fashion is the spread of toxin away from the administration site and into surrounding tissue or systemic circulation. The diffusion of toxin away from the target tissue is believed to be responsible for undesirable side effects that in extreme cases may be life threatening. This can be a particular concern when using clostridial toxin therapeutics (such as BoNT therapeutics) at high doses, concentrations and injection volumes. Adverse effects associated with this problem that have been reported for commercial BoNT/A therapeutics include asthenia, generalised muscle weakness, diplopia, ptosis, dysphagia, dysphonia, dysarthria, urinary incontinence, and breathing difficulties. Swallowing and breathing difficulties can be life threatening and there have been reported deaths related to the spread of toxin effects.

There is therefore a need in the art for clostridial toxins which have properties of increased tissue retention at the site of administration, and which accordingly exhibit a reduction in diffusion away from the administration site, as compared to known clostridial toxins.

The present disclosure solves the above problem by providing engineered clostridial toxins, as specified in the claims.

In one aspect, the present disclosure provides an engineered clostridial toxin comprising at least one amino acid modification, wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.2 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification; wherein said at least one amino acid modification is located in the clostridial toxin receptor binding domain (H_C domain); wherein said engineered clostridial toxin demonstrates increased tissue retention, potency and/or duration of action when compared to an otherwise identical clostridial toxin lacking said at least one amino acid modification; and wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s).

In another aspect, the present disclosure provides use of an engineered clostridial toxin receptor binding domain (H_C domain) comprising at least one amino acid modification for increasing tissue retention and reducing diffusion away from a site of administration of an engineered clostridial toxin comprising said engineered clostridial toxin receptor binding domain (HC domain), wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.2 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification, and wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s).

In another aspect, the present disclosure provides a nucleic acid comprising a nucleic acid sequence encoding an engineered clostridial toxin as disclosed above.

In another aspect, the present disclosure provides a method of producing a single-chain engineered clostridial toxin protein having a light chain and a heavy chain, the method comprising expressing a nucleic acid as disclosed above in a suitable host cell, lysing the host cell to provide a host cell homogenate containing the single-chain engineered clostridial toxin protein, and isolating the single-chain engineered clostridial toxin protein.

In another aspect, the present disclosure provides a method of activating an engineered clostridial toxin, the method comprising providing a single-chain engineered clostridial toxin protein obtainable by the method as disclosed above, contacting the polypeptide with a protease that cleaves the polypeptide at a recognition site (cleavage site) located between the light chain and heavy chain, and converting the polypeptide into a di-chain polypeptide wherein the light chain and heavy chain are joined together by a disulphide bond.

In another aspect, the present disclosure provides an engineered clostridial toxin as disclosed above, for use in medicine.

In another aspect, the present disclosure provides use of an engineered clostridial toxin as disclosed above in the manufacture of a medicament for the prevention or treatment of a disease or condition selected from: strabismus, blepharospasm, squint, dystonia (e.g. spasmodic dystonia, oromandibular dystonia, focal dystonia, tardive dystonia, laryngeal dystonia, limb dystonia, cervical dystonia), torticollis (e.g. spasmodic torticollis), neuromuscular disorder or condition of ocular motility (e.g. concomitant strabismus, vertical strabismus, lateral rectus palsy, nystagmus, dysthyroid myopathy), writer's cramp, blepharospasm, bruxism, Wilson's disease, tremor, tics, segmental myoclonus, spasms, spasticity due to chronic multiple sclerosis, spasticity resulting in abnormal bladder control, animus, back spasm, charley horse, tension headaches, levator pelvic syndrome, spina bifida, tardive dyskinesia, Parkinson's disease, stuttering, hemifacial spasm, eyelid disorder, cerebral palsy, focal spasticity, spasmodic colitis, neurogenic bladder, anismus, limb spasticity, tics, tremors, bruxism, anal fissure, achalasia, dysphagia, lacrimation, hyperhydrosis, excessive salivation, excessive gastrointestinal secretions, muscle pain (e.g. pain from muscle spasms), headache pain (e.g. tension headache), cancer, uterine disorders, uro-genital disorders, urogenital-neurological disorders, chronic neurogenic inflammation, and a smooth muscle disorder.

In another aspect, there is provided a method for cosmetic treatment, wherein said cosmetic treatment benefits from cell/muscle incapacitation, said method comprising administration of an engineered clostridial toxin as disclosed above.The disclosure provides an engineered clostridial toxin comprising at least one amino acid modification, wherein said at least one amino acid modification increases the isoelectric point (pi) of the engineered clostridial toxin to a value that is at least 0.2 (for example, at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1) pi units higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification. In one embodiment, said at least one amino acid modification increases the pi of the engineered clostridial toxin to a value that is at least 0.4 pi units higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification. In one embodiment, said at least one amino acid modification increases the pi of the engineered clostridial toxin to a value that is at least 0.5 pi units higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification. In one embodiment, said at least one amino acid modification increases the pi of the engineered clostridial toxin to a value that is at least 0.6 pi units higher than the pi of an otherwise identical clostridial toxin lacking said at least one amino acid modification. In one embodiment, said at

CLAIMS

1. An engineered clostridial toxin comprising at least one amino acid modification, wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.2 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification;

wherein said at least one amino acid modification is located in the clostridial toxin receptor binding domain (H_C domain);

wherein said engineered clostridial toxin demonstrates increased tissue retention, potency and/or duration of action when compared to an otherwise identical clostridial toxin lacking said at least one amino acid modification; and

wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s).

2. Use of an engineered clostridial toxin receptor binding domain (H_C domain) comprising at least one amino acid modification for increasing tissue retention and reducing diffusion away from a site of administration of an engineered clostridial toxin comprising said engineered clostridial toxin receptor binding domain (H_C domain), wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.2 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification, and wherein said engineered clostridial toxin retains the abilities of target cell binding, translocation, and cleavage of target SNARE protein(s).

3. The engineered clostridial toxin of claim 1 or the use according to claim 2, wherein the engineered clostridial toxin has a Safety Ratio of at least 8, wherein the Safety Ratio is calculated as: dose of toxin required for -10% bodyweight change measured as pg/mouse divided by DAS ED_{50} measured as pg/mouse, wherein ED_{50} = dose required to produce a DAS score of 2.

4. The engineered clostridial toxin or the use according to any one of the preceding claims, wherein said at least one amino acid modification increases the

isoelectric point (pI) of the engineered clostridial toxin to a value that is at least 0.5 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

5. The engineered clostridial toxin or the use according to any one of the preceding claims, wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least one pI unit higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

6. The engineered clostridial toxin or the use according to any one of the preceding claims, wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is at least two pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

7. The engineered clostridial toxin or the use according to any one of the preceding claims, wherein said at least one amino acid modification increases the isoelectric point (pI) of the engineered clostridial toxin to a value that is between 2 and 5 pI units higher than the pI of an otherwise identical clostridial toxin lacking said at least one amino acid modification.

8. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the engineered clostridial toxin has a pI of at least 6.

9. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the engineered clostridial toxin has a pI of between 6 and 10.

10. The engineered clostridial toxin or the use according to claim 9, wherein the engineered clostridial toxin has a pI of between 7 and 9, preferably between 8 and 9.

11. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the at least one amino acid modification is selected from: an amino acid substitution, an amino acid insertion, and an amino acid deletion.

12. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the at least one amino acid modification is selected from:
i. substitution of an acidic amino acid residue with a basic amino acid residue;
ii. substitution of an acidic amino acid residue with an uncharged amino acid residue;
iii. substitution of an uncharged amino acid residue with a basic amino acid residue;
iv. insertion of a basic amino acid residue; and
v. deletion of an acidic amino acid residue.

13. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the engineered clostridial toxin comprises between 1 and 80 amino acid modifications.

14. The engineered clostridial toxin or the use according to claim 13, wherein the engineered clostridial toxin comprises between 4 and 40 amino acid modifications.

15. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the at least one amino acid modification is located in the clostridial toxin H_{CN} domain.

16. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the at least one amino acid modification is a modification of a surface exposed amino acid residue.

17. The engineered clostridial toxin or the use according to any of the preceding claims, wherein the at least one amino acid modification comprises modification of an amino acid residue selected from: an aspartic acid residue, a glutamic acid residue, a histidine residue, a serine residue, a threonine residue, an asparagine residue, a glutamine residue, a cysteine residue, or a tyrosine residue.

18. The engineered clostridial toxin or the use according to claim 17, wherein the amino acid residue is substituted with a lysine residue or an arginine residue.

19. A nucleic acid comprising a nucleic acid sequence encoding an engineered clostridial toxin according to any one of claims 1 and 3 to 18.

20. A method of producing a single-chain engineered clostridial toxin protein having a light chain and a heavy chain, the method comprising expressing a nucleic acid according to claim 19 in a suitable host cell, lysing the host cell to provide a host cell homogenate containing the single-chain engineered clostridial toxin protein, and isolating the single-chain engineered clostridial toxin protein.

21. A method of activating an engineered clostridial toxin, the method comprising providing a single-chain engineered clostridial toxin protein obtainable by the method of claim 20, contacting the polypeptide with a protease that cleaves the polypeptide at a recognition site (cleavage site) located between the light chain and heavy chain, and converting the polypeptide into a di-chain polypeptide wherein the light chain and heavy chain are joined together by a disulphide bond.

22. An engineered clostridial toxin according to any one of claims 1 and 3-18, for use in medicine.

23. Use of an engineered clostridial toxin according to any one of claims 1 and 3-18 in the manufacture of a medicament for the prevention or treatment of a disease or condition selected from: strabismus, blepharospasm, squint, dystonia (e.g. spasmodic dystonia, oromandibular dystonia, focal dystonia, tardive dystonia, laryngeal dystonia, limb dystonia, cervical dystonia), torticollis (e.g. spasmodic torticollis), neuromuscular disorder or condition of ocular motility (e.g. concomitant strabismus, vertical strabismus, lateral rectus palsy, nystagmus, dysthyroid myopathy), writer's cramp, blepharospasm, bruxism, Wilson's disease, tremor, tics, segmental myoclonus, spasms, spasticity due to chronic multiple sclerosis, spasticity resulting in abnormal bladder control, animus, back spasm, charley horse, tension headaches, levator pelvic syndrome, spina bifida, tardive dyskinesia, Parkinson's disease, stuttering, hemifacial spasm, eyelid disorder, cerebral palsy, focal spasticity, spasmodic colitis, neurogenic bladder, anismus, limb spasticity, tics, tremors, bruxism, anal fissure, achalasia, dysphagia, lacrimation, hyperhydrosis, excessive salivation, excessive gastrointestinal secretions, muscle pain (e.g. pain from muscle spasms), headache pain (e.g. tension headache), cancer, uterine disorders, uro-genital disorders, urogenital-neurological disorders, chronic neurogenic inflammation, and a smooth muscle disorder.

24. A method for cosmetic treatment, wherein said cosmetic treatment benefits from cell/muscle incapacitation, said method comprising administration of an engineered clostridial toxin according to any one of claims 1 and 3-18.

25. A method according to claim 24, wherein the cosmetic treatment is the treatment of brow furrows or skin wrinkles.