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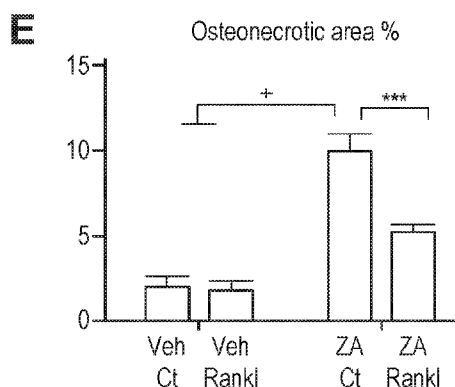
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(54) Title: METHOD OF TREATING OSTEONECROSIS



(57) Abstract: The present invention relates to methods of treating osteonecrosis, particularly Medication Related Osteonecrosis of the Jaw, in a subject, and to delivery devices and vehicles for use in such methods.

METHOD OF TREATING OSTEONECROSIS

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims benefit of U.S. Provisional Application No. 62/802,423, filed February 7, 2019, which is incorporated herein by reference in its entirety.

GOVERNMENT INTEREST STATEMENT

[002] This invention was made in whole or in part with government support under Grant Number DE019465, awarded by the National Institutes of Health. The government may have certain rights in the invention.

FIELD OF THE INVENTION

[003] The present invention relates to methods of treating osteonecrosis, particularly Medication Related Osteonecrosis of the Jaw, in a subject, and to delivery devices and vehicles for use in such methods.

BACKGROUND OF THE INVENTION

[004] Osteonecrosis, also referred to as avascular necrosis or aseptic necrosis, is the death of bone cells due to decreased blood flow. It can occur in almost any bone in the body. Osteonecrosis of the jaw (ONJ) occurs when the jawbone is necrotic and exposed to the oral cavity. ONJ can also occur in the absence of bone exposure, (Stage 0 or non-exposed variant).

[005] The term Medication related osteonecrosis of the Jaw (MRONJ) was introduced in 2014 and is used interchangeably with the term ONJ. MRONJ is a rare but serious side effect of antiresorptive medications and can cause significant morbidity to patients. MRONJ is characterized by areas of

exposed bone in the oral cavity for a period of more than 8 weeks. The most common instigating local factors of MRONJ is tooth extraction or other dental surgical procedure, such as implant placement. However, MRONJ can occur even in the absence of surgical intervention. A classic presentation of the disease is when a tooth is extracted and the extraction socket does not heal. However, tooth extraction in patients undergoing antiresorptive treatment often is unavoidable.

[006] The fear of MRONJ has been identified as a main contributor leading osteoporotic patients to not be compliant with their antiresorptive medications. Osteoporosis is the most common metabolic bone disease with 43.4 million people affected by osteoporosis or osteopenia in the USA, representing 44% of the people aged 50 and older. Indeed, reports in the scientific and public literature express serious concern about not treating osteoporosis more aggressively.

[007] Antiresorptive medications are mainly used for the management of osteoporosis, and less frequently but at higher doses in patients with bone malignancy. Antiresorptive medications target osteoclasts, the cells that resorb bone. Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) is a key molecule for the production of osteoclasts. Indeed, certain antiresorptive treatments target RANKL as a way to inhibit osteoclast formation, and thus bone resorption. If a successful intervention that could minimize the MRONJ risk could be identified, it would be beneficial for millions of osteoporotic patients that need to undergo a dental surgical procedure, such as tooth extraction.

SUMMARY OF THE INVENTION

[008] In an embodiment, provided herein is a method of treating osteonecrosis in a subject, comprising contacting an area of osteonecrosis in the subject with a therapeutically effective amount of a Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL).

[0009] In one embodiment, osteonecrosis comprises osteonecrosis of the jaw (ONJ). In one embodiment, ONJ is Medication Related Osteonecrosis of the Jaw (MRONJ). In one embodiment, MRONJ is associated with administration of an antiresorptive treatment in the subject. In one embodiment, the antiresorptive treatment comprises administration of a bisphosphonate.

[0010] In one embodiment, the antiresorptive treatment comprises an antibody to RANKL. In one embodiment, the antibody is a monoclonal antibody. In one embodiment, the monoclonal antibody comprises denosumab.

[0011] In one embodiment, the subject has osteoporosis. In one embodiment, the subject has osteopenia. In one embodiment, the subject has a bone malignancy. In one embodiment, the subject has undergone a dental surgical procedure. In one embodiment, the dental surgical procedure comprises a tooth extraction or an implant placement.

[0012] In any of the foregoing embodiment, contacting comprises local delivery to a bone surgical area or local delivery to an extraction socket, or local delivery to a dental implant site.

[0013] In any of the foregoing embodiments, RANKL is comprised on or in a substrate. In one embodiment the substrate is adapted for placement within the extraction socket. In one embodiment, the substrate comprises a sponge. In one embodiment, the sponge comprises collagen. In one embodiment, the substrate comprises a resorbable tape. In one embodiment, the resorbable tape comprises collagen.

[0014] In any of the foregoing embodiments, the substrate comprises a hydrogel. In any of the foregoing embodiments, the contacting results in localized activation of osteoclasts in the area. In any of the foregoing embodiments, the contacting results in a reduction in necrosis in the area. In any of the foregoing embodiments, the contacting results in improved healing in the area.

[0015] In any of the foregoing embodiments, the subject is a human.

[0016] In another embodiment, provided herein is a device for delivering a RANKL to an area of osteonecrosis in a subject, comprising a substrate adapted for local delivery to an area of osteonecrosis and a RANKL.

[0017] In one embodiment, the area comprises an extraction socket or a bone surgical area or an implant site. In one embodiment, the substrate comprises a sponge. In one embodiment, the sponge comprises collagen. In one embodiment the substrate comprises a resorbable tape. In one embodiment, the resorbable tape comprises collagen. In one embodiment, the substrate comprises a hydrogel.

[0018] Other features and advantages of the present invention will become apparent from the following detailed description examples and figures. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The subject matter regarded as the invention is particularly pointed out and distinctly claimed in the concluding portion of the specification. The invention, however, both as to organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings in which:

[0020] **Figure 1A-F.** RANKL immunohistochemistry of veh or ZA treated rats with or without RANKL treatment. (1A-D). Yellow arrows in onsets point to RANKL stain on osteoclasts. Quantification of RANKL positive cells over soft tissue area (1E). Serum TRAP levels 3 days before

and 3 days after surgery in veh and ZA treated rats (1F). **= statistically significant with a p value <0.01 . ***= statistically significant with a p value <0.001.

[0021] **Figure 2A-J.** Clinical images of mandibles 3 and 12 days after surgery. Images of the mandibular mucosa of veh or ZA treated rats with or without RANKL treatment 3 days after surgery. Mucosal defects and ongoing mucosal healing is seen. (2A-D, red circles). Quantification of unhealed mucosal area over total mucosal area 3 days after surgery. Image of the mandibular mucosa of veh or ZA treated rats with or without RANKL treatment 12 days after surgery. Image of the mandibular mucosa of a veh-treated rat without RANKL treatment (2F). Complete mucosal healing is seen (blue circle). Image of the mandibular mucosa of a veh-treated rat with RANKL treatment (2G). Complete mucosal healing is seen (blue circle). Image of the mandibular mucosa of a ZA treated rats without RANKL treatment (2H). A large mucosal defect and bone exposure are noted (red circle). Image of the mandibular mucosa of a ZA-treated rat with RANKL treatment (2I). Complete mucosal healing is seen (blue circle). Quantification of unhealed mucosal area over total mucosal area 12 days after surgery (2J). *=statistically significant with a p value<0.05.

[0022] **Figure 3A-H.** Histologic sections of extraction sockets of vehicle or ZA treated rats with or without local RANKL treatment 3 days after tooth extraction and RANKL delivery. Histologic section of the mandible of a veh-treated rat without RANKL treatment (3A, A1). Inflammatory cells and collagen fibers are seen overlying the alveolar bone (white arrow). Histologic section of the mandible of a veh-treated rat with RANKL treatment (3B, B1). Inflammatory cells, collagen fibers and epithelium are seen overlying the alveolar bone (white arrow). Histologic section of the mandible of a ZA-treated rat without RANKL treatment (3C, C1). Absence of soft tissue overlying the alveolar bone is seen. Significant areas of bone exposure and osteonecrosis are noted (black arrow). Histologic section of the mandible of a ZA-treated rat with RANKL treatment. Inflammatory cells, collagen

fibers and epithelium are seen overlying the alveolar bone (3D, D1, white arrow). Small areas of necrosis are noted. TRAP staining of veh or ZA treated sites with or without RANKL treatment (3A2-D2). Quantification of osteonecrotic area over total bone area (3E), empty osteocytes over bone area (3F), TRAP positive cells over bone length (3G) and attached osteoclasts over bone length (3H) *= statistically significant with a p value <0.05.

[0023] **Figure 4A-H.** Histologic sections of extraction sockets of vehicle or ZA treated rats with or without local RANKL treatment 12 days after tooth extraction and RANKL delivery. Extraction socket of a veh-treated rat without RANKL treatment. Intact epithelium, absence of inflammatory infiltrate (orange arrow) and minimal osteonecrosis are noted (4A, A1). Extraction socket of a veh-treated rat with RANKL treatment (4B, B1). Intact epithelium, absence of inflammatory infiltrate (orange arrow) and minimal osteonecrosis are noted. Extraction socket of a ZA-treated rat without RANKL treatment (4C, C1). Epithelial migration, and extensive bone exposure (black arrow). Bony sequestration and osteonecrosis are noted (blue arrow). Extraction socket of a ZA-treated rat with RANKL treatment. Intact epithelium, absence of significant inflammation or bone exposure and reduced osteonecrosis are seen. (4D, D1). TRAP staining of veh or ZA treated rats with or without RANKL treatment (4A2-D2). Quantification of osteonecrotic area over total bone area (4E), empty osteocytes over bone area (4F), TRAP positive cells over bone length (4G) and attached osteoclasts over bone length (4H) *** = statistically significant with a p value <0.001, + = statistically significant with a p value <0.0001.

[0024] **Figure 5A-H.** Picrosirius red stain of veh or ZA treated sites with or without RANKL treatment. (bright field-5A-D, polarized light-5A1-D1). Blue arrows point to insertion of collagen fibers in the bone. White arrows point to lack of collagen fiber insertion in the bone. Cytokeratin 14

stain of veh or ZA treated sites with or without RANKL treatment (5E-H). Black arrows points to epithelial disruption red arrow point to necrotic bone.

[0025] **Figure 6A-E.** MicroCT assessment (axial view) of veh or ZA treated sites with or without RANKL treatment (Fig. 6A-D). Quantification of bone volume over tissue volume in the distal root of the first molar (Fig 6E). *** = statistically significant with a p value <0.001.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0026] The present subject matter may be understood more readily by reference to the following detailed description which forms a part of this disclosure. It is to be understood that this invention is not limited to the specific products, methods, conditions or parameters described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed invention.

[0027] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0028] As employed above and throughout the disclosure, the following terms and abbreviations, unless otherwise indicated, shall be understood to have the following meanings.

[0029] In the present disclosure, the singular forms "a," "an," and "the" include the plural reference, and reference to a particular numerical value includes at least that particular value, unless the context clearly indicates otherwise. Thus, for example, a reference to "a compound" is a reference to one or more of such compounds and equivalents thereof known to those skilled in the art, and so forth. The term "plurality", as used herein, means more than one. When a range of

values is expressed, another embodiment includes from the one particular and/or to the other particular value.

[0030] Similarly, when values are expressed as approximations, by use of the antecedent "about," it is understood that the particular value forms another embodiment. All ranges are inclusive and combinable. In the context of the present disclosure, by "about" a certain amount it is meant that the amount is within $\pm 20\%$ of the stated amount, or preferably within $\pm 10\%$ of the stated amount, or more preferably within $\pm 5\%$ of the stated amount.

[0031] As used herein, the terms "treat", "treatment", or "therapy" (as well as different forms thereof) refer to therapeutic treatment, including prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change associated with a disease or condition. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of the extent of a disease or condition, stabilization of a disease or condition (i.e., where the disease or condition does not worsen), delay or slowing of the progression of a disease or condition, amelioration or palliation of the disease or condition, and remission (whether partial or total) of the disease or condition, whether detectable or undetectable. Those in need of treatment include those already with the disease or condition as well as those prone to having the disease or condition or those in which the disease or condition is to be prevented.

[0032] As used herein, the terms "component," "composition," "formulation", "composition of compounds," "compound," "drug," "pharmacologically active agent," "active agent," "therapeutic," "therapy," "treatment," or "medicament," are used interchangeably herein, as context dictates, to refer to a compound or compounds or composition of matter which, when administered to a subject (human or animal) induces a desired pharmacological and/or physiologic

effect by local and/or systemic action. A personalized composition or method refers to a product or use of the product in a regimen tailored or individualized to meet specific needs identified or contemplated in the subject.

[0033] The terms "subject," "individual," and "patient" are used interchangeably herein, and refer to an animal, for example a human, to whom treatment with a composition or formulation in accordance with the present invention, is provided. The term "subject" as used herein refers to human and non-human animals. The terms "non-human animals" and "non-human mammals" are used interchangeably herein and include all vertebrates, e.g., mammals, such as non-human primates, (particularly higher primates), sheep, dog, rodent, (e.g. mouse or rat), guinea pig, goat, pig, cat, rabbits, cows, horses and non-mammals such as reptiles, amphibians, chickens, and turkeys. The compositions described herein can be used to treat any suitable mammal, including primates, such as monkeys and humans, horses, cows, cats, dogs, rabbits, and rodents such as rats and mice. In one embodiment, the mammal to be treated is human. The human can be any human of any age. In an embodiment, the human is an adult. In another embodiment, the human is a child. The human can be male, female, pregnant, middle-aged, adolescent, or elderly. According to any of the methods of the present invention and in one embodiment, the subject is human. In another embodiment, the subject is a non-human primate. In another embodiment, the subject is murine, which in one embodiment is a mouse, and, in another embodiment is a rat. In another embodiment, the subject is canine, feline, bovine, equine, laprine or porcine. In another embodiment, the subject is mammalian. In another embodiment, the subject is any organism susceptible to osteonecrosis, osteopenia, and/or osteoporosis.

[0034] Conditions and disorders in a subject for which a particular drug, compound, composition, formulation (or combination thereof) is said herein to be "indicated" are not restricted to conditions

and disorders for which that drug or compound or composition or formulation has been expressly approved by a regulatory authority, but also include other conditions and disorders known or reasonably believed by a physician or other health or nutritional practitioner to be amenable to treatment with that drug or compound or composition or formulation or combination thereof.

[0035] Medication related osteonecrosis of the Jaws (MRONJ) is a severe complication of antiresorptive and anti-angiogenic medications prescribed to patients with osteoporosis or bone malignancies. Osteoclast inhibition is central in MRONJ pathogenesis. In the Example herein, as described in more detail below, 30 Wistar-Hun rats were i.p. treated with saline or 66 µg/kg zoledronic acid (ZA) for a week. Then, mandibular molars were extracted bilaterally. Collagen tapes infused with water or RANKL were placed in the extraction sockets of 60 hemimandibles of veh (veh/RANKL-, veh/RANKL+) or ZA treated rats (ZA/RANKL-, ZA/RANKL+). ZA delivery continued for 3 or 12 days after surgery, rats were euthanized, and clinical, radiographic and histologic assessments were performed.

[0036] RANKL immunostain showed increased signal in the RANKL treated sites of veh or ZA rats compared to the non-RANKL treated sites. At the 12-day timepoint, sockets of veh/RANKL- and veh/RANKL+ rats showed intact mucosa, while mucosal defects were noted in ZA/RANKL- rats. Interestingly, ZA/RANKL+ sockets showed absence of bone exposure. Histologically, at the 3-day timepoint, ZA/RANKL- sockets demonstrated extensive bone exposure and osteonecrosis. In contrast, ZA/RANKL+ rats showed soft tissue coverage and significantly reduced osteonecrosis, similar to the veh groups. Importantly, in the ZA/RANKL+ group, osteoclasts attached to the bone surface and osteoclast numbers were higher compared to ZA/RANKL- sites. At the 12-day timepoint, persistent osteonecrosis and bone exposure were detected in the sockets of ZA/RANKL- animals. Contrary, ZA/RANKL+ rats demonstrated socket epithelialization and reduced osteonecrosis.

Significantly more total and bony attached osteoclasts persisted in the ZA/RANKL+ vs the ZA/RANKL- group. A disruption was noted in the connective tissue-bone interface and the epithelium of the ZA/RANKL- group. In contrast, an intact collagen network and epithelium were seen in ZA/RANKL+ sites, resembling the veh groups. We present, *inter alia*, a novel approach towards improving socket healing, in the presence of ZA, by enhancing osteoclastic activity through local RANKL delivery. The approaches described herein are clinically applicable and could improve treatment outcomes of patients on high-dose ZA therapy.

[0037] It is provided herein that, *inter alia*, RANKL can be delivered locally within or to the extraction socket or bone surgical area or dental implant site of patients, including those on antiresorptive medications (such as a bisphosphonate (BP)), to, *inter alia*, enhance the local generation of osteoclasts, mitigate the local antiresorptive medication effect on resorption and/or improve socket healing without discontinuing the systemic antiresorptive treatment.

[0038] Provided herein are methods of treating osteonecrosis in a subject, including, *inter alia*, osteonecrosis of the jaw (ONJ), such as, for example, Medication Related Osteonecrosis of the Jaw (MRONJ). Osteonecrosis, ONJ, and MRONJ have the meanings ordinarily understood in the art.

[0039] In embodiments, the methods, compositions, and delivery devices or vehicles described herein include contacting or otherwise administering to an area in a subject a Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL). RANKL is a type II homotrimeric transmembrane protein that belongs to the TNF cytokine family, and is expressed as a membrane-bound and a secreted protein, which is derived from the membrane form as a result of either proteolytic cleavage or alternative splicing. RANKL binds to the RANK receptor of pre-osteoclastic cells, mediates the fusion of neighboring osteoclast precursors, causing them to become multinucleated under the

influence of other genes, such as DC-STAMP. RANKL also mediates the transcription of several osteoclast-specific genes such as TRAP, cathepsin K and calcitonin receptor committing the cells to an osteoclastic phenotype. RANKL may be expressed in different molecular forms, including a trimeric transmembrane protein, a primary secreted form, and a truncated ectodomain.

[0040] RANKL is known and readily available to those of ordinary skill in the art and in embodiments described herein can be obtained from any suitable source. In an embodiment, the RANKL is isolated. In an embodiment, the RANKL is recombinantly produced. Techniques for isolating, recombinantly producing, synthesizing, or otherwise obtaining proteins are well known to those of ordinary skill in the art.

[0041] In embodiments described herein, RANKL may be derived from the same species of animal as that for which the therapy is intended or may be another species' RANKL that cross reacts. Nonlimiting examples of RANKL suitable for use in the invention include rat sRANK ligand comprising the amino acid sequence set forth as: PAMMEGSWLD VARRGKPEAQ PFAHLTINAA DIPSGSHKVS LSSWYHDRGW AKISNMTLSN GKLRVNQDGF YYLYANICFR HHETSGSVPA DYLQLMVYVV KTSIKIPSSH NLMKGGSTKN WSGNSEFHFY SINVGFFFKL RAGEEISVQV SNPSLLDPDQ DATYFGAFKV QDID (SEQ ID NO: 1); and human sRANK ligand comprising the amino acid sequence set forth as MEKAMVDGSW LDLAKRSKLE AQPFAHLTIN ATDIPSGSHK VSLSSWYHDR GWAKISNMTF SNGKLIVNQD GFYYLYANIC FRHHETSGDL ATEYLQLMVY VTKTSIKIPS SHTLMKGGST KYWSGNSEFH FYSINVGFF KLRSGEEISI EVSNPSLLDP DQDATYFGAF KVRDID (SEQ ID NO: 2).

[0042] RANKL suitable for use in the present invention may also include any active variant or fragment thereof and/or amino acid modifications such as, for example, pegylation, or any other

modification intended to increase the clinical utility of peptides, including, for example those intended to reduce susceptibility to proteases or other degradation.

[0043] In an embodiment, provided herein is a method of treating osteonecrosis in a subject, comprising contacting an area of osteonecrosis in the subject with a therapeutically effective amount of a Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL). In an embodiment, the osteonecrosis comprises osteonecrosis of the jaw (ONJ). In an embodiment, the ONJ is Medication Related Osteonecrosis of the Jaw (MRONJ). In another embodiment, the MRONJ is associated with administration of an antiresorptive treatment in the subject. In another embodiment, the MRONJ is associated with administration of an antiangiogenic treatment in the subject.

[0044] In an embodiment, the antiresorptive treatment comprises administration of a bisphosphonate. In another embodiment, the antiresorptive treatment comprises an antibody to RANKL. In an embodiment, the antibody is a monoclonal antibody. In another embodiment, the monoclonal antibody comprises denosumab (e.g. Xgeva or Prolia). The antiresorptive treatment also can include any other suitable antibody, including any suitable monoclonal antibody, to RANKL.

[0045] In embodiments of the methods, compositions, devices, vehicles, or other aspects described herein, the antiresorptive treatment can comprise any other antiresorptive treatment, including any bisphosphonate, such as, without limitation alendronate (e.g. Fosamax), risedronate (e.g. Actonel or Atelvia), ibandronate (e.g. Boniva), zoledronic acid (e.g. Reclast), pamidronate (e.g. Aredia), clodronate, etidronate; any other monoclonal antibody, such as, without limitation , romosozumab (e.g. Evenity), any antiangiogenic agent, including, without limitation, bevacizumab, sunitinib, sorafenib, pazopanib, axitinib, any m-TOR inhibitor, including, without limitation, everolimus or temsirolimus, or any combination of the above, or any other medication or treatment that presently or in the future is associated with MRONJ.

[0046] In an embodiment, the subject has osteoporosis. In another embodiment, the subject has osteopenia. In another embodiment, the subject has a benign bone tumor. In another embodiment, the subject has a bone malignancy. The bone malignancy can include any bone malignancy, including, without limitation, multiple myeloma, osteosarcoma, Ewing's sarcoma, or chondrosarcoma. The malignancy can be a primary or secondary malignancy. In one embodiment, the subject has hypercalcemia of malignancy. In one embodiment, the subject has Paget's disease. In one embodiment, the subject has giant cell tumor. In one embodiment, the subject has a systemic disease that requires antiresorptive treatment.

[0047] In another embodiment, the subject has undergone a dental surgical procedure. In an embodiment, the dental surgical procedure comprises a tooth extraction. In another embodiment, the dental surgical procedure comprises an implant placement. In one embodiment, the patient has dentures. In one embodiment, the patient has periodontal disease. In one embodiment, the patient has tooth abscess. In one embodiment, the patient has jaw infection. In one embodiment, the patient has trauma to the oral mucosa. In one embodiment, the patient has spontaneous bone exposure.

[0048] In another embodiment, the dental surgical procedure comprises apicoectomy. In another embodiment, the dental surgical procedure comprises periodontal surgery. In another embodiment, the dental surgical procedure comprises grafting placement. In another embodiment, the dental surgical procedure comprises debridement. In another embodiment, the dental surgical procedure comprises treatment of peri-implantitis.

[0049] In another embodiment, the contacting comprises local delivery to a bone surgical area. In an embodiment, the contacting comprises local delivery to an extraction socket. In an embodiment, the contacting comprises local delivery to a dental implant site. In embodiments described herein, the RANKL can be administered to or contacted with any area of osteonecrosis in the subject. In an

embodiment, the RANKL can be administered concurrently with the antiresorptive or antiangiogenic or other treatment. In an embodiment, the RANKL can be administered prior to the antiresorptive or antiangiogenic or other treatment. In an embodiment, the RANKL can be administered subsequent to the antiresorptive or antiangiogenic or other treatment.

[0050] In an embodiment, the RANKL is comprised on or in a substrate. In an embodiment, the substrate is adapted for placement within the extraction socket. In another embodiment, the substrate comprises a sponge. In an embodiment, the sponge comprises collagen.

[0051] In another embodiment, the substrate comprises a resorbable tape. In an embodiment, the resorbable tape comprises collagen. In another embodiment, the substrate comprises a hydrogel. In another embodiment, the substrate comprises a collagen sponge. In another embodiment, the substrate comprises a PLGA scaffold. In another embodiment, the substrate comprises a platelet concentrate (e.g. autologous, allogeneic, xenogeneic platelet concentrate). In another embodiment, the substrate comprises any resorbable carrier or scaffold. In another embodiment, the substrate comprises a gel, tape, or sponge customarily used in dental surgical procedures. In an embodiment, the substrate is loaded with about 0.1 to about 100 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 10 to about 90 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 20 to about 80 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 30 to about 50 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 0.1 to about 0.5 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 0.5 to about 1 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 0.1 to about 1 mg/ml of RANKL. In another embodiment, the substrate is loaded with 0.1 to 1 mg/ml of RANKL.

[0052] In an embodiment, the contacting results in localized activation of osteoclasts in the area. In an embodiment, the contacting results in a reduction in necrosis in the area. In an embodiment, the

contacting results in improved healing in the area. In an embodiment, the contacting results in improved patient symptoms. In an embodiment, the contacting results in improved clinical signs. In an embodiment, the contacting results in improved radiographic findings.

[0053] In any of the embodiments, here, the substrate containing RANKL provides for a timed or slow delivery or release of RANKL to the site. Any means of provided slow or timed release is embodied herein, such as but not limited to the use of a collagen or hydrogel substrate or matrix comprising the RANKL, a polymer coated, liposomal or otherwise encapsulated RANKL comprising a biodegradable membrane or scaffold. In one embodiment, a porous collagen-hydroxyapatite scaffold comprises RANKL. In one embodiment RANKL is bound to a carrier that releases RANKL over time.

[0054] In an embodiment, the subject is a mammal. In an embodiment, the mammal is a human.

[0055] In another embodiment, provided herein is a device for delivering a RANKL to an area of osteonecrosis in a subject, comprising a substrate adapted for local delivery to an area of osteonecrosis and a RANKL. Any suitable delivery device or vehicle of any configuration, material, or shape that performs the desired functions described herein, including delivering the RANKL to the desired location in the subject, is within the scope of the invention. In an embodiment, the area comprises an extraction socket. In another embodiment, the area comprises a bone surgical area. In another embodiment, the area is any area in the body in need of the treatments or improvements in accordance with those described herein.

[0056] In an embodiment, the substrate comprises a sponge. In another embodiment, the sponge comprises collagen. In another embodiment, the substrate comprises a collagen sponge. In another embodiment, the substrate comprises a PLGA scaffold. In another embodiment, the substrate comprises a platelet concentrate (e.g. autologous, allogeneic, xenogeneic platelet concentrate). In

another embodiment, the substrate comprises any resorbable carrier or scaffold. In another embodiment, the substrate comprises a gel, tape, or sponge customarily used in dental surgical procedures.

[0057] In an embodiment, the substrate is loaded with about 0.1 to about 100 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 10 to about 90 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 20 to 80 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 30 to about 50 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 0.1 to about 0.5 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 0.5 to about 1 mg/ml of RANKL. In another embodiment, the substrate is loaded with about 0.1 to about 1 mg/ml of RANKL. In another embodiment, the substrate is loaded with 0.1 to 1 mg/ml of RANKL.

[0058] In another embodiment, the substrate comprises a resorbable tape. In an embodiment, the resorbable tape comprises collagen. In another embodiment, the substrate comprises a hydrogel. In embodiments, the substrate can be any other suitable substrate, as described herein.

[0059] In embodiments herein, a therapeutically effective amount of a compound or agent, such as, for example, RANKL, is administered to a subject. Any suitable formulation, device, or delivery vehicle for delivering the agent and/or achieving the desired therapeutic result can be employed. Thus, included herein are pharmaceutical compositions comprising compounds of the invention and one or more pharmaceutically acceptable carriers. "Pharmaceutically acceptable carriers" include any excipient which is nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. The pharmaceutical composition may include one or more therapeutic agents.

[0060] Thus, as used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0061] Moreover, "Pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem complications commensurate with a reasonable benefit/risk ratio. The term "pharmaceutically acceptable" also includes those carriers approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals and, more particularly, in humans.

[0062] In an embodiment, compositions containing the therapeutic agent or agents described herein, can be, in one embodiment, administered to a subject by any method known to a person skilled in the art and suitable to obtain the desired result.

[0063] Carriers may be any of those conventionally used, as described above, and are limited only by chemical-physical considerations, such as solubility and lack of reactivity with the compound of

the invention, and by the route of administration. The choice of carrier will be determined by the particular method used to administer the pharmaceutical composition.

[0064] The compositions and formulations as described herein may be administered alone or with other biologically-active agents.

[0065] In one embodiment, the compositions are formulated in a unit dosage form. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, optionally in association with a suitable pharmaceutical excipient. The abbreviation "veh" is used herein to refer to "vehicle."

Effective doses

[0066] Effective doses of the compositions of the present invention, for treatment of conditions or diseases vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human, but non-human mammals including transgenic mammals can also be treated. Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy. The pharmaceutical compositions of the invention thus may include a "therapeutically effective amount." A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of a molecule may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the molecule to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the molecule are outweighed by the therapeutically beneficial effects.

[0067] Furthermore, a skilled artisan would appreciate that the term "therapeutically effective amount" may encompass total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

[0068] The amount of a compound of the invention that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition and can be determined by standard clinical techniques. It is foreseen that in practice, the amount of RANKL to be used will depend on multiple factors, including, without limitation, patient weight, gender, size of the defect, number of extractions, number of implants, dose of antiresorptive medication, duration of antiresorptive treatment, and/or systemic disease.

[0069] In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. In one embodiment, the dosage is within the range of about 0.01 to about 50 $\mu\text{g/kg}$ of body weight. In an embodiment, the dosage is within a range of about 0.1 to about 5 $\mu\text{g/kg}$ of body weight. In an embodiment, the dosage is within a range of about 0.5 to about 10 $\mu\text{g/kg}$ of body weight. In an embodiment, the dosage is within a range of about 10 to about 40 $\mu\text{g/kg}$ of body weight. In an embodiment, the dosage is within a range of

about 20 to about 30 $\mu\text{g/kg}$ of body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test bioassays or systems.

[0070] Moreover, suitable doses may also be influenced by permissible daily exposure limits (PDE) of any compound included in a formulation or method as described herein. Such limits are readily available, including, for example, from industry guidance recommendations provided periodically from the U.S. Food and Drug Administration, and the evaluation of these limits are within the knowledge and understanding of one of ordinary skill in the art.

[0071] As used herein, the term "administering" refers to bringing in contact with a compound of the present invention. Administration can be accomplished to cells or tissue cultures, or to living organisms, for example humans. In one embodiment, the present invention encompasses administering the compounds and compositions of the present invention to a human subject.

[0072] In one embodiment, methods of the present invention comprise the step of contacting one or more cells or tissues of said subject with a compound or a composition as described herein. In one embodiment, contacting one or more cells or tissues of a subject with a compound described herein comprises the step of administering a composition comprising said compound to said subject.

[0073] In an embodiment, any of the therapeutic or prophylactic drugs or compounds described herein may be administered simultaneously. In another embodiment, they may be administered at different timepoint than one another. In one embodiment, they may be administered within a few minutes of one another. In another embodiment, they may be administered within a few hours of one another. In another embodiment, they may be administered within 1 hour of one another. In another embodiment, they may be administered within 2 hours of one another. In another embodiment, they may be administered within 5 hours of one another. In another embodiment, they may be administered

within 12 of one another. In another embodiment, they may be administered within 24 hours of one another.

[0074] The compounds and compositions of the invention may be administered only once, or may be administered multiple times. For multiple dosages, the composition may be, for example, administered three times a day, twice a day, once a day, once every two days, twice a week, weekly, once every two weeks, or monthly. Suitable dosage ranges and schedules can vary.

[0075] It is to be noted that dosage values and amounts and ratios of individual components of the compositions described herein also may vary with the type and severity of the condition to be alleviated and other factors. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0076] In embodiments of the methods described herein, patients, the drug may be delivered immediately after tooth extraction. In embodiments, delivery can occur in clinical cases where socket healing is delayed, to accelerate the process. In other embodiments, delivering the medication in patients with established MRONJ lesions that require surgical intervention can be employed. In other embodiments, the medication can be delivered during any surgical procedure on a patient at risk of developing MRONJ.

[0077] The pharmaceutical compositions, devices, and delivery vehicles described and contemplated herein can be included in a container, pack, or dispenser together with instructions for administration.

[0078] In one embodiment, and as described herein, treating may include directly affecting or curing, suppressing, inhibiting, preventing, reducing an incidence, reducing the severity of, delaying the onset

of, reducing symptoms associated with the disease, disorder or condition, or a combination thereof. Thus, in one embodiment, “treating” refers, *inter alia*, to delaying progression, expediting remission, inducing remission, augmenting remission, speeding recovery, increasing efficacy of or decreasing resistance to alternative therapeutics, or a combination thereof. In another embodiment, treating refers to reducing the pathogenesis of, ameliorating the symptoms of, ameliorating the secondary symptoms of, or prolonging the latency to a relapse of a disorder in a subject. In one embodiment, “preventing” refers, *inter alia*, to delaying the onset of symptoms, preventing relapse to a disease, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, or a combination thereof. In one embodiment, “suppressing” or “inhibiting”, refers *inter alia* to reducing the severity of symptoms, reducing the severity of an acute episode, reducing the number of symptoms, reducing the incidence of disease-related symptoms, reducing the latency of symptoms, ameliorating symptoms, reducing secondary symptoms, reducing secondary infections, prolonging patient survival, or a combination thereof.

[0079] All patents, patent applications, and scientific publications cited herein are hereby incorporated by reference in their entirety.

[0080] The following examples are provided to supplement the prior disclosure and to provide a better understanding of the subject matter described herein. These examples should not be considered to limit the described subject matter. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be apparent to persons skilled in the art and are to be included within, and can be made without departing from, the true scope of the invention.

EXAMPLE

Materials and methods

Animal care

[0081] Thirty Wistar-Hun 7-week-old rats were randomly assigned to receive saline or 66 µg/kg of zoledronic acid (ZA), (LKT laboratories, St. Paul, MN). Rats were injected intraperitoneally once a week in morning hours. Animals were kept and treated according to guidelines of the UCLA Chancellor's Animal Research Committee.

[0082] After a week of pretreatment with saline or ZA, the first and second mandibular molars of both sides were extracted in all rats. Two hours after tooth extraction, resorbable collagen tapes (ACE Surgical Supply, Brockton, MA) containing water or recombinant rat RANKL (Peprotech, Rocky Hill, NJ) were placed in the extraction sockets. Four µl of water or aqueous RANKL solution (0.1mg/ml) were placed in each collagen tape. Water or RANKL loaded collagen tapes were placed in the extraction sockets of 30 hemi-mandibles of veh-treated rats and 30 hemimandibles of ZA- treated rats (a total of 60 hemimandibles were used). Each rat received a veh infused tape on one side of the mandible and a RANKL infused tape on the other. Sides were alternated to account for possible discrepancies in surgical technique.

[0083] ZA treatment was continued and rats were euthanized 3 or 12 days after tooth extraction utilizing CO₂. Twelve rats were euthanized 3 days after surgery (6 veh treated and 6 ZA treated) and 18 rats were euthanized 12 days after surgery (9 veh treated and 9 ZA treated). Mandibles were dissected and photographs of the specimens were obtained utilizing a digital optical microscope (Keyence VHX-1000, Osaka, Japan). Areas of unhealed mucosa were measured with Image J (NIH, imagej.nih.gov) and were normalized over the total mucosal area of the first and second molars.

Micro CT scanning was performed, as described Bone volume-total volume ratio measurements were performed in the distal root of the first molar, as we have previously described.

Histology

[0084] Mandibles were fixed for 48 h in 4% paraformaldehyde and then decalcified in 14% EDTA for 4 weeks. Samples were paraffin embedded and 5 µm-thick cross sections were made perpendicular to the long axis of the alveolar ridge at the area of the mucosal defect. If the mucosa was completely healed, sections were made at the area between the first and second molars, approximately 2mm mesial to the mesial cusp of the third molar. H&E stained slides were digitally scanned utilizing the Aperio AT automated slide scanner and automated image analysis was performed using the Aperio Image Scope software (Aperio Technologies, Inc., Vista, CA, USA). The osteonecrotic area(s) and empty osteocytes over total bone area (s) were quantified.

TRAP assay, Picrosirius red staining, immunohistochemistry

[0085] For enumeration of osteoclasts, tartrate-resistant acid phosphatase (TRAP) staining was performed (387A-IKT Sigma Aldrich, St. Louis, MO, USA). Osteoclast numbers were normalized over bone length. Acid phosphatase assay (ab83370, Abcam) was used to measure serum TRAP levels 3 days before and 3 days after surgery. Picrosirius red (Pc red) stain was used to study collagen organization. Anti-RANKL (sc-7628, Santa Cruz) and Anti-Cytokeratin 14 (ab51054, Abcam) were used for immunohistochemistry.

Statistics

[0086] Raw data were analyzed using the GraphPad Prism Software (GraphPad Software, Inc. La Jolla, CA). Descriptive statistics were used to calculate the mean and the standard error of the mean (SEM). Data were analyzed by a two-way ANOVA and post-hoc Tukey's test for multiple comparisons among the various groups and t-test for a single comparison with a statistical significance of $p < 0.05$.

Results

RANKL immunohistochemistry

[0087] Three days after surgery, RANKL immunohistochemistry revealed increased signal in the sockets of veh/RANKL+ and ZA/RANKL+ rats compared to the non RANKL treated groups. RANKL signal was noted in the soft tissue of the sockets (Figure 1A, B, C, D, E, and Figure 1B, D, onsets, yellow arrows) 3 days after surgery. No significant differences were seen in the RANKL signal in the soft tissue of the mandibles 12 days after surgery (data not shown).

Serum TRAP assay

[0088] Serum TRAP assay was performed to ensure absence of off-target effects in rats. TRAP levels 3 days before and 3 days after surgery were compared in veh and ZA treated animals. TRAP levels before and after extraction and local RANKL delivery did not show a statistically significant increase neither in the vehn or in the ZA treated groups (Figure 1F).

Radiographic assessment 3 and 12 days after surgery

[0089] BV/TV values were less than 10% in all specimens from all groups 3 days after surgery (data not shown). Partial healing with woven bone was observed in the veh/RANKL- and veh/RANKL+ sites 12 days after surgery (Figure 6A, B). Significantly decreased BV/TV was seen in the extraction sockets of the ZA/RANKL- and ZA/RANKL+ groups compared to the veh treated groups. (Figure 6 C, D, E).

Specimen photographs 3 and 12 days after surgery

[0090] Specimen photographs 3 days after tooth extraction showed mucosal defects with granulation tissue in all groups and ongoing mucosal healing (Figure 2A-E). Specimen photographs 12 days after tooth extraction revealed an intact alveolar mucosa in the mandibles of veh/RANKL- and veh/RANKL+ rats (Figure 2F,G). Alveolar mucosal defects, granulation tissue and exposed bone were noted in the alveoli of ZA/RANKL- (Figure 2H). Interestingly, intact mucosa was noted in most of the ZA/RANKL+ hemi-mandibles (Figure 2I). Areas of unhealed mucosa were significantly smaller in ZA/RANKL+ rats compared to ZA/RANKL- rats (Figure 2J).

Histologic assessment of extraction sockets 3 days after surgery

[0091] Histologic evaluation 3 days after surgery showed inflammatory infiltrate and sparse collagen fibers overlying the sockets of veh/RANKL- or veh/RANKL+ rats (Fig 3A, B, white arrows). Absence of complete epithelialization of the wound was noted in both groups. In the ZA/RANKL- group, extensive bone exposure was revealed (Fig 3C, black arrows). There was absence of soft tissue overlying the alveolar bone and prominent areas of bone exposure were revealed. Approximately 20% of bone in the extraction sockets of this group was necrotic (Figure 3C, C1, E). Contrary,

ZA/RANKL+ sockets showed thin epithelium and soft tissue covering the sockets. (Fig 3D, E, white arrows). Importantly, osteonecrosis and empty osteocytes over bone area were significantly reduced compared to the ZA/RANKL- group (Figure 3D, D1, E).

TRAP staining in extraction sockets 3 days after surgery

[0092] TRAP staining revealed higher numbers of osteoclasts in the sockets of veh/RANKL+ vs veh/RANKL- groups (Figure 3A2, B2, G). Of note, statistically significantly more osteoclasts were also seen in the extraction sockets of ZA/RANKL+ compared to the ZA/RANKL- group (Figure 3C2, D2, G). Many osteoclasts in ZA treated rats showed an altered, round morphology with pyknotic nuclei and were not in contact with the bone surface. Interestingly, higher numbers of osteoclasts attached to the bone surface were seen in the extraction sockets of ZA/RANKL+ vs ZA/RANKL- sites. (Figure 3H).

Histologic assessment of extraction sockets 12 days after surgery

[0093] Intact epithelium with rete peg formation was detected in the alveoli of veh/RANKL- and veh/RANKL+ rats. Connective tissue showed interval resolution of the inflammatory infiltrate observed in the 3 days timepoint (4A, B, orange arrows). Significant amount of woven bone was seen occupying the extraction sockets in both groups of veh-treated animals. Osteonecrosis and empty osteocytes were minimal in the mandibles of both veh treated groups. (Figure 4A, A1, B, B1, E, F). Histologic evaluation of ZA/RANKL- sockets showed areas of epithelial disruption, bone exposure (4C, black arrow) and bony sequestration (4C1, blue arrow). Significant amount of persistent osteonecrosis and empty osteocytes were also detected (Figure 4C, C1, E). In contrast, the extraction sockets of ZA/RANKL+ treatment demonstrated continuous keratinized epithelium with no evidence

of bone exposure. Of note, statistically significantly less osteonecrosis and empty osteocytes were seen in the alveoli of these animals the ZA/RANKL- group (Figure 4D, D1, E).

TRAP staining in extraction sockets 12 days after surgery

[0094] Twelve days after tooth extraction, comparable numbers of osteoclasts were seen in the sockets of veh/RANKL- and veh/RANKL+ groups (Figure 4A2, B2, G). Importantly, abundance of osteoclasts was noted in the alveoli of ZA/RANKL+ rats. Statistically significant increase in total osteoclast numbers was detected in this group compared to the ZA/RANKL- group. Of note, significantly higher numbers of osteoclasts attached to the bone surface were seen in the alveoli of ZA-treated rats with RANKL vs ZA treated rats without RANKL treatment (Figure 4F).

Picrosirius red and cytokeratin 14

[0095] Pc red revealed an organized collagen network in the veh treated groups with strongly birefringent collagen fibers extending from the submucosal soft tissue and inserting within the vital bone (Fig 5A, B, A1, B1, blue arrows). Contrary, in ZA/RANKL- rats disruption of the bone-soft tissue interface was noted with absence of collagen fiber insertion in the alveolar bone (Fig 5C, C1, white arrow). In the ZA/RANKL+ group, however, intact collagen network with strong collagen birefringence and collagen fiber insertion in the bone were noted. (Fig 3D, D1, blue arrows).

[0096] Cytokeratin 14 showed intact epithelium in veh groups (Fig 5E, F). Contrary, epithelial disruption (5G, black arrow) adjacent to the necrotic bone (5G, red arrow) were noted in ZA/RANKL- mandibles (Fig 5G). The ZA/RANKL+ sockets showed intact, continuous epithelium covering the extraction socket, akin the veh treated rats (Fig 5H).

Discussion

[0097] Medication related osteonecrosis of the jaws is a severe complication of antiresorptive and antiangiogenic medications that can considerably deteriorate the quality of life of already compromised patients. Although progress in animal and clinical research has increased our understanding on MRONJ development, treatment for the disease remains largely empirical.

[0098] Several hypotheses regarding MRONJ development exist and include bone remodeling inhibition, angiogenesis inhibition, local infection/inflammation, soft tissue toxicity, or immunity disruption. Interestingly, MRONJ has been reported with a similar prevalence and severity in patients with a history of bisphosphonates and denosumab (a monoclonal antibody to RANKL). Even though these two groups of drugs act by entirely distinct pharmacologic mechanisms, they both target osteoclasts and result in reduced bone resorption and suppressed bone turnover. Data herein indicate that local restoration of osteoclastic function in a setting of BP treatment can reduce osteonecrosis and improve mucosal healing after tooth extraction. This present study, therefore, provides evidence in support of the bone remodeling inhibition hypothesis.

[0099] Our data showed no difference in TRAP serum levels before and after surgery, suggesting RANKL activity was mainly local and osteoclastogenesis was not stimulated in distant skeletal sites. Interestingly, serum TRAP levels were similar between the veh and ZA treated groups.

[00100] The present study included two different time-points. Three days after extraction, a considerable amount of osteonecrosis was noted in the mandibles of veh-treated rats with or without RANKL treatment. This is likely due to the early time-point elected after tooth extraction, which causes significant trauma to the soft and bony tissues. As expected, osteonecrosis in veh treated rats was minimal 12 days after tooth extraction, likely because of osteoclast activation and resorption of necrotic bone.

[00101] In ZA/RANKL- rats, mucosal defects, bone exposure and extensive bone necrosis were noted. Prominent osteonecrotic areas were seen 3 days after tooth extraction and persisted in the 12-day time-point. Contrary, ZA treated animals with RANKL treatment consistently demonstrated smaller osteonecrotic areas which coincided with an increase in osteoclastic activity. Our data suggest that local enhancement of osteoclastic activity resulting in resorption of necrotic bone can improve wound healing after tooth extraction despite the presence of systemic antiresorptive treatment.

[00102] Bisphosphonate treatment does not result in osteoclast elimination but rather in a change in their morphology which eventually results in apoptosis. Bisphosphonates alter the mevalonate pathway by inhibiting farnesyl pyrophosphate synthase. This results in the impairment of small GTPases, such as Ras and Rho which are essential in maintaining osteoclast morphology, migration and cell survival. Histologically, osteoclasts demonstrate a round shape with pyknotic nuclei and are seen at a distance from the bone, suggesting bone resorption is impaired. Interestingly, in ZA/RANKL+ rats, we appreciated more osteoclasts that were attached to the bony surface, suggesting they maintained their resorptive ability. RANKL has been shown to decrease apoptosis in osteoclasts treated with bisphosphonates in vitro. It is possible that the increased bone resorption observed in ZA/RANKL+ sites was due to both an increase in osteoclast numbers and an increased survival of the osteoclasts already exposed to zoledronic acid.

[00103] In both veh groups and in ZA/RANKL+ rats, we were able to appreciate collagen fiber insertion within the alveolar bone, while in ZA/RANKL- animals the collagen network was discontinuous around the necrotic bone areas. The disruption in the interface between soft tissue and bone may play an important role in the initiation of epithelial migration which can result in sequestration and bone exposure. We have described altered socket healing after extraction of periodontally compromised teeth in rats treated with bisphosphonates. In particular, we noticed a

disorganized collagen network with weak collagen bundle birefringence and lack of insertion of collagen fibers in the necrotic bone. It is possible that the restoration of bone resorption and the ongoing remodeling of vital bone seen in ZA/RANKL+ sites might have helped accommodate the insertion of the periodontium fibers after tooth extraction. Importantly, our study showed improvement of MRONJ lesions without the need for antiresorptive treatment discontinuation. The bisphosphonate dose (66 ug/kg) we utilized parallels the dose of zoledronic acid prescribed to oncologic patients. Nonetheless, the therapeutic approach we describe may also be efficient in osteoporotic patients, given that the bisphosphonate doses they receive are generally lower compared to cancer patients. In fact, zoledronic acid (Reclast) is prescribed to osteoporotic patients in 5mg once every 12 months whereas 4mg of zoledronic acid is used every 3-4 weeks in oncologic patients. This can be important because MRONJ amelioration can be achieved without the employment of 'a drug holiday', which could potentially compromise the patient's skeletal health.

[00104] Herein is presented a clinically relevant application of local RANKL delivery demonstrating the utility of a collagen sponge (a very commonly used substrate during extractions) could be infused with RANKL and inserted within the extraction socket of patients on antiresorptives, in an effort to accelerate wound healing and/or minimize the incidence of MRONJ. The intervention described herein would have beneficial effects, not only for minimizing MRONJ occurrence, but also to alleviate the fear of osteoporotic patients and thus improve compliance with antiresorptive medications. Additionally, it would allow clinicians to more easily elect extraction of hopeless teeth in patients at risk of MRONJ and therefore, minimize complications from persistent dental infection.

[00105] While certain features of the invention have been illustrated and described herein, many modifications, substitutions, changes, and equivalents will now occur to those of ordinary skill in

the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

CLAIMS

What is claimed is:

1. A method of treating osteonecrosis in a subject, comprising:

contacting an area of osteonecrosis in the subject with a therapeutically effective amount of a Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL).
2. The method of claim 1, wherein the osteonecrosis comprises osteonecrosis of the jaw (ONJ).
3. The method of claim 2, wherein the ONJ is Medication Related Osteonecrosis of the Jaw (MRONJ).
4. The method of claim 3, wherein the MRONJ is associated with administration of an antiresorptive treatment in the subject.
5. The method of claim 4, wherein the antiresorptive treatment comprises administration of a bisphosphonate.
6. The method of claim 4, wherein the antiresorptive treatment comprises an antibody to RANKL.
7. The method of claim 6, wherein the antibody is a monoclonal antibody.
8. The method of claim 7, wherein the monoclonal antibody comprises denosumab.
9. The method of claim 1, wherein the subject has osteoporosis.
10. The method of claim 1, wherein the subject has osteopenia.

11. The method of claim 1, wherein the subject has a bone malignancy.
12. The method of claim 1, wherein the subject has undergone a dental surgical procedure.
13. The method of claim 12, wherein the dental surgical procedure comprises a tooth extraction.
14. The method of claim 12, wherein the dental surgical procedure comprises an implant placement.
15. The method of any of claims 1-14, wherein the contacting comprises local delivery to a bone surgical area.
16. The method of claim 1, wherein the contacting comprises local delivery to an extraction socket.
17. The method of any of claims 1-16, wherein the RANKL is comprised on or in a substrate.
18. The method of claim 17, wherein the substrate is adapted for placement within the extraction socket.
19. The method of any of claims 17-18, wherein the substrate comprises a sponge.
20. The method of claim 19, wherein the sponge comprises collagen.
21. The method of claim 17, wherein the substrate comprises a resorbable tape.
22. The method of claim 21, wherein the resorbable tape comprises collagen.
23. The method of any of claims 17-18, wherein the substrate comprises a hydrogel.
24. The method of any of claims 1-23, wherein the contacting results in localized activation of osteoclasts in the area.

25. The method of any of claims 1-24, wherein the contacting results in a reduction in necrosis in the area.
26. The method of any of claims 1-25, wherein the contacting results in improved healing in the area.
27. The method of any of claims 1-26, wherein the subject is a human.
28. A device for delivering a RANKL to an area of osteonecrosis in a subject, comprising:
a substrate adapted for local delivery to an area of osteonecrosis; and
a RANKL.
29. The device of claim 28, wherein the area comprises an extraction socket.
30. The device of claim 28, wherein the area comprises a bone surgical area.
31. The device of claim 28, wherein the substrate comprises a sponge.
32. The device of claim 31, wherein the sponge comprises collagen.
33. The device of claim 28, wherein the substrate comprises a resorbable tape.
34. The device of claim 33, wherein the resorbable tape comprises collagen.
35. The device of claim 28, wherein the substrate comprises a hydrogel.

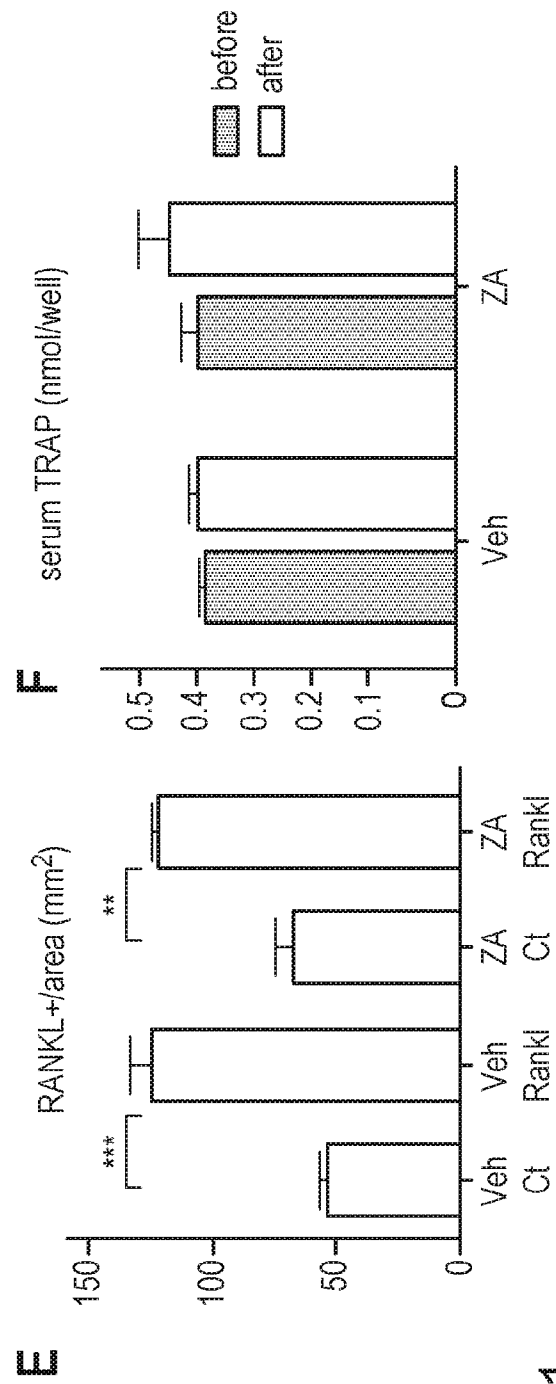
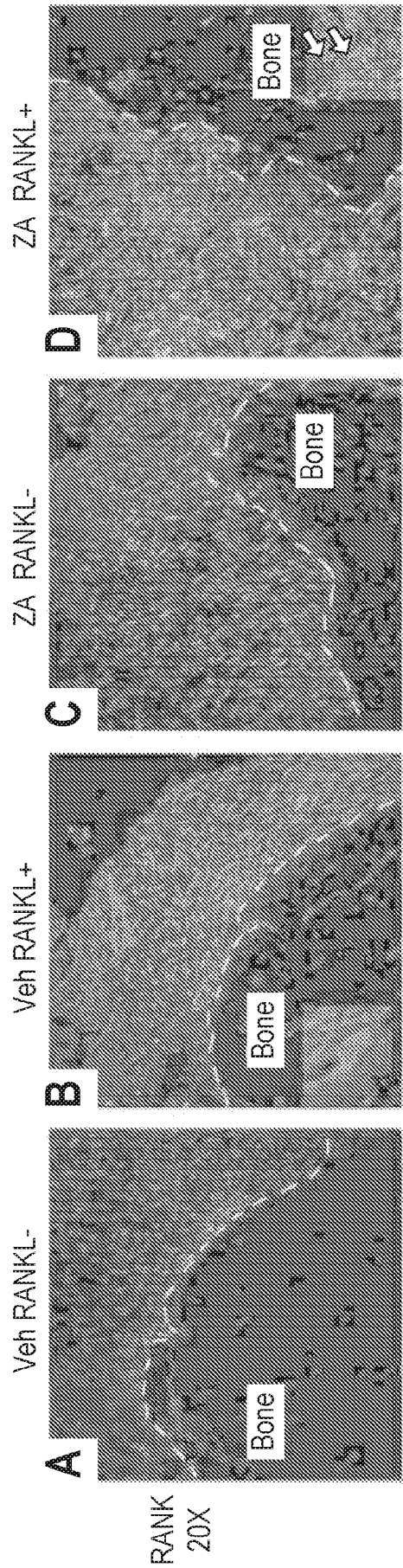


Figure 1

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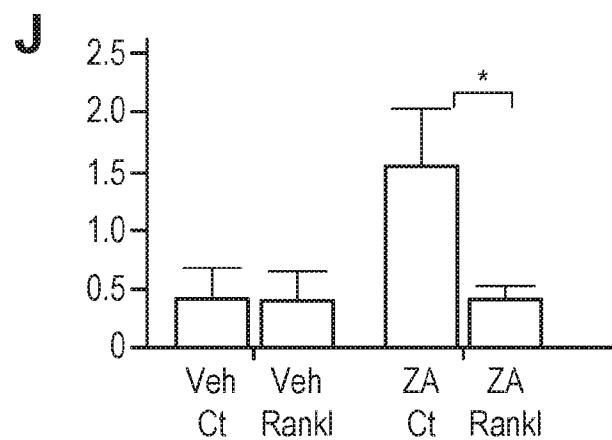
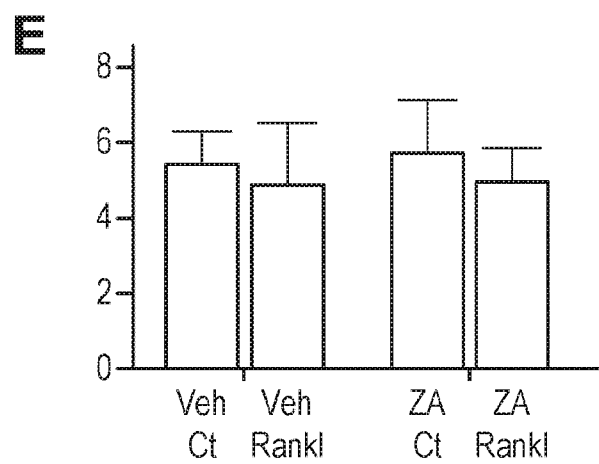
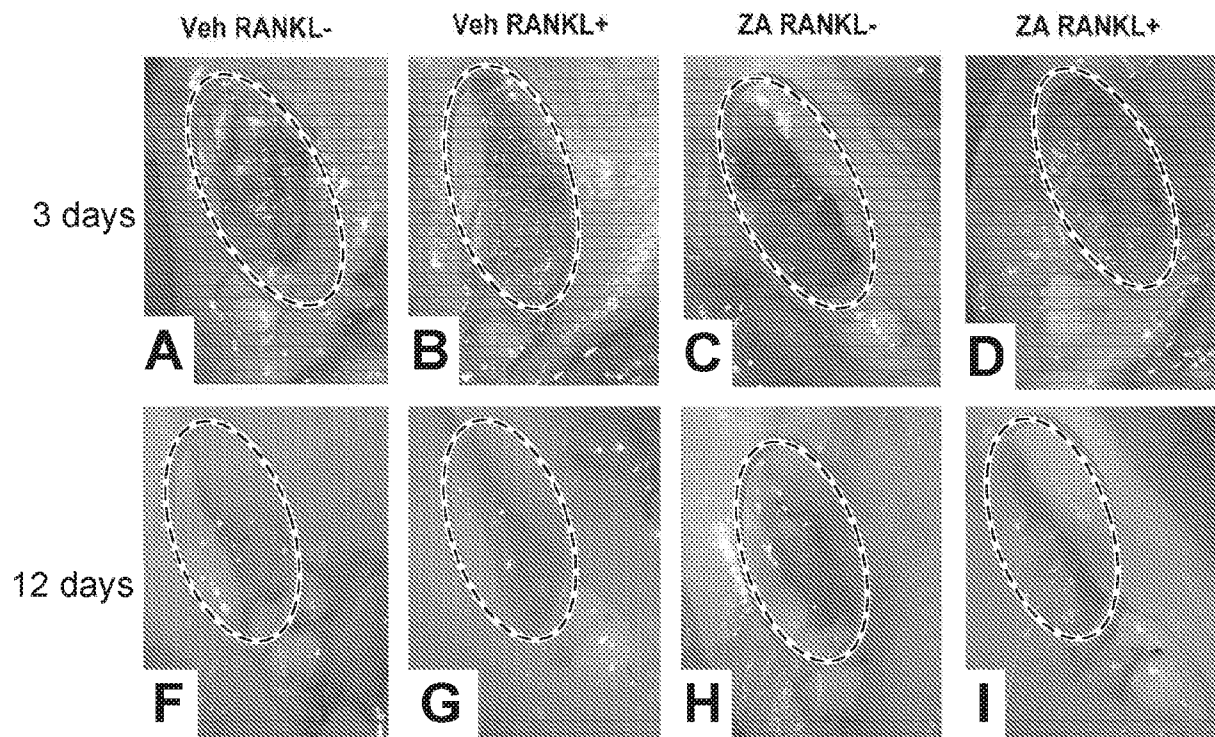


Figure 2

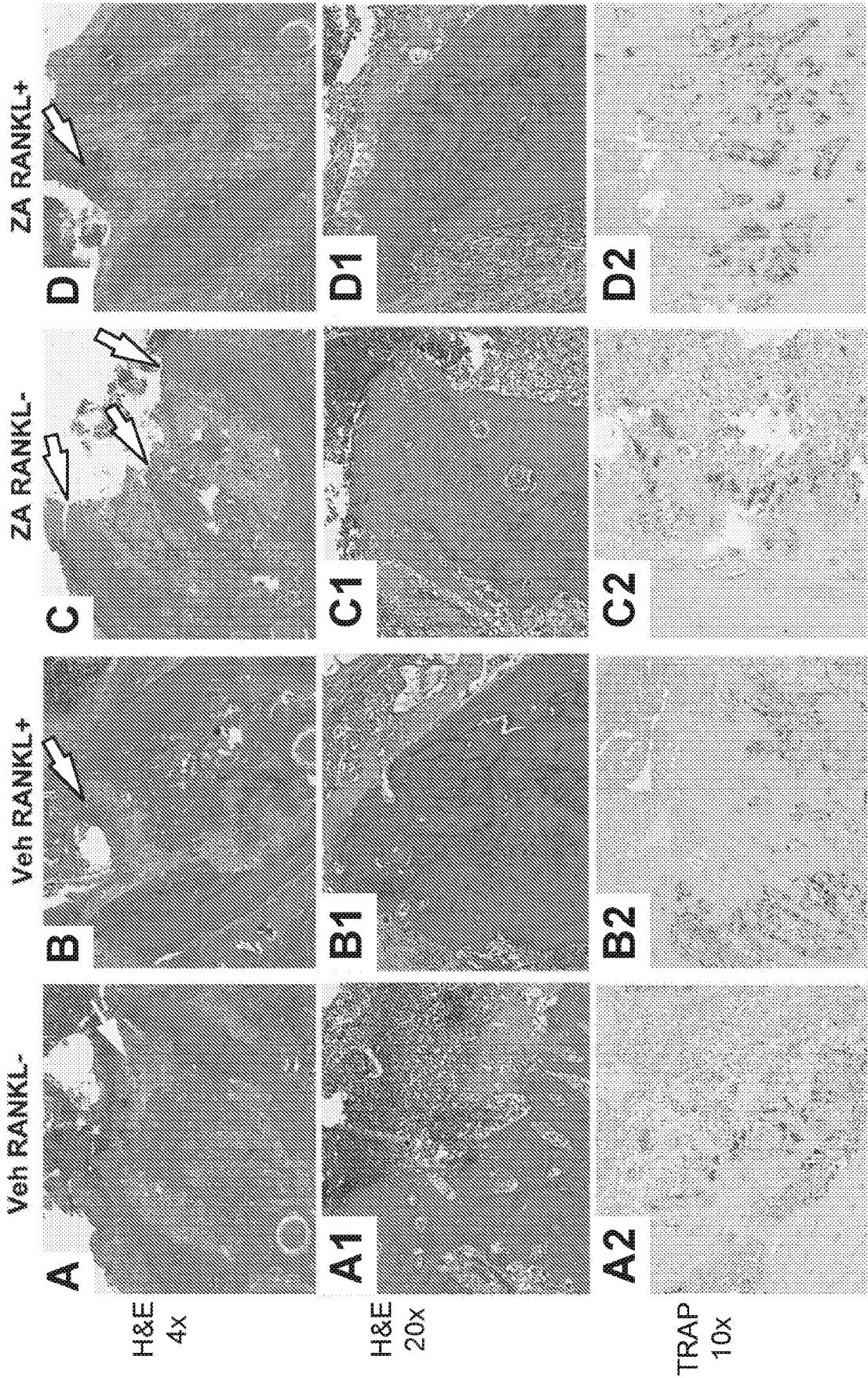


Figure 3

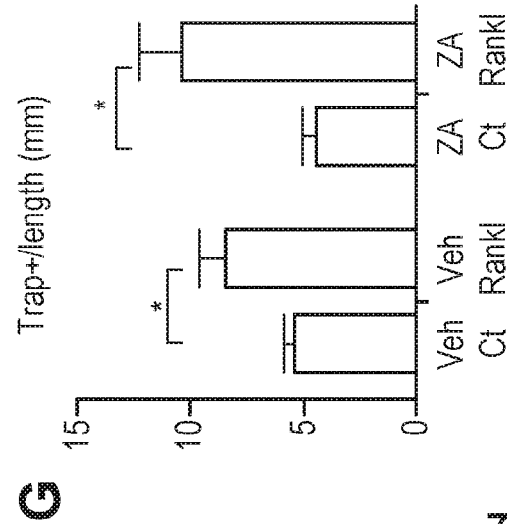
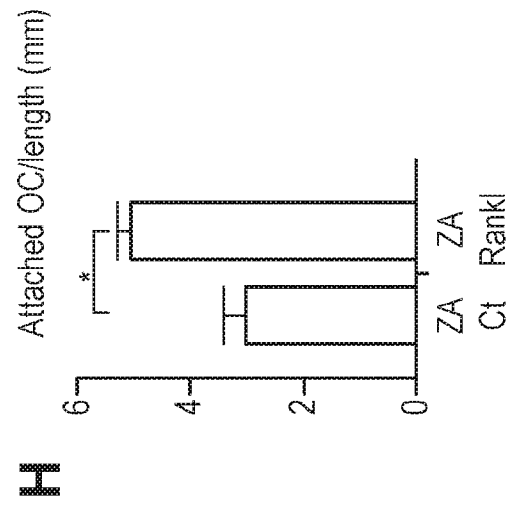
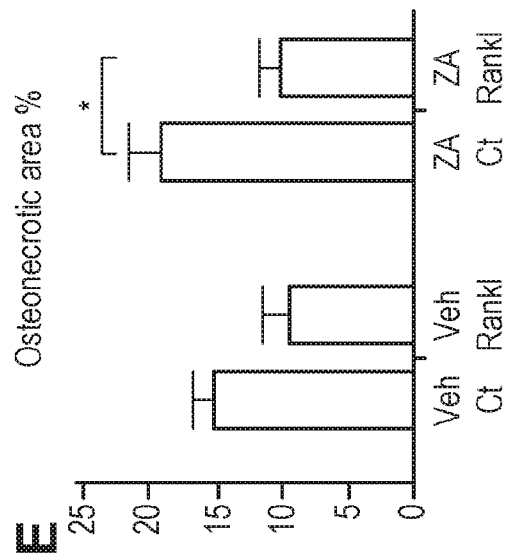
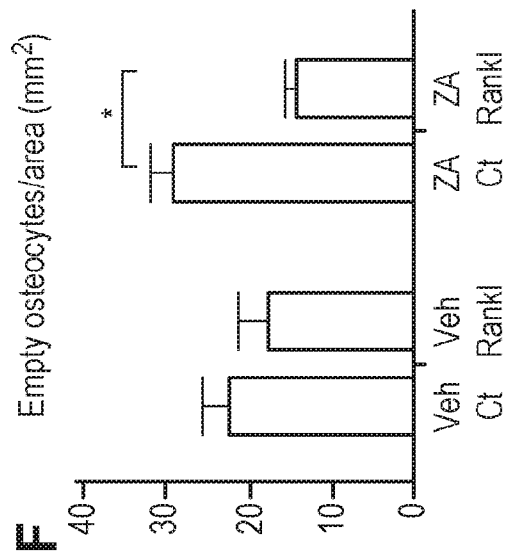
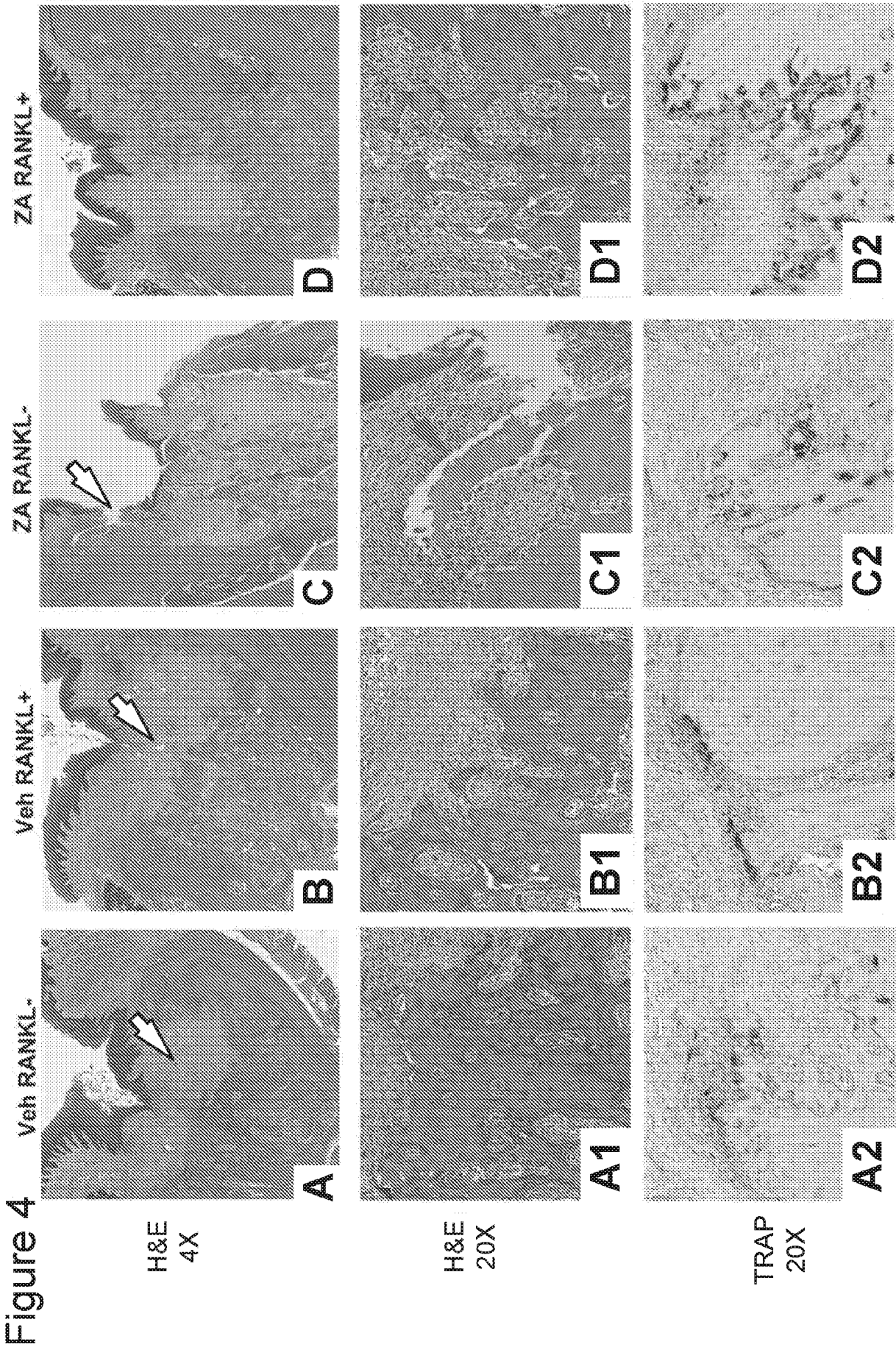


Figure 3 continued

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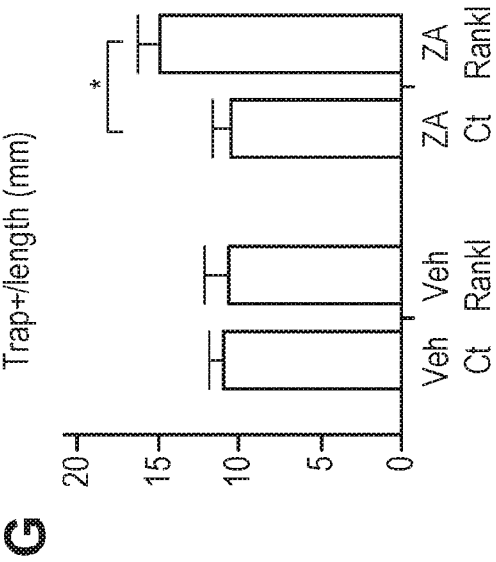
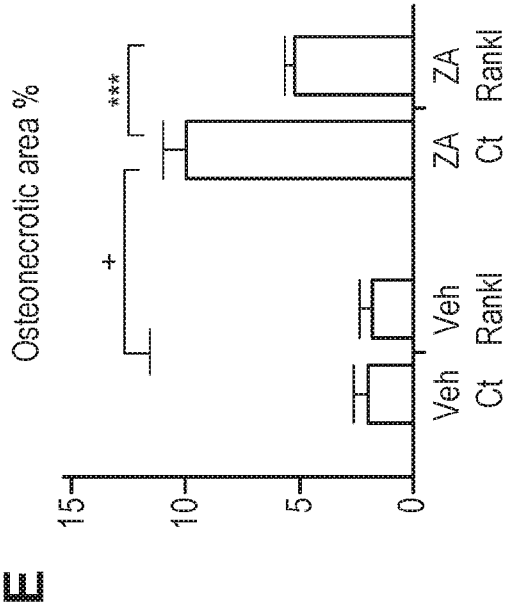
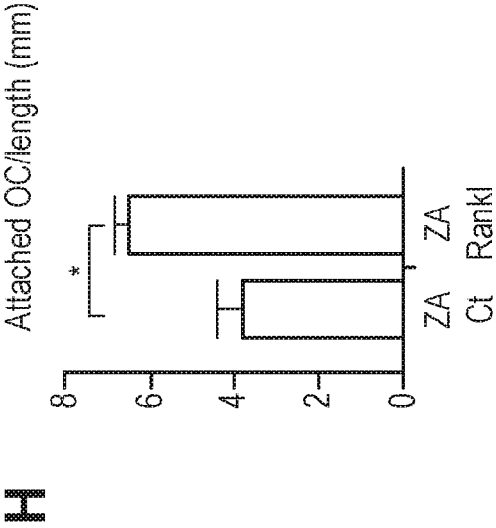
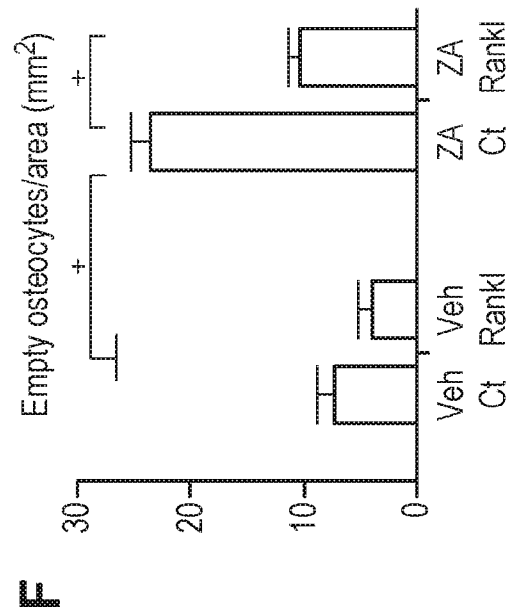


Figure 4 continued

Figure 5

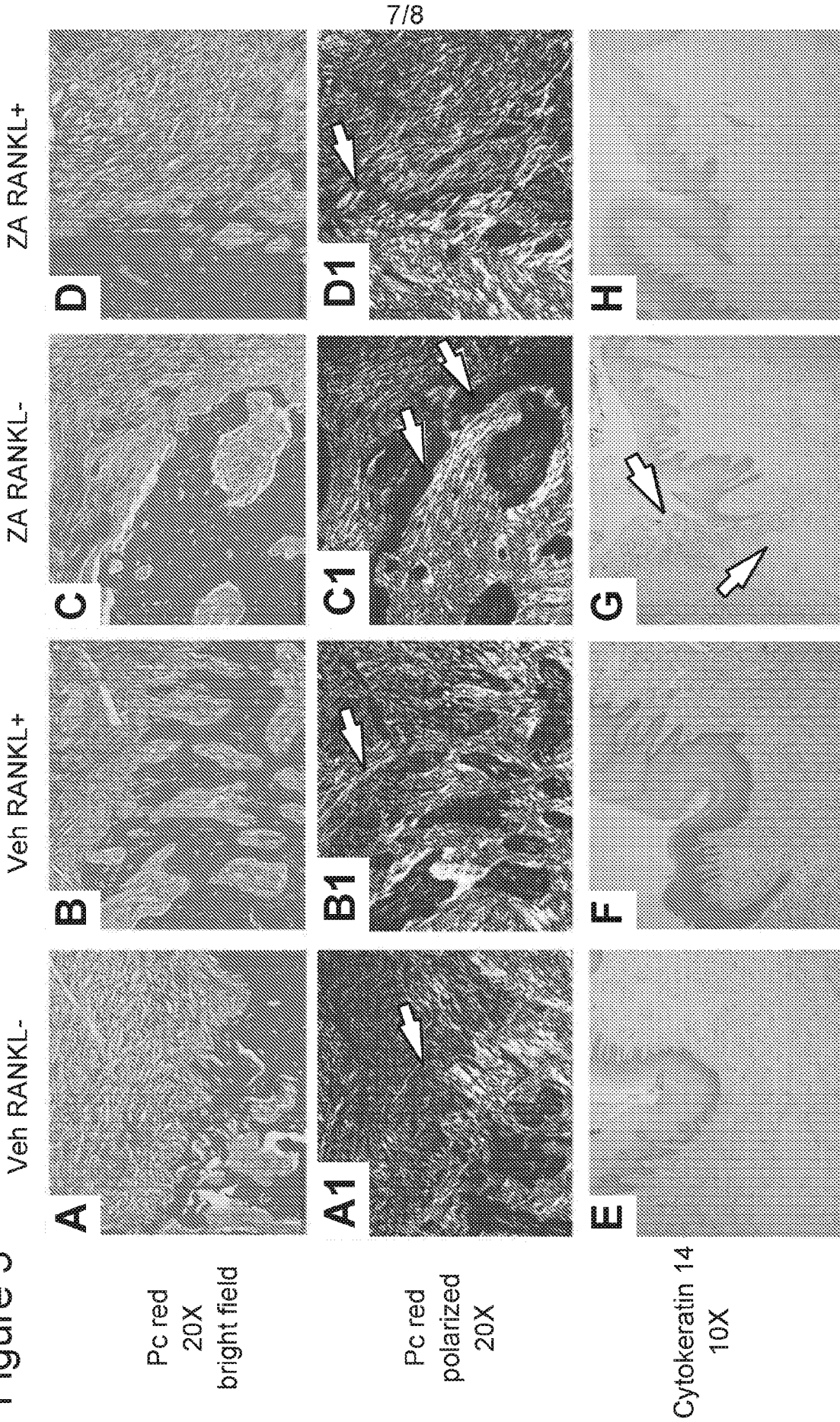
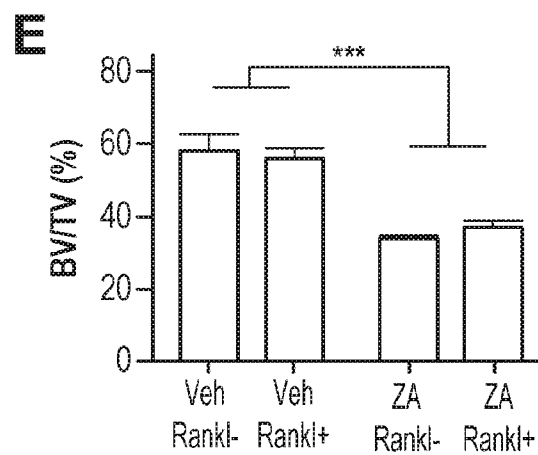
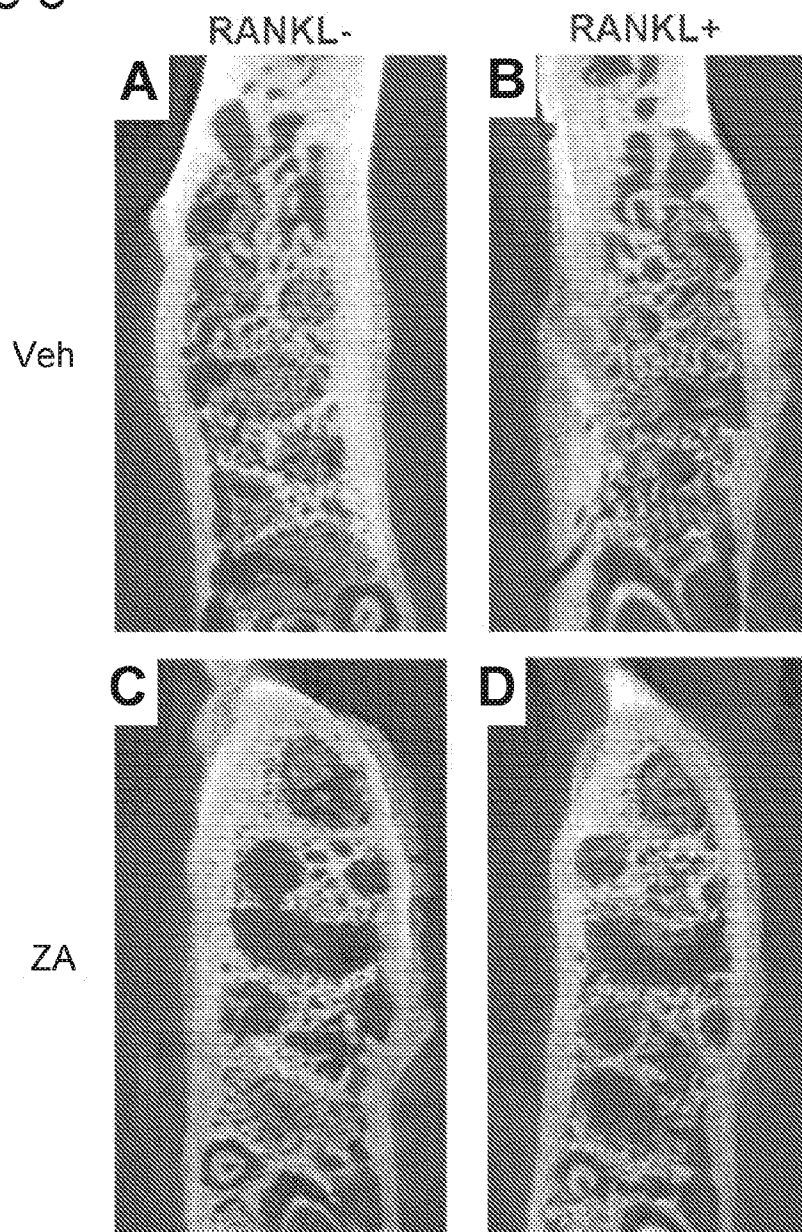


Figure 6

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/017294

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 9/006; A61K 38/00; A61L 15/44; C07K 14/78; C07K 16/24 (2020.01)

CPC - A61K 9/006; A61K 38/00; A61K 38/1875; A61L 15/44; C07K 14/70575; C07K 14/78; C07K 16/24; G01N 33/6863 (2020.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 435/375; 435/377; 514/1.1; 514/16.7; 514/16.9 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2003/0013651 A1 (LAM et al) 16 January 2003 (16.01.2003) entire document	1, 9-11, 15 ----- 2-8, 12-14, 16
X -- Y	US 2015/0037386 A1 (WARSAW ORTHOPEDIC, INC.) 05 February 2015 (05.02.2015) entire document	28, 30-32, 35 ----- 29, 33, 34
Y	WO 2010/115932 A1 (NOVARTIS AG et al) 14 October 2010 (14.10.2010) entire document	2-8
Y	US 2014/0170167 A1 (ABLYNX N.V.) 19 June 2014 (19.06.2014) entire document	6-8
Y	US 2010/0316685 A1 (WILLOUGHBY) 16 December 2010 (16.12.2010) entire document	12-14, 16, 29, 33, 34
A	US 2010/0105612 A1 (TOMIMORI et al) 29 April 2010 (29.04.2010) entire document	1-16, 28-35



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 March 2020

Date of mailing of the international search report

28 APR 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/017294

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 17-27
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.