(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 June 2019 (06.06.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/108321 A1

(51) International Patent Classification:
A61K 9/14 (2006.01) *C12N 15/00* (2006.01)
A61K 48/00 (2006.01) *C07H 21/04* (2006.01)
C12N 15/11 (2006.01) *A61K 38/00* (2006.01)

(21) International Application Number:
PCT/US2018/056972

(22) International Filing Date:
23 October 2018 (23.10.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/592,566 30 November 2017 (30.11.2017) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2019/108321 A1

(54) **Title:** METHOD FOR RESTORING HAIR FOLLICLES AND HAIR GROWTH

(57) **Abstract:** The disclosure provides methods of enhancing hair growth and hair follicle restoration using a siRNA or shRNA directed against a human Fidgetin like-2 nucleic acid. Specifically, the disclosure provides compositions comprising siRNA or shRNA against a human Fidgetin like-2 nucleic acid, and the methods of using the compositions. Further disclosed are siRNA sequences against a human Fidgetin like-2 nucleic acid and the formulations of the compositions.

METHOD FOR RESTORING HAIR FOLLICLES AND HAIR GROWTH

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 62/592,566, filed November 30, 2017, the contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] The disclosures of all publications, patents, patent application publications and books referred to in this application are hereby incorporated by reference in their entirety into the subject application to more fully describe the art to which the subject invention pertains.

[0003] Hair loss is a major esthetic and cosmetic concern to both men and women. Improved methods to restore hair follicles and/or increase hair growth are desirable.

[0004] The present invention addresses this need.

SUMMARY OF THE INVENTION

[0005] A method is provided for enhancing hair follicle growth in skin comprising directly administering to the skin an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to enhance hair follicle growth in skin.

[0006] Also provided is a method for increasing hair growth in skin comprising directly administering to the skin an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase hair growth in skin.

[0007] Also provided is a method for increasing Cytokeratin 14 expression in skin comprising directly administering to the skin an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase Cytokeratin 14 expression in skin.

[0008] A shampoo composition is provided comprising (i) an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to enhance hair follicle growth in skin and (ii) a surfactant.

BRIEF DESCRIPTION OF FIGURES

[0009] Fig. 1. Poloxamer FL2-siRNA was applied to mouse skin portion from which hair follicles have been removed. Representative H&E staining of bisected skin sections for Control-siRNA and FL2-siRNA groups taken at 8 days. Epithelial hyperplasia is present in the epidermis of the control-siRNA group while FL2-siRNA treated animals show cytokeratin 14 positive hair follicles within the portion from which hair follicles have been removed. Double-headed arrows designate observable areas initially treated to remove follicles.

[0010] Fig. 2. 2-photon confocal microscopy imaging shows nanoparticle encapsulated FL2 siRNA enhances hair follicle formation in skin previously treated to remove hair follicles.

[0011] Fig. 3. FL2 siRNA stimulates the restoration of hair follicles to burn wound sites. Animal skin was subjected to third degree thermal burns under anesthesia and then topically treated with FL2-siRNA. Following sacrifice on day 56, wounds were sectioned, stained with H&E and examined for adnexal structures. Hair follicles (indicated by the black arrow) were noted within the wound zone in FL2 siRNA treated conditions, while none were present in controls.

DETAILED DESCRIPTION OF THE INVENTION

[0012] A method is provided for enhancing hair follicle growth in skin comprising directly administering to the skin an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to enhance hair follicle growth in skin.

[0013] Also provided is a method for increasing hair growth in skin comprising directly administering to the skin an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase hair growth in skin.

[0014] Also provided is a method for increasing Cytokeratin 14 expression in skin comprising directly administering to the skin an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase Cytokeratin 14 expression in skin.

[0015] In embodiments, the Fidgetin like-2 comprises the amino acid set forth in SEQ ID NO:2

[0016] In embodiments, the siRNA is administered.

[0017] In embodiments, the shRNA is administered.

[0018] In embodiments, the siRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

[0019] In embodiments, the shRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

[0020] In embodiments, the siRNA or shRNA is directed against an mRNA encoding the human Fidgetin-like 2.

[0021] In embodiments, the siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2.

[0022] In embodiments, the siRNA comprises a sequence set forth in SEQ ID NOS:3, 4, 5, 6, 7, 8, 9, or 10.

[0023] In an embodiment of the methods, the siRNA comprises a sequence set forth in SEQ ID NOS:3, 4, 5, 6, 7, 8, 9, or 10.

[0024] In an embodiment, the Fidgetin like-2 comprises the amino acid set forth in SEQ ID NO:2. In an embodiment, the siRNA is administered. In an embodiment, the shRNA is administered. In an embodiment, the siRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification. In an embodiment, the shRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification. In an embodiment, the siRNA or shRNA is directed against an mRNA encoding the human Fidgetin-like 2. In an embodiment, the siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like In an embodiment, the siRNA comprises a sequence set forth in SEQ ID NOS:3, 4, 5, 6, 7, 8, 9, or 10.

[0025] In an embodiment, siRNA or shRNA administration is effected by administering liposomes containing the siRNA or shRNA. In an embodiment, siRNA or shRNA administration is effected by administering a nanoparticle encapsulating the siRNA or shRNA. In an embodiment, siRNA or shRNA administration is effected by administering a poloaxomer-comprising nanoparticle encapsulating the siRNA or shRNA.

[0026] In an embodiment, the inhibitor of Fidgetin-like 2 is a siRNA directed against a DNA or RNA encoding a human Fidgetin like-2. In an embodiment, the inhibitor of Fidgetin-like 2 is a shRNA directed against a DNA or RNA encoding a human Fidgetin like-2.

[0027] In an embodiment, the inhibitor of Fidgetin-like 2 is administered topically to the skin. In an embodiment, the inhibitor of Fidgetin-like 2 is administered from a reservoir that elutes the inhibitor, for example an eluting skin patch. In an embodiment, the inhibitor

of Fidgetin-like 2 is administered from microneedle patch, wherein the miconeedles deliver the inhibitor of Fidgetin-like 2, such as the siRNA, into the skin when placed on the skin or adhered onto the skin.

[0028] In an embodiment, the Fidgetin-like 2 is human Fidgetin-like 2.

[0029] The dosage of the inhibitor administered in treatment will vary depending upon factors such as the pharmacodynamic characteristics of a specific inhibitor and its mode and route of administration; the age, sex, metabolic rate, absorptive efficiency, health and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment being administered; the frequency of treatment with the inhibitor and the desired therapeutic effect.

[0030] A dosage unit of the inhibitor may comprise a single compound, or a mixture of the compound with, for example, one or more hair growth-stimulating compound(s).

[0031] In an embodiment, the siRNA (small interfering RNA) as used in the methods or compositions described herein comprises a portion which is complementary to an mRNA sequence encoding a Fidgetin-like 2 protein. In an embodiment, the Fidgetin-like 2 protein is a human Fidgetin-like 2 protein. In an embodiment, the mRNA is encoded by the DNA sequence NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1), and the siRNA is effective to inhibit expression of Fidgetin-like 2 protein. In an embodiment, the Fidgetin-like 2 protein comprises consecutive amino acid residues having the sequence set forth in SEQ ID NO:2.

[0032] In an embodiment, the siRNA comprises a double-stranded portion (duplex). In an embodiment, the siRNA is 19-25 nucleotides in length. In an embodiment, the siRNA is 20-25 nucleotides in length. In an embodiment the siRNA comprises a 19-21 core RNA duplex with a one or two nucleotide 3' overhang on, independently, either one or both strands. In an embodiment the siRNA comprises a 19-25 RNA duplex with a one or two nucleotide 3' overhang on, independently, either one or both strands. The siRNA can be 5' phosphorylated, or not, and may be modified with any of the known modifications in the art to improve efficacy and/or resistance to nuclease degradation. In an embodiment the siRNA can be administered such that it is transfected into one or more cells. In an embodiment, the siRNA is 5' phosphorylated. In an embodiment, the whole length of the non-overlapping portion of the siRNA is fully complementary to a portion of a mRNA encoding a Fidgetin-like 2 protein.

[0033] In an embodiment, the 5' terminal residue of a strand of the siRNA is phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is phosphorylated. In one embodiment, a siRNA of the invention comprises a double-stranded RNA wherein one strand of the double-stranded RNA is 80, 85, 90, 95 or 100% complementary to a portion of an RNA transcript of a gene encoding Fidgetin-like 2 protein. In an embodiment, the RNA transcript of a gene encoding Fidgetin-like 2 protein is an mRNA. In an embodiment, the Fidgetin-like 2 protein is a human Fidgetin-like 2 protein. In an embodiment, a siRNA of the invention comprises a double-stranded RNA wherein one strand of the RNA comprises a portion having a sequence the same as a portion of 18-25 consecutive nucleotides of an RNA transcript of a gene encoding Fidgetin-like 2 protein . In an embodiment, the Fidgetin-like 2 protein is a human Fidgetin-like 2 protein. In yet another embodiment, a siRNA of the invention comprises a double-stranded RNA wherein both strands of RNA are connected by a non-nucleotide linker. Alternately, a siRNA of the invention can comprise a double-stranded RNA wherein both strands of RNA are connected by a nucleotide linker, such as a loop or stem loop structure. In an embodiment, both of the strands of RNA are not connected by a nucleotide linker, such as a loop or stem loop structure.

[0034] In one embodiment, a single strand component of a siRNA of the invention is from 14 to 50 nucleotides in length. In another embodiment, a single strand component of a siRNA of the invention is 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 21 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 22 nucleotides in length. In yet another embodiment, a single strand component of a siRNA of the invention is 23 nucleotides in length. In one embodiment, a siRNA of the invention is from 28 to 56 nucleotides in length. In another embodiment, a siRNA of the invention is 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 nucleotides in length.

[0035] In another embodiment, an siRNA of the invention comprises at least one 2'-sugar modification. In another embodiment, an siRNA of the invention comprises at least one nucleic acid base modification. In another embodiment, an siRNA of the invention comprises at least one phosphate backbone modification. In embodiments, an siRNA of the invention comprises at least one 2'-O-methyl modification. In embodiments, an siRNA of the invention comprises at least one phosphorodithioate (PS2).

[0036] As used herein, "at least one" means one or more.

[0037] In one embodiment, RNAi inhibition of Fidgetin-like 2 protein is effected by a short hairpin RNA ("shRNA"). The shRNA can be introduced into the appropriate cell by transduction with a vector. In an embodiment, the vector is a lentiviral vector. In an embodiment, the vector comprises a promoter. In an embodiment, the promoter is a U6 or H1 promoter. In an embodiment the shRNA encoded by the vector is a first nucleotide sequence ranging from 19-29 nucleotides complementary to the target gene/mRNA, in the present case the mRNA encodes Fidgetin-like 2 protein. In an embodiment the Fidgetin-like 2 protein is a human Fidgetin-like 2 protein. In an embodiment the shRNA encoded by the vector also comprises a short spacer of 4-15 nucleotides (a loop, which does not hybridize) and a 19-29 nucleotide sequence that is a reverse complement of the first nucleotide sequence. In an embodiment the siRNA resulting from intracellular processing of the shRNA has overhangs of 1 or 2 nucleotides. In an embodiment the siRNA resulting from intracellular processing of the shRNA overhangs has two 3' overhangs. In an embodiment the overhangs are UU.

[0038] In an embodiment, the FL2 is encoded by NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1) (nucleic acid encoding Human Fidgetin-like 2)

1 agtgagctat ggggacacta ctgcactgta gcctgggcaa cagagcaaga ccttgtctca

61 aaaatgtata tatattttgg gctttttttc ctaaaacggg aactacaaca gcatatttgc

121 gagctgatga gagtgaccca gcagagaggg aaatggatca gctctgttga agatgcactg

181 gacaccagaa cacgcccagc ccctcaacca gtggccagag cagcacctgg acgtctcctc

241 caccaccccg tcgccggccc acaagttgga gttgcccccc gggggtcgcc aacgctgcca

301 ctacgcttgg gcacacgacg acatctcagc cctcactgcc tccaacctcc taaagcgcta

361 tgcagagaag tactctgggg tcttggattc tccctacgag cgtccggccc tgggcgggta

421 cagcgacgcc tccttcctca acggcgccaa aggggatccc gagccctggc cagggccgga

481 gccaccctac cccttggcct cactccacga aggcctccca ggaaccaaat cgggcggtgg

541 cggcggttcc ggggccctgg ggggctcccc agttttagcc gggaacctcc ctgaacccct

601 ctacgccggc aatgcgtgcg ggggcccatc ggcggcgccc gagtacgcgg ccggctacgg

661 cgggggggtac ctggcgccgg gttactgcgc gcagacgggc gccgcgctgc ccccgccgcc

721 cccggccgcg ctcctgcagc ccccaccgcc tccggggtac gggccctcag cgccgctgta

781 caactatccc gcaggggggct acgcagcgca gcccggctat ggcgcgctcc cgccgccccc

841 aggcccaccc ccggcccccct acctgacccc gggcctgccc gcgcccacgc ccctgcccgc

901 gccggcaccg cccaccgcct atggcttccc cacggccgcg ccgggtgccg aatccgggct

961 gtcgctgaag cgcaaggccg ccgacgaggg gcccgagggc cgctaccgca agtacgcgta
1021 cgagcccgcc aaggcccccg tggctgacgg agcctcctac cccgccgcgg acaacggcga
1081 atgtcggggc aacgggttcc gggccaagcc gccaggagcc gcggaggagg cgtcgggcaa
1141 gtacggtggc ggcgtccccc tcaaggtcct gggctccccc gtctacggcc cgcaactgga
1201 gcccctttgaa aagttcccgg agcgggcccc ggctcctcgt ggggggttcg ccgtgccgtc
1261 gggggagact cccaaaggcg tggaccctgg ggccctggag ctggtgacga gcaagatggt
1321 ggactgcggg cccccggtgc agtgggcgga tgtggcgggc cagggcgcgc tcaaggcggc
1381 gctggaggag gagctggtgt ggcccctgct caggccgccc gcctacccgg gcagcctgcg
1441 cccgccgcgg accgtcctgc tctttgggcc gcggggcgcg ggcaaagcgc tgctgggccg
1501 ctgcctcgcc acgcagctgg gcgccacgct gttgcgcctg cgcggcgcga ccctggctgc
1561 gcccggcgcc gccgagggcg cgcgcctcct ccaggccgcc ttcgcggccg cgcgctgccg
1621 cccaccctcc gtactcctca tcagcgagct agaggcgctg ctccccgccc gggacgacgg
1681 cgcggcggca gggggcgcgc tgcaggtgcc gctcctggcc tgcctggacg ggggctgcgg
1741 cgcgggggct gacggcgtgc tggttgtggg caccacctcg cggcccgcgg ctctggacga
1801 ggcgacccgc cggcgcttct ctctccgctt ctacgtggcg ctgcccgaca gcccggcccg
1861 cgggcagatc ctgcagcggg cgctggccca gcagggctgc gcgctcagtg agcgggaact
1921 ggcggcgctg gtgcagggca cgcagggctt ctctgggggc gagctggggc agctgtgcca
1981 gcaggcggcg gccggggcgg gcctcccggg gctgcagcgc cccctctcct acaaggacct
2041 ggaggcggcg ctggccaagg tgggccctag ggcctctgcc aaggaactgg actcgttcgt
2101 ggagtgggac aaaatgtacg gctccggaca ctgacggcgc gcgggggagg ccgcgggagc
2161 cgcagtccct ccgtccccgc cgcctccgcg tgggagggat gtcactgact aaacccggct
2221 ggcaggggct ggagtggtga atgtgggatc ggggacagga ggggtctgcc ggtggatatt
2281 ttttttttcg tgggaaggaa aatgcttctg ccaggcagat gccatatgcg ccgtgtactc
2341 aggtttttcc tatttattgt ggactggaag ctcgccatct ccgcccggca gaccgggcag
2401 atccggcatg ggctggcacc cggggcctta agaactcctg ctctcttgcc acaacgcttt
2461 tgtctcctcg ctatctgaat ggcaccctcc ttctccctca ctctctccat cccattctct
2521 gcattctctt ggttttctct ccctttgct ttgtcgctga cacccctgcc cacccatgc
2581 tggccctgtt tctctcctgc ccctccctcc ccagctctcc atccctcacc ctctgtgctt
2641 ctgtctccat ccctggctct ccagcgtccc tggccttttg gtccctgagc tttaatgcct
2701 ttccctgcct tctgttctta tttggactgc agtggccctt tgcaggagct ctggaggccc
2761 aggggctgag gaggagggtt acccctctac ccatctgaaa cctagggtct agggggatca
2821 aggaaaaaaa gtccccaaag aaggggaatt ttttgtttgt ttttgagggg agatcccaga
2881 aatgtagctt gtttcatatt ttagtcttct tatttttgta aaatgtgtag aatttgctgt

2941 ttttcttttt cttttgacaa ctcaggaaga aactgacctc agaaagaatg ttagactttg
3001 gctgctctcc tgtgtgcccc tcacacctgc cccctccccc ccactccatc caggggacca
3061 aattctccca gacactcaaa aaatgagact tacggggaag gggagaggaa gacccagagg
3121 cctcagtgaa accccagcta ttcctggtca gaagcagaat gtattcctaa gggcttcctc
3181 cccagggccg aggcctaggc atgaatgtgg ggagtgggct gtggggtttg agagaaggga
3241 ggccttattc ctctcctgct gctccccacc ccctgcccca cccaaccect ccgctgagtg
3301 ttttctgtga agggctatcc agagttagga tgcccttgcc caattccttc ctgagaccca
3361 gaaggtaggg tgggagggcc caaatgggaa ggtgacctaa gcagaaagtc tccagaaagg
3421 tcatgtcccc tggccctgcc ttggcagagg tccccagtga cttatgctag gaggattcca
3481 tctgggtaga cagtctggcc acaaaatcag ctactggacc tcagccatct ctgctggagg
3541 ctctgaggag gagtgagcat ccctcacttg tgggggctct gtgaggaaat gtgccttccc
3601 cattcccccg gagtcctagg tctggagctc cagggctggg agagggtgag ggagatgggc
3661 aggggtgttt tctctgacct tgggggctta gtctcagtcc tgcctgaact ttccactagg
3721 cttggaaccc ttccaagaac catatttctc tccttcccac caattttccc ttgatgaggc
3781 tttagcagtt tgctcccacc acccccagcc catttcacaa ctctgatctt agtccaaagc
3841 aggggacacg ccccccccacc accacttttt ctctctccca tctcagcctc ctgtgcagtt
3901 ccttgcctgc ccgtgcattt cctagagtct actgcctccc ccctggctgg gagggtgtct
3961 gggggggatc tttcaggggc cctggcaccc agggcctgtg ctggcctagg agtgctgacc
4021 agaaggctgc tctgttcccc cccacccccg ttgctttctg gccccctctt tggagccagc
4081 cacccacagg gctttggtgc ctcagaagca gtgggctgcc gggtcacagc cgcaggctgc
4141 aaaagaccct cggagggagc atggagtgag gggttctctc tcaggtgtgt atgtattggg
4201 gggtgggggt gggtggaggg tgtcagggaa gttggggtgg gatcccagcc ttcccttcaa
4261 gaggcaggga gctctgggag gtggagtccc caccgctttc tctactaggc tcctcctgtt
4321 ccccaggctt ggggagcttt gcacaaggag actgccccca gcctagtggc acctacctca
4381 tgggctctgg ggcaggtagg ggaagggcca gtccagctct ggtaatgctg gggggaggca
4441 taccaaagaa tccaggggca gggagtgggg agggtgactt ccgagctggc ctctcccctt
4501 cctctaccca gactggggct gggatcctct cctcccgctg taaccatttc tacctcattt
4561 tgctgcgtgt tgtacatgga cgtatttatc tcctgtctga cgatgctctg cagttgtggt
4621 ctgtctacct cagaagagac tgtattttaa aagaaagtat tacacagtat taaagcgatg
4681 acatgtggtt tgcaaaaaaa aaaaaaaaaa a.

[0039] In an embodiment, the FL2 protein sequence comprises:

MHWTPEHAQPLNQWPEQHLDVSSTTPSPAHKLELPPGGRQRCHYAWAHDDISALT
ASNLLKRYAEKYSGVLDSPYERPALGGYSDASFLNGAKGDPEPWPGPEPPYPLASL
HEGLPGTKSGGGGGSGALGGSPVLAGNLPEPLYAGNACGGPSAAPEYAAGYGGGY
LAPGYCAQTGAALPPPPPAALLQPPPPPGYGPSAPLYNYPAGGYAAQPGYGALPPPP
GPPPAPYLTPGLPAPTPLPAPAPPTAYGFPTAAPGAESGLSLKRKAADEGPEGRYRK
YAYEPAKAPVADGASYPAADNGECRGNGFRAKPPGAAEEASGKYGGGVPLKVLG
SPVYGPQLEPFEKFPERAPAPRGGFAVPSGETPKGVDPGALELVTSKMVDCGPPVQ
WADVAGQGALKAALEEELVWPLLRPPAYPGSLRPPRTVLLFGPRGAGKALLGRCL
ATQLGATLLRLRGATLAAPGAAEGARLLQAAFAAARCRPPSVLLISELEALLPARD
DGAAAGGALQVPLLACLDGGCGAGADGVLVVGTTSRPAALDEATRRRFSLRFYV
ALPDSPARGQILQRALAQQGCALSERELAALVQGTQGFSGGELGQLCQQAAAGAG
LPGLQRPLSYKDLEAALAKVGPRASAKELDSFVEWDKMYGSGH (SEQ ID NO:2).

[0040] In an embodiment, the FL2 is naturally occurring variant having 95% or greater identity with NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1). In an embodiment, the FL2 is naturally occurring variant having 96% or greater identity with NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1). In an embodiment, the FL2 is naturally occurring variant having 97% or greater identity with NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1). In an embodiment, the FL2 is naturally occurring variant having 98% or greater identity with NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1). In an embodiment, the FL2 is naturally occurring variant having 99% or greater identity with NCBI Reference Sequence: NM_001013690.4 (SEQ ID NO:1).

[0041] In embodiments, the siRNA comprise one of the following pairs of sense/antisense sequences:

Sense: UUACACAGUAUUAAAGCGAUU (SEQ ID NO:3)
Antisense: 5' UCGCUUUAAUACUGUGUAAUU(SEQ ID NO:4); or

Sense: CAUCUGAAACCUAGGGUCUUU(SEQ ID NO:5)
Antisense: 5' AGACCCUAGGUUUCAGAUGUU(SEQ ID NO:6); or

Sense: GUGACUUAUGCUAGGAGGAUU (SEQ ID NO:7)
Antisense: 5' UCCUCCUAGCAUAAGUCACUU (SEQ ID NO:8); or

Sense: GGUCAGAAGCAGAAUGUAUUU(SEQ ID NO:9)
Antisense: 5' AUACAUUCUGCUUCUGACCUU (SEQ ID NO:10).

[0042] In an embodiment, the siRNA is double-stranded and comprises SEQ ID NO:3 and 4; SEQ ID NO:5 and 6; SEQ ID NO:7 and 8; or SEQ ID NO:9 and 10.

[0043] In an embodiment, the 5' terminal residue of a strand of the siRNA is phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is phosphorylated. In an embodiment, the 5' terminal residue of a strand of the siRNA is not phosphorylated. In an embodiment the 5' terminal residue of the antisense strand of the siRNA is not phosphorylated.

[0044] In an embodiment the inhibitor of Fidgetin-like 2 is provided in a vehicle suitable for application to the skin. In an embodiment the inhibitor of Fidgetin-like 2 is provided in a dermatologically acceptable carrier.

[0045] In an embodiment the inhibitor of Fidgetin-like 2 is provided in a bulk-eroding system such as polylactic acid and glycolic acid (PLGA) copolymer based microspheres or microcapsules systems containing the inhibitor of Fidgetin-like 2. In an embodiment, blends of PLGA:ethylcellulose systems may be used as an appropriate carrier. A further medicament in accordance with this aspect of the invention may be formulated in a surface-eroding system wherein the inhibitor of Fidgetin-like 2 is embedded in an erodible matrix such as the poly(ortho) ester and polyanhydride matrices wherein the hydrolysis of the polymer is rapid. A medicament in accordance with this aspect of the invention may also be formulated by combining a pulsatile delivery system as described above and an immediate release system such as a lyophilized injectable composition described above.

[0046] The inhibitor may be used in a composition with additives. Examples of suitable additives are sodium alginate, as a gelatinizing agent for preparing a suitable base, or cellulose derivatives, such as guar or xanthan gum, inorganic gelatinizing agents, such as aluminum hydroxide or bentonites (termed thixotropic gel-formers), polyacrylic acid derivatives, such as Carbopol®, polyvinylpyrrolidone, microcrystalline cellulose and carboxymethylcellulose. Amphiphilic low molecular weight and higher molecular weight compounds, and also phospholipids, are also suitable. The gels can be present either as water-based hydrogels or as hydrophobic organogels, for example based on mixtures of low and high molecular weight paraffin hydrocarbons and vaseline. The hydrophilic organogels can be prepared, for example, on the basis of high molecular weight polyethylene glycols. These gelatinous forms are washable. Hydrophobic organogels are also suitable. Hydrophobic additives, such as petroleum jelly, wax, oleyl alcohol, propylene glycol monostearate and/or propylene glycol monopalmitostearate, in particular isopropyl

myristate can be included. In an embodiment the inhibitor is in a composition comprising one or more dyes, for example yellow and/or red iron oxide and/or titanium dioxide for the purpose of matching as regards color. Compositions may be in any suitable form including gels, lotions, balms, pastes, sprays, powders, bandages, wound dressing, emulsions, creams and ointments of the mixed-phase or amphiphilic emulsion systems (oil/water-water/oil mixed phase), liposomes and transfersomes or plasters/band aid-type coverings. Emulsifiers which can be employed in compositions comprising the inhibitor of Fidgetin-like 2 include anionic, cationic or neutral surfactants, for example alkali metal soaps, metal soaps, amine soaps, sulphurated and sulphonated compounds, invert soaps, higher fatty alcohols, partial fatty acid esters of sorbitan and polyoxyethylene sorbitan, e.g. lanette types, wool wax, lanolin or other synthetic products for preparing the oil/water and/or water/oil emulsions.

[0047] Compositions comprising the inhibitor of Fidgetin-like 2 can also comprise vaseline, natural or synthetic waxes, fatty acids, fatty alcohols, fatty acid esters, for example as monoglycerides, diglycerides or triglycerides, paraffin oil or vegetable oils, hydrogenated castor oil or coconut oil, hog fat, synthetic fats (for example based on caprylic acid, capric acid, lauric acid or stearic acid, such as Softisan®), or triglyceride mixtures, such as Miglyol®, can be used as lipids, in the form of fatty and/or oleaginous and/or waxy components for preparing the ointments, creams or emulsions of the compositions comprising the inhibitor of fidgetin-like 2 used in the methods described herein.

[0048] Osmotically active acids and alkaline solutions, for example hydrochloric acid, citric acid, sodium hydroxide solution, potassium hydroxide solution, sodium hydrogen carbonate, may also be ingredients of the compositions and, in addition, buffer systems, such as citrate, phosphate, tris buffer or triethanolamine, for adjusting the pH. It is possible to add preservatives as well, such as methyl benzoate or propyl benzoate (parabens) or sorbic acid, for increasing the stability.

[0049] Pastes, powders and solutions are additional forms of compositions comprising the inhibitor of Fidgetin-like 2 which can be applied topically. As consistency-imparting bases, the pastes frequently contain hydrophobic and hydrophilic auxiliary substances, preferably, however, hydrophobic auxiliary substances containing a very high proportion of solids. In order to increase dispersity, and also flowability and slipperiness, and also to prevent agglomerates, the powders or topically applicable powders can, for example, contain starch species, such as wheat or rice starch, flame-dispersed silicon dioxide or siliceous earth, which also serve as diluent.

[0050] In an embodiment, the compositions of the invention comprise further active ingredients suitable for stimulating hair growth. In an embodiment, compositions of the invention do not comprise further active ingredients suitable for stimulating hair growth.

[0051] In an embodiment of the methods and compositions described herein the subject is a mammal. In an embodiment the subject is human.

[0052] In some embodiments, excluded from the present invention is a method performed on skin which has a wound in the area of the skin being treated, i.e. a gross break or discontinuity in the structure of the skin tissue. Examples of wounds include ulcerations, bedsores, grazes, tears, cuts, and punctures. In some embodiments, excluded from the present invention is a method performed on skin which has been subjected to cosmetic laser treatment.

[0053] In an embodiment, the cytokeratin 14 is human. In an embodiment, the cytokeratin 14 has the following sequence:

MTTCSRQFTS SSSMKGSCGI GGGIGGGSSR ISSVLAGGSC RAPSTYGGGL SVSSSRFSSG GACGLGGGYG GGFSSSSSSF GSGFGGGYGG GLGAGLGGGF GGGFAGGDGL LVGSEKVTMQ NLNDRLASYL DKVRALEEAN ADLEVKIRDW YQRQRPAEIK DYSPYFKTIE DLRNKILTAT VDNANVLLQI DNARLAADDF RTKYETELNL RMSVEADING LRRVLDELTL ARADLEMQIE SLKEELAYLK KNHEEEMNAL RGQVGGDVNV EMDAAPGVDL SRILNEMRDQ YEKMAEKNRK DAEEWFFTKT EELNREVATN SELVQSGKSE ISELRRTMQN LEIELQSQLS MKASLENSLE ETKGRYCMQL AQIQEMIGSV EEQLAQLRCE MEQQNQEYKI LLDVKTRLEQ EIATYRRLLE GEDAHLSSSQ FSSGSQSSRD VTSSSRQIRT KVMDVHDGKV VSTHEQVLRT KN (SEQ ID NO:11)

[0054] In an embodiment, the subject has experienced hair loss caused by androgenetic alopecia. In an embodiment, the subject has experienced hair loss caused by alopecia areata. In an embodiment, the subject has experienced hair loss caused by telogen effluvium. In an embodiment, the subject has experienced hair loss caused by ringworm. In an embodiment, the subject has experienced hair loss caused by scarring alopecia. In an embodiment, the subject has not experienced scarring alopecia. In an embodiment, the subject has experienced hair loss caused by cosmetic overprocessing. In an embodiment, the subject has not experienced hair loss caused by cosmetic overprocessing.

[0055] A shampoo composition is provided comprising (i) an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2, as described herein, effective to enhance hair follicle growth in skin and (ii) a surfactant.

[0056] In embodiments, the shampoo composition comprises one or more of sodium lauryl sulfate, sodium laureth sulfate, and cocamidopropyl betaine.

[0057] A composition is provided comprising (i) an amount of siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2 effective to enhance hair follicle growth in skin contained (ii) in a microneedle array.

[0058] In embodiments, the microneedle array comprises a structure made of one or more of dextran, hyaluronic acid and PVP.

[0059] All combinations of the various elements described herein are within the scope of the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0060] This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

EXPERIMENTAL DETAILS

Example 1

[0061] Initially, inhibition of FL2 is performed in mouse model where a portion of the skin has been removed or trated to effect removal of hair follicles. The skin is subsequently treated at one location with siRNA or shRNA directed to FL2 and at a second location with control. The skin is then examined at the FL2 treatment site and control site at 8 days or 14 days subsequent to treating.

[0062] Skin treated with FL2 siRNA in a nanoparticle-encapsulated form (e.g. poloaxomer nanoparticle) showed increased hair follicle numbers and groeth (and cytokertin 14 staining) compared to control. (See Figs. 1 and 2).

Example 2

[0063] A visibly hairless portion of skin on a human, which previously during the life of the human had exhibited hair at some point, is treated with a topically applied siRNA or

shRNA which inhibits Fidgetin-like 2. The topically applied siRNA or shRNA is effective to increase the rate and extent of hair follicle growth in the skin compared to control, and subsequently produces hair.

Example 3

[0064] A visibly hairless portion of skin on a human, which previously at some point during the life of the human had exhibited hair, is treated with a topically applied siRNA or shRNA which inhibits Fidgetin-like 2. The topically applied siRNA or shRNA is effective to increase Cytokeratin 14 expression in the skin portion treated.

Example 4

[0065] FL2 siRNA stimulates the restoration of hair follicles to burn wound sites: Pigs were administered anesthesia and subjected to third degree thermal burns and then topically treated with FL2-siRNA. Following sacrifice on day 56, wounds were sectioned, stained with H&E and examined for adnexal structures. Hair follicles (indicated by the black arrow in Fig. 3) were noted within the wound zone in FL2 siRNA-treated conditions, while none were present in controls. FL2 siRNA effected hair follicle growth.

What is claimed is:

1. A method of enhancing hair follicle growth in skin comprising directly administering to the skin an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to enhance hair follicle growth in skin.

2. A method for increasing hair growth in skin comprising directly administering to the skin an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase hair growth in skin.

3. A method for increasing Cytokeratin 14 expression in skin comprising directly administering to the skin an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to increase Cytokeratin 14 expression in skin.

4. The method of any of Claims 1-3, wherein the Fidgetin like-2 comprises the amino acid set forth in SEQ ID NO:2

5. The method of any of Claims 1-4, wherein the siRNA is administered.

6. The method of any of Claims 1-4, wherein the shRNA is administered.

7. The method of Claim 5, wherein the siRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

8. The method of Claim 6, wherein the shRNA directed against a DNA or RNA encoding human Fidgetin-like 2 has at least one 2' sugar modification.

9. The method of any of Claims 1-8, wherein the siRNA or shRNA is directed against an mRNA encoding the human Fidgetin-like 2.

10. The method of any of Claims 1-9, wherein the siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2.

11. The method of any of Claims 1-5, 7, or 9-10, wherein the siRNA comprises a sequence set forth in SEQ ID NOS:3, 4, 5, 6, 7, 8, 9, or 10.

12. The method of any of Claims 1-5, 7, or 9-11, wherein the siRNA is nanoparticle-encapsulated.

13. The method of any of Claims 1-5, 7, or 9-12, wherein the siRNA is poloaxomer nanoparticle-encapsulated.

14. A shampoo composition is provided comprising (i) an amount of a siRNA or shRNA directed against a DNA or RNA encoding a human Fidgetin like-2 effective to enhance hair follicle growth in skin and (ii) a surfactant.

15. The shampoo of Claim 14 comprising one or more of sodium lauryl sulfate, sodium laureth sulfate, and cocamidopropyl betaine.

16. A composition comprising (i) an amount of siRNA or shRNA is directed against an DNA encoding the human Fidgetin-like 2 effective to enhance hair follicle growth in skin contained (ii) in a microneedle array.

17. The composition of Claim 16, wherein the microneedle array comprises a structure made of one or more of dextran, hyaluronic acid and PVP.

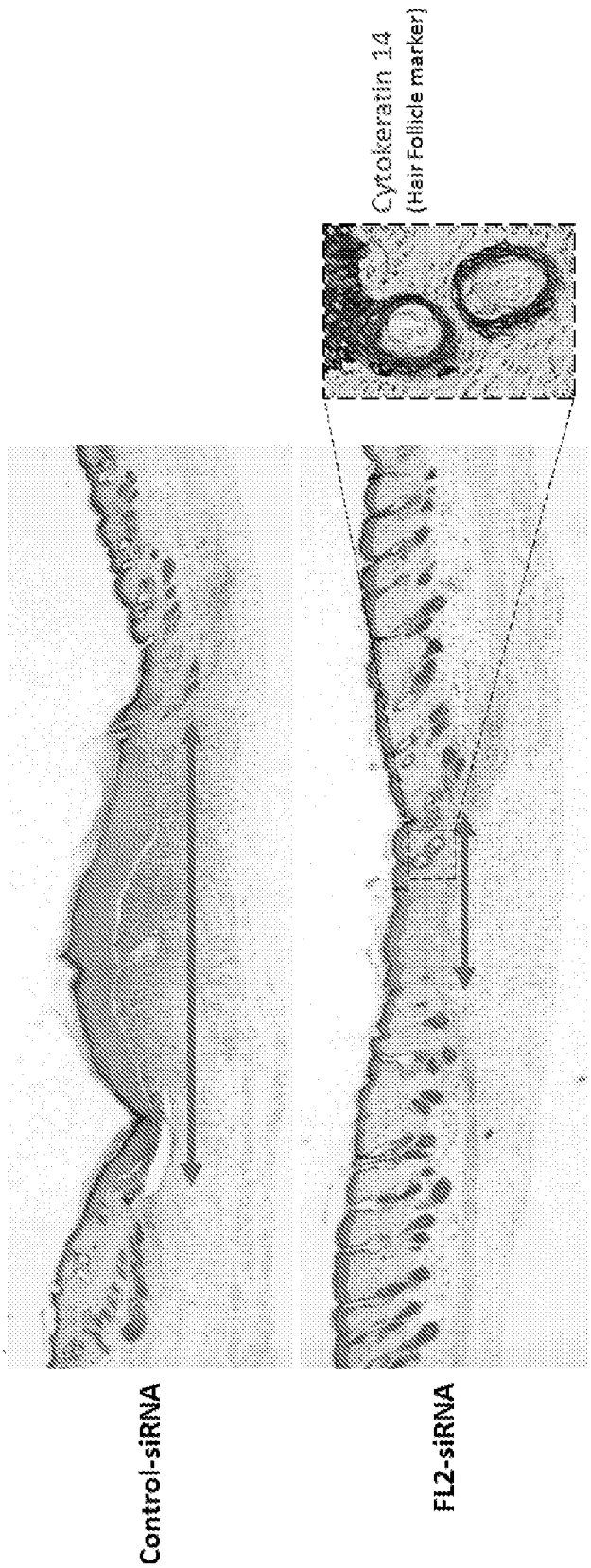
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Fig. 1

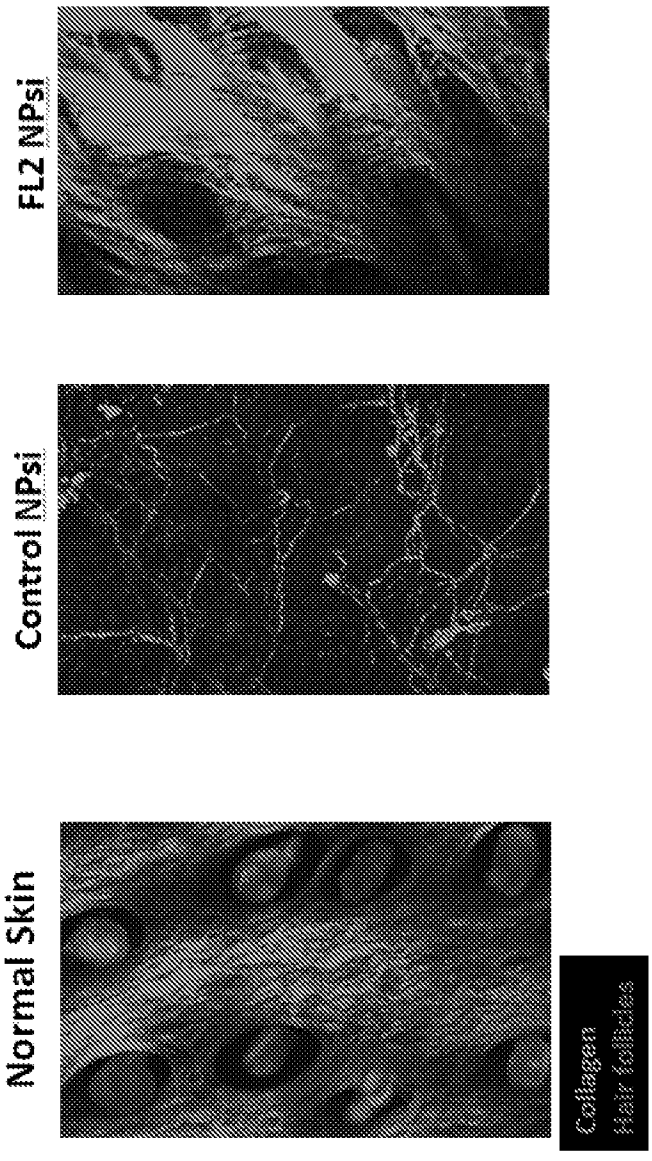
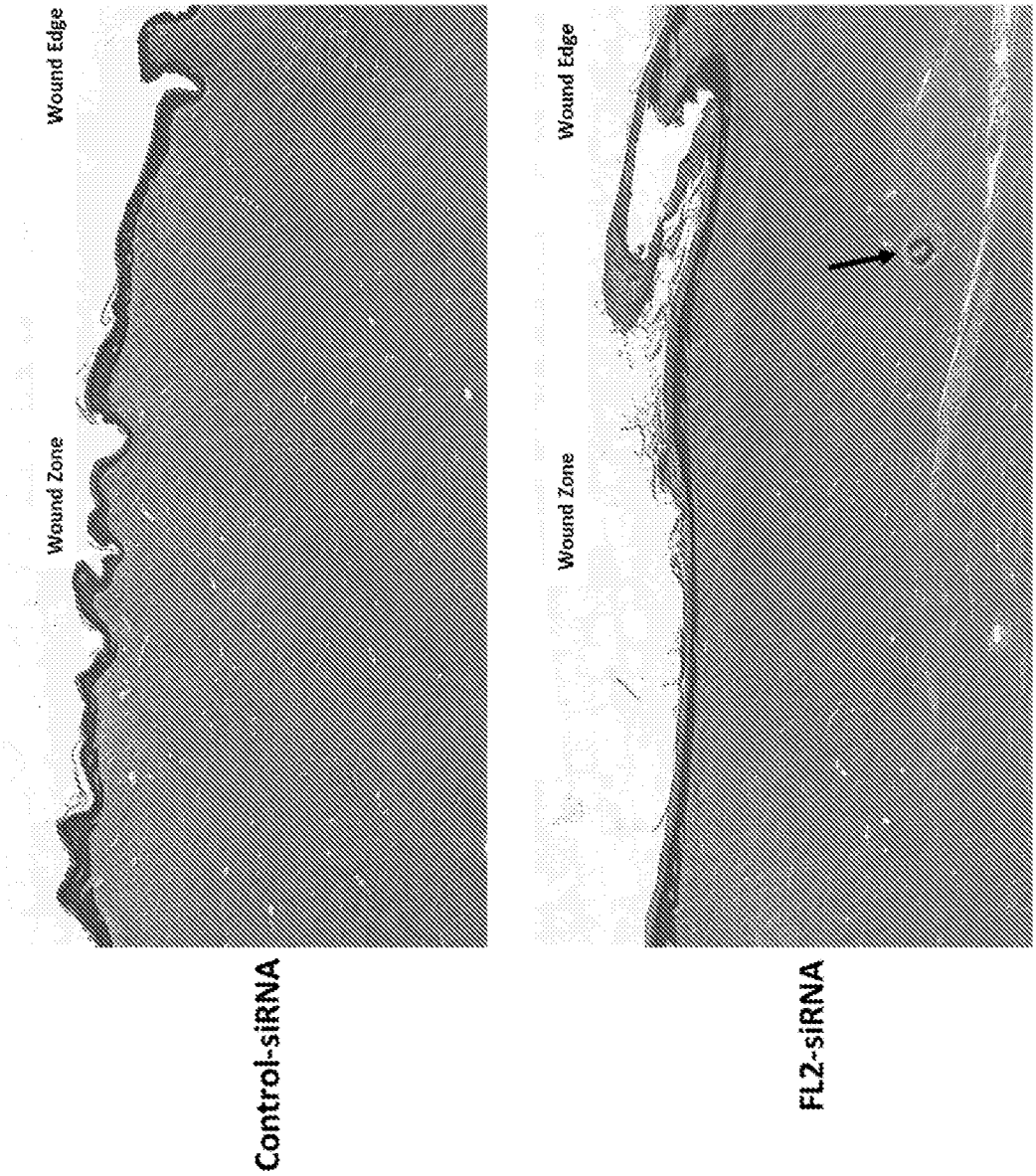


Fig. 2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Wound Edge
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FL2-siRNA

Fig. 3

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Met Thr Thr Cys Ser Arg Gln Phe Thr Ser Ser Ser Ser Met Lys Gly
1 5 10 15

Ser Cys Gly Ile Gly Gly Gly Ile Gly Gly Gly Ser Ser Arg Ile Ser
20 25 30

Ser Val Leu Ala Gly Gly Ser Cys Arg Ala Pro Ser Thr Tyr Gly Gly
35 40 45

Gly Leu Ser Val Ser Ser Ser Arg Phe Ser Ser Gly Gly Ala Cys Gly
50 55 60

Leu Gly Gly Gly Tyr Gly Gly Gly Phe Ser Ser Ser Ser Ser Ser Phe
65 70 75 80

Gly Ser Gly Phe Gly Gly Gly Tyr Gly Gly Gly Leu Gly Ala Gly Leu
85 90 95

Gly Gly Gly Phe Gly Gly Gly Phe Ala Gly Gly Asp Gly Leu Leu Val
100 105 110

Gly Ser Glu Lys Val Thr Met Gln Asn Leu Asn Asp Arg Leu Ala Ser
115 120 125

Tyr Leu Asp Lys Val Arg Ala Leu Glu Glu Ala Asn Ala Asp Leu Glu
130 135 140

Val Lys Ile Arg Asp Trp Tyr Gln Arg Gln Arg Pro Ala Glu Ile Lys
145 150 155 160

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Asp Tyr Ser Pro Tyr Phe Lys Thr Ile Glu Asp Leu Arg Asn Lys Ile
                165                 170                 175

Leu Thr Ala Thr Val Asp Asn Ala Asn Val Leu Leu Gln Ile Asp Asn
            180                 185                 190

Ala Arg Leu Ala Ala Asp Asp Phe Arg Thr Lys Tyr Glu Thr Glu Leu
        195                 200                 205

Asn Leu Arg Met Ser Val Glu Ala Asp Ile Asn Gly Leu Arg Arg Val
    210                 215                 220

Leu Asp Glu Leu Thr Leu Ala Arg Ala Asp Leu Glu Met Gln Ile Glu
225                 230                 235                 240

Ser Leu Lys Glu Glu Leu Ala Tyr Leu Lys Lys Asn His Glu Glu Glu
                245                 250                 255

Met Asn Ala Leu Arg Gly Gln Val Gly Gly Asp Val Asn Val Glu Met
            260                 265                 270

Asp Ala Ala Pro Gly Val Asp Leu Ser Arg Ile Leu Asn Glu Met Arg
        275                 280                 285

Asp Gln Tyr Glu Lys Met Ala Glu Lys Asn Arg Lys Asp Ala Glu Glu
    290                 295                 300

Trp Phe Phe Thr Lys Thr Glu Glu Leu Asn Arg Glu Val Ala Thr Asn
305                 310                 315                 320

Ser Glu Leu Val Gln Ser Gly Lys Ser Glu Ile Ser Glu Leu Arg Arg
                325                 330                 335

Thr Met Gln Asn Leu Glu Ile Glu Leu Gln Ser Gln Leu Ser Met Lys
            340                 345                 350

Ala Ser Leu Glu Asn Ser Leu Glu Glu Thr Lys Gly Arg Tyr Cys Met
        355                 360                 365
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Gln Leu Ala Gln Ile Gln Glu Met Ile Gly Ser Val Glu Glu Gln Leu
    370                 375                 380

Ala Gln Leu Arg Cys Glu Met Glu Gln Gln Asn Gln Glu Tyr Lys Ile
385                 390                 395                 400

Leu Leu Asp Val Lys Thr Arg Leu Glu Gln Glu Ile Ala Thr Tyr Arg
                405                 410                 415

Arg Leu Leu Glu Gly Glu Asp Ala His Leu Ser Ser Ser Gln Phe Ser
            420                 425                 430

Ser Gly Ser Gln Ser Ser Arg Asp Val Thr Ser Ser Ser Arg Gln Ile
        435                 440                 445

Arg Thr Lys Val Met Asp Val His Asp Gly Lys Val Val Ser Thr His
    450                 455                 460

Glu Gln Val Leu Arg Thr Lys Asn
465                 470
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