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(54) Title: COMPOSITIONS AND METHODS FOR IMPROVING SKIN APPEARANCE

(57) Abstract: Disclosed herein are injectable compositions and methods of treating skin to improve hydration, elasticity and/or texture. The compositions are based on crosslinked hyaluronic acid matrices made with low molecular weight hyaluronic acids.

COMPOSITIONS AND METHODS FOR IMPROVING SKIN APPEARANCE

By Inventors: Pierre Lebreton and Olivier Guetta

[0001] The present invention generally relates to injectable compositions and more specifically relates to hyaluronic acid-based compositions for treatment of fine lines in skin.

Current injectable treatment options for improving skin quality over the full-face or other significant surface area of skin require multiple treatment sessions (typically 3-4 treatments, every three to four weeks) and have a relatively short duration of effect.

Skin is composed of the epidermis and the dermis. The outermost epidermis is made up of stratified squamous epithelium with an underlying basement membrane. It contains no blood vessels, and is nourished by diffusion from the dermis. The main type of cells which make up the epidermis are keratinocytes, with melanocytes and langerhans cells being also present. This layer of skin is responsible for keeping water in the body and keeping harmful chemicals and pathogens out.

[0002] The dermis lies below the epidermis and contains a number of structures including blood vessels, nerves, hair follicles, smooth muscle, glands and lymphatic tissue. The dermis (or corium) is typically 3-5 mm thick and is the major component of human skin. It is composed of a network of connective tissue, predominantly collagen fibrils providing support and elastic tissue providing flexibility. The main cell types are fibroblasts, adipocytes (fat storage) and macrophages. Hyaluronic acid (HA) is a part of the dermis composition and is a major component of the extra cellular matrix.

[0003] Facial aging occurs as the result of several factors: inherent changes within the skin, effects of gravity, facial muscles acting on the skin (dynamic lines), soft tissue loss or shift and bone loss and loss of tissue elasticity. The skin ages when the epidermis begins to thin, causing the junction with the dermis to flatten. Collagen decreases as a person ages and the bundles of collagen, which gives the skin turgor, become looser and lose strength. When the skin loses elasticity, it is less able to resist stretching. Coupled with gravity, muscle pull and tissue changes, the

skin begin to wrinkle. Water loss and breakdown of bonds between cells also reduces the barrier function of the skin, which can cause the skin's pore size to increases.

[0004] It is well known that the eyes are often the first facial feature to show signs of aging. Skin changes around the eyes occur earlier than in the rest of the face since the skin is thinner around the eyes. The skin here contains fewer glands and is subjected to constant blinking, squinting, rubbing, and pulling. The midface ages when the cheeks begin to droop, causing nasolabial folds. Nasolabial folds are the lines that run from the sides of the nose to the corners of the mouth. In the lower face area, as the face ages, facial tissues descend. This results in the so-called "laugh lines". These and other folds and wrinkles are now commonly treated with subdermal and dermal injections of aesthetic facial fillers which add lost volume to the skin thereby reducing the appearance of the folds and wrinkles.

[0005] Hyaluronic acid (HA), also known as hyaluronan, is now one of the most commonly used components of dermal fillers. Hyaluronic acid is a naturally occurring, water soluble polysaccharide, specifically a glycosaminoglycan, which is a major component of the extra-cellular matrix and is widely distributed in animal tissues. The identical structure of hyaluronic acid in all species and tissues makes this polysaccharide an ideal substance for use as a bio-material in health and medicine.

[0006] HA has excellent biocompatibility and, unlike collagen, does not require any skin testing before implantation. In addition, HA has the ability to bind to large amounts of water, making it an excellent volumizer of soft tissues.

[0007] To enhance its longevity in vivo, the HA in dermal fillers is commonly crosslinked. Chemically crosslinked HA is formed by reacting uncrosslinked HA with a crosslinking agent under suitable reaction conditions.

[0008] It is generally accepted that HA-based dermal fillers having a high viscosity, for example, those that are highly crosslinked and/or made of high molecular weight HA and/or having a high HA concentration tend to last longer in the body. Conversely, it is generally accepted that HA-based dermal fillers having a low

viscosity, for example, those that are more lightly crosslinked and/or made up of low molecular weight HA and/or have a low HA concentration, may have a shorter duration in the body. Naturally, injection of a high viscosity material through a needle is relatively more difficult, and generally requires a lower gauge needle (for instance, 21G or 23G compared to 27G or 30G) than injection of a relatively low viscosity material. It has proven difficult to develop an HA based composition that is both easy to inject through a high gauge needle (i.e. thin needle) and which has extended duration in the body.

Summary

[0009] The present invention relates to injectable compositions, and more specifically, to injectable compositions for intradermal injection into skin. The compositions and methods provide improved skin appearance and quality by filling of superficial skin depressions, and/or improving skin quality and appearance. In one aspect, the compositions and methods provide at least one of improved skin texture, increased skin hydration and increased elasticity.

[0010] In one aspect, the present compositions are based on hyaluronic acid (HA) and pharmaceutically acceptable salts of HA, for example, sodium hyaluronate (NaHA). Many of the long lasting, highly injectable compositions of the present invention include a crosslinked HA matrix made with relatively low molecular weight HA. In some embodiments, the compositions have a relatively low concentration of HA. Advantageously, many of the compositions provided herein have an extended duration of effect. For example, rather than requiring multiple repeated treatments every three to four weeks, as is common with conventional intradermal injection treatment methods, many of the present compositions and methods provided herein have a duration of effect of three months, four months, six months, to a year or more.

[0011] In a broad aspect of the invention, a composition is provided which generally comprises an HA gel containing, or consisting essentially of, a low molecular weight HA material. The HA component includes more than 50%, for example, at least 70%, for example, about 90% by weight of the low molecular weight HA. The low molecular weight HA material has a weight average molecular

weight of no greater than about 0.20 MDa and about 0.99 MDa such as about 0.4 MDa to about 0.8 MDa.

[0012] In some embodiments, the HA gel may further contain a high molecular weight HA, that is, a HA material having a molecular weight of at least about 1.0 MDa to about 4.0 MDa. Generally, in embodiments of the invention including high molecular weight HA material, the weight average molecular weight of the high molecular weight HA material is at least twice that of the low molecular weight HA material.

[0013] The HA of the HA gel may be crosslinked. For example, the HA may be chemically crosslinked by a suitable crosslinking agent. In some embodiments, the crosslinking agent is selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), or 1,4-bis(2,3-epoxypropoxy)butane, or 1,4-bisglycidyloxybutane (all of which are commonly known as BDDE), 1,2-bis(2,3-epoxypropoxy)ethylene and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane.

[0014] In some embodiments, the composition has an HA concentration of about 10.0 mg/g up to about 17.0 mg/g. In some embodiments, the HA concentration is less than about 17.0 mg/g, for example, less than about 15.0 mg/g. In some embodiments, the HA concentration is between about 10.0 mg/g and about 14.0 mg/g. In some embodiments, the HA concentration is about 10.0 mg/g, about 11.0 mg/g, about 12.0 mg/g, about 13.0mg/g, or about 14.0 mg/g.

[0015] In another aspect of the invention, methods of treating skin using the present compositions are provided. For example, methods of improving one or more qualities of skin, or improving appearance or texture of skin, are provided.

[0016] In one aspect, methods of treating dryness, texture or roughness, and/or elasticity in skin are provided. The methods generally comprise treating an area of skin by introducing, into the area, multiple, spaced apart injections of a composition comprising a hyaluronic acid (HA) gel containing crosslinked HA, wherein the treated skin maintains an improved hydration, smoother texture or increased elasticity, due to the treatment for an extended duration, for example, for at least about 3 months to about a year or more. In a particularly advantageous embodiment, the step of

introducing is performed in only a single treatment session, thereby eliminating the need for repeated treatments to maintain the duration of effect.

[0017] In some embodiments, the step of introducing comprises introducing the composition in injections spaced apart by a distance of between about 2 mm to about 30 mm. For example, the step of introducing comprises introducing the composition in injections spaced apart by a distance of between about 5 mm to about 20 mm, or about 10 mm to about 15 mm. In some embodiments, the compositions are introduced at an injection depth of between about 500 microns and about 2000 microns, for example, a depth of about 1000 microns. In preferred embodiments, the compositions are introduced at an injection depth of between about 0.5 mm to about 5.0 mm, preferably about 1.0 mm to about 4.0 mm, more preferably from about 1.5 mm to about 3.0 mm. In general, a deeper injection provides improved hydration results.

[0018] In another aspect, the invention provides a method of treating roughness in skin comprising treating an area of skin by introducing, into the area, multiple, spaced apart injections of a composition comprising a hyaluronic acid (HA) gel containing crosslinked HA, wherein the treated skin maintains a smoother texture due to the treatment for at least about 3 months, at least about 4 months, at least about 6 months, or at least about 12 months.

Detailed Description

[0019] The term "about" in the context of numerical values will be readily understood by a person skilled in the art, and preferably means that specific values may be modified by +/- 10%. As regards endpoints of ranges, the modifier "about" preferably means that the lower endpoint may be reduced by 10% and the upper endpoint increased by 10%. It is also contemplated that each numerical value or range disclosed in this application can be absolute, i.e. that the modifier "about" can be deleted.

[0020] All numbers herein expressing "molecular weight" of HA are to be understood as indicating the weight average molecular weight (Mw) in Daltons.

[0021] The molecular weight of HA is calculated from an intrinsic viscosity measurement using the following Mark Houwink relation:

$$\text{Intrinsic Viscosity } (m^3/kg) = 9.78 \times 10^{-5} \times Mw^{0.690}$$

[0022] The intrinsic viscosity is measured according to the procedure defined European Pharmacopoeia (HA monograph N°1472, 01/2009).

[0023] High molecular weight HA as used herein describes a HA material having a molecular weight of at least about 1.0 million Daltons ($Mw \geq 10^6$ Da or 1 MDa) to about 5.0 MDa. For example, the high molecular weight HA in the present compositions may have a molecular weight in the range about 1.5 MDa to about 3.0 MDa, or the high molecular weight HA may have a weight average molecular weight of about 2.0 MDa. In another example, the high molecular weight HA may have a molecular weight of about 3.0 MDa. In another example, the high molecular weight HA may have a molecular weight of about 1 MDa which corresponds to an intrinsic viscosity of 1.35 m^3/kg when calculated according to the Mark Houwink relation as indicated above, 1.5 MDa (1.35 m^3/kg), 2 MDa (2.18 m^3/kg), 3 MDa (2.88 m^3/kg), 5 MDa (4.10 m^3/kg), 0.2 MDa (0.44 m^3/kg), 0.4 MDa (0.72 m^3/kg), 0.8 MDa (1.1 m^3/kg), 0.99 MDa (1.34 m^3/kg).

[0024] Low molecular weight HA as used herein describes a HA material having a molecular weight of less than about 1.0 MDa. Low molecular weight HA can have a molecular weight of between about 200,000 Da (0.2 MDa) to less than 1.0 MDa, for example, between about 400,000 Da and about 800,000 Da, for example about 386,000 Da (0.386 MDa) to about 740,000 Da (0.74 MDa). In some embodiments the low molecular weight HA used to make the crosslinked HA matrix does not exceed 0.99 MDa.

[0025] Preferably, the mixture of the low molecular weight HA and high molecular weight HA has a bimodal molecular weight distribution. The mixture may also have a multi-modal distribution.

[0026] In one aspect of the invention, the compositions comprise HA having a high molecular weight component and a low molecular weight component, and the

high molecular weight component has a weight average molecular weight at least twice the weight average molecular weight of the low molecular weight component.

[0027] For example, a composition in accordance with this aspect of the invention may include a low molecular weight component having a weight average molecular weight of about 500,000 Da, and a high molecular weight component having a weight average molecular weight of about, or at least about, 1.0 MDa.

[0028] In another example, a composition in accordance with the invention may include a low molecular weight component having a weight average molecular weight of about 800,000 Da, and a high molecular weight component having a weight average molecular weight of about, or at least about, 1.6 MDa.

[0029] Degree of crosslinking is measured by the final weight ratio of crosslinker to HA disaccharide units.

[0030] Uncrosslinked HA as used herein refers to individual HA polymer molecules that are not crosslinked to one another. Uncrosslinked HA generally remains water soluble.

[0031] Provided herein are compositions for treating skin, for example, injectable compositions that can be introduced intradermally to improve the appearance or quality of skin, for example, to improve hydration, texture and/or elasticity. The compositions may also be effective for treatment of fine lines in skin and for reducing superficial cutaneous depressions. Methods of making these compositions are also provided, as well as methods of treatment using these compositions. The compositions are based on hyaluronic acids (HA) and pharmaceutically acceptable salts of HA, for example, sodium hyaluronate (NaHA).

[0032] As used herein, hyaluronic acid (HA) can refer to any of its hyaluronate salts, and includes, but is not limited to, sodium hyaluronate (NaHA), potassium hyaluronate, magnesium hyaluronate, calcium hyaluronate, and combinations thereof. Both HA and pharmaceutically acceptable salts thereof can be used in this invention.

[0033] Generally, the concentration of HA in some of the present compositions is about 10.0 mg/g up to about 17.0 mg/g. In some embodiments, the HA concentration is less than about 17.0 mg/g, for example, less than about 15.0 mg/g. In some embodiments, the HA concentration is between about 10.0 mg/g and about 14.0 mg/g. In some embodiments, the HA concentration is about 10.0 mg/g, about 11.0 mg/g, about 12.0 mg/g, about 13.0mg/g, or about 14.0 mg/g.

[0034] Some of the compositions of the invention include additional agents, for example, anesthetic agents in an amount effective to mitigate pain experienced upon injection of the composition. The anesthetic agent may be selected from the group of ambucaine, amolanone, amylocaine, benoxinate, benzocaine, betoxycaine, biphenamine, bupivacaine, butacaine, butamben, butanilicaine, butethamine, butoxycaine, carticaine, chloroprocaine, cocaethylene, cocaine, cyclomethycaine, dibucaine, dimethisoquin, dimethocaine, diperodon, dicyclomine, ecgonidine, ecgonine, ethyl chloride, etidocaine, beta-eucaine, euprocin, fenalcomine, formocaine, hexylcaine, hydroxytetracaine, isobutyl p-aminobenzoate, leucinocaine mesylate, levoxadrol, lidocaine, mepivacaine, meprylcaine, metabutoxycaine, methyl chloride, myrtecaine, naepaine, octocaine, orthocaine, oxethazaine, parethoxycaine, phenacaine, phenol, piperocaine, piridocaine, polidocanol, pramoxine, prilocaine, procaine, propanocaine, proparacaine, propipocaine, propoxycaine, pseudococaine, pyrrocaine, ropivacaine, salicyl alcohol, tetracaine, tolycaine, trimecaine, zolamine, and salts thereof. In one embodiment, at least one anesthetic agent is lidocaine, such as in the form of lidocaine HCl. The compositions described herein may have a lidocaine concentration of between about 0.1% and about 5% by weight of the composition, for example, about 0.2% to about 1.0% by weight of the composition. In one embodiment, the composition has a lidocaine concentration of about 0.3% by weight (w/w %) of the composition.

[0035] In some embodiments, the compositions further comprise a beneficial additive, for example, an antioxidant. In some embodiments, the compositions include, for example, mannitol . The mannitol may be present in an amount of between about 0.1% w/w to about 2.0% w/w, or between about 0.3% to about 0.9% w/w. In some embodiments, the mannitol is present in an amount of less than, no greater than, or about 1.0% w/w. In some embodiments, the mannitol is present in

an amount of about 0.9 % w/w. In other embodiments, the mannitol is present in an amount of about 0.1% w/w, or about 0.2% w/w, or about 0.3% w/w, or about 0.4% w/w, or about 0.5% w/w, or about 0.6% w/w, or about 0.7% w/w, or about 0.8% w/w, or about 0.9% w/w, or about 1.0% w/w. In other embodiments, the mannitol is present in an amount of greater than about 1.0% w/w. In some embodiments, the mannitol is present in an amount of between about 1.0% w/w to about 5.0% w/w.

[0036] In some embodiments, the compositions further comprise a vitamin, for example, Vitamin C. In a more preferred embodiment, the vitamin is a derivative or a stabilized form of Vitamin C, for example, ascorbic acid 2-glucoside. The vitamin may be present in an amount of between about 0.1% to about 2.0% w/w, or between about 0.2% and about 1.0% w/w, or between about 0.3% to about 0.6% w/w. In some embodiments, the Vitamin C is present in an amount of about 0.6% w/w. In other embodiments, the Vitamin C derivative is present in an amount of about 0.1% w/w, or about 0.2% w/w, or about 0.3% w/w, or about 0.4% w/w, or about 0.5% w/w, or about 0.6% w/w, or about 0.7% w/w, or about 0.8% w/w, or about 0.9% w/w, or about 1.0% w/w. In other embodiments, the Vitamin C derivative is present in an amount of greater than 1.0%. In some embodiments, the Vitamin C derivative is present in an amount of between about 1.0% w/w to about 5.0% w/w.

[0037] In some embodiments, the compositions further comprise a combination of mannitol and ascorbic acid 2-glucoside. In some of these embodiments, the mannitol is present in an amount of no greater than 1.0% w/w, for example, 0.9 % w/w and the ascorbic acid 2-glucoside is present in an amount of about 0.6% w/w.

[0038] In some embodiments, the compositions do not include an antioxidant or a vitamin. For example, in some embodiments, the compositions comprise or consist essentially of hyaluronic acid crosslinked with a crosslinking agent and water. These compositions may or may not include an anesthetic agent such as lidocaine.

[0039] The present products and compositions are preferably provided in a sterile form. The compositions may be sterilized using conventional methods, for example, autoclaving. For example, the compositions may be sterilized by exposing the compositions to temperatures of at least about 120°C to about 130°C and/or

pressures of at least about 12 pounds per square inch (PSI) to about 20 PSI for a period of at least about 1 minute to about 15 minutes.

[0040] Method of making the composition comprise the steps of providing raw HA material in the form of dry HA fibers or powder. The raw HA material may be HA, its salts and/or mixtures thereof. In a preferred embodiment, the HA material comprises fibers or powder of NaHA, for example, bacterial-sourced NaHA fibers. In some aspects of the present description, the HA material may be animal derived. The HA material may be a combination of raw materials including HA and at least one other polysaccharide, for example, glycosaminoglycan (GAG).

[0041] In a broad aspect of the invention, the HA material of the compositions may comprise a crosslinked HA matrix made with between about 5% to about 95% low molecular weight HA with the balance of the HA material including high molecular weight HA.

[0042] In some embodiments of the invention, the HA material used to make the present compositions nearly entirely comprises or consists of low molecular weight HA. In some embodiments, nearly 100% of the HA material used to make the present compositions may be low molecular weight HA as defined above. In other embodiments, the HA material used to make the compositions comprises a combination of relatively high molecular weight HA and relatively low molecular weight HA, as defined above. In certain embodiments, at least about 50% w/w, for example, at least about 70% w/w, for example, at least about 90% w/w or greater of the HA material in the compositions is a low molecular weight HA as defined above, with the remaining portion of HA being high molecular weight HA. In one embodiment, the compositions are made using a 90:10 ratio of low molecular weight HA to high molecular weight HA. That is, the compositions of these embodiments are made with a combination of high and low molecular weight HA, with about 90% w/w of the HA being the low molecular weight HA.

[0043] In one embodiment, the pure, dry NaHA fibers are hydrated in an alkaline solution to produce an uncrosslinked NaHA gel. Any suitable alkaline solution may be used to hydrate the NaHA in this step, for example, but not limited to aqueous solutions containing sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium

bicarbonate ($NaHCO_3$), lithium hydroxide (LiOH), and the like. In another embodiment, the suitable alkaline solution is aqueous solutions containing NaOH. The resulting alkaline gel will have a pH above 7.5. The pH of the resulting alkaline gel can have a pH greater than 9, or a pH greater than 10, or a pH greater than 11, or a pH greater than 12, or a pH greater than 13.

[0044] The next step in the manufacturing process involves the step of crosslinking the hydrated, alkaline NaHA gel with a suitable crosslinking agent. The crosslinking agent may be any agent known to be suitable for crosslinking polysaccharides and their derivatives via their hydroxyl groups. Suitable crosslinking agents include but are not limited to, 1,4-butanediol diglycidyl ether (or 1,4-bis(2,3-epoxypropoxy)butane or 1,4-bisglycidyloxybutane, all of which are commonly known as BDDE), 1,2-bis(2,3-epoxypropoxy)ethylene and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane. The use of more than one crosslinking agent or a different crosslinking agent is not excluded from the scope of the present disclosure. In one embodiment, the HA gels described herein are crosslinked using BDDE.

[0045] The step of crosslinking may be carried out using any means known to those of ordinary skill in the art. Those skilled in the art appreciate how to optimize conditions of crosslinking according to the nature of the HA, and how to carry out crosslinking to an optimized degree.

[0046] In another embodiment, the crosslinking of the HA is accomplished during hydration of the HA fibers, by hydrating the combined high and low molecular weight fibers in an alkaline solution containing a crosslinking agent, for example, BDDE.

[0047] The degree of crosslinking in the HA component of the present compositions is at least about 1% and is up to about 20% BDDE/HA, w/w, for example, between about 4% and about 12% w/w, for example, about 10 % w/w, for example, about 8% w/w, for example, about 6% w/w, for example, about 5% w/w, for example, about 4% w/w.

[0048] The hydrated crosslinked, HA gels may be swollen to obtain the desired HA concentration. This step can be accomplished by neutralizing the crosslinked, hydrated HA gel, for example by adding an aqueous solution containing of an acid,

such as HCl. The gels are then swelled in a phosphate buffered saline (PBS) solution.

[0049] The gels may be purified by conventional means such as, dialysis against a phosphate buffer, or alcohol precipitation, to recover the crosslinked material, to stabilize the pH of the material and to remove any un-reacted crosslinking agent. The compositions are mixed to achieve homogeneity. Preferably, the homogenization step comprises mixing, stirring, or beating the gels with a controlled shearing force obtaining substantially homogenous mixtures. In some embodiments, during or after the mixing, a phosphate buffer is added to reach the desired concentration of HA in the final composition.

[0050] In some embodiments, lidocaine (e.g. in the form of lidocaine HCl), or another suitable anesthetic, is added to the compositions. For example, the pH of the purified, substantially pH neutral, gel is adjusted to cause the gel to become slightly alkaline such that the gels have a pH of greater than about 7.2, for example, about 7.5 to about 8.0. Alternatively, the gel is adjusted to cause the gel to become alkaline such that the gels have a pH of greater than about 9, for example, about 10.0 to about 11.0. This step may be accomplished by any suitable means, for example, by adding to the gels a suitable amount of dilute NaOH, KOH, $NaHCO_3$ or LiOH, or any other alkaline molecule, solution and/or buffering composition know by one skilled in the art. For example, in some embodiments, the lidocaine HCl is provided in a powder form which is solubilized using water for injection (WFI). The lidocaine is then added to the (slightly) alkaline gel. Uncrosslinked HA may then be added to the lidocaine-containing gel, if desired. For example, in one embodiment, the desired HA concentration is about 12.0 mg/g. The compositions including lidocaine may have a lidocaine concentration of between about 0.1% and about 5% by weight of the composition, for example, about 0.3% by weight (or w/w), based on the total weight of the composition. The compositions including uncrosslinked HA may have an uncrosslinked HA concentration of less than 10 % w/w or less than 5 % w/w such as preferably between about 0.5% and about 1.5% by weight of the composition, for example, about 0.9 to 1.0%, based on the total weight of the composition. The uncrosslinked HA has preferably a high molecular weight.

[0051] The compositions are introduced into delivery devices, for example, syringes. Syringes useful according to the present description include any syringe known in the art capable of delivering viscous dermal filler compositions. The syringes may have an internal volume of about 0.4 mL to about 3 mL, between about 0.5 mL and about 1.5 mL or between about 0.8 mL and about 1.0 mL.

[0052] In other embodiments, the compositions are introduced into injection devices suitable for delivering the compositions using multiple microdepot injections, into relatively shallow, superficial, surfaces of skin.

[0053] The gauges of needles used to deliver the present compositions include gauges of between about 18G and about 40G. In some embodiments, the needles for delivering the compositions are between about 25G to about 33G such as between about 31G to about 33G or about 32G to about 33G. In some embodiments, the compositions are delivered using a needle having a gauge of 28G, 29G, 30G, 32G or 33G.

[0054] In another aspect of the invention, methods of treating skin using the present compositions are provided. For example, methods of improving one or more qualities of skin, or improving appearance or texture of skin, are provided.

[0055] In one aspect, methods of treating dryness, texture or roughness, and/or elasticity in skin are provided. The methods generally comprise treating an area of skin by introducing, into the area, multiple, spaced apart injections of a composition comprising a hyaluronic acid (HA) gel containing crosslinked HA, wherein the treated skin maintains an improved hydration, smoother texture or increased elasticity, due to the treatment for an extended duration, for example, for at least about 3 months to about a year or more.

[0056] In a particularly advantageous embodiment, the step of introducing is performed in only a single treatment session, thereby eliminating the need for repeated treatments to maintain the duration of effect.

[0057] In one aspect of the invention, a method of treating skin in provided comprising introducing the composition into the skin during a treatment session comprising multiple injections of the composition into a skin region.

[0058] In one embodiment, a treatment session comprises a single visit by the patient to a practitioner. During the treatment session, multiple injections into the skin, for example into a particular skin region, may be administered.

[0059] The multiple injections of a single treatment session may comprise, for example, from 2 to about 500 injections, from about 50 to about 200 injections. In some embodiments, a treatment session comprises, for example, at least 2 injections, at least 10 injections, at least 20 injections, at least 40 injections, at least 60 injections, at least 80 injections, at least 100 injections, at least 140 injections, at least 180 injections, at least 200 injections, at least 300 injections, at least 400 injections at least 500 injections, or more, into the skin region.

[0060] In some embodiments, the treatment session takes no longer than about 45 minutes, no longer than about 30 minutes, no longer than about 15 minutes, or no longer than about 10 minutes per treatment area. Treatment area is defined as a skin region being treated with the present compositions and methods. The treatment area may comprise or consist of a skin region of at least one of a face, a neck, or a décolletage. The treatment area may also comprise or consist of a region of skin other than a face, neck or décolletage, for example, a skin region of the top of the hand, a knee, an elbow, a forearm, a calf, a thigh, a back, or any other region of skin that can be treated using the present compositions and methods and can be benefitted or improved thereby.

[0061] In some embodiments, the step of introducing comprises introducing the composition in injections spaced apart by a distance of between about 2 mm to about 30 mm. For example, the step of introducing comprises introducing the composition in injections spaced apart by a distance of between about 5 mm to about 20 mm. In some embodiments, the step of introducing comprises introducing the composition in injections spaced apart by a distance of between about 10 mm to about 15 mm.

[0062] In some embodiments, the injections are provided at a very superficial depth in the skin. For example, in some embodiments, the compositions are introduced at a depth of no greater than about 2000 microns. For example, the injections may be provided at a depth of about 500 microns to about 2000 microns,

about 800 microns to about 1600 microns, about 1000 microns to about 1200 microns. In preferred embodiments, the compositions are introduced at an injection depth of between about 0.5 mm to about 5.0 mm, preferably about 1.0 mm to about 4.0 mm, more preferably from about 1.5 mm to about 3.0 mm. In one embodiment, the injections are introduced in an amount of about 1 µL to about 200 µL, for example, about 5 µL to about 100 µL per injection, for example, between about 20 µL to about 80 µL, for example, about 40 µL to about 60 µL per injection. In some embodiments, the injections are introduced in an amount of about 5 µL to about 500 µL per injection, about 10 µL to about 400 µL, about 50 µL to about 200 µL, or about 100 µL per injection.

[0063] In some embodiments, the injections are delivered through a needle having a gauge of at least 27 G, for example, 28 G, 30 G or 32 G.

[0064] Advantageously, method of treatment may comprise or consist of a single treatment session lasting a relatively short amount of time. In some embodiments, the treatment session covering the region of skin being treated, comprises multiple, intradermal injections into the skin region, and takes no longer than about 45 minutes. In some embodiments, the treatment session takes no longer than about 30 minutes. In yet other embodiments, the treatment session takes no longer than about 20 minutes, or no longer than about 15 minutes, or no longer than about 10 minutes.

[0065] A method of treating roughness in skin is also provided, wherein the method comprises treating an area of skin by introducing, into the area, multiple, spaced apart injections of a composition comprising a hyaluronic acid (HA) gel containing crosslinked HA wherein the treated skin maintains a smoother texture due to the treatment for at least about 3 months, for at least about 4 months, for at least about 6 months, for at least about 9 months, or for at least about 12 months.

[0066] In a particular embodiment, the compositions of the invention comprise a sterile physiological solution of hyaluronic acid (HA) gel of non-animal origin cross-linked with 1,4-Butanediol Diglycidyl ether (BDDE) at a concentration of 12mg/mL. The compositions are useful for treatment of superficial skin depressions as

measured by improvement in skin texture and improvement of skin quality, such as smoothness, hydration and elasticity, as compared to non-treated superficial skin depressions. In a particular embodiment, methods of treatment include injections, for example, depot injections, into the dermis using a 32G needle across the target anatomic region(s). The target anatomic regions include skin regions of the face, and skin regions of the neck. The improvement of skin appearance, for example, reduced appearance of depressions, fine lines and uneven or rough texture as compared to untreated skin, may be markedly noticeable by the patient, and the improvement may last for a duration of at least 4 months, 6 months, 9 months or even 12 months, after a single treatment session lasting only 45 minutes, or only 30 minutes, or only 20 minutes, or only 15 minutes, or less. Improvement of skin texture can be evaluated using appropriate scales. Skin quality measurements of hydration and elasticity can be performed, using appropriate instrumentation, and compared to baseline, i.e. untreated skin. Other evaluation methods, for example, FACE Q and GAIS can be utilized to assess subject and investigator satisfaction, respectively. In a specific embodiment, the duration of action of the product after the treatment is at least about 4 months for example, about 6 months.

[0067] The addition of lidocaine to the compositions, in some embodiments, reduces pain in the treatment area. However, in some embodiments, the compositions do not include lidocaine, in order to address patient need with regard to allergy to lidocaine and pain sensitivity.

[0068] Another embodiment provides a method of increasing at least one of smoothness, hydration, and elasticity in skin comprising introducing, into a skin region at a depth of between about 500 microns and about 5000 microns, in a single treatment session, multiple, spaced apart injections of a composition comprising a hyaluronic acid (HA) gel containing BDDE-crosslinked HA and uncrosslinked HA, wherein the injections are introduced in an amount of about 5 µL to about 100 µL per injection, wherein the injections are delivered through a needle having a gauge of from 28 G to 33 G, wherein the injections are spaced apart by a distance of between about 5 mm to about 20 mm.

[0069] In some embodiments, the skin region maintains an increased smoothness, hydration, and/or elasticity due to the treatment for at least about 3 months, about 4 months, about 5 months, about 6 months, 9 months, 12 months, or longer.

[0070] In another embodiment, a composition for increasing at least one of smoothness, hydration, and elasticity in skin is provided comprising a hyaluronic acid (HA) gel comprising a crosslinked HA matrix made with a low molecular weight HA material having a weight average molecular weight of between about 400,000 Da and about 800,000 Da; wherein the HA concentration of the composition is from about 10.0 mg/g to about 14.0 mg/g; and wherein the composition maintains the increased smoothness, hydration, and/or elasticity due to the treatment for at least about 6 months from being introduced into the skin in a single treatment session comprising multiple, spaced apart injections of the composition in which the injections are introduced in an amount of about 5 µL to about 100 µL per injection, delivered through a needle having a gauge of from 28 G to 33 G and are spaced apart by a distance of between about 5 mm to about 20 mm. The skin region may maintain an increased smoothness, hydration, and/or elasticity due to the treatment for at least about 3 months, about 4 months, about 5 months, about 6 months, 9 months, 12 months, or longer. The compositions may further include at least one of a mannitol and a Vitamin C. In some embodiments, both mannitol and a Vitamin C derivative are present in the compositions.

[0071] The compositions are associated with a gel hardness G' of between about 50 to 200 Pa, such as 100-150 Pa or 120 Pa, when measured at 0.1 Hz or between about 100 to 300 Pa, such as 150 to 200 Pa or 175 Pa, when measured at 5 Hz. The method for measuring the gel hardness is known in the art. The gel hardness is indicative for the dermal filler's softness.

[0072] The compositions are further associated with a gel viscosity G'' of between about 10 to 100 Pa, such as 15-40 Pa or 20 Pa, when measured at 0.1 Hz or between about 10 to 100 Pa, such as 20-40 Pa or 30 Pa, when measured at 5 Hz. The method for measuring the visosity is known in the art.

[0073] The compositions are also associated with a compression of between about 5 to 20 N, such as 10-15 N or 12 N. The method for measuring the compression is known in the art. The compression is indicative for the dermal filler's resistance to deformation. Generally, the lower the compression, the lower the filler's lift spreadibility, i.e. the filler is then more suitable for treating superficial lines and folds in the face or neck as compared to fillers having a higher compression which are more suitable for deeper injections and volume restorations.

EXAMPLE 1

The free radical degradation test allows to evaluate the resistance of a gel sample toward the degradation of the HA chains by the free radicals (one of the main degradation pathway of HA). Free radical degradation tests was carried out on 3 different batches of the present compositions. The results obtained are presented on the Table1. All measures were found to conform (CVr<10%).

Gel		initial viscosity (Pa.s)	degradation time (s)	Conformity
LBA2-214		17.7	4572	CF
		15.5	4858	
		16.1	5044	
	Mean	**16.4**	**4825**	
	STD	1.1	238	
	CVr (%)	7%	5%	
LBA2-263		12.5	5377	CF
		13.8	5009	
		13.9	4882	
	Mean	**13.4**	**5090**	
	STD	0,8	257	
	CVr (%)	6%	5%	
LBA2-288		13.7	5057	CF
		14.1	5189	

	13.7	4878	
Mean	**13.8**	**5041**	
STD	0.2	156	
CVr (%)	2%	3%	

TABLE 1 Free radical degradation results for the present compositions

The inter-batch variability is around 3% on the 3 different batches and the average value of degradation time is 4985 s (Table 2).

	LBA2-214	**LBA2-288**	**LBA2-263**	
mes1	4572	5057	5377	
mes2	4858	5189	5009	
mes3	5044	4878	4882	
mean	4825	5041	5089	**4985**
STD	238	156	257	**141**
CVr	5%	3%	5%	**3%**

TABLE 2 Inter-batch variability for the present compositions degradation times

EXAMPLE 2

[0074] A 37-year old woman presents with rough, dry facial skin due to aging, dry climate and/or sun/wind exposure over the course of her lifetime. The physician administers, by intradermal, micro-depot injections, the compositions described herein. The treatment consists of from 10 to about 100 shallow injections per skin region, with a 32 G/4 mm needle. The skin regions treated are the face, neck, and décolleté. The treatment session, over the total of all of the skin regions of the patient, lasts about 40 minutes from the initial injection to the final injection. Each treatment region receives an appropriate amount of the composition in spaced apart injections. The facial region for example, receives about 2 mL to about 3 mL of the composition, administered by shallow single injections spaced apart approximately every 10 mm to about 15 mm. The neck is treated with about 1 mL to 2 mL of the

composition, the injections spaced apart approximately every 15 mm to about 20 mm. After the treatment, the areas of the skin treated are examined by the senses of sight, touch and pressure. A photographic evaluation is performed at the beginning and at the end of the treatment. The patient reports to the physician that the treatment has caused only minimum discomfort. The patient returns to her everyday activities immediately after the treatment. In a small area on the skin of her treated right hand, ecchymosis is found, but this resolves a few days after the application of an anti-inflammatory cream. The patient returns to the physician 4 months after the treatment for a follow up evaluation. Prior to the follow up evaluation visit, no further injections of fillers or mesotherapy treatments are performed on the patient. On objective examination at the follow up visit, the treatment has resulted in an improvement in the epidermal texture, and decreased dryness, and improved brightness of the skin. These improvements are evidenced in part by the photographic documentation. The treated skin regions are gently palpated and seem to have an increase in hydration, suppleness, elasticity and tone. The patient expresses a high degree of satisfaction with the treatment via the completion of a self-assessment questionnaire. The patient claims the treated areas have improved and she is very happy with the results. Interestingly, these good results are achieved based on only the single treatment session, with no repeated injections, "top-ups", or further injection treatment between the time of the treatment session and the follow up visit.

EXAMPLE 3

[0075] The hydration of the injectable compositions on the epidermal and dermal structures of human living skin explants has been evaluated by corneometry measurements: Using a CM825 Corneometer® (COURAGE & KHAZAKA) the humidity level of the most external cutaneous layers of the stratum corneum have been determined. The action principle of the Corneometer® is based on the modification of the electrical capacities of the detector which is designed in the form of a condenser. The surface of the measurement head, in contact with the skin, modifies its electrical capacity according to the humidity level of the skin. On D0 (=day 0), the electric epidermal capacitance expressed in AU (arbitrary unit) is an

index of the skin hydration. The following composition according to the present disclosure has been determined:

	Composition according to present disclosure (P)
NaHA concentration (mg/g)	10.5 – 13.5
Lidocaine content (% by weight)	0.27 - 0.33
Uncrosslinked HA content having a high molecular weight (% by weight)	0.95

TABLE 3

The product has been stored at room temperature within the duration of the study.

[0076] Explant preparation: On an abdominal plasty coming from a 45-year-old Caucasian woman, 9 explants were prepared. The explants were kept in survival in BEM medium (BIO-EC's Explants Medium) at 37°C in a humid, 5 %-CO_2 atmosphere. The explants were distributed in 3 batches (Nb=Number):

Analysis	Batch	Designation	Treatment	Nb and size of explants	Sampling time
Corneo-metry	**T-C1**	Untreated control (Blank)	/	3; 1.5 x 2 cm	D9
	P-C1	Product P	Composition according to present disclosure	3; 1.5 x 2 cm	D9
	P-C2	Product P	Composition according to present disclosure	3; 1.5 x 2.5 cm	D9

TABLE 4

[0077] Product application: The explants of the batch C1 used for corneometry were treated with 4x10 µl of the injectable product (square injection) with each point of injection separated by 0.5 cm (see Figure 1). The explants of the batch C2 used for corneometry were treated with 4x10 µl of the injectable product (square injection)

with each point of injection separated by 1 cm (see Figure 1). The untreated controls did not receive any treatment.

[0078] Corneomtry: An index of skin hydration, epidermal capacitance, was assessed using the CM825 Corneometer® (COURAGE & KHAZAKA) on the explants at D0, D2, D7 and D9. The measures have been performed at the centre of the square explants using a probe of 1 cm of diameter. Ten measurements are performed and the average value is calculated by the corneometer.

[0079] Sampling: On D0, three explants from the batch T0 were collected and cut in 2 parts: half was frozen at -80°C and half was fixed in formol. On D2, D7 and D9, 3 explants from each batch were collected and processed in the same way.

[0080] Statistical analysis: The statistical analysis is performed according the Student t-test. Student t test gives the probability "p" for two batches to be significantly different. The difference between two batches is significant if $p<0.05$(*), so a probability of 95% for two batches to be significantly different; or if $p<0.01$(**), so a probability of 99% for two batches to be significantly different.

[0081] RESULTS: Measurement of corneometry for each batch (see also Figures 2 and 3):

	D0		D2		D7		D9	
	Average	SD	Average	SD	Average	SD	Average	SD
T	**78.9**	1.7	**82.7**	1.3	**82.5**	1.0	**79.5**	3.9
Pc1	**77.6**	1.1	**115.5**	2.9	**108.3**	2.4	**112.0**	2.2
Pc2	**77.1**	0.8	**104.6**	6.8	**110.5**	3.7	**110.1**	5.0

TABLE 5: Corneometry data

[0082] The corneometry shows that with the product Pc1, the corneometry values are higher by 44.0%** vs T on D2, 34.9%** vs T on D7, and 43.6%** vs T on D9. With the product Pc2, the corneometry values are higher by 30.7%* vs T on D2, 38.6%** vs T on D7, and 41.9%** vs T on D9. (According to Student t-test: * significant with $p<0.05$ (95%); ** significant with $p<0.01$ (99%)). The compositions according to the present disclosure show a good hydration activity whatever the condition tested (4 injections of 10 µl each separated by 0.5 cm or 1 cm for Pc1 and

Pc2 respectively), it induces a strong increment of epidermal capacitance values which increases the skin hydration.

EXAMPLE 3

[0083] The skin hydration of the injectable compositions according to the present disclosure on the epidermal and dermal structures of human living skin explants has been evaluated by additional corneometry measurements. The following compositions have been determined:

	Composition including uncrosslinked HA only (P1)	**Composition according to present disclosure (P2)**
NaHA concentration (mg/g)	11.5 - 15.5	10.5 – 13.5
Lidocaine content	/	0.27% - 0.33%
Mannitol content	0.70% - 1.10%	/
Uncrosslinked HA content having a high molecular weight (% by weight)	100%	0.95%

TABLE 6

The products have been stored at room temperature within the duration of the study.

[0084] Explant preparation: For the first donor, 9 explants of 1.5 cm x 2 cm in size were prepared on an abdominal plasty coming from a 59-year-old Caucasian woman. For the second donor, 9 explants of 1.5 cm x 2 cm in size were prepared on an abdominal plasty coming from a 42-year-old Caucasian woman. For the third donor, 9 explants of 1.5 cm x 2 cm in size were prepared on an abdominal plasty coming from a 52-year-old Caucasian woman. For each donor, 9 explants were distributed in 3 batches as follows:

Batch	**Designation**	**Treatment**	**Number of explants**	**Sampling time**
T	Untreated Control (Blank)	/	**3**	D8

P1	Product 1	Composition according to present disclosure	**3**	D8
P2	Product 2	Composition including uncrosslinked HA only	**3**	D8

TABLE 7

[0085] Product application: On D0, 3 x 50µL of the products P1 or P2 were injected with a needle into the dermis of the rectangular explants (1.5 x 2 cm). The untreated controls did not receive any treatment.

[0086] Half of the culture medium (1 ml) was refreshed on D1, D2, D5 and D7.

[0087] Corneometry: An index of skin hydration, epidermal capacitance, was assessed using the CM825 Corneometer® (COURAGE & KHAZAKA) on the explants at D0, D1, D2 and D8.

[0088] RESULTS: The results of the corneometry measurements for the three donors and expressed as an average of three values (see also Figures 4 and 5):

		Day of measurement			
	AU	**D0**	**D1**	**D2**	**D8**
Product	**T**	61.00 (SD : 5.9)	63.45 (SD : 6.0)	62.42 (SD : 5.6)	61.98 (SD : 7.5)
	P1	64.76 (SD : 4.3)	74.64 (SD : 7.3)	76.54 (SD : 5.6)	79.03 (SD : 8.2)
	P2	64.10 (SD : 5.5)	87.90 (SD : 10.5)	92.88 (SD : 8.1)	95.37 (SD : 10.1)

AU (Arbitrary Units of corneometry)

TABLE 8

[0089] For the 3 donors, the corneometry shows that the product P1 induces an increase of 15% on D1 of corneometry value compared to D0, continues to increase to 18% on D2 and increases to 22% on D8. The product P2 induces a quick increase of 37% increase from D0 to D1 and continues to increase from D2 to D8 to reach 49%. According to these experimental conditions and compared to the blank batch on D8 (TJ8), the following may be concluded:

vs TJ8	P1	P2
Corneometry D8	+ 22%	+ 49%

TABLE 9

[0090] Overall, under the experimental conditions of this study, the results show that the product according to the present disclosure (P2) shows an increased skin hydration in the stratum corneum on day 8 (D8) as compared to the product P1 and the untreated control T.

[0091] Although the invention has been described and illustrated with a certain degree of particularity, it is understood that the present disclosure has been made only by way of example, and that numerous changes in the combination and arrangement of parts, steps and elements can be resorted to by those skilled in the art without departing from the scope of the invention, as hereinafter claimed.

[0092] The invention may be further illustrated by the following embodiments:

1. An injectable composition useful for reducing the appearance of superficial depressions in the skin, the composition comprising:

a hyaluronic acid (HA) gel comprising a crosslinked HA matrix made with a low molecular weight HA material having a weight average molecular weight of between 0.20 about MDa and about 0.99 MDa;

wherein the HA concentration of the composition is less than about 17.0 mg/g;

wherein the composition maintains the reduced appearance of superficial depressions in the skin for at least about 3 months from being introduced into the skin.

2. The composition of item 1 wherein the composition maintains the reduced appearance of superficial depressions in the skin for at least about 6 months from being introduced into the skin.

3. The composition of item 1 wherein the composition maintains the reduced appearance of superficial depressions in the skin for at least about 9 months from being introduced into the skin.

4. The composition of item 1 further comprising at least one of mannitol and a vitamin C derivative.

5. The composition of item 4 wherein the mannitol is present in an amount of between about 0.3% to about 0.9% w/w.

6. The composition of item 4 wherein the vitamin C derivative is ascorbic acid 2-glucoside.

7. The composition of item 6 wherein the ascorbic acid 2-glucoside is present in an amount of between about 0.3% to about 0.6%w/w.

8. The composition of item 1 further comprising about 0.9% w/w mannitol and about 0.6% w/w ascorbic acid 2-glucoside.

9. The composition of item 1 wherein the weight average molecular weight of the low molecular weight HA material is between about 400,000 Da and about 800,000 Da.

10. The composition of item 1 wherein the crosslinked HA matrix is crosslinked with a crosslinking agent selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), 1,4-bis(2,3-epoxypropoxy)butane, 1,4-bisglycidyloxybutane, 1,2-bis(2,3-epoxypropoxy)ethylene and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane.

11. The composition of item 1 wherein the HA concentration is about 10.0 mg/g to about 14.0 mg/g.

12. The composition of item 1 wherein the HA concentration is about 12.0 mg/g.

13. An injectable composition useful for reducing the appearance of superficial depressions in the skin or for improving skin quality, the composition comprising:

a hyaluronic acid (HA) gel comprising a crosslinked HA matrix;

at least one of a mannitol and a Vitamin C derivative;

wherein the HA concentration of the composition is less than about 17.0 mg/g;

wherein the composition maintains the reduced appearance of superficial depressions in the skin or the improved skin quality for at least about 3 months from being introduced into the skin.

14. The composition of item 13 wherein the composition maintains the reduced appearance of superficial depressions in the skin or the improved skin quality for at least about 6 months from being introduced into the skin.

15. The composition of item 13 wherein the composition maintains the reduced appearance of superficial depressions in the skin or the improved skin quality for at least about 9 months from being introduced into the skin.

16. The composition of item 13 wherein the mannitol is present in an amount of between about 0.3% to about 0.9% w/w.

17. The composition of item 13 wherein the vitamin C derivative is ascorbic acid 2-glucoside.

18. The composition of item 17 wherein the ascorbic acid 2-glucoside is present in an amount of between about 0.3% to about 0.6% w/w.

19. The composition of item 13 wherein the at least one of a mannitol and a Vitamin C derivative comprises both mannitol present at about 0.9% w/w and a Vitamin C derivative present at about 0.6% w/w.

20. The composition of item 19 wherein the Vitamin C derivative is ascorbic acid 2-glucoside.

21. The composition of item 13 wherein the HA concentration is about 10.0 mg/g to about 14.0 mg/g.

22. The composition of item 13 wherein the HA concentration is about 12.0 mg/g.

23. A method of improving at least one of texture, hydration and elasticity of skin, the method comprising;

treating an area of skin by introducing into a skin region, in a single treatment session, multiple, spaced apart injections of a composition comprising a hyaluronic acid (HA) gel containing crosslinked HA;

wherein the HA concentration of the composition is less than about 17.0 mg/g;

wherein the treated skin maintains at least one of an improved texture, hydration and elasticity due to the treatment for at least about 3 months.

24. The method of item 23 wherein the treated skin maintains the at least one of an improved texture, hydration and elasticity due to the treatment for at least about 6 months.

25. The method of item 23 wherein the treated skin maintains the at least one of an improved texture, hydration and elasticity due to the treatment for at least about 12 months.

26. The method of item 23 wherein the HA concentration is about 10.0 mg/g to about 14.0 mg/g.

27. The method of item 23 wherein the HA concentration is about 12.0 mg/g.

28. The method of item 23 wherein the injections are spaced apart by a distance of between about 5 mm to about 20 mm.

29. The method of item 23 wherein the injections are introduced in an amount of about 5 µL to about 100 µL per injection.

30. The method of item 23 wherein the treatment takes no longer than about 45 minutes.

31. The method of item 23 wherein the injections are delivered through a needle having a gauge of from 28 G to 33 G.

32. The method of item 23 wherein the composition further comprising at least one of mannitol and a vitamin C derivative.

33. The method of item 23 wherein the mannitol is present in an amount of between about 0.3% and about 0.9% w/w.

34. The method of item 23 wherein the vitamin C derivative is ascorbic acid 2-glucoside.

35. The method of item 34 wherein the ascorbic acid 2-glucoside is present in an amount of between about 0.3% and about 0.6% % w/w.

36. A method of increasing at least one of smoothness, hydration, and elasticity in skin comprising;

introducing, into a skin region at a depth of between about 0.5 to about 4.0 mm or about 0.5 to about 5.0 mm, in a single treatment session, multiple, spaced apart injections of a composition comprising a hyaluronic acid (HA) gel containing BDDE-crosslinked HA and uncrosslinked HA;

wherein the injections are introduced in an amount of about 5 µL to about 100 µL per injection;

wherein the injections are delivered through a needle having a gauge of from 28 G to 33 G;

wherein the injections spaced apart by a distance of between about 5 mm to about 20 mm; and

wherein the skin region maintains an increased smoothness, hydration, and/or elasticity due to the treatment for at least about 6 months.

37. The method of item 36 wherein the composition further comprises at least one of mannitol and a vitamin C derivative.

38. A composition for increasing at least one of smoothness, hydration, and elasticity in skin comprising:

a hyaluronic acid (HA) gel comprising a crosslinked HA matrix made with a low molecular weight HA material having a weight average molecular weight of between about 400,000 Da and about 800,000 Da;

wherein the HA concentration of the composition is from about 10.0 mg/g to about 14.0 mg/g; and

wherein the composition maintains the increased smoothness, hydration, and/or elasticity due to the treatment for at least about 6 months from being introduced into the skin in a single treatment session comprising multiple, spaced apart injections of the composition in which the injections are introduced in an amount of about 5 μL to about 100 μL per injection, delivered through a needle having a gauge of from 28 G to 33 G and are spaced apart by a distance of between about 5 mm to about 20 mm.

39. The composition of item 38 wherein the composition further comprises at least one of mannitol and a vitamin C derivative.

What is claimed is:

1. An injectable composition for reducing the appearance of superficial depressions in the skin, the composition comprising:

a hyaluronic acid (HA) gel comprising a crosslinked HA matrix made with more than 50% by weight, at least 70% by weight or at least about 90 % by weight of a low molecular weight HA material having a weight average molecular weight of between 0.20 about MDa and about 0.99 MDa, preferably between about 400,000 Da and about 800,000 Da, based on the total weight of the HA material;

wherein the HA concentration of the composition is less than about 17.0 mg/g such as about 10.0 mg/g to about 14.0 mg/g, preferably about 12 mg/g;

wherein the crosslinked HA matrix is crosslinked with a crosslinking agent selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), 1,2-bis(2,3-epoxypropoxy)ethylene and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane;

wherein the composition does preferably not include an antioxidant or a vitamin;

wherein the composition maintains the reduced appearance of superficial depressions in the skin for at least about 3 months from being introduced into the skin.

2. The composition of claim 1 wherein the composition maintains the reduced appearance of superficial depressions in the skin for at least about 6 months from being introduced into the skin, preferably for at least about 9 months or 12 months.

3. The composition of claims 1 or 2 wherein the composition includes uncrosslinked HA, preferably in an amount of less than 10 % w/w or less than 5 % w/w, based on the total weight of the composition.

4. The composition of claims 1-3 further comprising at least one of mannitol and a vitamin C derivative or further comprising lidocaine, preferably in an amount of between about 0.1% and about 5% by weight of the composition such as 0.3% by weight.

5. The composition of claim 4 wherein the mannitol is present in an amount of between about 0.3% to about 0.9% w/w, based on the total weight of the composition.

6. The composition of claim 4 wherein the vitamin C derivative is ascorbic acid 2-glucoside.

7. The composition of claim 6 wherein the ascorbic acid 2-glucoside is present in an amount of between about 0.3% to about 0.6% w/w, based on the total weight of the composition.

8. The composition of claims 1-4 further comprising about 0.9% w/w mannitol and about 0.6% w/w ascorbic acid 2-glucoside, based on the total weight of the composition.

9. An injectable composition for reducing the appearance of superficial depressions in the skin or for improving skin quality, the composition comprising:

a hyaluronic acid (HA) gel comprising a crosslinked HA matrix;

at least one of a mannitol and a Vitamin C derivative;

wherein the HA concentration of the composition is less than about 17.0 mg/g such as about 10.0 mg/g to about 14.0 mg/g, preferably about 12.0 mg/g;

wherein the composition maintains the reduced appearance of superficial depressions in the skin or the improved skin quality for at least about 3 months from being introduced into the skin.

10. The injectable composition of claim 9, wherein the crosslinked HA matrix is made with more than 50% by weight, at least 70% by weight or at least about 90 % by weight of a low molecular weight HA material having a weight average molecular weight of between 0.20 about MDa and about 0.99 MDa, preferably a weight average molecular weight of between about 400,000 Da and about 800,000 Da, based on the total weight of the HA material.

11. The injectable composition of claims 9-11, wherein the crosslinked HA matrix is crosslinked with a crosslinking agent selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), 1,2-bis(2,3-epoxypropoxy)ethylene and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane.

12. The composition of claims 9-11 wherein the composition maintains the reduced appearance of superficial depressions in the skin or the improved skin quality for at least about 6 months, at least about 9 months or at least about 12 months from being introduced into the skin.

13. The composition of claims 9-12 wherein the composition further comprises lidocaine, preferably in an amount of between about 0.1% and about 5% by weight of the composition such as 0.3% by weight.

14. The composition of claims 9-13 wherein the mannitol is present in an amount of between about 0.3% to about 0.9% w/w, based on the total weight of the composition.

15. The composition of claims 9-14 wherein the vitamin C derivative is ascorbic acid 2-glucoside.

16. The composition of claim 15 wherein the ascorbic acid 2-glucoside is present in an amount of between about 0.3% to about 0.6% w/w, based on the total weight of the composition.

17. The composition of claims 9-13 wherein the at least one of a mannitol and a Vitamin C derivative comprises both mannitol present at about 0.9% w/w and a Vitamin C derivative present at about 0.6% w/w, based on the total weight of the composition.

18. The composition of claim 17 wherein the Vitamin C derivative is ascorbic acid 2-glucoside.

19. A composition for use in a method of improving at least one of texture, hydration and elasticity of skin, the method comprising;

treating an area of skin by introducing into a skin region, in a single treatment session, multiple, spaced apart injections, of said composition comprising a hyaluronic acid (HA) gel containing crosslinked HA matrix made with more than 50% by weight, at least 70% by weight or at least about 90 % by weight of a low molecular weight HA material having a weight average molecular weight of between 0.20 about MDa and about 0.99 MDa, preferably between about 400,000 Da and about 800,000 Da, based on the total weight of the crosslinked HA matrix;

wherein the crosslinked HA matrix is crosslinked with a crosslinking agent selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), 1,2-bis(2,3-epoxypropoxy)ethylene and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane;

wherein the HA concentration of the composition is less than about 17.0 mg/g such as about 10.0 mg/g to about 14.0 mg/g, preferably about 12.0 mg/g;

wherein the injections are spaced apart by a distance of between about 5 mm to about 20 mm such as 5 mm or 10 mm;

wherein the treated skin maintains at least one of an improved texture, hydration and elasticity due to the treatment for at least about 3 months.

20. A non-therapeutic method of improving at least one of texture, hydration and elasticity of skin, the method comprising;

treating an area of skin by introducing into a skin region, in a single treatment session, multiple, spaced apart injections, of a composition comprising a hyaluronic acid (HA) gel containing crosslinked HA matrix made with more than 50% by weight, at least 70% by weight or at least about 90 % by weight of a low molecular weight HA material having a weight average molecular weight of between 0.20 about MDa and about 0.99 MDa, preferably between about 400,000 Da and about 800,000 Da, based on the total weight of the HA material;

wherein the crosslinked HA matrix is crosslinked with a crosslinking agent selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), 1,2-bis(2,3-epoxypropoxy)ethylene and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane;

wherein the HA concentration of the composition is less than about 17.0 mg/g such as about 10.0 mg/g to about 14.0 mg/g, preferably about 12.0 mg/g;

wherein the injections are spaced apart by a distance of between about 5 mm to about 20 mm such as 5 mm or 10 mm;

wherein the treated skin maintains at least one of an improved texture, hydration and elasticity due to the treatment for at least about 3 months.

21. The composition for use of claim 19, or the non-therapeutic method of claim 20 wherein the treated skin maintains the at least one of an improved texture, hydration and elasticity due to the treatment for at least about 6 months, at least about 9 months or at least about 12 months.

22. The composition for use of claims 19 or 21, or the non-therapeutic method of claims 20 or 21 wherein the treated skin maintains the at least one of an improved texture, hydration and elasticity due to the treatment for at least about 6 months, at least about 9 months or at least about 12 months.

23. The composition for use of claims 19 and 21-22, or the non-therapeutic method of claims 20-22 wherein the injections are introduced in an amount of about 5 µL to about 100 µL per injection.

24. The composition for use of claims 19 and 21-23, or the non-therapeutic method of claims 20-23 wherein the treatment takes no longer than about 45 minutes.

25. The composition for use of claims 19 and 21-24, or the non-therapeutic method of claims 20-24 wherein the injections are delivered through a needle having a gauge of from 28 G to 33 G.

26. The composition for use of claims 19 and 21-25, or the non-therapeutic method of claims 20-25 wherein the composition further comprises at least one of mannitol and a vitamin C derivative or wherein the composition does not include an antioxidant or a vitamin and/or wherein the composition further comprises lidocaine, preferably in an amount of between about 0.1% and about 5% by weight of the composition such as 0.3% by weight.

27. The composition for use of claims 19 and 21-26, or the non-therapeutic method of claims 20-26 wherein the mannitol is present in an amount of between about 0.3% and about 0.9% w/w, based on the total weight of the composition.

28. The composition for use of claims 19-27, or the non-therapeutic method of claims 20-27 wherein the vitamin C derivative is ascorbic acid 2-glucoside.

29. The composition for use of claim 28, or the non-therapeutic method of claim 28 wherein the ascorbic acid 2-glucoside is present in an amount of between about 0.3% and about 0.6% w/w, based on the total weight of the composition.

30. A composition for use in a method of increasing at least one of smoothness, hydration, and elasticity in skin wherein the method comprises:

introducing, into a skin region at an injection depth of between about 0.5 mm to about 5.0 mm, preferably about 1.0 mm to about 4.0 mm, more preferably from about 1.5 mm to about 3.0 mm, in a single treatment session, multiple, spaced apart injections of said composition comprising a hyaluronic acid (HA) gel containing BDDE-crosslinked HA and uncrosslinked HA;

wherein the HA concentration of the composition is less than about 17.0 mg/g such as from about 10.0 mg/g to about 14.0 mg/g, preferably about 12.0 mg/g;

wherein the injections are introduced in an amount of about 5 µL to about 100 µL per injection;

wherein the injections are delivered through a needle having a gauge of from 28 G to 33 G;

wherein the injections spaced apart by a distance of between about 5 mm to about 20 mm such as 5 mm or 10 mm; and

wherein the skin region maintains an increased smoothness, hydration, and/or elasticity due to the treatment for at least about 6 months, at least about 9 months or at least about 12 months.

31. A non-therapeutic method of increasing at least one of smoothness, hydration, and elasticity in skin wherein the method comprises:

introducing, into a skin region at an injection depth of between about 0.5 mm to about 5.0 mm, preferably about 1.0 mm to about 4.0 mm, more preferably from about 1.5 mm to about 3.0 mm, in a single treatment session, multiple, spaced apart injections of a composition comprising a hyaluronic acid (HA) gel containing BDDE-crosslinked HA and uncrosslinked HA;

wherein the HA concentration of the composition is less than about 17.0 mg/g such as from about 10.0 mg/g to about 14.0 mg/g, preferably about 12.0 mg/g;

wherein the injections are introduced in an amount of about 5 µL to about 100 µL per injection;

wherein the injections are delivered through a needle having a gauge of from 28 G to 33 G;

wherein the injections spaced apart by a distance of between about 5 mm to about 20 mm such as 5 mm or 10 mm; and

wherein the skin region maintains an increased smoothness, hydration, and/or elasticity due to the treatment at least about 6 months, at least about 9 months or at least about 12 months.

32. The composition for use of claim 30, or the non-therapeutic method of claim 31 wherein the composition further comprises at least one of mannitol and a vitamin C derivative or wherein the composition does not include an antioxidant or a vitamin and/or wherein the composition further comprises lidocaine, preferably in an amount of between about 0.1% and about 5% by weight of the composition such as 0.3% by weight.

33. A composition for increasing at least one of smoothness, hydration, and elasticity in skin comprising:

a hyaluronic acid (HA) gel comprising a crosslinked HA matrix made with a low molecular weight HA material having a weight average molecular weight of between 0.20 about MDa and about 0.99 MDa, preferably between about 400,000 Da and about 800,000 Da;

wherein the HA concentration of the composition is less than about 17.0 mg/g such as from about 10.0 mg/g to about 14.0 mg/g, preferably 12.0 mg/g; and

wherein the composition maintains the increased smoothness, hydration, and/or elasticity due to the treatment at least about 6 months, at least about 9 months or at least about 12 months from being introduced into the skin in a single treatment session comprising multiple, spaced apart injections of the composition in which the injections are introduced in an amount of about 5 µL to about 100 µL per injection, delivered through a needle having a gauge of from 28 G to 33 G, preferably 32 G, and are spaced apart by a distance of between about 5 mm to about 20 mm such as 5 mm or 10 mm.

34. The composition of claim 33 wherein the composition further comprises at least one of mannitol and a vitamin C derivative or wherein the composition does not include an antioxidant or a vitamin and/or wherein the composition further comprises lidocaine, preferably in an amount of between about 0.1% and about 5% by weight of the composition such as 0.3% by weight.

35. The composition of claims 33-34, wherein the crosslinked HA matrix is made with more than 50% by weight, at least 70% by weight or at least about 90 % by weight of said low molecular weight HA material, based on the total weight of the HA material.

36. The composition of claims 33-35, wherein the crosslinked HA matrix is crosslinked with a crosslinking agent selected from the group consisting of 1,4-butanediol diglycidyl ether (BDDE), 1,2-bis(2,3-epoxypropoxy)ethylene and 1-(2,3-epoxypropyl)-2,3-epoxycyclohexane.

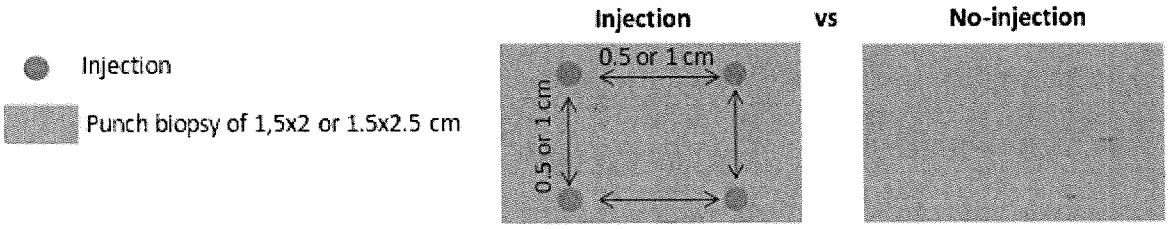

Figure 1

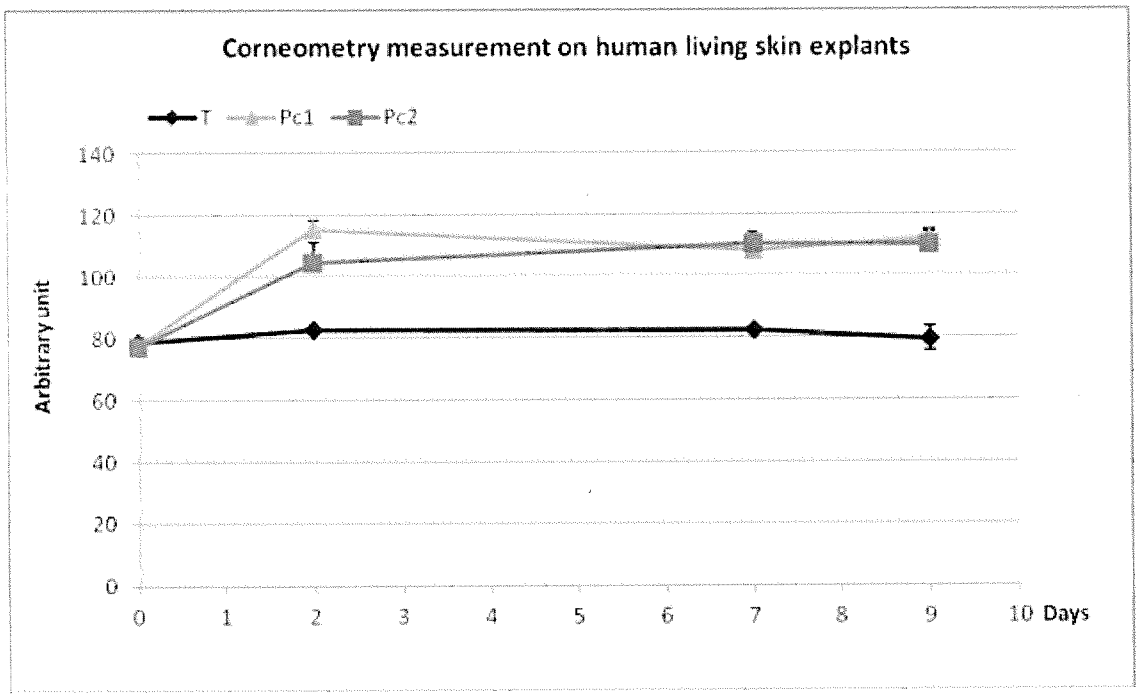

Figure 2

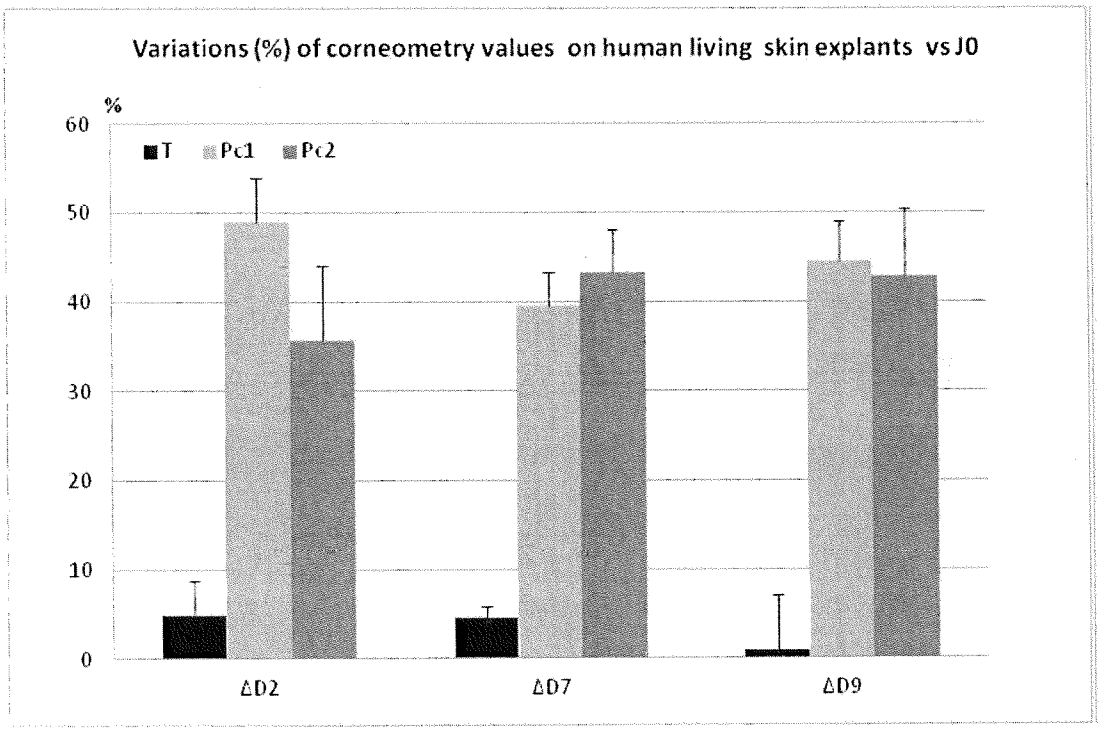

Figure 3

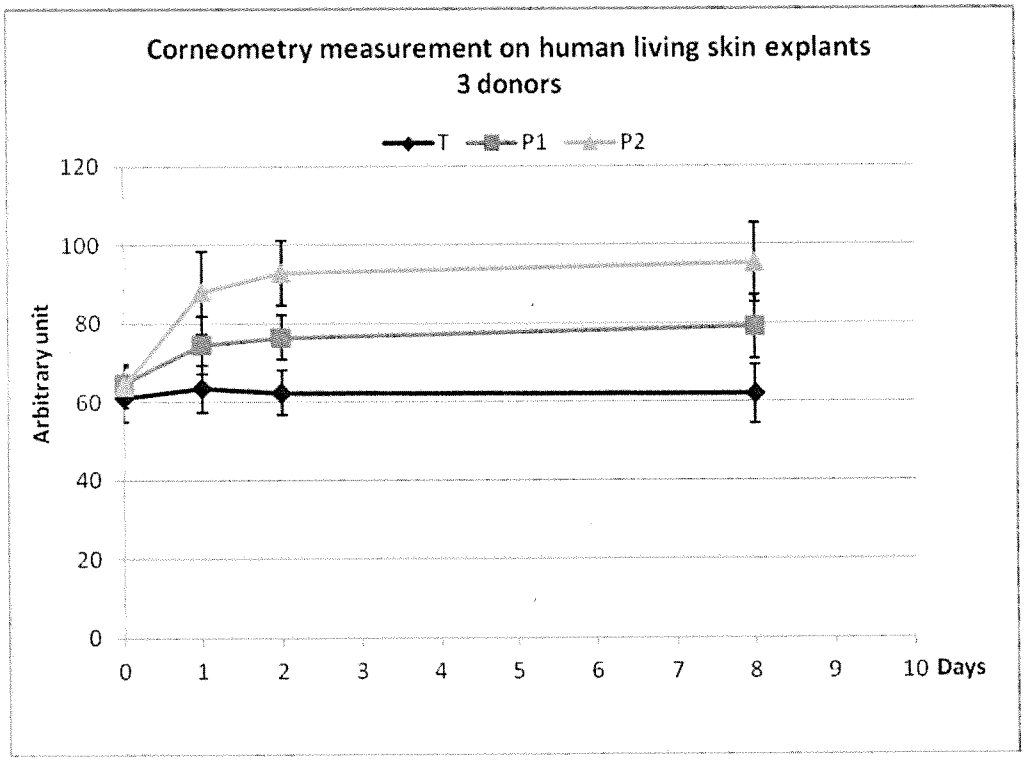

Figure 4

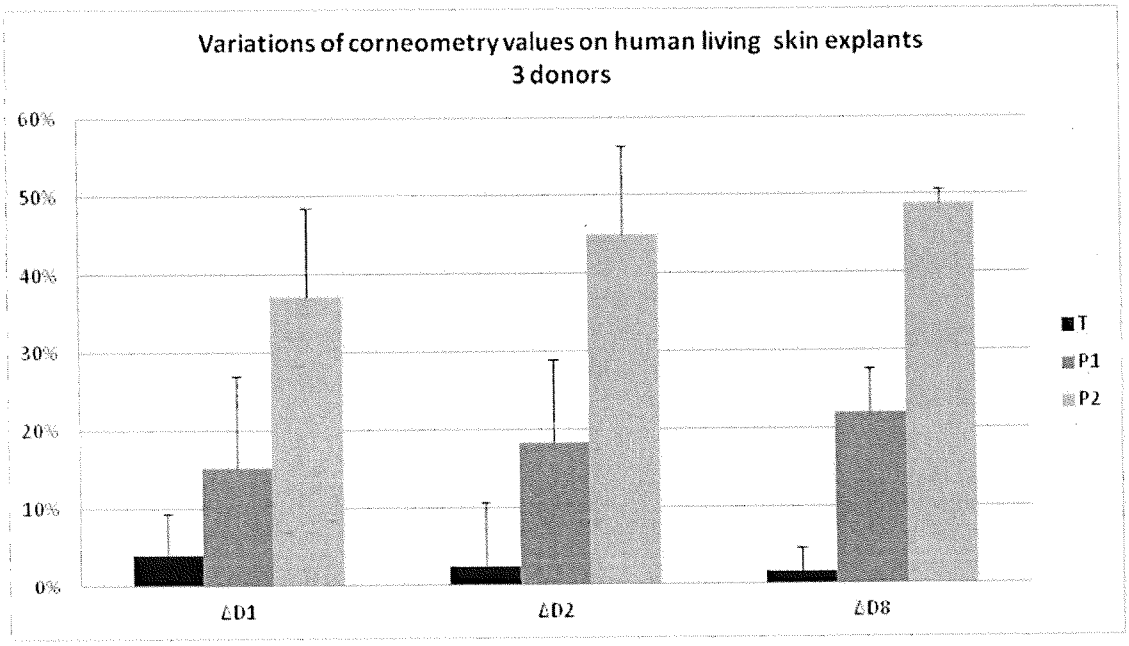
Variations of corneometry values on human living skin explants
3 donors
60%
50%
40%
30%
20%
10%
0%
ΔD1
ΔD2
ΔD8
T
P1
P2

Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/052682

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-8, 19, 30, 33-36(completely); 11, 21-29, 32(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/052682

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K8/34 A61K8/60 A61K8/73 A61Q19/08 C08B37/08
C08J3/075 A61K8/67
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 C08B C08J

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED) I. GENERAL INFORMATION Device Generic Name: Injectable Dermal Filler Device Trade Name: Restylane Silk Injectable Gel Device Procode: LMH Applicant's Name and Address: Valeant Pharmaceuticals North America", , 13 June 2014 (2014-06-13), pages 1-33, XP055210777, Retrieved from the Internet: URL:http://www.accessdata.fda.gov/cdrh_docs/pdf4/P040024S072b.pdf [retrieved on 2015-09-02] Device Description, Preclinical Studies ----- -/--	1,2,19, 21-26, 30,32-36

[X] Further documents are listed in the continuation of Box C.

[X] 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
14 April 2016	08/07/2016

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	Skulj, Primoz

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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.
X	Robert Kasten: "Volumisierung des Mittelgesichts", , 1 April 2014 (2014-04-01), XP055262134, Retrieved from the Internet: URL:http://www.zwp-online.info/archiv/pub/sim/fa/2014/fa0414/fa0414_10_14_kasten.pdf [retrieved on 2016-04-01]	1-4,19, 21-26, 30,32-36
Y	table 1 pages 12,13	1
T	Dean Tomasello: "Juvéderm Voluma for Cheek Enhancement ¦ ClearSkinMD.net", , 12 March 2016 (2016-03-12), XP055262203, Retrieved from the Internet: URL:https://www.clearskinmd.net/juvederm/ [retrieved on 2016-04-01]	
T	"Juvederm family of products", , 15 June 2015 (2015-06-15), XP055262127, Retrieved from the Internet: URL:https://nebula.wsimg.com/b27e8501ebd5e8924a6a745fd990f753?AccessKeyId=854699786DC957AB88BF&disposition=0 [retrieved on 2016-04-01]	
X	Alsoufi: "New and Innovative Developments in Hyaluronic Acid Fillers for Lip Enhancement and Contouring", European Dermatology, vol. 5 1 January 2011 (2011-01-01), pages 50-53, XP055210897, Retrieved from the Internet: URL:http://www.adoderm.com/literature/2010 0428 Eur Derm Vol. 5 Issue 1, New Innovative Developm..., Dr. Alsoufi.pdf [retrieved on 2015-09-02]	1,19, 21-26, 30,32-36
Y	the whole document	1
X	Weidmann: "Varioderm - Eine Innovation im Bereich der Hyaluronsäuren", Kosmetische Medizin, 30 June 2007 (2007-06-30), XP055210966, Retrieved from the Internet: URL:http://www.adoderm.com/literature/kosmetische-medizin-varioderm-dr.weidmann,06-2007.pdf [retrieved on 2015-09-03] page 275	1,19, 21-26, 30,32-36

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J U: "VARIODERM es", , 31 December 2011 (2011-12-31), XP055210889, Retrieved from the Internet: URL:http://www.cirugiadeavanzada.com/PDF/VARIODERM.PDF [retrieved on 2015-09-02] the whole document	1,19, 21-26, 30,32-36
X	"The European Aesthetic Guide", , 31 December 2011 (2011-12-31), XP055210886, Retrieved from the Internet: URL:http://cdn.coverstand.com/3708/63217/63217.5.pdf [retrieved on 2015-09-02] pages 98-99	1,19, 21-26, 30,32-36
X	MARCIA RAMOS-E-SILVA ET AL: "STYLAGE : a range of hyaluronic acid dermal fillers containing mannitol. Physical properties and review of the literature", CLINICAL, COSMETIC AND INVESTIGATIONAL DERMATOLOGY, 1 October 2013 (2013-10-01), page 257, XP055211011, DOI: 10.2147/CCID.S35251	1,19, 21-26, 30,32-36
Y	the whole document	5,27
X	US 2006/194758 A1 (LEBRETON PIERRE [FR]) 31 August 2006 (2006-08-31) claims 1,9,10,12,13,16	1-3,19, 21-26, 30,32-36
X	US 2011/118206 A1 (LEBRETON PIERRE F [FR]) 19 May 2011 (2011-05-19) paragraphs [0013] - [0016], [0018], [0024], [0036], [0045], [0050]; claims 1,5,10,15	1-3,19, 21-26, 30,32-36
X	WO 2013/040242 A2 (ALLERGAN INC [US]; NJIKANG GABRIEL N [US]; YU XIAOJIE [US]; LIU FUTIAN) 21 March 2013 (2013-03-21)	1-4,6-8, 11,19, 21-26, 28-30, 32-36
Y	pages 3,7,39; claims 37,34,38,39,36 pages 15,17,22 page 24	5,27

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/052682

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006194758 A1	31-08-2006	BR PI0409762 A	09-05-2006
		CA 2521961 A1	28-10-2004
		EP 1611160 A2	04-01-2006
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		ES 2536564 T3	26-05-2015
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		JP 4827727 B2	30-11-2011
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		US 2012295870 A1	22-11-2012
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		US 2014148406 A1	29-05-2014
		US 2015045321 A1	12-02-2015
WO 2013040242 A2	21-03-2013	NONE	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-8, 19, 30, 33-36(completely); 11, 21-29, 32(partially)

   HA dermal filler compositions with the concentration of HA below 17 mg/g made with a crosslinked HA matrix predominantlymade with a low molecular weight HA material having a weight average molecular weight of about 0.20 - 0.99 MDa and their uses

   ---

2. claims: 9, 10, 12-18(completely); 11(partially)

   HA dermal filler compositions with the concentration of HA below 17 mg/g comprising a crosslinked HA matrix and further comprising mannitol or/and vitamin C

   ---

3. claims: 20(completely); 21-29(partially)

   The administration technique of the HA dermal filler compositions with the concentration of HA below 17 mg/g comprising a crosslinked HA matrix predominantly made with a low molecular weight HA material having a weight average molecular weight of about 0.20 - 0.99 MDa defined by the injection spacings

   ---

4. claims: 31(completely); 32(partially)

   The administration technique of the HA dermal filler compositions with the concentration of HA below 17 mg/g comprising a crosslinked HA and a non-crosslinked HA matrix, defined by the injection depth.

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(19)中华人民共和国国家知识产权局

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(30)优先权数据
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(85)PCT国际申请进入国家阶段日
2017.08.07

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PCT/EP2016/052682 2016.02.09

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(71)申请人 阿勒根工业有限公司
地址 法国普兰日

(72)发明人 P·勒布雷东 O·格塔

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代理人 张广育 田婕

(51)Int.Cl.
A61K 8/73(2006.01)
A61K 8/34(2006.01)
A61K 8/67(2006.01)
A61Q 19/08(2006.01)
C08B 37/08(2006.01)

权利要求书4页 说明书16页 附图3页

(54)发明名称

用于改善皮肤外观的组合物和方法

(57)摘要

本发明公开了处理皮肤以改善水合、弹性和/或纹理的可注射组合物和方法。所述组合物基于由低分子量透明质酸制成的交联透明质酸基质。

CN 107205911 A

1.一种用于减少皮肤浅表凹陷外观的可注射组合物,所述组合物包含:

透明质酸(HA)凝胶,其含有由大于50重量%,至少70重量%或至少约90重量%的低分子量HA材料制成的交联HA基质,所述低分子量HA材料的重均分子量为约0.20MDa至约0.99MDa,优选约400000Da至约800000Da,基于HA材料的总重量计;

其中组合物的HA浓度小于约17.0mg/g,例如约10.0mg/g至约14.0mg/g,优选约12mg/g;

其中交联HA基质由选自以下的交联剂交联:1,4-丁二醇二缩水甘油醚(BDDE)、1,2-双(2,3-环氧丙氧基)乙烯和1-(2,3-环氧丙基)-2,3-环氧环己烷;

其中所述组合物优选不包含抗氧化剂或维生素;

其中所述组合物从被引入到皮肤中起,维持皮肤浅表凹陷外观的减少至少约3个月。

2.权利要求1的组合物,其中组合物从被引入到皮肤中起,维持皮肤浅表凹陷外观的减少至少约6个月,优选至少约9个月或12个月。

3.权利要求1或2的组合物,其中组合物包含未交联HA,优选以小于10%w/w或小于5%w/w的量包含未交联HA,基于组合物的总重量计。

4.权利要求1-3的组合物,其还包含甘露醇和维生素C衍生物中的至少一种或者还包含利多卡因,优选以组合物的约0.1重量%至约5重量%的量包含,例如0.3重量%。

5.权利要求4的组合物,其中甘露醇以约0.3%至约0.9%w/w的量存在,基于组合物的总重量计。

6.权利要求4的组合物,其中维生素C衍生物为抗坏血酸2-葡糖苷。

7.权利要求6的组合物,其中抗坏血酸2-葡糖苷以约0.3%至约0.6%w/w的量存在,基于组合物的总重量计。

8.权利要求1-4的组合物,其还包含约0.9%w/w的甘露醇和约0.6%w/w的抗坏血酸2-葡糖苷,基于组合物的总重量计。

9.一种用于减少皮肤浅表凹陷外观或用于改善皮肤质量的可注射组合物,所述组合物包含:

含有交联HA基质的透明质酸(HA)凝胶;

甘露醇和维生素C衍生物中的至少一种;

其中所述组合物的HA浓度小于约17.0mg/g,例如约10.0mg/g至约14.0mg/g,优选约12.0mg/g;

其中所述组合物从被引入到皮肤中起,维持皮肤浅表凹陷外观的减少或皮肤质量的改善至少约3个月。

10.权利要求9的可注射组合物,其中交联HA基质由大于50重量%,至少70重量%或至少约90重量%的低分子量HA材料制成,所述低分子量HA材料的重均分子量为约0.20MDa至约0.99MDa,优选地,重均分子量为约400000Da至约800000Da,基于HA材料的总重量计。

11.权利要求9-11的可注射组合物,其中交联HA基质由选自以下的交联剂交联:1,4-丁二醇二缩水甘油醚(BDDE)、1,2-双(2,3-环氧丙氧基)乙烯和1-(2,3-环氧丙基)-2,3-环氧环己烷。

12.权利要求9-11的组合物,其中组合物从被引入到皮肤中起,维持皮肤浅表凹陷外观的减少或皮肤质量的改善至少约6个月、至少约9个月或至少约12个月。

13.权利要求9-12的组合物,其中组合物还包含利多卡因,优选以组合物的约0.1重

量％至约5重量％的量包含利多卡因，例如0.3重量％。

14.权利要求9-13的组合物，其中甘露醇以约0.3％至约0.9％w/w的量存在，基于组合物的总重量计。

15.权利要求9-14的组合物，其中维生素C衍生物为抗坏血酸2-葡糖苷。

16.权利要求15的组合物，其中抗坏血酸2-葡糖苷以约0.3％至约0.6％w/w的量存在，基于组合物的总重量计。

17.权利要求9-13的组合物，其中甘露醇和维生素C衍生物中的至少一种包含以约0.9％w/w存在的甘露醇和以约0.6％w/w存在的维生素C衍生物，基于组合物的总重量计。

18.权利要求17的组合物，其中维生素C衍生物为抗坏血酸2-葡糖苷。

19.一种用于改善皮肤的纹理、水合和弹性中的至少一种的方法中使用的组合物，所述方法包括：

通过在单次处理疗程中向皮肤区域引入组合物的多次间隔隔开的注射来处理皮肤区域，所述组合物包含含有交联HA基质的透明质酸(HA)凝胶，所述交联HA基质由大于50重量％，至少70重量％或至少约90重量％的低分子量HA材料制成，所述低分子量HA材料的重均分子量为约0.20MDa至约0.99MDa，优选约400000Da至约800000Da，基于交联HA基质的总重量计；

其中交联HA基质由选自以下的交联剂交联：1,4-丁二醇二缩水甘油醚(BDDE)、1,2-双(2,3-环氧丙氧基)乙烯和1-(2,3-环氧丙基)-2,3-环氧环己烷；

其中组合物的HA浓度小于约17.0mg/g，例如约10.0mg/g至约14.0mg/g，优选约12.0mg/g；

其中注射以约5mm至约20mm例如5mm或10mm的距离间隔隔开；

其中经处理的皮肤因处理带来的改善的纹理、水合和弹性中的至少一种维持至少约3个月。

20.一种改善皮肤纹理、水合和弹性中的至少一种的非治疗性方法，该方法包括：

通过在单次处理疗程中向皮肤区域引入组合物的多次间隔隔开的注射来处理皮肤区域，所述组合物包含含有交联HA基质的透明质酸(HA)凝胶，所述交联HA基质由大于50重量％，至少70重量％或至少约90重量％的低分子量HA材料制成，所述低分子量HA材料的重均分子量为约0.20MDa至约0.99MDa，优选约400000Da至约800000Da，基于HA材料的总重量计；

其中交联HA基质由选自以下的交联剂交联：1,4-丁二醇二缩水甘油醚(BDDE)、1,2-双(2,3-环氧丙氧基)乙烯和1-(2,3-环氧丙基)-2,3-环氧环己烷；

其中组合物的HA浓度小于约17.0mg/g，例如约10.0mg/g至约14.0mg/g，优选约12.0mg/g；

其中注射以约5mm至约20mm例如5mm或10mm的距离间隔隔开；

其中经处理的皮肤因处理带来的改善的纹理、水合和弹性中的至少一种维持至少约3个月。

21.权利要求19的用于所述用途的组合物，或权利要求20的非治疗性方法，其中经处理的皮肤因处理带来的改善的纹理、水合和弹性中的至少一种维持至少约6个月、至少约9个月或至少约12个月。

22.权利要求19或21的用于所述用途的组合物，或权利要求20或21的非治疗性方法，其中经处理的皮肤因处理带来的改善的纹理、水合和弹性中的至少一种维持至少约6个月、至少约9个月或至少约12个月。

23.权利要求19和21-22的用于所述用途的组合物，或权利要求20-22的非治疗性方法，其中注射以约5μL至约100μL每次注射的量引入。

24.权利要求19和21-23的用于所述用途的组合物，或权利要求20-23的非治疗性方法，其中处理不超过约45分钟。

25.权利要求19和21-24的用于所述用途的组合物，或权利要求20-24的非治疗性方法，其中注射通过28G至33G的规格的针递送。

26.权利要求19和21-25的用于所述用途的组合物，或权利要求20-25的非治疗性方法，其中组合物还包含甘露醇和维生素C衍生物中的至少一种，或者其中组合物不包含抗氧化剂或维生素和/或其中组合物还包含利多卡因，优选以组合物的约0.1重量％至约5重量％的量包含，例如0.3重量％。

27.权利要求19和21-26的用于所述用途的组合物，或权利要求20-26的非治疗性方法，其中甘露醇以约0.3％至约0.9％w/w的量存在，基于组合物的总重量计。

28.权利要求19-27的用于所述用途的组合物，或权利要求20-27的非治疗性方法，其中维生素C衍生物为抗坏血酸2-葡糖苷。

29.权利要求28的用于所述用途的组合物，或权利要求28的非治疗性方法，其中抗坏血酸2-葡糖苷以约0.3％至约0.6％w/w的量存在，基于组合物的总重量计。

30.一种用于增加皮肤光滑性、水合和弹性中的至少一种的方法中使用的组合物，其中所述方法包括：

以单次处理疗程，向皮肤区域约0.5mm至约5.0mm、优选约1.0mm至约4.0mm、更优选约1.5mm至约3.0mm深度处引入组合物的多次间隔隔开的注射，所述组合物包含含有BDDE-交联HA和未交联HA的透明质酸(HA)凝胶；

其中组合物的HA浓度小于约17.0mg/g，例如约10.0mg/g至约14.0mg/g，优选约12.0mg/g；

其中注射以约5μL至约100μL每次注射的量引入；

其中注射通过28G至33G的规格的针递送；

其中注射以约5mm至约20mm例如5mm或10mm的距离间隔隔开；并且

其中皮肤区域因处理带来的增加的光滑性、水合和/或弹性维持至少约6个月、至少约9个月或至少约12个月。

31.一种增加皮肤光滑性、水合和弹性中的至少一种的非治疗性方法，其中所述方法包括：

以单次处理疗程，向皮肤区域约0.5mm至约5.0mm、优选约1.0mm至约4.0mm、更优选约1.5mm至约3.0mm深度处引入组合物的多次间隔隔开的注射，所述组合物包含含有BDDE-交联HA和未交联HA的透明质酸(HA)凝胶；

其中组合物的HA浓度小于约17.0mg/g，例如约10.0mg/g至约14.0mg/g，优选约12.0mg/g；

其中注射以约5μL至约100μL每次注射的量引入；

其中注射通过28G至33G的规格的针递送；

其中注射以约5mm至约20mm例如5mm或10mm的距离间隔隔开；并且

其中皮肤区域因处理带来的增加的光滑性、水合和/或弹性维持至少约6个月、至少约9个月或至少约12个月。

32.权利要求30的用于所述用途的组合物，或权利要求31的非治疗性方法，其中组合物还包含甘露醇和维生素C衍生物中的至少一种，或者其中组合物不包含抗氧化剂或维生素和/或其中组合物还包含利多卡因，优选以组合物的约0.1重量％至约5重量％的量包含，例如0.3重量％。

33.一种用于增加皮肤光滑性、水合和弹性中的至少一种的组合物，其包含：

透明质酸(HA)凝胶，所述透明质酸(HA)凝胶含有由低分子量HA材料制成的交联HA基质，所述低分子量HA材料的重均分子量为约0.20MDa至约0.99MDa，优选约400000Da至约800000Da；

其中组合物的HA浓度小于约17.0mg/g，例如约10.0mg/g至约14.0mg/g，优选12.0mg/g；并且

其中从以单次处理疗程向皮肤中引入组合物起，组合物维持因处理带来的增加的光滑性、水合和/或弹性至少约6个月、至少约9个月或至少约12个月，所述单次处理疗程包括组合物的多次间隔隔开的注射，其中注射以约5μL至约100μL每次注射的量引入，通过28G至33G优选32G的规格的针递送，并且以约5mm至约20mm例如5mm或10mm的距离间隔隔开。

34.权利要求33的组合物，其中组合物还包含甘露醇和维生素C衍生物中的至少一种，或者其中组合物不包含抗氧化剂或维生素和/或其中组合物还包含利多卡因，优选以组合物的约0.1重量％至约5重量％的量包含，例如0.3重量％。

35.权利要求33-34的组合物，其中交联HA基质由大于50重量％，至少70重量％或至少约90重量％的所述低分子量HA材料制成，基于HA材料的总重量计。

36.权利要求33-35的组合物，其中交联HA基质由选自以下的交联剂交联：1,4-丁二醇二缩水甘油醚(BDDE)、1,2-双(2,3-环氧丙氧基)乙烯和1-(2,3-环氧丙基)-2,3-环氧环己烷。

用于改善皮肤外观的组合物和方法

[0001] 本发明主要涉及可注射组合物，更具体地，涉及用于处理皮肤细纹的基于透明质酸的组合物。

[0002] 目前，用于改善全脸或其他重要皮肤表面区域的皮肤质量的可注射处理选项需要多个处理疗程(通常3-4次处理，每次3至4周)，并且具有较短的效果持续时间。

[0003] 皮肤由表皮和真皮构成。最外面的表皮由具有下层基膜的复层扁平上皮组成。表皮不含血管，通过真皮的扩散获得营养。构成表皮的主要细胞类型是角化细胞，也存在黑素细胞和朗格汉斯细胞。这层皮肤负责维持身体内的水分，并阻挡有害化学物质和病原体。

[0004] 真皮位于表皮下方并且包含许多结构，包括血管、神经、毛囊、平滑肌、腺体和淋巴组织。真皮(dermis或corium)的厚度通常为3-5mm，是人皮肤的主要组成部分。真皮由结缔组织网络组成，主要是提供支撑的胶原纤维和提供弹性的弹性组织。主要细胞类型是成纤维原细胞、脂肪细胞(储存脂肪)和巨噬细胞。透明质酸(HA)是真皮组分的一部分，并且是细胞外基质的主要成分。

[0005] 面部老化的发生是多种因素的结果：皮肤内的固有变化、重力效应、面部肌肉对皮肤的作用(动力性皱纹(dynamic lines))、软组织损失或移位以及骨损失和组织弹性损失。当表皮开始变薄时，皮肤老化，导致与真皮的结合处变平。随着人变老，胶原减少，并且胶原束(其使皮肤充盈)变得松散并丧失强度。当皮肤失去弹性时，其较少能够耐受拉伸。加上重力、肌肉拉力和组织改变，皮肤开始起皱纹。水分流失和细胞之间的键的断裂也降低皮肤的屏障功能，这会导致皮肤的孔径增大。

[0006] 众所周知，眼睛通常是第一个显示老化迹象的面部特征。因为眼睛周围的皮肤较薄，所以眼睛周围的皮肤变化比脸的其余部分更早发生。眼睛周围的皮肤含有较少的腺体，并经受不断的眨眼、眯眼、摩擦和拉动。当脸颊开始下垂时面中部老化，引起鼻唇沟皱褶。鼻唇沟皱褶是从鼻子的两侧到嘴角的皱纹。随着面部老化，在面部较低区域，面部组织下降。这导致所谓的“笑纹”。这些以及其他皱褶和皱纹目前通常用皮下和真皮注射面部美容填充剂来处理，面部美容填充剂增加皮肤所失去的体积，从而减少皱褶和皱纹外观。

[0007] 透明质酸(HA)(hyaluronic acid，也称为hyaluronan)，目前是真皮填充剂中最常使用的组分之一。透明质酸是天然存在的水溶性多糖，特别是糖胺聚糖(glycosaminoglycan)，其为细胞外基质的主要成分，广泛分布在动物组织中。透明质酸在所有物种和组织中的结构相同，这使得该多糖成为用作健康和医药中的生物材料的理想物质。

[0008] HA具有优异的生物相容性，并且与胶原蛋白不同，其在植入前不需要任何皮肤测试。此外，HA具有结合大量水的能力，使其成为优异的软组织丰盈剂(volumizer)。

[0009] 为了提高HA在体内的寿命，真皮填充剂中的HA通常是交联的。化学交联HA通过在合适的反应条件下将未交联HA与交联剂进行反应而形成。

[0010] 通常认为粘度高的基于HA的真皮填充剂——例如高度交联和/或由高分子量HA制成和/或具有高HA浓度的填充剂——倾向于在体内持续更长时间。相反地，通常认为粘度低的基于HA的真皮填充剂——例如较轻度交联和/或由低分子量HA制成和/或具有低HA浓度

的基于HA的真皮填充剂——可能具有较短的体内持续时间。自然地，通过针注射高粘度材料相对更加困难，通常比注射较低粘度的材料需要更低规格(gauge)的针(例如，相比于27G或30G，需要21G或23G的)。已经证明难以开发一种既容易通过高规格针(即细针)注射又具有延长的体内持续时间的基于HA的组合物。

发明内容

[0011] 本发明涉及可注射组合物，更具体而言，涉及用于皮内注射到皮肤中的可注射组合物。该组合物和方法通过填充浅表皮肤凹陷、和/或改善皮肤质量和外观而提供改善的皮肤外观和质量。一方面，该组合物和方法提供改善的皮肤纹理、增加的皮肤水合和增加的弹性中的至少一种。

[0012] 一方面，本发明的组合物是基于透明质酸(HA)和HA的药学上可接受的盐，例如透明质酸钠(NaHA)。许多长效、高度可注射的本发明组合物包含由较低分子量HA制成的交联HA基质。在一些实施方案中，组合物具有较低的HA浓度。有利地，本文中提供的许多组合物具有延长的效果持续时间。例如，本文中提供的许多本发明组合物和方法具有三个月、四个月、六个月、至一年或以上的效果持续时间，而不需要每三至四个星期进行多次重复处理(如采取常规皮内注射处理方法所常见地)。

[0013] 在本发明的宽的方面，提供一种组合物，所述组合物主要包含HA凝胶，所述HA凝胶包含低分子量HA材料或基本上由低分子量HA材料组成。HA组分包含多于50重量％(例如至少70重量％，例如约90重量％)的低分子量HA。低分子量HA材料的重均分子量不超过约0.20MDa和约0.99MDa，例如为约0.4MDa至约0.8MDa。

[0014] 在一些实施方案中，HA凝胶可以还包含高分子量HA，即分子量为至少约1.0MDa至约4.0MDa的HA材料。一般而言，在包含高分子量HA材料的本发明实施方案中，高分子量HA材料的重均分子量是低分子量HA材料的重均分子量的至少两倍。

[0015] HA凝胶的HA可以交联。例如，HA可通过合适的交联剂化学交联。在一些实施方案中，交联剂选自：1,4-丁二醇二缩水甘油醚(BDDE)、或1,4-双(2,3-环氧丙氧基)丁烷、或1,4-双缩水甘油基氧基丁烷(它们全部通常称为BDDE)，1,2-双(2,3-环氧丙氧基)乙烯和1-(2,3-环氧丙基)-2,3-环氧环己烷。

[0016] 在一些实施方案中，组合物具有约10.0mg/g至最高达约17.0mg/g的HA浓度。在一些实施方案中，HA浓度小于约17.0mg/g，例如小于约15.0mg/g。在一些实施方案中，HA浓度为约10.0mg/g至约14.0mg/g。在一些实施方案中，HA浓度为约10.0mg/g、约11.0mg/g、约12.0mg/g、约13.0mg/g或约14.0mg/g。

[0017] 在本发明的另一方面，提供使用本发明组合物处理皮肤的方法。例如，提供改善皮肤的一种或多种特性或改善皮肤外观或纹理的方法。

[0018] 一方面，提供处理皮肤干燥、纹理或粗糙和/或弹性的方法。所述方法一般包括通过向皮肤区域引入组合物的多次间隔隔开的注射来处理皮肤区域，所述组合物包含含有交联HA的透明质酸(HA)凝胶，其中经处理的皮肤因处理带来的改善的水合、更光滑的纹理或增加的弹性维持延长的持续时间，例如，至少约3个月至约一年或以上。在一个特别有利的实施方案中，引入步骤仅以单次处理疗程(session)进行，从而消除对重复处理以维持效果持久的需要。

[0019] 在一些实施方案中，引入步骤包括以约2mm至约30mm距离间隔隔开的注射而引入组合物。例如，引入步骤包括以约5mm至约20mm、或约10mm至约15mm距离间隔隔开的注射而引入组合物。在一些实施方案中，组合物以约500微米至约2000微米的注射深度引入，例如约1000微米的深度。在优选实施方案中，组合物以约0.5mm至约5.0mm，优选约1.0mm至约4.0mm，更优选约1.5mm至约3.0mm的注射深度引入。一般而言，较深的注射提供改善的水合结果。

[0020] 另一方面，本发明提供一种处理皮肤粗糙的方法，包括通过向皮肤区域引入组合物的多次间隔隔开的注射来处理皮肤区域，所述组合物包含含有交联HA的透明质酸(HA)凝胶，其中经处理的皮肤因处理带来的更光滑的纹理维持至少约3个月、至少约4个月、至少约6个月或至少约12个月。

具体实施方式

[0021] 在数值的上下文中，术语“约”将是本领域技术人员容易理解的，优选意为那个特定值可以调整+/-10％。关于范围的端点，修饰词“约”优选意为较低的端点可减小10％，较高的端点增加10％。还可想到，在本申请中公开的各数值或范围可以是绝对的，即可以删除修饰词“约”。

[0022] 在本文中，表示HA的“分子量”的所有数值都应被理解作指示以道尔顿为单位的重均分子量(Mw)。

[0023] HA的分子量使用以下Mark Houwink关系由特性粘度测量值来计算：

[0024] 特性粘度$(m^3/kg) = 9.78 \times 10^{-5} \times Mw^{0.690}$

[0025] 特性粘度根据European Pharmacopoeia(HA专论N°1472,01/2009)规定的程序来测量。

[0026] 如本文中所使用的高分子量HA描述的是具有至少约1百万道尔顿($Mw \geq 10^6$Da或1MDa)至约5.0MDa分子量的HA材料。例如，在本发明组合物中的高分子量HA可具有约1.5MDa至约3.0MDa范围内的分子量，或该高分子量HA可具有约2.0MDa的重均分子量。在另一实例中，高分子量HA可具有约3.0MDa的分子量。在另一实例中，高分子量HA的分子量可为约1MDa(根据如上所述的Mark Houwink关系计算时，其对应于1.35m^3/kg的特性粘度)、1.5MDa(1.35m^3/kg)、2MDa(2.18m^3/kg)、3MDa(2.88m^3/kg)、5MDa(4.10m^3/kg)、0.2MDa(0.44m^3/kg)、0.4MDa(0.72m^3/kg)、0.8MDa(1.1m^3/kg)、0.99MDa(1.34m^3/kg)。

[0027] 如本文中所使用的低分子量HA描述的是具有小于约1.0MDa的分子量的HA材料。低分子量HA可具有约200000Da(0.2MDa)至小于1.0MDa的分子量，例如，约400000Da至约800000Da，例如约386000Da(0.386MDa)至约740000Da(0.74MDa)。在一些实施方案中，用于制备交联HA基质的低分子量HA不超过0.99MDa。

[0028] 优选地，低分子量HA和高分子量HA的混合物具有双峰分子量分布(bimodal molecular weight distribution)。混合物也可具有多峰分布。

[0029] 在本发明的一个方面，组合物包含具有高分子量组分和低分子量组分的HA，并且高分子量组分的重均分子量是低分子量组分的重均分子量的至少两倍。

[0030] 例如，根据本发明的该方面的组合物可包含具有约500000Da的重均分子量的低分子量组分，和具有约或至少约1.0MDa的重均分子量的高分子量组分。

[0031] 在另一实例中，根据本发明的组合物可包含具有约800000Da的重均分子量的低分子量组分，和具有约或至少约1.6MDa的重均分子量的高分子量组分。

[0032] 交联度由交联剂与HA二糖单元的最终重量比来度量。

[0033] 如本文中所使用的未交联HA，其意指彼此未交联的单个HA聚合物分子。未交联HA通常仍然可溶于水。

[0034] 在本文中提供了用于处理皮肤的组合物——例如可经皮内引入的可注射组合物——以改善皮肤外观或皮肤质量，例如改善水合、纹理和/或弹性。该组合物也可有效地处理皮肤细纹并减少浅表皮肤凹陷。还提供了制备这些组合物的方法，以及使用这些组合物的处理方法。该组合物是基于透明质酸(HA)和HA的药学上可接受的盐，例如透明质酸钠(NaHA)。

[0035] 如本文中所用，透明质酸(HA)可以意指任何其透明质酸盐，包括但不限于：透明质酸钠(NaHA)、透明质酸钾、透明质酸镁、透明质酸钙及其组合物。HA及其药学上可接受的盐均可被用于本发明中。

[0036] 一般而言，在一些本发明组合物中，HA的浓度为约10.0mg/g至最高达约17.0mg/g。在一些实施方案中，HA浓度小于约17.0mg/g，例如小于约15.0mg/g。在一些实施方案中，HA浓度为约10.0mg/g至约14.0mg/g。在一些实施方案中，HA浓度为约10.0mg/g、约11.0mg/g、约12.0mg/g、约13.0mg/g或约14.0mg/g。

[0037] 本发明的一些组合物包含额外的试剂，例如，有效量的缓解注射组合物所经受疼痛的麻醉剂。麻醉剂可选自氨布卡因、阿莫拉酮、阿米卡因、丁氧普鲁卡因(benoxinate)、苯佐卡因、贝托卡因、苯柳胺酯(biphenamine)、布比卡因、布他卡因、氨苯丁酯、布坦卡因、丁胺卡因、丁托西卡因(butoxycaine)、卡替卡因(carticaine)、氯普鲁卡因、可卡乙碱(cocaethylene)、可卡因、环美卡因、辛可卡因、二甲异喹(dimethisoquin)、二甲卡因、地哌冬、双环胺(dicyclomine)、去水芽子碱(ecgonidine)、芽子碱(ecgonine)、氯乙烷、依替卡因、β-优卡因、尤普罗辛、非那可明、福莫卡因(formocaine)、海克卡因、羟丁卡因、对氨苯酸异丁酯、甲磺酸亮氨卡因、左沙屈尔、利多卡因、马比佛卡因、美普卡因、美布卡因、氯甲烷、麦替卡因、纳依卡因、奥妥卡因(octocaine)、奥索卡因、奥昔卡因(oxethazaine)、对乙氧卡因、芬那卡因、苯酚、皮珀罗卡因、哌啶卡因、聚多卡醇、普莫卡因、丙胺卡因、普鲁卡因、丙泮卡因、丙美卡因、丙哌卡因、丙氧卡因、假可卡因、吡咯卡因、罗哌卡因、水杨醇、丁卡因、托利卡因、三甲卡因、佐拉敏以及它们的盐。在一个实施方案中，至少一种麻醉剂是利多卡因，例如为盐酸利多卡因的形式。本文所述的组合物的利多卡因浓度可为组合物的约0.1重量%至约5重量%，例如，组合物的约0.2重量%至约1.0重量%。在一个实施方案中，组合物的利多卡因浓度为组合物的约0.3重量%(w/w%)。

[0038] 在一些实施方案中，组合物还包含有益添加剂，例如抗氧化剂。在一些实施方案中，组合物包含例如甘露醇。甘露醇的存在量可为约0.1%w/w至约2.0%w/w，或约0.3%至约0.9%w/w。在一些实施方案中，甘露醇的存在量小于、不大于或约1.0%w/w。在一些实施方案中，甘露醇的存在量为约0.9%w/w。在其它实施方案中，甘露醇的存在量为约0.1%w/w，或约0.2%w/w，或约0.3%w/w，或约0.4%w/w，或约0.5%w/w，或约0.6%w/w，或约0.7%w/w，或约0.8%w/w，或约0.9%w/w，或约1.0%w/w。在其它实施方案中，甘露醇的存在量大于约1.0%w/w。在一些实施方案中，甘露醇的存在量为约1.0%w/w至约5.0%w/w。

[0039] 在一些实施方案中，组合物还包含维生素，例如，维生素C。在一个更优选的实施方案中，该维生素是维生素C的衍生物或维生素C的稳定形式，例如抗坏血酸2-葡糖苷。维生素的存在量可为约0.1％至约2.0％w/w，或约0.2％至约1.0％w/w，或约0.3％至约0.6％w/w。在一些实施方案中，维生素C的存在量为约0.6％w/w。在其它实施方案中，维生素C衍生物的存在量为约0.1％w/w，或约0.2％w/w，或约0.3％w/w，或约0.4％w/w，或约0.5％w/w，或约0.6％w/w，或约0.7％w/w，或约0.8％w/w，或约0.9％w/w，或约1.0％w/w。在其它实施方案中，维生素C衍生物的存在量大于1.0％。在一些实施方案中，维生素C衍生物的存在量为约1.0％w/w至约5.0％w/w。

[0040] 在一些实施方案中，组合物还包含甘露醇和抗坏血酸2-葡糖苷的混合物。在这些实施方案的一些中，甘露醇的存在量不大于1.0％w/w，例如为0.9％w/w，并且抗坏血酸2-葡糖苷的存在量为约0.6％w/w。

[0041] 在一些实施方案中，组合物不包含抗氧化剂或维生素。例如，在一些实施方案中，组合物包含经交联剂交联的透明质酸及水、或基本上由经交联剂交联的透明质酸及水组成。这些组合物可包含或可不包含麻醉剂如利多卡因。

[0042] 本发明的产品和组合物优选以无菌形式提供。组合物可采用常规方法灭菌，例如高压灭菌。例如，可通过将组合物暴露于至少约120℃至约130℃的温度和/或至少约12磅/平方英寸(PSI)至约20PSI的压力至少约1分钟至约15分钟的时间而将组合物灭菌。

[0043] 制备组合物的方法包括提供干燥HA纤维或粉末形式的原料HA材料的步骤。原料HA材料可以是HA，其盐和/或它们的混合物。在一个优选实施方案中，该HA材料包括NaHA的纤维或粉末，例如细菌来源的NaHA纤维。在本说明书的一些方面，该HA材料可以是动物来源的。该HA材料可以是包括HA和至少一种其它多糖如糖胺聚糖(GAG)的原料材料的混合物。

[0044] 在本发明的广义方面，组合物的HA材料可包含交联的HA基质，其由约5％至约95％低分子量HA及其余的包含高分子量HA的HA材料制成。

[0045] 在本发明的一些实施方案中，用于制备本发明组合物的HA材料几乎完全包含低分子量HA或由低分子量HA组成。在一些实施方案中，几乎100％的用于制备本发明组合物的HA材料都可以是如上文定义的低分子量HA。在其它实施方案中，用于制备组合物的HA材料包含较高分子量HA和较低分子量HA的混合物，如上文定义的。在某些实施方案中，组合物中至少约50％w/w，例如至少约70％w/w，例如至少约90％w/w或更多的HA材料是如上文定义的低分子量HA，其余部分的HA是高分子量HA。在一个实施方案中，组合物是使用90:10比例的低分子量HA和高分子量HA制成。也就是说，这些实施方案的组合物是由高分子量HA和低分子量HA的混合物制成，其中约90％w/w的HA是低分子量HA。

[0046] 在一个实施方案中，使纯的干燥NaHA纤维在碱性溶液中与水化合以生成未交联的NaHA凝胶。在该步骤中可使用任何合适的碱性溶液使NaHA与水化合，例如，但不限于含有以下物质的水溶液：氢氧化钠(NaOH)、氢氧化钾(KOH)、碳酸氢钠($NaHCO_3$)、氢氧化锂(LiOH)等。在另一实施方案中，合适的碱性溶液是含有NaOH的水溶液。所得碱性凝胶将具有高于7.5的pH。所得碱性凝胶的pH可为大于9的pH，或大于10的pH，或大于11的pH，或大于12的pH，或大于13的pH。

[0047] 制备方法的下一步包括用合适的交联剂交联该水合的碱性NaHA凝胶的步骤。交联剂可为已知适于使多糖及其衍生物通过它们的羟基进行交联的任何试剂。适合的交联剂包

括但不限于1,4-丁二醇二缩水甘油醚(或1,4-双(2,3-环氧丙氧基)丁烷、或1,4-双缩水甘油基氧基丁烷,其全部通常称为BDDE)、1,2-双(2,3-环氧丙氧基)乙烯和1-(2,3-环氧丙基)-2,3-环氧环己烷。本公开内容的范围不排除使用多于一种交联剂或使用不同的交联剂。在一个实施方案中,本文中所述的HA凝胶是使用BDDE进行交联。

[0048] 交联步骤可采取本领域普通技术人员已知的任何手段进行。本领域技术人员了解如何根据HA的性质来优化交联条件,以及如何使交联进行至最佳程度。

[0049] 在另一实施方案中,HA的交联在HA纤维的水合过程中完成,其通过使混合的高分子量和低分子量纤维在含有交联剂如BDDE的碱性溶液中与水化合而进行。

[0050] 本发明组合物的HA组分的交联度为至少约1%BDDE/HA至最高达约20%BDDE/HA(w/w),例如约4%至约12%w/w,例如约10%w/w,例如约8%w/w,例如约6%w/w,例如约5%w/w,例如约4%w/w。

[0051] 可以使水合交联的HA凝胶溶胀,以获得所需的HA浓度。该步骤可通过中和交联的水合HA凝胶,例如通过加入含有酸如HCl的水溶液来完成。然后使凝胶在磷酸盐缓冲盐水(PBS)溶液中溶胀。

[0052] 可通过常规方式如对磷酸盐缓冲液透析、或醇沉淀而将凝胶纯化,以回收交联材料、稳定材料的pH并除去任何未反应的交联剂。将组合物混合以达到均匀。优选地,均化步骤包括用可控的剪切力混合、搅拌或拍打凝胶,获得基本均匀的混合物。在一些实施方案中,在混合期间或之后,加入磷酸盐缓冲液,以使在最终的组合物中达到所需的HA浓度。

[0053] 在一些实施方案中,向组合物中加入利多卡因(例如以盐酸利多卡因的形式)或另一合适的麻醉剂。例如,将经纯化的基本上pH中性的凝胶的pH调节至使凝胶变成微碱性,使得凝胶的pH值大于约7.2,例如,约7.5至约8.0。或者,将凝胶调节至使凝胶变成碱性,使得凝胶的pH大于约9,例如约10.0至约11.0。该步骤可通过任何合适的手段来完成,例如通过向凝胶中加入适量的稀的NaOH、KOH、$NaHCO_3$或LiOH,或本领域技术人员已知的任何其它碱性分子、溶液和/或缓冲组合物。例如,在一些实施方案中,提供粉末形式的盐酸利多卡因,将其用注射用水(WFI)溶解。然后将该利多卡因加入到(微)碱性凝胶中。如果需要,然后可向含利多卡因的凝胶中加入未交联的HA。例如,在一个实施方案中,期望的HA浓度为约12.0mg/g。包含利多卡因的组合物的利多卡因浓度可为组合物的约0.1%至约5重量%,例如约0.3重量%(或w/w),基于组合物的总重量计。包含未交联HA的组合物的未交联HA的浓度可小于10%w/w或小于5%w/w,例如优选为组合物的约0.5重量%至约1.5重量%,例如约0.9至1.0%,基于组合物的总重量计。未交联HA优选具有高分子量。

[0054] 将组合物引入递送装置,例如注射器中。根据本说明书,有用的注射器包括能够递送粘性真皮填充组合物的本领域已知的任何注射器。注射器的内体积可为约0.4mL至约3mL,约0.5mL至约1.5mL或约0.8mL至约1.0mL。

[0055] 在其它实施方案中,将组合物引入适于递送该组合物的注射装置中,采取多次微滴注射进入到较浅的浅表皮肤表面。

[0056] 用于递送本发明组合物的针的规格包括约18G至约40G的规格。在一些实施方案中,用于递送组合物的针为约25G至约33G,例如约31G至约33G,或约32G至约33G。在一些实施方案中,使用28G、29G、30G、32G或33G的规格的针递送组合物。

[0057] 在本发明的另一方面,提供使用本发明组合物处理皮肤的方法。例如,提供改善皮

肤的一种或多种质量或改善皮肤外观或纹理的方法。

[0058] 在一个方面，提供处理皮肤干燥、纹理或粗糙、和/或弹性的方法。所述方法一般包括通过向皮肤区域引入组合物的多次间隔隔开的注射来处理皮肤区域，所述组合物包含含有交联HA的透明质酸(HA)凝胶，其中经处理的皮肤因处理带来的改善的水合、更光滑的纹理或增加的弹性维持延长的持续时间，例如至少约3个月至约一年或以上。

[0059] 在一个特别有利的实施方案中，引入步骤仅以单次处理疗程进行，从而消除对重复处理以维持效果持久的需要。

[0060] 在本发明的一个方面，所提供的皮肤处理方法包括在一次处理疗程期间将组合物引入到皮肤中，所述一次处理疗程包括向皮肤区域中多次注射组合物。

[0061] 在一个实施方案中，一次处理疗程包括病人单次到访医师。在该处理疗程期间，可向皮肤，例如向特定皮肤区域中给予多次注射。

[0062] 单次处理疗程的多次注射可包括，例如，2至约500次注射，约50至约200次注射。在一些实施方案中，一次处理疗程包括向皮肤区域中的例如至少2次注射、至少10次注射、至少20次注射、至少40次注射、至少60次注射、至少80次注射、至少100次注射、至少140次注射、至少180次注射、至少200次注射、至少300次注射、至少400次注射、至少500次注射或更多次。

[0063] 在一些实施方案中，处理疗程不超过约45分钟、不超过约30分钟、不超过约15分钟、或不超过约10分钟/每个处理区域。处理区域定义为被本发明组合物和方法处理的皮肤区域。处理区域可包括面部、颈部或胸部(décolletage)中的至少一个皮肤区域，或由面部、颈部或胸部中的至少一个皮肤区域组成。处理区域也可包括除了面部、颈部或胸部之外的皮肤区域，或由除了面部、颈部或胸部之外的皮肤区域组成，例如手、膝盖、肘部、前臂、小腿、大腿、背部的顶部的皮肤区域或任何其它皮肤区域，所述任何其它皮肤区域是可使用本发明组合物和方法处理并从而受益或改善的皮肤区域。

[0064] 在一些实施方案中，引入步骤包括以约2mm至约30mm的距离间隔隔开的注射而引入组合物。例如，引入步骤包括以约5mm至约20mm的距离间隔隔开的注射而引入组合物。在一些实施方案中，引入步骤包括以约10mm至约15mm的距离间隔隔开的注射而引入组合物。

[0065] 在一些实施方案中，在皮肤中的非常浅的深度处提供注射。例如，在一些实施方案中，在不大于约2000微米的深度处引入组合物。例如，在约500微米至约2000微米，约800微米至约1600微米，约1000微米至约1200微米的深度处提供注射。在优选实施方案中，在约0.5mm至约5.0mm、优选约1.0mm至约4.0mm、更优选约1.5mm至约3.0mm的注射深度处引入组合物。在一个实施方案中，以约1μL至约200μL例如约5μL至约100μL每次注射、例如约20μL至约80μL、例如约40μL至约60μL每次注射的量引入注射。在一些实施方案中，以约5μL至约500μL每次注射、约10μL至约400μL、约50μL至约200μL、或约100μL每次注射的量引入注射。

[0066] 在一些实施方案中，注射通过至少27G，例如28G、30G或32G的规格的针递送。

[0067] 有利地，处理方法可包括持续较短时间量的单次处理疗程或由持续较短时间量的单次处理疗程组成。在一些实施方案中，处理疗程覆盖被处理的皮肤区域，包括向皮肤区域中多次皮内注射，并且进行不超过约45分钟。在一些实施方案中，处理疗程不超过约30分钟。在又一些实施方案中，处理疗程不超过约20分钟，或不超过约15分钟，或不超过约10分钟。

[0068] 还提供一种处理皮肤粗糙的方法，其中所述方法包括通过向皮肤区域引入组合物的多次间隔隔开的注射来处理皮肤区域，所述组合物包含含有交联HA的透明质酸(HA)凝胶，其中经处理的皮肤因处理带来的更光滑的纹理维持至少约3个月、至少约4个月、至少约6个月、至少约9个月、或至少约12个月。

[0069] 在一个特定的实施方案中，本发明的组合物包含用1,4-丁二醇二缩水甘油醚(BDDE)交联的非动物来源的透明质酸(HA)凝胶的无菌生理溶液，浓度为12mg/mL。该组合物可用于处理浅表皮肤凹陷，以与未经处理的浅表皮肤凹陷相比在皮肤纹理上的改善和皮肤质量如光滑度、水合和弹性的改善作为量度。在一个特定的实施方案中，处理方法包括使用32G的针穿过目标组织区域向真皮内注射，例如积存注射(depot injection)。目标组织区域包括面部皮肤区域和颈部皮肤区域。病人可以明显注意到与未经处理的皮肤相比皮肤外观的改善，如减少的凹陷、细纹和不均匀或粗糙纹理的外观，并且在仅持续45分钟、或仅30分钟、或仅20分钟、或仅15分钟或更短的单次处理疗程之后，改善可持续至少4个月、6个月、9个月或甚至12个月的时间。皮肤纹理的改善可以使用适当的尺度(scale)来评估。水合和弹性的皮肤质量测量可使用适当的仪器进行，并与基线(即未经处理的皮肤)比较。其他评估方法，例如，FACE Q和GAIS可分别用于评定受试者和调查者的满意度。在一个特定的实施方案中，在处理之后产品作用的持续时间为至少约4个月，例如约6个月。

[0070] 在一些实施方案中，在组合物中添加利多卡因可减轻处理区域的疼痛。然而，在一些实施方案中，组合物不包含利多卡因，以解决病人关于利多卡因过敏及疼痛敏感的需要。

[0071] 另一实施方案提供增加皮肤光滑度、水合和弹性中的至少一种的方法，包括在单次处理疗程中，向皮肤区域约500微米至约5000微米深度处引入组合物的多次间隔隔开的注射，所述组合物包含含有经BDDE交联的HA和未交联HA的透明质酸(HA)凝胶，其中以约5μL至约100μL每次注射的量引入注射，其中注射通过28G至33G的规格的针递送，其中注射以约5mm至约20mm的距离间隔隔开。

[0072] 在一些实施方案中，皮肤区域因处理带来的增加的光滑度、水合和/或弹性维持至少约3个月、约4个月、约5个月、约6个月、9个月、12个月或更长。

[0073] 在另一实施方案中，提供用于增加皮肤光滑度、水合和弹性中的至少一种的组合物，其包含透明质酸(HA)凝胶，所述透明质酸(HA)凝胶包含由重均分子量约400000Da至约800000Da的低分子量HA材料制成的交联HA基质；其中组合物的HA浓度为约10.0mg/g至约14.0mg/g；并且其中从以包括组合物的多次间隔隔开的注射的单次处理疗程被引入到皮肤中起，组合物使因处理带来的增加的光滑度、水合和/或弹性维持至少约6个月，其中以约5μL至约100μL每次注射的量引入注射，通过28G至33G的规格的针递送，并且以约5mm至约20mm的距离间隔隔开。皮肤区域因处理带来的增加的光滑度、水合和/或弹性可维持至少约3个月、约4个月、约5个月、约6个月、9个月、12个月或更长。组合物还可包含甘露醇和维生素C中的至少一种。在一些实施方案中，在组合物中既存在甘露醇和又存在维生素C衍生物。

[0074] 如果在0.1Hz下测量，组合物具有约50至200Pa，例如100-150Pa或120Pa的凝胶硬度G'，如果在5Hz下测量，约100至300Pa，例如150至200Pa或175Pa。测量凝胶硬度的方法是本领域已知的。凝胶硬度是真皮填充剂柔软度的指示。

[0075] 此外，如果在0.1Hz下测量，组合物具有约10至100Pa，例如15-40Pa或20Pa的凝胶粘度G"，或者如果在5Hz下测量，约10至100Pa，例如20-40Pa或30Pa。测量粘度的方法是本领

域已知的。

[0076] 组合物还具有约5至20N，例如10-15N或12N的压缩性(compression)。测量压缩性的方法是本领域已知的。压缩性是真皮填充剂的抗变形性的指示。一般而言，压缩性越低，填充剂的可提升展开性(lift spreadibility)越低，即与更适合于较深注射和体积复原的具有较高压缩性的填充剂相比，所述填充剂更适合于处理面部或颈部的浅表细纹和褶皱。

[0077] 实施例1

[0078] 自由基降解测试使得可以评估凝胶样品对HA链被自由基降解(HA的主要降解途径之一)的耐受性。对3个不同批次的本发明组合物进行自由基降解测试。所得结果显示在表1中。发现所有的测量值都是一致的(CVr<10％)。

[0079]

凝胶		初始粘度（Pa.s）	降解时间（s）	一致性
LBA2-214		17.7	4572	CF
		15.5	4858	
		16.1	5044	
	平均值	16.4	4825	
	STD	1.1	238	
	CVr（％）	7%	5%	
LBA2-263		12.5	5377	CF
		13.8	5009	
		13.9	4882	
	平均值	13.4	5090	
	STD	0.8	257	
	CVr（％）	6%	5%	
LBA2-288		13.7	5057	CF
		14.1	5189	
		13.7	4878	
	平均值	13.8	5041	
	STD	0.2	156	
	CVr (%)	2%	3%	

[0080] 表1本发明组合物的自由基降解结果

[0081] 3个不同批次的批次间差异为约3％，且降解时间平均值为4985s(表2)。

[0082]

	LBA2-214	LBA2-288	LBA2-263
mes1	4572	5057	5377
mes2	4858	5189	5009
mes3	5044	4878	4882

[0083]

平均值	4825	5041	5089	4985
STD	238	156	257	141
CVr	5%	3%	5%	3%

[0084] 表2本发明组合物降解时间的批次间差异

[0085] 实施例2

[0086] 一名37岁的女性在其生命过程中因老化、暴露于干燥的气候和/或太阳/风而表现出粗糙、干燥的面部皮肤。医师通过皮内微-积存注射(micro-depot injection)给予本文所述的组合物。处理由每个皮肤区域10至约100次浅表注射构成，用32G/4mm的针进行。所处理的皮肤区域为面部、颈部和胸部。从最初注射至最后注射，对患者所有皮肤区域的全部的处理疗程持续约40分钟。各处理区域以间隔隔开的注射接受合适量的组合物。例如，面部区域接受约2mL至约3mL的组合物，通过约每10mm至约15mm的间隔隔开的浅表单次注射而给予。颈部用约1mL至2mL的组合物处理，约每15mm至约20mm间隔隔开地注射。处理之后，通过视觉、触觉和压力感来检验经处理的皮肤区域。处理开始时和结束时进行照相评估。患者向医师报告：处理仅引起极小的不适。处理之后患者立即回到其日常活动中。在其经处理的右手皮肤的一小块区域中，发现了瘀斑，但是这在施用抗炎霜后几天就消散了。在处理之后4个月，病人返回医师处进行随访评价。在随访评价访问之前，患者没有进行进一步的填充剂注射或美索疗法处理(mesotherapy treatments)。在随访访问时的客观检查中，处理已引起表皮纹理改善、干燥度降低并且皮肤亮度提高。通过照片资料部分地证明了这些改善。轻轻触摸经处理的皮肤区域，似乎在水合、柔软度、弹性和色调方面有所增进。通过完成自我评估调查表，患者对处理表示高度满意。患者声称经处理的区域已经改善，并且她对结果非常满意。有趣的是，这些良好的结果基于仅单次处理疗程而实现，在处理疗程和随访访问之间的时间内没有重复注射、“增补(top-up)”或进一步注射处理。

[0087] 实施例3

[0088] 可注射组合物对人存活皮肤外植体的表皮和真皮结构的水合作用通过皮肤湿度(corneometry)测量来评价：使用CM825**Corneometer®**(COURAGE&KHAZAKA)，对角质层最外皮肤层的湿度水平进行测定。**Corneometer®**的作用原理是基于以冷凝器形式设计的检测器电容的改变。与皮肤接触的测量头的表面，根据皮肤湿度水平，其电容发生改变。在D0(＝第0天)，以AU(任意单位)表示的表皮电容是皮肤水合的指标。测定了根据本公开内容的以下组合物：

[0089]

	根据本公开内容的组合物（P）
NaHA 浓度（mg/g）	10.5 - 13.5
利多卡因含量（重量%）	0.27 - 0.33
具有高分子量的未交联 HA 含量（重量%）	0.95

[0090] 表3

[0091] 在研究期间，产品是在室温下储存着的。

[0092] 外植体制备：在45岁白种女性的腹部整形术中，制备了9个外植体。在BEM培养基(BIO-EC's Explants Medium)中，在37℃下，在潮湿的5%-CO_2气氛中使外植体保持存活。将外植体分成3个批次(Nb＝数目)：

[0093]

分析	批次	名称	处理	外植体的 Nb 和大小	采样时间
皮肤湿度	T-C1	未经处理的对照（空白）	/	3; 1.5 × 2 cm	D9
	P-C1	产品 P	根据本公开内容的组合物	3; 1.5 × 2 cm	D9
	P-C2	产品 P	根据本公开内容的组合物	3; 1.5 × 2.5 cm	D9

[0094] 表4

[0095] 产品应用：用于皮肤湿度测量的C1批次的外植体是用4×10μl的可注射产品进行处理(方形注射)，各注射点间隔0.5cm(参见图1)。用于皮肤湿度测量的C2批次的外植体是用4×10μl的可注射产品进行处理(方形注射)，各注射点间隔1cm(参见图1)。未经处理的对照未接受任何处理。

[0096] 皮肤湿度：在D0、D2、D7和D9，使用CM825 Corneometer® (COURAGE&KHAZAKA)在外植体上进行皮肤水合指标、表皮电容评价。测量是使用1厘米直径的探针，在方形外植体的中心进行的。进行十次测量，并通过皮肤湿度测定仪(corneometer)计算平均值。

[0097] 采样：在D0，收集T0批次的3个外植体，并切成2个部分，一半在-80℃冷冻，一半固定在甲醛中。在D2、D7和D9，收集每批次的3个外植体并以相同的方式处理。

[0098] 统计分析：根据Student t检验进行统计分析。Student t检验给出了两个批次显著不同的概率“p”。如果$p<0.05$(*)两个批次之间差异显著，那么两个批次95%概率地显著不同；或者如果$p<0.01$(**)，那么两个批次99%概率地显著不同。

[0099] 结果：各批次的皮肤湿度的测量值(也参见图2和3)：

[0100]

	D0		D2		D7		D9	
	平均值	SD	平均值	SD	平均值	SD	平均值	SD
T	78.9	1.7	82.7	1.3	82.5	1.0	79.5	3.9
Pc1	77.6	1.1	115.5	2.9	108.3	2.4	112.0	2.2
Pc2	77.1	0.8	104.6	6.8	110.5	3.7	110.1	5.0

[0101] 表5:皮肤湿度数据

[0102] 皮肤湿度测量显示,使用产品Pc1,在D2,皮肤表面测量值比T高44.0%**,在D7比T高34.9%**,在D9比T高43.6%**。使用产品Pc2,在D2皮肤湿度测量值比T高30.7%*,在D7比T高38.6%**,在D9比T高41.9%**。(Student t检验:*显著性为$p<0.05$(95%);**显著性为$p<0.01$(99%))。无论测试条件如何,本公开内容的组合物都显示出良好的水合活性(Pc1和Pc2分别注射4次,每间隔0.5cm或1cm注射10μl),其诱导表皮电容值极大的增长,这提高了皮肤水合。

[0103] 实施例3

[0104] 本公开内容的可注射组合物对人存活皮肤外植体的表皮和真皮结构的皮肤水合作用是通过额外的皮肤湿度测量而评价的。测定了以下组合物:

[0105]

	仅包含未交联 HA 的组合物(P1)	本公开内容的组合物(P2)
NaHA 浓度(mg/g)	11.5 - 15.5	10.5 - 13.5
利多卡因含量	/	0.27% - 0.33%
甘露醇含量	0.70% - 1.10%	/
具有高分子量的未交联 HA 含量(重量%)	100%	0.95%

[0106] 表6

[0107] 在研究期间,产品是在室温下储存的。

[0108] 外植体制备:对于第一个供体,从一个59岁的白种女性,在腹部整形术中制备9个1.5cm×2cm尺寸的外植体。对于第二个供体,从一个42岁的白种女性,在腹部整形术中制备9个1.5cm×2cm尺寸的外植体。对于第三个供体,从一个52岁的白种女性,在腹部整形术中制备9个1.5cm×2cm尺寸的外植体。对于每个供体,将9个外植体分为如下3个批次:

[0109]

批次	名称	处理	外植体数目	采样时间
T	未经处理的对照（空白）	/	3	D8
P1	产品 1	本公开内容的组合物	3	D8
P2	产品 2	仅包含未交联 HA 的组合物	3	D8

[0110] 表7

[0111] 产品应用：在D0，用针将3×50μL的产品P1或P2注射到矩形(1.5×2cm)外植体的真皮中。未经处理的对照未接受任何处理。

[0112] 在D1、D2、D5和D7更新一半培养基(1ml)。

[0113] 皮肤湿度：在D0、D1、D2和D8，使用CM825Corneometer® (COURAGE&KHAZAKA)在外植体上进行皮肤水合指标、表皮电容评估。

[0114] 结果：得出三个供体的皮肤湿度测量结果，并表示为三个值的平均值(也参见图4和5)：

[0115]

		测量日			
	AU	D0	D1	D2	D8
产品	T	61.00（SD：5.9）	63.45（SD：6.0）	62.42（SD：5.6）	61.98（SD：7.5）
	P1	64.76（SD：4.3）	74.64（SD：7.3）	76.54（SD：5.6）	79.03（SD：8.2）
	P2	64.10（SD：5.5）	87.90（SD：10.5）	92.88（SD：8.1）	95.37（SD：10.1）

[0116] AU(皮肤湿度的任意单位)

[0117] 表8

[0118] 对于3个供体，皮肤湿度测量显示，产品P1导致在D1皮肤湿度测量值比在D0增加15％，在D2继续增加到18％，在D8增加到22％。产品P2导致从D0至D1迅速增加37％，并从D2至D8继续增加达49％。根据这些实验条件，并在D8(TJ8)与空白批次相比，可以得出以下结论：

[0119]

vs TJ8	P1	P2
皮肤湿度D8	+22％	+49％

[0120] 表9

[0121] 总体而言，在本研究的实验条件下，结果表明，在第8天(D8)，与产品P1和未经处理的对照T相比，本公开内容的产品(P2)在角质层(stratum corneum)中显示出增加的皮肤水合作用。

[0122] 虽然已经以一定具体的程度对本发明进行了描述和说明，但应理解，本公开内容仅以示例的方式进行了公开，本领域技术人员可以采取部分、步骤和元素的组合和排列的许多变化，而不背离本发明的范围，如下文所要求保护的。

[0123] 本发明还可由以下实施方案说明：

[0124] 1.一种用于减少皮肤浅表凹陷外观的可注射组合物，所述组合物包含：

[0125] 透明质酸(HA)凝胶，其含有由重均分子量约0.20MDa至约0.99MDa的低分子量HA材料制成的交联HA基质；

[0126] 其中组合物的HA浓度小于约17.0mg/g；

[0127] 其中组合物从被引入到皮肤中起，维持皮肤浅表凹陷外观的减少至少约3个月。

[0128] 2.第1项的组合物，其中组合物从被引入到皮肤中起，维持皮肤浅表凹陷外观的减少至少约6个月。

[0129] 3.第1项的组合物，其中组合物从被引入到皮肤中起，维持皮肤浅表凹陷外观的减少至少约9个月。

[0130] 4.第1项的组合物，其还包含甘露醇和维生素C衍生物中的至少一种。

[0131] 5.第4项的组合物，其中甘露醇以约0.3％至约0.9％w/w的量存在。

[0132] 6.第4项的组合物，其中维生素C衍生物为抗坏血酸2-葡糖苷。

[0133] 7.第6项的组合物，其中抗坏血酸2-葡糖苷以约0.3％至约0.6％w/w的量存在。

[0134] 8.第1项的组合物，其还包含约0.9％w/w的甘露醇和约0.6％w/w的抗坏血酸2-葡糖苷。

[0135] 9.第1项的组合物，其中低分子量HA材料的重均分子量为约400000Da至约800000Da。

[0136] 10.第1项的组合物，其中交联HA基质用选自以下的交联剂交联：1,4-丁二醇二缩水甘油醚(BDDE)、1,4-双(2,3-环氧丙氧基)丁烷、1,4-双缩水甘油基氧基丁烷、1,2-双(2,3-环氧丙氧基)乙烯和1-(2,3-环氧丙基)-2,3-环氧环己烷。

[0137] 11.第1项的组合物，其中HA浓度为约10.0mg/g至约14.0mg/g。

[0138] 12.第1项的组合物，其中HA浓度为约12.0mg/g。

[0139] 13.一种用于减少皮肤浅表凹陷外观或用于改善皮肤质量的可注射组合物，所述组合物包含：

[0140] 含有交联HA基质的透明质酸(HA)凝胶；

[0141] 甘露醇和维生素C衍生物中的至少一种；

[0142] 其中所述组合物的HA浓度小于约17.0mg/g；

[0143] 其中所述组合物从被引入到皮肤中起，维持皮肤浅表凹陷外观的减少或皮肤质量的改善至少约3个月。

[0144] 14.第13项的组合物，其中组合物从被引入到皮肤中起，维持皮肤浅表凹陷外观的减少或皮肤质量的改善至少约6个月。

[0145] 15.第13项的组合物，其中组合物从被引入到皮肤中起，维持皮肤浅表凹陷外观的减少或皮肤质量的改善至少约9个月。

[0146] 16.第13项的组合物，其中甘露醇以约0.3％至约0.9％w/w的量存在。

[0147] 17.第13项的组合物，其中维生素C衍生物为抗坏血酸2-葡糖苷。

[0148] 18.第17项的组合物，其中抗坏血酸2-葡糖苷以约0.3％至约0.6％w/w的量存在。

[0149] 19.第13项的组合物，其中甘露醇和维生素C衍生物的至少一种包含以约0.9％w/w存在的甘露醇和以约0.6％w/w存在的维生素C衍生物。

[0150] 20.第19项的组合物，其中维生素C衍生物为抗坏血酸2-葡糖苷。

[0151] 21.第13项的组合物，其中HA浓度为约10.0mg/g至约14.0mg/g。

[0152] 22.第13项的组合物，其中HA浓度为约12.0mg/g。

[0153] 23.一种改善皮肤纹理、水合和弹性中的至少一种的方法，所述方法包括：

[0154] 通过在单次处理疗程中向皮肤区域引入组合物的多次间隔隔开的注射来处理皮肤区域，所述组合物包含含有交联HA的透明质酸(HA)凝胶；

[0155] 其中组合物的HA浓度小于约17.0mg/g；

[0156] 其中经处理的皮肤因处理带来的改善的纹理、水合和弹性中的至少一种维持至少约3个月。

[0157] 24.第23项的方法，其中经处理的皮肤因处理带来的改善的纹理、水合和弹性中的至少一种维持至少约6个月。

[0158] 25.第23项的方法，其中经处理的皮肤因处理带来的改善的纹理、水合和弹性中的至少一种维持至少约12个月。

[0159] 26.第23项的方法，其中HA浓度为约10.0mg/g至约14.0mg/g。

[0160] 27.第23项的方法，其中HA浓度为约12.0mg/g。

[0161] 28.第23项的方法，其中注射以约5mm至约20mm的距离间隔隔开。

[0162] 29.第23项的方法，其中注射以约5μL至约100μL每次注射的量引入。

[0163] 30.第23项的方法，其中处理不超过约45分钟。

[0164] 31.第23项的方法，其中注射通过28G至33G的规格的针递送。

[0165] 32.第23项的方法，其中组合物还包含甘露醇和维生素C衍生物中的至少一种。

[0166] 33.第23项的方法，其中甘露醇以约0.3％至约0.9％w/w的量存在。

[0167] 34.第23项的方法，其中维生素C衍生物为抗坏血酸2-葡糖苷。

[0168] 35.第34项的方法，其中抗坏血酸2-葡糖苷以约0.3％至约0.6％w/w的量存在。

[0169] 36.一种增加皮肤光滑性、水合和弹性中的至少一种的方法，包括：

[0170] 在单次处理疗程中向皮肤区域约0.5至约4.0mm或约0.5至约5.0mm深度处引入组合物的多次间隔隔开的注射，所述组合物包含含有BDDE-交联HA和未交联HA的透明质酸(HA)凝胶；

[0171] 其中注射以约5μL至约100μL每次注射的量引入；

[0172] 其中注射通过28G至33G的规格的针递送；

[0173] 其中注射以约5mm至约20mm的距离间隔隔开；并且

[0174] 其中皮肤区域因处理带来的增加的光滑性、水合和弹性维持至少约6个月。

[0175] 37.第36项的方法，其中组合物还包含甘露醇和维生素C衍生物中的至少一种。

[0176] 38.一种用于增加皮肤光滑性、水合和弹性中的至少一种的组合物，其包含：

[0177] 透明质酸(HA)凝胶，所述透明质酸(HA)凝胶含有由低分子量HA材料制成的交联HA基质，所述低分子量HA材料的重均分子量为约400000Da至约800000Da；

[0178] 其中组合物的HA浓度为约10.0mg/g至约14.0mg/g；并且

[0179] 其中从以单次处理疗程向皮肤引入组合物起，组合物因处理带来的增加的光滑性、水合和/或弹性维持至少约6个月，所述单次处理疗程包括组合物的多次间隔隔开的注射，其中注射以约5μL至约100μL每次注射的量引入，通过28G至33G的规格的针递送，并且以约5mm至约20mm的距离间隔隔开。

[0180] 39.第38项的组合物，其中组合物还包含甘露醇和维生素C衍生物中的至少一种。

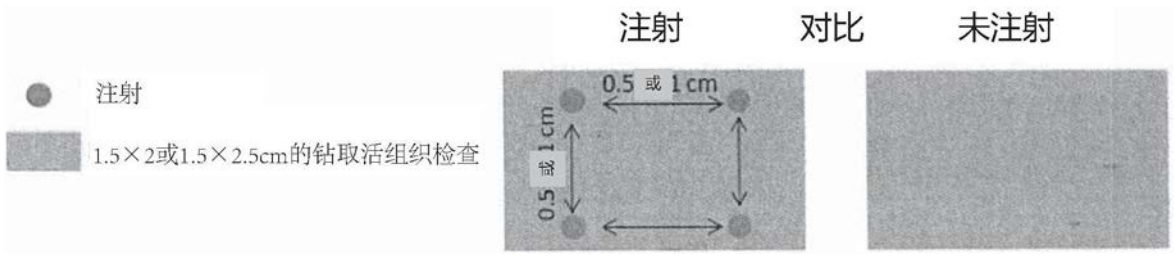
注射
对比
未注射
注射
1.5×2或1.5×2.5cm的钻取活组织检查
0.5 或 1 cm
0.5 或 1 cm

图1

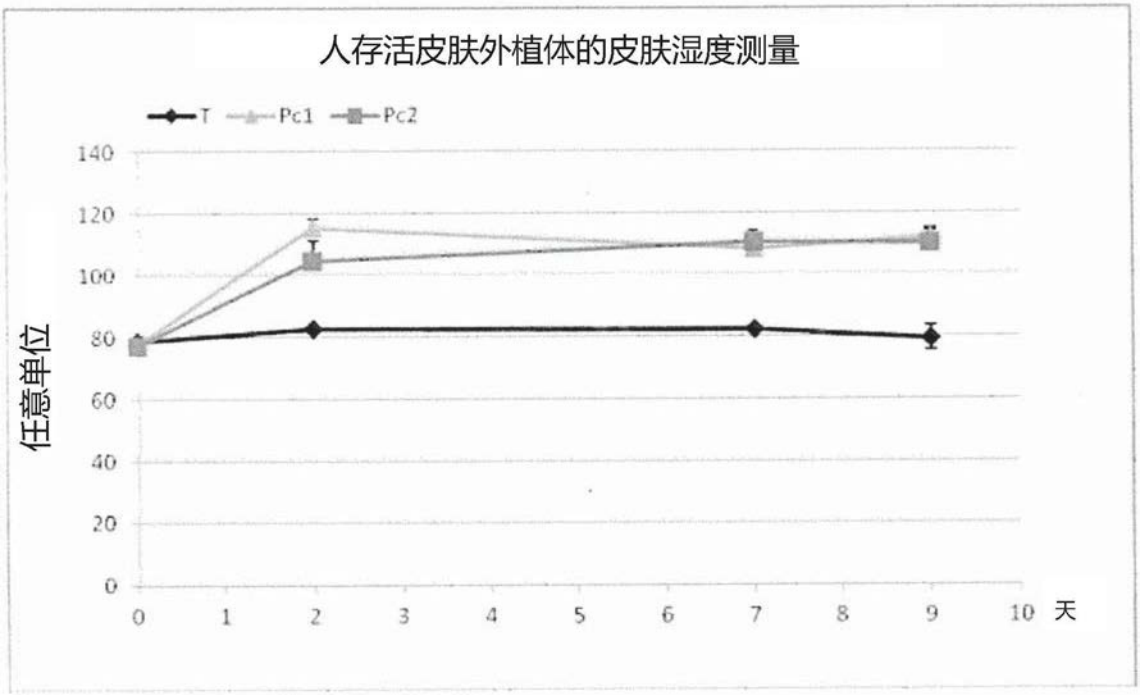
人存活皮肤外植体的皮肤湿度测量
T
Pc1
Pc2
任意单位
140
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
天

图2

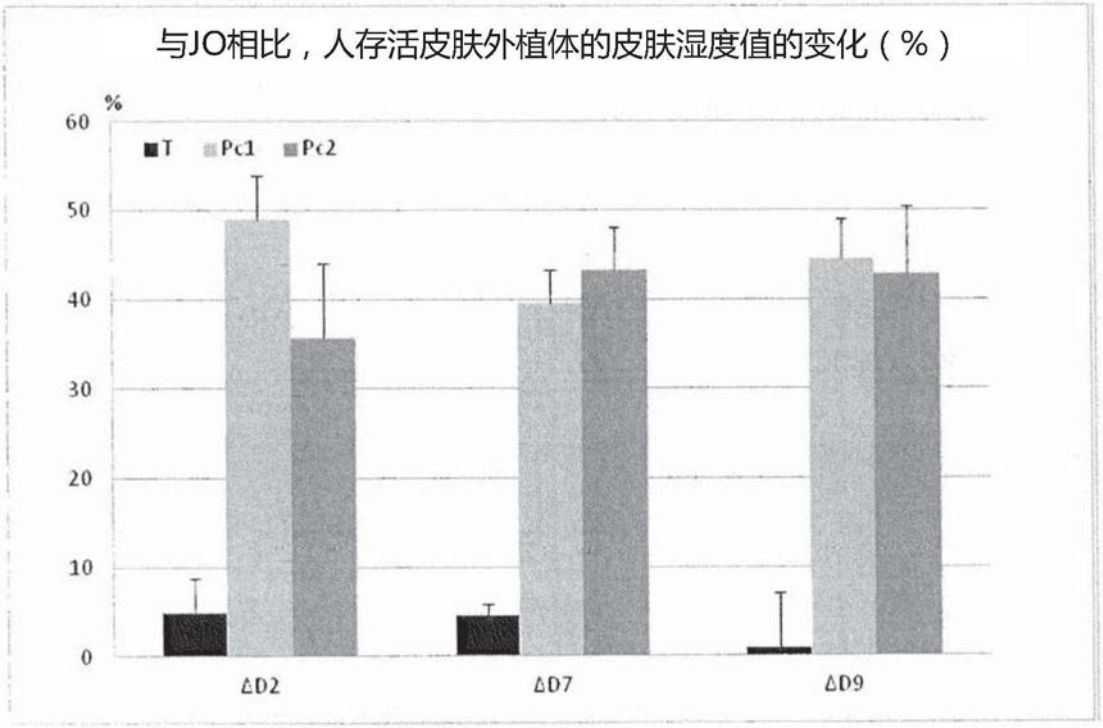
与JO相比，人存活皮肤外植体的皮肤湿度值的变化（%）
%
60
50
40
30
20
10
0
T
Pc1
Pc2
ΔD2
ΔD7
ΔD9

图3

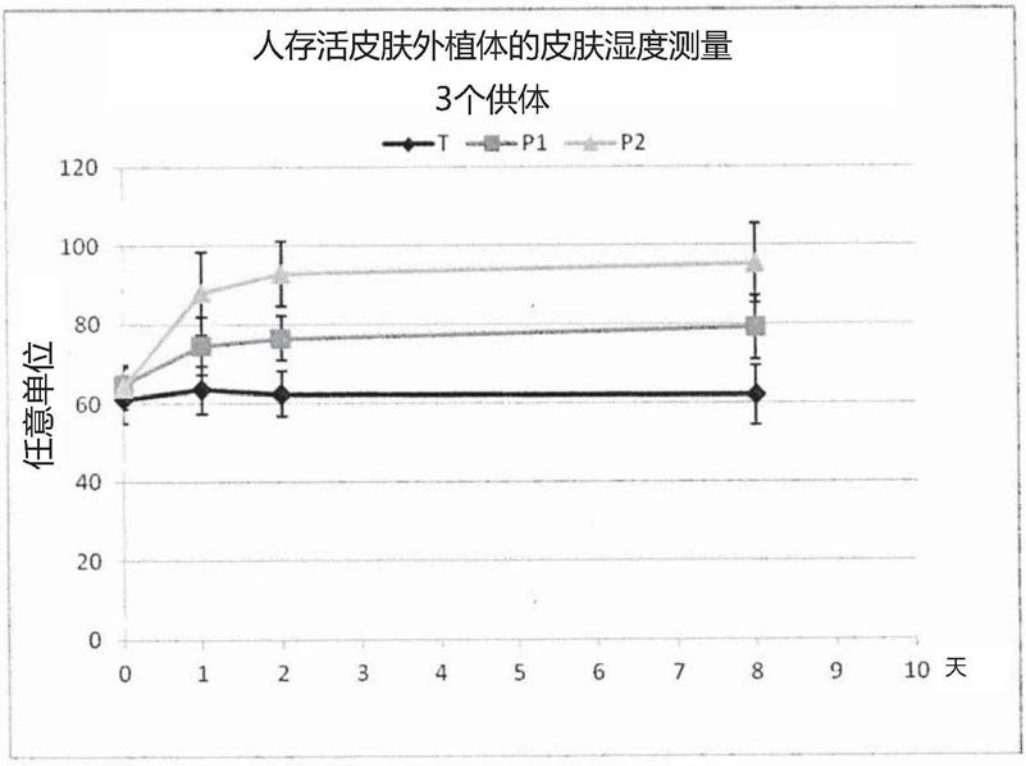
人存活皮肤外植体的皮肤湿度测量
3个供体
T
P1
P2
任意单位
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
天

图4

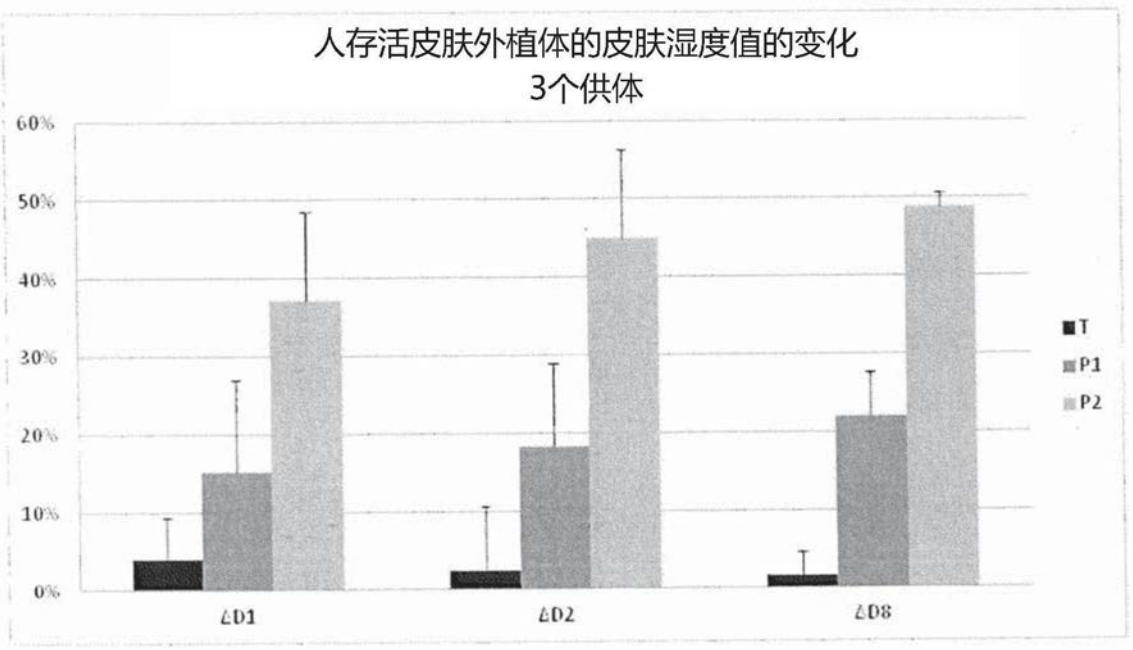

图5