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(71) Applicant(s)
Allergan Industrie, SAS

(72) Inventor(s)
Lebreton, Pierre;Guetta, Olivier

(74) Agent / Attorney
Davies Collison Cave Pty Ltd, Level 14 255 Elizabeth St, Sydney, NSW, 2000, AU

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(71) Applicant: **ALLERGAN INDUSTRIE, SAS** [FR/FR]; Route de Promery, Zone Artisanale de Pre-Mairy, 74370 Pringy (FR).

(72) Inventors: **LEBRETON, Pierre**; 7 rue Louis Revon, 74000 Annecy (FR). **GUETTA, Olivier**; 74 Boulevard du Fier, 74000 Annecy (FR).

(74) Agents: **KLUSMANN, Peter** et al.; (HOFFMANN EITLE PATENT- UND RECHTSANWÄLTE PARTMBB), Arabellastraße 30, 81925 Munich (DE).

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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.

[0018a] Another aspect of the invention provides 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 about 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 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 from about 10.0 mg/g to 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; and 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.

[0018b] Another aspect of the invention provides use of a composition in the manufacture of a medicament for improving at least one of texture, hydration and elasticity of skin, wherein the medicament is to be administered by introducing into a skin region, in a single treatment session, multiple, spaced apart injections of said medicament; wherein the injections are spaced apart by a distance of between about 5 mm to about 20 mm; and 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.

[0018c] Another aspect of the invention provides 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, wherein the injections are spaced apart by a distance of between about 5 mm to about 20 mm, and 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.

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 34

[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.

[0093] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0094] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as, an acknowledgement or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

The claims defining the invention are as follows:

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 about 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 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 from about 10.0 mg/g to 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; and 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 4 months, 6 months, 9 months or 12 months from being introduced into the skin.

3. The composition of claim 1 or 2, wherein the composition includes uncrosslinked HA.

4. The composition of claim 3, wherein the uncrosslinked HA is present in an amount of less than about 10 % w/w or less than about 5 % w/w, based on the total weight of the composition.

5. The composition of any one of claims 1 to 4, further comprising at least one of mannitol, a vitamin C derivative and lidocaine.

6. The composition of claim 5, wherein the lidocaine is present in an amount of between about 0.1% and about 5% w/w, based on the total weight of the composition.

7. The composition of claim 6, wherein the lidocaine is present in an amount of about 0.3% w/w, based on the total weight of the composition.

8. The composition of claim 5, 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.

9. The composition of claim 5, wherein the vitamin C derivative is ascorbic acid 2-glucoside.

10. The composition of claim 9, 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.

11. The composition of any one of claims 1 to 5, 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.

12. The composition of any one of claims 1 to 11, wherein the HA matrix is 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.

13. Use of a composition of any one of claims 1 to 12 in the manufacture of a medicament for improving at least one of texture, hydration and elasticity of skin, wherein the medicament is to be administered by introducing into a skin region, in a single treatment session, multiple, spaced apart injections of said medicament; wherein the injections are spaced apart by a distance of between about 5 mm to about 20 mm; and 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.

14. 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 of any one of claims 1 to 12, wherein the injections are spaced apart by a distance of between about 5 mm to about 20 mm, and 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.

15. The method of claim 14, wherein the injections are spaced apart by a distance of about 5 mm or about 10 mm.

16. The method of claim 14 or 15, 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.

17. The method of any one of claims 14 to 16, wherein the injections are introduced with an amount of about 5 µL to about 100 µL per injection.

18. The method of any one of claims 14 to 17, wherein the treatment takes no longer than about 45 minutes.

19. The method of any one of claims 14 to 18, wherein the injections are delivered through a needle having a gauge of from 28 G to 33 G.

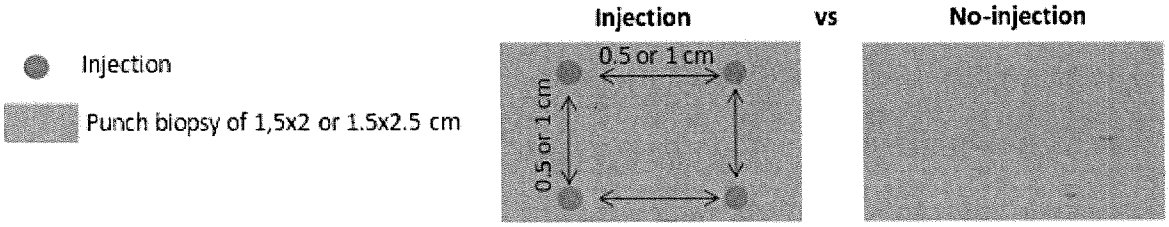

Figure 1

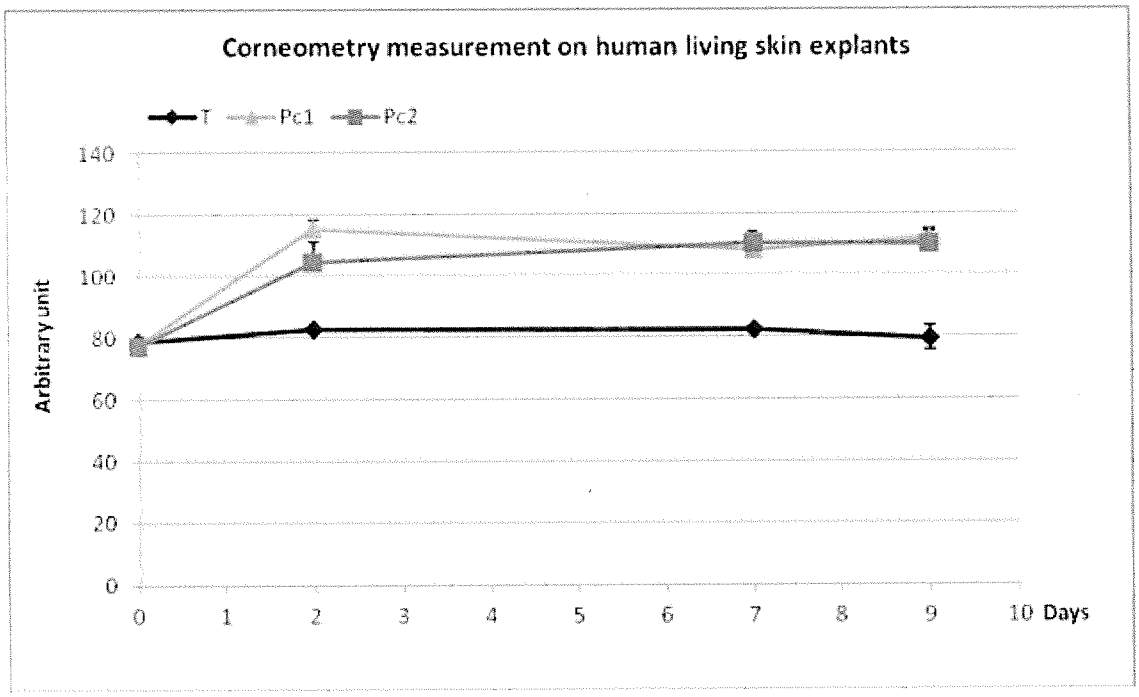

Figure 2

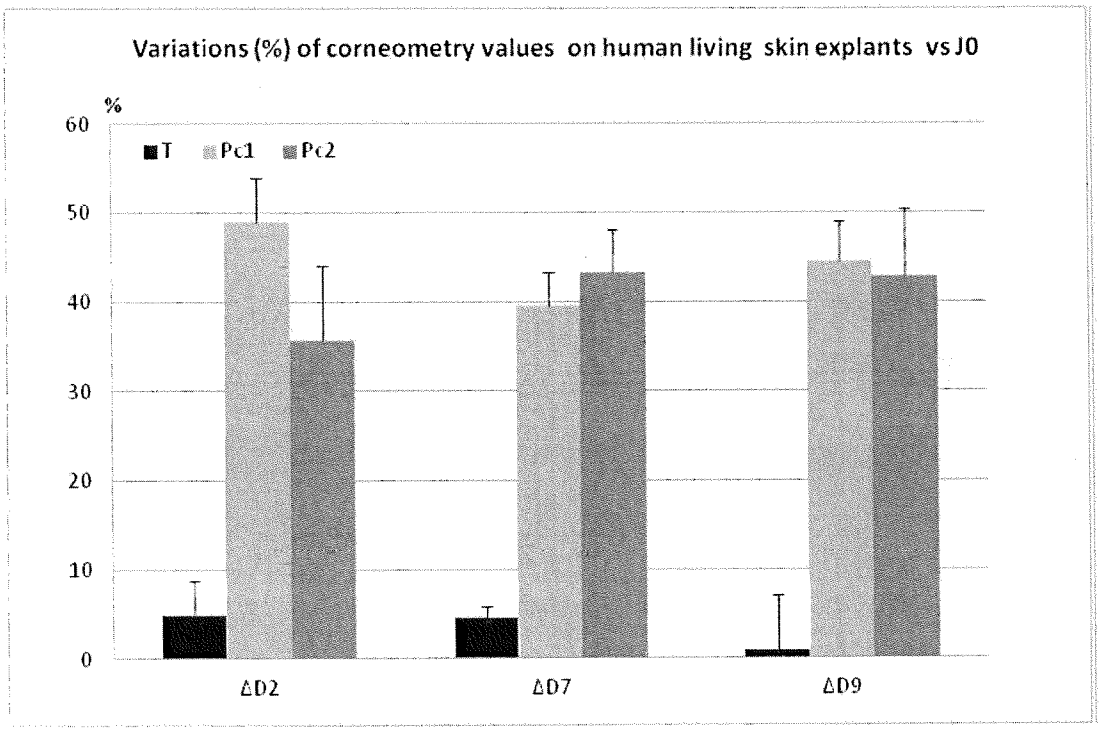

Figure 3

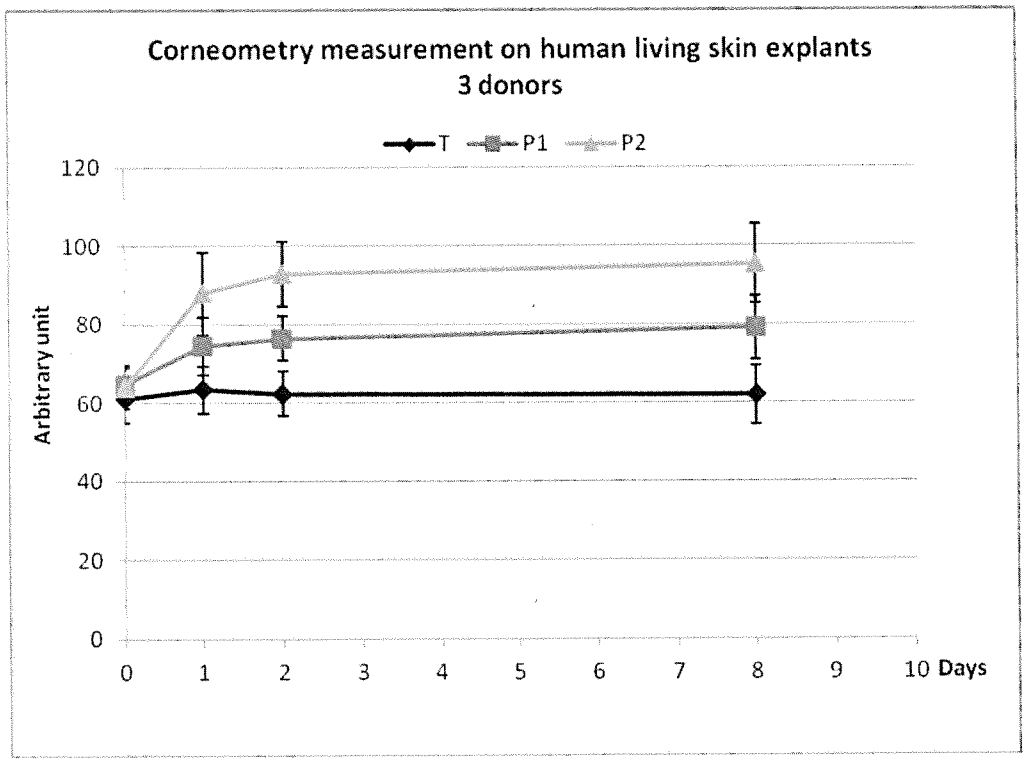

Figure 4

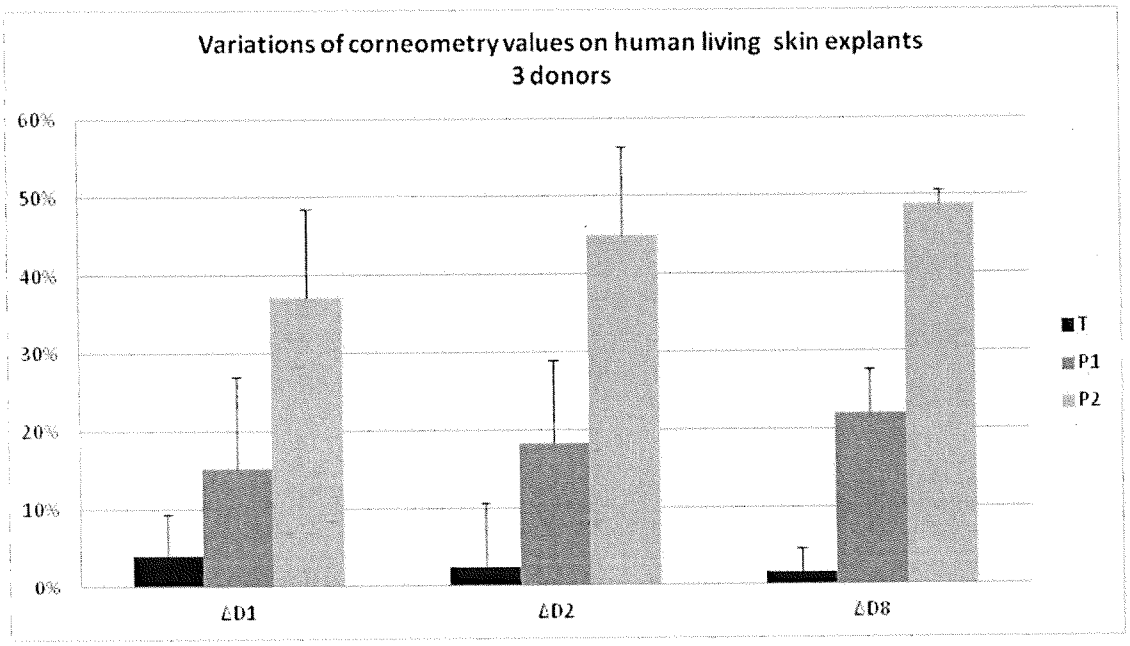
Variations of corneometry values on human living skin explants
3 donors
60%
50%
40%
30%
20%
10%
0%
ΔD1
ΔD2
ΔD8
T
P1
P2

Figure 5