The healthy cornea has a limited water influx (leak) into the stroma which is counter-balanced by water efflux due to hydrogen carbonate, lactate and NaCl solutes across the Descemet layer and the endothelial monolayer. Oxidative stress occurs as a result of the tear break-up followed by disruption of the balance between the anti-oxidant system and the pro-oxidant system and damages the ocular surface and plays a critical role in dry eye disease due to increased oxidized protein levels in accordance with the elevated inflammatory activity during these diseases. In addition, lack of corneal epithelial integrity leads to water influx into the stroma. The described condition needs to be treated adequately and efficiently, in particular epithelial defects need to be closed quickly and the tear film stabilized, in order to avoid long-term damage and worsening of dry eye disease.

However, the pharmaceutical products known in the art do not provide a sufficient regenerative capacity, i.e. by healing corneal epithelial defects. Accordingly, there is an unmet therapeutic need for novel products that are more active against the dry eye disease and related pathologies.

The above objects are solved by the subject-matter of the claims and by the subject-matter described here below.

Summary

In a first aspect, the disclosure relates to a multi-donor human serum product comprising insulin-like growth factors (IGFs) bound in macromolecular complexes of a molecular size of 30 kDa or more, the product comprising 20 ng/ml IGF-1 or more, the product comprising less than 70 mg/dl monosaccharides, wherein the product is optionally an isotonic solution or an isotonic gel.

In a related example, the disclosure relates to a human serum product comprising insulin-like growth factors (IGFs) bound in macromolecular complexes of a molecular size of 30 kDa or more, the product comprising 20 to 100 ng/ml IGF-1, the product comprising less than 70 mg/dl monosaccharides, wherein the product is optionally an isotonic solution or an isotonic gel.

The inventors have discovered that human serum comprises insulin-like growth factors (IGFs) bound in macromolecular complexes of a molecular size of at least 30 kDa, and that these macromolecular complexes provide for an improved wound healing when they are left intact and incorporated into a pharmaceutical product. Human serum contains specific epitheliotrophic factors such as IGF-1, EGF, and others, in addition to a high concentration of proteins such as albumin and fibronectin. Serum directly supports the proliferation and migration of epithelial cells or indirectly enhances epithelial viability by binding and neutralising in-

flammatory cytokines. Growth factors and cytokines are important regulators that stimulate growth, proliferation, migration, differentiation, adhesion, as well as ECM deposition and proteinase regulation of cells involved in wound healing.

In another aspect, the disclosure relates to the human serum product for use in medicine.

In a related example, the disclosure relates to the human serum product for use in the treatment of dry eye disease.

In another aspect, there is provided the use of the product as defined above in the manufacture of a medicament for the treatment of dry eye disease.

In another aspect, there is provided an ophthalmic composition, such as eye drops or gels, comprising the product as defined above.

In another aspect, the disclosure relates to a method for manufacturing a human serum product, comprising the steps of

- providing human allogeneic blood volume portions from a multitude of donors,
- selecting volume portions from at least two donors,
- deriving serum from the selected volume portions to obtain serum portions,
- pooling the selected serum portions to obtain pooled serum, wherein optionally the selected serum portions are from donors all having an age of 45 years or less;
- obtaining an intermediate product from the pooled serum, the intermediate product comprising 100 ng/ml IGF-1 or more; and
- obtaining the human serum product according to this disclosure from the intermediate product.

The method relies on providing whole blood volume portions from a multitude of donors which are selected in such a way to ensure a sufficient IGF concentration. IGF-1 concentrations in human blood differ between individuals. Some volume portions may have very high IGF, others very low IGF-1 concentrations. By selecting volume portions with adequate IGF-1 concentrations, a desired IGF-1 concentration in the product can be obtained. For example, it was found that the IGF-1 concentration in human serum decreases with an increasing lifetime of the donors. Hence, restricting the donor age of the multitude of donors to 45 years or less contributes to an efficient production method as less blood has to be discarded. The method provides for obtaining an intermediate product, wherein the intermediate product comprises 100 ng/ml IGF-1 or more. The

obtained product comprises insulin-like growth factors (IGFs) bound in macromolecular complexes of a molecular size of 30 kDa or more. Advantageously, the method is versatile with respect to choosing variants, parameters and conditions to obtain the product according to the disclosure from the intermediate product, and

thus provides a product which has improved therapeutic properties, e.g. for repairing corneal epithelial defects and anti-inflammation properties.

In an example related to the product, this disclosure provides an intermediate product comprising insulin-like growth factors (IGFs) bound in macromolecular complexes of a molecular size of 30 kDa or more, wherein the intermediate product comprises 100 to 500 ng/ml IGF-1.

Brief description of the drawings

Figure 1A and **Figure 1B**, respectively, show the age-dependant IGF-1 and IGF-BP3 concentrations in human serum. The age is given in years.

Figures 2A and **2B** show the activity of serum fractions in an *in vitro* wound closure assay.

Figures 3A to **3D**, respectively, show the effect of four different human recombinant (hr) growth factors, i.e. EGF, IGF-1, PDGF and KGF, on wound closure when combined with the high-molecular weight fraction or the low-molecular weight fraction of ultra-filtered multi-donor human serum, respectively.

Figure 4 shows the anti-inflammatory effect of three high-molecular weight fraction samples in a (LPS)-TNF-α inhibition assay.

Detailed description

Product

In a first aspect, the invention relates to a multi-donor human serum product comprising insulin-like growth factors (IGFs) bound in macromolecular complexes of a molecular size of 30 kDa or more, the product comprising 20 ng/ml IGF-1 or more, the product comprising less than 70 mg/dl monosaccharides, wherein the product is optionally an isotonic solution or an isotonic gel.

In a related aspect, the invention relates to a human serum product comprising insulin-like growth factors (IGFs) bound in macromolecular complexes of a molecular size of 30 kDa or more, the product comprising 20 to 100 ng/ml IGF-1, the product comprising less than 70 mg/dl monosaccharides, wherein the product is optionally an isotonic solution or an isotonic gel.

If this disclosure refers to a ‘human serum product’, it refers equally to the ‘multi-donor human serum product’ as well as the ‘human serum product’.

Claims

1. Multi-donor human serum product comprising insulin-like growth factors (IGFs) bound in macromolecular complexes of a molecular size of 30 kDa or more, the product comprising 20 ng/ml IGF-1 or more,

the product comprising less than 70 mg/dl monosaccharides,

wherein the product is optionally an isotonic solution or an isotonic gel.

2. Product according to claim 1, wherein the product comprises less than 40 mg/dl total cholesterol, wherein total cholesterol is the sum of LDL (low-density lipoprotein) and HDL (high-density lipoprotein).

3. Product according to claim 1 or claim 2, wherein the product has a wound-closure performance in an *in vitro* wound-closure assay of 50% or more, normalised to an area of injury, at 35 hours.

4. Product according to any one of the preceding claims, wherein the product has an anti-inflammatory performance in an inflammation assay, wherein the anti-inflammatory performance is normalised to a standardised expression of LPS-induced TNF-α (as a reference base value), wherein the anti-inflammatory performance is 50% or more,

and/or wherein the product comprises less than 0.2 mg/dl C-reactive protein (CRP).

5. Product according to any one of the preceding claims, comprising one or more growth factors selected from the list of EGF, IGF-1, IGF-BP3, IGF-2, PDGF-BB, Alpha 2-Macroglobulin, KGF, and PEDF.

6. Product according to any one of the preceding claims, comprising one or more growth factors in the following concentration ranges:

- 50 to 500 pg/ml EGF, or 20 to 200 pg/ml EGF;

- 50 to 250 ng/ml IGF-1, or 20 to 100 ng/ml IGF-1;

- 650 to 4000 ng/ml IGF-BP3, or 260 to 1600 ng/ml IGF-BP3;

- 450 to 6000 ng/ml ALS, or 180 to 2400 ng/ml ALS;

- 0.37 to 1.49 g/l Alpha 2-Macroglobulin, or 0.15 to 0.6 g/l Alpha 2-Macroglobulin;

- 471 to 3683 pg/ml PDGF-BB, or 188.4 to 1473.2 pg/ml PDGF-BB;

- 2 to 100 pg/ml KGF;

- 1 to 30 ng/ml TGF-β1, or 0.4 to 12 ng/ml TGF-β1;

- 30 to 222.5 pg/ml VEGF, or 12 to 89 pg/ml VEGF;

- 60 to 5000 ng/ml PEDF, or 24 to 2000 ng/ml PEDF; and

- 1750 to 2600 mg/dl albumin, or 700 to 1040 mg/dl albumin.

7. Product according to any one of claims 1 to 6 for use in medicine.

8. Use of the product according to any one of claims 1 to 6 in the manufacture of a medicament for the treatment of dry eye disease.

9. The use according to claim 8 in the manufacture of a medicament for the treatment of dry eye disease, wherein the human serum composition is derived from allogeneic blood.

10. Ophthalmic composition, such as eye drops or gels, comprising the product according to any one of claims 1 to 6.

11. Method for manufacturing a product, comprising the steps of

- providing human allogeneic blood volume portions from a multitude of donors,

- selecting volume portions from at least two donors,

- deriving serum from the selected volume portions to obtain serum portions,

- pooling the selected serum portions to obtain pooled serum, wherein optionally the selected serum portions are from donors all having an age of 45 years or less;

- obtaining an intermediate product from the pooled serum, the intermediate product comprising 100 ng/ml IGF-1 or more; and

- obtaining the human serum product according to any one of claims 1 to 6 from the intermediate product.

12. Method according to claim 11, wherein

(i) the intermediate product is serum, wherein obtaining the intermediate product comprises preparing serum from the human allogeneic blood; or

(ii) the intermediate product is a retentate of an ultrafiltration process, wherein obtaining the intermediate product comprises preparing serum from the human allogeneic blood, ultrafiltrating the serum to obtain a filtrate and a retentate, and selecting the retentate.

13. Method according to claim 12, wherein the ultrafiltration process of step (ii) is carried with a molecular cut-off membrane of at least 20 kDa, or at least 30 kDa, or at

least 50 kDa, or at least 100 kDa, or wherein the step of obtaining the human serum product further comprises diluting the intermediate product.

14. Method according to any one of claims 11 to 13,

wherein the intermediate product comprises less than 140 mg/dl monosaccharides, and/or 10 to 200 mg/dl total cholesterol, wherein total cholesterol is the sum of LDL (low-density lipoprotein), HDL (high-density lipoprotein) and VLDL (very low-density lipoprotein).