

[CLAIMS]

[Claim 1]

Long-acting insulin, wherein a biocompatible material capable of prolonging a half-life is linked to an insulin analog,

wherein the insulin analog includes the A chain of SEQ ID NO: 37 represented by Formula 1 below and the B chain of SEQ ID NO: 38 represented by Formula 2 below (with the proviso that the peptide where the A-chain coincides with SEQ ID NO: 39 while the B-chain also coincides with SEQ ID NO: 40 is excluded):

[Formula 1]

Xaa1-Xaa2-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-Cys-Ser-Leu-Xaa3-Gln-Leu-Glu-Asn-Xaa4-Cys-Asn (SEQ ID NO: 37)

wherein:

Xaa1 is glycine or alanine,

Xaa2 is isoleucine or alanine,

Xaa3 is tyrosine, and

Xaa4 is tyrosine or alanine; and

[Formula 2]

Phe-Val-Asn-Gln-His-Leu-Cys-Xaa5-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Xaa6-Xaa7-Xaa8-Tyr-Thr-Pro-Lys-Thr (SEQ ID NO: 38)

wherein:

Xaa5 is glycine or alanine,

Xaa6 is glycine or alanine,

Xaa7 is phenylalanine or alanine, and

Xaa8 is phenylalanine or alanine.

[Claim 2]

The long-acting insulin of claim 1, wherein the biocompatible material is selected from the group consisting of polyethylene glycol, fatty acid, cholesterol, albumin and a fragment thereof, an albumin-binding material, a polymer of repeating units of a particular amino acid sequence, an antibody, an antibody fragment, an FcRn-binding material, *in-vivo* connective tissue or a derivative thereof, a nucleotide, fibronectin, transferrin, saccharide, and a polymer.

[Claim 3]

The long-acting insulin of claim 1, wherein the insulin analog and the biocompatible material are linked to each other by a peptide bond.

[Claim 4]

The long-acting insulin of claim 1, wherein the insulin analog and the biocompatible material are linked by a linker selected from the group consisting of polyethylene glycol, fatty acid, saccharide, a polymer, a low molecular weight compound, a nucleotide, and a combination thereof.

[Claim 5]

The long-acting insulin of claim 1, wherein the insulin analog and the biocompatible material are linked by a linker interposed therebetween, and the biocompatible material is an FcRn-binding material, wherein the linker is a peptide linker, or a non-peptide linker selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxytheyleated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ether, a biodegradable polymer, a lipid-polymer, chitins, hyaluronic acid, oligonucleotide, and a combination thereof.

[Claim 6]

The long-acting insulin of claim 5, wherein the FcRn-binding material is a polypeptide comprising an immunoglobulin Fc region.

[Claim 7]

The long-acting insulin of claim 5, wherein each end of the non-peptide linker is respectively linked to an amine group or thiol group of the biocompatible material and the insulin analog.

[Claim 8]

The long-acting insulin of claim 1, wherein the insulin analog comprises:

- (i) the A-chain, wherein, in Formula 1, Xaa1 is alanine, Xaa2 is isoleucine, Xaa3 is tyrosine, and Xaa4 is tyrosine; and the B-chain, wherein, in Formula 2, Xaa5 is glycine, Xaa6 is glycine, Xaa7 is phenylalanine, and Xaa8 is phenylalanine;
- (ii) the A-chain, wherein, in Formula 1, Xaa1 is glycine, Xaa2 is alanine, Xaa3 is tyrosine, and Xaa4 is tyrosine; and the B-chain, wherein, in Formula 2, Xaa5 is glycine, Xaa6 is glycine, Xaa7 is phenylalanine, and Xaa8 is phenylalanine;
- (iii) the A-chain, wherein, in Formula 1, Xaa1 is glycine, Xaa2 is isoleucine, Xaa3 is

- tyrosine, and Xaa4 is alanine; and the B-chain, wherein, in Formula 2, Xaa5 is glycine, Xaa6 is glycine, Xaa7 is phenylalanine, and Xaa8 is phenylalanine;
- (iv) the A-chain, wherein, in Formula 1, Xaa1 is glycine, Xaa2 is isoleucine, Xaa3 is tyrosine, and Xaa4 is tyrosine; and the B-chain, wherein, in Formula 2, Xaa5 is alanine, Xaa6 is glycine, Xaa7 is phenylalanine, and Xaa8 is phenylalanine;
 - (v) the A-chain, wherein, in Formula 1, Xaa1 is glycine, Xaa2 is isoleucine, Xaa3 is tyrosine, and Xaa4 is tyrosine; and the B-chain, wherein, in Formula 2, Xaa5 is glycine, Xaa6 is alanine, Xaa7 is phenylalanine, and Xaa8 is phenylalanine;
 - (vi) the A-chain, wherein, in Formula 1, Xaa1 is glycine, Xaa2 is isoleucine, Xaa3 is tyrosine, and Xaa4 is tyrosine; and the B-chain, wherein, in Formula 2, Xaa5 is glycine, Xaa6 is glycine, Xaa7 is alanine, and Xaa8 is phenylalanine; or
 - (vii) the A-chain, wherein, in Formula 1, Xaa1 is glycine, Xaa2 is isoleucine, Xaa3 is tyrosine, and Xaa4 is tyrosine; and the B-chain, wherein, in Formula 2, Xaa5 is glycine, Xaa6 is glycine, Xaa7 is phenylalanine, and Xaa8 is alanine.

[Claim 9]

The long-acting insulin of claim 1, wherein the insulin analog comprises:

(a) in Formula 1, Xaa1 is glycine, Xaa2 is isoleucine, Xaa3 is tyrosine, and Xaa4 is tyrosine; and the B-chain, wherein, in Formula 2, Xaa5 is glycine, Xaa6 is glycine, Xaa7 is alanine, and Xaa8 is phenylalanine; or

(b) the A-chain, wherein, in Formula 1, Xaa1 is glycine, Xaa2 is isoleucine, Xaa3 is tyrosine, and Xaa4 is tyrosine; and the B-chain, wherein, in Formula 2, Xaa5 is glycine, Xaa6 is glycine, Xaa7 is phenylalanine, and Xaa8 is alanine.

[Claim 10]

A method for preparing the long-acting insulin of claim 1, comprising:

linking an insulin analog to a biocompatible material,

wherein the insulin analog includes the A chain of SEQ ID NO: 37 represented by Formula 1 below and the B chain of SEQ ID NO: 38 represented by Formula 2 below (with the proviso that the peptide where the A-chain coincides with SEQ ID NO: 39 while the B-chain also coincides with SEQ ID NO: 40 is excluded):

[Formula 1]

Xaa1-Xaa2-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-Cys-Ser-Leu-Xaa3-Gln-Leu-Glu-Asn-Xaa4-Cys-Asn (SEQ ID NO: 37)

wherein:

Xaa1 is glycine or alanine,

Xaa2 is isoleucine or alanine,
Xaa3 is tyrosine, and
Xaa4 is tyrosine or alanine; and

[Formula 2]

Phe-Val-Asn-Gln-His-Leu-Cys-Xaa5-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-
Cys-Gly-Glu-Arg-Xaa6-Xaa7-Xaa8-Tyr-Thr-Pro-Lys-Thr (SEQ ID NO: 38)

wherein:

Xaa5 is glycine or alanine,
Xaa6 is glycine or alanine,
Xaa7 is phenylalanine or alanine, and
Xaa8 is phenylalanine or alanine.

[Claim 11]

The long-acting insulin of claim 1, wherein the long-acting insulin is represented by the following Chemical Formula 1:

[Chemical Formula 1]

X-La-F;

wherein X is the insulin analog,

L is a linker,

a is 0 or a natural number, with the proviso that each L is independent from each other when a is 2 or higher, and

F is a biocompatible material capable of prolonging a half-life ;

wherein the insulin analog includes the A chain of SEQ ID NO: 37 represented by Formula 1 below and the B chain of SEQ ID NO: 38 represented by Formula 2 below (with the proviso that the peptide where the A-chain coincides with SEQ ID NO: 39 while the B-chain also coincides with SEQ ID NO: 40 is excluded):

[Formula 1]

Xaa1-Xaa2-Val-Glu-Gln-Cys-Cys-Thr-Ser-Ile-Cys-Ser-Leu-Xaa3-Gln-Leu-Glu-Asn-
Xaa4-Cys-Asn (SEQ ID NO: 37)

wherein:

Xaa1 is glycine or alanine,
Xaa2 is isoleucine or alanine,
Xaa3 is tyrosine, and
Xaa4 is tyrosine or alanine; and

[Formula 2]

Phe-Val-Asn-Gln-His-Leu-Cys-Xaa5-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly-Glu-Arg-Xaa6-Xaa7-Xaa8-Tyr-Thr-Pro-Lys-Thr (SEQ ID NO: 38)

wherein:

Xaa5 is glycine or alanine,

Xaa6 is glycine or alanine,

Xaa7 is phenylalanine or alanine, and

Xaa8 is phenylalanine or alanine.

[Claim 12]

The long-acting insulin of claim 11, wherein X and F are linked by L through a covalent chemical bond, a non-covalent chemical bond, or a combination thereof.

[Claim 13]

The long-acting insulin of claim 11, wherein F is an IgG Fc region.

[Claim 14]

A long-acting insulin formulation with improved *in-vivo* duration and stability comprising the long-acting insulin of claims 1 to 13.

[Claim 15]

A long-acting insulin formulation for the treatment of diabetes comprising the long-acting insulin of claims 1 to 13.