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(71) **Applicant** *(for all designated States except US)*: DESMOS, INC. [US/US]; 3550 General Atomics Court, San Diego, CA 92121 (US).

(72) **Inventor; and**
(75) **Inventor/Applicant** *(for US only)*: TUCKER, Bryan, J. [US/US]; 12743 War Horse Street, San Diego, CA 92129 (US).

(74) **Agent:** ALTMAN, Daniel, E.; Knobbe, Martens, Olson and Bear, LLP, 16th floor, 620 Newport Center Drive, Newport Beach, CA 92660 (US).

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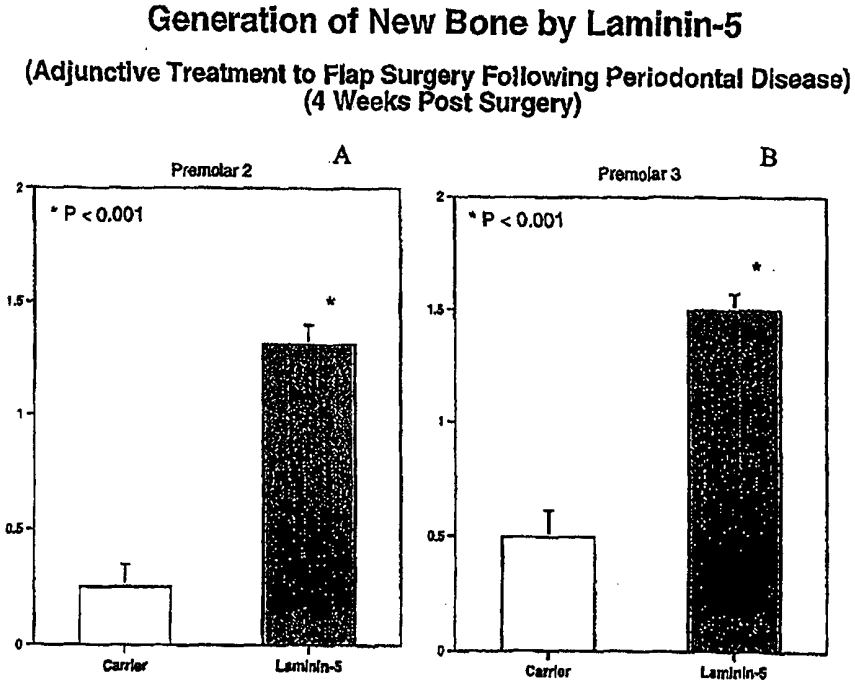

(57) **Abstract**

A method of treating a bone defect by administering to the bone defect a laminin 5 composition. Laminin 5 promotes bone growth and regeneration. The composition may also include one or more osteogenic and/or angiogenic factors. The growth and differentiation of bone marrow cells is also induced by laminin 5.

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LAMININ 5 FOR TREATMENT OF BONE DEFECTS

Field of the Invention

The present invention relates to the treatment of bone defects. More specifically, the invention relates to the treatment of bone defects, such as those caused by degeneration or trauma, with the extracellular matrix protein laminin 5.

Background of the Invention

Bone repair and/or regeneration is required to treat various bone defects which are caused by disease or trauma. For example, defects in bone regeneration and repair are associated with various human bone diseases and disorders, including osteoporosis and osteogenesis imperfecta. Current treatments for these disorders involve daily estrogen administration, increased intake of calcium-rich foods and calcium supplements and increased exercise. Although bone loss is diminished and bone density is increased in some individuals, the treatment is not completely satisfactory and bone loss often resumes.

Most fractured bones are still treated by one or more of the following procedures: applying casts, inserting pins and performing bone grafts. The bone repair is then allowed to occur naturally without further surgical or non-surgical intervention. However, fractured bones often do not completely heal or re-knit which often requires further surgical intervention. Bone cements and fibrin glue compositions have also been used to repair bone as described in U.S. Patent Nos. 5,514,137 and 5,651,982. However, these materials are somewhat tedious to manufacture, are not entirely natural products and are not entirely efficacious.

Laminins are heterotrimeric extracellular matrix proteins consisting of three subunits: α, β and γ. There are at least five known α subunits (α_1,α_2,α_3,α_4,α_5), three known β subunits (β_1,β_2,β_3) and two known γ (γ_1,γ_2) subunits (Miner et al., *J. Cell. Biol.* **137**:685-701, 1997). Laminin 5 is an $\alpha_3\beta_3\gamma_2$ heterotrimer which is typically associated with epithelial cell adhesion and sometimes with hemidesmosome formation. The designation "laminin 5" was coined by Burgeson et al. (*Matrix Biol.* **14**:209-211, 1994) to refer to a protein which is secreted into the culture medium by human keratinocytes and enhances keratinocyte attachment (Rousselle et al., *J. Cell Biol.* **114**:567-576, 1991; International Publication Nos. WO92/17498 and WO94/0531). A similar protein was also identified by Carter et al. (*Cell* **65**:599-619, 1991; International Publication No. WO95/06660) and called epiligrin. This protein is similar to the 425 kDa basement membrane glycoprotein recognized by the GB_3 antibody in human keratinocyte culture medium called nicein (Hsi et al., *Placenta* 8:209-217, 1987).

Laminin 5 is also produced by 804G and NBT-II rat bladder carcinoma cells (U.S. Patent Nos. 5,541,106; U.S. Patent No. 5,422,264). This rat laminin 5 differs functionally from kalinin and epiligrin, in that the former induces hemidesmosome formation while the latter do not. A human epithelial cell line, MCF-10A, produces an extracellular matrix which also induces hemidesmosome formation. This extracellular matrix is described in U.S. Patent No. 5,770,448.

U.S. Patent Numbers 5,422,264 and 5,541,106 describe the isolation of rat laminin 5 and its ability to induce adhesion and hemidesmosome formation in epithelial cells. The purification of soluble laminin 5 is described in U.S. Patent No. 5,760,179. U.S. Patent Nos. 5,510,263 and 5,681,587 disclose the successful passaging of fetal and adult islet cells when plated on a rat laminin 5-coated substrate. U.S. Patent No. 5,672,361 discloses the growth of pancreatic islet cells on human laminin 5-coated substrates. U.S. Patent No. 5,585,267 discloses the growth of epithelial cells on trans-epithelial appliances coated with rat laminin 5.

The patents described in the preceding paragraph all relate to the ability of laminin 5 to promote epithelial and islet cell adhesion and growth. The growth of other cell types both *in vitro* and *in vivo* is also highly desirable. For example, enhancement of bone growth and regeneration is desirable in disorders in which bone loss has occurred due to disease or trauma. However, bone cells have very different lineages, morphology and growth requirements than do epithelial cells or islet cells.

There is a constant need for compositions and methods for inducing bone repair, growth and/or regeneration. The present invention provides natural compositions for this purpose.

Summary of the Invention

One embodiment of the present invention is a method of treating a bone defect in a vertebrate in need thereof, comprising the step of administering to a bone affected by said defect, or to a tooth in the vicinity of said periodontal bone, an effective amount of a bone growth-inducing composition comprising laminin 5, or a peptide, fragment or derivative thereof. Preferably, the vertebrate is a mammal. More preferably, the mammal is a human. Advantageously, the laminin 5 is rat laminin 5. Alternatively, the laminin 5 is human laminin 5. Preferably, the laminin 5 is recombinant. The composition may further comprise an osteogenic factor and/or angiogenic factor. In one aspect of this preferred embodiment, the bone defect is selected from the group consisting of a bone fracture, joint fracture, non-union, delayed union, percutaneous arthrodesis, pseudo-arthritis, pseudo-arthrosis, osteoporosis or osteogenesis imperfecta.

Preferably, the bone defect is periodontal bone loss. Advantageously, the laminin 5 is kalinin, epiligrin, nicein or ladsin. In one aspect of this preferred embodiment, the adminstering step comprises topical administration of laminin 5 to the bone. In another aspect of this preferred embodiment, the contacting step comprises injection of the laminin 5 into or in the vicinity of the bone. Preferably, for topical administration, the effective amount is between about 1 $\mu g/cm^2$ and 1 mg/cm^2. More preferably, for topical administration, the effective amount is between about 10 $\mu g/cm^2$ and 100 $\mu g/cm^2$. Preferably, for injection, the effective amount is between about 0.1 μg and about 1 mg. More preferably, for injection, the effective amount is between about 100 μg and about 1 mg.

The present invention also provides a method for stimulating periodontal bone growth, comprising the steps of: identifying an individual suffering from periodontal bone loss; and administering to the periodontal bone, or to a

tooth in the vicinity of the bone, an effective bone growth-inducing amount of composition comprising laminin 5, or a peptide, fragment or derivative thereof. Preferably, the laminin 5 is rat laminin 5.

Alternatively, the laminin 5 is human laminin 5. The method may further comprise the step of performing flap surgery or scaling and root planing in the vicinity of the bone loss. Preferably, the contacting step comprises topical administration of said laminin 5 to the periodontal bone. In another aspect of this preferred embodiment, the contacting step comprises injection of the laminin 5 into or in the vicinity of the periodontal bone.

Preferably, for topical administration, the effective amount is between about 1 $\mu g/cm^2$ and 1 mg/cm^2. More preferably, for topical administration, the effective amount is between about 10 $\mu g/cm^2$ and 100 $\mu g/cm^2$. Preferably, for injection, the effective amount is between about 0.1 μg and about 1 mg. More preferably, for injection, the effective amount is between about 100 μg and about 1 mg.

The present invention also provides a composition comprising laminin 5 in combination with a bone cement material. Preferably, the bone cement material comprises hydroxyapatite or fibrin glue.

Another embodiment of the invention is a method for stimulating growth and differentiation of bone marrow cells, comprising contacting the bone marrow cells with laminin 5.

The present invention also provides a composition comprising laminin 5 in the preparation of a medicament for treating a bone defect in a vertebrate in need thereof. Preferably, the vertebrate is a mammal. More preferably, the mammal is a human. In one aspect of this preferred embodiment, the laminin 5 is rat laminin 5. Preferably, the laminin 5 is human laminin 5. The composition may further comprise an osteogenic factor and/or angiogenic factor. Advantageously, the bone defect is selected from the group consisting of a bone fracture, joint fracture, non-union, delayed union, percutaneous arthrodesis, pseudo-arthritis, pseudo-arthrosis, osteoporosis or osteogenesis imperfecta. Preferably, the bone defect is periodontal bone loss. In another aspect of this preferred embodiment, the human laminin 5 is kalinin, epiligrin, nicein or ladsin.

The present invention also provides the use of a composition comprising laminin 5 for stimulating periodontal bone growth. Preferably, the laminin 5 is rat laminin 5. In another aspect of this preferred embodiment, the laminin 5 is human laminin 5.

In another embodiment of the invention, there is provided the use of a composition comprising laminin 5 for stimulating growth and differentiation of bone marrow cells. Preferably, the laminin 5 is rat laminin 5. In another aspect of this preferred embodiment, the laminin 5 is human laminin 5.

Brief Description of the Drawing

Figures 1A-B show the stimulation of bone formation by laminin 5 when used as an adjunctive treatment to flap surgery in a dog following periodontal disease (4 weeks post surgery) on premolar 2 (Figure 1A) and premolar 3 (Figure 1B).

Detailed Description of the Preferred Embodiments

The present invention includes the observation that laminin 5 is capable of stimulating bone growth and regeneration in a vertebrate, preferably a mammal, more preferably a human. As defined herein, the term "laminin 5" encompasses a family of closely related heterotrimeric ($\alpha_3\beta_3\gamma_2$) extracellular matrix proteins which promote adhesion of epithelial cells. Laminin 5 encompasses soluble and insoluble forms of: the extracellular matrix proteins such as those secreted by 804G and NBT-II rat bladder carcinoma cells described in U.S. Patent Nos. 5,422,264, 5,541,106 and 5,658,789; keratinocyte attachment-inducing proteins such as those secreted by human keratinocytes such as kalinin (Rousselle et al., *supra.*), epiligrin (Carter et al., *supra.*) and nicein (Hsi et al., *supra.*); and a large cell-adhesive scatter factor such as ladsin (Miyazaki et al., *Proc. Natl. Acad. Sci. U.S.A.* **90**:11767-11771, 1993), and the hemidesmosome-inducing extracellular matrix protein such as that secreted by human MCF-10A epithelial cells which is described in U.S. Patent No. 5,770,448. Laminin 5 produced by 804G, NBT-II and MCF-10A cells also induces formation of cell adhesion structures called hemidesmosomes in epithelial cells cultured in the presence of this protein.

The present invention includes the use of both soluble and insoluble laminin 5 proteins, including those obtained from or obtainable from, but not limited to, such cells as 804G rat bladder carcinoma cells, NBT-II rat bladder carcinoma cells, human keratinocytes and MCF-10A cells, for treating bone defects in a mammal, preferably a human by promoting bone growth, repair and regeneration.

In a preferred embodiment, laminin 5 is purified from 804G, NBT-II, MCF 10-A, or human keratinocyte cell culture supernatant as described in U.S. Patent No. 5,760,179. In an alternative embodiment, kalinin is immunopurified from keratinocyte conditioned medium using an immunoaffinity column directed against its BM165 antigen as described by Burgeson et al. (PCT WO92/17498). Epiligrin is also present in the cell matrix secreted by a three-step extraction procedure comprising 1% w/v TRITON X-100 to solubilize membrane and cytoplasmic components, 2 M urea and 1 M NaCl to remove nuclear and cytoskeletal components; and 8 M urea to solubilize residual components. Sodium dodecyl sulfate (SDS, 0.5% w/v) is then added and the matrix is removed by scraping (Carter et al., *Cell* **65**:599-610, 1991).

The individual protein components of laminin 5 may be isolated or recombinantly produced by methods well known in the art (see, for example, U.S Patent No. 5,658,789; Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York, 1997). For example, cDNA sequences encoding laminin 5 polypeptides, or fragments thereof, are inserted into either conventional prokaryotic or eukaryotic expression vectors, widely available from many commercial sources including Stratagene (La Jolla, CA) and Invitrogen (San Diego, CA) using routine techniques, transfected into cells and the expressed protein purified according to well known methods (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). The vectors typically include a sequence which, when transcribed, is operably linked to the cDNA sequences and acts as a translation initiation sequence. Preferably, the vector includes a selectable marker such as neomycin resistance, hygromycin resistance or the herpes simplex virus thymidine kinase (TK) gene, as well as one or more restriction sites and a translation termination sequence.

In general, recombinant vector constructs are prepared by selecting a plasmid with a strong promoter, and appropriate restriction sites for insertion of DNA sequences if interest downstream from the promoter. As noted above, the vector construct may have a gene encoding antibiotic resistance for selection as sell as termination and polyadenylation signals. Additional elements may include enhancers and introns with functional splice donor and acceptor sites.

Derivatives of any of the three polypeptide chains of laminin 5 may also be used, including oligopeptides, peptides or polypeptides contained within laminin 5 which are capable of promoting bone growth, repair or regeneration. These peptides are produced by standard solid phase peptide synthesis using a commercially available peptide synthesizer, such as those available from Applied Biosystems, Foster City, CA. These peptides and polypeptides can also be produced recombinantly using the methods described above. The ability of any desired laminin 5 derivative to promote bone growth can be determined using standard bone growth assays or the assay described in Example 1.

Laminin 5 may be used to treat any defect in bone tissue as is desirable. Bone defects include, but are not limited to, bone fractures, joint fractures, non-unions and delayed unions, percutaneous arthrodesis, pseudo-arthritis, pseudo-arthrosis and bone defects resulting from congenital defects, trauma, tumor, infection, degenerative disease and other causes of loss of skeletal mass or integrity.

In a particularly preferred embodiment, laminin 5 is used for stimulating periodontal bone growth in individuals suffering from periodontal bone loss due to gingivitis and periodontal disease which largely result from microbial and plaque buildup between the teeth and gums. Laminin 5 can be used alone, or as an adjuvant to other periodontal therapies such as flap surgery or scaling and root planing, which are techniques well known in the periodontal art. Laminin 5 is administered to periodontal bone or to a tooth in the vicinity of the bone, either topically or by injection into or in the vicinity of (i.e., adjacent) the tooth or bone. In an alternative embodiment, rather than administering laminin 5 to bone, a molecule which specifically binds laminin 5 (i.e., an antibody generated against laminin 5),is applied to bone or a tooth to promote binding of endogenous laminin 5.

Laminin 5 compositions are also useful for prosthesis implantation and enhancement of prosthesis stability, enhancement of osteointegration or implant materials used for internal fixation procedures, stabilization of dental implant materials, healing acceleration of ligament insertion, and spine or other joint fusion procedures. Thus, laminin 5 is useful in methods to induce bone formation at a selected site of a defect in bone tissue of a mammal, preferably a human.

The present methods may be performed by well known arthroscopic, open surgical or percutaneous procedures (Sabiston, D. C. Jr., M.D., *Textbook of Surgery: the Biological Basis of Modern Surgical Practice*, 15th edition, W. B. Saunders Co., Philadelphia, 1997). To perform the methods of treating bone defects described herein, a bone defect is identified, prepared and contacted with the laminin 5 compositions of the present invention.

In one preferred embodiment of the present invention, laminin 5 is topically applied to a damaged bone. This may be accomplished using, for example, a brush, cotton swab or other type of applicator. In another preferred

embodiment, laminin 5 is injected into or in the vicinity of a damaged bone (e.g., adjacent to a site of natural or disease-induced bone loss or demineralization, or to a fractured bone), using standard surgical procedures, to stimulate bone growth and/or regeneration at that site. Laminin 5 is also used to induce healing of "green stick" fractures, in which no actual separation of bone has occurred, by surgically exposing the bone using well known techniques, and contacting the fracture with laminin 5.

In another preferred embodiment, laminin 5 is used as an adjuvant to other bone growth inducing and/or regenerating therapies to treat natural or disease-induced bone loss or demineralization, or bone fractures, such as casts, bone grafts and the fibrin glue compositions described in U.S. Patent No. 5,651,982. In addition, laminin 5 can be formulated with one or more osteogenic factors to further promote bone formation. The term "osteogenic factor" refers to any peptide, polypeptide, protein or any other compound or composition which induces or stimulates the formation of bone. The osteogenic factor induces differentiation of bone repair cells into bone cells, such as osteoblasts or osteocytes. The bone tissue formed from bone cells will contain bone specific substances such as type I collagen fibrils, hydroxyapatite mineral and various glycoproteins and bone proteoglycans. For example, osteogenic factors useful in the invention include proteins such as transforming growth factor-β (TGF-β; Sampath et al., *J. Biol. Chem.* **65**:13198-13205, 1990), osteogenin (Luyten et al., *J. Biol. Chem.* **264**:13377-13380, 1989), bone morphogenetic protein (BMP; Wang et al., *Proc. Natl. Acad. Sci. U.S.A.* **87**:2220-2224, 1990), and TGF-β combined with epidermal growth factor. In a preferred embodiment, the ratio of laminin 5 to osteogenic factor is from about 10,000:1 to about 1:100, more preferably form about 1,000:1 to about 1:10 and most preferably from about 100:1 to about 1:1.

The differentiation of mesenchymal cells induced by an osteogenic factor may include the formation of intermediary tissues such as fibrous, hyaline, and calcified cartilage; and endochondral ossification, which leads to the formation of woven bone tissue, which will become remodeled and transformed into mature lamellar bone tissue.

One or more angiogenic factors may also be added to the laminin 5 compositions of the invention to stimulate the formation and ingrowth of blood vessels and associated cells (e.g., endothelial, perivascular, mesenchymal and smooth muscle cells) and of basement membranes in the area of the bone defect. The term "angiogenic factor" refers to any agent capable of stimulating vascularization throughout the deposited matrix in the area of the bone defect. Angiogenic factors useful in the compositions and methods of the present invention include, but are not limited to, basic fibroblast growth factor (bFGF), TGF-β, platelet-derived growth factor (PDGF) and angiogenin. Heparin sulfate has been found to enhance the angiogenic activity of bFGF. In a preferred embodiment, the ratio of laminin 5 to angiogenic factor is from about 10,000:1 to about 1:100, more preferably form about 1,000:1 to about 1:10 and most preferably from about 100:1 to about 1:1.

In another preferred embodiment of the invention, laminin 5 is used to induce the regeneration of bone marrow. In this embodiment, prior to bone marrow transplantation into a recipient, bone marrow from the recipient or bone marrow from a donor which has been type matched, is combined with soluble laminin 5, then injected into a recipient. The laminin 5 promotes growth and differentiation of the bone marrow cells to increase the yield of cells

repopulating the bones of the recipient. Typically, between about 10 micrograms and 1,000 micrograms of laminin 5 is contacted with about 1 x 10^6 bone marrow stem cells. More preferably, between about 100 micrograms and 500 micrograms of laminin 5 is contacted with about 1 x 10^6 bone marrow stem cells. The bone marrow cells may be either allogeneic or autologous. Thus, less donor marrow is required due to the enhancement of bone marrow growth promoted by laminin 5.

Laminin 5, through its communication with integrins and other cell surface structures, stimulates osteoblast activity to form new bone both *in vitro, ex vivo* and *in vivo*. Bone produced ex *vivo* may be transplanted *in vivo*. Laminin 5 can be used in conjunction with any known ex *vivo* bone growth method to stimulate osteoblast activity and subsequent bone growth. Examples of such methods are described by Kuznetsov et al. (*The Hematopoietic Microenvironment*, Keystone Symposia, Taos, New Mexico, Abstract 308, p. 52, February 1996) and Gundle et al. (*Bone* **16**:597-601, 1995).

In a preferred embodiment, laminin 5 is used to bind bone fragments. The bone-binding ability of laminin 5 is very useful in bone reconstruction, as in plastic surgery or in the repair of major bone breaks.

The amount of laminin 5 administered topically onto the area of bone damage or demineralization, or injected into or in the vicinity of (i.e., adjacent to) the bone, will vary depending on the type of disorder to be treated, extent of demineralization, severity of the fracture and size of the bone. In a preferred embodiment, in treatment of a long bone fracture in an adult human, laminin 5 is topically administered in a pharmaceutically acceptable excipient or diluent in an amount ranging from about 1 $\mu g/cm^2$ to about 1 mg/cm^2. In a particularly preferred embodiment, the amount administered is between about 10 $\mu g/cm^2$ and about 100 $\mu g/cm^2$. Either single or multiple dosages may be administered. Laminin 5 may also be directly injected into the bone, typically in an amount ranging from about 0.1 μg to about 1 mg, preferably between about 100 μg and about 1 mg. Pharmaceutical formulations containing laminin 5 can be prepared by conventional techniques, e.g. as described in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, 1985. Pharmaceutically acceptable carriers or diluents are those which are nontoxic to recipients at the dosages and concentrations employed.

Suitable carriers, excipients or diluents for the preparation of solutions and syrups include water, polyols, sucrose, invert sugar and glucose. Suitable excipients for the preparation of injectable solutions include water, alcohols, polyols, glycerol and vegetable oils. Pharmaceutical compositions for injection into or adjacent to bone comprise pharmaceutically acceptable sterile aqueous or non-aqueous liquids, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. These formulations can additionally contain preservatives, solubilizers, stabilizers, viscosity agents, wetting agents, emulsifiers, buffers, antioxidants and diluents.

The laminin 5 composition may advantageously be in a form suitable for topical administration to bone, such as an ointment, foam, spray, gel, cream, paste, suspension or dispersion. The topical composition is applied with a brush or applicator.

Laminin 5 may also be provided in a controlled release composition for implantation at a site where bone growth and/or regeneration is desired. For example, polylactic acid, polygalactic acid, regenerated collagen, poly-L-lysine, sodium alginate, gellan gum, chitosan, agarose, multilamellar liposomes and many other conventional depot formulations comprise bioerodible or biodegradable materials that can be formulated with biologically active compositions. These materials, when implanted or injected at a fracture site or a site of bone loss, gradually break down and release the active material to the surrounding tissue. For example, one method of encapsulating laminin 5 comprises the method disclosed in U.S. Patent No. 4,391,909. The use of bioerodible, biodegradable and other depot formulations is expressly contemplated in the present invention. Typically these compositions comprise laminin 5 combined with the carrier or depot material in a ratio of from about 1:1 to about 1:10,000, more preferably from about 1:10 to about 1:1,000. The use of infusion pumps and matrix entrapment systems for delivery of laminin 5 is also within the scope of the present invention.

Laminin 5 may also advantageously be enclosed in or combined with micelles or liposomes. Liposome encapsulation technology is well known in the art (i.e. Radin et al., *Meth. Enzymol.* **98**:613-618, 1983). For example, a bone fracture can be sealed with the laminin 5-liposome composition which can also be formulated to contain bone-specific growth factors. Upon slow dissolution of laminin 5 at the site of the bone fracture, the liposomes release their entrapped growth factors and thus improve the rate and quality of the healing bone. This method is described with regard to fibrin glue containing liposomes in U.S. Patent No. 5,651,982.

Laminin 5 may also be combined with a completely or partially resorbable bone cement material. Many such materials are well known to one of ordinary skill in the art, including hydroxyapatite and fibrin glue. Bone cement materials are described in U.S. Patent No. 5,514,137. The laminin 5-containing cement material is then implanted at a bone site where bone regeneration and/or regrowth is desired.

The study described below demonstrates the ability of laminin 5 to stimulate new bone formation in the periodontal diseased dog model which is an art-accepted animal model of human periodontal disease and bone growth. In this model system, a portion of existing bone was deliberately removed, disease was induced and allowed to progress, and, following debridement treatment for the disease, laminin 5 was applied directly to the bone.

Example 1

Promotion of periodontal bone growth by laminin 5

After one week of acclimation, six adult beagle dogs under 5 years of age were anesthetized and the teeth cleaned with an ultrasonic instrument. Impressions of the mandibular teeth were taken for fabrication of stints for standardized radiography. The mandibular second, third and fourth premolars and first molars were designated as test teeth. Mucoperiosteal flaps were elevated extending from the mesial of the first premolars through the distal of the first molars. Using a straight fissure bur, horizontal cuts were made around the second premolar 3 mm apical to the bone margin, around the third premolar 3 mm from the bone margin and around the fourth premolar 4 mm from the bone margin. Using a k13 chisel, buccal and lingual alveolar bone was removed. using a round bur and curettes, furcal bone was removed. Rubber base impression material was injected into the open furcation areas and the mucogingival

flaps sutured at the level of the alveolar bone margin. After six weeks, the rubber base material was removed and another four weeks were allowed before the treatment was initiated.

After anesthetizing the animals, standardized radiographs and 35 mm color photographs were taken of the test teeth. Mucoperisteal flaps were reflected and the root surfaces of the test teeth thoroughly cleaned. Notches were cut into the root surfaces at the level of the alveolar bone crest to serve as reference points. The test teeth were isolated with gauze. Laminin 5 was purified from 804G cell culture supernatant as described in U.S. Patent No. 5,760,179. Laminin 5 at 100 μg/ml in a buffered solution containing 57 mM Na_2PO_4, 100 mM NaCl and 0.0025% Tween-20 was applied to the roots of the test teeth and to the marginal bone in the test quadrant. The flaps were sutured into place. The gauze prevented the experimental drug from contaminating the control quadrant. After treatment, the gauze was removed. Buffered solution alone was applied to the roots and marginal bone in the contralateral control quadrant and the flaps sutured into place.

After two weeks, three animals were euthanized and the segments of the mandible containing the test teeth harvested and fixed with 10% buffered formalin. The specimens were decalcified, paraffin embedded and sections prepared for morphometric analysis. The same procedure was followed for the remaining three dogs at week four.

With direct application of laminin 5 to the bone and tooth surfaces, an approximate 3-fold increase in new bone growth was observed after 4 weeks in adult beagle dogs (Figure 1). This increase in new bone growth appears to be normal regrowth from mechanical and disease-induced losses rather than immobility and consolidation of a joint due to disease, injury or surgical procedure (ankylosis).

Example 2

Treatment of bone fractures with laminin 5

An individual with an acute fracture of the radius is admitted for surgery and an x-ray is performed to determine the location of the fracture. The fractured area is exposed by standard surgical procedures or laparoscopic surgery is performed. Prior to closing the incision, laminin 5 is combined with a pharmaceutically acceptable carrier and injected into or topically applied to the fractured area in an amount ranging from about 1 $\mu g/cm^2$ to 1 mg/cm^2. The incision is closed and healing of the fracture is monitored over the next several months. Healing occurs significantly faster compared to the average fracture in the absence of laminin 5.

Example 3

Treatment of periodontal bone loss with laminin 5 controlled release formulation

An individual with significant periodontal bone loss is identified and a laminin 5 controlled release formulation containing 5 mg laminin 5 and 10 μg TGF-β in 10 grams of polylactic acid putty is prepared using well known methods, then implanted into the bone by standard surgical procedures at the site of the most significant demineralization. The controlled release composition gradually breaks down and delivers laminin 5 and the osteogenic factor to the area of bone loss over a time period of several days or weeks, promoting bone mineralization and regrowth in the demineralized areas.

Example 4

Use of laminin 5 in conjunction with bone grafting

A damaged femur in which an area of bone has been lost is exposed by standard surgical procedures and the damaged surface is coated with laminin 5 in a pharmaceutically acceptable carrier. Autologous bone is surgically obtained from the hip bone, coated with the laminin 5 composition, and grafted onto the damaged bone area by standard procedures. The laminin 5 composition enhances bone growth and regeneration, facilitating fusion of the autologous hip bone to the damaged femur.

It should be noted that the present invention is not limited to only those embodiments described in the Detailed Description. Any embodiment that retains the spirit of the present invention should be considered to be within its scope. However, the invention is only limited by the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating a bone defect in a vertebrate in need thereof, comprising the step of administering to a bone affected by said defect, or to a tooth in the vicinity of said periodontal bone, an effective amount of a bone growth-inducing composition comprising laminin 5, or a peptide, fragment or derivative thereof.

2. The method of Claim 1, wherein said vertebrate is a mammal.

3. The method of Claim 2, wherein said mammal is a human

4. The method of Claim 1, wherein said laminin 5 is rat laminin 5.

5. The method of Claim 1, wherein said laminin 5 is human laminin 5.

6. The method of Claim 1, wherein said laminin 5 is recombinant.

7. The method of Claim 1, wherein said composition further comprises an osteogenic factor and/or angiogenic factor.

8. The method of Claim 1, wherein said bone defect is selected from the group consisting of a bone fracture, joint fracture, non-union, delayed union, percutaneous arthrodesis, pseudo-arthritis, pseudo-arthrosis, osteoporosis or osteogenesis imperfecta.

9. The method of Claim 1, wherein said bone defect is periodontal bone loss.

10. The method of Claim 1, wherein said human laminin 5 is kalinin, epiligrin, nicein or ladsin.

11. The method of Claim 1, wherein said administering step comprises topical administration of laminin 5 to said bone.

12. The method of Claim 1, wherein said contacting step comprises injection of said laminin 5 into or in the vicinity of said bone.

13. The method of Claim 11 wherein said effective amount is between about 1 $\mu g/cm^2$ and 1 mg/cm^2.

14. The method of Claim 13, wherein said effective amount is between about 10 $\mu g/cm^2$ and 100 $\mu g/cm^2$.

15. The method of Claim 12, wherein said effective amount is between about 0.1 μg and about 1 mg.

16. The method of Claim 15, wherein said effective amount is between about 100 μg and about 1 mg.

17. A method for stimulating periodontal bone growth, comprising the steps of:

identifying an individual suffering from periodontal bone loss; and

administering to said periodontal bone, or to a tooth in the vicinity of said bone, an effective bone growth-inducing amount of composition comprising laminin 5, or a peptide, fragment or derivative thereof.

18. The method of Claim 17 wherein said laminin 5 is rat laminin 5.

19. The method of Claim 17 wherein said laminin 5 is human laminin 5.

20. The method of Claim 17 further comprising the step of performing flap surgery or scaling and root planing in the vicinity of said bone loss.

21. The method of Claim 17, wherein said contacting step comprises topical administration of said laminin 5 to said periodontal bone.

22. The method of Claim 17, wherein said contacting step comprises injection of said laminin 5 into or in the vicinity of said periodontal bone.

23. The method of Claim 21 wherein said effective amount is between about 1 $\mu g/cm^2$ and 1 mg/cm^2.

24. The method of Claim 23, wherein said effective amount is between about 10 $\mu g/cm^2$ and 100 $\mu g/cm^2$.

25. The method of Claim 22, wherein said effective amount is between about 0.1 μg and about 1 mg.

26. The method of Claim 25, wherein said effective amount is between about 100 μg and about 1 mg.

27. A composition comprising laminin 5 in combination with a bone cement material.

28. The composition of Claim 27, wherein said bone cement material comprises hydroxyapatite or fibrin glue.

29. A method for stimulating growth and differentiation of bone marrow cells, comprising contacting said bone marrow cells with laminin 5.

30. Use of a composition comprising laminin 5 in the preparation of a medicament for treating a bone defect in a vertebrate in need thereof.

31. The use of Claim 30, wherein said vertebrate is a mammal.

32. The use of Claim 31, wherein said mammal is a human.

33. The use of Claim 30, wherein said laminin 5 is rat laminin 5.

34. The use of Claim 30, wherein said laminin 5 is human laminin 5.

35. The use of Claim 30, wherein said composition further comprises an osteogenic factor and/or angiogenic factor.

36. The use of Claim 30, wherein said bone defect is selected from the group consisting of a bone fracture, joint fracture, non-union, delayed union, percutaneous arthrodesis, pseudo-arthritis, pseudo-arthrosis, osteoporosis or osteogenesis imperfecta.

37. The use of Claim 30, wherein said bone defect is periodontal bone loss.

38. The method of Claim 34, wherein said human laminin 5 is kalinin, epiligrin, nicein or ladsin.

39. Use of a composition comprising laminin 5 for stimulating periodontal bone growth.

40. The use of Claim 39, wherein said laminin 5 is rat laminin 5.

41. The use of Claim 39, wherein said laminin 5 is human laminin 5.

42. Use of a composition comprising laminin 5 for stimulating growth and differentiation of bone marrow cells.

43. The use of Claim 42, wherein said laminin 5 is rat laminin 5.

44. The use of Claim 42, wherein said laminin 5 is human laminin 5.

Generation of New Bone by Laminin-5

(Adjunctive Treatment to Flap Surgery Following Periodontal Disease) (4 Weeks Post Surgery)

FIG 1A

FIG 1B