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(54) Title: RECOMBINANT HUMAN FIBRONECTIN FRAGMENT FOR CELL CULTURE

(57) Abstract: The present invention discloses protein fragment including a cell binding region of fibronectin and methods of using the same for cell culture. Additionally, surfaces modified with such protein fragment and compositions including the same are provided. Desirably, such surfaces allow various cells to adhere thereto yet avoid issues associated with animal-derived products.

RECOMBINANT HUMAN FIBRONECTIN FRAGMENT FOR CELL CULTURE

FIELD OF THE INVENTION

[0001] The present invention relates to protein fragment and surfaces modified therewith which provide cell attachment for various cells.

BACKGROUND OF THE INVENTION

[0002] Extracellular matrix (ECM) protein coated surfaces have been widely used in cell culture. In particular, fibronectin is a commonly used ECM protein used in cell culture that supports cell attachment. ECM proteins used for coating surfaces for cell culture are generally of human or other animal origin and often poorly defined. The use of such ECM proteins can be problematic, for example, in human therapeutic applications where having a defined and animal-free culture is desirable. Thus, there is a need for better defined and animal-component free surfaces that support cell attachment for cell culture.

SUMMARY OF THE INVENTION

[0003] The present invention provides protein fragment including at least a cell binding region of fibronectin and surfaces wherein at least a portion thereof is modified with such protein fragment. Advantageously, surfaces modified with such protein fragment support cell attachment yet avoid issues associated with animal-derived products which are poorly defined and/or may elicit an immune response in therapeutic applications. Additionally, the present invention provides cell culture vessels and methods of culturing cells including a surface modified with a protein fragment of the present invention.

[0004] In one aspect, the present invention provides methods of culturing cells using a surface wherein at least a portion of the surface is modified with a protein fragment including amino acid sequence PLS PPTNLHLEAN PDTGVLTVSW ERSTTPDITG YRITTTPTNG QQGNSLEEVV HADQSSCTFD NLSPGLEYNV SVYTVKDDKE SVPISDTIIP AVPPPTDLRF TNIGPDTMRV TWAPPPSIDL TNFLVRYSPV KNEEDVAELS ISPSDNAVVL TNLLPGTEYV VSVSSVYEQH ESTPLRGRQK TGLDSPTGID FSDITANSFT VHWIAPRATI TGYRIRHHPE HFSGRPREDR VPHSRNSITL TNLTPGTEYV VSIVALNGRE ESPLLIGQQS

TVSDVPRDLE VVAATPTSLL ISWDAPAVTV RYYRITYGET GGNSPVQEFT VPGSKSTATI SGLKPGVDYT ITVYAVTGRG DSPASSKPIS INYRT (SEQ ID NO: 1).

[0005] In one embodiment, the cells are cultured in serum-free media.

[0006] In one embodiment, the cells are derived from a human.

[0007] In one embodiment, the protein fragment further comprises a purification tag. In one embodiment, the purification tag comprises a histidine tag having 5-10 histidines (*e.g.*, amino acid sequence HHHHHH (SEQ ID NO: 2)).

[0008] In embodiments, the cells are LNCaP cells, mesenchymal cells, bone marrow-derived human mesenchymal stem cells, embryonic stem cells or induced pluripotent stem cells. In an embodiment, the cells are human embryonic stem cells such as human embryonic stem cell line H9.

[0009] In another aspect, the present invention provides a protein fragment including amino acid sequence MHHHHHHPLS PPTNLHLEAN PDTGVLTVSW ERSTTPDITG YRITTTPTNG QQGNSLEEVV HADQSSCTFD NLSPGLEYNV SVYTVKDDKE SVPISDTIIP AVPPPTDLRF TNIGPDTMRV TWAPPPSIDL TNFLVRYSPV KNEEDVAELS ISPSDNAVVL TNLLPGTEYV VSVSSVYEQH ESTPLRGRQK TGLDSPTGID FSDITANSFT VHWIAPRATI TGYRIRHHPE HFSGRPREDR VPHSRNSITL TNLTPGTEYV VSIVALNGRE ESPLLIGQQS TVSDVPRDLE VVAATPTSLL ISWDAPAVTV RYYRITYGET GGNSPVQEFT VPGSKSTATI SGLKPGVDYT ITVYAVTGRG DSPASSKPIS INYRT (SEQ ID NO: 3).

[0010] In one embodiment, the present invention provides a surface wherein at least a portion of the surface is modified with a protein fragment including amino acid sequence SEQ ID NO: 3. In one embodiment, the surface is comparable to or exceeds one or more functional characteristics of a fibronectin coated surface. In one embodiment, the fibronectin coated surface includes fibronectin of human-origin.

[0011] In one embodiment, the present invention provides compositions including a surface modified with a protein fragment including the amino acid sequence SEQ ID NO: 3. In one embodiment, the composition is a cell culture vessel or microcarrier. In one embodiment, the cell culture vessel is a dish, a flask, a multi-well plate, or a microscopic slide.

[0012] In another aspect, the present invention provides methods of culturing cells using a surface wherein at least a portion of the surface is modified with a protein fragment including amino acid sequence MHHHHHHPLS PPTNLHLEAN PDTGVLTVSW ERSTTPDITG YRITTTPTNG QQGNSLEEVV HADQSSCTFD NLSPGLEYNV SVYTVKDDKE SVPISDTIIP AVPPPTDLRF TNIGPDTMRV TWAPPPSIDL TNFLVRYSPV KNEEDVAELS ISPSDNAVVL TNLLPGTEYV VSVSSVYEQH ESTPLRGRQK TGLDSPTGID FSDITANSFT VHWIAPRATI TGYRIRHHPE HFSGRPREDR VPHSRNSITL TNLTPGTEYV VSIVALNGRE ESPLLIGQQS TVSDVPRDLE VVAATPTSLL ISWDAPAVTV RYYRITYGET GGNSPVQEFT VPGSKSTATI SGLKPGVDYT ITVYAVTGRG DSPASSKPIS INYRT (SEQ ID NO: 3).

[0013] In one embodiment, the cells are derived from a human. In one embodiment, the cells are embryonic stem cells. In one embodiment, the cells are human embryonic stem cells. In one embodiment, the cells are LNCaP cells. In one embodiment, the cells are bone marrow-derived human cells. In one embodiment, the cells are human embryonic cell line H9. In one embodiment, the cells are induced pluripotent stem cells. In one embodiment, cells are cultured in serum-free media.

[0014] In one embodiment, the cells exhibit comparable or improved cell attachment as compared to a surface coated with fibronectin of human-origin.

[0015] These and other features of the invention will be better understood through a study of the following detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0016] **Figure 1A** is an image of LNCaP human prostate cancer cells attached to a surface that is coated by covalent immobilization of a recombinant protein fragment having the amino acid sequence of SEQ ID NO: 3.

[0017] **Figure 1B** is an image of LNCaP human prostate cancer cells attached to a tissue culture surface that has a coating of human fibronectin thereon.

[0018] **Figure 1C** is an image of LNCaP human prostate cancer cells present on a tissue culture surface without any extracellular matrix protein coating thereon.

[0019] **Figure 1D** is an image of LNCaP human prostate cancer cells attached to a tissue culture surface that is passively coated by a recombinant protein fragment having the amino acid sequence of SEQ ID NO: 3.

[0020] **Figures 2A and 2B** are images of bone marrow-derived human mesencyhmal stem cells (MSCs) of either Donor "A" or Donor "B" attached to a tissue culture surface that is coated with human-origin Fibronectin (HFN).

[0021] **Figures 2C and 2D** are images of bone marrow-derived human MSCs attached to a tissue culture surface that is coated with a recombinant protein fragment having the amino acid sequence of SEQ ID NO: 3.

[0022] **Figure 3** is a graph of the cell yield of bone marrow-derived human MSCs of either Donor "A" or Donor "B" for passages 2 cultured in Mosiac medium on a tissue culture surface coated with either human-origin Fibronectin or a recombinant protein fragment having the amino acid sequence of SEQ ID NO: 3.

[0023] **Figures 4A and 4B** are images of induced pluripotent stem cells (iPSC) cultured in StemPro media on a surface coated with either (A) Matrigel™ (by Becton, Dickinson & Company) or (B) a recombinant protein fragment having the amino acid sequence of SEQ ID NO: 3.

[0024] **Figures 5A and 5B** are images of human embryonic cell line H9 cultured in StemPro media on a surface coated with either (A) a recombinant protein fragment having the amino acid sequence of SEQ ID NO: 3 or (B) Matrigel™.

[0025] **Figures 6A and 6B** are images of induced pluripotent stem cells (iPSC) cultured in mTeSR1 media on a surface coated with either (A) a recombinant protein fragment having the amino acid sequence of SEQ ID NO: 3 or (B) Matrigel™.

DETAILED DESCRIPTION OF THE INVENTION

[0026] As used herein the following terms shall have the definitions set forth below.

[0027] As used herein, the phrase "one or more functional characteristics of a fibronectin coated surface" includes, but is not limited to, cell attachment, growth, migration and differentiation. In one embodiment, one or more functional characteristics of a fibronectin coated surface are characterized using LNCaP cells. In another embodiment, one or more functional characteristics of a fibronectin coated surface are characterized using bone marrow-derived human mesencyhmal stem cells. In yet another embodiment, one or more functional characteristics of a fibronectin coated surface are characterized using induced pluripotent stem cells. In still yet another embodiment, one or more functional characteristics of a fibronectin coated

surface are characterized using human embryonic cell line H9 cells. Desirably, the surface is comparable to or exceeds cell attachment of a fibronectin coated surface.

[0028] As used herein, the phrase "comparable to or exceeds" with regard to the comparison of a cell culture surface coated with a compound of the present invention with a cell culture surface coated with fibronectin refers to the relative similarity or improvement in one or more functional characteristics being assessed. Desirably, quantification thereof would reveal at least 90% similarity in at least one functional characteristic.

[0029] As used herein, the phrase "purification tag" refers to a moiety that facilitates purification of a biological material, such as a protein fragment. Exemplary purification tags include a histidine-tag which includes 5-10 histidines. In one embodiment, the purification tag is a histidine-tag having amino acid sequence HHHHHH (SEQ ID NO: 2).

[0030] Fibronectin is a high-molecular weight (~440kDa) ECM glycoprotein that binds to membrane-spanning receptor proteins called integrins as well as ECM components collagen, fibrin and heparan sulfate proteoglycans (*e.g.*, syndecans). Fibronectin supports cell attachment, growth, migration and differentiation and has been shown to play a key role in wound healing, embryonic development and certain types of carcinogenesis.

[0031] Structurally, fibronectin exists as a dimer, consisting of two nearly identical polypeptide fragment chains linked by a pair of C-terminal disulfide bonds. Each fibronectin monomer has a molecular weight of 230-250 kDa and contains three types of modules: type I, II, and III. The modules are arranged into several functional and protein-binding domains along the length of a fibronectin monomer. There are four fibronectin-binding domains, allowing fibronectin to associate with other fibronectin molecules. One of these fibronectin-binding domains, $I_{1\text{-}5}$, is referred to as the "assembly domain", and it is required for the initiation of fibronectin matrix assembly. Modules $III_{8\text{-}10}$ correspond to the "cell-binding domain" of fibronectin. The RGD sequence (Arg–Gly–Asp) is located in III_{10} and is the site of cell attachment via $\alpha5\beta1$ and $\alpha V\beta3$ integrins on the cell surface. The "synergy site" is in III_9 and has a role in modulating fibronectin's association with $\alpha5\beta1$ integrins. Fibronectin also contains domains for fibrin-binding ($I_{1\text{-}5}$, $I_{10\text{-}12}$), collagen-binding ($I_{6\text{-}9}$), fibulin-1-binding ($III_{13\text{-}14}$), heparin-binding and syndecan-binding ($III_{12\text{-}14}$).

[0032] Though not meant to be limited by any theory with the subject invention, the protein fragment of the present invention includes a recombinant fibronectin fragment wherein regions therein are believed to interact with one another in a manner that provides one or more functional characteristics of fibronectin. In particular, surfaces coated with such protein fragment are comparable to or exceed cell attachment of a human-origin fibronectin coated surface.

[0033] The protein fragment of the present invention may be produced using conventional recombinant technologies. Similarly, such protein fragment may be purified using conventional techniques to a degree suitable for a given application.

[0034] It is understood that one of skill in the art could substitute one or more amino acids of the protein fragment described herein without compromising the ability of the resultant protein fragment when coated on a surface to support cell culture.

[0035] In particular, protein fragment of the present invention may have conservative substitution of one or more amino acids. A conservative substitution being defined as the side chain of the respective amino acid being replaced by a side chain of similar chemical structure and polarity, the side chain being derived from a genetically coded or not genetically coded amino acid. Families of amino acids of this kind having similar side chains are known in the art. They comprise for instance amino acids having basic side chains (lysine, arginine, histidine), acidic side chains (aspartic acid, glutamic acid), uncharged polar side chains (glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (threonine, valine, isoleucine) and aromatic side chains (tyrosine, phenylalanine, tryptophane, histidine).

[0036] Protein fragment of the present invention is useful for applications where one or more functional properties of fibronectin are desirable or where modification of one or more signaling properties associated with fibronectin are desirable. For example, protein fragment of the present invention may be employed as a therapeutic to inhibit cell signaling. Antimetastatic effects of protein fragments derived from fibronectin are described for example, in Kato *et al.*, *Clinical Cancer Research*, 8:2455-2462 (2002).

[0037] Likewise, surfaces modified using the protein fragment of the present invention are useful for applications where one or more functional properties of fibronectin are desirable or where modification of one or more signaling properties associated with fibronectin are desirable. Such applications include *in vitro* cell culture as well as fostering cell growth *in vivo*. For example, prosthetic devices coated with fibronectin are desirable to foster growth and migration of new epithelial tissue. Preparation of fibronectin coated prosthetic devices are well known to one of skill in the art (*see, e.g.,* U.S. Patent No. 5,171,318).

[0038] Surfaces modified with a protein fragment of the present invention may employ either passive (*i.e.*, non-covalent) coating, covalent immobilization of the compound or any other method of deposition of the compound.

[0039] Surfaces modified with a protein fragment of the present invention for use in cell culture include cell culture vessels and microcarriers. Suitable cell culture vessels for use in the present invention are well known to one of skill in the art. Examples of suitable vessels include, but are not limited to, dishes, flasks, multi-well plates, and microscopic slides. Microcarriers suitable for cell culture are also well known to one of skill in the art. *See, e.g.*, Nie, *Biotechnol. Prog.*, 25(1):20-31 (2009).

[0040] Advantageously, cells cultured using the surfaces of the present invention are suitable for therapeutic application (*e.g.*, in wound healing) and avoid problems inherent to the use of isolated fibronectin from a different source which may otherwise elicit an immunogenic response and even lead to rejection of transplanted cells.

EXAMPLES

[0041] The protein fragment of the present invention having amino acid sequence (SEQ ID NO: 3) was produced using a commercially available service. This protein fragment was then added on a surface to modify the surface. The surface of a cell culture vessel was modified by a protein fragment of the present invention using well established techniques known to a skilled artisan. In brief, covalent immobilization of a protein fragment was done by EDC/NHS Chemistry, protein fragment solution was added onto the surface followed by an incubation and wash step. Alternatively, for passive adsorption of a protein fragment, the protein fragment was diluted in phosphate buffered saline and the resultant solution applied to a tissue

culture surface. Incubation was allowed at room temperature for at least 2 hrs after which the surface was washed and the tissue culture surface used. For human embryonic stem cells and induced pluripotent stem cells, tissue culture treated surface was coated for 2 hrs after which the coating was removed prior to seeding cells.

[0042] Cell cultures were established based on established protocols are well known to a skilled artisan.

[0043] To explore the ability of a surface modified by a protein fragment of the present invention to support cell culture and/or provide one or more functional characteristics of a fibronectin coated surface, cells were seeded and monitored on both such surfaces under the same culture conditions.

[0044] In brief, LNCaP cells, bone marrow-derived human mesencyhmal stem cells, human embryonic cell line H9 available from ATCC, Lonza, and Wicell were cultured according to supplier's instructions. Likewise, human induced pluripotent stem cells (*i.e.*, Gibco® Episomal human induced pluripotent stem cell line) were cultured according to supplier's instructions.

[0045] For cell attachment assays, cells were seeded in serum-free media. For MSC, cells were cultured in Mosaic formulation for MSC culture with media supplied by BD Technologies (BDT). For human embryonic stem cell culture (*i.e.*, H9 cell line and human induced pluripotent stem cells (Gibco® Episomal human induced pluripotent stem cell line)), cells were cultured in StemPro human embryonic stem cell serum-free media (Life Technologies) or defined mTeSR1 media (Stem Cell Technologies).

[0046] Cell attachment was monitored by visual inspection under the microscope and images captured. Quantification was performed by measuring cell count using techniques well known to a skilled artisan.

[0047] As reflected in Figures 1A and D, cell attachment and spreading of LNCaP human prostate cancer cells was evident on surfaces coated by either covalent modification or passive adsorption of with a protein fragment having the amino acid sequence of SEQ ID NO: 3. In fact, cell attachment and spreading of LNCaP human prostate cancer cells on surfaces modified with a protein fragment of SEQ ID NO: 3 was comparable to that exhibited on a surface coated with human fibronectin which served as a positive control (*see* Figure 1B). In contrast, cell attachment and

spreading was significantly reduced in tissue culture surface without a coating thereon which served as a negative control (*see* Figure 1C).

[0048] Similarly, surfaces modified by a protein fragment having the amino acid sequence of SEQ ID NO: 3 supported cell attachment and spreading of bone marrow-derived MSCs in a manner comparable to that of a surface coated with human-origin fibronectin as reflected upon a visual inspection (*see* Figures 2A-D). Further, a quantitative analysis of the cell yield of bone-marrow derived MSC from two different donors (Donor "A" and Donor "B") for passages 2 cultured in Mosiac medium on either a surface modified by a protein fragment having the amino acid sequence of SEQ ID NO: 3 or a surface coated with human-origin fibronectin also revealed comparable % cell yield between the different coated surfaces for each donor (*see* Figure 3).

[0049] Additionally, comparable morphology was exhibited by induced pluripotent stem cells cultured in StemPro media on a surface coated with Matrigel™ or a surface modified with a protein fragment having the amino acid of SEQ ID NO: 3 (*see* Figures 4A and 4B).

[0050] Likewise, comparable morphology was exhibited by human embryonic stem cell line H9 cultured in StemPro media on a surface modified with a protein fragment having the amino acid of SEQ ID NO: 3 or a surface coated with Matrigel™ (*see* Figures 5A and 5B).

[0051] Similarly, comparable morphology was exhibited by induced pluripotent stem cells cultured in mTeSR1 media on a surface modified with a protein fragment having the amino acid of SEQ ID NO: 3 or a surface coated with Matrigel™ (*see* Figures 6A and 6B).

[0052] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but is intended to cover modifications that are within the spirit and scope of the invention, as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method of culturing cells using a surface wherein at least a portion of the surface is modified with a protein fragment comprising amino acid sequence PLS PPTNLHLEAN PDTGVLTVSW ERSTTPDITG YRITTTPTNG QQGNSLEEVV HADQSSCTFD NLSPGLEYNV SVYTVKDDKE SVPISDTIIP AVPPPTDLRF TNIGPDTMRV TWAPPPSIDL TNFLVRYSPV KNEEDVAELS ISPSDNAVVL TNLLPGTEYV VSVSSVYEQH ESTPLRGRQK TGLDSPTGID FSDITANSFT VHWIAPRATI TGYRIRHHPE HFSGRPREDR VPHSRNSITL TNLTPGTEYV VSIVALNGRE ESPLLIGQQS TVSDVPRDLE VVAATPTSLL ISWDAPAVTV RYYRITYGET GGNSPVQEFT VPGSKSTATI SGLKPGVDYT ITVYAVTGRG DSPASSKPIS INYRT (SEQ ID NO: 1); wherein the cells are cultured in serum-free media.

2. The method of Claim 1 wherein the cells are derived from a human.

3. The method of Claim 1, wherein the cells are LNCaP cells, bone marrow-derived mesencyhmal stem cells, embryonic cell line H9 or induced pluripotent stem cells.

4. The method of Claim 1, wherein said cells are embryonic stem cells.

5. A protein fragment comprising amino acid sequence MHHHHHHPLS PPTNLHLEAN PDTGVLTVSW ERSTTPDITG YRITTTPTNG QQGNSLEEVV HADQSSCTFD NLSPGLEYNV SVYTVKDDKE SVPISDTIIP AVPPPTDLRF TNIGPDTMRV TWAPPPSIDL TNFLVRYSPV KNEEDVAELS ISPSDNAVVL TNLLPGTEYV VSVSSVYEQH ESTPLRGRQK TGLDSPTGID FSDITANSFT VHWIAPRATI TGYRIRHHPE HFSGRPREDR VPHSRNSITL TNLTPGTEYV VSIVALNGRE ESPLLIGQQS TVSDVPRDLE VVAATPTSLL ISWDAPAVTV RYYRITYGET GGNSPVQEFT VPGSKSTATI SGLKPGVDYT ITVYAVTGRG DSPASSKPIS INYRT (SEQ ID NO: 3).

6. A surface wherein at least a portion of the surface is modified with the protein fragment of Claim 5.

7. The surface of Claim 6, wherein the surface is comparable to or exceeds one or more functional characteristics of a fibronectin coated surface.

8. The surface of Claim 7, wherein the fibronectin coated surface comprises fibronectin of human-origin.

9. A composition comprising the surface of Claim 6 thereon.

10. The composition of Claim 9 which is a cell culture vessel or microcarrier.

11. The composition of Claim 10, wherein the cell culture vessel is a dish, a flask, a multi-well plate, or a microscopic slide.

12. A method of culturing cells using a surface wherein at least a portion of the surface is modified with a protein fragment comprising amino acid sequence MHHHHHHPLS PPTNLHLEAN PDTGVLTVSW ERSTTPDITG YRITTTPTNG QQGNSLEEVV HADQSSCTFD NLSPGLEYNV SVYTVKDDKE SVPISDTIIP AVPPPTDLRF TNIGPDTMRV TWAPPPSIDL TNFLVRYSPV KNEEDVAELS ISPSDNAVVL TNLLPGTEYV VSVSSVYEQH ESTPLRGRQK TGLDSPTGID FSDITANSFT VHWIAPRATI TGYRIRHHPE HFSGRPREDR VPHSRNSITL TNLTPGTEYV VSIVALNGRE ESPLLIGQQS TVSDVPRDLE VVAATPTSLL ISWDAPAVTV RYYRITYGET GGNSPVQEFT VPGSKSTATI SGLKPGVDYT ITVYAVTGRG DSPASSKPIS INYRT (SEQ ID NO: 3).

13. The method of Claim 12, wherein the cells are derived from a human.

14. The method of Claim 12, wherein said cells are embryonic stem cells.

15. The method of Claim 13, wherein said cells are embryonic stem cells.

16. The method of Claim 12, wherein said cells are LNCaP cells.

17. The method of Claim 12, wherein said cells are bone marrow-derived human mesencyhmal stem cells.

18. The method of Claim 12, wherein said cells are human embryonic cell line H9.

19. The method of Claim 12, wherein said cells are induced pluripotent stem cells.

20. The method of Claim 12, wherein said cells exhibit comparable or improved cell attachment as compared to a surface coated with fibronectin of human-origin.

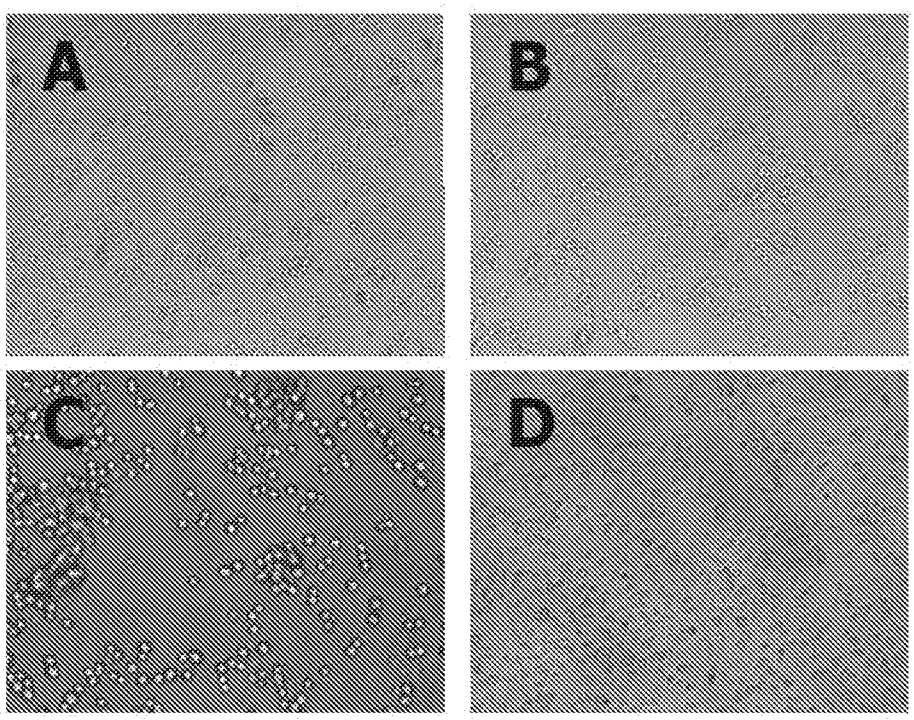

FIG. 1

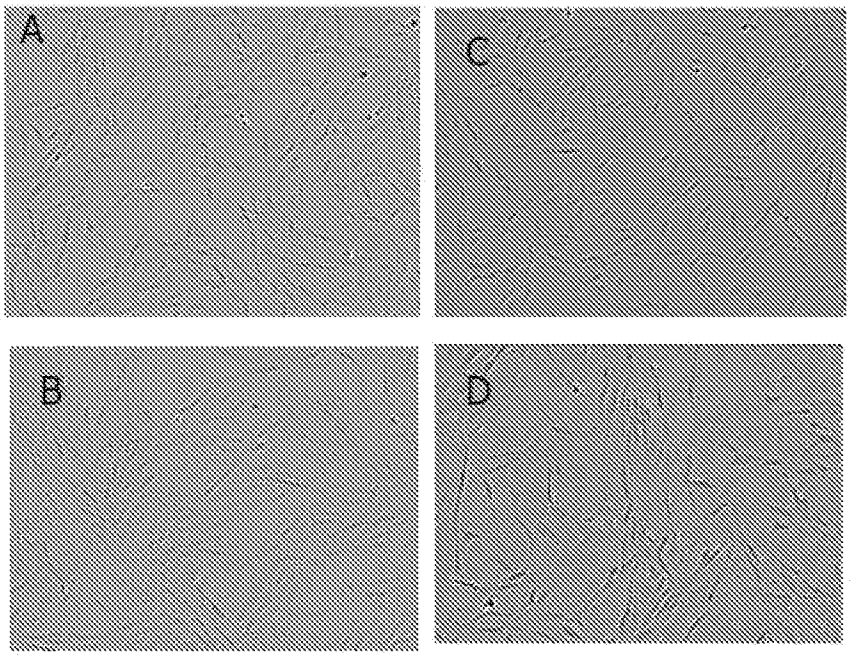

FIG. 2

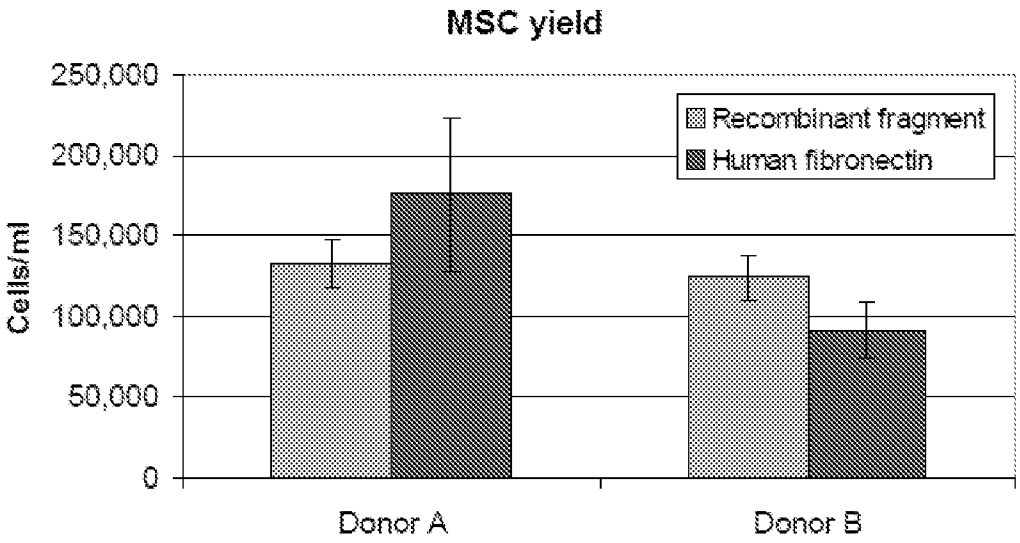
MSC yield
250,000
200,000
150,000
100,000
50,000
0
Cells/ml
Recombinant fragment
Human fibronectin
Donor A
Donor B

FIG. 3

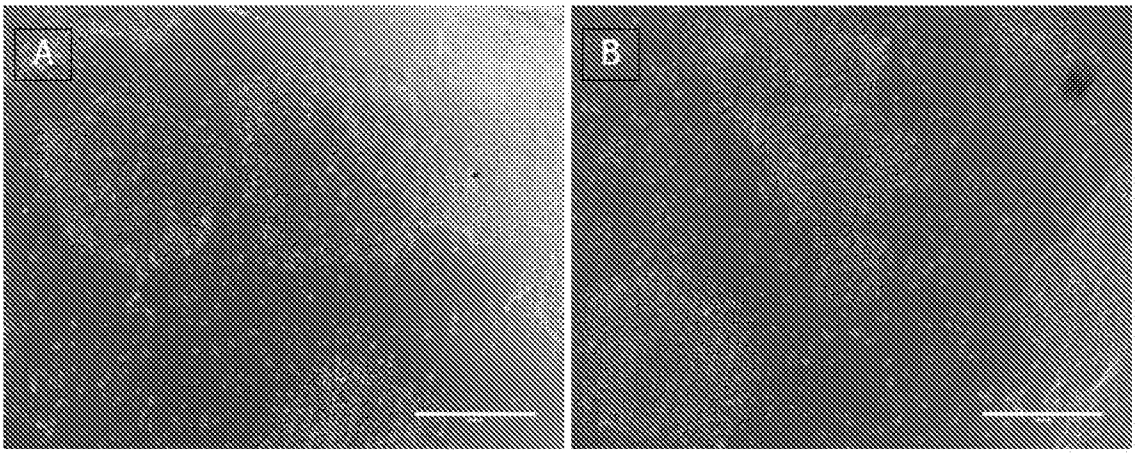

FIG. 4

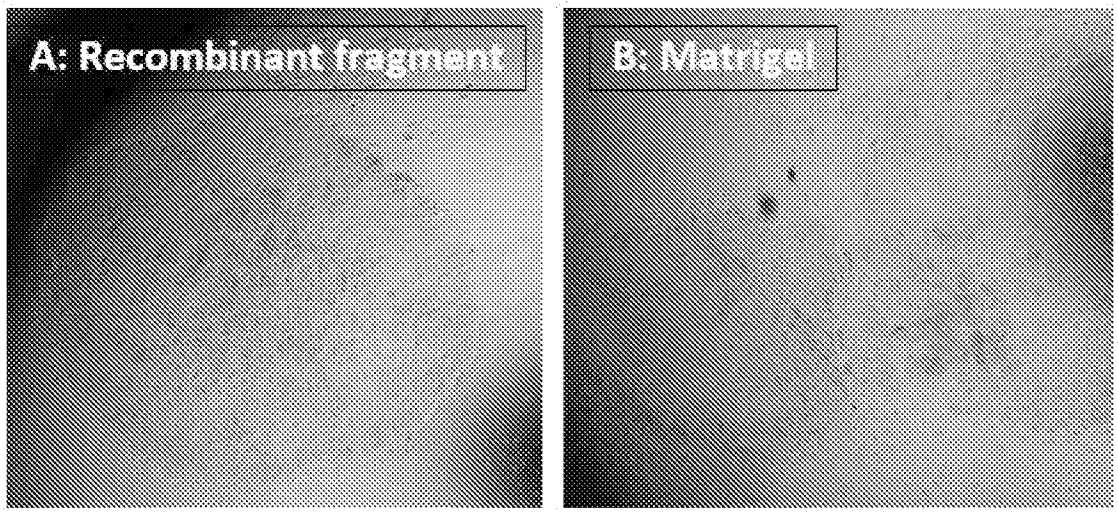

FIG. 5

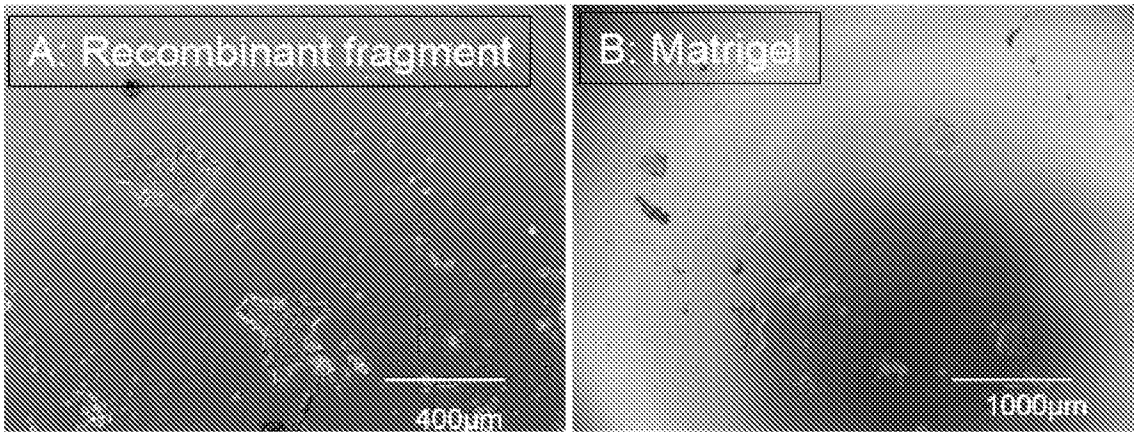

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/066138

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/131425 A1 (GARCIA ANDRES J [US] ET AL) 5 June 2008 (2008-06-05) paragraphs [0055], [0089]; examples 4,6; sequence 2	1-20
X	US 4 589 881 A (PIERSCHBACHER MICHAEL D [US] ET AL) 20 May 1986 (1986-05-20)	1-4
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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18 December 2013	07/01/2014

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/066138

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KIMIZUKA F ET AL: "Production and characterization of functional domains of human fibronectin expressed in Escherichia coli", JOURNAL OF BIOCHEMISTRY, JAPANESE BIOCHEMICAL SOCIETY / OUP, TOKYO; JP, vol. 110, no. 2, 1 August 1991 (1991-08-01), pages 284-291, XP009130307, ISSN: 0021-924X	1-4
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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