HU000026830T2

(19) **HU**

(11) Lajstromszám: **E 026 830**

(13) **T2**

MAGYARORSZÁG
Szellemi Tulajdon Nemzeti Hivatala

EURÓPAI SZABADALOM
SZÖVEGÉNEK FORDÍTÁSA

(21) Magyar ügyszám: **E 14 170084**
(22) A bejelentés napja: **2010. 08. 25.**

(96) Az európai bejelentés bejelentési száma:
EP 20100170084
(97) Az európai bejelentés közzétételi adatai:
EP 2772273 A2 **2014. 09. 03.**
(97) Az európai szabadalom megadásának meghirdetési adatai:
EP 2772273 B1 **2015. 12. 02.**

(51) Int. Cl.: ***A61L 27/26*** *(2006.01)*
A61L 27/52 *(2006.01)*
A61L 27/58 *(2006.01)*
C08B 37/00 *(2006.01)*
C08L 5/08 *(2006.01)*

(30) Elsőbbségi adatok:
PD20090246 **2009. 08. 27.** **IT**

(72) Feltaláló(k):
D'Este, Matteo, 35031 Abano Terme (PD) (IT)
Renier, Davide, 35031 Abano Terme (PD) (IT)

(73) Jogosult(ak):
FIDIA FARMACEUTICI S.p.A., 35031 Abano Terme (PD) (IT)

(74) Képviselő:
Danubia Szabadalmi és Jogi Iroda Kft., Budapest

(54) **Viszkoelasztikus gélek mint új kitöltőanyagok**

Az európai szabadalom ellen, megadásának az Európai Szabadalmi Közlönyben való meghirdetésétől számított kilenc hónapon belül, felszólalást lehet benyújtani az Európai Szabadalmi Hivatalnál. (Európai Szabadalmi Egyezmény 99. cikk(1))

A fordítást a szabadalmas az 1995. évi XXXIII. törvény 84/H. §-a szerint nyújtotta be. A fordítás tartalmi helyességét a Szellemi Tulajdon Nemzeti Hivatala nem vizsgálta.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 772 273 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
02.12.2015 Bulletin 2015/49

(21) Application number: **14170084.9**

(22) Date of filing: **25.08.2010**

(51) Int Cl.:
A61L 27/26 *(2006.01)* ***A61L 27/52*** *(2006.01)*
A61L 27/58 *(2006.01)* ***C08L 5/08*** *(2006.01)*
C08B 37/00 *(2006.01)*

(54) Viscoelastic gels as novel fillers

Viskoelastische Gele als neue Füller

Nouveaux gels viscoélastiques comme nouveaux remplissages

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR

(30) Priority: **27.08.2009 IT PD20090246**

(43) Date of publication of application:
03.09.2014 Bulletin 2014/36

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
10750046.4 / 2 470 230

(73) Proprietor: **FIDIA FARMACEUTICI S.p.A.**
35031 Abano Terme (PD) (IT)

(72) Inventors:
- **D'Este, Matteo**
  35031 Abano Terme (PD) (IT)
- **Renier, Davide**
  35031 Abano Terme (PD) (IT)

(74) Representative: **Bertuccio, Silvia**
BIANCHETTI BRACCO MINOJA S.R.L.
Via Plinio, 63
20129 Milano (IT)

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WO-A1-2009/073437 **WO-A2-2005/112888**

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EP 2 772 273 B1

Printed by Jouve, 75001 PARIS (FR)

Description

FIELD OF INVENTION

[0001] Hyaluronic acid (HA) is a heteropolysaccharide consisting of alternating residues of D-glucuronic acid and N-acetyl-D-glucosamine.

[0002] HA is a straight-chain polymer with a molecular weight ranging between 50,000 and 13 x 10^6 Da, depending on the source from which it is obtained and the preparation methods used.

[0003] HA is present in nature in pericellular gels, in the ground substance of the connective tissue of vertebrates (of which it is one of the main components), in the vitreous humour and in the umbilical cord.

[0004] HA plays an important part in the biological organism as a structural and mechanical support for the tissues, and as an active component in the cell physiology of tissues such as skin, tendons, muscles and cartilage.

[0005] It is one of the main molecules in cartilage matrix, and also represents the main non-protein constituent of synovial fluid. As it is a strongly hydrophilic viscoelastic molecule, it gives the synovial fluid lubricant properties; HA has therefore been used in osteoarthritis for over 30 years, mainly to treat the associated pain.

[0006] HA also plays a crucial role in the tissue repair process from the structural standpoint (in the organisation of the extracellular matrix and regulation of its hydration), and as stimulating/regulating substance of a wide range of physiological processes wherein said polysaccharide acts directly and/or indirectly (clot formation, phagocyte activity, fibroblast proliferation, neovascularisation, re-epithelialisation, etc.) (Weigel P. et al., J Theoretical Biol, 1986:219-234; Abatangelo G. et al., J Surg Res, 1983, 35:410-416; Goa K. et al., Drugs, 1994, 47:536-566). As these properties have long been recognised, HA is also used to prepare dressings for the care of wounds, ulcers and skin lesions of various origins.

[0007] Hyaluronic acid is also used as a filler for wrinkles, furrows and small depressed areas of the face, and to increase the volume of the lips and cheeks, because it is immunologically inert, non-toxic, biodegradable and bioresorbable.

[0008] Treatment based on hyaluronic acid is indicated for the correction of:

- lip volume and contours
- furrows (e.g. nasolabial folds)
- remodelling of facial contours (e.g. cheeks and chin)
- wrinkles (e.g. glabellar lines and oral commissures)
- periorbital wrinkles
- fibrous post-acne scars
- fibrous post-traumatic scars
- soft tissue blemishes
- rhinoplasty scars.

[0009] Hyaluronic acid is not a permanent filler. This means that once injected, the product is gradually metabolised and resorbed by the body in times varying according to the area treated and the type of preparation used. The effect of filling and increased volume (or attenuation of wrinkles) is immediate, and only lasts a few weeks. The main products present on the market can be classified under the following categories, based on their different resorption times:

- rapid-resorption fillers (2-3 months),
- medium-term resorption fillers (5-6 months),
- slow-resorption fillers (1 year) such as Restylane Sub Q (QMed, EP0839159).

[0010] In the dermis, HA performs hydrating functions due to its high capacity to bind water, and structural functions as "scaffolding" because, by binding to other substances, it forms macromolecular complexes which render the skin compact.

[0011] The action mechanism therefore consists of immediate volumetric filling due to the viscoelastic properties of the product, and new collagen synthesis due to stimulation of the cutaneous fibroblasts.

[0012] However, HA is a natural polysaccharide which is rapidly broken down by the hyaluronidase enzymes present in connective tissue; in order to obtain fillers whose effect lasts for several months, HA is therefore subjected to crosslinking processes which improve its viscoelastic properties and increase its residence time. The fillers thus formed are crosslinked, for example, through BDDE (1,4-butanediol diglycidyl ether, Restylane®, BELOTERO® and Regenyal Idea) or DVS (divinyl sulphone, Hylaform®), which create bridges between the polymer molecules. However, increasing the degree of crosslinking progressively denatures the HA to the extent of profoundly modifying its chemical, physical and biological properties. Excessively crosslinked HA matrices present as particulate solids which are no longer recognised by the cells (and especially by the immune system) as HA; the polysaccharide is therefore perceived as a foreign body, which triggers inflammatory reactions with the formation of fibrotic capsules around it. Moreover, excessively crosslinked HA is unable to stimulate the dermal/cutaneous tissue regeneration induced, as known from well-established scientific results, by HA fragments (especially those with a low molecular weight) which have the effect of stimulating collagen synthesis by the cutaneous fibroblasts.

[0013] Fillers are also classified as resorbable or permanent. The resorbable type are the most biocompatible; they consist of hyaluronic acid or collagen, either modified or present in their native form, and are consequently resorbed within a year at most. The permanent type consist of synthetic polymers such as polyacrylamides, particular crosslinked molecules which form a stable gel when combined with water. The permanent type always remain *in situ* and are very useful for filling the lips, but their use is not recommended because acute inflammations are increasingly often caused by their cutaneous

insertion, leading to the formation of fibrotic capsules around the filler, which is perceived as a foreign body and therefore toxic.

[0014] WO2008068297 discloses injectable implants / cutaneous fillers comprising BDDE crosslinked hyaluronic acid and hyaluronic acid /salts.

[0015] WO2009073437 discloses filling gels comprising BDDE crosslinked hyaluronic acid and sulfated hyaluronic acid.

[0016] WO2006122638 discloses that the residence time of fillers comprising hyaluronic acid or derivatives (including *inter alia*HYADD) is increased when structured with/in liposomes.

[0017] The applicant has perfected a novel type of biomaterial as new filler and/or as new product for body shaping, formed by mixing two HA derivatives crosslinked in different but complementary ways, to obtain a skin/tissue substitute which allows immediate hydration (and consequently immediate filling) of the treated skin/tissue, while maintaining very long *in vivo* breakdown times to eliminate the need for repeated injections, thus reducing the side effects.

[0018] The novel biomaterials to which the present invention relates present particular characteristics of biocompatibility identical to those of hyaluronic acid as such, but their biodegradability is different; when implanted *in vivo,* their residence time is much longer than that of unmodified HA, thus allowing immediate regeneration/reconstruction of dermal/cutaneous tissue which has lost its original compactness.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The applicant has perfected a novel type of biomaterial as new filler and/or as new product for body shaping based on mixing two HA derivatives with different but complementary characteristics to obtain a novel product for injection in the treatment of skin blemishes, in dermatology, in dermocosmetology and/or in aesthetic surgery, which produces:

1. immediate dermal/cutaneous hydration
2. immediate filling of the treated tissue
3. very long breakdown times *in vivo*
4. reduced side effects.

The novel biomaterials consist of:

- HA hexadecylamide (HYADD), mixed with
- hyaluronic acid crosslinked with BDDE (HBC).

[0020] HA hexadecylamide (HYADD) is prepared as described in EP 1095064 and EP1853279, preferably using HA with a mean molecular weight (MW) of 500-730 KDa, with a mean degree of final amidation/substitution of between 1 and 3% in moles.

[0021] HYADD is the HA derivative responsible for the immediate hydration (leading to instant dermal filling) elicited by the intradermal injection of the filler to which the present invention relates.

[0022] HA crosslinked with BDDE (a molecule containing epoxy groups for the formation of ethers on the primary hydroxyls of HA) contains the crosslinking molecule, and is therefore more resistant to enzymatic degradation as it possesses ether bonds which stabilise the polysaccharide, giving the product obtained a long residence time.

[0023] Mixing of the two species of crosslinked HA leads to the formation of a novel biomaterial which has biocompatibility characteristics identical to those of native hyaluronic acid, but a different biodegradability so that, when implanted *in vivo,* its residence time is much longer than that of unmodified HA, thus allowing the regeneration/reconstruction of dermal tissue which has lost its original compactness. The Applicant has also demonstrated that their association quite unexpectedly leads to an *in vivo* breakdown time much longer than that of the commercial reference fillers formed by the same type of HA crosslinked with BDDE, with a consequent increase in residence time. Finally, the Applicant claims the use of the novel biomaterials as fillers and/or as new products for body shaping in the treatment of skin blemishes, in dermatology, in dermocosmetology and/or in aesthetic surgery.

[0024] The chemically heterogeneous nature of the novel biomaterial allows the properties of the end product to be modulated by suitably varying the weight ratio between the constituents. The two HAs can be mixed in the HYADD :HBC ratio of 10:90 to 90:10: the weight ratio will be selected on the basis of the desired final viscosity, which will depend on the site treated. If areas requiring implantation of large amounts of biomaterial are to be treated, as in the case of filling of the breasts, buttocks, cheeks or chin, or deep expression wrinkles, the biomaterial used will preferably present good compactness, and therefore a viscosity suitable to obtain a gel with an excellent consistency and a low biodegradability rate; in this case the HYADD :HBC mixture will be between 10:90 and 50:50, and preferably 25:75, because the product obtained by increasing the weight fraction of HBC is more suitable to perform a longer-lasting volume-enhancing effect. However, if lip furrows or fine forehead wrinkles are to be treated, the HYADD :HBC ratio will preferably be between 90:10 and 50: 50 HYADD/HBC composition being equal, the properties of the biomaterial can also be suitably modulated by means of a targeted selection of the vehicle in which it is prepared. The viscoelastic properties of the material consequently affect the performance of the product.

[0025] The present invention also relates to the two biomaterials preparation processes described above: process A and process B.

[0026] The novel processes A and B are divided into two steps:

1. process for the production of the HBC derivative,

and

2. process for mixing it with the HYADD derivative.

[0027] The two steps lead to the production of products with a very high degree of purity. With the methods normally used for the production of HA crosslinked with BDDE, the purifications are performed by washing the mass of gel obtained, or by dialysis. In both cases, optimum purification efficiency may not be achieved due the nature of the gel matrix which, in view of its tendency to swell, incorporates large amounts of solvent. These gels have low mobility and transport capacity, and tend to precipitate as gelatinous gums. The precipitate thus obtained, isolated as a solid, has different solubility and rheology properties when rehydrated, especially swelling capacity, elasticity and homogeneity (essential characteristics for a filler), from the gel before purification.

[0028] However, the method hereinafter described by the Applicant as process A precipitates the product in the form of a finely divided powder, which is consequently easily washable. Moreover, the careful choice of reaction conditions produces, after isolation by precipitation and washing, a product with gel reconstruction capacity by means of rehydration and sterilisation which gives rise to a biomaterial having reproducible, well standardised characteristics of elasticity and homogeneity.

[0029] Process B does not include the step of precipitation of the HBC product as a powder; the purification and homogenisation of the gel (obtained after mixing HBC with HYADD) is effected at the crushing step, which involves passing it through a filter with a particulate matter retention coefficient of between 25 and 150 μm. This step purifies the final gel and makes it perfectly homogenous.

[0030] The HA used in the present invention to prepare the derivatives described above (HBC, HYADD) can derive from any source, such as extraction from cockscombs or fermentation, and have a mean molecular weight of between 400 and $3x10^6$ Da, preferably between $1x\ 10^5$ Da and $1x\ 10^6$ Da, and even more preferably between 200,000 and $1x\ 10^6$ Da.

[0031] Novel manufacturing process A comprises the following steps:

Synthesis of crosslinked HBC

[0032]

1. Dissolution in alkaline solution (preferably 0.15M - 0.35M NaOH) of diepoxide BDDE in a stoichiometric ratio of between 2.5 and 25% in moles, preferably between 5 and 15% in moles (depending on the intended use of the product; the higher the percentage of BDDE, the longer the residence time) of the repetitive units of hyaluronic acid, followed by
2. dispersion of HA in the solution referred to in the preceding paragraph, at room temperature. The HA concentration must be between 80 and 300 mg/ml, and the homogenisation time between 30 and 300 minutes.
3. Triggering of the reaction by heat activation, said solution being heated at a temperature of between 35 and 55°C for between 2 and 36 hours.
4. Extrusion of the mass obtained through a metal sieve, to reduce it to particles with a size of approx. 600 μm.
5. Hydration of gel by diluting it with water by a factor of 3 to 25, for a time of between 4 and 48 hours at a temperature of 4 to 24°C.
6. Correction of pH to neutral with an aqueous HCl solution having a concentration of 0.5 to 5 moles/l, preferably 1 to 2 moles/l.
7. Addition of 2.5 volumes of water-soluble organic solvent such as ethanol, methanol, isopropanol, n-propanol, dioxane, acetonitrile, acetone and/or mixtures thereof (preferably ethanol and acetone), until the product is obtained in the form of a precipitated powder.
8. Washing with organic solvents such as ethanol, methanol, isopropanol, n-propanol, dioxane, acetonitrile, acetone and/or mixtures thereof (preferably ethanol and acetone), containing a water fraction of under 35%.
9. Drying under vacuum at a temperature of between 30 and 45°C for between 2 and 7 days, and in any event until elimination of the residual solvents under 400 ppm, to obtain a white HBC powder.

Mixing of HYADD with HBC

[0033]

10. Mixing of the HBC powder with HYADD powder in a ratio of between 10:90 and 90:10 (depending on the use chosen, as previously described).
11. Hydration with saline solution or phosphate buffer, preferably saline solution (which may contain further excipients such as lidocaine), leading to a total HA concentration of between 12 and 27 mg/ml, preferably between 20 and 25 mg/ml, at a temperature of between 0 and 26°C.
12. Extrusion through a sieve with a mesh of between 50 and 500 μm, preferably between 100 and 250 μm. Said filtration is performed at room temperature, or at a temperature of between 25 and 65°C, preferably between 40 and 60°C.
13. Filling of syringes, preferably made of glass or polymer material, with the product obtained.
14. Heat sterilisation with saturated steam at a temperature of between 120 and 124°C (preferably 121.5 ±1°C) for at least 10 min.

[0034] Novel manufacturing process B comprises the following steps:

Synthesis of crosslinked HBC

[0035]

1. Dissolution in alkaline solution (preferably 0.15M - 0.35M NaOH) of diepoxide BDDE in a stoichiometric ratio of 2.5 to 25% in moles, preferably between 5 and 15% in moles (depending on the intended use of the product) of the repetitive units of hyaluronic acid, followed by.
2. dispersion of HA in the solution referred to in the preceding paragraph, at room temperature. The HA concentration must be between 80 and 300 mg/ml, and the homogenisation time between 30 and 300 minutes.
3. Triggering of the reaction by heat activation, said solution being heated at a temperature of between 35 and 55°C for between 2 and 36 hours.
4. Correction of pH to neutral with an aqueous HCl solution having a concentration of 0.05 to 1 moles/l, preferably 0.1 moles/l.
5. Hydration of gel by diluting it with water by a factor of 3 to 20 for a time of between 4 and 48 hours at a temperature of 4 to 24°C. This solution may contain further excipients, such as NaCl, phosphoric acid sodium or potassium salts, and lidocaine, preferably in the form of hydrochloride salt. Sodium salts (chloride or phosphate) have the function of maintaining the appropriate osmolarity of the product, and maintaining the pH at a value compatible with the tissues. In a preferred embodiment of the invention, NaCl is added in an amount such that the final solution contains a concentration of between 0.8 and 1.0% thereof, preferably 0.9%; the lidocaine hydrochloride, if present, is added in an amount such that the final formulation contains an amount of between 2.2 and 3.2 mg/ml thereof, preferably 2.7 mg/ml.

Mixing of HYADD with HBC

[0036]

6. Mixing of the HBC gel with HYADD powder in the HYADD :HBC ratio of between 10:90 and 90:10 (in weight of the active ingredient) depending on the use chosen for the novel filler, as previously described. Alternatively, the HYADD can be mixed with HBC starting with both components in gel form, using a suitable stirring system (preferably with an orbital blade) for a time of between 30 minutes and 24 hours at a temperature of between 0 and 26°C.
7. Crushing and homogenisation by passing through a filter with a particulate matter retention coefficient of between 25 and 150 μm, preferably between 40 and 110 μm. If the viscosity is excessive, the operation can be performed hot, at a temperature of between 25 and 65°C.
8. Filling of syringes, made of glass or polymer material, with the product obtained.
9. Sterilisation by heat from saturated steam at a temperature of between 120 and 124°C (preferably 121.5 ± 1°C) for at least 10 min.

[0037] Some examples of preparation of the novel filler according to the invention are described below, by way of example and not of limitation.

Example 1: Synthesis of HBC 500 (HA 500-730 kDa)

process A

[0038] 0.075 moles of HA with a molecular weight of 500-730 kDa, produced by fermentation, are dispersed in 215 ml of an 0.25M NaOH solution containing 1.41 ml of BDDE. The mixture is then heated to 42°C and reacted for 3 hours. The mixture is then hydrated for 24h with 300 ml of a solution containing a stoichiometric amount of HCl to adjust the pH to neutral. The total volume is made up to 750 ml and precipitated with 2.5 volumes of ethanol to obtain a filterable, decantable precipitate. The mixture is washed with 75% ethanol until exhaustive purification, verified by measuring the specific conductivity of the washing solvents, which should be under 30 uS/cm, and dried under vacuum at 40°C for 5 days. The HBC 500 product is obtained with a weight yield of 87%.

Example 2: Synthesis of HBC 1000 (HA 1MDa)

process A

[0039] 1.60 g of HA with a mean molecular weight of 1 MDa, produced by fermentation, is dispersed in 20 ml of an 0.25M NaOH solution containing 75 μl of BDDE. The mixture is then heated to 42°C and reacted for 2 hours. The mixture is then hydrated for 24h with 20 ml of a solution containing a stoichiometric amount of HCl to adjust the pH to neutral. The total volume is made up to 75 ml and HBC is precipitated with 2.5 volumes of ethanol to obtain a filterable, decantable precipitate. The mixture is washed with 75% ethanol until exhaustive purification, verified by measuring the specific conductivity of the washing solvents, which should be under 30 uS/cm, and dried under vacuum at 40°C for 5 days. The product HBC 1000 is obtained with a weight yield of 90%.

Example 3: Synthesis of HBC 200 (HA 200 kDa)

process A

[0040] 2.55 g of HA with a mean molecular weight of 200 KDa, produced by fermentation, is dispersed in 20 ml of an 0.25M NaOH solution containing 63 μl of BDDE. The mixture is then heated to 42°C and reacted for 150 minutes. The mixture is then hydrated for 24h with 20 ml of a solution containing a stoichiometric amount of HCl. The total volume is made up to 75 ml and precipitated

with 2.5 volumes of ethanol to obtain a filterable, decantable precipitate. The mixture is washed with 75% ethanol until exhaustive purification, verified by measuring the specific conductivity of the washing solvents, which should be under 30 uS/cm, and dried under vacuum at 40°C for 5 days. The product HBC 200 is obtained with a weight yield of 85%.

Example 4: preparation of ACP:HBC 500 gel, in the ratio of 50:50

process A

process A (comparative example, not forming part of the invention)

[0041] 1.00 g of HBC 500, prepared as described in example 1, is mixed with 1.00 g of HA ACP internal ester. The powder is hydrated with 100 ml of 0.9% weight/volume sterile saline solution at the temperature of 8°C for 16 hours. The gel obtained is heated to 48°C and filtered through a metal sieve with a mesh of 0.17 mm, and then distributed between 1 ml glass syringes, which subsequently undergo a sterilisation cycle with saturated steam at the temperature of 121°C for 10 minutes. A homogenous sterile gel suitable for local administration is obtained.

Example 5: preparation of ACP:HBC 1000 gel, in the ratio of 30:70

process A (comparative example, not forming part of the invention)

[0042] 1.40 g of HBC 1000, prepared as described in example 2, is mixed with 0.60 g of HA ACP internal ester. The powder is hydrated with 100 ml of 0.9% w/v sterile saline solution at the temperature of 8°C for 16 hours. The gel obtained is heated to 48°C and filtered through a metal sieve with a mesh of 0.17 mm, and then distributed between 1 ml glass syringes, which subsequently undergo a sterilisation cycle with saturated steam at the temperature of 121°C for 10 minutes. A homogenous sterile gel suitable for local administration is obtained.

Example 6: preparation of ACP:HBC 500 gel, in the ratio of 25:75

process A (comparative example, not forming part of the invention)

[0043] 1.875 g of HBC 500, prepared as described in example 1, is mixed with 0.625 g of HA internal ester ACP. The powder is hydrated with 100 ml of 0.9% w/v sterile saline solution at the temperature of 8°C for 16 hours. The gel obtained is heated to 48°C and filtered through a metal sieve with a mesh of 0.19 mm, and then distributed between 1 ml glass syringes, which subsequently undergo a sterilisation cycle with saturated steam at the temperature of 121 °C for 12 minutes. A homogenous sterile gel suitable for local administration is obtained.

Example 7: preparation of ACP:HBC 1000 gel, in the ratio of 75:25

process A (comparative example, not forming part of the invention)

[0044] 0.50 g of HBC 1000, prepared as described in example 2, is mixed with 1.50 g of HA internal ester ACP. The powder is hydrated with 100 ml of 0.9% w/v sterile saline solution at the temperature of 8°C for 24 hours. The gel obtained is heated to 42°C and filtered through a metal sieve with a mesh of 0.17 mm, and then distributed between 2 ml glass syringes, which subsequently undergo a sterilisation cycle with saturated steam at the temperature of 121 °C for 12 minutes. A homogenous sterile gel suitable for local administration is obtained.

Example 8: preparation of HYADD:HBC 500 gel, in the ratio of 60:40

process A

[0045] 1.20 g of HBC 500 prepared as described in example 1 is mixed with 0.80 g of HA hexadecylamide (HYADD). The powder is hydrated with 100 ml of 0.9% w/v sterile saline solution at the temperature of 8°C for 24 hours. The gel obtained is heated to 52°C and filtered through a metal sieve with a mesh of 0.17 mm, and then distributed between 1 ml glass syringes, which subsequently undergo a sterilisation cycle with saturated steam at the temperature of 121 °C for 11 minutes. A homogenous sterile gel suitable for local administration is obtained.

Example 9: preparation of HYADD:HBC 500 gel, in the ratio of 40:60

process A

[0046] 8.0 g of HA sodium salt with a mean molecular weight of 500-730 kDa, produced by fermentation, is dispersed in 40 ml of an 0.25M NaOH solution containing 0.44 ml of BDDE. The mixture is heated at 41.5°C for 2 hours 40 minutes. It is then hydrated overnight with 100 ml of an 0.1 M HCl solution and 200 ml of water. 50 ml of a saturated solution of NaCl is added, and the mixture is left to swell overnight. The next day, 170 ml of acetone and 30 ml of saturated NaCl solution are added, and the mixture is precipitated by slowly adding one litre of ethanol. The precipitate is washed with the same solvent until the NaCl residues have been eliminated, then stove dried at 35°C under vacuum until the residual solvents have been eliminated. The HBC powder thus obtained

is mixed in the ratio of 5:3 with HYADD, prepared as described in patent EP1853279. The mixed powders are hydrated with saline solution, leading to a total concentration of 20 mg/ml (corresponding to 12.5 mg/ml of HBC and 7.5 mg/ml of HYADD4). The product is left to swell overnight at 5°C, and the next day is filtered through a flat membrane with a nominal particulate matter retention rate of 100 μm. 1 ml glass syringes are filled with the product thus obtained and sterilised in a cycle with F0=13 at 121.5°C.

Example 10: Cutaneous filling and tolerability of HYADD:HBC gel in the intradermal rabbit administration model

[0047] The purpose of the experiment was to evaluate cutaneous filling, the onset of any macroscopic adverse events, and the tissue response elicited by HYADD:HBC gel (prepared as described in example 9) injected into the intradermal tissue of the rabbit, by comparison with the commercial filler BELOTERO®.

[0048] For said evaluation, the gels tested were administered intradermally to male NZW-KBL rabbits weighing 1.8-2.3 kg.

Experiment design:

[0049] The animals were anaesthetised by intravenous administration of ketamine and xylazine. 3 animals were used for each filler tested.
day 0: T0

- Injection of samples (1 ml of hydrogel per sample) after shaving of the rabbits' backs;
- Measurement of the swelling on all rabbits and macroscopic observation for adverse events.

Day 7: T7

- Measurement of swelling volume and macroscopic observation for adverse events.

[0050] The swelling volume was calculated with the formula:

$$(2/3 \times \pi) \times (r1) \times (r2) \times (r3)$$

where: (r1), (r2) and (r3) represent the width, length and height of the swelling respectively, measured with a caliper.

Results:

[0051] The novel filler did not cause any inflammatory event in the treated dermis.

[0052] The results obtained for the residence time are shown in Figure 1: the amount of swelling evaluated in the first week's treatment (expressed as mm^3) demonstrated that the gel according to the invention is capable of inducing a larger skin swelling volume than the control, which remains high even after 7 days, again to a much greater extent than the commercial filler used as comparator. This finding clearly confirms that the novel fillers immediately produce significant dermal hydration, and this effect is attributable to the presence of the HYADD derivative which, due to its chemical/rheological characteristics, has proved essential to promote immediate cutaneous filling which remains stable over time.

Example 11: Synthesis of HBC 500 (HA 500-730 kDa)

process B

[0053] 18.75 g of HA sodium salt with a molecular weight of 500-730 kDa, produced by fermentation, is dispersed in 133 ml of an 0.25M solution of NaOH containing 885 μl of BDDE. The mixture is then heated at 45°C for 2.5 hours. The mixture is hydrated overnight with 0.62 1 of a solution containing a stoichiometric amount of HCl, 2.65g of NaCl and 2.7g of lidocaine hydrochloride, under slow stirring.

Example 12: preparation of ACP:HBC 500 gel, in the ratio of 25:75

process B (comparative example, not forming part of the invention)

[0054] 6.25 g of internal ester of hyaluronic acid ACP 200 is solubilised in 250 ml of a solution containing 4.4 g of NaCl under slow stirring. When hydration has been completed, the gel is combined with the gel obtained according to example 11 in a mixer equipped with a system for mixing semisolids, until homogenous. The gel obtained is extruded through a flat membrane filter with a nominal particulate matter retention rate of 70 μm. The product thus obtained is introduced into glass syringes and sterilised in a cycle with F0=13 at 121.5°C.

Example 13: preparation of HYADD:HBC 500 gel, in the ratio of 25:75

process B

[0055] 6.25 g of HYADD hexadecylamide is solubilised in 250 ml of a solution containing 4.4 g of NaCl under slow stirring. When hydration has been completed, the gel is combined with the gel obtained according to example 11 in a mixer equipped with an orbital mixing system, until homogenous. The gel obtained is extruded through a flat membrane filter with a nominal particulate matter retention rate of 70 μm. The product thus obtained is introduced into glass syringes and sterilised in a cycle with F0=13 at 121.5°C.

Example 14: Synthesis of HBC 500 (HA 500-730 kDa)

process B

[0056] 125 g of HA sodium salt with a molecular weight of 500-730 kDa, produced by fermentation, is dispersed in 1.33 1 of an 0.25M NaOH solution containing 9.4 ml of BDDE. The mixture is heated at 45°C for 2.5 hours. The mixture is hydrated overnight with 6.2 1 of a solution containing a stoichiometric amount of HC1, 26.5 g of NaCl and 27 g of lidocaine hydrochloride, under slow stirring.

Example 15: preparation of ACP:HBC 500 gel, in the ratio of 50:50

process B (comparative example, not forming part of the invention)

[0057] 125 g of internal ester of hyaluronic acid ACP200 is solubilised in 2.5 1 of a solution containing 44 g of NaCl under slow stirring. When hydration has been completed, the gel is combined with the gel obtained according to example 14 in a mixer equipped with an orbital mixing system with buffle and scraper. The gel obtained is extruded through a flat membrane filter with a nominal particulate matter retention rate of 45 μm. The product thus obtained is introduced into glass syringes and sterilised in a cycle with F0=13 at 121.5°C.

Example 16: Cutaneous filling and tolerability of ACP:HBC gel in the intradermal rabbit administration model (comparative example, not forming part of the invention)

[0058] The experiment was performed as described in example 10, using gel prepared as described in examples 11-12, and comparing it with the Belotero® control and with a second commercial filler, Regenyal Idea.

[0059] For this experiment, the Applicant not only determined the skin swelling volume caused by the treatment but also evaluated the total residence time of the gel/filler according to the invention by comparison with two well-known commercial fillers which represent the final comparator because both consist of HA crosslinked with BDDE.

[0060] The skin swelling in the treated rabbits was measured fortnightly (with macroscopic observation for adverse events) for a maximum of 96 days.

Results:

[0061] Figure 2 shows the results obtained: the findings described above were confirmed, namely immediate hydration of the treated dermis (mainly within the first 7 days) to a surprisingly greater extent than in the controls; moreover, the size of the skin swelling was more evident and the residence time longer than those of the two commercial comparators. At the end of the experiment, the novel filler according to the invention was still present, whereas the two controls had almost disappeared.

Claims

1. Biomaterials obtainable by mixing

   - hyaluronic acid hexadecylamide (HYADD) with
   - the derivative (HBC) of hyaluronic acid crosslinked with 1,4-butanediol diglycidyl ether (BDDE)

   in the weight ratio of between 10:90 and 90:10 as novel fillers and/or as body shaping products.

2. Biomaterials as claimed in claim 1, wherein the weight ratio is of 90:10 to 50:50, as biorevitalising fillers.

3. Biomaterials as claimed in claim 1, wherein the weight ratio is of 10:90 to 50:50 with a volume-enhancing effect.

4. Biomaterials as claimed in claim 3, wherein the weight ratio is of 25:75.

5. Process of mixing HYADD with HBC prepared by a process comprising the following steps:

   a. dissolution in alkaline solution of diepoxide BDDE in a stoichiometric ratio from 2.5 to 25% in moles of the repetitive units of hyaluronic acid, followed by
   b. dispersion of hyaluronic acid (HA) in the solution referred to in step a), at room temperature;
   c. triggering of the reaction by heat activation, the solution referred to in step b) being heated at a temperature of between 35 and 55°C for between 2 and 36 hours;
   d. extrusion of the mass obtained through a metal sieve, to reduce it to particles with a size of approx. 600 μm;
   e. hydration of the gel obtained by diluting it with water by a factor of 3 to 20;
   f. correction of pH to neutral with an aqueous solution of HCl;
   g. precipitation with a water-soluble organic solvent until the product is obtained in powder form;
   h. washing with organic solvents containing water;
   i. drying under vacuum until the residual solvents under 400 ppm have been eliminated and an HBC powder is obtained;

   wherein the mixing process comprises the following steps:

j. mixing of HYADD powder with HBC powder in the HYADD:HBC ratio of between 90:10 and 10:90;
k. hydration with saline solution or phosphate buffer, leading to a total HA concentration of between 12 and 27 mg/ml;
l. extrusion at a temperature of between 25 and 65°C through a sieve with a mesh of between 50 and 500 μm;
m. syringe filling;
n. sterilisation by heat from saturated steam at a temperature of between 120 and 124°C for at least 10 min.

6. Process as claimed in claim 5, wherein the water-soluble organic solvent of step g) is selected from the group consisting of ethanol, methanol, isopropanol, n-propanol, dioxane, acetonitrile, acetone and/or mixtures thereof.

7. Process as claimed in claim 5, wherein the organic solvents of step h) are selected from the group consisting of ethanol, methanol, isopropanol, n-propanol, dioxane, acetonitrile, acetone and/or mixtures thereof.

8. Process of mixing HYADD with HBC prepared by a process comprising the following steps:

a. dissolution in alkaline solution of diepoxide BDDE in a stoichiometric ratio from 2.5 to 25% in moles of the repetitive units of hyaluronic acid, followed by
b. dispersion of HA in the solution referred to in step a), at room temperature;
c. triggering of the reaction by heat activation, the solution referred to in step b) being heated at a temperature of between 35 and 55°C for between 2 and 36 hours;
d. correction of pH to neutral with an aqueous solution of HCl;
e. hydration of the gel obtained by diluting it with water by a factor of 3 to 20;

wherein the mixing process comprises the following steps:

f. mixing of HYADD gel or powder with HBC in gel form in a HYADD:HBC ratio of between 90:10 and 10:90;
g. crushing and homogenisation by passing through a filter with a particulate matter retention coefficient of between 25 and 150 μm;
h. syringe filling;
i. heat sterilisation with saturated steam at a temperature of between 120 and 124°C for at least 10 min.

9. Process as claimed in claim 5 or 6, wherein the HA used for the preparation of the HBC and HYADD derivatives has a mean molecular weight of between 400 and $3x10^6$ Da.

10. Process as claimed in claim 9, wherein the HA has a mean molecular weight between $1x10^5$ Da and $1x10^6$ Da.

11. Process as claimed in claim 9, wherein the HA has a mean molecular weight between 200,000 and $1x10^6$ Da.

12. Biomaterials as claimed in the preceding claims, wherein the vehicle is saline solution.

13. Biomaterials as claimed in the preceding claims, containing lidocaine.

14. Biomaterials as claimed in claims 12 and 13, containing lidocaine, wherein the vehicle consists of saline solution.

15. Biomaterials as claimed in the preceding claims for use as new fillers and/or as new products for body shaping in the treatment of skin blemishes, in dermatology, in dermocosmetology and/or in aesthetic surgery.

Patentansprüche

1. Biomaterialien, erhältlich durch Mischen

- von Hyaluronsäurehexadecylamid (HYADD) mit
- dem Derivat (HBC) von Hyaluronsäure, das mit 1,4-Butandioldiglycidylether (BDDE) vernetzt ist,

im Gewichtsverhältnis von zwischen 10:90 und 90:10, als neue Füllstoffe und/oder als Körperformungsprodukte.

2. Biomaterialien, wie in Anspruch 1 beansprucht, in denen das Gewichtsverhältnis 90:10 bis 50:50 ist, als biorevitalisierende Füllstoffe.

3. Biomaterialien, wie in Anspruch 1 beansprucht, in denen das Gewichtsverhältnis 10:90 bis 50:50 ist, mit einer Volumen erhöhenden Wirkung.

4. Biomaterialien, wie in Anspruch 3 beansprucht, in denen das Gewichtsverhältnis 25:75 ist.

5. Verfahren zum Mischen von HYADD mit HBC, das durch ein Verfahren hergestellt wurde, das die folgenden Schritte umfasst:

a. Auflösung von Diepoxid BDDE in alkalischer Lösung in einem stöchiometrischen Verhältnis von 2,5 bis 25 Mol% der Wiederholungseinheiten von Hyaluronsäure, gefolgt von
b. Dispersion von Hyaluronsäure (HA) in der Lösung, die in Absatz a) genannt ist, bei Raumtemperatur;
c. Auslösen der Reaktion durch Wärmeaktivierung, wobei die in Absatz b) genannte Lösung bei einer Temperatur von zwischen 35 und 55°C für zwischen 2 und 36 Stunden erwärmt wird;
d. Extrusion der erhaltenen Masse durch ein Metallsieb, um sie auf Partikel mit einer Größe von etwa 600 μm zu verringern;
e. Hydratisierung des erhaltenen Gels durch Verdünnen desselben mit Wasser mit einem Faktor von 3 bis 20;
f. Korrektur des pH auf neutral mit wässriger HCl - Lösung;
g. Präzipitation mit einem wasserlöslichen organischen Lösungsmittel, bis das Produkt in Pulverform erhalten wird;
h. Waschen mit organischen Lösungsmitteln, die Wasser enthalten;
i. Trocknung unter Vakuum, bis die restlichen Lösungsmittel unter 400 ppm entfernt wurden und ein HBC-Pulver erhalten wird;

wobei das Mischverfahren die folgenden Schritte umfasst:

j. Mischen von HYADD-Pulver mit HBC-Pulver im Verhältnis HYADD:HBC von zwischen 90:10 und 10:90;
k. Hydratisierung mit Kochsalzlösung oder Phosphatpuffer, was zu einer Gesamt-HA-Konzentration von zwischen 12 und 27 mg/ml führt;
l. Extrusion bei einer Temperatur von zwischen 25 und 65°C durch ein Sieb mit einer Maschenweite von zwischen 50 und 500 μm;
m. Spritzenfüllung;
n. Sterilisation durch Hitze aus gesättigtem Dampf bei einer Temperatur von zwischen 120 und 124°C für wenigstens 10 min.

6. Verfahren, wie in Anspruch 5 beansprucht, wobei das wasserlösliche organische Lösungsmittel von Schritt g) aus der Gruppe, bestehend aus Ethanol, Methanol, Isopropanol, n-Propanol, Dioxan, Acetonitril, Aceton und/oder Gemischen davon, ausgewählt wird.

7. Verfahren, wie in Anspruch 5 beansprucht, wobei die organischen Lösungsmittel von Schritt h) aus der Gruppe, bestehend aus Ethanol, Methanol, Isopropanol, n-Propanol, Dioxan, Acetonitril, Aceton und/oder Gemischen davon, ausgewählt werden.

8. Verfahren zum Mischen von HYADD mit HBC, das durch ein Verfahren hergestellt wurde, das die folgenden Schritte umfasst:

a. Auflösung von Diepoxid BDDE in alkalischer Lösung in einem stöchiometrischen Verhältnis von 2,5 bis 25 Mol% der Wiederholungseinheiten von Hyaluronsäure, gefolgt von
b. Dispersion von HA in der Lösung, die in Absatz a) genannt ist, bei Raumtemperatur;
c. Auslösen der Reaktion durch Wärmeaktivierung, wobei die in Absatz b) genannte Lösung bei einer Temperatur von zwischen 35 und 55°C für zwischen 2 und 36 Stunden erwärmt wird;
d. Korrektur des pH auf neutral mit einer wässrigen HCl-Lösung;
e. Hydratisierung des erhaltenen Gels durch Verdünnen desselben mit Wasser mit einem Faktor von 3 bis 20;

wobei das Mischverfahren die folgenden Schritte umfasst:

f. Mischen von HYADD-Gel oder -Pulver mit HBC in Gelform in einem Verhältnis HYADD:HBC von zwischen 90:10 und 10:90;
g. Zerkleinerung und Homogenisierung durch Durchleiten durch ein Filter mit einem Retentionskoeffizienten für partikuläres Material von zwischen 25 und 150 μm;
h. Spritzenfüllung;
i. Hitzesterilisation mit gesättigtem Dampf bei einer Temperatur von zwischen 120 und 124°C für wenigstens 10 min.

9. Verfahren, wie in Anspruch 5 oder 6 beansprucht, wobei die für die Herstellung der HBC- und HYADD-Derivate verwendete HA ein mittleres Molekulargewicht von zwischen 400 und $3x10^6$ Da hat.

10. Verfahren, wie in Anspruch 9 beansprucht, wobei die HA ein mittleres Molekulargewicht von zwischen $1x10^5$ Da und $1x10^6$ Da hat.

11. Verfahren, wie in Anspruch 9 beansprucht, wobei die HA ein mittleres Molekulargewicht von zwischen 200.000 und $1x10^6$ Da hat.

12. Biomaterialien, wie in den vorangehenden Ansprüchen beansprucht, in denen das Vehikel physiologische Kochsalzlösung ist.

13. Biomaterialien, wie in den vorangehenden Ansprüchen beansprucht, die Lidocain enthalten.

14. Biomaterialien, wie in Anspruch 12 und 13 beansprucht, die Lidocain enthalten, wobei das Vehikel aus physiologischer Kochsalzlösung besteht.

15. Biomaterialien, wie in den vorangehenden Ansprüchen beansprucht, zur Verwendung als neue Füllstoffe und/oder als neue Produkte zur Körperformung in der Behandlung von Hautschönheitsfehlern, in der Dermatologie, in der Dermokosmetologie und/oder in der Schönheitschirurgie.

Revendications

1. Biomatériaux que l'on peut obtenir en mélangeant

   - l'hexadécylamide d'acide hyaluronique (HYADD) avec
   - le dérivé (HBC) d'acide hyaluronique réticulé avec du 1,4-butanediol-diglycidyléther (BDDE)

   dans un rapport pondéral compris entre 10:90 et 90:10 en tant que nouveaux matériaux de remplissage et/ou en tant que produits de remodelage du corps.

2. Biomatériaux selon la revendication 1, dans lesquels le rapport pondéral est de 90:10 à 50:50, en tant que matériaux de remplissage biorevitalisants.

3. Biomatériaux selon la revendication 1, dans lesquels le rapport pondéral est de 10:90 à 50:50, avec un effet d'amélioration du volume.

4. Biomatériaux selon la revendication 3, dans lesquels le rapport pondéral est de 25:75.

5. Procédé de mélange d'HYADD avec du HBC préparés par un procédé comprenant les étapes suivantes :

   a. dissolution en solution alcaline du diépoxyde BDDE dans un rapport stoechiométrique de 2,5 à 25 % en moles des unités répétitives d'acide hyaluronique, suivie par
   b. dispersion d'acide hyaluronique (HA) dans la solution citée dans l'étape a), à température ambiante ;
   c. déclenchement de la réaction par activation à chaud, la solution citée dans l'étape b) étant chauffée à une température comprise entre 35 et 55 °C pendant 2 à 36 heures ;
   d. extrusion de la masse obtenue à travers un tamis métallique, pour la réduire en particules ayant une taille d'environ 600 μm ;
   e. hydratation du gel obtenu, par dilution de celui-ci avec de l'eau d'un facteur 3 à 20 ;
   f. correction du pH pour l'amener à un pH neutre avec une solution aqueuse de HCl ;
   g. précipitation avec un solvant organique hydrosoluble jusqu'à ce que le produit soit obtenu sous forme de poudre ;
   h. lavage avec des solvants organiques contenant de l'eau ;
   i. séchage sous vide jusqu'à ce que les solvants résiduels sous 400 ppm aient été éliminés et qu'une poudre de HBC soit obtenue ;

   le procédé de mélange comprenant les étapes suivantes :

   j. mélange de poudre d'HYADD avec de la poudre de HBC dans un rapport HYADD:HBC compris entre 90:10 et 10:90 ;
   k. hydratation avec une solution saline ou un tampon phosphate, conduisant à une concentration totale de HA comprise entre 12 et 27 mg/ml ;
   l. extrusion à une température comprise entre 25 et 65 °C à travers un tamis ayant une maille comprise entre 50 et 500 μm ;
   m. remplissage d'une seringue ;
   n. stérilisation par la chaleur à partir de vapeur d'eau saturée à une température comprise entre 120 et 124 °C pendant au moins 10 min.

6. Procédé selon la revendication 5, dans lequel le solvant organique hydrosoluble de l'étape g) est choisi parmi le groupe comprenant l'éthanol, le méthanol, l'isopropanol, le n-propanol, le dioxane, l'acétonitrile, l'acétone et/ou les mélanges de ceux-ci.

7. Procédé selon la revendication 5, dans lequel les solvants organiques de l'étape h) sont choisis parmi le groupe comprenant l'éthanol, le méthanol, l'isopropanol, le n-propanol, le dioxane, l'acétonitrile, l'acétone et/ou les mélanges de ceux-ci.

8. Procédé de mélange d'HYADD avec du HBC préparés par un procédé comprenant les étapes suivantes :

   a. dissolution en solution alcaline du diépoxyde BDDE dans un rapport stoechiométrique de 2,5 à 25 % en moles des unités répétitives d'acide hyaluronique, suivie par
   b. dispersion de HA dans la solution citée dans l'étape a), à température ambiante ;
   c. déclenchement de la réaction par activation à chaud, la solution citée dans l'étape b) étant chauffée à une température comprise entre 35 et 55 °C pendant 2 à 36 heures ;
   d. correction du pH pour l'amener à un pH neutre avec une solution aqueuse de HCl ;
   e. hydratation du gel obtenu, par dilution de celui-ci avec de l'eau d'un facteur 3 à 20 ;

   le procédé de mélange comprenant les étapes suivantes :

f. mélange de gel ou de poudre d'HYADD avec du HBC sous forme de gel dans un rapport HYADD:HBC compris entre 90:10 et 10:90 ;
g. broyage et homogénéisation par passage à travers un filtre ayant un coefficient de rétention de matière particulaire compris entre 25 et 150 μm;
h. remplissage d'une seringue ;
i. stérilisation par la chaleur avec de la vapeur d'eau saturée à une température comprise entre 120 et 124 °C pendant au moins 10 min.

9. Procédé selon les revendications 5 ou 6, dans lequel le HA utilisé pour la préparation des dérivés HBC et HYADD a une masse moléculaire moyenne comprise entre 400 et $3x10^6$ Da.

10. Procédé selon la revendication 9, dans lequel le HA a une masse moléculaire moyenne comprise entre $1x10^5$ Da et $1x10^6$ Da.

11. Procédé selon la revendication 9, dans lequel le HA a une masse moléculaire moyenne comprise entre 200 000 et $1x10^6$ Da.

12. Biomatériaux selon les revendications précédentes, dans lesquels le véhicule est une solution saline.

13. Biomatériaux selon les revendications précédentes, contenant de la lidocaïne.

14. Biomatériaux selon les revendications 12 et 13, contenant de la lidocaïne, dans lesquels le véhicule est constitué d'une solution saline.

15. Biomatériaux selon les revendications précédentes pour une utilisation en tant que nouveaux matériaux de remplissage et/ou en tant que nouveaux produits pour le remodelage du corps dans le traitement des imperfections de la peau, en dermatologie, en dermocosmétologie et/ou en chirurgie esthétique.

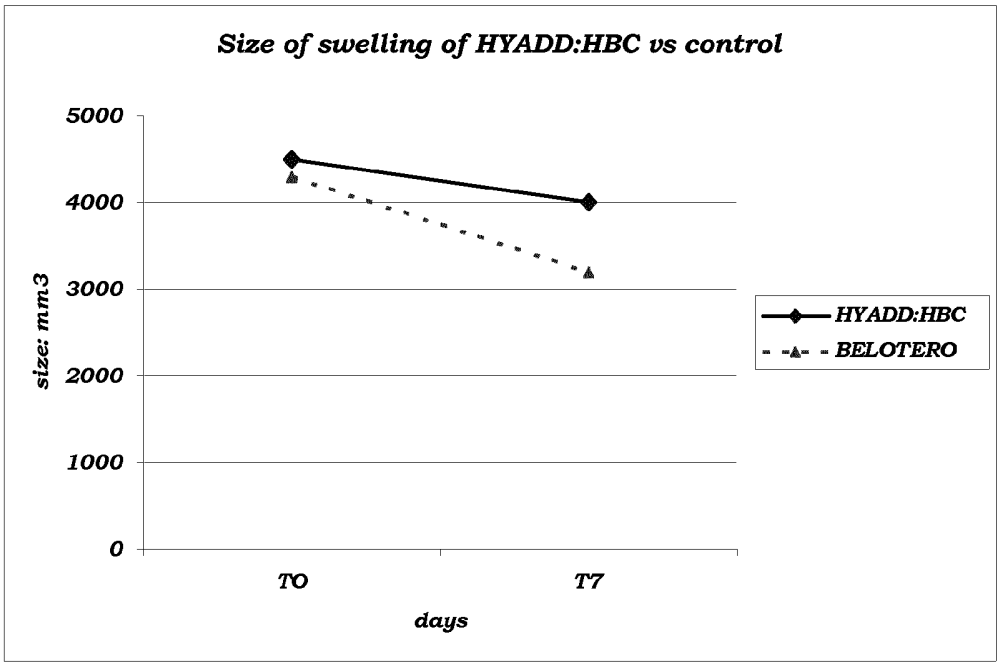

Figure 1

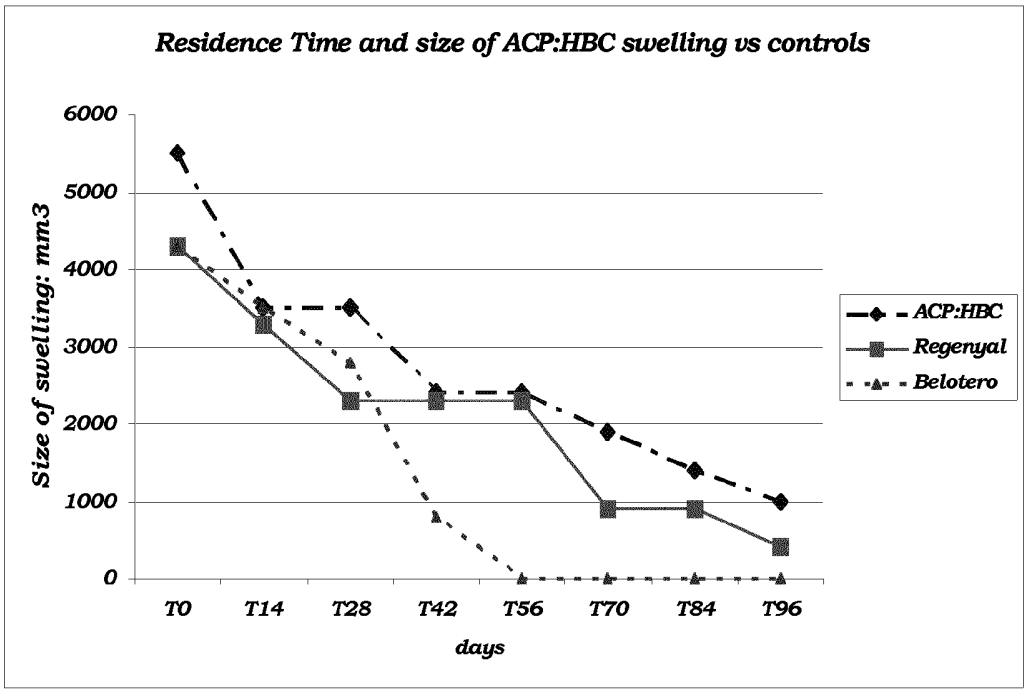

Figure 2

REFERENCES CITED IN THE DESCRIPTION

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- **WEIGEL P. et al.** *J Theoretical Biol,* 1986, 219-234 **[0006]**
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Szabadalmi igénypontok

1. Bioanyagok, amelyek

- hialuronsav-hexadecilamidnak (HYADD-nak)

-1,4-bután̈diol-diglicidil-éterrel (BDDE-vel) keresztkötött hialuronsav-származékkal (HBC-vel)

10:90-90:10 tömegarányban történő keverésével állíthatók elő, mint új kitöltőanyagok és/vagy testformáló termékek.

2. Az 1. igénypont szerinti bioanyagok, ahol a tömegarány 90:10-50:50, mint biorevitalizáló kitöltőanyagok.

3. Az 1. igénypont szerinti bioanyagok, ahol a tömegarány 10:90-50:50, amelyek térfogatnövelő hatásúak.

4. A 3. igénypont szerinti bioanyagok, ahol a tömegarány 25:75.

5. Eljárás HYADD és a következő lépéseket magában foglaló eljárással előállított HBC összekeverésére:

a. BDDE-diepoxid oldása lúgos oldatban a hialuronsav ismétlődő egységeinek móljaira számított 2,5-25%-os sztöchiometrikus arányban, ezután

b. a hialuronsav (HA) diszpergálása az a) lépésben meghatározott oldatban, szobahőmérsékleten,

c. a reakció kiváltása hőaktiválással: a b) lépésben meghatározott oldat 2-36 órán keresztül 35-55°C-ra melegítésével,

d. a kapott massza fémszűrőn keresztül történő sajtolása, ezáltal mintegy 600 µm méretű részecskékre csökkentése,

e. a kapott gél hidrálása 3-20-szoros, vízzel történő hígítással,

f. semleges pH beállítása HCL vizes oldatával,

g. vízoldékony szerves oldószerrel történő kicsapás a termék por formában történő előállításáig,

h. vizet tartalmazó szerves oldószerekkel történő mosás,

i. szárítás vákuum alatt, amíg a maradék, 400 ppm alatti oldószer eliminálódik és HBC-port kapunk,

ahol a keverési eljárás magában foglalja a következő lépéseket:

j. HYADD-por keverése HBC-porral 90:10 és10:90 közötti HYADD:HBC arányban,

k. hidrálás sóoldattal vagy foszfátpufferrel, ami 12-27 mg/ml teljes HA-koncentrációt eredményez,

l. sajtolás 25-65°C közötti hőmérsékleten 50-500 µm szemméretű rostán keresztül,

m. fecskendőtöltés,

n. sterilizálás telített gőzzel 120-124°C közötti hőmérsékleten legalább 10 percen keresztül.

6. Az 5. igénypont szerinti eljárás, ahol a g) lépés vízoldékony szerves oldószere a következőkből álló csoportból van kiválasztva etanol, metanol, izopropanol, n-propanol, dioxán, acetonitril, aceton és ezek elegyei.

7. Az 5. igénypont szerinti eljárás, ahol a h) lépés szerves oldószerei következőkből álló csoportból vannak kiválasztva etanol, metanol, izopropanol, n-propanol, dioxán, acetonitril, aceton és ezek elegyei.

8. Eljárás HYADD és a következő lépéseket magában foglaló eljárással előállított HBC összekeverésére:

a. BDDE-diepoxid oldása lúgos oldatban a hialuronsav ismétlődő egységeinek móljaira számított 2,5-25%-os sztöchiometrikus arányban, majd

b. a hialuronsav diszpergálása az a) lépésben meghatározott oldatban, szobahőmérsékleten,

c. a reakció kiváltása hőaktiválással: a b) lépésben meghatározott oldat 2-36 órán keresztül 35-55°C-ra melegítésével,

d. semleges pH beállítása HCL vizes oldatával,

e. a kapott gél hidrálása 3-20-szoros, vízzel történő hígítással,

ahol a keverési eljárás magában foglalja a következő lépéseket:

f. HYADD-gél vagy -por keverése gél formájú HBC-vel 90:10 és 10:90 közötti HYADD:HBC arányban,

g. zúzás és homogenizálás szűrőn keresztül történő átnyomással, amelynek finomszemcsés anyagra vonatkozó retenciós együtthatója 25 és 150 μm közötti,

m. fecskendőtöltés,

n. sterilizálás telített gőzzel 120-124°C közötti hőmérsékleten legalább 10 percen keresztül.

9. Az 5. vagy 6. igénypont szerinti eljárás, ahol a HBC- és HYADD-származékok előállítására alkalmazott HA átlagos molekulatömege 400 és $3x10^6$ Da közötti,

10. A 9. igénypont szerinti eljárás, ahol a HA átlagos molekulatömege $1x10^5$ Da és $1x10^6$ Da közötti.

11. A 9. igénypont szerinti eljárás, ahol a HA átlagos molekulatömege 200,000 and $1x10^6$ Da közötti.

12. Az előző igénypontok bármelyike szerinti bioanyagok, ahol a vivőanyag sóoldat.

13. Az előző igénypontok bármelyike szerinti bioanyagok, amelyek tartalmaznak lidokaint.

14. A 12. vagy 13. igénypont szerinti, lidokaint tartalmazó bioanyagok, ahol a vivőanyag sóoldatból áll.

15. Az előző igénypontok bármelyike szerinti bioanyagok új kitöltőanyagként és/vagy testformálásra szolgáló új termékként történő dermatológiai, dermokozmetológiai alkalmazásra, bőrhibák kezelésében és/vagy esztétikai műtétekben történő alkalmazásra.

A meghatalmazott:
DANUBIA
Szabadalmi és Jogi Iroda Kft.

Dr. Pethő Árpád
szabadalmi ügyvivő