

**TREATMENT OF HYPERPIGMENTATION CONDITIONS BY BOTULINUM TOXIN****FIELD OF THE INVENTION**

[001] The present invention relates to the use of botulinum toxin for the treatment of hyperpigmentation conditions. The use comprises the intradermal injection of botulinum toxin in the skin of a patient affected with the hyperpigmentation condition at a specified depth, volume and dose, thereby specifically targeting the epidermal and upper dermal layers. For example, the intradermal injection of botulinum toxin according to the present invention may be used in the treatment of melasma.

**BACKGROUND OF THE INVENTION**

[002] Hyperpigmentation is a common condition which occurs when patches of skin become darker in color than the surrounding skin due to the production of excess melanin. Melanin is produced by specialized cells called melanocytes. These cells are located in the basal layer (stratum basale) of the epidermis, the deepest epidermal skin layer forming an interface (dermal-epidermal junction (DEJ)) with the dermal layer below. The synthesis of melanin begins with the amino acid tyrosine, which is converted into dihydroxyphenylalanine (DOPA) by the enzyme tyrosinase. DOPA is then converted into dopaquinone, which is the precursor for both eumelanin and pheomelanin, two types of melanin that contribute to skin color. Eumelanin exhibits black color and is responsible for darker skin tones, while pheomelanin exhibits brown-red color and is responsible for lighter skin tones. The relative amounts of eumelanin and pheomelanin produced by melanocytes determine skin color. Once melanin is produced, it is transferred from the melanocytes to neighboring skin cells called epidermal keratinocytes. Melanin is then distributed evenly throughout the skin, where it absorbs and scatters UV radiation from the sun, helping to protect the skin from damage.

[003] The prevalence of hyperpigmentation varies widely depending on the underlying cause, geographic location, and other factors. For example, studies have shown that up to 90% of people may experience some form of hyperpigmentation

during their lifetime, with varying degrees of severity. This can include age spots, melasma, post-inflammatory hyperpigmentation, and other types of hyperpigmentation. For instance, melasma is estimated to affect up to 5-10% of people in the United States, with a higher prevalence among women, particularly those who are pregnant or taking oral contraceptives.

[004] While hyperpigmentation is not harmful to one's health, it can be a significant cosmetic concern for many people and affect their self-esteem and quality of life. Currently, there are different treatment options available for hyperpigmentation, including topical creams containing ingredients like hydroquinone, kojic acid and retinoids, chemical peels, laser therapy, cryotherapy, and other procedures. However, these hyperpigmentation treatments may have several side effects, such as skin irritation, hypopigmentation, allergic reactions and sun sensitivity, and the effectiveness of these treatments is limited resulting in unsatisfactory outcomes.

[005] Furthermore, botulinum toxin (botulinum neurotoxin or BoNT) has been suggested as an alternative treatment option for hyperpigmentation conditions. Botulinum toxin is a neurotoxin from *Clostridium botulinum* which blocks the release of acetylcholine into the synaptic cleft, thereby preventing cholinergic neuromuscular innervation. For example, WO 2018/222652 A1 discloses methods for treating hyperpigmentation by administering a composition comprising a botulinum neurotoxin BoNT/DC. According to the examples, BoNT/DC reduces melanin content in a human skin equivalent model and in human explant skin. The examples further describe the intraepidermal treatment of solar lentigines by injecting 50  $\mu$ l BoNT/DC toxin per injection point using a 30 G needle as well as the intraepidermal treatment of melasma by injecting 2  $\mu$ l BoNT/DC toxin per injection point using a multineedle dermal injector system.

[006] Generally, BoNT is locally administered by subcutaneous or intramuscular injection. The treatment of hyperpigmentation, however, requires more shallow injections, typically injection in the dermis (intradermal injections). Such intradermal injections have been used in the past years in an increasing number of applications. However, when using a conventional syringe for the injection of botulinum toxin,

intra dermal 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 intra dermal 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 OF THE INVENTION**

[008] The present invention 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 invention 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] In a first aspect, the present invention 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  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0010] In a preferred embodiment, 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 invention, 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] In a second aspect, the present invention 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  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0013] In a third and fourth aspect, the present invention 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  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0014] Within the present invention, 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 embodiment, 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 1/2" 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.

### **DETAILED DESCRIPTION OF THE INVENTION**

[0019] The discovery that led to the present invention is that the depth-controlled injection of relatively low volumes of BoNT in specific layers of the dermis allows for the reliable targeting of BoNT in epidermal and upper dermal layers, in particular the stratum basale, for various applications. Surprisingly, it was found that the injection bolus in the upper dermis is redirected to the upper skin layers, i.e., the upper dermal layer and the stratum basale (the deepest epidermal layer). This makes it possible to specifically address cells located in said upper skin layers, which are involved in a variety of diseases, disorders and conditions. For example, melanocytes contained in the stratum basale may be specifically addressed to treat hyperpigmentation in general and melasma in particular.

[0020] The precise injection of low volumes of BoNT at an accurate depth not only enables the specific delivery of BoNT to skin layer(s) containing target cells such as melanocytes but also allows for the use of reduced amounts of BoNT, thereby generating significant cost savings. Furthermore, the optimal dosing leads to a reduced risk of overdosing and, thus, minimized risk of adverse effects. Thus, in sum, the treatment of a disease, disorder or condition by such an injection method is more accurate, more reliable and less costly in comparison to a normal intradermal injection using a standard needle which is associated with a high variability in terms of injection depth and volume.

[0021] The term "hyperpigmentation", as used herein, is intended to refer to skin conditions that occur as a result of increased production of melanin and is characterized by patches of skin that are darker in color than the surrounding skin.

[0022] The term "epidermis", as used herein, has the meaning commonly used in the art, unless otherwise stated. It is the outermost of the three layers of the skin, the middle layer being the dermis and the innermost layer being the hypodermis. The epidermis is composed of 4 or 5 layers, wherein these layers from outermost to innermost are: stratum corneum, stratum lucidum (only on the palms and soles),

stratum granulosum, stratum spinosum, and stratum basale. The stratum basale contains keratinocytes, melanocytes and Merkel cells. The thickness of the epidermis of human skin differs by region. Within the context of the present invention, the stratum corneum generally has roughly a thickness of about 10  $\mu\text{m}$  to about 40  $\mu\text{m}$ , typically about 20  $\mu\text{m}$ , and the viable epidermal layers may have a thickness of about 30  $\mu\text{m}$  to about 150  $\mu\text{m}$ , typically about 40  $\mu\text{m}$  to about 100  $\mu\text{m}$ . The term "viable epidermis", as used herein, refers to all epidermal layers including the stratum basale, except for the stratum corneum.

[0023] The term "dermis", as used herein, generally refers to the skin layer below the epidermis and above the hypodermis (subcutaneous tissue containing fat and connective tissue). The thickness of the dermis varies by its location on the body. In the context of the present invention, the thickness is typically between 1500  $\mu\text{m}$  and 4000  $\mu\text{m}$ . The dermis can be further differentiated between papillary dermis and reticular dermis. The papillary dermis is the thin, upper layer that contains capillaries. The thickness of the papillary dermis is typically about 300  $\mu\text{m}$  to about 400  $\mu\text{m}$ . The reticular dermis is the lower layer that is much thicker than the papillary dermis and contains connective tissues and dense collagen bundles.

[0024] The term "dermal-epidermal junction", "DEJ" or "dermoepidermal junction" refers to a structure at the interface between the epidermis and the dermis. It comprises a dense collagen network that creates undulations of the dermal-epidermal junction (DEJ). The DEJ measures about 80 nm and assures several role within the skin, namely, ensuring a good cohesion between the epidermis and the dermis to maintain skin integrity, providing a physical boundary layer between fibroblasts and keratinocytes.

[0025] The terms "upper dermis" or "upper dermal layer(s)", as used herein, refer to the layers of the dermis that are closer to the epidermis than to the hypodermis. These layers usually include the papillary layer and a limited part of the reticular layer of the dermis.

[0026] The term "injection site", as used herein, means the location where the injection device is placed on the skin and botulinum toxin is injected into the dermis via one or more needles. If the injection device used for injection of botulinum toxin is a syringe with a single needle, the injection site is the site on the skin where the single needle enters the skin, and which may also be referred to herein as "injection point". If the injection device has multiple needles, i.e., two, three, four or more, the injection site is the location on the skin where the multiple needles enter the skin.

[0027] The term "distance between injection sites", as used herein, refers to the distance between an injection site and another adjacent injection site. If the injection device used for injection of botulinum toxin is a syringe with a single needle, the distance between injection sites is the distance between the site on the skin where the single needle enters the skin in a first injection and the site on the skin where the single needle enters the skin in a second adjacent injection. If the injection device has multiple needles, i.e., two, three, four or more, the distance between injection sites is the shortest distance between any one of the multiple needles of the injection device entering the skin in a first injection and any one of the multiple needles of the injection device entering the skin in a second adjacent injection.

[0028] The term "injection depth", as used herein, refers to the depth of injection as measured from the skin surface to the end of needle tip entering the skin to reach the dermis.

[0029] The terms "volume per injection site" and "dose per injection site" refer to the volume and toxin dose (in Units) of the botulinum toxin injected at one injection site. If an injection device is used that has multiple needles, i.e., two, three, four or more, the terms "volume per injection site" and "dose per injection site" refer to the volume and dose of botulinum toxin injected at one site through all needles, not only through a single needle of the multi-needle injection device.

[0030] The term "injection device", as used herein, refers to any device, such as a syringe, which is suitable for injecting botulinum toxin into the dermis. The injection device may be a conventional syringe equipped with a single hollow needle or,

preferably, a microneedle device. A "microneedle device", as used herein, refers to device having multiple needles (also referred to as "microneedles"), e.g., two, three, four, five or more needles. The needles are generally hollow and in fluid contact with a reservoir of the device filled with the botulinum toxin solution to be injected. The needles may be made of different materials such as stainless steel or silicone. A microneedle device that is suitable for use with the present invention is, for example, the MicronJet™ 600 silicon microneedle device available from NanoPass Technologies Ltd.

[0031] The term "subject", as used herein, is not particularly limited but generally refers to a human receiving therapeutic or cosmetic treatment. Herein, the subject is preferably a human receiving a cosmetic treatment. The term "subject" may be interchangeably used herein with the term "patient".

[0032] The term "effective amount", as used herein, means the amount of botulinum toxin which is generally sufficient to affect a desired change in the subject. For example, where the desired effect is a reduction in melanin, an effective amount of botulinum toxin is that amount which causes at least a substantial reduction of melanin, and without resulting in significant toxicity.

[0033] The terms "a" and "an" and "the" and similar reference used in the context of the present invention are to be construed to cover both the singular and the plural and, thus, may also relate to "at least one" or "more than one", unless otherwise indicated herein or clearly contradicted by the context.

[0034] The term "comprising", like the terms "including" and "containing", and any variations thereof such as "comprises", "includes" and "contains", are intended to refer to a non-exclusive inclusion, such that a process, method, product-by-process, composition or formulation that comprises, includes, or contains an element or list of elements does not include only those elements but can include other elements not expressly listed for such process, method, product-by-process, composition or formulation. In addition, within the framework of the present invention, it is intended that each of the terms "comprise," "comprising", "includes", "including", "contains",

"containing", and any variations thereof, can be replaced by the term "consists" or "consisting", or any variation thereof, which will be understood to refer to an exclusive inclusion of the elements indicated.

[0035] Within the meaning of the present invention, numerical values without decimal places shall be understood to include all numerical values with one or more decimal places that, applying common rules of rounding, give the numerical value without decimal places. For example, the numerical value 3 includes 2.5 or 2.50 (which are rounded up to 3) and 3.4 or 3.49 (which are rounded off to 3), and all numerical values in between. As regards endpoints of ranges, the same applies, i.e., a range of 3 to 5 may mean, e.g., 2.5-3.4 to 4.5-5.4.

[0036] In a first aspect, the present invention 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  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0037] Preferably, the injection depth is 0.45 mm to 0.90 mm, more preferably 0.50 mm to 0.80 mm, still more preferably 0.55 mm to 0.70 mm, and most preferably 0.60 mm. Due to the administration by injection no botulinum toxin is applied onto the skin and the selected injection depth results in no or only minimal backflow of botulinum toxin from the injection channel. Further, in accordance with the present invention the injection depth is reached in a controlled and reliable manner. For example, at least 80%, preferably at least 85%, more preferably at least 90%, yet more preferably at least 95% of the injections in accordance with the present invention have an injection depth of  $X \pm 50 \mu\text{m}$ , with X being 450  $\mu\text{m}$  to 950  $\mu\text{m}$ . Preferably at least 80%, more preferably at least 85%, still more preferably at least 90%, and yet more preferably at least 95% of the injections in accordance with the present invention have an injection depth of  $X \pm 50 \mu\text{m}$ , wherein X is 550  $\mu\text{m}$  to 750  $\mu\text{m}$ . Furthermore, preferably at least 80%, more preferably at least 85%, still more preferably at least 90%, and yet more

preferably at least 95% of the injections in accordance with the present invention have an injection depth of  $X \pm 50 \mu\text{m}$ , wherein  $X$  is  $600 \mu\text{m}$  to  $650 \mu\text{m}$ .

[0038] The volume per injection site is preferably  $15 \mu\text{l}$  to  $30 \mu\text{l}$  or  $10 \mu\text{l}$  to  $25 \mu\text{l}$ , more preferably  $15 \mu\text{l}$  to  $25 \mu\text{l}$ , and most preferably  $20 \mu\text{l}$  to  $25 \mu\text{l}$ . The dose per injection site is preferably  $0.50 \text{ U}$  to  $10.0 \text{ U}$ , more preferably  $1.0 \text{ U}$  to  $8.0 \text{ U}$ , still more preferably  $1.5 \text{ U}$  to  $6 \text{ U}$ , and yet more preferably  $2.0 \text{ U}$  to  $4.0 \text{ U}$ .

[0039] In accordance with the present invention, the distance between injection sites is preferably at least  $5 \text{ mm}$  and more preferably  $5 \text{ mm}$  to  $20 \text{ mm}$ , e.g.,  $5 \text{ mm}$ ,  $6 \text{ mm}$ ,  $7 \text{ mm}$ ,  $8 \text{ mm}$ ,  $9 \text{ mm}$ ,  $10 \text{ mm}$ ,  $11 \text{ mm}$ ,  $12 \text{ mm}$ ,  $13 \text{ mm}$ ,  $14 \text{ mm}$ ,  $15 \text{ mm}$ ,  $16 \text{ mm}$ ,  $17 \text{ mm}$ ,  $18 \text{ mm}$ ,  $19 \text{ mm}$ , and  $20 \text{ mm}$ . Still more preferably, the distance between injection sites is  $5 \text{ mm}$  to  $15 \text{ mm}$ , yet still more preferably  $10 \text{ mm}$  to  $20 \text{ mm}$ , and most preferably  $10 \text{ mm}$  to  $15 \text{ mm}$ . Moreover, the injection density, i.e., the number of injections per area treated, is typically  $1$  to  $16$  injections per  $4 \text{ cm}^2$ . Preferably, the number of injections per area treated is  $1.5$  to  $8$  injections per  $4 \text{ cm}^2$ , more preferably  $2$  to  $4$  injections per  $4 \text{ cm}^2$ .

[0040] Within the context of the present invention, the hyperpigmentation conditions to be treated are preferably selected from melasma, freckles, solar lentigines, café-au-lait macules, post-inflammatory hyperpigmentation (PIH), moles and hyperpigmented nevi. A preferred hyperpigmentation condition to be treated herein is melasma.

[0041] These hyperpigmentation conditions are cosmetic conditions, i.e., conditions that affect the appearance of a subject rather than the subject's health and does not require any therapeutic treatment. However, since cosmetic conditions refer to aesthetic characteristics of the body, they may cause a person to feel unhappy with their appearance and affect a person's self-esteem and quality of life. As a result, individuals may choose to seek cosmetic treatments to improve their appearance and boost their confidence. Accordingly, the use of botulinum toxin for the treatment of hyperpigmentation conditions of the present invention is for purely cosmetic purposes, i.e., botulinum toxin is used for improving or enhancing physical appearance rather than for therapeutic reasons, i.e., to treat or prevent a medical condition.

[0042] As used herein, the term "melasma" refers to an acquired hypermelanosis that occurs in sun-exposed areas. It is a type of hyperpigmentation that is characterized by dark patches on the face, particularly on the forehead, cheeks, upper lip, and occasionally on the neck, and rarely on the forearms. It is more common in women and is often associated with hormonal changes, such as those that occur during pregnancy or with the use of birth control pills.

[0043] The term "freckles" (also known as "ephelides"), as used herein, refers to small, flat, dark (typically brown) spots that are often hereditary and typically appear on the face, chest, and arms. Freckles are more common in people with fair skin. They are caused by an increase in melanin production in response to sun exposure.

[0044] The term "solar lentigines" (also known as "age spots" or "liver spots"), as used herein, refers to flat, brown spots that appear on the skin as a result of aging and exposure to the sun. They are most common in areas of the skin that are frequently exposed to the sun, such as the face, hands, and arms.

[0045] The term "café-au-lait macules", as used herein, mean a type of skin pigmentation condition characterized by flat, light to dark brown spots on the skin that are oval or irregularly shaped. These macules can be present at birth or develop later in childhood.

[0046] The term "postinflammatory hyperpigmentation" (PIH), as used herein, is intended to refer to a condition that occurs after the skin has been irritated or injured. It is characterized by the appearance of darkened patches or spots on the skin that are typically brown, black, or red-brown in color. In response to the irritation or injury of the skin, the body produces excess melanin, which leads to the darkening of the affected area. Common causes of PIH include acne, eczema, psoriasis, insect bites, burns, cuts, and surgical scars. While PIH is not a serious medical condition and does not pose any health risks, it is often of significant cosmetic concern for affected individuals.

[0047] The term "moles", as used herein, refers to pigmented areas of the skin that are usually round or oval in shape and can vary in size, color, and texture. Moles are usually brown or black and can be flat or raised. Moles can appear at any age, but most people develop them during childhood or adolescence. Specific examples of moles include congenital moles and acquired moles (usually caused by sun exposure).

[0048] The term "hyperpigmented nevi", as used herein, relates to pigmented areas of skin that can be present at birth or acquired later in life. Examples include congenital melanocytic nevus, acquired melanocytic nevus, halo nevus, spitz nevus, blue nevus, Becker's nevus, Hori's nevus.

[0049] The term "therapeutic disorder", as used herein, refers to a disorder or disease that affects the subject's health and which is treated by therapy. In the context of the present invention, the term "therapy" relates to the treatment of a disorder or disease to restore health by curing the disease or to a prophylactic treatment aimed at maintaining health by preventing ill effects that would otherwise arise or to the alleviation of the symptoms of pain and suffering. In other words, the term "therapeutic" or "therapy" relates to restoring the human body from a pathological to its original (healthy) condition, or to prevent pathology, whereas cosmetic treatment takes as its starting point a normal state in general or a healthy in the narrower sense.

[0050] The botulinum toxin used within the present invention is not particularly limited and includes botulinum toxin of any serotype (BoNT/A-H) in a form that is free of complexing proteins or in the form of a complex that contains complexing proteins. Preferably, the botulinum toxin is of serotype A or B (BoNT/A, BoNT/B), with serotype A (BoNTA) being particularly preferred. More preferably, the botulinum toxin is of serotype A, still more preferably of serotype A1 (BoNT/A1), and most preferably BoNT/A1 produced by *Clostridium botulinum* Hall strain. Preferably, the botulinum toxin is of type A and in a form that is free of complexing proteins or is in the form of a complex that contains complexing proteins and, more preferably, is botulinum toxin of type A in a form that is free of complexing proteins.

[0051] As used herein, the term "botulinum toxin" ("BT") is synonymously used with the term "botulinum neurotoxin" ("BoNT"). These terms are intended to refer to a toxin form that is free of complexing proteins, i.e., the (active) neurotoxic polypeptide that ultimately inhibits acetylcholine release (also referred to herein as the "pure botulinum neurotoxin", "neurotoxic component", "150 kDa neurotoxin" or "Clostridium botulinum neurotoxin (150 kD)") and a toxin form that is a complex containing complexing proteins (i.e., a complex of the neurotoxic component and complexing proteins). The botulinum toxin complex is a high-molecular complex of the neurotoxic component and a set of complexing proteins (NAPs), including the 900 kDa, 500 kDa, and 300 kDa *C. botulinum* type A toxin complexes. The complexing proteins are nontoxic nonhaemagglutinin (NTNHA) and, in strains of serotype A-D, different haemagglutinins (HAs). For example, the 900 kDa complex is included in onabotulinumtoxin A (Botox®/Vistabel®, Allergan, Inc., Irvine, CA, USA), and also abobotulinumtoxin A (Dysport®, Azzalure®, Ipsen, Paris, France), Alluzience® (Ipsen/Galderma) and Innotox® (Medytox) contain a toxin complex as active agent. Preferably, the botulinum toxin is the pure botulinum neurotoxin that is contained in Xeomin® or is Xeomin®, or is the toxin complex contained in Botox® or Dysport® or is Botox® or Dysport®.

[0052] Within the present invention, the botulinum toxin may be a natural neurotoxin obtainable from the bacteria *Clostridium botulinum* or any other botulinum toxin such as a botulinum toxin obtainable from alternative sources, including recombinant technologies and genetic or chemical modification. Chimeric or genetically modified botulinum toxins, i.e., botulinum toxins containing mutations including substitutions, deletions and insertions, are also encompassed by the terms "botulinum toxin", "neurotoxic component" and the like. Preferably, the mutation does not compromise any of the biological activities of botulinum toxin. However, it is also envisaged to use mutations to modulate the biological activity of the botulinum toxin. Also included are botulinum toxins containing chemically modified amino acids, for example one or more amino acids which are glycosylated, acetylated or otherwise modified, which may be beneficial to the uptake or stability of the toxin. Particularly preferred is the lipidation of the neurotoxic component.

[0053] In the context of the present invention, the dose is expressed in biological units because the used botulinum toxin may contain, for example, variable percentages of inactive toxin that contribute to the overall protein load without contributing to efficacy. Within the context of the present invention, the biological potency of botulinum toxin is determined using the mouse bioassay (MBA). The MBA determines the mean lethal dose (LD<sub>50</sub>) of toxin/neurotoxin after intraperitoneal injection in mice, i.e., the dose of toxin/neurotoxin capable of killing 50% of a group of mice. On this basis, 1 unit (U) of toxin/neurotoxin, as used herein, is defined as one mouse LD<sub>50</sub> (1.0 LD<sub>50</sub> = 1.0 U). The LD<sub>50</sub> mouse bioassay is the gold standard among various biological, chemical or immunological detection and activity determination methods for botulinum toxin and is known to those skilled in the art (see, e.g., Pearce, L.B.; Borodic, G.E.; First, E.R.; MacCallum, R.D. Measurement of botulinum toxin activity: Evaluation of the lethality assay. *Toxicol. Appl. Pharmacol.* 1994, 128, 69-77).

[0054] Another useful method for determining the biological activity (biological potency) of a botulinum neurotoxin is a cell-based potency assay as it is disclosed, for example, in WO2009/114748, WO 2013/049508 or WO 2014/207109. The activity results obtained with such cell-based assays correspond to the activity values obtained in the mouse i.p. LD<sub>50</sub> assay because the values are calibrated using the LD<sub>50</sub> reference standard.

[0055] Due to differences in the LD<sub>50</sub> tests used by manufacturers of commercial botulinum toxin formulations, the unit potencies indicated by the manufacturers for their commercial botulinum toxin formulations is proprietary and cannot easily be compared. Therefore, within the framework of the present invention, the conversion rates provided below are used to establish the comparative potencies of incobotulinumtoxinA ("INCO"; Xeomin®, Bocouture®; botulinum toxin serotype A, free of complexing proteins; Merz Pharmaceuticals GmbH), onabotulinumtoxinA ("ONA"; Botox®, Vistabel®; botulinum toxin complex of serotype A; Allergan Inc.), abobotulinumtoxinA ("ABO"; Dysport®, Azzalure®; botulinum toxin complex of serotype A; Medicis Pharmaceutical Corp., Galderma Lab.), rimabotulinumtoxinB ("RIM"; Myobloc®, NeuroBloc®; botulinum toxin serotype B; Solstice Neurosciences Inc.), and PurTox®

("TBD"; botulinum toxin serotype A; Mentor Worldwide LLC). For use herein, the conversion rate of ONA and INCO is 1:1. The conversion rate of ONA/INCO:ABO is 1:2.5. The conversion rate of ONA/INCO:RIM is 1:50, and the conversion rate of ONA/INCO:TBD is 1:1.5. Furthermore, and preferably, within the context of the present invention, 1 U of INCO (Xeomin®) and 1 U of onabotulinumtoxinA ("ONA"; Botox®) shall be deemed to correspond to one mouse LD50 (1.0 LD50), or 1 U, measured as described above.

[0056] Generally, the botulinum toxin used within the present invention is in the form of a liquid composition. The liquid composition can be formulated by various techniques dependent on the desired application, as known in the art. It may be provided as a ready-to-use liquid formulation or in the form of a lyophilized powder that is to be reconstituted, typically in physiological saline, prior to use. Preferably, the botulinum toxin used within the present invention is in the form of an aqueous solution, more preferably a saline solution or a physiological saline solution, and most preferably a phosphate buffered physiological saline solution. The aqueous solution may additionally comprise one or more pharmaceutically acceptable substances. Suitable pharmaceutically acceptable substances comprise those well known in the art, see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania.

[0057] In particular, the aqueous botulinum toxin solution or composition may include other carriers or non-toxic, non-therapeutic, non-immunogenic stabilizers and the like. Thus, the aqueous botulinum toxin composition may contain glycerol, protein stabilizers such as human serum albumin (HSA) or non-protein stabilizers such as polyvinyl pyrrolidone (PVP), hyaluronic acid or free amino acids, e.g., methionine or histidine. Suitable non-proteinaceous stabilizers are disclosed in WO 2005/007185 or WO 2006/020208. Also, it may be free of amino acids and/or free of stabilizing peptides (e.g., consisting of 5 to 50 amino acids, 10 to 40 amino acids or 15 to 30 amino acids). The botulinum toxin composition can also include non-ionic or ionic surfactant, e.g., polysorbate or poloxamer. A suitable formulation for HSA-stabilized formulation comprising a botulinum toxin according to the present invention is, for example, disclosed in US 8,398,998 B2.

[0058] Preferably, the botulinum toxin used within the present invention is in the form of an aqueous solution comprising sodium chloride (NaCl), preferably in the form of a physiological saline solution (i.e. a solution including sodium chloride in physiological concentration, e.g., about 9 g/l NaCl), which further comprises one or more of the following (i) to (ix): (i) no other excipient (except NaCl), (ii) human serum albumin (HSA) and a sugar, in particular a monosaccharide or a disaccharide, (iii) human serum albumin (HSA) and lactose, (iv) human serum albumin (HSA) and sucrose, (v) a monosaccharide and/or a disaccharide (e.g. lactose and/or sucrose), (vi) no buffer, (vii) no single amino acids, (viii) no human serum albumin (HSA), sodium chloride and lactose or no HSA, sodium chloride and sucrose, or (ix) no HSA and sodium chloride, or any combination of (i) to (ix).

[0059] Particularly preferred, the botulinum toxin is in the form of an aqueous formulation comprising botulinum toxin, sodium chloride and human serum albumin, or an aqueous formulation comprising botulinum toxin, sodium chloride, human serum albumin and lactose, or an aqueous formulation comprising botulinum toxin, sodium chloride, human serum albumin and sucrose. Another particularly preferred aqueous formulation comprises botulinum toxin, sodium chloride, human serum albumin and histidine. The botulinum toxin can be as defined herein above.

[0060] In a second aspect, the present invention the present invention relates to a method for the treatment of hyperpigmentation disorders, 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  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0061] The hyperpigmentation conditions are not particularly limited and include the hyperpigmentation conditions as defined above.

[0062] The method according to the second aspect of the present invention is closely related to the use according to the first aspect of the present invention. Thus, all

definitions, explanations and advantages given herein with respect to the use according to the first aspect equally apply to the method according to the second aspect. In particular, the injection scheme and dosing regimen used in connection with the method according to the second aspect of the present invention is as disclosed herein above in relation to the use according to the first aspect of the present invention.

[0063] Within the present invention, 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 embodiment, is of type A and in the form of a complex that contains complexing proteins.

[0064] In a third and fourth aspect, the present invention 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  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0065] The term "rosacea", as used herein, relates to a chronic skin condition that causes redness, flushing, and visible blood vessels in the face, especially the cheeks, chin, nose and mid forehead. It is not a life-threatening condition but of cosmetic concern and can cause enormous social and psychological problems. In other words, rosacea is considered to be a cosmetic disorder or state, rather than a therapeutic disorder. The mechanism of action of botulinum toxin in the treatment of rosacea is not yet fully understood. Among the hypotheses is the inhibition of the release of the neuropeptides associated with vasodilation and inflammation, such as substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and acetylcholine (ACh) from the presynaptic vesicle. Rosacea is considered to be a cosmetic condition, i.e., a condition that affects the appearance of a subject rather than the subject's health, as explained above in relation to the hyperpigmentation conditions.

[0066] The use and method according to the third and fourth aspects of the present invention are closely related to the use according to the first aspect of the present invention and the method according to the second aspect of the present invention, respectively, and differ from these only in that the condition to be treated is rosacea rather than a hyperpigmentation disorder. Thus, all definitions, explanations and advantages given herein with respect to the use according to the first and second aspects equally apply to the use according to the third aspect and the method of the fourth aspect of the present invention. In particular, any disclosure regarding the injection scheme and dosing regimen set out above in relation to the first and second aspect of the present invention is to be understood to also represent a disclosure for the third and fourth aspect of the present invention.

[0067] In the following, preferred administration regimens of the first to fourth aspects of the present invention are described.

[0068] In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, and at a dose of 0.25 U to 12.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 20

μl to 25 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 20 μl to 25 μl per injection site, and at a dose of 0.25 U to 12.0 U per injection site.

[0069] In a preferred embodiment, 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 0.50 U to 10.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 0.50 U to 10.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15 μl to 30 μl per injection site, and at a dose of 0.50 U to 10.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15 μl to 30 μl per injection site, and at a dose of 0.50 U to 10.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15 μl to 25 μl per injection site, and at a dose of 0.50 U to 10.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15 μl to 25 μl per injection site, and at a dose of 0.50 U to 10.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 20 μl to 25 μl per injection site, and at a dose of 0.50 U to 10.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 20 μl to 25 μl per injection site, and at a dose of 0.50 U to 10.0 U per injection site.

[0070] In a preferred embodiment, 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 1.0 U to 6.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 10 μl to 30 μl per injection site, and at a dose of 1.0 U to 6.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected

at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 1.0 U to 6.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, and at a dose of 1.0 U to 6.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, and at a dose of 1.0 U to 6.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, and at a dose of 1.0 U to 6.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, and at a dose of 1.0 U to 6.0 U per injection site. In a preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, and at a dose of 1.0 U to 6.0 U per injection site.

[0071] In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.25 U to 12.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.25 U to 12.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.25 U to 12.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.25 U to 12.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection

depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.25 U to 12.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.25 U to 12.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.25 U to 12.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.25 U to 12.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm.

[0072] In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.50 U to 10.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.50 U to 10.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.50 U to 10.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.50 U to 10.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection

depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.50 U to 10.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.50 U to 10.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.50 U to 10.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.50 U to 10.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm.

[0073] In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 1.0 U to 6.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 1.0 U to 6.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 1.0 U to 6.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 1.0 U to 6.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection

depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 1.0 U to 6.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 1.0 U to 6.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 1.0 U to 6.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm. In another preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 1.0 U to 6.0 U per injection site, and at an injection distance of 5 mm to 20 mm, preferably 10 mm to 20 mm, and more preferably 15 mm to 20 mm.

[0074] In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.25 U to 1.0 U per injection site, and at an injection distance of 5 mm to 10 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.25 U to 1.0 U per injection site, and at an injection distance of 5 mm to 10 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.25 U to 1.0 U per injection site, and at an injection distance of 5 mm to 10 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 0.25 U to 1.0 U per injection site, and at an injection distance of 5 mm to 10 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.25 U to 1.0 U per injection site, and at an injection distance of 5 mm to 10 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an

injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.25 U to 1.0 U per injection site, and at an injection distance of 5 mm to 10 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.25 U to 1.0 U per injection site, and at an injection distance of 5 mm to 10 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 0.25 U to 1.0 U per injection site, and at an injection distance of 5 mm to 10 mm.

[0075] In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 1.0 U to 8.0 U, preferably 2.0 U to 7.0 U, more preferably 3.0 U to 6.0 U, per injection site, and at an injection distance of 10 mm to 20 mm or 10 mm to 15 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 1.0 U to 6.0 U, preferably 2.0 U to 7.0 U, more preferably 3.0 U to 6.0 U, per injection site, and at an injection distance of 10 mm to 20 mm or 10 mm to 15 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 1.0 U to 8.0 U, preferably 2.0 U to 7.0 U, more preferably 3.0 U to 6.0 U, per injection site, and at an injection distance of 10 mm to 20 mm or 10 mm to 15 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 1.0 U to 8.0 U, preferably 2.0 U to 7.0 U, more preferably 3.0 U to 6.0 U, per injection site, and at an injection distance of 10 mm to 20 mm or 10 mm to 15 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 1.0 U to 8.0 U, preferably 2.0 U to 7.0 U, more preferably 3.0 U to 6.0 U, per injection site, and at an injection distance of 10 mm to 20 mm or 10 mm to 15 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 1.0 U to

8.0 U, preferably 2.0 U to 7.0 U, more preferably 3.0 U to 6.0 U, per injection site, and at an injection distance of 10 mm to 20 mm or 10 mm to 15 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 1.0 U to 8.0 U, preferably 2.0 U to 7.0 U, more preferably 3.0 U to 6.0 U, per injection site, and at an injection distance of 10 mm to 20 mm or 10 mm to 15 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 1.0 U to 8.0 U, preferably 2.0 U to 7.0 U, more preferably 3.0 U to 6.0 U, per injection site, and at an injection distance of 10 mm to 20 mm or 10 mm to 15 mm.

[0076] In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 8.0 U to 12.0 U per injection site, and at an injection distance of 15 mm to 20 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 10  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 8.0 U to 12.0 U per injection site, and at an injection distance of 15 mm to 20 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 8.0 U to 12.0 U per injection site, and at an injection distance of 15 mm to 20 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 30  $\mu$ l per injection site, at a dose of 8.0 U to 12.0 U per injection site, and at an injection distance of 15 mm to 20 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 8.0 U to 12.0 U per injection site, and at an injection distance of 15 mm to 20 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 15  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 8.0 U to 12.0 U per injection site, and at an injection distance of 15 mm to 20 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.40 mm to 1.00 mm, at a volume of 20  $\mu$ l to 25  $\mu$ l per injection site, at a dose of 8.0 U to 12.0 U per injection site, and at an

injection distance of 15 mm to 20 mm. In a particularly preferred embodiment, the botulinum toxin is intradermally injected at an injection depth of 0.50 mm to 0.80 mm, at a volume of 20 µl to 25 µl per injection site, at a dose of 8.0 U to 12.0 U per injection site, and at an injection distance of 15 mm to 20 mm.

## **EXAMPLES**

### **EXAMPLE 1**

#### *Distribution of fluorescence labelled BoNT in an ex vivo human skin model*

[0077] The distribution of fluorescence labelled inactive BoNT was determined using an ex vivo human skin model. The inactive BoNT (triple mutation in amino acid sequence of the light chain) was labeled with Alexa Fluor 647 and solved in PBS puffer (pH 7.4) at a concentration of 0.58 mg/mL. The skin model consisted of ex vivo abdominal human skin. The hypodermis of the skin was nearly completely removed. The trimmed skin was clamped under tension and incubated at 37°C. The model was kept hydrated by a cloth soaked in PBS puffer (pH 7.4), which was in contact to the reticular dermis. Low volumes (0.01 mL) of BoNT solution (~10.000 Units) were intradermally injected into the human skin model using (1) a MicronJet™ 600 silicon microneedle device for intradermal injections (NanoPass Technologies Ltd.; in the following "MicronJet600"), (b) a silicon microneedle for intradermal delivery called Bella-mu 1.4 mm (U-Needle B.V.; in the following "U-needle"), and (c) a standard steel 30G x ½" hypodermic needle (in the following "G30½ needle"). After incubation for 4 hours, the distribution (spread and diffusion) of the fluorescence labelled BoNT was determined by measuring the fluorescence signal of cryo-sections of the human skin model.

[0078] As shown in FIG. 1, the MicronJet600 resulted in a BoNT concentration halo of around the depth of 1.0 mm, with a maximum BoNT concentration at a shallow injection depth. In contrast, the U-needle shows the highest BoNT concentration in a depth of around 1.4 mm (see FIG. 2) and addresses therefore the mid-dermal region. As expected, the standard G30½ needle resulted in a low injection accuracy with random injection depths (see FIG. 3; two exemplary injections are shown). In other

words, the injections using a standard G30½ needle address the whole dermal layer randomly.

[0079] Furthermore, as can be seen from FIG. 1, the MicronJet600 injection bolus is surprisingly redirected to the upper skin layers, i.e., the upper dermal region including the papillary region (thin top layer of the dermis) as well as the stratum basale (the deepest epidermal layer). This allows to specifically address the target cells, e.g., the melanocytes involved in pigmentation, which are located in the dermoepidermal junction (the interface between the basal epidermis and the papillary dermis). Furthermore, as opposed to injections with the standard G30½ needle, the BoNT distribution was consistent in all injections with MicronJet600, thereby providing high injection accuracy and reliability.

[0080] It was further concluded from dye experiments that the specific Micronjet targeting effect is limited to a lower injection volume of, e.g., about 0.05 mL. More specifically, a blue dye Toluidine blue was used as a surrogate for a BoNT solution and injected at different volumes into the dermis of the previously described *ex vivo* human skin model using the MicronJet600. Immediately after injection, the vertical spread of the dye was then determined by gross cryo-sectioning of the human skin model. Using an injection volume of 0.0125 ml, the maximal vertical spread was found to be 1.5 mm. With a volume of 0.025 ml, the maximal vertical spread increased to 1.9 mm. With a higher volume of 0.050 ml, the observed maximal vertical spread was 2.0 mm, which means that the whole dermis (max. dermal vertical dimension/length was in the particular case around 2 mm) is filled with the solution from the upper dermis to the lower dermis.

## EXAMPLE 2

### *Dosing regimen and injection scheme for the cosmetic treatment of melasma*

[0081] The botulinum toxin solution is administered using the exemplary dosing regimens according to the present invention as shown in **Table 1**.

**Table 1.** Dosing regimens

| Group No. | Reconstitution            | Melasma                 |                           |
|-----------|---------------------------|-------------------------|---------------------------|
|           |                           | Dose per injection site | Volume per injection site |
| 4         | 25 U/ml (100 U : 4.0 ml)  | 0.25 U                  | 0.01 ml                   |
| 5         |                           | 0.5 U                   | 0.02 ml                   |
| 6         |                           | 0.75 U                  | 0.03 ml                   |
| 7         | 50 U/ml (200 U : 4.0 ml)  | 0.5 U                   | 0.01 ml                   |
| 8         |                           | 1 U                     | 0.02 ml                   |
| 9         |                           | 1.5 U                   | 0.03 ml                   |
| 10        | 200 U/ml (200 U : 1.0 ml) | 2 U                     | 0.01 ml                   |
| 11        |                           | 4 U                     | 0.02 ml                   |
| 12        |                           | 6 U                     | 0.03 ml                   |
| 13        | 400 U/ml (200 U : 0.5 ml) | 4 U                     | 0.01 ml                   |
| 14        |                           | 8 U                     | 0.02 ml                   |
| 15        |                           | 12 U                    | 0.03 ml                   |

[0082] Generally, the injection dose increases with the injection distance. For example, if the dose per injection point is 0.25 U to 2 U, the injection distance may be for example 5 mm. If the dose per injection point is 2 U to 8 U, the injection distance may be for example 10 mm, and if the dose per injection point is 4 U to 16 U, the injection distance may be for example 20 mm.

[0083] The injected botulinum toxin is typically a 0.9% saline solution. A microneedle device is used to deliver the botulinum toxin to the upper dermis. For example, a syringe equipped with a MicronJet600 microneedle device can be used. This device can be used for multiple injections without the need of exchanging the device after each injection.

[0084] With respect to the injection scheme, the botulinum toxin is preferably injected using an injection scheme that includes multiple injections. The number of injections depends on the size of the area to be treated (e.g., melasma area). Typically, the injections are arranged in a grid-like pattern, e.g., in a square grid. Based on

experimental results using fluorescence labelled BoNT, the lateral distribution of BoNT is about  $5.4 \pm 0.3$  mm after 4 hours. Therefore, depending on the dose per injection site, a suitable distance between injection sites is about 5 mm or higher.

**CLAIMS**

1. 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  $\mu$ l to 30  $\mu$ l 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  $\mu$ l to 30  $\mu$ l or 20  $\mu$ l to 25  $\mu$ l.
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 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  $\mu$ l to 30  $\mu$ l 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 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  $\mu$ l to 30  $\mu$ l 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 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  $\mu$ l to 30  $\mu$ l 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.