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(54) **Titre : APPLICATION DE LAMININE A UNE CULTURE DE CELLULES ENDOTHELIALES DE LA CORNEE**
(54) **Title: APPLICATION OF LAMININ TO CORNEAL ENDOTHELIAL CELL CULTURE**

(57) **Abrégé/Abstract:**

The present invention provides a method of culturing corneal endothelial cells. More specifically, the present invention provides a composition for culturing or growing corneal endothelial cells, comprising at least one agent consisting of laminins and fragments thereof which express in corneal endothelial cells. Specifically, the present invention can comprise laminin 511 (alpha5 beta1 gamma1) and laminin 512 (alpha5 beta2 gamma 1). The present invention further provides a culture container for corneal endothelial cells, which is coated with the composition of the present invention. Furthermore, the present invention provides a method for culturing corneal endothelial cells comprising the step of using the composition or the container of the present invention to culture the corneal endothelial cells.



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(54) Title: APPLICATION OF LAMININ TO CORNEAL ENDOTHELIAL CELL CULTURE

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culture the corneal endothelial cells.



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Description

[Title of Invention]

APPLICATION OF LAMININ TO CORNEAL ENDOTHELIAL CELL CULTURE

[Detailed Description of Invention]

5 [Technical Field]

[0001]

The present invention relates to use of laminin as a component for culturing or growing corneal endothelial cells culture. Specifically, the present invention is related to a composition, container, culturing method and the like for culturing or growing of corneal endothelial cells comprising laminin.

[Background Art]

[0002]

Human corneal endothelial cells are present at a density of about 3000 cells per square millimeter at birth. However, once impaired, human corneal endothelial cells do not have an ability to regenerate. As such, corneal endothelial cells are deemed difficult to culture. Due to the current state where it is difficult to culture or grow corneal endothelial cells in transplant techniques, a treatment or surgery on a corneal endothelium is practically impossible. There is a shortage of donation of corneas in Japan, where approximately 1700 cases of corneal transplants are annually performed domestically in comparison to about 2600 patients awaiting a corneal transplant.

25 [Citation List]

[Patent Literature]

[0003]

[PTL 1] Japanese Laid-Open Publication No. 2011-78370

[PTL 2] WO 2013/047763

30 [PTL 3] WO 2011/024070

[PTL 4] WO 2010/140464

[Non-Patent Literature]

[NPTL 1] Journal of the Medical Society of Toho University Vol.56, No.1, Page.39 (01.01.2009)

35 [NPTL 2] Nippon Ganka Gakkai Zasshi [Journal of Japanese Ophthalmological Society] Vol.105, extra edition, Page.196 (03.15.2001)

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[NPTL 5] Cell Adh Migr. Jan-Feb 2013; 7(1):142-9. doi: 10.4161/cam.22125. Epub Oct 17, 2012

5 [NPTL 6] J Cell Biochem. Feb 15, 2007; 100(3):545-56.

[SUMMARY OF INVENTION]

[Solution to Problem]

[0004]

10 The inventors of the present application have consummated the present invention by discovering that a specific laminin is useful in culturing and growing corneal endotheliums. Thus, the present invention provides the following representative items:

(1) A composition for culturing or growing corneal endothelial cells, comprising at least one agent consisting of laminins and fragments thereof which express in corneal endothelial cells.

(2) The composition according to item 1, wherein the laminins comprise laminin 511 (alpha5 beta1 gamma1) and laminin 512 (alpha5 beta2 gamma 1).

20 (3) The composition according to item 1 or 2, wherein the fragments has cell adhesion capability of corneal endothelial cells.

(4) The composition according to any one of items 1-3, wherein the agent is laminin 511, laminin 521 or laminin 511-E8 fragment.

(5) The composition according to any one of items 1-4, wherein the corneal endothelial cells are from human.

25 (6) A medium for culturing corneal endothelial cells, comprising the composition according to any one of items 1-5.

(7) A culture container for corneal endothelial cells, which is coated with the composition according to item 1.

30 (8) A method for culturing corneal endothelial cells comprising the step of using the composition according to any one of items 1-5.

(9) A method for culturing corneal endothelial cells comprising the step of using the medium according to item 6.

35 (10) A method for culturing corneal endothelial cells comprising the step of using the container according to item 7.

It is understood that the above mentioned feature(s) may be used in combination. Further embodiments and advantages of the present invention will be appreciated by the skilled in the art upon reading

and understanding the detailed description of the invention as provided below as necessary.

[Advantageous Effects of Invention]

[0005]

- 5 The present invention provides a component that enables culture, maintenance, and growth of corneal endothelial cells (in particular, human corneal endothelial cells).

[Brief Description of Drawings]

[0006]

- 10 [Fig. 1] Figure 1 is a diagram showing mRNA expression of various laminin chains in human corneal endothelial cells. The diagram, from the left, shows a molecular weight marker, a laminin $\alpha 1$ chain, $\alpha 2$ chain, $\alpha 3$ chain, $\alpha 4$ chain, $\alpha 5$ chain, $\beta 1$ chain, $\beta 2$ chain, $\beta 3$ chain, $\beta 4$ chain, $\gamma 1$ chain, $\gamma 2$ chain, and $\gamma 3$ chain.

- 15 [Fig. 2] Figure 2 shows mRNA expression of various integrin chains in human corneal endothelial cells. The top row, from the left, shows integrin $\alpha 1$ chain, $\alpha 2$ chain, $\alpha 3$ chain, $\alpha 4$ chain, $\alpha 5$ chain, $\alpha 6$ chain, $\alpha 7$ chain, $\alpha 8$ chain, $\alpha 9$ chain, $\alpha 10$ chain, $\alpha 11$ chain, αE chain, αV chain, and αL chain. The bottom row, from the left, shows
20 integrin αM chain, αX chain, αD chain, αIIb chain, $\beta 1$ chain, $\beta 2$ chain, $\beta 3$ chain, $\beta 4$ chain, $\beta 5$ chain, $\beta 6$ chain, $\beta 7$ chain, and $\beta 8$ chain.

- [Fig. 3A] Figure 3A-Figure 3C collectively show the expression analysis of various integrin chains in human corneal endothelial
25 cells by flow cytometry. Figure 3A shows the expression analysis of various integrin chains in human corneal endothelial cells by flow cytometry. The top row, from the left, shows integrin $\alpha 1$, $\alpha 2$, and $\alpha 3$. The bottom row, from the left, shows integrin $\alpha 4$, $\alpha 5$, and $\alpha 6$.

- 30 [Fig. 3B] Figure 3B also shows the expression analysis of various integrin chains in human corneal endothelial cells by flow cytometry. The top row, from the left, shows integrin αE , αV , and αL . The bottom row, from the left, shows integrin αM , αX , and αIIb (CD41a).

- [Fig. 3C] Figure 3C also shows the expression analysis of various
35 integrin chains in human corneal endothelial cells by flow cytometry. The top row, from the left, shows integrin αIIb (CD41b), $\beta 1$, and $\beta 2$. The bottom row, from the left, shows integrin $\beta 3$, $\beta 4$, and $\beta 7$.

[Fig. 4] Figure 4 is a picture showing that laminin 511 and laminin

521 promote cell adhesion of human corneal endothelial cells. The top row, from the left, shows laminin 511, laminin 521, and laminin 211. The bottom row, from the left, shows no coating, FNC coating, and gelatin coating. The scale is 50 μm .

5 [Fig. 5]Figure 5 is a graph showing that laminin 511 and laminin 521 promote cell adhesion of human corneal endothelial cells. The y-axis indicates the cell numbers (% control). The x-axis, from the left, indicates no coating (control), laminin 511, laminin 521, laminin 211, FNC coating, gelatin coating, and laminin 511-E8
10 fragment.

[Fig. 6]Figure 6 is a graph showing that a laminin 511-E8 fragment promotes cell adhesion of human corneal endothelial cells. The y-axis indicates the number of cells (% control). The x-axis, from the left, indicates no coating (control), each concentration of laminin 511-E8 fragments (in order: 0.001 $\mu\text{g}/\text{cm}^2$, 0.01 $\mu\text{g}/\text{cm}^2$, 0.1 $\mu\text{g}/\text{cm}^2$,
15 0.5 $\mu\text{g}/\text{cm}^2$, 1.0 $\mu\text{g}/\text{cm}^2$, and 1.5 $\mu\text{g}/\text{cm}^2$), and FNC coating mix.

[Fig. 7]

Figure 7 is a graph showing that laminin 511, laminin 521, and laminin 511-E8 fragments promote cell adhesion of human corneal endothelial
20 cells. The y-axis indicates the relative value (%) of BrdU absorbance with respect to a control. The x-axis, from the left, indicates no coating (control), laminin 511, laminin 521, laminin 211, FNC coating mix, and laminin 511-E8 fragments (from the left, 0.5 $\mu\text{g}/\text{cm}^2$, 1.0 $\mu\text{g}/\text{cm}^2$, and 1.5 $\mu\text{g}/\text{cm}^2$).

25 [Fig. 8]Figure 8 is a picture from a phase-contrast microscope on day 2 of culture, showing that laminin 511 and laminin 521 enhance the efficiency of culturing human corneal endothelial cells. The top left shows laminin 511, top right shows laminin 521, bottom left shows laminin 211, and bottom right shows no coating. The bar
30 indicates 100 μm .

[Fig. 9]Figure 9 is a picture from a phase-contrast microscope on day 20 of culture, showing that laminin 511 and laminin 521 enable culturing human corneal endothelial cells at a high cellular density. Top left shows laminin 511, top right shows laminin 521, bottom
35 left shows laminin 211, and bottom right shows no coating. The bar indicates 100 μm .

[Fig. 10]Figure 10 is a picture showing that laminin 511 and laminin 521 enable culturing human corneal endothelial cells at a high

cellular density. The red dye indicates Na^+/K^+ -ATPase and the blue dye indicates DAPI. The top left shows laminin 511, top right shows laminin 521, bottom left shows laminin 211, and bottom right shows no coating. The bar indicates 100 μm .

5 [Fig. 11]Figure 11 is a picture showing that laminin 511 and laminin 521 enable culturing human corneal endothelial cells at a high cellular density. The green dye indicates ZO-1 and the blue dye indicates DAPI. The top left shows laminin 511, top right shows laminin 521, bottom left shows laminin 211, and bottom right shows
10 no coating. The bar indicates 100 μm .

[Fig. 12]Figure 12 is a graph indicating that cell adhesion of a human corneal endothelial cell is promoted even in a culture in which laminin 521 and laminin 511-E8 fragment are added to a culture medium. The y-axis indicates the number of cells (% control). The
15 x-axis, from the left, indicates no coating (control), each concentration of laminin 521 (in order: 1.0 $\mu\text{g}/\text{cm}^2$, 2.0 $\mu\text{g}/\text{cm}^2$ and 4.0 $\mu\text{g}/\text{cm}^2$), each concentration of laminin 511-E8 fragments (in order: 1.0 $\mu\text{g}/\text{cm}^2$, 2.0 $\mu\text{g}/\text{cm}^2$ and 4.0 $\mu\text{g}/\text{cm}^2$), and FNC coating mix.

[Description of Embodiments]

20 [0007]

The present invention is described below. Throughout the present specification, an expression in a singular form should be understood as encompassing the plural form of the concept unless specifically stated otherwise. Thus, a singular article (e.g., "a", "an", "the" and the like in English) should be understood as encompassing the
25 plural form of the concept unless specifically stated otherwise. Further, the terms used herein should be understood as being used in the meaning as conventionally used in the art unless specifically stated otherwise. Thus, unless defined otherwise, all the specific
30 technical terms and scientific terminology used herein bears the same meaning as generally understood by those skilled in the art to which the present invention belongs. In a case of a contradiction, the present specification (including the definitions) takes precedence.

35 [0008]

(Definition)

As used herein, "corneal endothelial cell" is used in the meaning as conventionally used in the art. A cornea is one of the laminar

tissues constituting an eye. A cornea is transparent and is located at the closest part to the outside environment. In humans, a cornea is considered as consisting of five layers, in order from the outside (body surface), a corneal epithelium, a Bowman's membrane, a substantia propia, a Descemet's membrane (corneal endothelial basement membrane), and a corneal endothelium. In particular, unless otherwise specified, the portions other than the epithelium and the endothelium may be referred together as the "corneal stroma" and is called as such herein. As used herein, "HCEC" is an abbreviation of human corneal endothelial cells. It is understood that for corneal endothelial cells used in the present invention, naturally occurring cells, as well as cells differentiated from a stem cell, i.e., differentiation-induced cells from iPS or the like, can be used. [0009]

As used herein, "isolated" refers to a state where the amount of materials that naturally come together with the cells in a normal environment is at least reduced, and preferably a state of being substantially free of such materials. Thus, an isolated cell, tissue or the like refers to a cell that is substantially free of other materials (e.g., other cells, protein, or nucleic acid) that comes together in the cell in a natural environment. [0010]

As used herein, "corneal endothelial formulation" refers to any formulation or medicinal agent comprising a corneal endothelium or a corneal endothelium cell. Since corneal endothelial cells that are produced and cultured with a method of the present invention can be formulated, a corneal endothelial formulation/agent can be manufactured using corneal endothelial cells that are cultured and produced with a method of the present invention. [0011]

As used herein, "extracellular matrix" is also called (ECM) and refers to a material that exists between somatic cells, regardless of whether the cell is an epithelial cell or a non-epithelial cell. Since an extracellular matrix is generally produced by cells, an extracellular matrix is a biological material. An extracellular domain is involved not only in supporting tissue but also in constituting the internal environment needed for the survival of all somatic cells. An extracellular matrix is generally produced

from connective tissue cells. However, some are secreted from the cells themselves that have a basement membrane, such as an epithelial cell or an endothelial cell. An extracellular matrix is roughly divided into fibrous components and a matrix that fills the space between the fibrous components. The fibrous components include collagenous fibers and elastic fibers. The basic constituent of the matrix is glucosaminoglycan (acid mucopolysaccharide), the majority of which forms a macromolecule of proteoglycans (acid mucopolysaccharide-protein complex) by binding with a non-collagenous protein. In addition, a matrix comprises laminin in the basement membrane, microfibril in the periphery of elastic fibers, fibers, and a glycoprotein such as fibronectin on the cell surface. The basic structure is the same in specialized tissue. For example, in hyaline cartilage, a cartilage matrix comprising a characteristically large amount of proteoglycans is produced by a chondroblast, and in bone, a bone matrix where calcinosis takes place is produced by an osteoblast. In this regard, representative materials constituting an extracellular matrix include, but not limited to, collagen I, collagen III, collagen IV, collagen V, elastin, collagen VIII, vitronectin, fibronectin, laminin, thrombospondin, and proteoglycans (e.g., decorin, biglycan, fibromodulin, lumican, hyaluronic acid, aggrecan and the like). Various extracellular matrices having a role in cell adhesion can be utilized in the present invention.

[0012]

As used herein, "laminin" is a protein constituting a basement membrane of an extracellular matrix. Laminin promotes multicellularity/tissue construction and maintenance thereof, cell adhesion, cell migration, and cell growth and has a close relationship with cancer cells. A laminin is considered to be expressed in the early stage (2-cell stage) of blastogenesis. Laminin is a heterotrimer consisting of each one of an α chain, a β chain and a γ chain. For the naming of a laminin, the nomenclature in the order of discovery (laminin-1, laminin-2, etc) is known. However, since correspondence to subunits is not considered, a newer naming method, in which the name of the subclasses α , β , or γ (a three digit number, the digit of the hundred indicates α , the digit of ten indicates β , and the digit of one indicates γ) is described

together, is employed herein. In case of $\alpha 1$, $\beta 1$ and $\gamma 1$, such a laminin is called laminin 111. For a laminin, five types of α chains, 3 types of β chains, and three types of γ chains have been discovered. Thus, the theoretic maximum number of combinations is $5 \times 3 \times 3 = 45$, and 45 types of laminin molecules are possible. However, it is believed that not all of the combinations exist in nature. Each subunit is called LAMA1, LAMA2, LAMA3, LAMA4, or LAMA5 for an α chain, LAMB1, LAMB2, or LAMB3 for a β chain, and LAMC1, LAMC2, or LAMC3 for a γ chain. Laminin proteins used in the present invention may be those in a natural form or those in a modified form where one or more amino acid residues are modified while retaining the biological activity, especially the cell adhesion promoting activity. Further, the laminin proteins in the present invention are not limited in the origin, production method thereof or the like, as long as the laminin protein has the features described herein. Thus, the laminin proteins used in the present invention may be of any naturally occurring proteins, proteins expressed from a recombinant DNA by a genetic engineering method, or chemically synthesized proteins. The origin of the laminin proteins used in the present invention is not particularly limited, but is preferably derived from a human. When culturing a human cell for the purpose of obtaining a medical material, it is preferable, but is not limited to, using a laminin derived from a human in order to avoid the use of a material derived from another animal.

[0013]

Binding molecules of a laminin are known. $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 2\beta 2$, $\alpha 3\beta 1$, $\alpha 6\beta 1$, $\alpha 6\beta 4$, $\alpha 7\beta 1$, $\alpha 9\beta 1$, $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 8$ are integrins known as laminin receptor.

[0014]

The following Table describes representative laminins and the explanation therefor.

trimer composition (name)	main expression site	integrin binding specificity
$\alpha 1 \beta 1 \gamma 1$ (laminin-1) $\alpha 1 \beta 2 \gamma 1$ (laminin-3)	Fetal tissue	$\alpha 5 \beta 1$
$\alpha 2 \beta 1 \gamma 1$ (laminin-2) $\alpha 2 \beta 2 \gamma 1$ (laminin-4) $\alpha 2 \beta 1 \gamma 3$ (laminin-12)	Muscles, nerves (Schwann cell)	$\alpha 7 \beta 1, \alpha 5 \beta 1, \alpha 3 \beta 1$
$\alpha 3 \beta 3 \gamma 2$ (laminin-5) $\alpha 3 \beta 1 \gamma 1$ (laminin-6) $\alpha 3 \beta 2 \gamma 1$ (laminin-7)	Skin, lung, and other epithelial tissue	$\alpha 3 \beta 1, \alpha 5 \beta 4$
$\alpha 4 \beta 1 \gamma 1$ (laminin-8) $\alpha 4 \beta 2 \gamma 1$ (laminin-9)	Blood vessel	$\alpha 5 \beta 1, \alpha 3 \beta 1$
$\alpha 5 \beta 1 \gamma 1$ (laminin-10) $\alpha 5 \beta 2 \gamma 1$ (laminin-11)	Blood vessel, liver, lung, and other epithelial tissue	$\alpha 3 \beta 1, \alpha 5 \beta 1$

[0015]

As used herein, " $\alpha 1$ chain" (LAMA1) is a subunit of a laminin protein of a cell adhesion molecule in an extracellular matrix, and is called

5 LAMA1, LAMA, S-LAM-alpha, or the like. For human LAMA1, the sequences of the gene and protein are registered as NCBI registration numbers NM_005559 and NP_005550, respectively. OMIM is identified with an accession number 150320. When used for the purpose herein, it is understood that " $\alpha 1$ chain" or "LAMA1" means not only a protein having

10 an amino acid sequence described in the specific sequence number or accession number (or a nucleic acid encoding the protein), but also a functionally active derivative, a functionally active fragment, or a homologue thereof, or a mutant encoded by a nucleic acid that hybridizes to a nucleic acid encoding a protein under

15 high or low stringency condition.

[0016]

As used herein, " $\alpha 2$ chain" (LAMA2) is a subunit of a laminin protein of a cell adhesion molecule in an extracellular matrix, and is called

20 LAMA2, LAMM, or the like. For human LAMA2, the sequences of the gene and protein are registered as NCBI registration numbers NM_000426 and NP_000417, respectively. OMIM is identified with an accession number 156225. When used for the purpose herein, it is understood that " $\alpha 2$ chain" or "LAMA2" means not only a protein having an amino acid sequence described in the specific sequence number

25 or accession number (or a nucleic acid encoding the protein), but also a functionally active derivative, a functionally active

fragment, or a homologue thereof, or a mutant encoded by a nucleic acid that hybridizes to a nucleic acid encoding a protein under high or low stringency condition.

[0017]

5 As used herein, " α 3 chain" (LAMA3) is a subunit of a laminin protein of a cell adhesion molecule in an extracellular matrix, and is called LAMA3, BM600, E170, LAMNA, LOCS, lama3a, or the like. For human LAMA3, the sequences of the gene and protein are registered as NCBI registration numbers NM_000227 and NP_000218, respectively. OMIM
10 is identified with an accession number 600805. When used for the purpose herein, it is understood that " α 3 chain" or "LAMA3" means not only a protein having an amino acid sequence described in the specific sequence number or accession number (or a nucleic acid encoding the protein), but also a functionally active derivative,
15 a functionally active fragment, or a homologue thereof, or a mutant encoded by a nucleic acid that hybridizes to a nucleic acid encoding a protein under high or low stringency condition.

[0018]

As used herein, " α 4 chain" (LAMA4) is a subunit of a laminin protein
20 of a cell adhesion molecule in an extracellular matrix, and is called LAMA4, LAMA3, LAMA4*-1, CMD1JJ or the like. For human LAMA4, the sequences of the gene and protein are registered as NCBI registration numbers NM_001105206 and NP_001098676, respectively. OMIM is identified with an accession number 600133. When used for the purpose
25 herein, it is understood that " α 4 chain" or "LAMA4" means not only a protein having an amino acid sequence described in the specific sequence number or accession number (or a nucleic acid encoding the protein), but also a functionally active derivative, a functionally active fragment, or a homologue thereof, or a mutant
30 encoded by a nucleic acid that hybridizes to a nucleic acid encoding a protein under high or low stringency condition.

[0019]

As used herein, " α 5 chain" (LAMA5) is a subunit of a laminin protein of a cell adhesion molecule in an extracellular matrix, and is called
35 LAMA5, KIAA1907, or the like. For human LAMA5, the sequences of the gene and protein are registered as NCBI registration numbers NM_005560 and NP_005551, respectively. OMIM is identified with an accession number 601033. When used for the purpose herein, it is

understood that " α 5 chain" or "LAMA5" means not only a protein having an amino acid sequence described in the specific sequence number or accession number (or a nucleic acid encoding the protein), but also a functionally active, a functionally active fragment, or a
5 homologue thereof, or a mutant encoded by a nucleic acid that hybridizes to a nucleic acid encoding a protein under high or low stringency condition.

[0020]

As used herein, " β 1 chain" (LAMB1) is a subunit of a laminin protein
10 of a cell adhesion molecule in an extracellular matrix, and is called LAMB1, CLM, LIS5, or the like. For human LAMB1, the sequences of the gene and protein are registered as NCBI registration numbers NM_002291 and NP_002282, respectively. OMIM is identified with an accession number 150240. When used for the purpose herein, it is
15 understood that " β 1 chain" or "LAMB1" means not only a protein having an amino acid sequence described in the specific sequence number or accession number (or a nucleic acid encoding the protein), but also a functionally active derivative, a functionally active fragment, or a homologue thereof, or a mutant encoded by a nucleic
20 acid that hybridizes to a nucleic acid encoding a protein under high or low stringency condition.

[0021]

As used herein, " β 2 chain" (LAMB2) (laminin S) is a subunit of a laminin protein of a cell adhesion molecule in an extracellular
25 matrix, and is called LAMB2, LAMS, NPHS5, or the like. For human LAMB2, the sequences of the gene and protein are registered as NCBI registration numbers NM_002292 and NP_002283, respectively. OMIM is identified with an accession number 150325. When used for the purpose herein, it is understood that " β 2 chain" or "LAMB2" means
30 not only a protein having an amino acid sequence described in the specific sequence number or accession number (or a nucleic acid encoding the protein), but also a functionally active derivative, a functionally active fragment, or a homologue thereof, or a mutant encoded by a nucleic acid that hybridizes to a nucleic acid encoding
35 a protein under high or low stringency condition.

[0022]

As used herein, " β 3 chain" (LAMB3) is a subunit of a laminin protein of a cell adhesion molecule in an extracellular matrix, and is called

LAMB3, BM600-125KDA, LAM5, LAMNB1, or the like. For human LAMB3, the sequences of the gene and protein are registered as NCBI registration numbers NM_000228 and NP_000219, respectively. OMIM is identified with an accession number 150310. When used for the purpose herein, it is understood that " β 3 chain" or "LAMB3" means not only a protein having an amino acid sequence described in the specific sequence number or accession number (or a nucleic acid encoding the protein), but also a functionally active derivative, a functionally active fragment, a homologue thereof, or a mutant encoded by a nucleic acid that hybridizes to a nucleic acid encoding a protein under high or low stringency condition.

[0023]

As used herein, " γ 1 chain" (LAMC1) is a subunit of a laminin protein of a cell adhesion molecule in an extracellular matrix, and is called LAMC1, LAMB2, or the like. For human LAMC1, the sequences of the gene and protein are registered as NCBI registration numbers NM_002293 and NP_002284, respectively. OMIM is identified with an accession number 150290. When used for the purpose herein, it is understood that " γ 1 chain" or "LAMC1" means not only a protein having an amino acid sequence described in the specific sequence number or accession number (or a nucleic acid encoding the protein), but also a functionally active derivative, a functionally active fragment, a homologue thereof, or a mutant encoded by a nucleic acid that hybridizes to a nucleic acid encoding a protein under high or low stringency condition.

[0024]

As used herein, " γ 2 chain" (LAMC2) is a subunit of a laminin protein of a cell adhesion molecule in an extracellular matrix, and is called LAMC2, B2T, BM600, CSF, EBR2, EBR2A, LAMB2T, LAMNB2, or the like. For human LAMC2, the sequences of the gene and protein are registered as NCBI registration numbers NM_005562 and NP_005553, respectively. OMIM is identified with an accession number 150292. When used for the purpose herein, it is understood that " γ 2 chain" or "LAMC2" means not only a protein having an amino acid sequence described in the specific sequence number or accession (or a nucleic acid encoding the protein), but also a functionally active derivative, a functionally active fragment, a homologue thereof, or a mutant encoded by a nucleic acid that hybridizes to a nucleic acid encoding

a protein under high or low stringency condition.

[0025]

As used herein, " γ 3 chain" (LAMC3) is a subunit of a laminin protein of a cell adhesion molecule in an extracellular matrix, and is called
5 LAMC3, OCCM, or the like. For human LAMC3, the sequences of the gene and protein are registered as NCBI registration numbers NM_006059 and NP_006050, respectively. OMIM is identified with an accession number 604349. When used for the purpose herein, it is understood that " γ 3 chain" or "LAMC3" means not only a protein having
10 an amino acid sequence described in the specific sequence number or accession number (or a nucleic acid encoding the protein), but also a functionally active derivative, a functionally active fragment, a homologue thereof, or a mutant encoded by a nucleic acid that hybridizes to a nucleic acid encoding a protein under
15 high or low stringency condition.

[0026]

As used herein, "laminin expressed in corneal endothelial cells" refers to a type of laminin gene which is expressed in a normal state, or preferably significantly expressed at the protein level,
20 in corneal endothelial cells. α 5, β 1, β 2, and γ 1 are confirmed as being expressed by the analysis herein (Fig 2). Thus, at least laminin 511 and laminin 521 are confirmed as being expressed in corneal endothelial cells. Dev.Dyn.218, 213-234, 2000, and J.Biol.Chem. 277(15), 12741-12748, 2002 have detailed description for laminin
25 511. Thus, the content disclosed in these documents is incorporated by reference. For laminin 511 or the like, it is possible to utilize those that are commercially available. For example, recombinant proteins of laminin 511 and laminin 521 are commercially available and obtainable from BioLamina AB.

30 [0027]

As used herein, "expression" of a gene, polynucleotide, polypeptide or the like refers to the gene or the like being subjected to a certain effect in vivo to be in another form. Preferably, the term refers to a gene, polynucleotide or the like being transcribed and
35 translated to be in a form of a polypeptide. However, the gene, polynucleotide or the like being transcribed to result in mRNA can be one form of expression. Still preferably, such a polypeptide form can be those receiving processing after translation (referred

to as a derivative herein). For example, the expression level of each laminin chain can be determined by any method. Specifically, the expression level of each laminin chain can be found by evaluating the amount of mRNA of each laminin chain, amount of protein of each laminin chain and biological activity of the protein of each laminin chain. The amount of mRNA or protein of each laminin chain can be determined by a method as described herein.

[0028]

As used herein, "functional equivalent" refers to anything that has the same target function but a different structure with respect to the original subject entity. Thus, it is understood that when referring to a "group consisting of a laminin or each laminin chain, or a functional equivalent thereof" or a "group consisting of a laminin, each laminin chain, and a functional equivalent thereof", the following is encompassed therein: a laminin or each laminin chain itself, as well as fragments, mutants or variants of the laminin or each laminin chain (e.g., amino acid sequence variant or the like) having one or more capabilities of cellular adhesion, differentiation regulation and/or growth promoting action on an eye cell or the like; and substances that can change into a laminin or each laminin chain itself, or a fragment, mutant or variant of the laminin or each laminin chain at the time of action (including, for example, a nucleic acid encoding a laminin or each laminin chain itself, or a fragment, mutant or variant of laminin or each laminin chain and a vector, cell or the like comprising such a nucleic acid). A representative example of the "group consisting of a laminin or each laminin chain, or a functional equivalent thereof" or "group consisting of a laminin, each laminin chain, and a functional equivalent thereof" includes at least one agent selected from the group consisting of lamini and fragments thereof. In the present invention, it is understood that a functional equivalent of a laminin or each laminin chain can be used similarly to a laminin or each laminin chain without any specific mention thereof.

[0029]

As used herein, "fragment" refers to a polypeptide or polynucleotide with a sequence length of 1 to n-1 with respect to the entire length of a polypeptide or polynucleotide (length n). The length of a fragment can be appropriately changed in accordance with the objective. For

example, for a polypeptide, the lower limit of the length thereof includes 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50 and more amino acids. In addition, lengths represented by an integer that is not specifically listed herein (e.g. 11 and the like) can also
5 be appropriate as the lower limit. Further, for a polynucleotide, the lower limit of the length thereof includes 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100 and more nucleotides. In addition, lengths represented by an integer that is not specifically listed herein (e.g. 11 and the like) can also be appropriate as the lower
10 limit. It is understood herein that fragments themselves of such a laminin chain, when functioning as a factor of activity, e.g., growth promotion or maintenance, are within the scope of the present invention. According to the present invention, as used herein, the term "activity" refers to a function of a molecule in the broadest
15 meaning. Activity generally encompasses, but is not intended to be limited to, biological function, biochemical function, physical function, or chemical function of a molecule. Activity encompasses, for example, enzymatic activity, ability to interact with another molecule and ability to activate, promote, stabilize, inhibit,
20 suppress, or destabilize a function of another molecule, stability, and ability to localize at a specific position in a cell. When applicable, the term is directed to a function of a protein complex in the broadest meaning. As used herein, "biological function", when a reference is made to a gene or a nucleic acid or polypeptide
25 related thereto, refers to a specific function that the gene, nucleic acid or polypeptide can have in a living organism. "Biological function" includes, but is not limited to, creation of a specific antibody, enzymatic activity, giving resistance and the like. As used herein, biological function can be exerted by "biological
30 activity". As used herein "biological activity" refers to activity that a factor (e.g., polynucleotide and protein) can have in a living organism, and activity exerting a variety of functions (e.g., transcription promoting activity) is encompassed therein. For example, activity of activating or deactivating a molecule from
35 interaction with another molecule is also encompassed. When two factors interact, the biological activity thereof can be thought of as the binding of the two molecules and the biological change resulting therefrom, e.g., in a case when two molecules are bound,

e.g., the two molecules are bound if they co-precipitate when using an antibody against either one of the molecules. Thus, a method of determination includes observing such co-precipitation. For example, when a factor is an enzyme, the biological activity thereof encompasses the enzymatic activity thereof. In another example, when a factor is a ligand, the binding to a receptor to which the ligand matches is encompassed. Such biological activity can be measured by a well-known technique in the art. Thus, "activity" refers to various measurable indicators that indicate or reveal the binding (either directly or indirectly) or an elicited response (i.e., having a measurable effect in response to some exposure or stimulation). For example, "activity" includes a compound that binds to a polypeptide or polynucleotide of the present invention, the amount of proteins affected upstream or downstream after some exposure or stimulation, or a measure of another analogous function.

[0030]

"Functionally active" as used herein refers to a polypeptide, a fragment or a derivative, having biochemical function, regulation function, or structural function of a protein such as biological activity in accordance with the embodiment associated with the polypeptide, a fragment or derivative, of the present invention.

[0031]

As used herein, a "fragment" of a laminin refers to any fragment of a laminin. As an agent used in the present invention, it is understood that not only the entire length of a laminin, but also a fragment of the laminin can be used as long as the fragment has the function of the entire length of the laminin, particularly the cell adhesion capability of an endothelial cell. Thus, a fragment of a laminin used in the present invention generally has at least one feature of the laminin. Such a feature can encompass cell adhesion capability of an endothelial cell in particular.

[0032]

The sequence of a laminin found to be expressed in corneal endothelial cells in the present invention will be described below. It is understood that these laminins indicate preferred representative examples of the present invention and the present invention is not limited to these specific laminin subtypes.

[0033]

A representative nucleotide sequence of a laminin $\alpha 5$ chain can be
(a) a polynucleotide having the base sequence described in SEQ ID
NO: 1 or a fragment sequence thereof;

(b) a polynucleotide encoding a polypeptide consisting of the amino
acid sequence described in SEQ ID NO: 2 or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide or a fragment
thereof in which one or more amino acids have a mutation selected
from the group consisting of substitution, addition and deletion
in the amino acid sequence described in SEQ ID NO: 2, wherein the
variant polypeptide has biological activity;

(d) a polynucleotide which is an allele or a splice mutant of the
base sequence described in SEQ ID NO: 1 or a fragment thereof;

(e) a polynucleotide encoding a species homolog of a polypeptide
consisting of the amino acid sequence described in SEQ ID NO: 2
or a fragment thereof;

(f) a polynucleotide which hybridizes with a polynucleotide of one
of (a)-(e) under a stringent condition and encodes a polypeptide
having biological activity; or

(g) a polynucleotide consisting of a base sequence with identity
of at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% to a
polynucleotide of one of (a) to (e) or a complementary sequence
thereof and encodes a polypeptide having biological activity. In
this regard, biological activity typically refers to activity of
a laminin $\alpha 5$ chain. Doi M et al., J.Biol.Chem. 277(15), 12741-12748,
2002 and US Patent No. 6,933,273 can be referenced for $\alpha 5$ chains.
[0034]

An amino acid sequence of a laminin $\alpha 5$ chain can be

(a) a polypeptide consisting of the amino acid sequence described
in SEQ ID NO: 2 or a fragment thereof;

(b) a polypeptide having biological activity and one or more amino
acids with a mutation selected from the group consisting of
substitution, addition and deletion in the amino acid sequence
described in SEQ ID NO: 2;

(c) a polypeptide encoded by an allele or a splice mutant of the
base sequence described in SEQ ID NO: 1;

(d) a polypeptide which is a species homolog of the amino acid sequence
described in SEQ ID NO: 2; or

(e) a polypeptide having an amino acid sequence with identity of

at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% to a polypeptide of one of (a) to (d). In this regard, biological activity typically refers to the activity of a laminin $\alpha 5$ chain. Doi et al., J. Biol. Chem. 277(15), 12741-12748, 2002 and US Patent No. 6,933,273 can be referenced for $\alpha 5$ chains.

[0035]

A representative nucleotide sequence of a laminin $\beta 1$ chain can be (a) a polynucleotide having the base sequence described in SEQ ID NO: 3 or a fragment sequence thereof;

10 (b) a polynucleotide encoding a polypeptide consisting of the amino acid sequence described in SEQ ID NO: 4 or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide or a fragment thereof in which one or more amino acids have a mutation selected from the group consisting of substitution, addition and deletion
15 in the amino acid sequence described in SEQ ID NO: 4, wherein the variant polypeptide has biological activity;

(d) a polynucleotide which is an allele or a splice mutant of the base sequence described in SEQ ID NO: 3 or a fragment thereof;

(e) a polynucleotide encoding a species homolog of a polypeptide
20 consisting of the amino acid sequence described in SEQ ID NO: 4 or a fragment thereof;

(f) a polynucleotide which hybridizes with a polynucleotide of one of (a)-(e) under a stringent condition and encodes a polypeptide having biological activity; or

25 (g) a polynucleotide consisting of a base sequence with identity of at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% to a polynucleotide of one of (a) to (e) or a complementary sequence thereof and encodes a polypeptide having biological activity. In this regard, biological activity typically refers to activity of
30 a laminin $\beta 1$ chain. Pillarainen et al., J. Biol. Chem. 262(22), 10454-10462, 1987 and US Patent No. 6,933,273 can be referenced for $\beta 1$ chains.

[0036]

An amino acid sequence of a laminin $\beta 1$ chain can be

35 (a) a polypeptide consisting of the amino acid sequence described in SEQ ID NO: 4 or a fragment thereof;

(b) a polypeptide having biological activity and one or more amino acids with a mutation selected from the group consisting of

substitution, addition and deletion in the amino acid sequence described in SEQ ID NO: 4;

(c) a polypeptide encoded by an allele or a splice mutant of the base sequence described in SEQ ID NO: 3;

5 (d) a polypeptide which is a species homolog of the amino acid sequence described in SEQ ID NO: 4; or

(e) a polypeptide having an amino acid sequence with identity of at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% to a polypeptide of one of (a) to (d). Pillarainen et al., J.Biol.Chem.262(22),
10 10454-10462, 1987 and US Patent No. 6,933,273 can be referenced for β 1 chains.

[0037]

A representative nucleotide sequence of a laminin β 2 chain can be

(a) a polynucleotide having the base sequence described in SEQ ID
15 NO: 5 or a fragment sequence thereof;

(b) a polynucleotide encoding a polypeptide consisting of the amino acid sequence described in SEQ ID NO: 6 or a fragment thereof;

(c) a polynucleotide encoding a variant polypeptide or a fragment thereof in which one or more amino acids have a mutation selected
20 from the group consisting of substitution, addition and deletion in the amino acid sequence described in SEQ ID NO: 6, wherein the variant polypeptide has biological activity;

(d) a polynucleotide which is an allele or a splice mutant of the base sequence described in SEQ ID NO: 5 or a fragment thereof;

25 (e) a polynucleotide encoding a species homolog of a polypeptide consisting of the amino acid sequence described in SEQ ID NO: 6 or a fragment thereof;

(f) a polynucleotide which hybridizes with a polynucleotide of one of (a)-(e) under a stringent condition and encodes a polypeptide
30 having biological activity; or

(g) a polynucleotide consisting of a base sequence with identity of at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% to a polynucleotide of one of (a) to (e) or a complementary sequence thereof and encodes a polypeptide having biological activity. In
35 this regard, biological activity typically refers to the activity of a laminin β 2 chain. Wewer UM et al., Genomics. Nov 15, 1994; 24(2):243-52., 1987 and US Patent No. 6,933,273 can be referenced for β 2 chains.

[0038]

An amino acid sequence of a laminin β 2 chain can be

(a) a polypeptide consisting of the amino acid sequence described in SEQ ID NO: 6 or a fragment thereof;

5 (b) a polypeptide having biological activity and one or more amino acids with a mutation selected from the group consisting of substitution, addition and deletion in the amino acid sequence described in SEQ ID NO: 6;

10 (c) a polypeptide encoded by an allele or a splice mutant of the base sequence described in SEQ ID NO: 5;

(d) a polypeptide which is a species homolog of the amino acid sequence described in SEQ ID NO: 6; or

15 (e) a polypeptide having an amino acid sequence with identity of at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% to a polypeptide of one of (a) to (d). In this regard, biological activity typically refers to the activity of a laminin β 2 chain. Wewer U Met al., Genomics. Nov 15, 1994; 24(2):243-52., 1987 and US Patent No. 6,933,273 can be referenced for α 5 chains.

[0039]

20 A representative nucleotide sequence of a laminin γ 1 chain can be (a) a polynucleotide having the base sequence described in SEQ ID NO: 7 or a fragment sequence thereof;

(b) a polynucleotide encoding a polypeptide consisting of the amino acid sequence described in SEQ ID NO: 8 or a fragment thereof;

25 (c) a polynucleotide encoding a variant polypeptide or a fragment thereof in which one or more amino acids have a mutation selected from the group consisting of substitution, addition and deletion in the amino acid sequence described in SEQ ID NO: 8, wherein the variant polypeptide has biological activity;

30 (d) a polynucleotide which is an allele or a splice mutant of the base sequence described in SEQ ID NO: 7 or a fragment thereof;

(e) a polynucleotide encoding a species homolog of a polypeptide consisting of the amino acid sequence described in SEQ ID NO: 8 or a fragment thereof;

35 (f) a polynucleotide which hybridizes with a polynucleotide of one of (a)-(e) under a stringent condition and encodes a polypeptide having biological activity; or

(g) a polynucleotide consisting of a base sequence with identity

of at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% to a polynucleotide of one of (a) to (e) or a complementary sequence thereof and encodes a polypeptide having biological activity. In this regard, biological activity typically refers to activity of a laminin γ 1 chain. Pillarainen et al., J.Biol.Chem.263(14), 6751-6758, 1988 and US Patent No. 6,933,273 can be referenced for γ 1 chains.

[0040]

An amino acid sequence of a laminin γ 1 chain can be

(a) a polypeptide consisting of the amino acid sequence described in SEQ ID NO: 8 or a fragment thereof;

(b) a polypeptide having biological activity and one or more amino acids with a mutation selected from the group consisting of substitution, addition and deletion in the amino acid sequence described in SEQ ID NO: 8;

(c) a polypeptide encoded by an allele or a splice mutant of the base sequence described in SEQ ID NO: 7;

(d) a polypeptide which is a species homolog of the amino acid sequence described in SEQ ID NO: 8; or

(e) a polypeptide having an amino acid sequence with identity of at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% to a polypeptide of one of (a) to (d). In this regard, biological activity typically refers to the activity of a laminin γ 1 chain. Pillarainen et al., J.Biol.Chem.263(14), 6751-6758, 1988 and US Patent No. 6,933,273 can be referenced for γ 1 chains.

[0041]

As used herein, "protein", "polypeptide", "oligopeptide" and "peptide" are interchangeably used with the same meaning, and they refer to a polymer of an amino acid sequence of any length. This polymer may be a linear chain or a branched chain, or a cyclic chain. Amino acids may be natural or non-natural, or may be altered amino acids. This term may also encompass those assembled into a complex of a plurality of polypeptide chains. This term also encompasses naturally or artificially-altered amino acid polymer. Such alteration encompasses, for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation or any other operation or alteration (e.g., conjugation with a labelled component). This definition also encompasses, for example,

polypeptide comprising one or more analogs of amino acids (e.g., including non-natural amino acid), peptide-like compounds (e.g., peptoid) and other alteration publicly known in the subject field. With regard to the protein of the present invention (e.g., each chain of laminin), a DNA encoding each targeted chain gene is incorporated into an appropriate vector, which is introduced into an eukaryote or prokaryotic cell using an expression vector which can express in either host, and a respective chain is allowed to be expressed, thus obtaining a desired protein. Host cells that can be used to express laminin include, without particular limitation, prokaryotic host cells such as E. coli and bacillus subtilis, and eukaryotic host cells such as yeast, fungus, insect cell, plant and plant cell, and mammalian cell. Vectors constructed to express a targeted laminin chain or the like can be introduced into the above-mentioned host cell, using transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technique, calcium phosphate precipitation, Agrobacterium method, direct microinjection or the like. Cells comprising vectors are grown in an appropriate culture medium to produce laminin chains used in the present invention, and the laminin chains are purified from the cells or culture medium, thus obtaining laminin chains or the like. The purification can be performed using size exclusion chromatography, HPLC, ion exchange chromatography, immunoaffinity chromatography or the like.

[0042]

As used herein, "amino acid" may be natural or non-natural as long as the objective of the present invention is fulfilled.

[0043]

As used herein, "polynucleotide", "oligonucleotide" and "nucleic acid" are interchangeably used with the same meaning, and they refer to polymer of nucleotide of any length. These terms also include "oligonucleotide derivative" or "polynucleotide derivative". The "oligonucleotide derivative" or "polynucleotide derivative" refers to oligonucleotide or polynucleotide which includes a derivative of nucleotide or in which the bonding between nucleotides is different from the normal bonding, and they are used interchangeably. As for such oligonucleotide, the following are specifically exemplified: 2'-O-methyl-ribonucleotide; an oligonucleotide derivative in which

phosphodiester bonding in an oligonucleotide is converted into phosphorothioate bonding; an oligonucleotide derivative in which phosphodiester bonding in an oligonucleotide is converted into N3'-P5' phosphoramidate bonding; an oligonucleotide derivative in which ribose and phosphodiester bonding in an oligonucleotide are converted into peptide nucleic acid bonding; an oligonucleotide derivative in which uracil in an oligonucleotide is substituted by C-5 propynyl uracil; an oligonucleotide derivative in which uracil in an oligonucleotide is substituted by C-5 thiazole uracil; an oligonucleotide derivative in which cytosine in an oligonucleotide is substituted by C-5 propynyl cytosine; an oligonucleotide derivative in which cytosine in an oligonucleotide is substituted by phenoxazine-modified cytosine; an oligonucleotide derivative in which ribose in DNA is substituted by 2'-O-propylribose; and an oligonucleotide derivative in which ribose in an oligonucleotide is substituted by 2'-methoxyethoxy ribose. Unless indicated otherwise, specific nucleic acid sequences are intended to encompass a conservatively altered variant (e.g., degenerate codon substitute) and a complementary sequence thereof, as similar to explicitly indicated sequences. Specifically, a degenerate codon substitute can be achieved by creating a sequence in which the third position of one or more selected (or all the) codons are substituted by a mixed base and/or deoxyinosine residue (Batzner et al., Nucleic Acid Res. 19:5081(1991); Ohtsuka et al., J. Biol. Chem. 260: 2605-2608(1985); Rossolini et al., Mol. Cell. Probes 8:91-98(1994)). As used herein, "nucleic acid" is interchangeably used with gene, cDNA, mRNA, oligonucleotide, and polynucleotide. As used herein, "nucleotide" may be natural or non-natural.

[0044]

As used herein, "gene" refers to an agent that defines a genetic trait. Normally, a gene is sequenced in a given order on a chromosome. A gene that defines a primary structure of protein is referred to as a structural gene, and a gene that influences the expression thereof is referred to as a regulator gene. Herein, "gene" may refer to "polynucleotide", "oligonucleotide" and "nucleic acid".

[0045]

Amino acids may be referred herein by publicly known three-letter codes thereof, or one-letter codes recommended by IUPAC-IUB

Biochemical Nomenclature Commission. Similarly, nucleotides may be referred by generally recognized one-letter codes. Herein, the comparison of the similarity, identity and homology of amino acid sequences and base sequences is calculated with default parameters, and with a sequence analysis tool, BLAST. Identity searches are conducted with, for example, BLAST 2.2.26 (issued on October 30, 2011) of NCBI. Values of identity used herein normally refer to values aligned under default conditions using the BLAST. However, if higher values are obtained due to changes in parameters, the highest value will be defined to be the value for identity. If identity is evaluated in a plurality of regions, the highest value in the regions is defined to be the value for identity. Similarity is a numerical value in which similar amino acids are taken into consideration for calculation in addition to identity.

[0046]

As used herein, "polynucleotide hybridizing under stringent conditions" refers to well-known conditions commonly used in the subject field. It is understood, with regard to laminin used in the present invention, that those encoded by "polynucleotide hybridizing under stringent conditions" with respect to nucleic acid sequences of respective specifically-disclosed laminins may also be used. With a polynucleotide selected from the polynucleotides of the present invention used as a probe, the use of a colony hybridization technique, a plaque hybridization technique or a southern blot hybridization technique allows such a polynucleotide to be obtained. Specifically, it means a polynucleotide obtained by conducting hybridization at 65°C in the presence of NaCl of 0.7 to 1.0M using a filter to which a colony or plaque-derived DNA is immobilized, and then by washing the filter under the condition of 65°C with a SSC (saline-sodium citrate) solution of 0.1 to 2 fold concentration (wherein the composition of the SSC solution of one fold concentration is 150 mM sodium chloride and 15 mM sodium citrate). Hybridization can be conducted according to methods described in experimental documents such as Molecular Cloning 2nd ed., Current Protocols in Molecular Biology, Supplement 1-38, DNA Cloning 1: Core Techniques, A Practical Approach, Second Edition, Oxford University Press (1995). Here, sequences comprising only an A sequence or a T sequence is preferably excluded from sequences

that hybridize under stringent conditions. Therefore, the polypeptides (e.g., transthyretin) used in the present invention also encompass a polypeptide encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding the polypeptide specifically described in the present invention. These low stringency conditions include conducting hybridization for 18 to 20 hours at 40°C in a buffer comprising 35% formamide, 5×SSC, 50mM Tris-HCl (pH 7.5), 5mMEDTA, 0.02% PVP, 0.02% BSA, 100 µg/ml denatured salmon sperm DNA, and 10% (weight/volume) dextran sulfate; washing 1 to 5 hours at 55°C in a buffer consisting of 2×SSC, 25 mM Tris-HCl (pH 7.4), 5m MEDTA, and 0.1% SDS; and washing for 1.5 hours at 60° C in a buffer consisting of 2×SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS.

[0047]

As for functional equivalents of the present invention, those in which one or a plurality of amino acids are inserted, substituted or deleted, or added to one or both ends in an amino acid sequence, can be used. Herein, "one or a plurality of amino acids are inserted, substituted or deleted, or added to one or both ends in an amino acid sequence" means that alteration is made by substitution or the like of a plurality of amino acids that could naturally occur, by a well-known technical method such as site-directed mutagenesis, or naturally-occurring mutation.

[0048]

Altered amino acid sequences such as each chain of laminin used in the present invention can be those in which, for example, 1 to 30, preferably 1 to 20, more preferably 1 to 9, still more preferably 1 to 5, particularly preferably 1 to 2 amino acids are inserted, substituted, or deleted, or added to one or both ends thereof. The altered amino acid sequences may be preferably such amino acid sequences having one or a plurality (preferably, 1 or several, or 1, 2, 3, or 4) of conservative substitutions in an amino acid sequence of each chain or the like of laminin. Herein, "conservative substitution" means to substitute one or a plurality of amino acid residues with different chemically similar amino acid residues so that the functions of protein will not be substantially altered. For example, such cases can be mentioned when a given hydrophobic residue is substituted with another hydrophobic residue, or when

a given polar residue is substituted with another polar residue having the same electric charge. Functionally similar amino acids capable of making such a substitution are publicly known in the subject field for each amino acid. Specific examples of non-polar (hydrophobic) amino acids include alanine, valine, isoleucine, leucine, proline, tryptophan, phenylalanine, methionine and the like. Specific examples of polar (neutral) amino acids include glycine, serine, threonine, tyrosine, glutamine, asparagine, cysteine and the like. Specific examples of (basic) amino acids having a positive electric charge include arginine, histidine, lysine and the like. Further, specific examples of (acidic) amino acids having a negative electric charge include asparagine acid, glutamic acid and the like.

[0049]

As used herein, "agent" may be, in a broad sense, any substance or any other elements (e.g., energy such as light, radioactivity, heat and electricity) as long as the substance can be exchangeably used and can achieve an intended objective. Such substances include, without limitation, for example, protein, polypeptide, oligopeptide, peptide, polynucleotide, oligonucleotide, nucleotide, nucleic acid (including, for example, DNA such as genome DNA and cDNA, RNA such as mRNA), polysaccharide, oligosaccharide, lipid, organic low molecule (e.g., hormone, ligand, messenger, organic low molecule, molecule synthesized by combinatorial chemistry, low molecule that can be used as a medicament (e.g., low molecular ligand) and the like), and complex molecules thereof. Agents specific to polynucleotide typically include, without limitation, polynucleotide having complementarity with given sequence homology to a sequence of the aforementioned polynucleotide (e.g., 70% or more sequence identity), polypeptide like a transcription factor binding to a promoter region, and the like. Agents specific to polypeptide typically include, without limitation, an antibody specifically directed to the polypeptide, or a derivative thereof or an analog thereof (e.g., single-stranded antibody), a specific ligand or receptor in a case when the polypeptide is a receptor or a ligand, a matrix thereof in a case when the polypeptide is enzyme, and the like.

[0050]

As used herein, "culture" refers to growing subject cells, and in a limited sense, it refers to a state where the condition of subject cells is maintained, which would otherwise be worsened (e.g., where the number of cells will not substantially decrease). In a limited sense, the term is used to mean the same meaning as maintenance of culture or maintenance.

[0051]

As used herein, "growth" refers to a state where the number of cells increases.

10 [0052]

As used herein, "growth capability" refers to a cell's capability to grow. Unless specifically stated herein, a state of growth refers to a possibility of growth in a steady state. The "steady state" refers to a normal condition for a living organism where the homeostasis of the living organism is maintained. Such a state can be readily determined by those skilled in the art. For example, such can be confirmed through cellular density analysis, where cellular density is almost constant with no change, or expression of a cellular growth marker is recognized, or the like. As used herein, "promotion of growth" refers to a growth state of a given cell. If a target cell was not growing at the beginning, the start of even a little growth would correspond to promotion of growth. If a cell was already growing and the growth level was maintained or was increased, and preferably increased, then it would correspond to promotion of growth.

25 [0053]

As used herein, "stem cell" refers to a cell which has both a capability to differentiate into a plurality of systems of cells (multilineage potential) and a capability to maintain multilineage potential even after cellular division (self-renewal capability). Stem cells include embryonic stem cell, germ cell, iPS cell, tissue stem cell and the like. The corneal endothelial cell aimed by the present invention may be such cells that are differentiated from stem cells. The differentiation from stem cells to corneal endothelial cells can be achieved using methods publicly known in the subject field.

35 [0054]

As used herein, "normal cellular function" of cell refers to a function which a cell originally possesses when a specific cell such as corneal

endothelial cells is referred. With regard to the corneal endothelial cell, such a function includes, without limitation, ZO-1 and Na⁺/K⁺-ATPase, adaptive capability to corneal transplant (Matsubara M, Tanishima T: Wound-healing of the corneal endothelium in the monkey: a morphometric study, Jpn J Ophthalmol 1982, 26:264-273; Matsubara M, Tanishima T: Wound-healing of corneal endothelium in monkey: an autoradiographic study, Jpn J Ophthalmol 1983, 27:444-450; Van Horn DL, Hyndiuk RA: Endothelial wound repair in primate cornea, Exp Eye Res 1975, 21:113-124 and Van Horn DL, Sendele DD, Seideman S, Bucu PJ: Regenerative capability of the corneal endothelium in rabbit and cat, Invest Ophthalmol Vis Sci 1977, 16:597-613), and the like.

[0055]

ZO-1 and Na⁺/K⁺-ATPase can be evaluated by observing the expression of proteins by immunological means or at an mRNA level such as RT-PCR. Confirmation of Na⁺/K⁺-ATPase and ZO-1 expression and/or function at the same level as normal cells allows for confirmation as to whether or not subject cells have a normal function.

[0056]

As to adaptive capability to corneal transplant, implantation tests of cultured cells can be conducted by mechanically curetting corneal endothelium as a bullous keratopathy model with experimental animals such as rabbits. However, since corneal endothelial cells of rabbits grow in vivo, it is not possible to deny the possibility of natural healing due to growth of corneal endothelial cells of hosts (Matsubara M, et al., Jpn J Ophthalmol 1982, 26:264-273; Matsubara M, et al., Jpn J Ophthalmol 1983, 27:444-450; Van Horn DL, et al., Exp Eye Res 1975, 21:113-124 and Van Horn DL, et al., Invest Ophthalmol Vis Sci 1977, 16:597-613). Therefore, in order to evaluate more accurate transplant adaptive capability, it is preferable to evaluate engraftment to primates. When transplant adaptive capability to humans is evaluated, adaptivity is evaluated in primates, such as crab-eating monkeys, after at least one month, preferably at least two months, more preferably at least three months, still more preferably at least six months, and further still more preferably at least twelve months, for example. Confirmation of transplant adaptive capability in primates such as monkeys is important in application to humans, in particular.

[0057]

As used herein, "BrdU" is an abbreviation of bromodeoxyuridine. Since BrdU is incorporated as an analog of dTTP in DNA synthesis, the DNA (cell nucleus) in which BrdU is incorporated can be detected
5 by an antibody specific to BrdU that is incorporated into DNA. Thus, BrdU is used as an index to show high growth capability/differentiation capability.

[0058]

As used herein, "BrdU positive" refers to a state where a cell marker,
10 BrdU, is expressed in a target cell.

[0059]

As used herein, "cultures" refers to those produced by culturing cells such as corneal endothelium. Thus, "corneal endothelium cultures" refers to the cultures of corneal endothelium, and the
15 term normally refers to those present in a different state from those present in vivo. The corneal endothelium cultures obtained by conventional methods were problematic in that such cultures had low growth capability and would be easily become transformed and lose functions (Peh GS, Beuerman RW, Colman A, Tan DT, Mehta JS
20 (2011) Human corneal endothelial cell expansion for corneal endothelium transplantation: an overview. Transplantation 91: 811-819., Okumura N, Kay E, Nakahara M, Hamuro J, Kinoshita S, et al. (2013) Inhibition of TGF- β signaling enables human corneal endothelial cell expansion in vitro for use in regenerative medicine.
25 PLoS One 8:e58000.). Therefore, from the viewpoint of cultures, such a cellular density could not be achieved for those that were cultured for a particularly long period of time or those that were subcultured. Specifically, the density of corneal endothelial cells is easily decreased through culturing. Corneal endothelial density
30 is one of the most clinically important indices for the degree of health. Thus, the culturing to high density is important from the point of regeneration medicine. Further, it is possible to increase the endothelial density in advance and then conduct administration in vivo, which can be an extremely important therapeutic agent.
35 In that sense, it is an important fact that the cellular density that was used to decrease through a normal culturing method can now be increase. The normal level of human corneal endothelium in vivo is within the range of about 2500-3000/mm². The present invention

is advantageous in that the present invention has provided a technique to bring the cellular density of the cultures to the above-mentioned level or to exceed the level.

[0060]

5 As used herein, "medium" refers to any medium capable of culturing or growing corneal endothelial cells, and the medium can take any form, such as liquid medium (culture medium), suspension medium and solid medium, as needs arise and as appropriate. Components for the medium used for such corneal endothelial cells include,
10 for example, DMEM (GIBCO BRL), OptiMEM (Life Technologies), serum (e.g., fetal bovine serum (FBS), human serum), proliferation factor/growth factor (e.g., b-FGF), antibiotic substance (e.g., penicillin, streptomycin, gentamicin) and the like.

[0061]

15 As used herein, "(culture) container" refers to a container for culturing corneal endothelial cells. The type of the culture containers is not particularly limited, and any containers can be used that are sterilized to prevent contamination by bacteria and that are of any material and any shape suitable for culturing cells.
20 Examples of such culture containers include, without limitation, culture dishes, culture flasks, culture Schales, culture plates such as 96-well, 48-well, 12-well, 6-well and 4-well, culture bottles, generally used in the subject field.

[0062]

25 (General Techniques)

The molecular biological technique, biochemical technique, microbiological process used herein are well-known and commonly used in the subject field, which are described in, for example, Sambrook J. et al.(1989).Molecular Cloning: A Laboratory Manual,
30 Cold Spring Harbor and its 3rd Ed.(2001); Ausubel, F. M.(1987).Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience; Ausubel, F. M.(1989). Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Greene Pub. Associates and
35 Wiley-Interscience; Innis, M. A. (1990). PCR Protocols: A Guide to Methods and Applications, Academic Press; Ausubel, F. M. (1992). Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Greene Pub. Associates;

Ausubel, F. M. (1995). Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Greene Pub. Associates; Innis, M. A. et al. (1995). PCR Strategies, Academic Press; Ausubel, F. M. (1999). Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Wiley, and annual updates; Sninsky, J. J. et al. (1999). PCR Applications: Protocols for Functional Genomics, Academic Press, Gait, M. J. (1985). Oligonucleotide Synthesis: A Practical Approach, IRL Press; Gait, M. J. (1990). Oligonucleotide Synthesis: A Practical Approach, IRL Press; Eckstein, F. (1991). Oligonucleotides and Analogues: A Practical Approach, IRL Press; Adams, R. L. et al. (1992). The Biochemistry of the Nucleic Acids, Chapman & Hall; Shabarova, Z. et al. (1994). Advanced Organic Chemistry of Nucleic Acids, Weinheim; Blackburn, G. M. et al. (1996). Nucleic Acids in Chemistry and Biology, Oxford University Press; Hermanson, G. T. (1996). Bioconjugate Techniques, Academic Press, and Bessatsu Jikken Igaku "Idenshi Dounyu & Hatsugen Kaiseki Jikken Hou" Yodosha Co., Ltd., 1997. With regard to corneal endothelial cells, the report by Nancy Joyce et al., {Joyce, 2004 #161} {Joyce, 2003 #7} is well-known; however, as mentioned earlier, transformation of fibroblast cells is due to long-term culture or subculture. Thus, studies for effective culture methods are still continued today. Associated portions (which may be the entire portions) thereof are incorporated by reference herein.

[Description of Embodiments]
[0063]

The description of the preferred embodiments will be described below. However, it should be understood that the embodiments are examples of the present invention and the scope of the present invention is not limited to such preferred embodiments. Further, it should be understood that those skilled in the art can readily practice modification, alteration or the like within the scope of the present invention while referring to the following preferred embodiments. Further, it should be understood that any embodiment may be combined.

[0064]

(Composition and culturing container for culturing or growing corneal endothelial cells)

In one aspect, the present invention provides a composition for

culturing or growing corneal endothelial cells, comprising at least one agent consisting of laminins and fragments thereof which express in corneal endothelial cells.

[0065]

5 Although an agent (laminin or the like) of the present invention can be included in a culture medium for use, the agent may be coated onto (or may cover) a culture dish for use. In order to conduct a primary culture and/or a subculture of a target cell, an appropriate medium (e.g., DMEM (Dulbecco's modified Eagle's medium) or OptiMEM)
10 is used and the cells, which are collected and separated, are seeded for the primary culture and subculture in a culture dish for culturing. In the present invention, a good growth result can be obtained even with an amount of serum added to a medium that is 10 % or less. Furthermore, in the present invention, in addition to a laminin
15 or in addition to a coating of a laminin, a cytokine (e.g., fibroblast growth factor (FGF)) can be added to a medium as an additive. An agent of the present invention is useful in an isolated cell as described in the Examples and in an endothelial cell induced from iPS or ES cells. Further, it is understood that such a agent is
20 effective for the induction itself.

[0066]

The concentration of a laminin that is used includes, for example, about 0.1 μg to about 500 $\mu\text{g}/\text{ml}$ in a culture medium solution (e.g. PBS). For coating, an amount of about 0.75 $\mu\text{g}/\text{cm}^2$ per unit area (e.g.
25 cm^2) may be used for the coating. For use in the treatment of a culture container, the amount of laminins of the present invention or fragments thereof is not particularly limited. A favorable result can be obtained when treated with a solution of laminin or fragments thereof, preferably in the amount of about 0.01 $\mu\text{g}/\text{ml}$ or more,
30 preferably about 0.01 to 10 $\mu\text{g}/\text{ml}$, still preferably about 0.01 to about 2 $\mu\text{g}/\text{ml}$. About 0.01 to 10 $\mu\text{g}/\text{ml}$ or about 0.01 to about 2 $\mu\text{g}/\text{ml}$ of laminin or fragments thereof corresponds to about 0.0015 to 1.5 $\mu\text{g}/\text{cm}^2$ or about 0.0015 to 0.3 $\mu\text{g}/\text{cm}^2$ as an amount of laminin or fragments thereof in a solid phase per area of a culture container.

35 [0067]

In a preferred embodiment, the laminin includes laminin 511 and laminin 521. Thus, in the present embodiment, an agent of the present invention can be laminin 511, laminin 521 or a fragment thereof.

Any fragment may be used as a fragment of laminin 511 or a fragment of laminin 521 of the present invention, as long as the fragment can be used in culturing (may be referred to as "maintaining" or "maintaining a culture" herein, but is used in the same meaning as culturing) or growing corneal endothelial cells. Such fragments include but are not limited to a laminin 511-E8 fragment and a laminin 521-fragment (respectively, sequence numbers 9, 10 (nucleic acid sequence, amino acid sequence) and sequence numbers 11, 12 (nucleic acid sequence, amino acid sequence)) (see Taniguchi Y, Ido H, Sanzen N, Hayashi M, Sato-Nishiuchi R, Futaki S, Sekiguchi K. The C-terminal region of laminin beta chains modulates the integrin binding affinities of laminins. J Biol Chem. 284:7820-7831, 2009. Available from Nippi, Incorporated). A laminin 511-E8 fragment and laminin 521 fragment are fragments obtained by elastase treatment and are comprised of a portion of a coiled-coil domain and three LG domains (LG1 to LG3) in the α chain C-terminal region of a heterotrimer. An E8 fragment is regarded as corresponding to an integrin binding site of a heterotrimer molecule in which an α chain, β chain and γ chain of a laminin assemble via a coiled-coil domain with one another. Thus, as a preferred fragment, those in which an integrin binding site is substantially retained in the entire length of a laminin can be used. It is understood that such a fragment can be made by an appropriate alteration based on information on laminin 511-E8 and laminin 521 fragments.

[0068]

In this regard, an E8 fragment of human laminin $\alpha 5\beta 1\gamma 1$ (also referred to as "human laminin 511-E8" herein) means a fragment of human laminin $\alpha 5\beta 1\gamma 1$ (also referred to as "human laminin 511" herein) corresponding to an E8 fragment of murine laminin $\alpha 1\beta 1\gamma 1$ (also referred to as "murine laminin 111-E8" herein). An E8 fragment of a laminin has been identified as a fragment with strong cell adhesion activity among fragments that are obtained by digesting murine laminin $\alpha 1\beta 1\gamma 1$ (hereinafter, described as "murine laminin 111") with elastase (Edgar D., Timpl R., Thoenen H. The heparin-binding domain of laminin is responsible for its effects on neurite outgrowth and neuronal survival. EMBO J., 3:1463-1468, 1984., Goodman SL., Deutzmann R., von der Mark K. Two distinct cell-binding domains in laminin can independently promote nonneuronal cell adhesion and spreading. J.

Cell Biol., 105: 589-598, 1987.). For human laminin 511 and human laminin 332, the presence of a fragment corresponding to murine laminin 111-E8 is presumed upon digestion with elastase. Human laminin 511-E8 used in the present invention is not required to
5 be an elastase digestion product of human laminin 511. Human laminin 511-E8 used in the present invention may be a fragment of human laminin 511 having similar cell adhesion activity, similar structure, and approximately the same molecular weight as murine laminin 111-E8. A method of manufacturing human laminin 511-E8 is not particularly
10 limited. For example, such a method includes a method of allowing the digestion of the entire length of human laminin 511 by a proteolytic enzyme such as elastase to fractionate and purify a target fragment, a method of manufacturing as a recombinant protein and the like. Manufacturing as a recombinant protein is preferred
15 from the viewpoint of quantity of manufacture, uniformity of quality, manufacturing cost and the like. Recombinant human laminin 511-E8 can be manufactured by appropriately using a known gene recombination technique. A method of manufacturing recombinant human laminin 511-E8, for example, can manufacture recombinant human laminin
20 511-E8 by obtaining DNA encoding the protein of each of α chain, β chain and γ chain of human laminin 511-E8, inserting each obtained DNA into an expression vector, expressing the resulting three kinds of expression vectors by cotransfecting into the appropriate host cells, and purifying the trimeric proteins by a known method (for
25 example, see Hiroyuki Ido, et al, "The requirement of the glutamic acid residue at the third position from the carboxyl termini of the laminin γ chains in integrin binding by laminins" The Journal of Biological Chemistry, 282, 11144-11154, 2007.). JP 2011-78370 can be referred to for a specific method for manufacturing. Similar
30 fragments may be produced by using human laminin 521, which is referred to as "laminin 521-E8 fragment." It is understood that such a fragment may be produced in a similar manner as laminin 511-E8, and that such retains similar activity to that of laminin 511-E8.

[0069]

35 In a preferred embodiment, the agent is laminin 511, laminin 521, laminin 511-E8 fragment or laminin 521-E8 fragment.

[0070]

In a preferred embodiment, the corneal endothelial cells are from

human.

[0071]

Endothelial cells targeted by the present invention can be prepared from an exfoliated cornea by removing the corneal endothelium native to a donor cornea without harming the integrity of a Descemet's membrane and the structure and function of a stromal layer (e.g., WO 2005/038015). An exfoliated cornea can be used as human corneal endothelial culturing cells.

[0072]

10 In another aspect, the present invention provides a culture container for corneal endothelial cells, coated with an agent or composition of the present invention.

[0073]

Preparation of a covered container or a culture plate for a laminin or the like for culturing corneal endothelial cells can be carried out by referring to a known method in the art. The preparation (coating) of a culture container can be performed as follows: For example, after a laminin solution diluted with phosphate buffer to 20 µg/mL is added to a culture dish and incubated for two hours at 37°C (5% CO₂), the solution can be removed and washed twice each with the phosphate buffer in a medium for use.

[0074]

Thus, in another aspect, the present invention is related to a composition for solid-phasing (coating) a cell culture container for culturing a cell in corneal endothelial cells with a system comprising at least one agent selected from the group consisting of laminins expressed in corneal endothelial cells or a fragment thereof (e.g., laminin 511, laminin 521 or a fragment thereof), or an agent for solid-phasing (coating) a cell culture container.

25 In one embodiment, a composition or an agent of the present invention is a coating composition or a coating agent. A treatment technique of solid-phasing laminins on a surface of a culture container is known in the art. Thus, those skilled in the art can use any culture container in accordance with the objective of the present invention and apply a treatment to the container to use the container in a method of the present invention.

[0075]

In another aspect, the present invention is further related to a

kit comprising the above-described composition or agent. A kit of the present invention may further comprise a cell culture medium, a cell culture container or the like. A cell culture container may be, for example, a precoated culture dish or a precoated culture plate. Alternatively, a cell culture container of a kit may be in a state where at least one agent selected from a group consisting of laminins expressed in corneal endothelial cells or a fragment thereof (e.g., laminin 511, laminin 521 or a fragment thereof) is in a solid-phase. A cell culture container, composition, agent, and kit of the present invention can be used in a method of culturing a mammalian cell in a system comprising at least one factor selected from a group consisting of laminins expressed in corneal endothelial cells or a fragment thereof (e.g., laminin 511, laminin 521 or a fragment thereof).

[0076]

(Method of culturing a corneal endothelial cell)

In another aspect, the present invention provides a method of culturing corneal endothelial cells, which uses a composition of the present invention. That is, the present invention provides a method of culturing or growing corneal endothelial cells, comprising a step of culturing the corneal endothelial cells by using an agent (laminin, a fragment thereof or the like) of the present invention. An agent (laminin, a fragment thereof, or the like) used in a method of present invention is understood to be able to use any form described herein. Further, any culture component can be used in a method of the present invention as long as the component can be used in culturing the corneal endothelium, and a culture component of any form described herein can be exemplified.

[0077]

In one embodiment, corneal endothelial cells cultured in the present invention are from primates. In a preferred embodiment, corneal endothelial cells cultured in the present invention are from a human.

[0078]

In a preferred embodiment, culturing target cells by a method of the present invention is for preventing or treating a corneal endothelial disorder, and can be used particularly for producing cells, tissues or the like for a transplant.

[0079]

The temperature condition upon culturing corneal endothelial cells are not particularly limited insofar as growing a corneal endothelial cells. However, for example, the temperature condition is about 25° C to about 45° C, or preferably about 30° C to about 40° C considering growth efficiency, or still preferably about 37° C. A culturing method is performed in a common cell culturing incubator under humidified environment with a CO₂ concentration of about 5 to 10%.

[0080]

Any component that can be used in culturing a corneal endothelium can be used as a culture component that may be used in the present invention. In addition, the culture component may be a medium component that has been conventionally sold and used or a component developed separately for corneal endothelium. Examples of such a medium component include but are not limited to OptiMEM, DMEM, M199, MEM and the like (they are available from INVITROGEN or the like).

[0081]

The present invention is characterized in elevating activity of a variety of specific laminins or fragments thereof by using a specific polypeptide and/or peptide in a cell culturing system comprising an agent (e.g., specific laminin or a fragment thereof) of the present invention in cell culturing. The polypeptide is selected from the group consisting of blood proteins other than an extracellular matrix protein, which is serum, serum albumin, prealbumin, immunoglobulin, α -globulin, β -globulin, α 1-antitrypsin (α 1-AT), haptoglobin (Hp), α 2-macroglobulin (α 2-M), α -fetoprotein (AFP), transferrin, retinol-binding protein (RBP) or adiponectin, and gelatin, protein of the tumor necrosis factor (TNF) family, and peptone. In one embodiment of the present invention, a polypeptide and/or peptide that can be used as an additional component is, but not limited to, serum albumin, protein of the tumor necrosis factor (TNF) family or peptone, or the polypeptide and/or peptide is immunoglobulin or gelatin.

[0082]

In the present invention, preferably a blood protein and still preferably a blood protein other than an extracellular matrix protein can be used with an agent (specific laminin or a fragment thereof) of the present invention. The blood protein is preferably selected from serum, serum albumin, prealbumin, immunoglobulin, α -globulin,

β -globulin, α 1-antitrypsin (α 1-AT), haptoglobin (Hp), α 2-macroglobulin (α 2-M), α -fetoprotein (AFP), transferrin, retinol-binding protein (RBP) or adiponectin, which are all blood proteins other than an extracellular matrix protein. "Extracellular matrix" is a substance that fills the extracellular space. At the same time, extracellular matrix has a skeletal role (e.g., cartilage or bone of an animal), a role of a scaffold in cell adhesion (e.g., basement membrane or fibronectin), a role in retaining or providing cell growth factor or the like (e.g., cell growth factor FGF that binds to heparan sulfate) and the like. Many of the individual cells constituting a multicellular organism that are recognized as living are buried in a bed or a nest of an extracellular matrix. Major components of an extracellular matrix of a vertebrate including humans are glycoproteins such as collagen, proteoglycan, fibronectin, and laminin (partially cell adhesion molecules). "Extracellular matrix protein" means a protein constituting such an extracellular matrix.

[0083]

"Blood proteins other than an extracellular matrix protein" in the present invention means blood proteins other than an extracellular matrix protein involved in cell adhesion or the like. They are all known proteins that those skilled in the art can appropriately obtain. Blood proteins other than an extracellular matrix protein are preferably but not limited to human serum albumin (HSA/ e.g. available from Nacalai Tesque), recombinant human serum albumin (rHSA/ e.g., available from SIGMA-ALDRICH), or bovine serum albumin (BSA/ e.g., available from SIGMA-ALDRICH). Further, "blood proteins other than an extracellular matrix protein" may be immunoglobulin. Immunoglobulins, including IgG, IgA, IgM, IgD, and IgE, are well-known to those skilled in the art. For example, a human immunoglobulin (IgG/ e.g., available from Oriental Yeast Co, Ltd.) can be used but is not limited thereto.

[0084]

"Gelatin" referred to herein is a substance extracted by adding heat to collagen, which is the main component of connective tissue such as skin, bone, or tendon of an animal. The main component of gelatin is protein.

[0085]

As an additional component, a protein of the tumor necrosis factor (TNF) family can be used herein. "Tumor Necrosis Factor, TNF)" is one type of cytokine. Narrowly defined, there are three types of TNF, i.e., TNF- α , TNF- β (lymphotoxin (LT)- α) and LT- β . "Proteins of the TNF family" includes at least 19 types of molecules such as a receptor activator NF κ B ligand (RANKL), Fas ligand and CD40 ligand. Preferably, a receptor activator NF κ B ligand (RANKL, sRANKL) can be used as an example of a protein of the TNF family used as an additional component used in the present invention.

10 [0086]

In the present invention, a peptone can be utilized as an additional component. "Peptone" is a protein obtained by dissolving a protein with a proteolytic enzyme. A protein is digested by pepsin in the stomach into a peptone in vivo, and the resulting peptone is further
15 digested into amino acid by pancreatic juice secreted by the pancreas and by intestinal juice secreted by the jejunum. Since a peptone is suitable as a nutritional source for microorganisms, it is often added to a medium. A peptone as such a nutritional source for a medium is obtained by hydrolyzing a protein into amino acid and
20 a low molecular weight peptide. In general, a peptone obtained from a protein of milk (milk casein) undergoing enzymolysis (using protease such as pancreatin extracted from the pancreas of a swine) is commonly used. A peptone derived from a plant is preferably used but are not limited thereto. For example, a peptone is selected
25 from the group consisting of a peptone derived from cottonseeds, a peptone derived from soy beans, a peptone derived from wheat, and a peptide derived from peas.

[0087]

(Corneal Endothelial Cells and Corneal Endothelium Formulation)

30 The present invention provides corneal endothelial cells cultured and produced by the method according to the present invention. The present invention can be considered as having characteristics that do not exist in conventional cells in that normally cultured or grown cells are obtained even when normal culturing is conducted
35 and also when subculturing is conducted. In addition, the most important characteristic is that the cells have corneal endothelial characteristics that are normal in their functions. Accordingly, the corneal endothelial cells that the present invention provides

may be provided as a formulation, which means that the present invention provides a corneal endothelium formulation. Accordingly, the present invention provides a method for manufacturing a corneal endothelium formulation, comprising a step of culturing corneal endothelial cells using a culture solution comprising the agent or composition according to the present invention or a container on which the agent or composition according to the present invention is coated

[0088]

10 In one aspect, the corneal endothelium formulation according to the present invention contains a base material, and corneal endothelial cells layer on the base material.

[0089]

The base material used in the present invention is not particularly limited as long as it may support a cultured corneal endothelial cell layer and maintain its shape in vivo for a given period of time, preferably at least three days, after transplantation. Further, the base material used in the present invention may be those having a role as a scaffold in culturing corneal endothelial cells in a test tube, or may be those having only a role to support the corneal endothelial cell layer after the culturing. Preferably, the base material used in the present invention is those having a role as a scaffold used for culturing corneal endothelial cells and directly subjected to transplantation after the completion of the culturing.

25 [0090]

The base material used in the present invention include, for example, high-polymer material derived from natural products such as collagen, gelatin and cellulose; synthetic macromolecular material such as polystyrene, polyester, polycarbonate and poly(N-isopropyl acrylamide); biodegradable polymer material such as polylactic acid and polyglycolic acid; hydroxyapatite, amnion and the like.

[0091]

The shape of the base material used in the present invention is not particularly limited as long as it supports the corneal endothelial cell layer and it is a shape suitable for transplantation. However, the shape is preferably a sheet. When the formulation according to the present invention is in a sheet shape, it can be cut and used into a size in accordance with an application site

at transplantation. Further, it is also possible to roll the sheet up tightly and insert it into a wound. As a preferable specific example, a circular shape is exemplified which covers about 80% of the area of an injured corneal endothelium. In addition, it is also preferable to incise the peripheral portion of the circle so as to be closely adhered to an application site.

[0092]

In a preferable embodiment, the example of the base material used in the present invention is collagen. As to collagen, the collagen sheet described in Japanese Laid-Open Publication No. 2004-24852 can be preferably used. The subject collagen sheet can be prepared from, for example, amnion in accordance with the method described in Japanese Laid-Open Publication No. 2004-24852.

[0093]

Hereinafter, preparation of corneal endothelial cells layer will be described as an example of a corneal endothelium formulation.

[0094]

The corneal endothelial cell layer used in the present invention preferably comprises at least one of the following characteristics.

More preferably, the corneal endothelial cell layer used in the present invention comprises two or more of the following characteristics. Still more preferably, the corneal endothelial cell layer used in the present invention comprises all of the following characteristics.

(1) The cell layer has a single layer structure. This is one of the characteristics that corneal endothelial cell layers of living organisms comprise.

(2) The cellular density in the cell layer is about 1,000 to about 4,000 cells/mm². In particular, the cellular density is preferably about 2,000 to about 3,000 cells/mm² when an adult is a recipient.

(3) The planar shape of cells constituting the cell layer is substantially hexagon. This is one of the characteristics that cells constituting corneal endothelial cells layer in living organisms comprises. The formulation of the present invention is similar to corneal endothelial cell layers of living organisms, which is capable of exerting a similar function as native corneal endothelial cell layers and is also capable of exerting growth capability in vivo.

(4) The cells are regularly arranged in the cell layer. In the corneal

endothelial cell layers of living organisms, cells constituting the layers are regularly arranged. Thus, normal functions and high transparency of corneal endothelial cells are considered to be maintained, and the moistening function of the cornea is appropriately exerted. Therefore, by comprising such morphological characteristics, the formulation according to the present invention is expected to exert a function similar to that of corneal endothelial cell layers in living organisms.

[0095]

The manufacturing method according to the present invention comprises a step of culturing corneal endothelial cells using the agent, composition or container according to the present invention, and can be carried out by, for example, the following method.

[0096]

<1> Harvesting and Culturing Corneal endothelial Cells in a Test Tube

Corneal endothelial cells are harvested from the cornea of a recipient himself or an appropriate donor using an ordinary method. In consideration of transplantation conditions in the present invention, corneal endothelial cells derived from the same race may be prepared. For example, the Descemet's membrane and endothelial cell layer of cornea tissues are exfoliated from parenchyma of the cornea, they are transferred to a culture dish and treated with Dispase or the like. Accordingly, corneal endothelial cells will fall off the Descemet's membrane. Corneal endothelial cells remaining on the Descemet's membrane can be fallen off by pipetting or the like. After the removal of the Descemet's membrane, the corneal endothelial cells are cultured in a culture solution according to the present invention. As for the culture or culture solution, for example, the following can be used: FBS(fetal bovine serum)(e.g., BIOWEST, catalogue number: S1820-500), b-FGF (basic fibroblast growth factor)(e.g., INVITROGEN, catalogue number: 13256-029), and an antibiotic substance, such as penicillin and streptomycin, may be appropriately added to commercially available DMEM (Dulbecco's Modified Eagle's Medium)(e.g., INVITROGEN, catalogue number :12320 or the like), followed by adding components of a culture normalizer according to the present invention. By coating the agent according

to the present invention to conduct culturing, the adhesion is promoted for corneal endothelial cells to the surface of a culture container, thereby conducting favorable growth. In addition, when culturing is conducted by adding laminin to the culture solution, it is preferable to use a culture dish, surface of which is coated with type I collagen, type IV collagen, fibronectin, laminin or extracellular matrix of bovine corneal endothelial cells or the like. Alternatively, it is possible to use an ordinary culture container which is treated with a commercially available coating agent such as FNC coating mix® (50 ml(AES-0407), ATHENA, catalogue number: 0407). The temperature conditions for culturing corneal endothelial cells are not particularly limited as long as corneal endothelial cells grow. For example, the temperature is within the range of about 25°C to about 45°C, and when the growth efficiency is taken into consideration, it is preferably about 30°C to about 40°C, and still preferably about 37 °C. The culturing method is conducted in such an environment of about 5 to 10% CO₂ concentration under humidification, in a normal cell culturing incubator.

[0097]

<2> Subculturing

After corneal endothelial cells subjected to culturing are grown, Subculturing may be conducted. Preferably, subculturing is conducted at the time of being sub-confluent or confluent. Subculturing may be conducted as follows. First, cells are treated with trypsin-EDTA or the like so that the cells are removed from the surface of a culture container. Then, the cells are collected. The culture normalizer or medium according to the present invention is added to the collected cells to obtain a cell suspension. It is preferable to conduct a centrifugal treatment when the cells are collected or after the collection. The subject centrifugal treatment allows for preparation of a cell suspension with a high cellular density. Preferable cellular density is about 1 to 2×10^6 cells/mL. Note that the conditions for the centrifugal treatment include, without limitation, for example, 500 rpm (30g) to 1000 rpm (70g), and 1 to 10 minutes.

[0098]

The cell suspension is seeded to a culture container similar to the above-mentioned initial culture, thus being subjected to

culturing. While the dilution rate at subculturing varies in accordance with the state of the cells, but it is about 1:2 to 1:4, and preferably 1:3. Subculturing may be conducted under culture conditions similar to the above-mentioned initial culture. The incubation time varies in accordance with the state of cells to be used or the like, but it is 7 to 30 days, for example. The above-mentioned subculturing may be conducted multiple times as needs arise. When a cell adhesion promoting agent (e.g., ROCK inhibitor or the like) is used in the agent, composition, medium or container according to the present invention, the cell adhesion in an initial period of the culture may be enhanced, making it possible to shorten the culture period.

[0099]

<3> Preparation of Corneal Endothelial Cell Layer

The cell suspension is seeded onto a base material such as a collagen sheet, to be subjected to culturing. At this stage, the number of cells to be seeded is adjusted so that a desired cellular density of a cell layer is formed in a corneal endothelium formulation that is manufactured in the end. Specifically, the cells are seeded so that a cell layer of a cellular density within the range of about 1,000 to about 4,000 cells/mm² is formed. The culturing may be conducted under conditions similar to the above-mentioned initial culturing. The incubation time varies in accordance with the state of cells to be used, but it is, for example, 3 to 30 days.

[0100]

By conducting the culture as described above, a corneal endothelium formulation is obtained, in which corneal endothelial cells layer cultured in the test tube is formed on the base material.

[0101]

In the present invention, the corneal endothelium formulation may comprise the agent or composition according to the present invention, or a medium comprising any of them, or the corneal endothelium formulation may be maintained in a container containing any of them, in order to culture or grow corneal endothelial cells. The corneal endothelium formulation may comprise the agent or composition according to the present invention, or a medium comprising any of them, or the corneal endothelium formulation may be maintained in a container containing any of them, until being subjected to

transplantation. The present invention may comprise a corneal endothelium formulation, the agent or composition according to the present invention, or a medium comprising any of them; and alternatively, the present invention provides a combination with
5 a container comprising any of them.

[0102]

The corneal endothelium formulation obtained by the manufacture method according to the present invention may be used as a graft in a treatment of diseases which require transplantation of corneal
10 endothelium, such as bullous keratopathy, corneal edema, corneal leukoma, in particular, cornea dystrophy, and bullous keratopathy caused by corneal endothelium disorder due to external injury or internal ophthalmic surgery. The cause of such bullous keratopathy, corneal endothelium disorder or the like includes Fuchs' corneal
15 endothelial dystrophy, pseudoexfoliation syndrome, corneal endotheliitis and the like, in addition to surgery.

[0103]

The subject for the administration of the corneal endothelium formulation according to the present invention includes mammals
20 (e.g., humans, mice, rats, hamsters, rabbits, cats, dogs, cows, sheep, monkeys and the like), and preferably, primate (e.g., humans).

[0104]

(Treatment or prevention of a corneal endothelial disease, disorder or condition)

25 The present invention provides a medicament for treating or preventing a corneal endothelial disease, disorder or condition, comprising corneal endothelial cells produced by a method for culturing or growing a corneal endothelial cell, comprising a step of culturing corneal endothelial cells using an agent, composition,
30 or medium or a container of the present invention. It is understood that an agent, composition, or medium or container of the present invention can be used in any form described herein. For example, the matters described herein, such as (Composition for culturing or growing a corneal endothelial cell), (Method of culturing a corneal
35 endothelial cell) and (Corneal endothelial cell and corneal endothelial formulation) can be considered. Further, it is understood that corneal endothelial cells used as a medicament can take any form used herein. For example, the matter described in

(Corneal endothelial cell and corneal endothelial formulation) can be considered.

[0105]

In one embodiment, a medicament of the present invention is for the purpose of treating or preventing a corneal endothelium of primates. Preferably, the subject of such treatment or prevention is a human corneal endothelium.

[0106]

In one embodiment, corneal endothelial cells used in a medicament of the present invention are from primates. Preferably, corneal endothelial cells used in a medicament of the present invention are from human.

[0107]

In one embodiment, a corneal endothelial disease, disorder or condition targeted by a medicament of the present invention is bullous keratopathy, corneal endotheliitis, corneal edema, leukoma and the like.

[0108]

In one embodiment, a medicament of the present invention is provided in a sheet form or as a suspension.

[0109]

In one embodiment, a medicament of the present invention further comprises a cell adhesion promoting agent. A cell adhesion promoting agent exerts adhesion promoting action on corneal endothelial cells separated from a corneal tissue or corneal endothelial cells separated and subcultured. Such a cell adhesion promoting agent can be provided together with or separately from corneal endothelial cells provided as a medicament. In a specific embodiment, a cell adhesion promoting agent used in a medicament of the present invention includes a Rho kinase inhibitor. A Rho kinase inhibitor includes compounds disclosed in the following references: US Patent No. 4678783, Japanese Patent No. 3421217, International Publication No. WO 95/28387, International Publication No. WO 99/2062, International Publication No. WO 99/6140, International Publication No. WO 02/076976, International Publication No. WO 02/076977, International Publication No. WO 2002/083175, International Publication No. WO 02/100833, International Publication No. WO 03/059913, International Publication No. WO 03/062227,

International Publication No. WO 2004/009555, International Publication No. WO 2004/022541, International Publication No. WO 2004/108724, International Publication No. WO 2005/003101, International Publication No. WO 2005/039564, International Publication No. WO 2005/034866, International Publication No. WO 2005/037197, International Publication No. WO 2005/037198, International Publication No. WO 2005/035501, International Publication No. WO 2005/035503, International Publication No. WO 2005/035506, International Publication No. WO 2005/080394, International Publication No. WO 2005/103050, International Publication No. WO 2006/057270, and International Publication No. WO 2007/026664. Such compounds can be manufactured by methods described in each of the disclosed references and include, for example, 1-(5-Isoquinolinesulfonyl)homopiperazine or a salt thereof (e.g., fasudil(1-(5-Isoquinolinesulfonyl)homopiperazine)), and (+)-trans-4-(1-aminoethyl)-1-(4-pyridylcarbamoyl)cyclohexanecarboxamide or a salt thereof (e.g., Y-27632((R)-(+)-trans-(4-pyridyl)-4-(1-aminoethyl)-cyclohexanecarboxamide dihydrochloride monohydrate.

20 [0110]

Targets of administration (transplant) of a medicament or method of the present invention include mammals (e.g., humans, mice, rats, hamsters, rabbits, cats, dogs, cows, sheep, monkeys and the like). However, primates are preferred and humans are preferred in particular. Satisfactory results have not been achieved in a corneal endothelial treatment for primates. In this sense, the present invention provides a ground-breaking therapeutic method and medicament.

[0111]

30 In another aspect, the present invention provides a method for treating or preventing a corneal endothelial disease, disorder or condition, comprising a step of using corneal endothelial cells produced by a method for culturing corneal endothelial cells in a normal manner, comprising a step of culturing corneal endothelial cells using an agent, composition, medium, or a container of the present invention.

[0112]

The mentioned references cited herein such as scientific

publications, patents, and patent applications are incorporated by reference herein in their entirety in the same manner as the contents of each reference are specifically described therein.

[0113]

5 As described above, the present invention has been described while presenting preferred embodiments to facilitate understanding. Hereinafter, the present invention will be described based on the Examples. However, the aforementioned description and the following Examples are provided for the sole purpose of exemplification and
10 are not provided for the purpose of limiting the present invention. Thus, the scope of the present invention is limited solely by the Claims, not by the embodiments or the Examples specifically described herein.

[0114]

15 [Examples]

Hereinafter, an example will be described where cells of corneal endothelial cells according to the present invention are normally cultured. When applicable, the standards set forth by Ministry of Health, Labour and Welfare, Ministry of Education, Culture, Sports,
20 Science and Technology, or the like were observed for the handling of biological samples or the like, and when applicable, the handling was conducted based on Helsinki Declaration or the ethical provision created based on Helsinki Declaration. With regard to donation of eyes for studies, letters of consent were obtained from close
25 relatives of all the deceased donors. The present study was approved by the ethical review of SightLife™ (Seattle, WA) eye bank.

[0115]

(Experimental Method: Human Corneal Tissue of Study Grade)

Twelve human donor corneas were each obtained from the SightLife™
30 eye bank, and all the corneas were preserved in a preservation medium (Optisol; Chiron Vision Corporation, Irvine, CA) at 4° C for a period of less than 14 days before the primary culture.

[0116]

(Statistics Analysis)

35 The statistically-significant difference (P value) in an average value of a comparison of two samples was determined using t-test of Student's t-test. The statistically-significant difference in a comparison of a plurality of sample sets was analyzed using Dunnett's

multiple comparison test. The values shown in the graph represent average $\pm$ SE.

[0117]

(Example 1: Expression of a laminin chain and an integrin chain in corneal endothelial cells and a Descemet's membrane)

In the present Example, expression of a laminin chain in a Descemet's membrane, which is a basement membrane of a corneal endothelial cell, was observed.

[0118]

10 (Materials and Methods)

Expression of laminin chain mRNA was conducted using a PCR method. Although data is not shown, expression of proteins is verified by immunostaining.

[0119]

15 A secondary antibody was diluted with PBS and the resultant solution was incubated for thirty minutes at room temperature. AlexaTM Fluor 488 labeled (conjugated) Goat Anti-Rabbit IgG (Catalog Number: A11034; 1:1500; Molecular Probe-Invitrogen) was used as the secondary antibody. After shaking and washing twice with 0.15% Triton/PBS and once with PBS, nuclear staining was performed with propidium iodide (Catalog Number: SP29004-41; PI; Nacalai Tesque, Inc. Kyoto, Japan) and the secondary antibody was embedded by covering with a cover glass. The fluorescence-labelled secondary antibody was observed with a confocal laser scanning microscope (Olympus Fluoview, Tokyo, Japan) and a picture thereof was taken.

[0120]

The sequences of primers of laminin chains used in a PCR method are shown in the following Table 1. The sequences of primers of integrin chains used in a PCR method are shown in the following Table 2. The primers were obtained from Life Technologies Japan Ltd (Catalog Number: 10336022).

Table 1. Oligonucleotide sequences for PCR

Gene	Sense primer	Anti-sense primer	Size (bp)
Laminin α 1	5'-GAGTCCGTCTCTCTGGACATAG-3' (SEQ ID NO: 9)	5'-CGTGGCATTACAGGGTTGAC-3' (SEQ ID NO: 10)	180
Laminin α 2	5'-TGCTAGAATTTACCTCCGCTCG-3' (SEQ ID NO: 11)	5'-GATCAAGTGGACAAGCCCTG-3' (SEQ ID NO: 12)	203
Laminin α 3	5'-CTCCAAAGGCCCAACTCAAG-3' (SEQ ID NO: 13)	5'-CCATAACTGCCTCCTTAGTCTC-3' (SEQ ID NO: 14)	304
Laminin α 4	5'-CTTACGCAACACCACCGGATTC-3' (SEQ ID NO: 15)	5'-CCTTCTTCCAAGCATTCTCCG-3' (SEQ ID NO: 16)	140

Laminin $\alpha 5$	5'-GAGGACTGAAGTGAAACTCAA-3' (SEQ ID NO: 17)	5'-CCACTGAAGTTGTAAATGGTG-3' (SEQ ID NO: 18)	221
Laminin $\beta 1$	5'-GATGGTGAACCTTGATGAAAAGT-3' (SEQ ID NO: 19)	5'-GGCTTATATCCTTTAGGAGTGA-3' (SEQ ID NO: 20)	258
Laminin $\beta 2$	5'-GATGATCGCATCCAAGGGAG-3' (SEQ ID NO: 21)	5'-GTCCAGAGTAGGGAGTCTCAG-3' (SEQ ID NO: 22)	150
Laminin $\beta 3$	5'-CCCAGATGGAGGAAGATGTC-3' (SEQ ID NO: 23)	5'-GTAGCTGAGTCTGTGGGCAG-3' (SEQ ID NO: 24)	144
Laminin $\beta 4$	5'-GGCAGGCTACTTTGGATTTC-3' (SEQ ID NO: 25)	5'-GCTTGAGGGATCATCTGGAC-3' (SEQ ID NO: 26)	204
Laminin $\gamma 1$	5'-GATGAGATGGTGACAGATCAAG-3' (SEQ ID NO: 27)	5'-TTCCAGTCTCTTCAATGGTAT-3' (SEQ ID NO: 28)	199
Laminin $\gamma 2$	5'-ATCGAAGGTTACTGCGGAATC-3' (SEQ ID NO: 29)	5'-GTAGCCAGAAGCACAATCCTG-3' (SEQ ID NO: 30)	193
Laminin $\gamma 3$	5'-GGGATACAAGAGGGAGATGC-3' (SEQ ID NO: 31)	5'-CATAGAAACCTGGCAAACAGC-3' (SEQ ID NO: 32)	157

Table 2. Oligonucleotide sequences for PCR

Gene	Sense primer	Anti-sense primer	Size (bp)
<i>Integrin $\alpha 1$</i>	5'-gaagaacctcctgaaaccttt-3' (SEQ ID NO: 33)	5'-tgatgcatattggggaatgaa-3' (SEQ ID NO: 34)	254
<i>Integrin $\alpha 2$</i>	5'-tgatgggacagaagtaacatgc-3' (SEQ ID NO: 35)	5'-lggaccaacatctcaaaactg-3' (SEQ ID NO: 36)	333
<i>Integrin $\alpha 3$</i>	5'-gctctgcctttggtttatcgt-3' (SEQ ID NO: 37)	5'-ttccactagaaggctctggga-3' (SEQ ID NO: 38)	257
<i>Integrin $\alpha 4$</i>	5'-atattcagtcggagctggcat-3' (SEQ ID NO: 39)	5'-gcattttgtcacttccaacga-3' (SEQ ID NO: 40)	338
<i>Integrin $\alpha 5$</i>	5'-tcctcagcaagaatcacaacaa-3' (SEQ ID NO: 41)	5'-gttgagtcctgaactctgggc-3' (SEQ ID NO: 42)	304
<i>Integrin $\alpha 6$</i>	5'-agcaaggcagatggaataatgt-3' (SEQ ID NO: 43)	5'-cagggtaggaattcgatcaag-3' (SEQ ID NO: 44)	275
<i>Integrin $\alpha 7$</i>	5'-caggtcacctctacctcatcc-3' (SEQ ID NO: 45)	5'-accgtgacctcatactgacct-3' (SEQ ID NO: 46)	262
<i>Integrin $\alpha 8$</i>	5'-atggaaaatgaaccaggatgg-3' (SEQ ID NO: 47)	5'-cagttatgaatgggcagaacaa-3' (SEQ ID NO: 48)	265
<i>Integrin $\alpha 9$</i>	5'-cacttcagcccatcaatatca-3' (SEQ ID NO: 49)	5'-acagtgctgtgttaggcaagaa-3' (SEQ ID NO: 50)	305
<i>Integrin $\alpha 10$</i>	5'-atcagtggttcagagggact-3' (SEQ ID NO: 51)	5'-gccctggctttagtattgtc-3' (SEQ ID NO: 52)	330
<i>Integrin $\alpha 11$</i>	5'-ggacactgctgactacgtgaag-3' (SEQ ID NO: 53)	5'-gcgtgtgctctctatgatgaag-3' (SEQ ID NO: 54)	294
<i>Integrin αE</i>	5'-tagcaggaagaagctgacgag-3' (SEQ ID NO: 55)	5'-tcttcaggaagacgacagtga-3' (SEQ ID NO: 56)	300
<i>Integrin αV</i>	5'-atctgtgaggtcgaaacaggat-3' (SEQ ID NO: 57)	5'-acctgccaataaaagclacca-3' (SEQ ID NO: 58)	255
<i>Integrin αL</i>	5'-gaaccattgacaccagaagtga-3' (SEQ ID NO: 59)	5'-ttctcaaaccccaactgtctt-3' (SEQ ID NO: 60)	341
<i>Integrin αM</i>	5'-gatcggctaagagaaggacaga-3' (SEQ ID NO: 61)	5'-cattgccacaattcttctcaa-3' (SEQ ID NO: 62)	330
<i>Integrin αX</i>	5'-ccaacatctgcctttacattga-3' (SEQ ID NO: 63)	5'-cgtgaagtatctctgagcatcg-3' (SEQ ID NO: 64)	331
<i>Integrin αD</i>	5'-ttaaccagatgaagggttgtt-3' (SEQ ID NO: 65)	5'-ggctttgtactctgcccattc-3' (SEQ ID NO: 66)	296
<i>Integrin αIIb</i>	5'-gaaaagactgaggaggctgaga-3' (SEQ ID NO: 67)	5'-gagaaaatalccgcaactggag-3' (SEQ ID NO: 68)	245

<i>Integrin β1</i>	5'-gctgaagactatcccatggacc-3' (SEQ ID NO: 69)	5'-attccagatatgcgctgtttt-3' (SEQ ID NO: 70)	321
<i>Integrin β2</i>	5'-tgatggacctctctactccat-3' (SEQ ID NO: 71)	5'-gaaactgggtggagttgttgg-3' (SEQ ID NO: 72)	258
<i>Integrin β3</i>	5'-tgtttaccactgatgccaagac-3' (SEQ ID NO: 73)	5'-tcccataagcatcaacaalgag-3' (SEQ ID NO: 74)	308
<i>Integrin β4</i>	5'-gcttcacacctattccctgtc-3' (SEQ ID NO: 75)	5'-gaaggaagggttcagatggatg-3' (SEQ ID NO: 76)	316
<i>Integrin β5</i>	5'-gctggtgttcacaacagatgat-3' (SEQ ID NO: 77)	5'-atcccagactgacaactccact-3' (SEQ ID NO: 78)	349
<i>Integrin β6</i>	5'-tgtgactgttggaatgtgtg-3' (SEQ ID NO: 79)	5'-caccagctagttgcactgtc-3' (SEQ ID NO: 80)	289
<i>Integrin β7</i>	5'-cacttcagacgacacattccat-3' (SEQ ID NO: 81)	5'-cccaactgcagacttaggaatc-3' (SEQ ID NO: 82)	250
<i>Integrin β8</i>	5'-gcattatgtcgaccaaactca-3' (SEQ ID NO: 83)	5'-attcttcaggctctcacgtc-3' (SEQ ID NO: 84)	255

[0121]

*PCR Method: A PCR method was performed on each laminin chain and integrin chain by RT-PCR (semiquantitative reverse transcription polymerase chain reaction). Primers were purchased from INVITROGEN, which is an oligonucleotide synthesizing company, and those on which a desalination treatment has been performed were used. RNEasy Mini Kit (QIAGEN GmbH, Catalog Number: 74106) was used for the extraction of total RNA from cells. A Descemet's membrane including corneal endothelial cells was exfoliated from a cornea for research-use that was purchased from the Seattle Eye Bank and the corneal endothelial cells were mechanically exfoliated with the basement membrane to use in RNA extraction from the corneal endothelial cells. A reverse transcription reaction (42° C, sixty minutes) was performed on the RNA with ReverTra Ace (Toyobo Co., Ltd. (Catalog Number: TRT-101)), and CD166 and CD73 were amplified with GAPDH as an internal standard by using a TAKARA Taq HotStart Version of DNA polymerase (Takara Bio Inc, Catalog Number: RR001A). The same amount of cDNA was amplified by a PCR device (GeneAmp 9700; Applied Biosystems) and the following primer pair. In the PCR reaction, primers shown in Table 1, Table 2 and those described below were used.

*GAPDH-F: GAGTCAACGGATTTGGTCGT (SEQ ID NO: 85)

*GAPDH-R: TTGATTTTGGAGGGATCTCG (SEQ ID NO: 86)

[0122]

25 An amplified cDNA fragment was electrophoresed with 1.5% agarose gel (Nacalai Tesque, Catalog Number: 01149-76) and detected by staining with ethidium bromide (Nacalai Tesque, Catalog Number: 14603-51).

[0123]

* Flow cytometry: A cultured human corneal endothelium was seeded in a culture dish coated with FNC Coating and cultured for about 14 days until reaching confluent state under the condition of 5% CO₂ at 37° C. Cells were exfoliated with TrypLE™ Select and collected. Then, analysis on a surface antigen of an integrin chain was conducted by using a flow cytometer (BD FACSCanto™ II (BD Biosciences, Franklin Lakes, NJ)) in accordance with the instruction manual while using Human Cell Surface Marker Screening Panel (BD Lyoplate™, BD Bio-sciences, Franklin Lakes, NJ).

[0124]

A human corneal endothelial cell was cultured as described below. A Descemet's membrane including corneal endothelial cells was exfoliated from a cornea for research-use that was purchased from the Seattle Eye Bank and the corneal endothelial cell was mechanically exfoliated with the basement membrane. After detaching (typically, treated for two hours at 37° C by using 1 mg/mL collagenase A (Roche Applied Science)) and collecting from the base membrane by using collagenase (ROCHE Catalog Number: 10 103 586 001), primary culture was conducted. For a medium, a medium in which the following was conditioned for a 3T3 feeder cell was used: Opti-MEM I Reduced-Serum Medium, Liquid (INVITROGEN, Catalog Number: 31985-070) + 8% fetal bovine serum (FBS) (BIOWEST, Catalog Number: S1820-500) + 200 mg/ml CaCl₂·2H₂O (SIGMA Catalog Number: C7902-500G) + 0.08% chondroitin sulfate (SIGMA Catalog Number: C9819-5G) + 20 µg/ml ascorbic acid (SIGMA Catalog Number: A4544-25G) + 50 µg/ml gentamicin (INVITROGEN Catalog Number: 15710-064) + 5 ng/ml EGF (INVITROGEN Catalog Number: PHG0311). Specifically, after digestion at 37° C, HCEC obtained from individual cornea is resuspended in a culture medium and plated in one well of a 12-well plate coated with FNC Coating Mix™. The culture medium was prepared in accordance of a published protocol to which a partial alteration was added. Briefly explained, a base culture medium was prepared, containing OptiMEM-I (Life Technologies), 8% FBS, 5ng/mL epidermal growth factor (EGF) (Sigma-Aldrich Co., St. Louis, MO), 1µM SB431542 (Merck Millipore), 20 µg/mL ascorbic acid (Sigma-Aldrich), 200 mg/L calcium chloride (Sigma-Aldrich), 0.08% chondroitin sulfate (Wako Pure Chemical Industries, Ltd., Osaka) and 50 µg/mL gentamicin. Next, a conditioned

medium was collected after culturing inactivated 3T3 fibroblasts. Inactivation of the 3T3 fibroblasts was performed as described above. Briefly explained, confluent 3T3 fibroblasts were incubated for two hours at 37° C under 5% CO₂ with 4 µg/mL mitomycin C (MMC) (Kyowa Hakko Kirin Co., Ltd., Tokyo) and then the resultant was treated with trypsin and was plated on a plastic plate at a density of 2×10⁴ cells/cm². HCEC was cultured under a humidified atmosphere at 37° C in 5% CO₂, and the culture medium was replaced every three days. When HCEC reaches confluent state in 14 to 28 days, HCEC was rinsed in Ca²⁺ and Mg²⁺ free PBS, treated with trypsin with 0.05% trypsin-EDTA for five minutes at 37° C, and then subcultured at a ratio of 1:2. [0125]

(Results)

When expression of the laminin chain in the Descemet's membrane (corneal endothelial cell basement membrane) was observed, expression of laminin α5 chain, laminin β1 chain, and laminin γ1 chain was prominent. On the other hand, expression of laminin α1 chain, laminin α2 chain, and laminin α3 chain was not apparent (data not shown).

[0126]

As shown in Figure 1, when mRNA expression of laminin chains of human corneal endothelial cells was observed, expression of laminin α5 chain, laminin β1 chain, laminin β2 chain, and laminin γ1 chain was prominent. On the other hand, expression of laminin α1 chain, laminin α2 chain, laminin α3 chain, laminin α4 chain, laminin β3 chain, laminin γ2 chain, and laminin γ3 chain was not apparent.

[0127]

As shown in Figure 2, expression was recognized in integrin α1 chain, integrin α2 chain, integrin α3 chain, integrin α6 chain, integrin α10 chain, integrin α11 chain, integrin β1 chain, integrin β5 chain, integrin β8 chain, and integrin αV chain. Slight expression was also recognized in integrin β3 chain, integrin β4 chain, and integrin β6 chain. Thus, the above matter suggests that corneal endothelial cells express at least one of α1β1, α2β1, α3β1, α6β1, α7β1, and α6β4, which are integrins known as a laminin binding integrin.

[0128]

As shown in Figure 3 (Fig. 3A, 3B and 3C), expression of integrin α1 chain, integrin α2 chain, integrin α3 chain, integrin α5 chain,

and integrin $\beta 1$ chain was recognized as an expression as a surface antigen.

[0129]

(Example 2: Promotion of Cell adhesion of Human Corneal Endothelial Cells)

In the present example, a culture container coated with laminin or the like was used to confirm as to whether or not cell adhesion would be made for human corneal endothelial cells.

[0130]

10 (Material and Method)

*laminin 511 (LN511, VERITAS Corporation)

*laminin 521(LN521, VERITAS Corporation)

*laminin 511-E8 fragment (382-02413, Nippi. Inc.)

*FNC coating mix® (50 ml(AES-0407), ATHENA, catalogue number: 0407)

15 *gelatine (G1890-500G, Sigma-Aldrich Co. LLC.)

*container (3526, CORNING)

[0131]

(Method) *Human Corneal Endothelial Cells (HCEC, Source and Culture Method): the culturing of HCEC was conducted as in Example 1 mentioned above. The cultured cells were rinsed in Ca^{2+} and Mg^{2+} free PBS, and trypsinized at 37°C for five minutes with 0.05% trypsin-EDTA, followed by seeding to 12-well plates coated with FNC Coating Mix®. The culture medium was prepared in accordance with a publicly-released protocol with a partial alteration added thereto. Briefly speaking, a fundamental culture medium was prepared which contained OptiMEM-I(Life Technologies), 8% FBS, 5ng/mL epidermal growth factor (EGF) (Sigma-Aldrich Co., St. Louis, MO), 10 μM SB431542 (Merck Millipore), 20 $\mu\text{g/mL}$ ascorbic acid (Sigma-Aldrich), 200mg/L calcium chloride (Sigma-Aldrich), 0.08% chondroitin sulfate (Wako Pure Chemical Industries, Ltd., Osaka) and 50 $\mu\text{g/mL}$ gentamicin. Then, after the culturing of inactivated 3T3 fibroblast cells, the conditioned medium was retrieved. Inactivation of the 3T3 fibroblast cells was conducted as described previously. Briefly speaking, confluent 3T3 fibroblast cells were incubated together with 4 $\mu\text{g/mL}$ mitomycin C (MMC) (Kyowa Hakko Kirin Col, Ltd, Tokyo) under 5% CO_2 at 37°C for two hours, followed by trypsin treatment, and plating with the density of 2×10^4 cells/cm² on a plastic plate. The HCEC were cultured in 5% CO_2 at 37°C in humidified atmosphere, and the

culture medium was replaced every three days.

[0132]

(Method) The HCEC were seeded to each well of 96-well plates coated with laminin 511, laminin 521, laminin 211, gelatine, FNC Coating Mix®, and the cell formed after 24 hours were observed using a phase-contrast microscope. Seeding was conducted with the seeding density of 5,000 cells/well to 96-well culture plates, and the number of adhered cells was examined at the point 24 hours after the cell seeding using CellTiter-Glo® Luminescent Cell Viability Assay (Promega Corporation, Madison, WI, USA). In addition, the cell adhesion was evaluated for culture plates coated with laminin 511-E8 fragment (0.001-1.5 µg/cm²). Further, various concentrations of laminin 521 and laminin 511-E8 fragments were added to media for seeding and the number of cells after 24 hours were similarly evaluated.

[0133]

(Measurement of Cell Growth by BrdU Uptake)

Similarly, BrdU uptake of HCEC coated with various matrices was evaluated by ELISA. Seeding was conducted to 96-well culture plates with 5,000 cells/well seeding density, followed by culturing overnight. Then, 5-bromo-2'-deoxyuridine (BrdU) was added to the medium, followed by culturing overnight. The medium was removed, and fixative solution (Amersham cell proliferation biotrak ELISA system, version 2) was added for incubation for 30 minutes at a room temperature. Then, the fixative solution was removed, and a blocking solution (Amersham cell proliferation biotrak ELISA system, version 2) was added, followed by an incubation of 30 minutes at a room temperature. Then, the blocking solution was removed, and peroxidase conjugated anti-BrdU antibody was added, followed by an incubation of 90 minutes at a room temperature. The plates were washed three times with washing buffer, and TMB (3,3',5,5'-tetramethyl benzidine) matrix (Amersham cell proliferation biotrak ELISA system, version 2) was added, followed by an incubation of 5 to 30 minutes. The reaction was stopped using 1M sulfuric acid, and absorbance at 450 nm was measured using a plate reader. The result was shown as an average value of five measurements ± standard error.

[0134]

(Result)

As shown in Figure 4, in the presence of laminin 511 and laminin 521, the adhesion and extension of corneal endothelial cells were favorable, while the growth was not favorable under other conditions.

5 [0135]

As shown in Figure 5, in the presence of laminin 511 and laminin 521, the cell adhesion of corneal endothelial cells was shown to be favorable compared to other conditions.

[0136]

10 As shown in Figure 6, in the presence of laminin 511-E8 fragment, the cell growth of corneal endothelial cells was shown to be favorable compared to other conditions. In particular, the cell adhesion was favorably promoted in the concentration of 0.1 to 1.5 $\mu\text{g}/\text{cm}^2$.

[0137]

15 As shown in Figure 7, in the presence of laminin 511, laminin 521 and laminin 511-E8 fragment, the cell growth of corneal endothelial cells was shown to be favorable compared to other conditions.

[0138]

(Example 3: Functional Analysis for laminin 511 and laminin 521
20 in Cell Culture of Human Corneal Endothelial Cells)

In the present example, functional analysis was conducted for laminin 511 and laminin 521 in cell culture of human corneal endothelial cells.

[0139]

25 (Material and Method)

*Human corneal endothelial cells (HCEC, Source and Culture Method):
Culturing of the HCEC was conducted as follows. Briefly speaking, Descemet's membrane including corneal endothelial cells were exfoliated from the corneas for research-use purchased from the
30 Seattle Eye Bank,; the basement membrane was then mechanically exfoliated together with the corneal endothelial cells, which were then exfoliated from the basement membrane using collagenase (ROCHE catalogue number: 10 103 586 001) (typically, they were treated for two hours using 1 mg/mL collagenase A (Roche Applied Science)
35 at 37°C). After the retrieval, the primary culture was conducted. At the primary culture, plating was conducted to 1 well out of 12-well plates coated with laminin 511, laminin 521, laminin 211, FNC Coating Mix®. For the medium, the same one as in Example 1 was used. Cell

observation was conducted over a period of time using a phase-contrast microscope.

[0140]

*Cell observing method for staining or the like (histological test):
5 after immobilizing the cultured HCEC, immunostaining was conducted using ZO-1, Na⁺/K⁺-ATPase as a function-related marker followed by observation using a fluorescence microscope. The HCEC were immobilized with 4% formaldehyde for 10 minutes at room temperature (RT), followed by incubation with 1% bovine serum albumin (BSA)
10 for 30 minutes. Immune tissue chemical analysis was conducted for tight junction-related protein, ZO-1, and protein related to pumping function, Na⁺/K⁺-ATPase. Each of the primary antibodies was used in a 1:200 dilution. For secondary antibody, 1:2000 dilution of Alexa Fluor™ 488 labelled or Alexa Fluor™ 594 labelled goat
15 anti-mouse IgG (Life Technologies) was used. Then, cell nuclei were stained with DAPI (Vector Laboratories, Inc., Burlingame, CA). Then, slides were observed using a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan).

[0141]

20 *Antibodies to Na⁺/K⁺-ATPase: those made by MILLIPORE (MILLIPORE catalogue number: 05-369) were used.

[0142]

*Antibodies to ZO-1: those made by Rabbit ZYMED LABORATORIES (ZYMED LABORATORIES catalogue number: 61-7300) were used.

25 [0143]

(Result)

As shown in Figure 8, the laminin 511 and laminin 521 were shown to make cell culturing of human corneal endothelial cells more efficient. The photographs show cells two days after the primary
30 culture through a phase-contrast microscope.

[0144]

As shown in Figure 9, the laminin 511 and laminin 521 were shown to allow for cell culturing of human corneal endothelial cells with high cell density. The photographs show cells twenty days after
35 the primary culture through a phase-contrast microscope.

[0145]

As shown in Figures 10 and 11, laminin 511 and laminin 521 were shown to retain the activity of ZO-1 and Na⁺/K⁺-ATPase, and it was

demonstrated that culturing using the method according to the present invention allows for growing while maintaining normal functions. The cell density in laminin 511 and laminin 521 was higher compared to laminin 211 and non-coated control.

5 [0146]

As shown in Figure 12, laminin 521 and laminin 511-E8 fragment of various concentrations were added to media to be seeded, thus promoting the cell adhesion of the corneal endothelial cells.

[0147]

10 (Example 4: exemplary formulations: Culture solution for preparing a corneal endothelial sheet)

In the present Example, as a formulation example, a culture solution for preparing a corneal endothelium sheet containing an agent of the present invention is manufactured as follows.

15 [0148]

A culture solution shown below is prepared with a conventional method. Laminin 511, laminin 521 and/or a fragment thereof ($0.75 \mu\text{g}/\text{cm}^2$)

Fetal bovine serum (FBS)	10 mL
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penicillin-streptomycin solution	1mL
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20 FGF basic	200 ng
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DMEM	appropriate amount
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total amount	100 mL
--------------	--------

[0149]

For example, BIOWEST (Catalog Number: S1820-500) or those
 25 manufactured by Invitrogen can be used for the FBS. For the penicillin-streptomycin solution, those manufactured by Nacalai Tesque (containing penicillin $5000 \mu\text{g}/\text{mL}$, streptomycin $5000 \mu\text{g}/\text{mL}$) can be used. In addition, for example, for the FGF basic, those manufactured by Invitrogen (INVITROGEN, Catalog Number: 13256-029)
 30 can be used. For SB431542, those manufactured by Tocris Cookson Ltd, and for SB203580, those of CALBIOCHEM brand can be used. For DMEM, those manufactured by Invitrogen can be used.

[0150]

(Example 5: exemplary formulations: composition for a container
 35 for preserving or amplifying a cornea)

In the present Example, as an example of a formulation, a solution for coating a container comprising an agent of the present invention is manufactured as follows.

[0151]

A preservation solution shown below is prepared by a conventional method.

Laminin 511, laminin 521 and/or a fragment thereof ($0.75 \mu\text{g}/\text{cm}^2$)

5 Appropriate buffer appropriate amount

Total amount 100 mL

[0152]

Each ingredient can be obtained similarly to Example 4.

[0153]

10 As described above, the present invention is exemplified by the use of preferred embodiments of the present invention. However, it is understood that the scope of the present invention should be interpreted solely based on the claims. Further, it is understood that any patent, any patent application and any references cited
15 herein should be incorporated by reference herein in the same manner as the contents are specifically described therein. The subject application claims priority on Japanese Patent Application No. 2013-244972 filed on November 27, 2013, which should be herein incorporated by reference in its entirety.

20 [Industrial Applicability]

[0154]

Culture components and a culture method for promoting growth of corneal endothelium cells are provided. A technique available in the industry associated with a technique related to corneal implants
25 (cell culturing industry, drug manufacturing and the like) is provided.

[0155]

(Sequence Listing Free Text)

SEQ ID NO: 1 laminin α 5 chain nucleic acid sequence (NM_005560)
30 SEQ ID NO: 2 laminin α 5 chain amino acid sequence (NP_005551)
SEQ ID NO: 3 laminin β 1 chain nucleic acid sequence (NM_002291)
SEQ ID NO: 4 laminin β 1 chain amino acid sequence (NP_002282)
SEQ ID NO: 5 laminin β 2 chain nucleic acid sequence (NM_002292)
SEQ ID NO: 6 laminin β 2 chain amino acid sequence (NP_002283)
35 SEQ ID NO: 7 laminin γ 1 chain nucleic acid sequence (NM_002293)
SEQ ID NO: 8 laminin γ 1 chain amino acid sequence (NP_002284)
SEQ ID NO: 9 laminin α 1 chain sense primer sequence:
5'-GAGTCCGTCTCTCTGGACATAG-3'

SEQ ID NO: 10 laminin α 1 chain antisense primer sequence:
5'-CGTGGCATTCACAGGGTTGAC-3'

SEQ ID NO: 11 laminin α 2 chain sense primer sequence:
5'-TGCTAGAATTTACCTCCGCTCG-3'

5 SEQ ID NO: 12 laminin α 2 chain antisense primer sequence:
5'-GATCAAGTGGACAAGCCCTG-3'

SEQ ID NO: 13 laminin α 3 chain sense primer sequence:
5'-CTCCAAAGGCCCAACTCAAG-3'

SEQ ID NO: 14 laminin α 3 chain antisense primer sequence:
10 5'-CCATAACTGCCTCCTTAGTCTC-3'

SEQ ID NO: 15 laminin α 4 chain sense primer sequence:
5'-CTTACGCAACACCACCGGATTC-3'

SEQ ID NO: 16 laminin α 4 chain antisense primer sequence:
5'-CCTTCTTCCAAGCATTCTCCG-3'

15 SEQ ID NO: 17 laminin α 5 chain sense primer sequence:
5'-GAGGACTGAAGTGAAAACCTCAA-3'

SEQ ID NO: 18 laminin α 5 chain antisense primer sequence:
5'-CCACTGAAGTTGTAAATGGTG-3'

SEQ ID NO: 19 laminin β 1 chain sense primer sequence:
20 5'-GATGGTGAACCTTGATGAAAAGT-3'

SEQ ID NO: 20 laminin β 1 chain antisense primer sequence:
5'-GGCTTATATCCTTTAGGAGTGA-3'

SEQ ID NO: 21 laminin β 2 chain sense primer sequence:
5'-GATGATCGCATCCAAGGGAC-3'

25 SEQ ID NO: 22 laminin β 2 chain antisense primer sequence:
5'-GTCCAGAGTAGGGAGTCTCAG-3'

SEQ ID NO: 23 laminin β 3 chain sense primer sequence:
5'-CCCAGATGGAGGAAGATGTC-3'

SEQ ID NO: 24 laminin β 3 chain antisense primer sequence:
30 5'-GTAGCTGAGTCTGTGGGCAG-3'

SEQ ID NO: 25 laminin β 4 chain sense primer sequence:
5'-GGCAGGCTACTTTGGATTTC-3'

SEQ ID NO: 26 laminin β 4 chain antisense primer sequence:
5'-GCTTGAGGGATCATCTGGAC-3'

35 SEQ ID NO: 27 laminin γ 1 chain sense primer sequence:
5'-GATGAGATGGTGACAGATCAAG-3'

SEQ ID NO: 28 laminin γ 1 chain antisense primer sequence:
5'-TTTCCAGTCTCTTCAATGGTAT-3'

SEQ ID NO: 29 laminin γ 2 chain sense primer sequence:

5'-ATCGAAGGTTACTGCGGAATC-3'

SEQ ID NO: 30 laminin γ 2 chain antisense primer sequence:

5'-GTAGCCAGAAGCACAAATCCTG-3'

5 SEQ ID NO: 31 laminin γ 3 chain sense primer sequence:

5'-GGGATACAAGAGGGAGATGC-3'

SEQ ID NO: 32 laminin γ 3 chain antisense primer sequence:

5'-CATAGAAACCTGGCAAACAGC-3'

SEQ ID NO: 33 integrin α 1 chain sense primer sequence:

10 5'-gaagaacctcctgaaacccttt-3'

SEQ ID NO: 34 integrin α 1 chain antisense primer sequence:

5'-tgatgtcatattgggggaatgaa-3'

SEQ ID NO: 35 integrin α 2 chain sense primer sequence:

5'-tgatgggacagaagtaacatgc-3'

15 SEQ ID NO: 36 integrin α 2 chain antisense primer sequence:

5'-tggaccaacatcttcaaaactg-3'

SEQ ID NO: 37 integrin α 3 chain sense primer sequence:

5'-gctctgcctttggtttatctgt-3'

SEQ ID NO: 38 integrin α 3 chain antisense primer sequence:

20 5'-ttcccactagaaggtctgggta-3'

SEQ ID NO: 39 integrin α 4 chain sense primer sequence:

5'-atattcagtcggagctggatcat-3'

SEQ ID NO: 40 integrin α 4 chain antisense primer sequence:

5'-gcatatttgtcacttccaacga-3'

25 SEQ ID NO: 41 integrin α 5 chain sense primer sequence:

5'-tcctcagcaagaatctcaacaa-3'

SEQ ID NO: 42 integrin α 5 chain antisense primer sequence:

5'-gttgagtcccgtactctgggtc-3'

SEQ ID NO: 43 integrin α 6 chain sense primer sequence:

30 5'-agcaaggcagatggaataatgt-3'

SEQ ID NO: 44 integrin α 6 chain antisense primer sequence:

5'-cagggtaggaatttcgatcaag-3'

SEQ ID NO: 45 integrin α 7 chain sense primer sequence:

5'-caggtcaccttctacatcc-3'

35 SEQ ID NO: 46 integrin α 7 chain antisense primer sequence:

5'-accgtgacctcatacttgacct-3'

SEQ ID NO: 47 integrin α 8 chain sense primer sequence:

5'-atggaaaatgtaaccaggatgg-3'

SEQ ID NO: 48 integrin $\alpha 8$ chain antisense primer sequence:
 5'-cagttatgaatgggcagaacaa-3'

SEQ ID NO: 49 integrin $\alpha 9$ chain sense primer sequence:
 5'-cactttcagcccatcaatatca-3'

5 SEQ ID NO: 50 integrin $\alpha 9$ chain antisense primer sequence:
 5'-acagtgtgctggttaggcaagaa-3'

SEQ ID NO: 51 integrin $\alpha 10$ chain sense primer sequence:
 5'-atcagtgtggttcagagggact-3'

SEQ ID NO: 52 integrin $\alpha 10$ chain antisense primer sequence:
 10 5'-gccctggctttgtagtattgtc-3'

SEQ ID NO: 53 integrin $\alpha 11$ chain sense primer sequence:
 5'-ggacactgctgactacgtgaag-3'

SEQ ID NO: 54 integrin $\alpha 11$ chain antisense primer sequence:
 5'-gcgtgtgctctctatgatgaag-3'

15 SEQ ID NO: 55 integrin αE chain sense primer sequence:
 5'-tagcagtgaagaagctgacgag-3'

SEQ ID NO: 56 integrin αE chain antisense primer sequence:
 5'-tctttcaggaagacgacagtga-3'

SEQ ID NO: 57 integrin αV chain sense primer sequence:
 20 5'-atctgtgaggtcgaaacaggat-3'

SEQ ID NO: 58 integrin αV chain antisense primer sequence:
 5'-accttgccaataaaagctacca-3'

SEQ ID NO: 59 integrin αL chain sense primer sequence:
 5'-gaaccattgacaccagaagtga-3'

25 SEQ ID NO: 60 integrin αL chain antisense primer sequence:
 5'-ttcttcaaaccccaactgtctt-3'

SEQ ID NO: 61 integrin αM chain sense primer sequence:
 5'-gatcggctaagagaaggacaga-3'

SEQ ID NO: 62 integrin αM chain antisense primer sequence:
 30 5'-cattgccacaattcttctcaaa-3'

SEQ ID NO: 63 integrin αX chain sense primer sequence:
 5'-ccaacatctgcctttacattga-3'

SEQ ID NO: 64 integrin αX chain antisense primer sequence:
 5'-cgtgaagtatctctgagcatcg-3'

35 SEQ ID NO: 65 integrin αD chain sense primer sequence:
 5'-ttaaccagatgaagggtttgt-3'

SEQ ID NO: 66 integrin αD chain antisense primer sequence:
 5'-ggtctttgtacttctgcccac-3'

SEQ ID NO: 67 integrin α Ib chain sense primer sequence:
 5'-gaaaagactgaggaggctgaga-3'

SEQ ID NO: 68 integrin α Ib chain antisense primer sequence:
 5'-gagaaaatatccgcaactggag-3'

5 SEQ ID NO: 69 integrin β 1 chain sense primer sequence:
 5'-gctgaagactatcccattgacc-3'

SEQ ID NO: 70 integrin β 1 chain antisense primer sequence:
 5'-atttccagatatgcgctgtttt-3'

SEQ ID NO: 71 integrin β 2 chain sense primer sequence:
 10 5'-tgatggacctctcctactccat-3'

SEQ ID NO: 72 integrin β 2 chain antisense primer sequence:
 5'-gaaactggttggagttgttggt-3'

SEQ ID NO: 73 integrin β 3 chain sense primer sequence:
 5'-tgtttaccactgatgccaagac-3'

15 SEQ ID NO: 74 integrin β 3 chain antisense primer sequence:
 5'-tcccataagcatcaacaatgag-3'

SEQ ID NO: 75 integrin β 4 chain sense primer sequence:
 5'-gcttcacacctatttccctgtc-3'

SEQ ID NO: 76 integrin β 4 chain antisense primer sequence:
 20 5'-gaaggaaggtttcagatggatg-3'

SEQ ID NO: 77 integrin β 5 chain sense primer sequence:
 5'-gctggtgttcacaacagatgat-3'

SEQ ID NO: 78 integrin β 5 chain antisense primer sequence:
 5'-atcccagactgacaactccact-3'

25 SEQ ID NO: 79 integrin β 6 chain sense primer sequence:
 5'-tgtgactgtggtgaatgtgtgt-3'

SEQ ID NO: 80 integrin β 6 chain antisense primer sequence:
 5'-caccagctagtttgcacttgtc-3'

SEQ ID NO: 81 integrin β 7 chain sense primer sequence:
 30 5'-cacttcagacgacacattccat-3'

SEQ ID NO: 82 integrin β 7 chain antisense primer sequence:
 5'-cccaactgcagacttaggaatc-3'

SEQ ID NO: 83 integrin β 8 chain sense primer sequence:
 5'-gcattatgtcgaccaaacttca-3'

35 SEQ ID NO: 84 integrin β 8 chain antisense primer sequence:
 5'-atttcttcaggcttctcacgtc-3'

SEQ ID NO: 85 GAPDH-F: GAGTCAACGGATTTGGTCGT

SEQ ID NO: 86 GAPDH-R: TTGATTTTGGAGGGATCTCG

CLAIMS

[Claim 1]

A composition for culturing or growing corneal endothelial cells, comprising at least one agent consisting of laminins and fragments thereof which express in corneal endothelial cells.

[Claim 2]

The composition according to claim 1, wherein the laminins comprise laminin 511 (alpha5 beta1 gamma1) and laminin 512 (alpha5 beta2 gamma 1)

10 [Claim 3]

The composition according to claim 1, wherein the fragments has cell adhesion capability of corneal endothelial cells.

[Claim 4]

15 The composition according to claim 1, wherein the agent is laminin 511, laminin 521 or laminin 511-E8 fragment.

[Claim 5]

The composition according to claim 1, wherein the corneal endothelial cells are from human.

[Claim 6]

20 A medium for culturing corneal endothelial cells, comprising the composition according to claim 1.

[Claim 7]

A culture container for corneal endothelial cells, which is coated with the composition according to claim 1.

25 [Claim 8]

A method for culturing corneal endothelial cells comprising the step of using the composition according to claim 1.

[Claim 9]

30 A method for culturing corneal endothelial cells comprising the step of using the medium according to claim 6.

[Claim 10]

A method for culturing corneal endothelial cells comprising the step of using the container according to claim 7.

Fig. 1

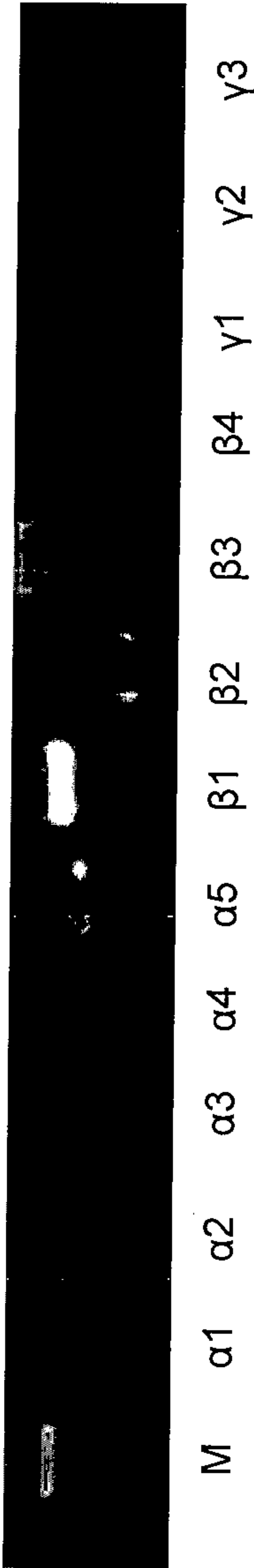
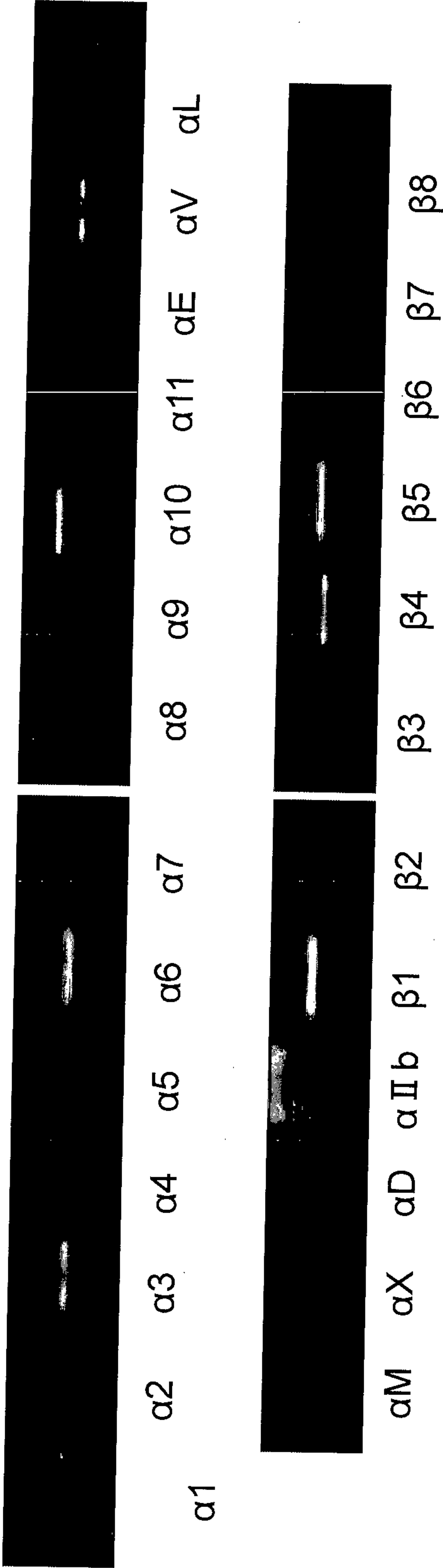
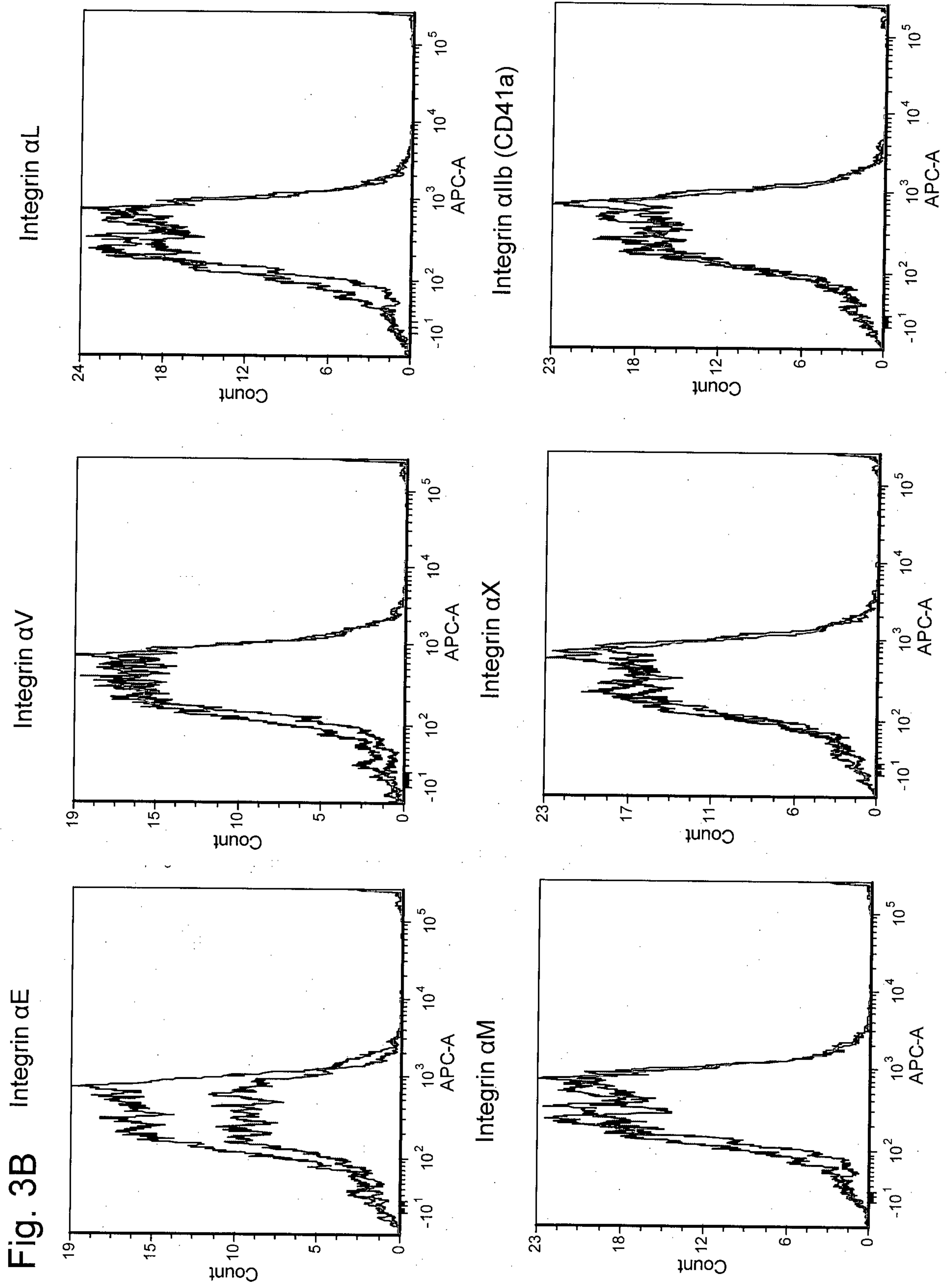


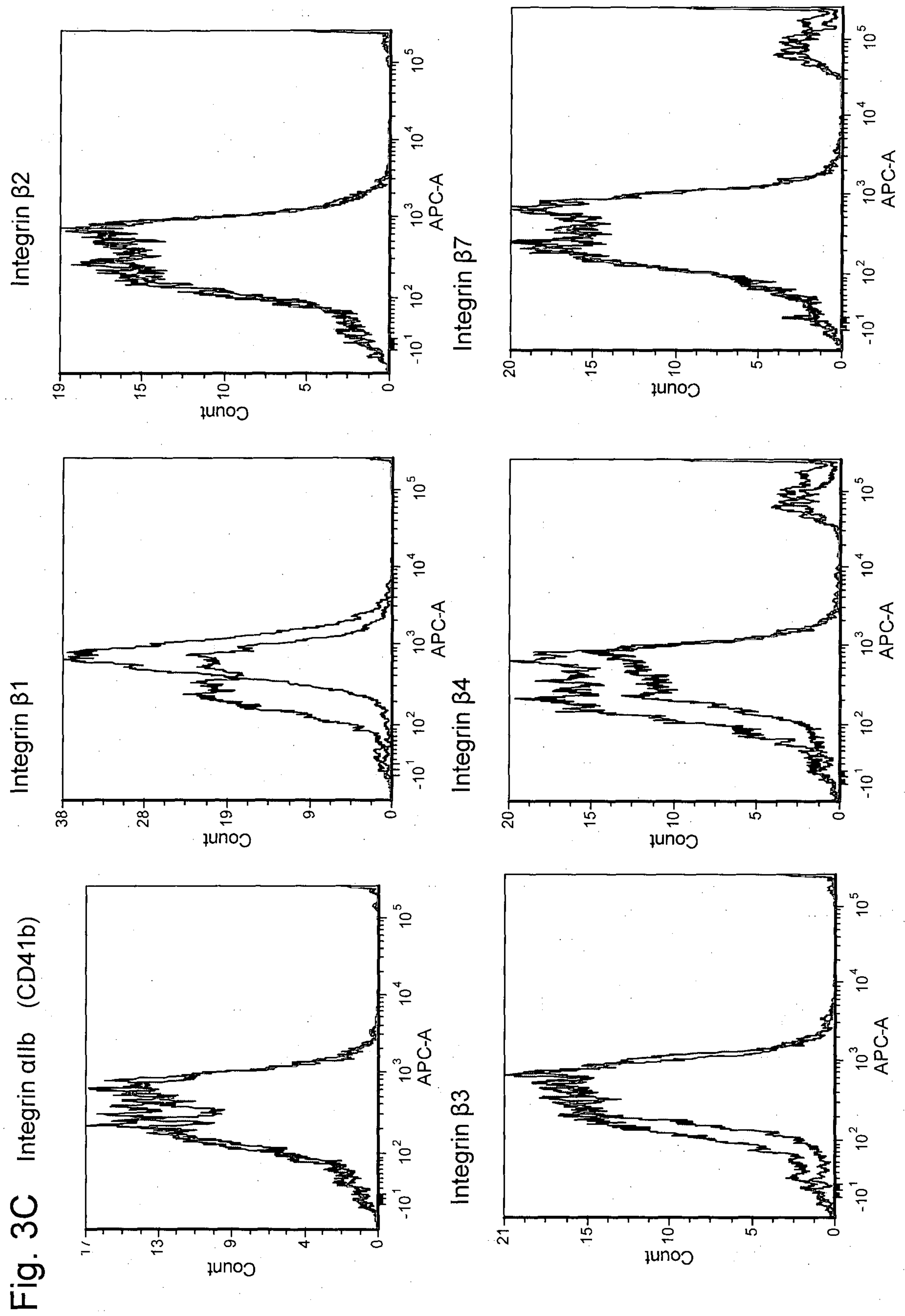
Fig. 2



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Fig. 4

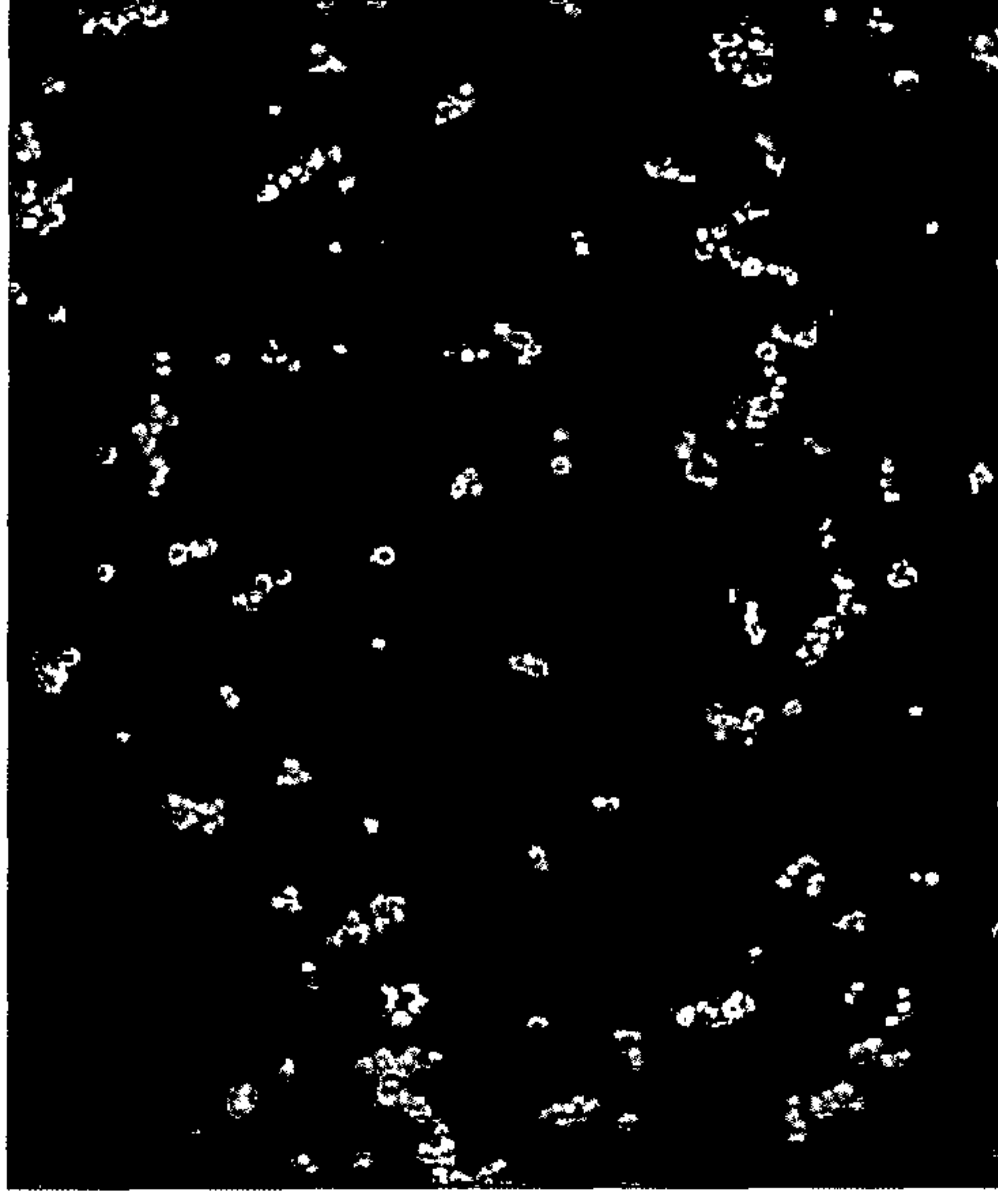
LM-511



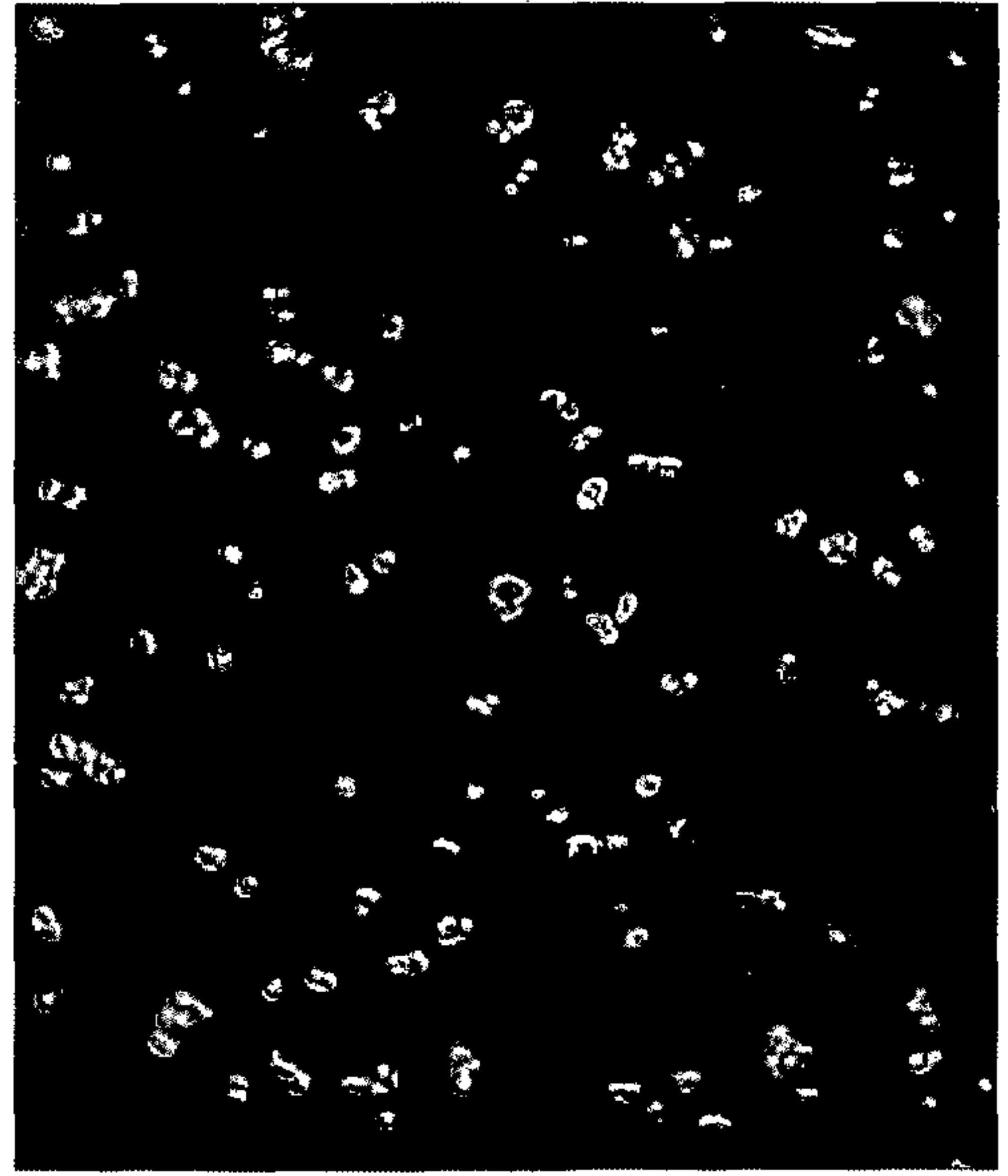
LM-521



LM-211



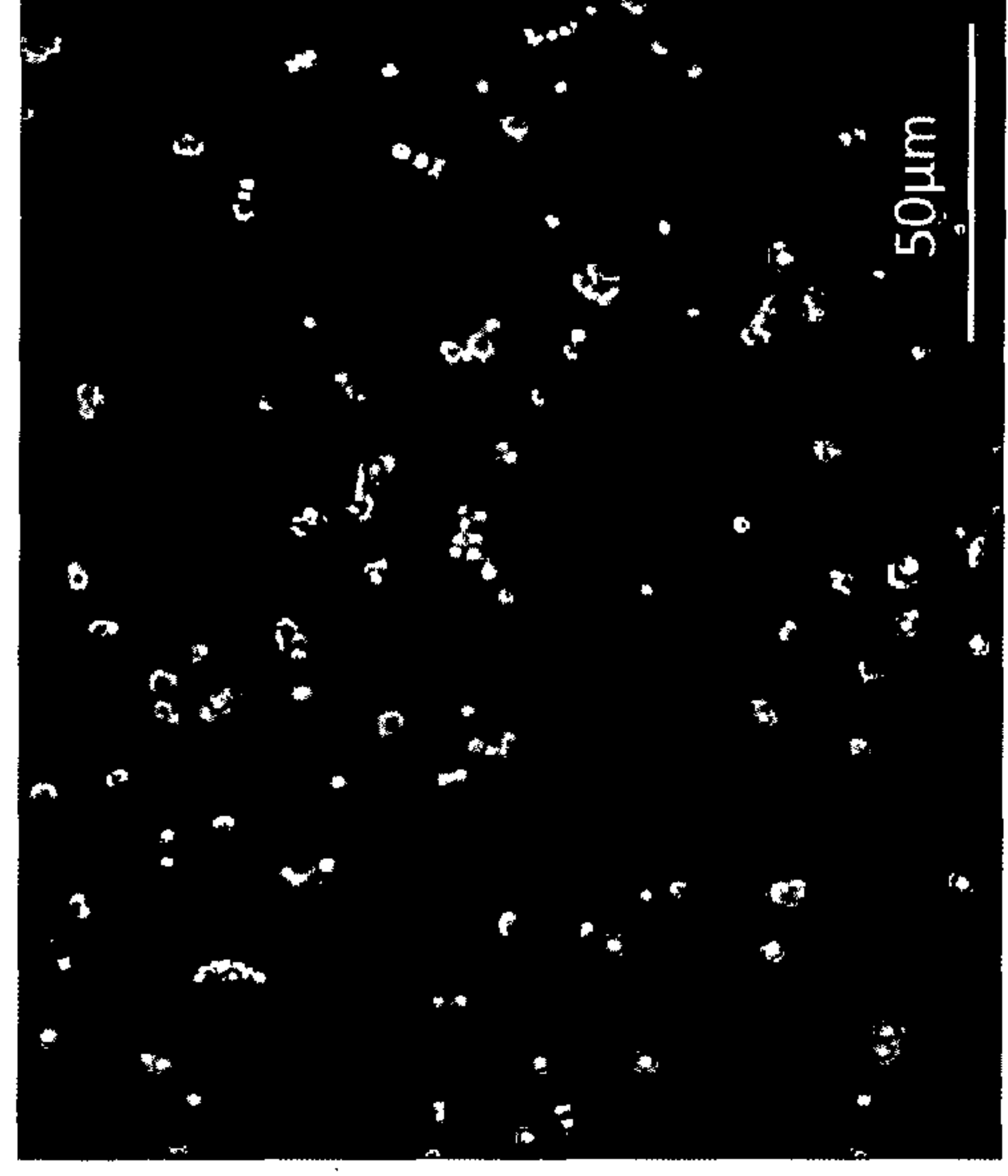
Non Coat



FNC



gelatin



7/14

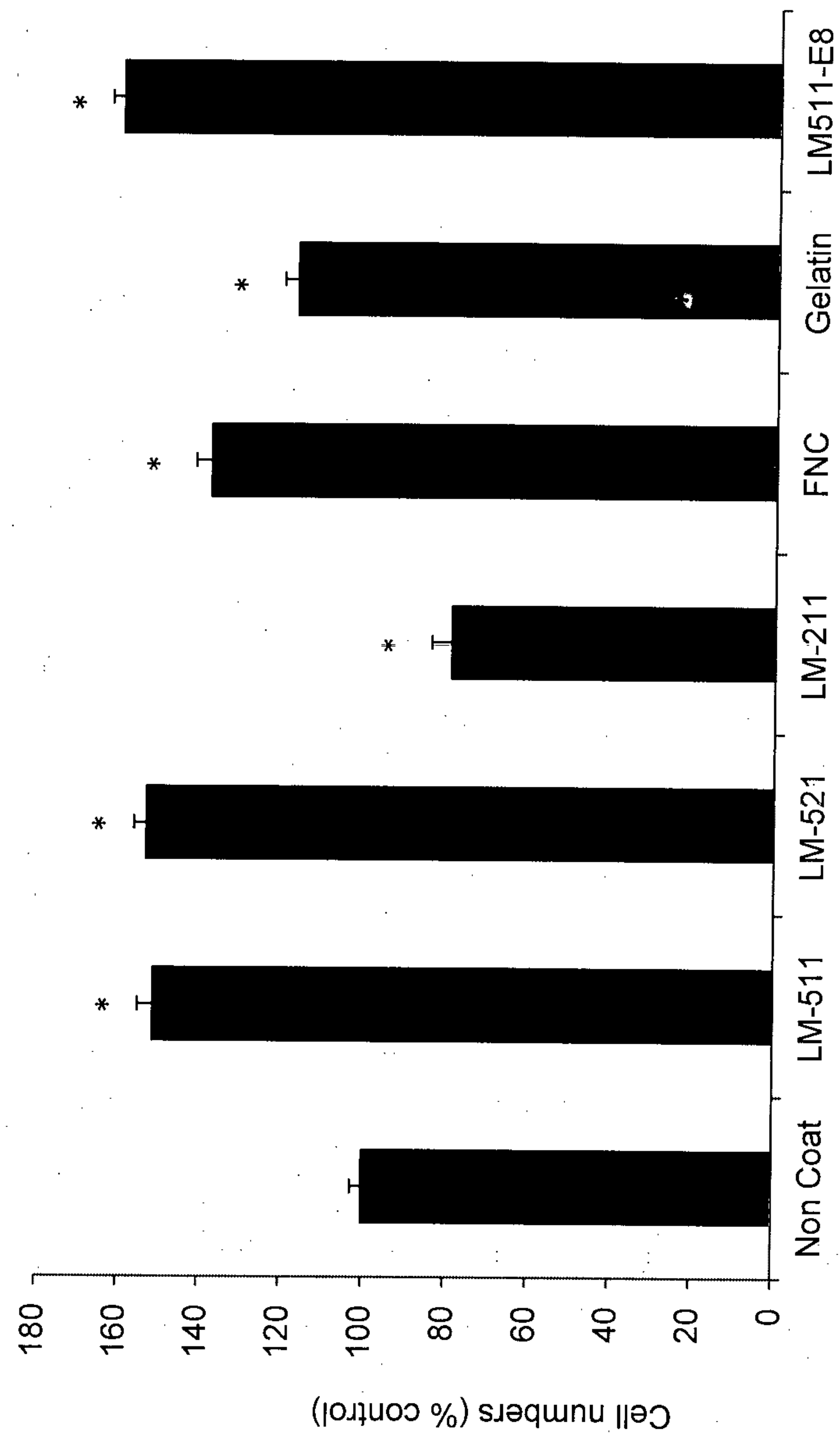
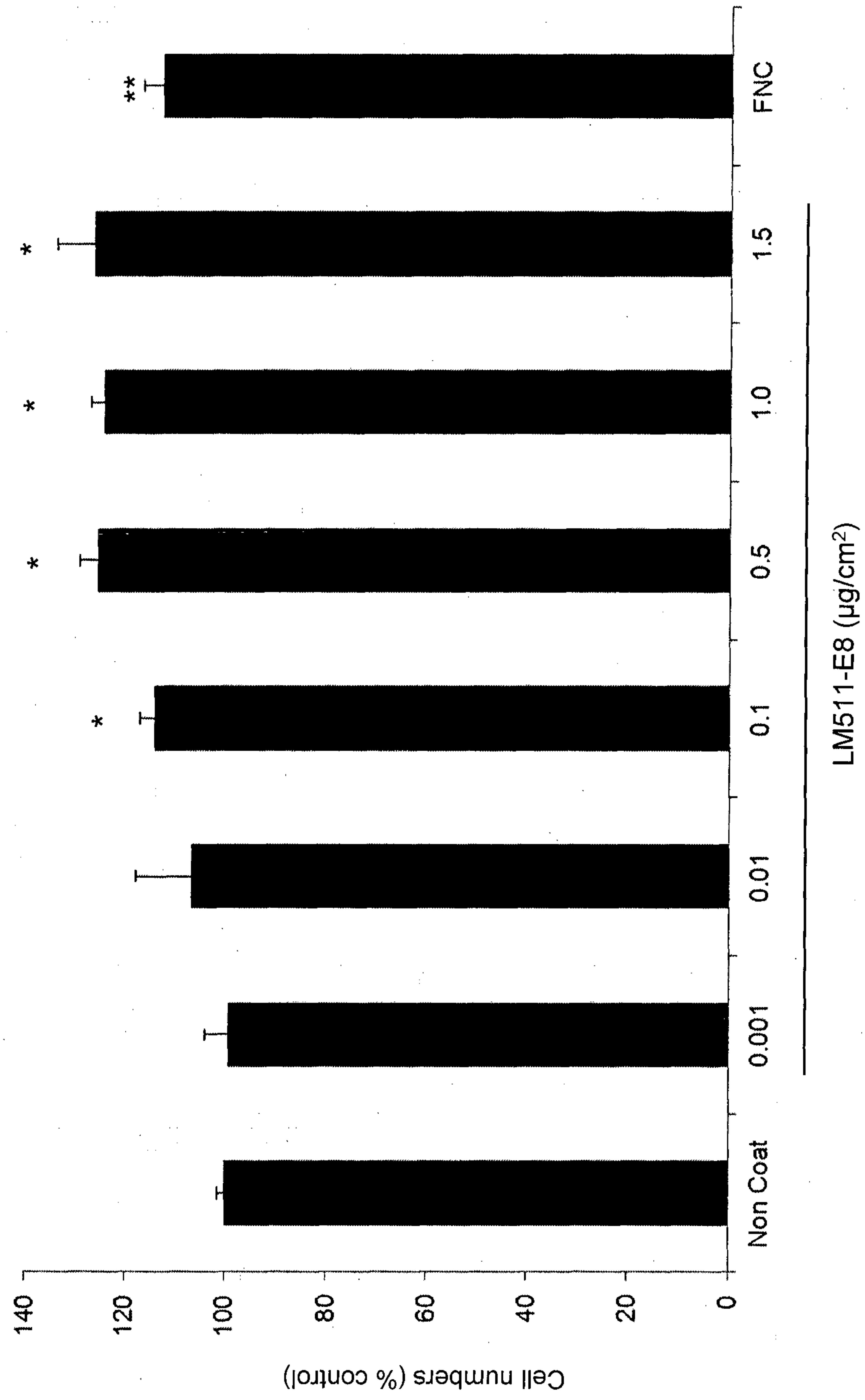


Fig. 5

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Fig. 6



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Fig. 7

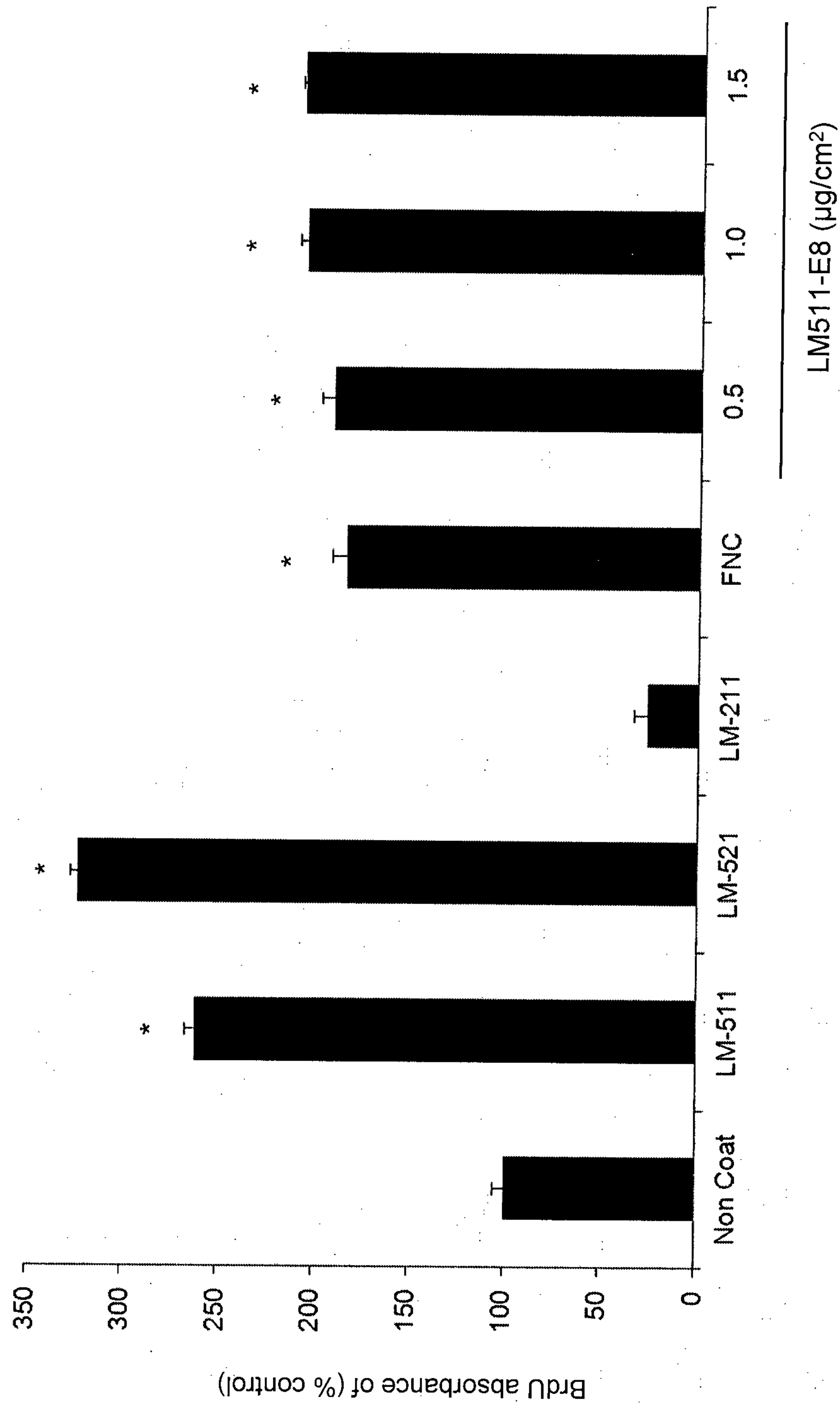
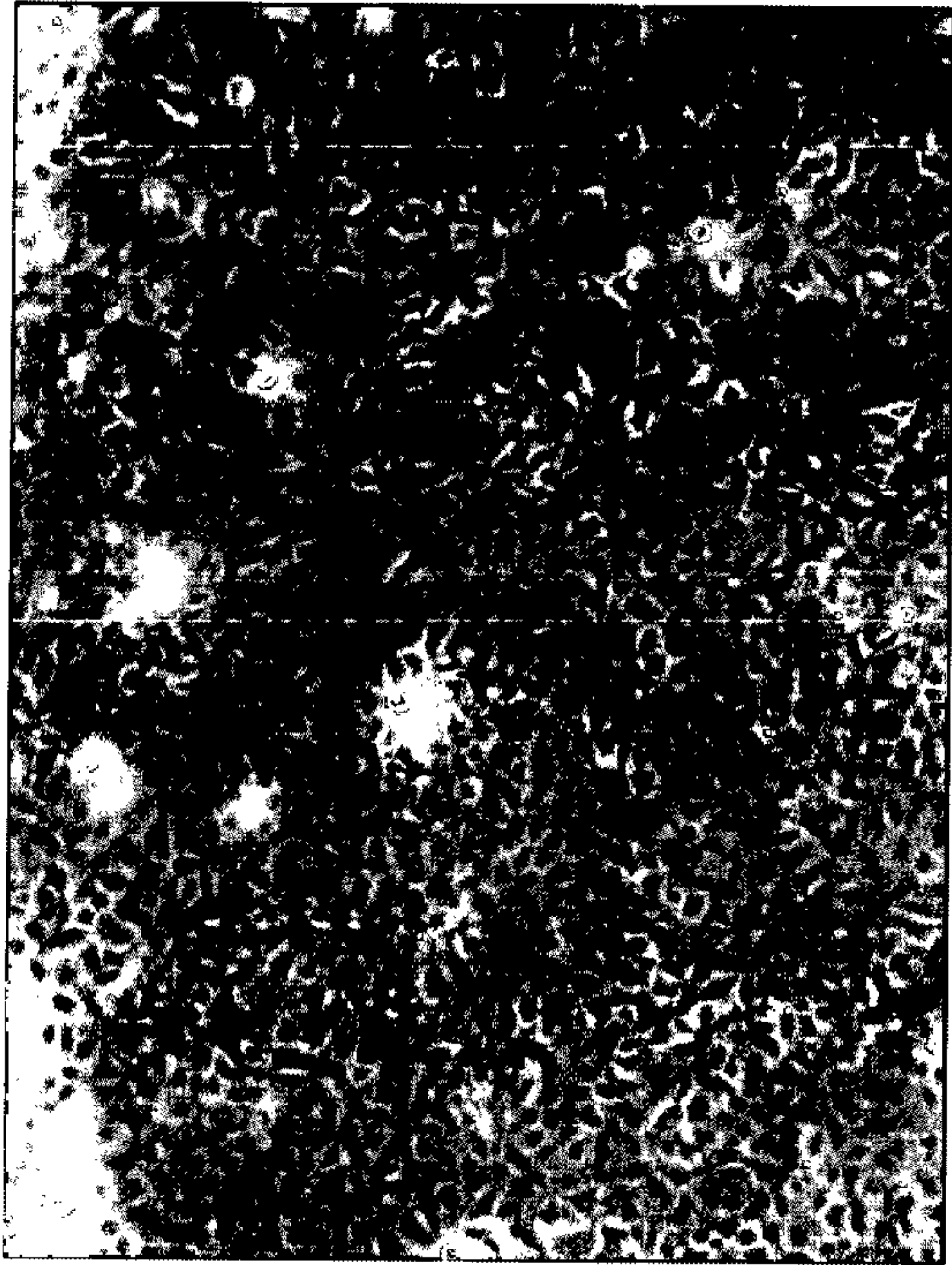
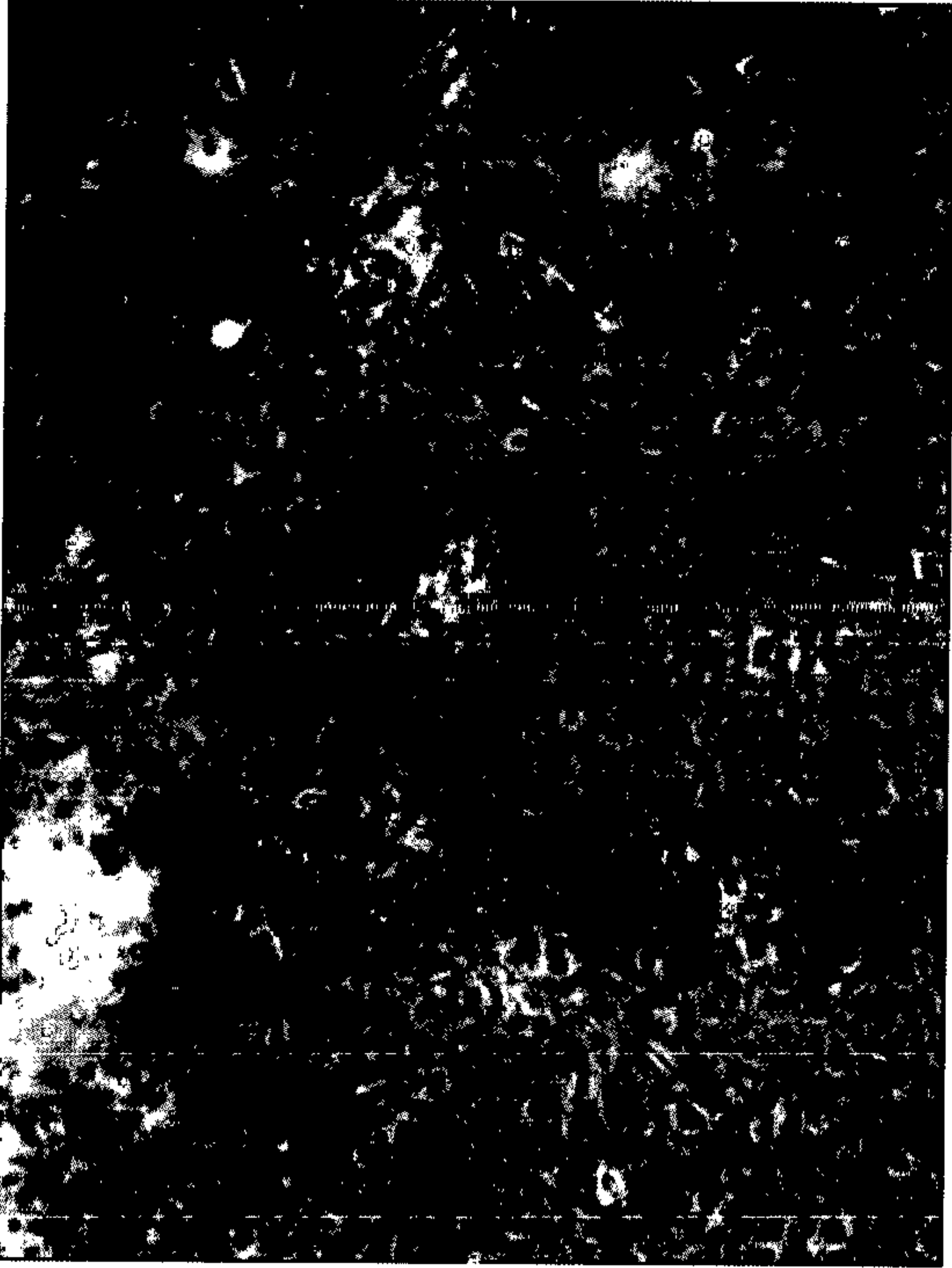


Fig. 8

LM-511



LM-521



LM-211

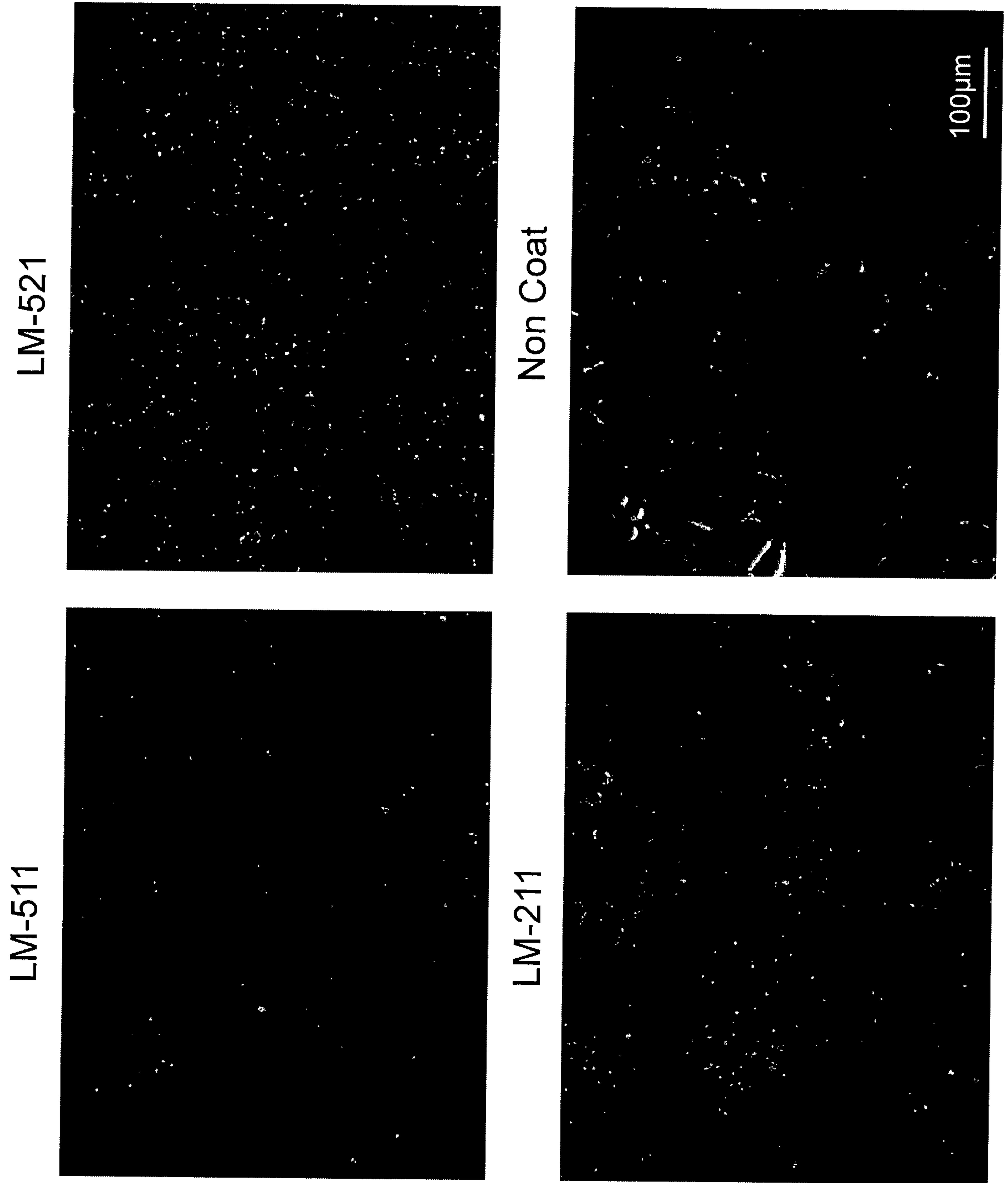


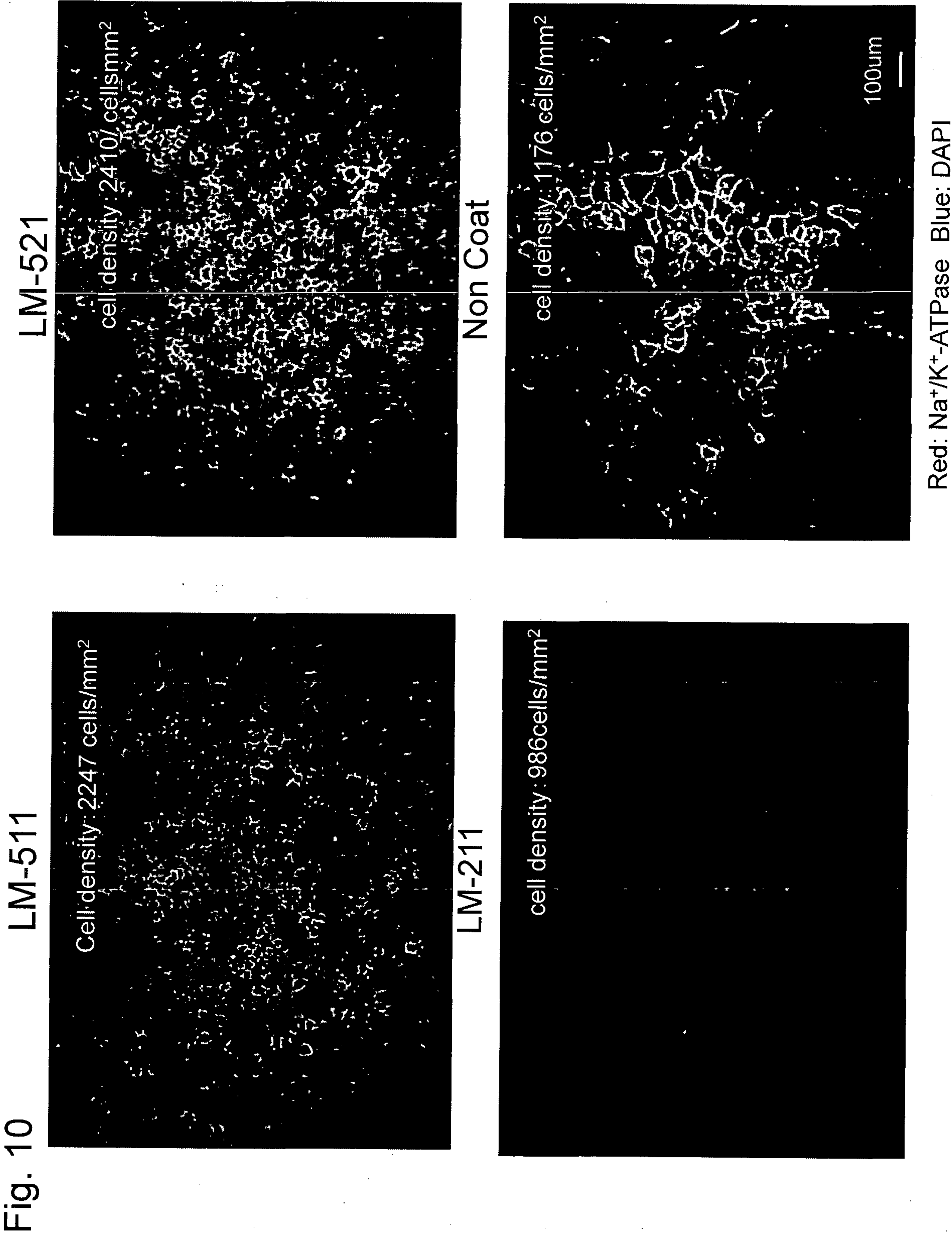
Non Coat

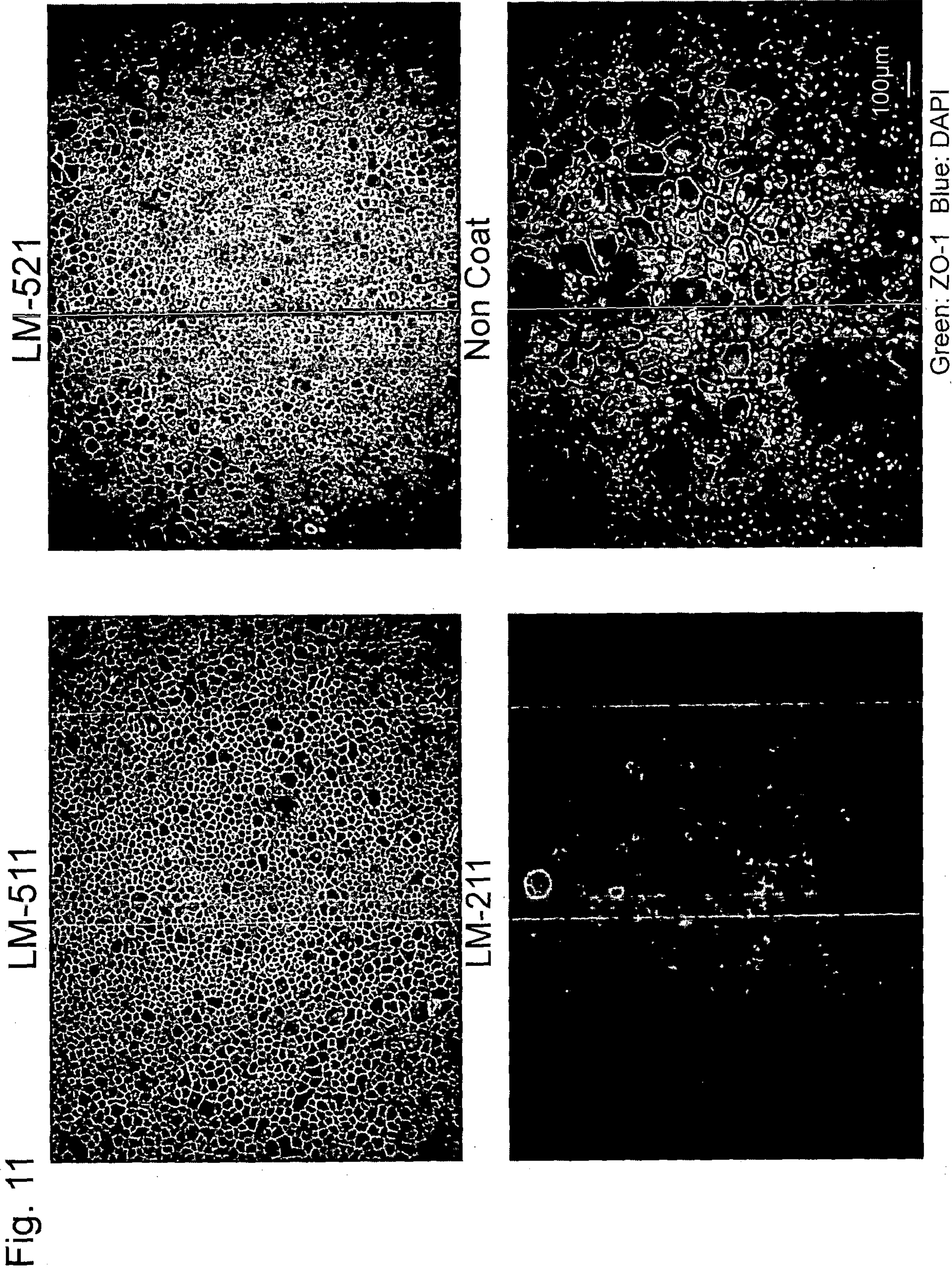


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Fig. 9







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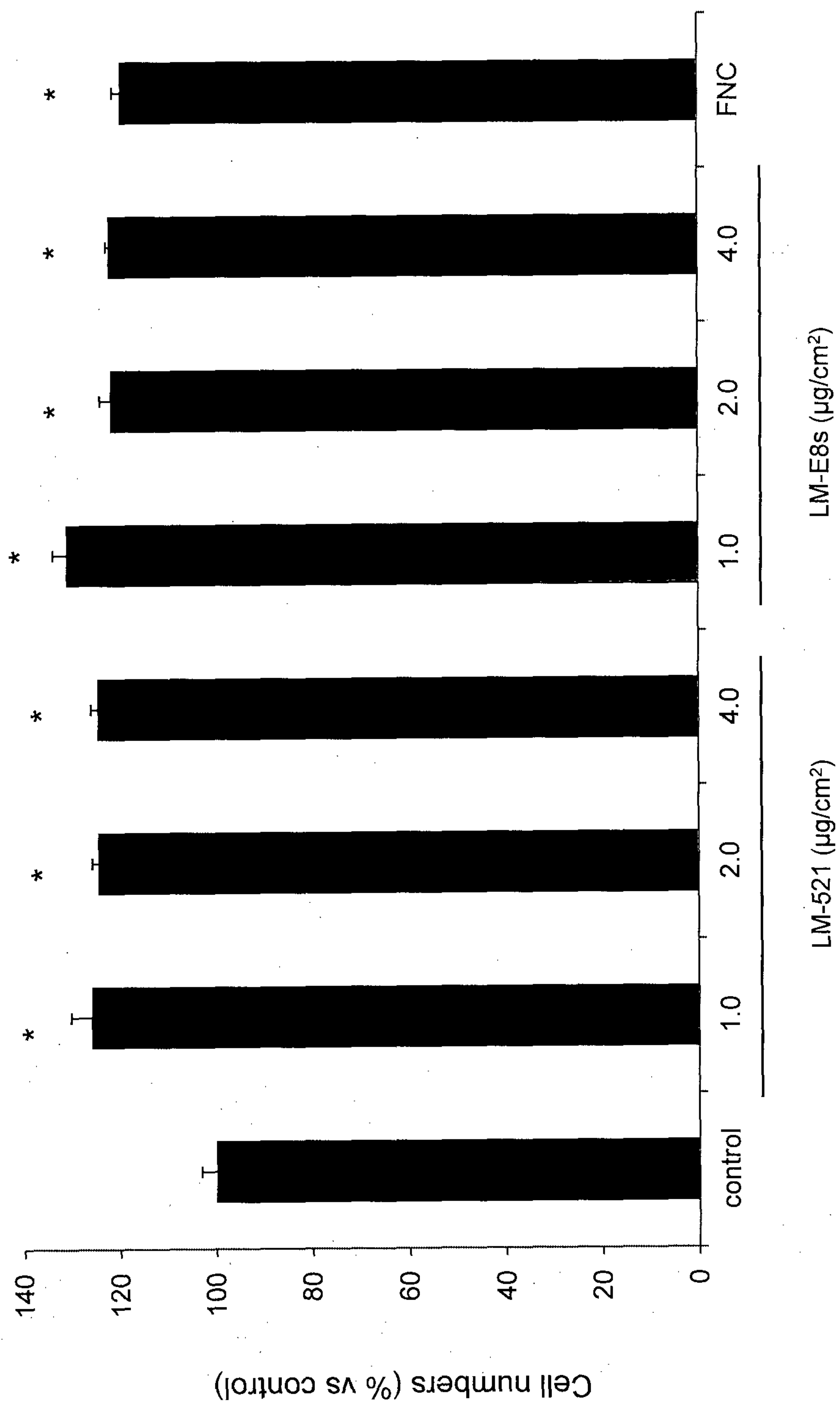


Fig. 12