WOUND HEALING COMPOSITION AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore provisional application No. 10202007138Y, filed 24 July 2020, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the fields of molecular biology and biochemistry. In particular, the present invention relates to compositions for the treatment of wounds.

BACKGROUND OF THE INVENTION

[0003] It is estimated that over 400 million people globally have diabetes (http://www.idf.org). In Singapore alone it is estimated that 11% of 20 – 80-year-olds have diabetes, second only to the United States, and this number is set to rise significantly.

[0004] A major complication of diabetes is non-healing wounds. What can start as a small scratch, can later become a large, non-healing wound that in some cases can only be treated with limb amputation. In Singapore, non-healing wounds are the leading cause of non-traumatic lower limb amputations with more than 4 lower limb amputations occurring daily. Non-healing wounds or ulcers can persist for 12 months or longer and have a very high recurrence rate of 65%. It is estimated that 33% of the annual diabetes budget is spent on diabetic foot ulcers. The inability to effectively treat non-healing wounds has resulted in dramatically increased wound care costs in Singapore in recent years, with current estimates being well over S$700 million annually.

[0005] Despite their prevalence and the significant healthcare burden of non-healing wounds, there remains no effective treatment. Thus, there is an unmet need for providing compositions and methods for treating non-healing wounds.

SUMMARY ~~OF THE INVENTION~~

[0006] In one aspect, the present disclosure provides ~~there is provided~~ a pharmaceutical composition comprising an Agrin fragment or derivative thereof, wherein the Agrin fragment or derivative thereof comprises

the LG3 domain of Agrin and an eight-amino-acid insert ELANEIPV (SEQ ID NO: 1) at the z-site of the LG3 domain.

[0007] In another example~~aspect~~, there is provided a vector comprising the nucleic acid molecule encoding for an Agrin fragment or derivative thereof, wherein the Agrin fragment or derivative thereof comprises the LG3 domain of Agrin and an eight-amino-acid insert ELANEIPV (SEQ ID NO: 1) at the z-site of the LG3 domain.

[0008] In another example~~aspect~~, there is provided a host cell comprising the vector as disclosed herein.

[0009] In another example~~aspect~~, there is provided a hydrogel or scaffold comprising the pharmaceutical composition as disclosed herein.

[0010] In another example~~aspect~~, there is provided the pharmaceutical, the hydrogel, or the scaffold as disclosed herein, for use in therapy.

[0011] In another example~~aspect~~, there is provided a method of treating a wound, the method comprising administering a pharmaceutically effective amount of the pharmaceutical composition, the hydrogel, or the scaffold as disclosed herein, to a subject in need thereof.

[0012] In one ~~yet another~~ aspect, the present disclosure provides a ~~there is provided~~ use of ~~a pharmaceutically effective amount of~~ the pharmaceutical composition~~, the hydrogel, or the scaffold~~ as disclosed herein, in the manufacture of a medicament for the treatment of wound. In another aspect, the present disclosure provides a use of a pharmaceutical composition in the manufacture of a medicament to treat a wound, wherein the pharmaceutical composition comprising an Agrin fragment or derivative thereof that comprises the LG3 domain of Agrin and an eight-amino-acid insert ELANEIPV (SEQ ID NO: 1) at the z-site of the LG3 domain. In yet another aspect, the present disclosure provides a use of a pharmaceutical composition comprising an Agrin fragment or derivative in the manufacture of a medicament to treat a wound, wherein the Agrin fragment or derivative thereof comprises the sequence LGREGTFCQTASGQDGSGPFLADFNGFSHLELRGLHTFARDLGEKMALEVVFLARGP SGLLLYNGQKTDGKGDFVSLALRDRRLEFRYDLGKGAAVIRSREPVTLGAWTRVSL ERNGRKGALRVGDGPRVLGESPVPHTVLNLKEPLYVGGAPDFSKLARAAAVSSGFD GAIQLVSLGGRQLLTPEHVLRQVDVTSFAGHPCTRASGHPCLNGASCVPREAAYVCL CPGGFSGPHCEKGLVEKSAGDVDTLAFDGRTFVEYLNAVTESELANEIPVEKALQSN HFELSLRTEATQGLVLWSGKATERADYVALAIVDGHLQLSYNLGSQPVVLRSTVPV

NTNRWLRVVAHREQREGSLQVGNEAPVTGSSPLGATQLDTDGALWLGGLPELPVGP ALPKAYGTGFVGCLRDVVVGRHPLHLLEDAVTKPELRPCPTP (SEQ ID NO: 8).

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

Fig. 1 shows results of assays investigating the effect of skin injury on the expressions of various proteins, including Agrin. (a) Gene-expression analysis of an ECM-based wound signature from single-cell transcriptomics of wounded cells in a wound-healing mouse model (Students 't' test, *$p<0.05$, **$p<0.005$, respectively). (b) Confluent cells were wound-scratched and allowed to migrate. After indicated time-points, mRNA isolated from migrating cells were analyzed by RT-PCR for the indicated genes. Data represented as mean+/- s.d. Multiple t tests were performed for all the genes comparing the respective phases post-wounding; p values represented in tabular form (values in grey signify reduction). (c) Western blot analysis of migrating skin cells (as in panel b) for Agrin

Claims:

1. A hydrogel or scaffold comprising a pharmaceutical composition comprising an Agrin fragment or derivative thereof, wherein the Agrin fragment or derivative thereof comprises the LG3 domain of Agrin and an eight-amino-acid insert ELANEIPV (SEQ ID NO: 1) at the z-site of the LG3 domain.

2. The hydrogel or scaffold~~pharmaceutical composition~~ of claim 1, wherein the LG3 domain of Agrin without any insert at the z-site comprises the sequence EYLNAVTESEKALQSNHFELSLRTEATQGLVLWSGKATERADYVALAIVDGH LQLSYNLGSQPVVLRSTVPVNTNRWLRVVAHREQREGSLQVGNEAPVTGSSP LGATQLDTDGALWLGGLPELPVGPALPKAYGTGFVGCLRDVVVGRHPLHLL EDAVTKPELRPC (SEQ ID NO: 5).

3. The hydrogel or scaffold~~pharmaceutical composition~~ of claim 1 or 2, wherein the Agrin fragment or derivative thereof comprises the sequence DTLAFDGRTFVEYLNAVTESELANEIPVEKALQSNHFELSLRTEATQGLVLWS GKATERADYVALAIVDGHLQLSYNLGSQPVVLRSTVPVNTNRWLRVVAHRE QREGSLQVGNEAPVTGSSPLGATQLDTDGALWLGGLPELPVGPALPKAYGTG FVGCLRDVVVGRHPLHLLEDAVTKPELRPCPTP (SEQ ID NO: 7).

4. The hydrogel or scaffold~~pharmaceutical composition~~ of any one of the preceding claims, wherein the Agrin fragment or derivative further comprises the LG2 domain of Agrin.

5. The hydrogel or scaffold~~pharmaceutical composition~~ of claim 4, wherein the LG2 domain of Agrin without any insert at the y-site comprises the sequence PFLADFNGFSHLELRGLHTFARDLGEKMALEVVFLARGPSGLLLYNGQKTDG KGDFVSLALRDRRLEFRYDLGKGAAVIRSREPVTLGAWTRVSLERNGRKGAL RVGDGPRVLGESPVPHTVLNLKEPLYVGGAPDFSKLARAAAVSSGFDGAIQL VSLGGRQLLTPEHVLRQVDVTSFAGHPC (SEQ ID NO: 2).

6. The hydrogel or scaffold~~pharmaceutical composition~~ of claim 4 or claim 5, wherein the Agrin fragment or derivative thereof comprises the sequence LGREGTFCQTASGQDGSGPFLADFNGFSHLELRGLHTFARDLGEKMALEVVF LARGPSGLLLYNGQKTDGKGDFVSLALRDRRLEFRYDLGKGAAVIRSREPVT LGAWTRVSLERNGRKGALRVGDGPRVLGESPVPHTVLNLKEPLYVGGAPDF SKLARAAAVSSGFDGAIQLVSLGGRQLLTPEHVLRQVDVTSFAGHPCTRASG HPCLNGASCVPREAAYVCLCPGGFSGPHCEKGLVEKSAGDVDTLAFDGRTFV EYLNAVTESELANEIPVEKALQSNHFELSLRTEATQGLVLWSGKATERADYV ALAIVDGHLQLSYNLGSQPVVLRSTVPVNTNRWLRVVAHREQREGSLQVGN EAPVTGSSPLGATQLDTDGALWLGGLPELPVGPALPKAYGTGFVGCLRDVVV GRHPLHLLEDAVTKPELRPCPTP (SEQ ID NO: 8).

7. The hydrogel or scaffold~~pharmaceutical composition~~ of any one of the preceding claims, wherein the Agrin fragment or derivative thereof is soluble.

~~8. A vector comprising the nucleic acid molecule encoding for an Agrin fragment or derivative thereof, wherein the Agrin fragment or derivative thereof comprises the LG3 domain of Agrin and an eight amino acid insert ELANEIPV (SEQ ID NO: 1) at the z-site of the LG3 domain.~~

~~9. A host cell comprising the vector of claim 8.~~

~~10. A hydrogel or scaffold comprising the pharmaceutical composition of any one of claims 1 to 7.~~

~~11.~~8. The hydrogel or scaffold of any one of claims ~~10~~1 to 7, wherein the hydrogel is an inert hydrogel or a thermoresponsive hydrogel.

~~12.~~9. The hydrogel or scaffold~~pharmaceutical composition~~ of any one of claims 1 to ~~7~~8, ~~or the hydrogel of claim 10 or 11, or the scaffold of claim 10,~~ for use in therapy.

~~13. A method of treating a wound, the method comprising administering a pharmaceutically effective amount of the pharmaceutical composition of any one of claims 1 to 7, or the hydrogel of claim 10 or 11, or the scaffold of claim 10, to a subject in need thereof.~~

~~14.~~10. Use of ~~a pharmaceutically effective amount of~~ the ~~pharmaceutical composition~~hydrogel or scaffold of any one of claims 1 to 8~~7, or the hydrogel of claim 10 or 11, or the scaffold of claim 10~~, in the manufacture of a medicament for the treatment of wound.

~~15.~~11. The ~~method of claim 13 or the~~ use of claim ~~14~~10, wherein the wound is a slow healing wound or a nonhealing wound.

12. Use of a pharmaceutical composition in the manufacture of a medicament to treat a wound, wherein the pharmaceutical composition comprising an Agrin fragment or derivative thereof that comprises the LG3 domain of Agrin and an eight-amino-acid insert ELANEIPV (SEQ ID NO: 1) at the z-site of the LG3 domain.

13. Use of a pharmaceutical composition comprising an Agrin fragment or derivative in the manufacture of a medicament to treat a wound, wherein the Agrin fragment or derivative thereof comprises the sequence LGREGTFCQTASGQDGSGPFLADFNGFSHLELRGLHTFARDLGEKMALEVVF LARGPSGLLLYNGQKTDGKGDFVSLALRDRRLEFRYDLGKGAAVIRSREPVT LGAWTRVSLERNGRKGALRVGDGPRVLGESPVPHTVLNLKEPLYVGGAPDF SKLARAAAVSSGFDGAIQLVSLGGRQLLTPEHVLRQVDVTSFAGHPCTRASG HPCLNGASCVPREAAYVCLCPGGFSGPHCEKGLVEKSAGDVDTLAFDGRTFV EYLNAVTESELANEIPVEKALQSNHFELSLRTEATQGLVLWSGKATERADYV ALAIVDGHLQLSYNLGSQPVVLRSTVPVNTNRWLRVVAHREQREGSLQVGN EAPVTGSSPLGATQLDTDGALWLGGLPELPVGPALPKAYGTGFVGCLRDVV VGRHPLHLLEDAVTKPELRPCPTP (SEQ ID NO: 8).