogen-free saline.

- According to some embodiments, the composition of the invention is in the form of  
10 gel or hydrogel suitable for subcutaneous or intradermal injection.

In some embodiments, the gel or hydrogel particles have a particle size equal to or less than 700  $\mu\text{m}$ . For example, from 50 to 700  $\mu\text{m}$ , or from 100 to 400  $\mu\text{m}$ , or from 200 to 300  $\mu\text{m}$ , measured as the mass median diameter by laser diffraction technique or by microscopic analysis.

- 15 According to other embodiments, the monophasic gel is almost free from gel particles.

According to some embodiments, the composition of the invention is in the form of a water-based suspension.

- According to some embodiments, the composition of the invention further  
20 comprises one or more physiologically acceptable excipients such as antioxidants, diluents, solvents, preservatives, bactericidal agents, stabilizers, emulsifiers, surfactants, buffers, humectants, dyes, or other excipients commonly used in cosmetic/pharmaceutical preparation techniques.

- Suitable excipients are sodium chloride, sodium phosphate monobasic and or  
25 dibasic, cellulose and its derivatives such as carboxymethylcellulose, hydroxymethylcellulose, hydroxypropyl methylcellulose, methylcellulose, hydroxypropylcellulose, hydroxyethylcellulose, carboxyethylcellulose, ethylhydroxyethylcellulose, carboxymethylcellulose, cellulose acetate butyrate, cellulose acetate phthalate, and mixtures thereof.

- 30 The composition of the invention may further contain one or more antioxidants such sodium bisulfite, glutathione, coenzyme Q and mixtures thereof or vitamins such as vitamin B6, vitamin C.

In some embodiments, the composition of the invention comprises further cosmetically active substances which can be administered by injection.

In accordance with an aspect of the invention, a cosmetic or aesthetic treatment method is provided that comprises application of a cosmetically acceptable  
5 amount of a composition as defined herein.

The cosmetic or aesthetic treatment method of the invention may be applied in the treatment of any area of the body, such as the face, the lips, the area around the eyes, breasts, buttocks, and in particular to improve the aesthetic quality of a anatomical feature.

10 According to certain embodiments, the composition of the invention is suitable for use for aesthetic purposes, in particular to increase the volume of areas of the body, for example the lips, around the eyes, or in the treatment, prevention, distension of wrinkles, skin folds or corrugations, particularly those located on the face.

15 In some embodiments, the cosmetic treatment method involves the intradermal or subcutaneous injection of a composition according to any one of the embodiments previously described for filling wrinkles, facial corrugations, skin depressions or scars.

In certain embodiments, the composition of the invention may contain a local  
20 anesthetic, such as lidocaine, tetracaine, mepivacaine, procaine, benzocaine, and mixtures thereof, analgesics such as non-steroidal anti-inflammatory drugs, antimicrobials such as antibiotics for local use and mixtures thereof.

In some embodiments, the active principles contained in the composition of the present invention can be combined or mixed as active principles in intimate  
25 admixture with a suitable carrier and/or excipient according to pharmaceutical techniques.

According to some aspects of the present invention, the filler or the filler composition of the invention can be applied in the field of aesthetic medicine.

In some embodiments, the filler or the composition of the invention are applied in  
30 reconstructive surgery, such as in maxillofacial or aesthetic surgery, for example in plastic surgery or conservative aesthetics, for example in blepharoplasty or rhinoplasty.

In accordance with other aspects of the present invention, the filler or the filler composition of the invention can be applied in cosmetic and/or aesthetic filed.

In accordance with an aspect of the invention, a method for the treatment of an area of the body of an individual for aesthetic purposes is provided comprising the application or administration to an individual of a filler or of a composition comprising a filler according to the invention.

#### Definitions

In the present document, the term "carrier" refers to an excipient, carrier, diluent or adjuvant which may be present in the composition of the invention. Any carrier and/or excipient suitable for the desired form of preparation for administration is envisaged in the uses described herein.

The term "cosmetically acceptable" as used herein means that the composition or components thereof are suitable for cosmetic use and when applied, do not cause toxicity, allergic response, redness, incompatibility, instability and similar undesired reactions.

The term "physiologically acceptable" means that the substance, when applied or administered to the human body, does not cause toxicity, allergic response, redness, incompatibility, instability and similar undesired reactions.

In general, the term "crosslinked" refers to intermolecular bonds that bind molecules or individual polymer chains such as HA or monomeric moieties thereof in a more stable structure.

Within the scope of invention, the term "monophasic gel" means a network of crosslinked viscoelastic gel comprising less than 20%, less than 10%, less than 5% by weight of gel particles.

The term biocompatible means a product or products that are physiologically acceptable and that when injected do not cause significant adverse reactions or anyway if they occur, they are of local type and are among the reactions and discomfort commonly encountered upon injection or implantation of products of known use.

The following examples are provided to illustrate the present invention.

#### EXAMPLE 1

Cosmetic composition having the following formulation

| INGREDIENT                        | AMOUNT % W/W |
|-----------------------------------|--------------|
| WATER FOR INJECTIONS              | q.b          |
| Sodium Hyaluronate LW P-FIL       | 0,01-2,000   |
| Sodium Carboxymethylcellulose MW  | 0,01-4,000   |
| Human Recombinant Type 1 Collagen | 0,001-1,000  |
| ALA-PRO                           | 0,001-4,00   |
| PRO-GLY                           | 0,001-4,00   |
| GLY-PRO                           | 0,001-4,00   |
| L-ALANINE                         | 0,01-4,00    |
| L-VALINE                          | 0,01-4,00    |
| L-GLYCINE                         | 0,01-4,00    |
| L-PROLINE                         | 0,01-4,00    |
| L-OH-PROLINE                      | 0,01-4,00    |
| ASPARTIC ACID                     | 0,01-4,00    |
| LISINE HCL                        | 0,01-4,00    |
| GLUTAMIC ACID                     | 0,01-4,00    |
| GLUTAMINE                         | 0,01-4,00    |
| ASPARAGINE                        | 0,01-4,00    |
| LEUCINE                           | 0,01-4,00    |
| VITAMIN B6                        | 0,01-3,500   |
| Soda Solution 30%                 | q.b to pH    |
| SODIUM CHLORIDE                   | 0,01-1,00    |
| BIPHASIC SODIUM PHOSPHATE         | 0,01-1,00    |
| MONOPHASIC SODIUM PHOSPHATE       | 0,01-1,00    |
|                                   | 100,000      |

## EXAMPLE 2

Composition having the following formulation

| COMPOSITION                                | %                |
|--------------------------------------------|------------------|
| Water ppi                                  | 91,825           |
| Ala-pro                                    | 0,001            |
| Pro-gly                                    | 0,001            |
| Gly-pro                                    | 0,001            |
| Alanine                                    | 0,130            |
| Valine                                     | 0,170            |
| Glycine                                    | 0,150            |
| Proline                                    | 0,090            |
| OH-Proline                                 | 0,090            |
| Aspartic Acid                              | 0,150            |
| Lisine HCl                                 | 0,100            |
| Glutamic Acid                              | 0,080            |
| Glutamine                                  | 0,080            |
| Asparagine                                 | 0,080            |
| Leucine                                    | 0,030            |
| Vitamin B6                                 | 2,000            |
| Hyaluronic Acid p-fil Lw                   | 2,000            |
| Sodium Carboxymethylcellulose              | 3,000            |
| Recombinant Type 1 Collagen                | 0,022            |
| Biphasic Sodium Phosphate<br>Dodecahydrate | 0,090            |
| Monophasic Sodium Phosphate<br>Dihydrate   | 0,017            |
| Sodium Chloride                            | 0,150            |
| Sodium Hydroxide Solution 30%              | q.b pH 6,50-7,50 |

The above composition is obtained by a process including the following steps:

- 1) weigh the ppi water needed.
- 2) add under stirring, respectively: Sodium Chloride, Biphasic Sodium Phosphate Dodecahydrate, Monophasic Sodium Phosphate Dihydrate. Let dissolve.
- 3) once dissolved, add all the amino acids in the same order of the formula.
- 4) dissolve the amino acids and bring the pH in a range from 6,5 to 7,5. Bring the pH around 7,10
- 5) add the Vitamin B and let dissolve
- 6) add powdered Sodium Hyaluronate LW, as defined above, till complete dispersion
- 7) add recombinant Collagen till dispersion
- 8) add sodium Carboxymethylcellulose till dispersion and swelling overnight.

#### EXAMPLE 3

Composition with the following formulation

| INGREDIENT                       | AMOUNT % W/W |
|----------------------------------|--------------|
| WATER FOR INJECTIONS             | q.b          |
| Sodium Hyaluronate LW P-FIL      | 0,01-2,000   |
| Sodium Carboxymethylcellulose MW | 0,01-4,000   |
| Recombinant Type 1 Collagen      | 0,001-1,000  |
| ALA-PRO                          | 0,001-4,00   |
| PRO-GLY                          | 0,001-4,00   |
| GLY-PRO                          | 0,001-4,00   |
| Soda Solution 30%                | q.b to pH    |
| SODIUM CHLORIDE                  | 0,01-1,00    |
| BIPHASIC SODIUM PHOSPHATE        | 0,01-1,00    |
| MONOPHASIC SODIUM PHOSPHATE      | 0,01-1,00    |

#### EXAMPLE 4

Experimental evidence of the cosmetic effect of the combined composition.

An open, clinical trial was performed on an individual of female gender of 60 years. The individual was affected by wrinkles on the face.

Two visits were performed: basal and 1 week after the injection.

5 The individual was treated by intradermal (subcutaneous) injection of a composition having the formulation of Example 1.

The intradermal injection was performed at time T0 (baseline) during the basal visit, after evaluations planned by the study procedure, in the malar area (zygomatic protuberance), by bolus technique using a needle. In the alternative, a cannula for aesthetic uses may be used for injecting the composition.

10 The amount of tested product injected, was determined by the investigator for each subject in 3 ml. This dosage may be varied from 2 to 5 ml.

The efficacy of the cosmetic effect was evaluated in a visit made 7 days (T7) after the day of the treatment.

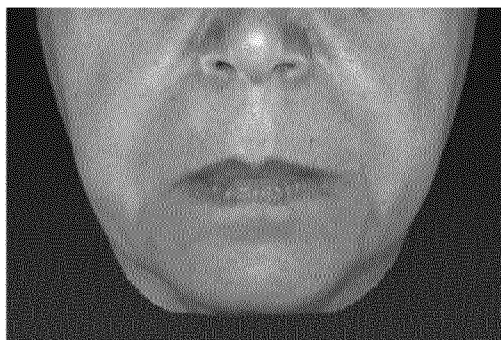
15 As can be seen in comparative Figures 1 and 2, after 7 days (T7) from the day T0 (baseline) of the cosmetic treatment, nasolabial folds were reduced and cheek volume increased (bluish area in 3D image elaboration). This outcome was confirmed by 3D image elaboration of the face of the individual made before and 7 days after the cosmetic treatment, as illustrated in Fig. 2.

## CLAIMS

1. A composition comprising a dermal filler component in combination with a peptide and/or amino acid having biological and antiaging activity on the skin, wherein the filler component comprises a hyaluronic acid or a salt thereof and a recombinant type 1 collagen protein.
2. The composition of claim 1 wherein the peptide is selected from AlaPro, ProGly, GlyPro and mixtures thereof.
3. The composition of claim 1 or 2 wherein the peptides are AlaPro, ProGly and GlyPro.
4. The composition according to anyone of claims 1-3 further comprising an amino acid selected from Alanine, Valine, Glycine, Proline, OH-Proline, Lisine, Glutamic Acid, Glutamine, Asparagine, Leucine and mixtures thereof.
5. The composition according to anyone of claims 1-3 comprising the amino acid Alanine, Valine, Glycine, Aspartic acid, Proline, OH-Proline, Lisine, Glutamic Acid, Glutamine, Asparagine, Leucine.
6. The composition according to anyone of claims 1-5 wherein the recombinant type 1 collagen is produced by transgenic silkworms.
7. The composition according to anyone of claim 1-6 wherein the hyaluronic acid is a low molecular weight from 0.8 to  $8 \times 10^5$  Da.
8. Cosmetic use of a composition according to anyone of claims 1-7 for improving an aesthetic aspect of an individual.
9. Cosmetic use according to claim 8 for increasing the volume or reshaping a soft tissue, especially skin of the face or neck.
10. Cosmetic use according to claim 8 for reducing the depth or ameliorating the aesthetic aspect of wrinkles, folds, corrugations, furrows, folds, in particular on the face or neck.
11. Composition comprising a dermal filler component in combination with a peptide and/or amino acid having biological and antiaging activity on the skin, wherein the filler component comprises a hyaluronic acid or a salt thereof and a recombinant type 1 collagen protein for use in the treatment of wrinkles, folds, corrugations, furrows, folds by a subcutaneous injection

of a cosmetically active amount of said composition.

FIG. 1A



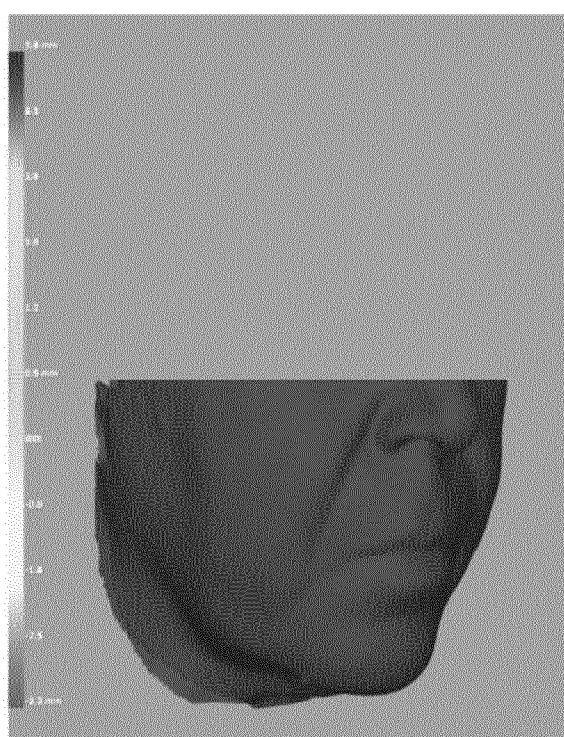
T0 (baseline)

FIG. 1B



T7  
(7 days after the injection procedure)

FIG. 2

 $\Delta T7$  vs  $T0$

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2019/070361

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A61K8/65 A61K8/73 A61Q19/08  
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 A61Q

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, Sequence Search, EMBASE, EMBL, INSPEC, CHEM ABS Data, SCISEARCH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents :

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"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

25 March 2020

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2019/070361

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International application No

PCT/EP2019/070361

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