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(54) Title: COMPOSITION AND METHOD FOR INTRADERMAL SOFT TISSUE AUGMENTATION

(57) Abstract: The present invention relates to biocompatible compositions for intradermal soft tissue augmentation comprising hyaluronic acid or a salt thereof and mannitol, having enhanced residence time and reduced risks of local inflammation; to the method for soft tissue augmentation comprising injecting this composition into a zone of the human body in need of such a treatment for augmenting the tissue at and around these zones; and to the kit for performing soft tissue augmentation.

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COMPOSITION AND METHOD FOR INTRADERMAL SOFT TISSUE AUGMENTATION

FIELD OF THE INVENTION

The present invention relates to a biocompatible composition for soft tissue augmentation comprising hyaluronic acid or salts thereof and mannitol, a method for soft tissue augmentation comprising injecting this composition into a zone of the human body in need of such a treatment, and a kit comprising this composition for providing soft tissue augmentation.

STATE OF THE ART

Since several years the correction and augmentation of wrinkles, furrows, soft tissue defects and scars is carried out by intradermal injection of implants and filler materials.

Many different types of filler have been used until today and many different methods of soft tissue augmentation are available, but no filler material seems to be at present completely safe and efficacious in providing a long term correction.

The alternative filler materials now available, suitable for use in soft tissue augmentation, can be of synthetic, biological or autologous origin.

The synthetic implants have the advantage to provide a permanent correction of defects, but their use include the risk of inflammation, infection, and migration of the implanted synthetic material to distant body parts wherein it may cause physiological and clinical problems. This is the case of liquid silicone, widely used in past but now prohibited by the FDA for the use in humans, and, even if less frequently, of the polymethylmethacrylate microspheres commercially available with the trade name Artecoll®, and of the expanded polytetrafluoroethylene known with the trade name Gore-Tex®, for which the above said complications are less common but in any case more likely than with biologically derived materials.

The filler materials of biological origin are in fact less likely to become infected or displaced than the synthetic ones.

Biological materials currently used intradermally for treating fine lines and wrinkles comprise bovine collagen, such as the commercial product Zyderm®, hyaluronic acid of natural origin possibly modified, such as the product known with the trade name Restylane®, that is a cross-linked hyaluronic acid in the form of crystal-clear

injectable gel, or the product Hyal-system®, that is a sterile viscous solution of hyaluronic acid having a molecular weight of 1,000,000 Daltons.

Nevertheless, the use of biological filler materials includes the risks of allergic reactions and of an only temporary correction. As a matter of fact, the lasting effects of the above cited materials of biological origin are shorter than 12 weeks, and then a repeated injection is needed.

Amongst the materials of biological origin, hyaluronic acid is one of the most interesting, because, despite its relatively short residence time, it would be the ideal filler material for soft tissue augmentation. In fact, in addition to its biocompatibility, easy availability from biological sources, and ability in supporting the dermal structures, the exogenous hyaluronic acid substitutes the endogenous hyaluronic acid naturally occurring in the skin, which has been degraded upon reaction with free radicals. It was in fact proven that endogenous hyaluronic acid acts as scavenger of free radicals, the reactive oxygen species which promote skin ageing and wrinkles formation (Trabucchi E. et al., *Int. J. Tissue React.* XXIV(2) 65-71 2002). Therefore, exogenous hyaluronic acid acts twice as much against ageing signs, both as filler material and as substitute of the endogenous hyaluronic acid, degraded by free radicals.

Therefore, many efforts have been made to increase stability of hyaluronic acid implants, for instance by chemical modifications on the hyaluronic acid molecule, but none of the so obtained hyaluronic acid derivatives completely satisfies the above said requisite of long lasting augmentation maintaining acceptable biocompatibility and tolerance by the human body tissue.

The problem of only temporary correction is even more felt for the autologous implants, i.e. for the materials removed from the patient, processed and re-implanted in the patient's body site to be augmented. On one hand, these materials are obviously completely safe from allergic reaction and infection, migration and rejection of such implants are extremely rare; but, on the other hand, these implants are very quickly absorbed, resulting in a temporary or limited correction. Further drawbacks in connection with the use of such implants are the limited availability of the material and the additional surgery necessary to obtain it from the patient.

Autologous materials include skin, dermal and fat grafts; an example of autologous material is Antologen, an injectable implant material made from the patient's own fat. A dramatic re-absorption of fat cells has been observed for this type of implant, its efficacy being thus severely limited.

In view of the above, the need of a filler material suitable for soft tissue augmentation which is completely safe and effective in providing long term correction, is deeply felt.

SUMMARY OF THE INVENTION

The Applicant has now found that hyaluronic acid or salts thereof are particularly effective as soft tissue augmentation material when used in combination with mannitol.

A sort of "synergistic effect" between hyaluronic acid or a salt thereof and mannitol has been observed. In fact, the efficacy in the intradermal treatments of hyaluronic acid or salts thereof, when used in combination with mannitol, appears to be advantageously increased not only in relation to the duration of residence in place of the augmentation material, but also in relation to the action as free radicals scavenger. Moreover, the use of mannitol reduces the risks of local inflammatory reactions that usually occur after the intradermal injection of a filler material.

Subject of the present invention is therefore a biocompatible composition for soft tissue augmentation comprising hyaluronic acid or a salt thereof and mannitol.

Subject of the invention is also the method for soft tissue augmentation, comprising injecting a composition comprising hyaluronic acid or a salt thereof and mannitol into a zone of the human body in need of such a treatment for augmenting the tissue at the said zone.

Further subject of the invention is the kit for performing soft tissue augmentation comprising a needle; a mean for injecting a solution through the said needle; and a biocompatible, sterile composition in the form of isotonic solution comprising hyaluronic acid or a salt thereof and mannitol.

Features and advantages of the present invention will be illustrated in detail in the following description.

DETAILED DESCRIPTION OF THE INVENTION

The present invention allows providing novel compositions directed to the soft tissue augmentation with enhanced properties thanks to the combination of hyaluronic acid or salts thereof with mannitol.

The compositions of the invention show in fact an unexpectedly higher stability when intradermally implanted, in comparison with similar compositions based on hyaluronic acid or salts thereof but devoid of mannitol, and act efficaciously also against free radicals.

Preferably, the present compositions are in the form of neutral isotonic aqueous solutions with a pH ranging between 7.0 and 7.5, preferably equal to 7.3.

In order to obtain such a solution, a phosphate salts based buffer may be added to the composition in an amount sufficient to produce the above said neutral solution.

The present composition may further comprise an isotonic salt solution comprising sodium chloride and sodium dihydrogenphosphate.

The concentration of hyaluronic acid or hyaluronic acid salt in the solution may range from 1.5% to 3.5%, and preferably is 2% by weight in respect of the volume of the solution; whereas the concentration of mannitol may range from 0.2% to 1%, and preferably is 0.5% weight by volume.

According to the present invention, compositions comprising sodium hyaluronate are preferred. Extractive (e.g. from cockscombs, umbilical cords, etc.) or fermentative sodium hyaluronate (e.g. from strains belonging to the genus Streptococcus, etc.) having a molecular weight ranging from 1,000,000 to 2,000,000 Daltons can be used in the present compositions.

The present compositions may comprise other pharmacologically acceptable diluents or excipients, besides those mentioned above, as well as pharmaceutically active principles or adjuvants, in particular active principles having anaesthetic or anti-inflammatory action.

According to a preferred embodiment of the invention, the present composition is as follows:

Components	**Quantity % (w/v)**
Sodium hyaluronate (M.W. 1,000,000 – 2,000,000)	1.5 - 3.5
Mannitol	0.2 – 1.0
Sodium chloride	0.4 – 1.2

Sodium dihydrogenphosphate · 2 H_2O	0.002 – 0.01
Sodium monohydrogenphosphate · 12 H_2O	0.02 – 0.1
Water for injection	up to 1 ml; pH 7.0-7.5

Particularly preferred is the following composition:

Components	**Quantity % (w/v)**
Sodium hyaluronate (M.W. 1,000,000 – 2,000,000)	2.0%
Mannitol	0.5%
Sodium chloride	0.77%
Sodium dihydrogenphosphate · 2 H_2O	0.005%
Sodium monohydrogenphosphate · 12 H_2O	0.06%
Water for injection	up to 1 ml; pH = 7.3

The present compositions have typically a viscosity ranging from 18 to 41 Pa · s (from 18,000 to 41,000 centipoises (cps)) at a share rate of 2 sec^{-1} and at 25°C.

The compositions of the present invention can be prepared according to well known procedures, mixing the components under aseptic conditions by means of techniques and equipment usual in the preparation of compositions for intradermal injection.

Intradermal soft-tissue augmentation using the compositions of the present invention has a number of advantages.

The present composition in the form of isotonic solution as above described has a viscosity sufficiently low to be easily injected into soft tissue with a needle and a suitable mean for injection, without previously heating the composition. A syringe corresponding to the needle is preferably used as the mean for injection, and more preferably a syringe fitted with a Luer-Lok system and equipped with an elastomer backstop.

The mean for injection to be used according to the present method may be pre-filled with the present biocompatible sterile composition, and packed in a sealed polypropylene pouch or blister.

These injectable compositions can be used for a variety of soft tissue augmentation operations for cosmetic or therapeutic effect, especially in facial and

neck tissues augmentation, for example in repairing post-surgical and post-traumatic defects, smoothing out age-related folds, lines, oral commissures, wrinkles, enhancing lips, and the like.

Pharmaceutically active principles or adjuvants can be administered together with the present composition, in particular active principles having anaesthetic, bactericidal or anti-inflammatory action.

The injection may be repeated after a certain period of time to provide for further soft tissue augmentation.

As mentioned above when describing the prior art materials, hyaluronic acid is already used as filler material in soft tissue augmentation. Nevertheless, it degrades rapidly and is absorbed by the surrounding tissues when implanted in a human body. The biocompatible implanted materials based on plain hyaluronic acid do not meet therefore the requisite of long lasting augmentation.

Now the Applicant has found that the addition of mannitol, a naturally occurring sugar alcohol found in animals and plants, increases the stability of hyaluronic acid and salts thereof when intradermally implanted, and prolong the average residence time of the augmentation from 12 weeks to 1 year, before a second injection is needed.

Moreover, mannitol acts as free radicals scavenger, and its presence in the injected composition strengthens the scavenger action against free radicals of the endogenous hyaluronic acid and reduces its degradation, thus acting both directly and indirectly against skin ageing.

Even the local inflammatory reactions that may occur after intradermal injection of the filler material are very limited when mannitol is added to hyaluronic acid or hyaluronic acid salt compositions.

In summary, the present compositions appear to fulfil the requirements of safety and efficacy required to a filler material for soft tissue augmentation, and show the following remarkable advantages over the prior art filler materials, even over those based on hyaluronic acid and derivatives thereof:

i) longer residence time;

ii) reduced risks of local inflammatory reactions;

iii) action as free radicals scavenger.

The following examples are reported to give a non-limiting illustration of the present invention.

EXAMPLE 1

The following composition according to the invention has been prepared in the form of an isotonic solution with a pH of 7.3, by mixing the components under aseptic conditions:

Components	**Quantity**
Sodium hyaluronate	20.0 mg
Mannitol	5.0 mg
Sodium chloride	7.7 mg
Sodium dihydrogenphosphate · 2 H_2O	0.05 mg
Sodium monohydrogenphosphate · 12 H_2O	0.6 mg
Water for injection q. s. ad	1 ml

The above composition, without adding any preservative, is stored for 2-8°C, possibly already contained in the mean to be used for injection, and maintained for 30 minutes at room temperature prior to use for soft tissue augmentation.

CLAIMS

1. A biocompatible composition for soft tissue augmentation comprising hyaluronic acid or a hyaluronic acid salt and mannitol.

2. The composition according to claim 1, wherein said hyaluronic acid or hyaluronic acid salt has a molecular weight ranging from 1,000,000 to 2,000,000 Daltons.

3. The composition according to claim 1, wherein the said hyaluronic acid salt is sodium hyaluronate.

4. The composition according to claim 1, in the form of an isotonic aqueous solution with a pH ranging from 7.0 to 7.5.

5. The composition according to claim 4, in the form of an isotonic aqueous solution with a pH of 7.3.

6. The composition according to claim 4, further comprising a phosphate salts buffer and an isotonic salt solution comprising sodium chloride and sodium dihydrogenphosphate.

7. The composition according to claim 1, wherein the concentration of the said hyaluronic acid or hyaluronic acid salt range from 1.5 to 3.5% by weight in respect of the volume of the solution, and the concentration of the said mannitol ranges from 0.2 to 1.0 % weight by volume.

8. The composition according to claim 5, wherein the concentration of the said hyaluronic acid or hyaluronic acid salt is 2.0% by weight in respect of the volume of the solution, and the concentration of the said mannitol is 0.5% weight by volume.

9. The composition according to claim 1, having the following composition:

Sodium hyaluronate (M.W. 1,000,000 – 2,000,000)	1.5 - 3.5%
Mannitol	0.2 – 1.0%
Sodium chloride	0.4 – 1.2%
Sodium dihydrogenphosphate · 2 H_2O	0.002 – 0.01%
Sodium monohydrogenphosphate · 12 H_2O	0.02 – 0.1%
Water for injection	up to 1 ml

10. The composition according to claim 9, having the following composition:

Sodium hyaluronate (M.W. 1,000,000 – 2,000,000)	2.0%
Mannitol	0.5%
Sodium chloride	0.77%
Sodium dihydrogenphosphate · 2 H_2O	0.005%
Sodium monohydrogenphosphate · 12 H_2O	0.06%
Water for injection	up to 1 ml

11. The composition according to claim 1, having a viscosity ranging from 18 to 41 Pa · s (18,000 – 41,000 cps) at a share rate of 2 sec^{-1} and at 25°C.

12. The composition according to claim 1, further comprising one or more active principles.

13. A cosmetic method for soft tissue augmentation, comprising injecting a composition comprising hyaluronic acid or a salt thereof and mannitol into a zone of the human body in need of such a treatment, for augmenting the soft tissue at and around the said zone.

14. A cosmetic method according to claim 13, for repairing post-surgical and/or post-traumatic defects, for smoothing out age-related folds, lines, oral commissures, or wrinkles, or for enhancing lips.

15. The cosmetic method according to claim 13, wherein the said injection is carried out through a syringe and needle.

16. The cosmetic method according to claim 13, comprising repeating the said injection after a certain period of time to provide for further soft tissue augmentation.

17. The cosmetic method according to claim 13, wherein the said portion of the human body is comprised in the facial and neck soft tissue.

18. Use of a composition comprising hyaluronic acid or a salt thereof and mannitol for the preparation of a medicament for soft tissue augmentation.

19. Use according to claim 18, for repairing post-surgical and/or post-traumatic defects, for smoothing out age-related folds, lines, oral commissures, or wrinkles, or for enhancing lips.

20. Use according to claim 18, wherein said composition is an injectable composition.

21. Use according to claim 18, wherein said soft tissue augmentation is performed in the facial and neck area.

22.A kit for performing soft tissue augmentation comprising a needle; a suitable mean for injecting a solution through the said needle; and a biocompatible, sterile composition in the form of isotonic solution comprising hyaluronic acid or a salt thereof and mannitol.

23. The kit according to claim 22, wherein the said mean for injection is a syringe corresponding to the said needle.

24. The kit according to claim 23, wherein the said mean for injection is pre-filled with the said biocompatible sterile composition, and packed in a sealed polypropylene pouch or blister.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/01641

A. CLASSIFICATION OF SUBJECT MATTER
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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)
IPC 7 A61L

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 practical, search terms used)
EPO-Internal, BIOSIS, PAJ, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 571 503 A (MAUSNER JACK) 5 November 1996 (1996-11-05) example 1; table 1 ---	1-12
X	US 5 087 446 A (FUJII MASAHIRO ET AL) 11 February 1992 (1992-02-11) examples 3,6 ---	1-12
X	MIYAZAKI T ET AL: "Ultrasonic depolymerization of hyaluronic acid" POLYMER DEGRADATION AND STABILITY, BARKING, GB, vol. 74, no. 1, 2001, pages 77-85, XP004305408 ISSN: 0141-3910 page 79, line 14 - line 20; figure 1 --- -/--	1-12

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in 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 document but published on or after the international filing date
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/EP 03/01641

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	LI MIN ET AL: "Degradation of hyaluronan by peroxynitrite" ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 341, no. 2, 1997, pages 245-250, XP002257790 ISSN: 0003-9861 page 248, line 1 - line 5 page 246, line 17 - line 19 ---	1-12
X	FRATI ELENA ET AL: "Degradation of hyaluronic acid by photosensitized riboflavin in vitro. Modulation of the effect by transition metals, radical quenchers, and metal chelators" FREE RADICAL BIOLOGY AND MEDICINE, vol. 22, no. 7, 1997, pages 1139-1144, XP002257793 ISSN: 0891-5849 page 1142; figure 4	1-12
Y	page 1141, line 11 - line 13 page 1143, line 21 - line 34 ---	1-24
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/01641

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