intradermal injections are more difficult to carry out than subcutaneous and intramuscular injection. This is especially true for the targeting of active substances to the epidermal and upper dermal layers of the skin. If a syringe with a normal injection needle is used for intradermal injection, this will give variable results in terms of application area and/or injection depths. Furthermore, relatively high volumes are typically used to ensure that the BoNT reaches the target cells by spread and diffusion of the toxin to the target skin layer. This in turn leads to increased costs due to the use of high amounts of BoNT. It also results in a higher risk of overdosing/adverse effects. To date, there is no established administration regimen for BoNT that allows for the reliable and efficacious treatment of hyperpigmentation at limited costs.

OBJECT OF THE INVENTION

[007] In view of the above, the object of the present invention is to provide an improved botulinum toxin treatment of hyperpigmentation conditions, which is efficacious, reliable and cost-effective.

SUMMARY

[008] The present disclosure is based on the finding that injection of low volumes of BoNT in the dermis in a depth-controlled manner allows to specifically address the layers of viable epidermis and upper dermal skin layers, more specifically the stratum basale and neighboring layers, in a safe, accurate and reliable manner. Since the addressed stratum basale contains target cells such as melanocytes, the BoNT injection of the present disclosure may be used to treat a variety of melanin-related afflictions, in particular hyperpigmentation conditions due to excess melanin production such as melasma. Also, the delivery of low volumes of BoNT specifically to viable epidermal and upper dermal layers allows for the use of reduced amounts of BoNT, resulting in significant cost savings.

[009] The present disclosure relates to the use of botulinum toxin for the treatment of hyperpigmentation conditions, comprising injecting botulinum toxin in a patient’s skin that is affected from the hyperpigmentation condition, wherein the

botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0009a] In an aspect, the present disclosure provides use of botulinum toxin in the manufacture of a medicament for the treatment of hyperpigmentation conditions, wherein the botulinum toxin is to be injected in a patient's skin that is affected from the hyperpigmentation condition, wherein the botulinum toxin is to be intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0010] In a preferred example, the distance between injection sites is at least 5 mm, more preferably 5 mm to 20 mm, still more preferably 5 mm to 15 mm, yet still more preferably 10 mm to 20 mm, and most preferably 10 mm to 15 mm.

[0011] In the context of the present disclosure, the hyperpigmentation condition to be treated is not particularly limited and may be a condition selected from the group consisting of melasma, freckles, solar lentigines, café-au-lait macules, post-inflammatory hyperpigmentation (PIH), moles and hyperpigmented nevi. A particularly preferred condition to be treated is melasma. The mentioned conditions are generally cosmetic in nature since they refer to aesthetic characteristics of the body that may cause a person to feel unhappy with their appearance but are non-threatening or do not cause substantial health problems that require therapeutic interventions.

[0012] The present disclosure relates to a method for the treatment of hyperpigmentation conditions, comprising injecting botulinum toxin in a patient's skin that is affected from the hyperpigmentation condition, wherein the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0012a] In another aspect, the present disclosure provides a non-therapeutic method for the treatment of hyperpigmentation conditions, comprising injecting botulinum toxin in a patient's skin that is affected from the hyperpigmentation disorder, wherein the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0013] The present disclosure relates to the use of botulinum toxin for the treatment of rosacea and a method for the treatment of rosacea, respectively. The use and method comprise injecting botulinum toxin in a patient's skin that is affected from rosacea, wherein the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0013a] In another aspect, the disclosure provides use of botulinum toxin in the manufacture of a medicament for the treatment of rosacea, wherein the botulinum toxin is to be injected in a patient's skin that is affected from rosacea, wherein the botulinum toxin is to be intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site, preferably wherein the use is defined as disclosed herein.

[0013b] In another aspect, the disclosure provides a non-therapeutic method for the treatment of rosacea, comprising injecting botulinum toxin in a patient's skin that is affected from rosacea, wherein the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site, preferably wherein the method is defined as disclosed herein.

[0014] Within the present disclosure, the botulinum toxin is preferably of type A. Further, the botulinum toxin may be in a form that is free of complexing proteins or is

in the form of a complex that contains complexing proteins. Preferably, the botulinum toxin is of type A and is in a form that is free of complexing proteins or, in an alternative preferred example, is of type A and in the form of a complex that contains complexing proteins.

[0015] Preferred embodiments are set forth in the appended dependent claims and in the following detailed description taken in connection with the examples provided therein and the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

[0016] **FIG. 1** shows the distribution of fluorescence labelled inactive BoNT in an *ex vivo* human skin model after injection using the MicronJet600 microneedle device (E = epidermis, D = dermis). All images were taken at the same magnification. Scale bar = 0.1 cm. (A): pictures showing an overlay of fluorescence microscopy (concentric structure of light to dark grey shades around injection site) and brightfield microscopy (grey). (B): graphs showing the BoNT distribution in terms of the BoNT signal (relative fluorescence intensity) as a function of skin depth.

[0017] **FIG. 2** shows the distribution of fluorescence labelled inactive BoNT in an *ex vivo* human skin model after injection using a 1.4 mm U-needle (E = epidermis, D = dermis). Scale bar = 0.1 cm. (A): picture showing an overlay of fluorescence microscopy (concentric structure of light to dark grey shades around injection site) and brightfield microscopy (grey). (B): graph showing the BoNT distribution in terms of the BoNT signal (relative fluorescence intensity) as a function of skin depth.

[0018] **FIG. 3** shows the distribution of fluorescence labelled inactive BoNT in an *ex vivo* human skin model after injection using a conventional 30G x ½″ needle (E = epidermis, D = dermis). All images were taken at the same magnification. Scale bar = 0.1 cm. (A): pictures showing an overlay of fluorescence microscopy (concentric structure of light to dark grey shades around injection site) and brightfield microscopy (grey). (B): graphs showing the BoNT distribution in terms of the BoNT signal (relative fluorescence intensity) as a function of skin depth.

TREATMENT OF HYPERPIGMENTATION CONDITIONS BY BOTULINUM TOXIN

CLAIMS

1. Use of botulinum toxin in the manufacture of a medicament for the treatment of hyperpigmentation conditions, wherein the botulinum toxin is to be injected in a patient's skin that is affected from the hyperpigmentation condition, wherein the botulinum toxin is to be intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

2. The use of claim 1, wherein the injection depth is 0.45 mm to 0.90 mm or 0.50 mm to 0.80 mm.

3. The use of claim 1 or 2, wherein the volume per injection site is 15 μl to 30 μl or 20 μl to 25 μl.

4. The use of any one of claims 1 to 3, wherein the dose per injection site is 0.5 U to 8.0 U or 1.0 U to 4.0 U.

5. The use of any one of claims 1 to 4, wherein the distance between injection sites is 5 mm to 20 mm or 5 mm to 15 mm or 10 mm to 20 mm or 10 mm to 15 mm.

6. The use of any one of claims 1 to 5, wherein the hyperpigmentation condition is selected from melasma, freckles, solar lentigines, café-au-lait macules, post-inflammatory hyperpigmentation (PIH), moles and hyperpigmented nevi.

7. The use of any one of claims 1 to 6, wherein the hyperpigmentation condition is melasma.

8. The use of any one of claims 1 to 7, wherein the botulinum toxin is (i) of type A (ii) in a form that is free of complexing proteins or in the form of a complex that contains complexing proteins, (iii) of type A and in a form that is free of

complexing proteins or in the form of a complex that contains complexing proteins, or (iv) of type A and in a form that is free of complexing proteins.

9. Use of botulinum toxin in the manufacture of a medicament for the treatment of rosacea, wherein the botulinum toxin is to be injected in a patient's skin that is affected from rosacea, wherein the botulinum toxin is to be intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site, preferably wherein the use is defined as in any one of claims 2 to 8.

10. A non-therapeutic method for the treatment of hyperpigmentation conditions, comprising injecting botulinum toxin in a patient's skin that is affected from the hyperpigmentation disorder, wherein the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

11. The method of claim 10, wherein the injection depth is as defined in claim 2 and/or the volume per injection is as defined in claim 3.

12. The method of claim 10 or 11, wherein the dose per injection site is as defined in claim 4 and/or the distance between injection sites is as defined in claim 5.

13. The method of any one of claims 10 to 12, wherein the hyperpigmentation condition is selected from melasma, freckles, solar lentigines, café-au-lait macules, post-inflammatory hyperpigmentation (PIH), moles and hyperpigmented nevi.

14. The method of any one of claims 10 to 13, wherein the botulinum toxin is (i) of type A (ii) in a form that is free of complexing proteins or in the form of a complex that contains complexing proteins, (iii) of type A and in a form that is free of complexing proteins or in the form of a complex that contains complexing proteins, or (iv) of type A and in a form that is free of complexing proteins.

15. A non-therapeutic method for the treatment of rosacea, comprising injecting botulinum toxin in a patient's skin that is affected from rosacea, wherein the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site, preferably wherein the method is defined as in claim 11 or 12.