formed skin layer. However, under the Acticoat™ series, only Acticoat™ Absorbent is minimally adhesive as its main function is to absorb exudate which allows gel formation and prevents attachment to newly formed skin layer. However, not all wounds release sufficient amount of exudate.

Other criteria that wound therapy agents should preferably possess is the ability to attract growth factors to the site of injury to spur cell regeneration, being flexible to facilitate application of therapeutics over awkward positions, having water proof feature and can be synthesised at low cost.

There is therefore a need to provide biomaterials that overcome, or at least ameliorate, one or more of the disadvantages described above.

Summary

There is provided a nanoparticle-containing hydrogel comprising:

metal nanoparticles, and

at least one peptide having the general formula:

$$Z\text{-}(X)_a\text{-}(Y)_b\text{-}Z'_c$$

wherein

Z is an N-terminal protecting group;

X is, at each occurrence, independently selected from the group consisting of aliphatic amino acids and aliphatic amino acid derivatives;

Y is, at each occurrence, independently selected from the group consisting of polar amino acids and polar amino acid derivatives;

Z' is a C-terminal protecting group;

a is an integer selected from 1 to 6, preferably 1 to 5;

b is 0, 1 or 2, preferably 1; and

c is 0 or 1.

In an aspect, there is provided a nanoparticle-containing hydrogel comprising

metal nanoparticles and

at least one peptide having the general formula:

$$Z\text{-}(X)_a\text{-}(Y)_b\text{-}Z'_c$$

wherein

Z is an N-terminal protecting group;

X is, at each occurrence, independently selected from the group consisting of aliphatic amino acids and aliphatic amino acid derivatives;

Y is, at each occurrence, independently selected from the group consisting of polar amino acids and polar amino acid derivatives, wherein said polar amino acids and polar amino acid derivatives have a polar group which is independently selected from a hydroxyl, an ether, an imido, an amido, an ester, an amino, a guanidino, a seleno, and a telluro group;

Z' is a C-terminal protecting group;

a is an integer selected from 1 to 6, preferably 1 to 5;

b is 0, 1 or 2, preferably 1; and

c is 0 or 1.

Advantageously, the peptide(s) may be able to self-assemble in water or physiologically relevant buffers to form hydrogels.

Further advantageously, the hydrogels may display superior mechanical strength. The metal nanoparticles may stabilize the fibrous structure of the hydrogel, therefore requiring a reduced concentration of peptide(s), which are considerably more expensive. Therefore advantageously, the disclosed hydrogels may be cost-effective.

According to another aspect, there is provided an *in situ* method of producing the disclosed nanoparticle-containing hydrogel, said method comprising the steps of:

(a) dissolving at least one disclosed peptide in an aqueous solution;

(b) adding a precursor of metal nanoparticles, preferably a metal salt solution;

(c) gel formation to obtain a peptide hydrogel;

(d) irradiating the peptide hydrogel resulting from step (c); and

(e) obtaining a nanoparticle-containing hydrogel.

According to another aspect, there is provided a nanoparticle-containing hydrogel prepared by the disclosed *in situ* method.

There is provided the use of the disclosed nanoparticle-containing hydrogel:

- as a device for metal delivery, preferably for sustained or controlled release metal delivery;

- as an antimicrobial agent;

- in a method of treatment of an infectious disease,

  wherein, preferably, said infectious disease is caused by Gram-positive bacteria, Gram-negative bacteria, yeast or fungi;

- as a wound dressing and for wound healing;

- in a surgical application;

- to prevent bacterial attachment and/or formation of microbial biofilm;

- for topical medical application;

- for bioimaging (by utilitzing the fluorescence of the metal nanoparticles);

- in combination with medical devices, such as stents, prostheses, catheters, dental implants, orthopedical devices, orthopedical implants;

- for application where the hydrogel is used as a (dried) membrane, such as filtration.

In another aspect, there is provided the use of the disclosed nanoparticle-containing hydrogel:

- as a device for metal delivery, preferably for sustained or controlled release metal delivery;

- as an antimicrobial agent;

- in the manufacture of a medicament for the treatment of an infectious disease,

wherein, preferably, said infectious disease is caused by Gram-positive bacteria, Gram-negative bacteria, yeast or fungi;

- as a wound dressing and for wound healing;
- in a surgical application;
- to prevent bacterial attachment and/or formation of microbial biofilm;
- for topical medical application;
- for bioimaging (by utilitzing the fluorescence of the metal nanoparticles);
- in combination with medical devices, such as stents, prostheses, catheters, dental implants, orthopedical devices, orthopedical implants;
- for application where the hydrogel is used as a (dried) membrane, such as filtration.

Advantageously, the disclosed hydrogels may not require additional chemical modification to display antibacterial and/or antimicrobial properties.

According to another aspect, there is provided a device for metal delivery, preferably sustained or controlled release metal delivery, comprising a disclosed nanoparticle-containing hydrogel.

treatment of an infectious disease wherein, preferably, said infectious disease is caused by Gram-positive bacteria, Gram-negative bacteria, yeast or fungi.

According to the present disclosure this object is solved by a method of treatment of a wound and for wound healing, said method comprising the step of applying an effective amount of a disclosed nanoparticle-containing hydrogel or a disclosed pharmaceutical composition to a wound.

Claims

1. A nanoparticle-containing hydrogel comprising

metal nanoparticles and

at least one peptide having the general formula:

$$Z\text{-}(X)_a\text{-}(Y)_b\text{-}Z'_c$$

wherein

Z is an N-terminal protecting group;

X is, at each occurrence, independently selected from the group consisting of aliphatic amino acids and aliphatic amino acid derivatives;

Y is, at each occurrence, independently selected from the group consisting of polar amino acids and polar amino acid derivatives, wherein said polar amino acids and polar amino acid derivatives have a polar group which is independently selected from a hydroxyl, an ether, an imido, an amido, an ester, an amino, a guanidino, a seleno, and a telluro group;

Z' is a C-terminal protecting group;

a is an integer selected from 1 to 6, preferably 1 to 5;

b is 0, 1 or 2, preferably 1; and

c is 0 or 1.

2. The nanoparticle-containing hydrogel according to claim 1, wherein the metal nanoparticles are selected from the group of metal nanoparticles, metal oxide nanoparticles, and combinations thereof, preferable selected from Ag nanoparticles, Au nanoparticles, Cu nanoparticles, CuO nanoparticles, Fe_2O_3 nanoparticles, Pt nanoparticles, Pd nanoparticles and combinations thereof.

3. The nanoparticle-containing hydrogel according to claim 1, wherein the metal nanoparticles are selected from the group of heterometallic nanoparticles, heterometallic oxide nanoparticles and combinations thereof.

4. The nanoparticle-containing hydrogel according to any one of claims 1 to 3, wherein the metal nanoparticles have a uniform size distribution in the hydrogel, preferably have a particle size of less than about 1 μm, more preferably about 10 to about 20 nm.

5. The nanoparticle-containing hydrogel according to any one of claims 1 to 4, wherein said aliphatic amino acids and aliphatic amino acid derivatives, and said polar amino acids and polar amino acid derivatives are either D-amino acids or L-amino acids.

6. The nanoparticle-containing hydrogel according to any one of claims 1 to 5, wherein said aliphatic amino acids are selected from the group consisting of alanine (Ala, A), homoallylglycine, homopropargylglycine, isoleucine (Ile, I), norleucine, leucine (Leu, L), valine (Val, V) and glycine (Gly, G), preferably from the group consisting of alanine (Ala, A), isoleucine (Ile, I), leucine (Leu, L), valine (Val, V) and glycine (Gly, G).

7. The nanoparticle-containing hydrogel according to claim 6, wherein all or a portion of said aliphatic amino acids are arranged in an order of decreasing amino acid size in the direction from N- to C-terminus, wherein the size of the aliphatic amino acids is defined as I = L > V > A > G.

8. The nanoparticle-containing hydrogel according to claim 7, wherein said aliphatic amino acids arranged in an order of decreasing amino acid size have a sequence selected from LIVAG (SEQ ID NO: 1), ILVAG (SEQ ID NO: 2), LIVAA (SEQ ID NO: 3), LAVAG (SEQ ID NO: 4), IVAG (SEQ ID NO: 5), LIVA (SEQ ID NO: 6), LIVG (SEQ ID NO: 7), IVA (SEQ ID NO: 23) and IV (SEQ ID NO: 24), wherein, optionally, there is an A preceding such sequence at the N-terminus.

9. The nanoparticle-containing hydrogel according to any one of claims 1 to 8, wherein said polar amino acids are selected from the group consisting of asparagine (Asn, N), glutamine (Gln, Q), 5-N-ethyl-glutamine (theanine), citrulline, serine (Ser, S), homoserine, ornithine (Orn), threonine (Thr, T), L-Dopa, tryptophan (Trp, W), thyroxine, allo-threonine, lysine

(Lys, K), 2,4-diaminobutyric acid (Dab), 2,3-diaminopropionic acid (Dap), N(6)-carboxymethyllysine, and tyrosine (Tyr, Y),
wherein said polar amino acid is preferably selected from the group consisting of asparagine, glutamine, serine, threonine, methionine, lysine, ornithine (Orn), 2,4-diaminobutyric acid (Dab), and 2,3-diaminopropionic acid (Dap).

10. The nanoparticle-containing hydrogel according to any one of claims 1 to 9, wherein $(X)_a$-$(Y)_b$ has a sequence selected from the group consisting of LIVAGK (SEQ ID NO: 14), ILVAGK (SEQ ID NO. 15), LIVAGT (SEQ ID NO: 16), AIVAGT (SEQ ID NO: 17), IIIK (SEQ ID NO: 22), and IY (SEQ ID NO: 39).

11. The nanoparticle-containing hydrogel according to any one of claims 1 to 10, wherein the C-terminal amino acid of the peptide is selected from the group consisting of a lysine (K), an ornithine (Orn), a 2,4-diaminobutyric acid (Dab), and a 2,3-diaminopropionic acid (Dap).

12. The nanoparticle-containing hydrogel according to any one of claims 1 to 11, wherein said N-terminal protecting group ***Z*** has the general formula –C(O)–R, wherein R is selected from the group consisting of H, unsubstituted or substituted alkyls, and unsubstituted or substituted aryls.

13. The nanoparticle-containing hydrogel according to claim 12, wherein R is selected from the group consisting of methyl, ethyl, propyl, isopropyl, butyl and isobutyl.

14. The nanoparticle-containing hydrogel according to any one of claims 1 to 13, wherein said N-terminal protecting group ***Z*** is an acetyl group.

15. The nanoparticle-containing hydrogel according to any one of claims 1 to 14, wherein said N-terminal protecting group ***Z*** is a peptidomimetic molecule, including natural and synthetic amino acid derivatives, wherein the N-terminus of said peptidomimetic molecule may be modified with a functional group selected from the group consisting of amide, alcohol, aldehyde, amine, imine, nitrile, an urea analog, phosphate, carbonate, sulfate, nitrate, maleimide, vinyl sulfone, azide, alkyne, alkene, carbohydrate, imide, peroxide, ester, aryl, ketone, sulphite, nitrite, phosphonate, and silane.

16. The nanoparticle-containing hydrogel according to any one of claims 1 to 15, wherein said C-terminal protecting group ***Z'*** is an amide group,
wherein, preferably, the C-terminus has the formula -CONHR or -CONRR', with R and R' being selected from the group consisting of H, unsubstituted or substituted alkyls, and unsubstituted or substituted aryls

17. The nanoparticle-containing hydrogel according to any one of claims 1 to 16, wherein said C-terminal protecting group ***Z'*** is an ester group,
wherein, preferably, the C-terminus has the formula $-CO_2R$, with R being selected from the group consisting of unsubstituted or substituted alkyls, and unsubstituted or substituted aryls.

18. The nanoparticle-containing hydrogel according to any one of claims 1 to 17, wherein said C-terminal protecting group is a peptidomimetic molecule, including natural and synthetic amino acid derivatives, wherein the C-terminus of said peptidomimetic molecule is optionally modified with a functional group selected from the group consisting of amide, alcohol, aldehyde, amine, imine, nitrile, an urea analog, phosphate, carbonate, sulfate, nitrate, maleimide, vinyl sulfone, azide, alkyne, alkene, carbohydrate, imide, peroxide, ester, aryl, ketone, sulphite, nitrite, phosphonate, and silane.

19. The nanoparticle-containing hydrogel according to any one of claims 1 to 18, wherein **a** is an integer selected from 1 to 5, preferably 2 to 5, and wherein, preferably, said aliphatic amino acids and aliphatic amino acid derivatives exhibit an overall decrease in hydrophobicity from the N-terminus to the C-terminus of said peptide.

20. The nanoparticle-containing hydrogel according to any one of claims 1 to 19, wherein said peptide is present at a concentration in the range of from 0.1 % to 30 % (w/w), preferably 0.1 % to 20 % (w/w), more preferably 0.1 % to 10 % (w/w), more preferably 0.1 % to 5 % (w/w), even more preferably 0.1 % to 3 % (w/w), with respect to the total weight of said nanoparticle-containing hydrogel.

21. The nanoparticle-containing hydrogel according to any one of claims 1 to 20, further comprising at least one additional substance.

22. The nanoparticle-containing hydrogel according to claim 21, wherein said at least one additional substance is encapsulated by said hydrogel, immobilized in the bulk phase of said hydrogel or conjugated to said peptide.

23. The nanoparticle-containing hydrogel according to claim 21 or 22, wherein said at least one additional substance is selected from the group consisting of dyes, pigments, quantum dot nanoparticles and semiconductor nanoparticles.

24. An *in situ* method of producing a nanoparticle-containing hydrogel according to any one of claims 1 to 23, said method comprising the steps of

(a) dissolving at least one peptide as defined in any one of claims 1 and 5 to 23 in an aqueous solution;

(b) adding a precursor of metal nanoparticles, preferably a metal salt solution;

(c) gel formation to obtain a peptide hydrogel;

(d) irradiating the peptide hydrogel resulting from step (c); and

(e) obtaining a nanoparticle-containing hydrogel.

25. The method of claim 24, wherein the metal salt is selected from $AgNO_3$, $AuCl_3$, $HAuCl_4$, $CuSO_4$, $CuSO_4 \cdot 5H_2O$, $Cu(NO_3)_2$ and its hydrates, $Cu(OAc)_2$ and its hydrates, $FeCl_3$ and its hydrates, $FeCl_2$ and its hydrates, K_2PtCl_4, $PdCl_2$, H_2PdCl_4 and $Pd(NO_3)_2$.

26. The method of claim 24 or 25 wherein the concentration of said metal salt precursor is in the range from 0.001 to 1M, preferably 1 to 100mM, even more preferred 10 to 50mM.

27. The method of any one of claims 24 to 26, wherein step (b) is carried out in water or in chloride and phosphate free buffer, preferable Tris buffer, either at physiological or at a basic pH, such as pH of about 7.4 or 8.5 (or from about pH 7 to about pH 9).

28. The method of any one of claims 24 to 27, characterized in that the metal nanoparticles are formed *in situ*.

29. The method of claim 28, wherein, after gelation (step (c)), the metal nanoparticles are formed *in situ* though irradiation with light, preferably UV light, for minutes to hours, preferably 1 to 60 minutes, even more preferred 1 to 10 minutes.

30. The method of claim 28 or 29, wherein *in situ* reduction is achieved through a reducing agent, preferably a biocompatible reducing agent, more preferably, citric acid or sodium ascorbate,
wherein the reducing agent is added before or after gelation (i.e. before or during step (c) or after (c)),
wherein, when a reducing agent is added, the irradiation step (d) can be omitted.

31. A nanoparticle-containing hydrogel prepared by the *in situ* method of any one of claims 24 to 30.

32. Use of a nanoparticle-containing hydrogel of any one of claims 1 to 23 and 31
- as a device for metal delivery, preferably for sustained or controlled release metal delivery;

- as an antimicrobial agent;
- in the manufacture of a medicament for the treatment of an infectious disease, wherein, preferably, said infectious disease is caused by Gram-positive bacteria, Gram-negative bacteria, yeast or fungi;
- as a wound dressing and for wound healing (such as after surgery, in treatment of chronic and large surface wounds, like diabetic ulcer or severe burns);
- in a surgical application;
- to prevent bacterial attachment and/or formation of microbial biofilm;
- for topical medical application;
- for bioimaging (by utilitzing the fluorescence of the metal nanoparticles);
- in combination with medical devices, such as stents, prostheses, catheters, dental implants, orthopedical devices, orthopedical implants;
- for application where the hydrogel is used as a (dried) membrane, such as filtration.

33. A device for metal delivery, preferably sustained or controlled release metal delivery, comprising a nanoparticle-containing hydrogel of any one of claims 1 to 23 and 31.

34. An antimicrobial agent comprising a nanoparticle-containing hydrogel of any one of claims 1 to 23 and 31.

35. A wound dressing or wound healing agent comprising a nanoparticle-containing hydrogel of any one of claims 1 to 23 and 31,
which is preferably used to prevent microbial infection and/or formation of microbial biofilm.

36. A pharmaceutical or cosmetic composition comprising a nanoparticle-containing hydrogel of any one of claims 1 to 23 and 31.

37. The pharmaceutical or cosmetic composition of claim 36, wherein the pharmaceutical or cosmetic composition is provided in the form of a topical gel or cream, a spray, a powder, or a sheet, patch or membrane.

38. The pharmaceutical or cosmetic composition of claim 36, wherein the pharmaceutical or cosmetic composition is provided in the form of an injectable solution.

39. The pharmaceutical or cosmetic composition of any one of claims 36 to 38, further comprising a pharmaceutically active compound and/or a pharmaceutically acceptable carrier.

40. A nanoparticle-containing hydrogel of any one of claims 1 to 23 and 31 or a pharmaceutical composition of any one of claims 36 to 38 for use in the treatment of an infectious disease,
wherein, preferably, said infectious disease is caused by Gram-positive bacteria, Gram-negative bacteria, yeast or fungi.

41. A nanoparticle-containing hydrogel of any one of claims 1 to 23 and 31 or a pharmaceutical composition of any one of claims 36 to 36 for use in the treatment of a wound and/or for wound healing.

42. A bioimaging device comprising a nanoparticle-containing hydrogel of any one of claims 1 to 23 and 31 for *in vitro* and/or *in vivo* use,

preferably for oral application, for injection and/or for topical application.

43. Use of a nanoparticle-containing hydrogel of any one of claims 1 to 23 and 31 for the manufacture of a medicament for the treatment of an infectious disease.

44. A kit, comprising

(i) at least one peptide as defined in any one of claims 1 and 5 to 23 optionally, in an aqueous solution;

(ii) a precursor of metal nanoparticles,

preferably a metal salt solution (such as $AgNO_3$);

(iii) instructions for gel formation to obtain a peptide hydrogel and for irradiating the peptide hydrogel resulting therefrom for obtaining a nanoparticle-containing hydrogel;

(iv) optionally, excipients.

45. A kit, comprising

(i) a hydrogel obtained from at least one peptide as defined in any one of claims 1 and 5 to 23,

preferably in a container to be protected from radiation;

(ii) a precursor of metal nanoparticles,

preferably a metal salt solution (such as $AgNO_3$);

(iii) instructions for irradiating the peptide hydrogel for obtaining a nanoparticle-containing hydrogel;

(iv) optionally, excipients.