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[Continued on next page]

(54) Title: DENTAL TREATMENT SYSTEMS AND USES THEREOF

(57) Abstract: Embodiments herein comprise the dental treatment system, dental laser system and applicators, and methods for their use in dental pulp capping, wound healing, bone healing, and the induction of differentiation of stem cells.

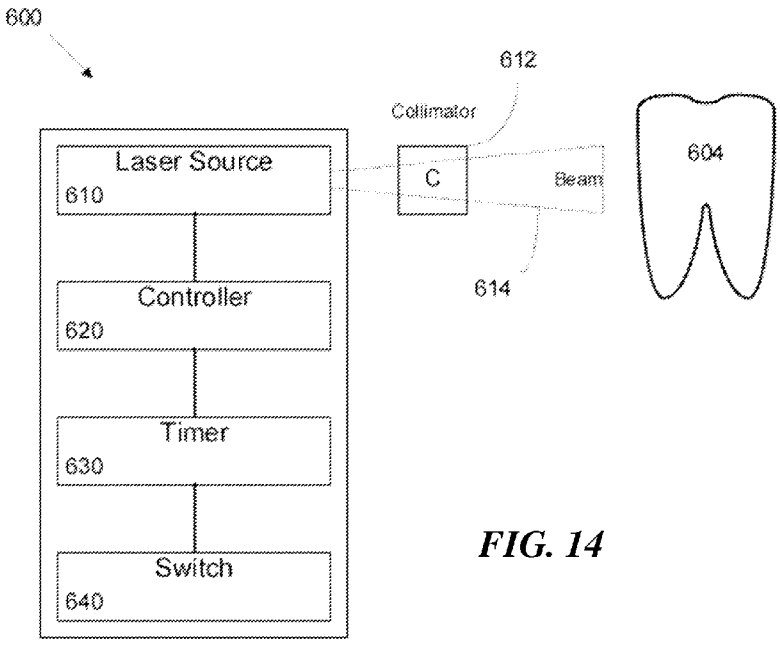

FIG. 14

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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DENTAL TREATMENT SYSTEMS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims benefit under 35 U.S.C. § 119(e) of the U.S. Provisional Application No. 61/449,249 filed March 4, 2011, the contents of which are incorporated herein by reference in its entirety.

GOVERNMENT SUPPORT

[0002] This invention was made with Government support under Grant No.: R1 DE019023-01 awarded by the National Institutes of Sciences. The Government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] Embodiments of the invention relates to dental pulp capping, odontogenesis, stem cell differentiation, tissue healing and tissue regeneration.

BACKGROUND OF THE INVENTION

[0004] Laser systems have many useful applications to the treatment of surfaces, in the industrial field as well as in the medical and dental field. Lasers have become valuable medical instruments, particularly in the field of dentistry. For example, dentists use lasers for a variety of purposes: (1) detection and treatment of tooth decay; (2) detection and treatment of gum disease, e.g. reshape the gum and crown lengthening; (3) for obtaining biopsy and removal; (4) for teeth whitening; (5) reduction of tooth sensitivity; (6) promoting nerve regeneration; (7) treatment of temporomandibular joint disease; and (8) treatment of sleep apnea.

[0005] Numerous laser devices and methods of use thereof are available for medical and dental applications, see e.g., U.S. Patent Nos. 4,461,294; 5,336,217; 5,388,987; 5,456, 603; 5,964,749; 6,120,497; 6,273,885; 7,665,467; 7,867,223; US Patent Publication No: 2011/0027744. Although the current devices and methods may work well for their intended purposes, they pose some drawbacks. With today's demand and wide variety of different applications, there is a strong desire to develop more versatile devices that can be applied with fewer drawbacks.

SUMMARY OF THE INVENTION

[0006] Embodiments of the present invention are based on the observations that low power laser (LPL) irradiations can induce stem cells to differentiate. The inventors demonstrated that an infrared (810 nm) low power laser can direct odontgenic differentiation of human dental stem cells; the differentiation was verified by activation of intracellular signaling, extracellular matrix production and calcium deposition. Furthermore, the inventors observed that the LPL induces multiple reactive oxygen species (ROS) that, in turn, is capable of activating the latent transforming growth factor β (LTGF-β) complex as a key signaling mediator, e.g., in oral wound healing. These observations indicate the utility of LPL as an alternative, non-invasive clinical tool in dental applications, e.g., dental pulp capping, and also for stem cell differentiation and related applications, e.g., in oral wound healing, general wound healing, healing of bone fractures or bone fusions.

[0007] It is the objective of this invention to provide a dental treatment system and a dental laser system for inducing, stimulating and/or promoting dentinogenesis, for inducing, stimulating and/or promoting stem cell differentiation, and for inducing, stimulating and/or promoting tissue repair or regeneration.

[0008] It is also the objective of this invention to provide a method of stimulating dentinogenesis in a subject.

[0009] In addition, it is the objective of this invention to provide a method of dental pulp capping in a subject.

[0010] Furthermore, it is the objective of this invention to provide a method for inducing, stimulating and/or promoting stem cell differentiation, and a method for inducing, stimulating and/or promoting tissue repair or regeneration.

[0011] Accordingly, in one embodiment, provided herein is a dental treatment system for inducing dentinogenesis, the system comprising: a source of irradiation, the source being adapted to produce irradiation having a wavelength in the range of 780 nm to 840 nm and a power output in the range of 5 mW to 300 mW; a control element controlling the source of irradiation to turn the source of laser irradiation on and to turn the source of laser irradiation off; and a timer operatively connected to the control element to activate the source of irradiation to produce irradiation for a pre-determined time period and then deactivate the source of irradiation

from producing radiation, resulting in the application of a pre-determined dose of irradiation to a target.

[0012] In one embodiment, the dental treatment system further comprising a collimator coupled to the source of irradiation and collimating the irradiation produced by the source of irradiation.

[0013] In one embodiment, the laser treatment system further comprises a switch element operatively connected to and controlling the timer.

[0014] In one embodiment, the switch element is a foot operated switch. The foot switch provides a "hands-free" approach for the operator using the laser treatment system, e.g., the operator is a dentist.

[0015] In one embodiment, the timer controls the source of irradiation to apply a dosage of between 1 – 10 J/cm^2 of irradiation to a target location. In one preferred embodiment, the dosage is 3 J/cm^2.

[0016] In one embodiment, the source of irradiation produces laser irradiation.

[0017] In one embodiment, the source of irradiation includes a diode laser.

[0018] In one embodiment, the source of irradiation includes a laser selected from the group including but is not limited to gas lasers, dye lasers, semiconductor lasers and solid state lasers.

[0019] In another embodiment, provided herein is a dental laser system for inducing dentinogenesis in a target, the system comprising: a source of laser irradiation, the source being adapted to produce laser irradiation having a wavelength of 810 nm; a control element controlling operation of the source of irradiation to turn the source of laser irradiation on and to turn the source of laser irradiation off; and a timer operatively connected to the control element to turn the source of laser irradiation on for a pre-determined time period and then turn the source of laser irradiation off and wherein the pre-determined time period is determined to apply a dose of 3 J/cm^2 on the target.

[0020] In one embodiment, the target is a living organism. In one embodiment, the target is a location on or in the organism. In another embodiment, the target comprises living tissues that can respond to the irradiation of the dental treatment or laser system. In another

embodiment, the target comprises living tissues that comprises a population of stem cells. In one embodiment, the stem cells can respond to the irradiation of the dental treatment or laser system, e.g., differentiate to a cell that is further along the lineage of that original stem cell. For example, the target is an exposed dental pulp having a population of dental stem cells within. Upon the irradiation of the dental treatment or laser system described, the dental stem cells differentiate to odontoblasts.

[0021] In another embodiment, provided herein is a method of stimulating dentinogenesis in a subject, the method comprising exposing a tissue comprising a population of dental pulp stem cells to a laser output from the dental treatment system or dental laser system described herein, whereby the dental pulp stem cells is induced to differentiate to odontoblasts.

[0022] In another embodiment, provided herein is a method of dental pulp capping in a subject, the method comprising exposing a tissue comprising an exposed dental pulp to a laser output from the dental treatment system or dental laser system described herein, whereby increased dentin matrix is secreted. The induced increase in secretion of dentin matrix is from inside of the tooth and the direction of secretion is outwards of the tooth. This "inside out" approach function to fill in space of the hole where the dental pulp is exposed to the exterior and eventually enclose the pulp. This "inside out" approach is advantageous over the current "outside in" approach because it precludes any bacteria from being trapped within the pulp after capping is completed.

[0023] In one embodiment, provided herein is a method for inducing and/or promoting stem cell differentiation, the method comprising: (a) contacting a tissue comprising a population of stem cells with an effective amount of a metal ion; and (b) exposing the tissue comprising the population of stem cells in step (a) to one or more irradiation, wherein the one or more irradiation are sufficient, individually or collectively, to induce differentiation of stem cells, wherein each dose of irradiation is of a pre-determined period of time resulting in the application of a pre-determined dose of irradiation to the tissue, whereby the irradiation induces the population of stem cells to differentiate.

[0024] In one embodiment, provided herein is a method of inducing differentiation of stem cells, the method comprising exposing a population of stem cells or a tissue comprising a population of stem cells to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of 780 nm to 840 nm ranges, and provides an irradiation dosage ranging

from 1 to 10 joule per cm^2 within a period of time of between 2 - 8 minutes, whereby the irradiation induces the population of stem cells to differentiate.

[0025] In another embodiment, provided herein is a method of inducing dentinogenesis in a subject, the method comprising exposing a tissue comprising a population of dental pulp stem cells to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from 1 to 10 joule per cm^2 within a period of time of between 2 - 8 minutes.

[0026] In one embodiment, provided herein is a method of dental pulp capping in a subject, the method comprising exposing a tissue comprising an exposed dentine pulp to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from 1 to 10 joule per cm^2 for a period of time of between 2 - 8 minutes.

[0027] In one embodiment of any of these methods described, the tissues are exposed to more than one irradiation dose.

[0028] In one embodiment of any of these methods described, the irradiation is laser irradiation.

[0029] In one preferred embodiment of any of these methods described, the dosage of laser irradiation is 3 joule per cm^2.

[0030] In one preferred embodiment of any of these methods described, the period of time is about 5 minutes.

[0031] In one preferred embodiment of any of these methods described, the laser irradiation has a wavelength is 810 nm.

[0032] In one embodiment of any of these methods described herein further comprising contacting the population of stem cells, the exposed dentine pulp of a tooth, or the population of odontoblasts with metal ions prior to exposure to the laser irradiation.

[0033] In one embodiment of any of these methods described, the metal ion is a divalent metal ion.

[0034] In one embodiment of any of these methods described, the divalent metal is selected from a group consisting of lithium, barium, magnesium, copper, iron, manganese, and zinc.

[0035] In one embodiment of any of these methods described herein further comprising contacting the population of stem cells, the exposed dentine pulp of a tooth, or the population of odontoblasts with at least one agent that activates or up-regulates the Wnt pathway. In one embodiment, the contacting is prior to exposure to the laser irradiation. In another embodiment, the contacting is after exposure to the laser irradiation. In the embodiments where multiple irradiation doses are to be applied, the contacting can take place in between the several irradiation doses.

[0036] In one embodiment, the "inside-out" induction of dentine formation in a tooth by the laser-based methods and/or treatment systems described herein served to reduce the sensitivity of the tooth. Accordingly, the treatment systems and methods described herein can be used for tooth desensitization.

[0037] In another embodiment, the "inside-out" induction of dentine formation in a tooth by the laser-based methods and/or treatment systems described herein served to reduce the sensitivity of the dentine of the tooth. Accordingly, the treatment systems and methods described herein can be used for dentine desensitization.

Definitions

[0038] As used herein, the term "stem cell" refers to a cell that has the ability to self-renew, i.e., to go through numerous cycles of cell division while maintaining the undifferentiated state, and has potency, i. e. the capacity to differentiate into specialized cell types, e.g. a nerve cell or a skin cell.

[0039] In one embodiment, the term "stem cell" as used herein, refers to an undifferentiated cell that is capable of proliferation and giving rise to more progenitor cells having the ability to generate a large number of mother cells that can in turn give rise to differentiated, or differentiable daughter cells. The ability to differentiate is the potential to develop into other cell types. A totipotent stem cell (e.g. fertilized egg) can develop into all cell types including the embryonic membranes. A pluripotent stem cell can develop into cells from all three germinal layers (e.g, cells from the inner cell mass). Other cells can be oligopotent, bipotent or unipotent (e.g. mast cell precursor and sperm stem cells) depending on their ability to

develop into few, two or one other cell type(s). The daughter cells themselves can be induced to proliferate and produce progeny that subsequently differentiate into one or more mature cell types, while also retaining one or more cells with parental developmental potential. In one embodiment, the term "stem cell" refers to a subset of progenitors that have the capacity or potential, under particular circumstances, to differentiate to a more specialized or differentiated phenotype, and which retains the capacity, under certain circumstances, to proliferate without substantially differentiating. In one embodiment, the term "stem cell" refers generally to a naturally occurring mother cell whose descendants (progeny) specialize, often in different directions, by differentiation, e.g., by acquiring completely individual characters, as occurs in progressive diversification of embryonic cells and tissues. Cellular differentiation is a complex process typically occurring through many cell divisions. A differentiated cell may derive from a multipotent cell which itself is derived from a multipotent cell, and so on. While each of these multipotent cells may be considered stem cells, the range of cell types each can give rise to may vary considerably. Some differentiated cells also have the capacity to give rise to cells of greater developmental potential. Such capacity may be natural or may be induced artificially upon treatment with various factors. In many biological instances, stem cells are also "multipotent" because they can produce progeny of more than one distinct cell type, but this is not required for "stem-ness."

[0040] The term "embryonic stem cell" is used to refer to the pluripotent stem cells of the inner cell mass of the embryonic blastocyst (see U.S. Patent Nos. 5,843,780; 6,200,806). Such cells can similarly be obtained from the inner cell mass of blastocysts derived from somatic cell nuclear transfer (see, for example, U.S. Patent Nos. 5,945,577; 5,994,619; 6,235,970). The distinguishing characteristics of an embryonic stem cell define an embryonic stem cell phenotype. Accordingly, a cell has the phenotype of an embryonic stem cell if it possesses one or more of the unique characteristics of an embryonic stem cell such that cell can be distinguished from other cells. Exemplary distinguishing embryonic stem cell characteristics include, without limitation, gene expression profile, proliferative capacity, differentiation capacity, karyotype, responsiveness to particular culture conditions, and the like.

[0041] The term "adult stem cell" is used to refer to any multipotent stem cell derived from non-embryonic tissue, including fetal, juvenile, and adult tissue. Stem cells have been isolated from a wide variety of adult tissues including blood, bone marrow, brain, olfactory epithelium, skin, pancreas, skeletal muscle, teeth and cardiac muscle. Each of these stem cells can be characterized based on gene expression, factor responsiveness, and morphology in

culture. Exemplary adult stem cells include liver stem cells, hair follicle stem cells, neural stem cells, neural crest stem cells, mesenchymal stem cells, hematopoietic stem cells, and pancreatic stem cells. As indicated above, stem cells have been found resident in virtually every tissue. "Adult stem cell" and "somatic stem cells" are used interchangeably.

[0042] As used herein, the term "mesenchymal stem cell" or abbreviated as "MSC" refers to a generalized cell that has pluripotency (descendants can specialize into different cell types), for example, an undifferentiated MSC that is capable of differentiating into more than one specific type of mesoderm-derived cells and regenerating into various tissues *in vivo*. Such cell also has unlimited proliferating and self-renewal capability and can differentiate into osteogenic, myogenic, adipogenic or chondrogenic, neurogenic, hepatogenic, nephrogenic, urogenic, isletogenic, pancreatogenic, gastroenterogenic, epitheliogenic, thyroidogenic, myocardiogenic, pneumogenic, retinogenic, gametogenic, endotheliogenic, or hematopoietic lineages. In one embodiment, the MSCs can be substantially positive for any one of the following cell surface markers CD73 (SH3), CD105 (SH2), CD44, CD29, CD 90, CD13, CD10, CD71, CD49d, CD49e, and/or HLA Class I (A, B, and C). Additionally, the MSCs are substantially negative for for any one of the following cell surface markers CD8, CD14, CD19, CD31, CD34, CD45, CD56, CD133, and/or HLA-DR).

[0043] The term "pluripotent" as used herein refers to a cell with the capacity, under different conditions, to differentiate to more than one differentiated cell type, and preferably to differentiate to cell types characteristic of all three germ cell layers. Pluripotent cells are characterized primarily by their ability to differentiate to more than one cell type, preferably to all three germ layers, using, for example, a nude mouse teratoma formation assay. Pluripotency is also evidenced by the expression of embryonic stem (ES) cell markers, although the preferred test for pluripotency is the demonstration of the capacity to differentiate into cells of each of the three germ layers. It should be noted that simply culturing such cells does not, on its own, render them pluripotent. Reprogrammed pluripotent cells also have the characteristic of the capacity of extended passaging without loss of growth potential, relative to primary cell parents, which generally have capacity for only a limited number of divisions in culture.

[0044] The term "multipotent" when used in reference to a "multipotent cell" refers to a cell that is able to differentiate into some but not all of the cells derived from all three germ layers. Thus, a multipotent cell is a partially differentiated cell. Multipotent cells are well known in the art, and examples of multipotent cells include adult somatic stem cells, such as for example, hematopoietic stem cells and neural stem cells, hair follicle stem cells, liver stem cells

etc. Multipotent means a stem cell can form many types of cells in a given lineage, but not cells of other lineages. For example, a multipotent blood stem cell can form the many different types of blood cells (red, white, platelets, etc..), but it cannot form neurons; cardiovascular progenitor cell (MICP) differentiation into specific mature cardiac, pacemaker, smooth muscle, and endothelial cell types; pancreas-derived multipotent progenitor (PMP) colonies produce cell types of pancreatic lineage (cells that produces insulin, glucagon, amylase or somatostatin) and neural lineage (cells that are morphologically neuron-like, astrocytes-like or oligodendrocyte-like).

[0045] The term "progenitor" cell are used herein refers to cells that have a cellular phenotype that is more primitive (i.e., is at an earlier step along a developmental pathway or progression than is a fully differentiated or terminally differentiated cell) relative to a cell which it can give rise to by differentiation. Often, progenitor cells also have significant or very high proliferative potential. Progenitor cells can give rise to multiple distinct differentiated cell types or to a single differentiated cell type, depending on the developmental pathway and on the environment in which the cells develop and differentiate. Progenitor cells give rise to precursor cells of specific determine lineage, for example, certain lung progenitor cells divide to give pulmonary epithelial lineage precursor cells. These precursor cells divide and give rise to many cells that terminally differentiate to pulmonary epithelial cells.

[0046] The term “precursor" cell are used herein refers to cells that have a cellular phenotype that is more primitive than a terminally differentiated cell but is less primitive than a stem cell or progenitor cells that is along its same developmental pathway. A “precursor" cell is typically progeny cells of a "progenitor" cell which are some of the daughter of “stem cells.” One of the daughters in a typical asymmetrical cell division assumes the role of the stem cell.

[0047] As used herein, the term “dental pulp stem cell”, “pulp cell”, “dental stem cell” refers to the mesenchymal or neural crest derived stem cell inside dental pulp. In some embodiments, “dental pulp stem cell” has the potential to differentiate into a variety of other cell types including: cardiomyocytes, neurons, myocytes, osteocytes, chondrocytes, adipocytes, and bone and tissue from the oral cavity. In otee embodiment, “dental pulp stem cell” is a mesenchymal stem cell.

[0048] In the context of cell ontogeny, the term "differentiate", or "differentiating" is a relative term meaning a "differentiated cell" is a cell that has progressed further down the developmental pathway than its precursor cell. For example, a muscle satellite cell is an adult

stem cell that had developed from a mesenchymal stem cell. In other words, the muscle satellite cell has differentiated from a mesenchymal stem cell, its precursor cell.

[0049] The term "differentiated cell" refers to any primary cell that is not, in its native form, pluripotent as that term is defined herein. The term a "differentiated cell" also encompasses cells that are partially differentiated, such as multipotent cells (e.g., adult somatic stem cells). It should be noted that placing many primary cells in culture can lead to some loss of fully differentiated characteristics. Thus, simply culturing such cells are included in the term differentiated cells and do not render these cells non-differentiated cells (e.g., undifferentiated cells) or pluripotent cells. The transition of a differentiated cell to pluripotency requires a reprogramming stimulus beyond the stimuli that lead to partial loss of differentiated character in culture. Reprogrammed cells also have the characteristic of the capacity of extended passaging without loss of growth potential, relative to primary cell parents, which generally have capacity for only a limited number of divisions in culture. In some embodiments, the term "differentiated cell" also refers to a cell of a more specialized cell type derived from a cell of a less specialized cell type (e.g., from an undifferentiated cell or a reprogrammed cell) where the cell has undergone a cellular differentiation process.

[0050] As used herein, the term "differentiation" with respect of "dental pulp stem cell", "pulp cell", "dental stem cell" refers to the dental pulp stem cell taking on the characteristics of an odontoblast, a cell type that is further down the developmental pathway than the original pulp stem cell's lineage. Odontoblasts express alkaline phosphatase, and secretes collagen and dentin matrix.

[0051] The term "lineages" is used herein describes a cell with a common ancestry or cells with a common developmental fate.

[0052] As used herein, the term "dentinogenesis" refers to the formation of dentin, a substance that forms the majority of teeth. Dentinogenesis is performed by odontoblasts.

[0053] As used herein, the term "odontoblast" refers to a specialized cell located in the dental pulp that secretes dentin matrix (organic) which further undergoes mineralization to form tertiary/ reparative dentin or osteodentin which comprises both organic and inorganic materials.

[0054] As used herein, the term "dentin" refers the mineralized substance which makes up the bulk of teeth that is found immediately beneath the enamel. It is a dense matrix of minerals, primarily calcium, and collagen. It serves to protect the sensitive pulp of the tooth and

create a base under the enamel, or outer coating of the tooth. It is found between the pulp and the enamel.

[0055] As used herein, the term “dental capping” refers to covering of an exposed dental pulp with some material to provide protection against external influences and to encourage healing.

[0056] As used herein, the term "comprising" or "comprises" is used in reference to methods, systems and and respective component(s) thereof, that are essential to the invention, yet open to the inclusion of unspecified elements, whether essential or not. The use of “comprising” indicates inclusion rather than limitation.

[0057] As used herein, the term “exposing” in the context of irradiation refers to directing or pointing the emitted irradiation at a target, e.g., a tissue comprising a population of stem cells, dental pulp cells or exposed dental pulp, i.e. shining the emitted irradiation upon the target which can be a population of stem cells, a population of dental pulp cells or an exposed dental pulp. In one embodiment, “exposing” refers to directing or pointing the emitted irradiation at a carious tooth or a dental caries.

[0058] The term “contacting” or “contact” as used herein as in connection with contacting a population of stem cells, dental pulp cells or exposed dental pulp with a composition comprising a metal ion as disclosed herein, includes subjecting the cells or expose pulp to a culture media or solution which comprises the metal ion and/or at least one agent that activates or up-regulates the Wnt pathway. In one embodiment, the “contacting” encompass rinsing the population of stem cells, dental pulp cells or exposed dental pulp with a solution comprising a metal ion. In another embodiment, the “contacting” encompass apply a gel composition comprising a metal ion on the population of stem cells, dental pulp cells or exposed dental pulp.

[0059] The term "isolated" as used herein signifies that the cells are placed into conditions other than their natural environment. The term "isolated" does not preclude the later use of these cells thereafter in combinations or mixtures with other cells.

[0060] As used herein, the term “expanding” refers to increasing the number of like cells through cell division (mitosis). The term “proliferating” and “expanding” are used interchangeably.

[0061] As used herein, the terms "tissue regeneration", "tissue engineering" and "regenerative medicine" are related terms and used interchangeably.

[0062] As used herein, the word "repair" means the replacement of worn, torn, lost or broken components with newly synthesized components. The word "healing", as used herein, means the returning of torn and broken organs and tissues (wounds) to wholeness. For example, an open wound on the skin or oral gums can be repaired with the laser irradiation and/or with the engineered tissues comprising stem cells that were previously irradiated to induce the differentiation of the stem cells. It is envisioned healing would be the eventual closing of the open wound with new growth of skin and underlying connective tissues.

[0063] As used herein, the adjective "reparative" means related to or of the nature of repairing. As used herein, the terms "repairative" and "reparative" are used interchangeably.

BRIEF DESCRIPTION OF THE DRAWINGS

[0064] Figure 1A shows the microcomputed tomographic imaging quantification for dentin induction following pulp exposure and control (cement alone) or Laser (LPL irradiation, 3J/cm2, and cement filling) at 12weeks. Red lines indicate mean in each group, n=7/8, * indicates $p<0.05$.

[0065] Figure 1B shows the quantification for non-decalcified sections evaluated for their mineral content by SEM-EDS.

[0066] Figure 2A shows the quantification of superoxide generation by LPL irradiation ($3J/cm^2$) of serum, n=3, * indicates $p<0.05$.

[0067] Figure 2B shows the hydrogen peroxide generation assessed with AMPLEX® ULTRARED following dose dependent LPL irradiation of serum. In one condition, samples were pre-incubated with NAC, n=3, * indicates $p<0.05$.

[0068] Figure 2C shows the LPL ($3J/cm^2$) generated hydroxyl radicals in serum assessed with proxylhydroxylamine, n=3, *indicates $p<0.05$.

[0069] Figure 2D shows the LPL irradiation ($3J/cm^2$) of serum diluted in either water or deuterium assessed with AMPLEX® ULTRARED. In one condition, samples were pre-incubated with NAC prior to LPL irradiation, n=3,* indicates $p<0.05$.

[0070] Figure 2E shows LPL irradiation (3J/cm^2) of Chelex 100 resin-treated serum assessed with AMPLEX® ULTRARED to detect hydrogen peroxide generation, n=3,* indicates $p<0.05$.

[0071] Figure 2F shows that low power laser (LPL) irradiation induces the ROS species superoxide (O), hydrogen peroxide (H_2O_2) and hydroxyl ions (OH-).

[0072] Figure 2G shows that LPL irradiation can induce hydrogen peroxide (H_2O_2) in a linear dose-dependent manner implying a non-limiting biophysical ROS induction reaction by LPL.

[0073] Figure 3A shows the experimental outline of free cysteines screen.

[0074] Figure 3B shows the assessment of free cysteines in serum and recombinant latent TGF-β1 following LPL irradiation (3J/cm^2), n=3, * indicates $p<0.05$.Latent TGF-β1 following LPL irradiation (3J/cm^2), n=3, * indicates $p<0.05$.

[0075] Figure 3C shows the gel electrophoresis and visualization with UV of IAEDANs labeled serum complexes following LPL irradiation, panel on right shows same samples subjected to immunoblotting for TGF-β.

[0076] Figure 3D shows the TGF-β1 activation assessed by ELISA following increasing fluence of LPL irradiation, n=3, *indicates $p<0.05$. Fluence (J/cm^2) was varied by changing irradiance (W/cm^2) while keeping the distance and time constant.

[0077] Figure 3E shows the biological activity of LPL activated TGF-β1 using a p3TP-lucifease reporter assay in Mv1Lu cells, n=3, * indicates $p<0.05$.

[0078] Figure 3F shows the TGF-β1 activation by LPL irradiation (3J/cm^2) and distinct ROS assessed with an ELISA, n=3, * indicates $p<0.05$.

[0079] Figure 3G shows the experimental outline for latent TGF-β1 constructs in MEF conditioned media.

[0080] Figure 3H shows the immunoblots to assess TGF-β signal transduction following LPL irradiation (3J/cm^2) of wild type and m253a mutant latent TGF-β1 MEFs-conditioned media.

[0081] Figure 3I shows the ability of laser to produce conformational change in associated proteins in serum samples. Conformational changes were determined by circular dichorism. Chemical and peroxide treatments are used were used as positive controls.

[0082] Figure 3J shows that LPL at various doses induces hydrogen peroxide (H_2O_2) specifically as demonstrated with AMPLEX® ULTRARED assay. The use of a ROS scavenger, N-acetylcysteine (NAC) results in decreased fluorescence.

[0083] Figure 3K shows that hydrogen peroxide (H_2O_2) generation following LPL in a dose dependent manner in MLEC using CM-DCFDA and MITOTRACKER RED™ to stain mitochondria. Fluorescence is seen as various shades of grey.

[0084] Figure 4A shows the immunoblot to assess TGF-β signal transduction following LPL irradiation ($3J/cm^2$) of human dental stem cells (hDSCs).

[0085] Figure 4B shows the immunoblot for stem cell markers CD44, CD106 and CD117 in hDSCs following LPL irradiation ($3J/cm^2$) or TGF-β1 treatment at 7 days.

[0086] Figure 4C shows the dentin matrix expression following LPL irradiation of hDSC at 7 days.

[0087] Figure 5A shows the immunoblot indicating LPL activated TGF-β1 responsiveness with phospho-smad2 in a pre-odontoblast cell line, MDPC23.

[0088] Figure 5B shows the specificity of LPL activated TGF-β activation evaluated with TGF-βRI and Smad3 inhibitors by immunoblotting.

[0089] Figure 5C shows the assessment of mineralized phenotype with alkaline phosphatase (ALP) assay following of dose dependent LPL irradiation of MDPC23 at 7days, n=3, * indicates $p<0.05$.

[0090] Figure 5D shows the role of ROS and TGF-β1 in mediating LPL-induced alkaline phosphatase (ALP) activity, n=3, * indicates $p<0.05$.

[0091] Figure 5E shows the quantification of luciferase reporter cell line, Mv1Lu, in 3D scaffolds following LPL irradiation ($3J/cm^2$) or TGF-β1 treatment at 24 hours assayed with a microplate reader, n=3,* indicates $p<0.05$.

[0092] Figure 5F shows the immunoblots for dentin matrix markers following LPL ($3J/cm^2$) irradiated MDPC-23 cells seeded in PLG scaffolds at 21days.

[0093] Figure 5G shows the LPL-induced mineralization assessed by alkaline phosphatase (ALP) activity of cells above, n=3, *indicates $p<0.05$.

[0094] Figure 5H shows the mineral content in the scaffolds assessed by SEM-EDS.

[0095] Figure 5I shows a histogram of ALP activity of cells LPL irradiated in the presence of increasing amount of serum. MDPC-23 cells were cultured as in Fig. 5F and the ALP was assayed as in Fig. 5C.

[0096] Figure 5J shows increased mineral deposition following laser irradiation in the scaffolds with MDPC-23 cells. Mineral deposition was assessed for calcium phosphate deposition by SEM-EDS.

[0097] Figure 6A shows the experimental design for rat experiments to investigate whether TGF-β mediates LPL-induced dentin *in vivo* differentiation and repair.

[0098] Figure 6B shows the immunoblots for dental stem cell markers following LPL irradiation ($3J/cm^2$) of exposed pulp after 7 days. The right panel shows LPL irradiation in the presence of TGF-βRII inhibitor.

[0099] Figure 6C shows the quantitation of dentin volume in above group in Fig. 6A and 6B by microcomputed tomorgraphy. The horizontal bars in graph indicate mean in each group, n=4, $p>0.05$.

[0100] Figure 6D shows the scheme of conditional knockout ($DSPP^{Cre}TGF\text{-}\beta RII^{f/f}$) generation for targeted deletion of TGF-β responsiveness of pulp-dentin cells.

[0101] Figure 6E shows the dentin volume estimated by uCT following LPL irradiation ($3J/cm^2$) in coKO mice ($DSPP^{Cre}TGF\text{-}\beta RII^{f/f}$) above, red line indicates mean, n=4/3, $p>0.05$.

[0102] Figure 7 shows a schematic depicting LPL generated ROS that, in turn, activates latent TGF-β1. The activated TGF-β1 induces relevant cells of the pulp-dentin complex to induce dentin differentiation. The laser dose directly modulates ROS generation that could be within a low therapeutic regimen, photobiomodulation (PBM), or higher destructive, photodynamic therapy (PDT) regimen.

[0103] Figure 8 shows the Raman signatures of mineralized tissues enamel, dentin, cementum and bone with specific peaks representing compositional elements are highlighted.

[0104] Figure 9A shows that Griess assay did not demonstrate significant nitric oxide generation following LPL irradiation (3J/cm^2) of Mv1Lu and MPC-23 cells, n=3.

[0105] Figure 9B shows the AMPLEX® ULTRARED observed a progressive increase in hydrogen peroxide generation following dose dependent LPL irradiation of serum, n=3, * indicates $p<0.05$. Fluence (J/cm^2) was varied by changing irradiance (W/cm^2) while keeping the distance and time constant.

[0106] Figure 10A shows that increased free cysteines were observed in serum following dose dependent LPL irradiation (3J/cm^2) with the IAEDAN assay, * indicates $p<0.05$.

[0107] Figure 10B shows that LPL irradiation (3J/cm^2) of serum in deuterium showed increased free cysteines as compared to dilution in water, *indicates $p<0.05$.

[0108] Figure 10C shows that hydrogen peroxide (100uM) treatment of Chelex 100 resin-treated serum showed decreased free cysteines, * indicates $p<0.05$.

[0109] Figure 10D shows that LPL irradiation (3J/cm^2) of Chelex 100 resin-treated serum showed decreased free cysteines, * indicates $p<0.05$.

[0110] Figure 10E shows that the activation of recombinant latent TGF-β1 was observed following various doses of LPL irradiation assessed with an ELISA, * indicates $p<0.05$.

[0111] Figure 10F shows that conditioned media (CM) from wild type (WT) and ROS-insensitive (m253a) latent TGF-β1 transfected MEFs were both activatable by routine chemical treatments (1N HCl followed by 1.2N NaOH with 0.5M HEPES) but m253a CM did not demonstrate increased levels after peroxide treatment (1mM) as seen with WT CM assessed using a TGF-β1 ELISA, n=3, * indicates $p<0.05$.

[0112] Figure 10G shows the immunoblot following hydrogen peroxide treatment (100 μM) showed reduced phospho-Smad2 levels in m253a latent TGF-β1 compared to WT constructs transfected TGF-$\beta^{+/+}$ and TGF-$\beta1^{-/-}$ MEFs.

[0113] Figure 10H shows that decreased phospho-Smad2 levels were observed following LPL irradiation (3J/cm^2) in m253a latent TGF-β1 compared to WT in TGF-$\beta1^{-/-}$ MEFs. An

increased baseline levels were observed in the m253a conditioned media that might be due to enhanced autocrine feedback of disrupted signaling.

[0114] Figure 11A shows the immunoblots of stem cell surface markers of isolated hDSC; stem cell surface markers decreased over prolonged passages. For comparison, osteoblast (7F2), pre-odontoblast (MDPC-23) and a mouse mesenchymal stem cell (D1) line were assessed for these markers. Cells in culture at passage 3 had either an Epitheloid (Ep3) or Fibroblastoid (Fp3) but were all stellate-fibroblastoid by later passages, noted here as EPp14. All cells were used in experiments in passage 3-7.

[0115] Figure 11B shows the immunoblots of CD106 expression in hDSC, mMSC and hMSCs demonstrated decreased expression after LPL irradiation (3J/cm2) and TGF-β1 treatment (2.5 ng/ml) at 7days. Incubation with NAC (1mM), SB431542 (10 μM) or Smad3 inhibitor (3 μM) prior to LPL irradiation were able to partially restore CD106 expression, CD44 and CD117 did not show a similar pattern of regulation.

[0116] Figure 12A shows that TGF-β1 (2.5 ng/ml) or hydrogen peroxide (100 μM) treatment increased alkaline phosphatase (ALP) activity in MDPC-23 cells at 3 days, * indicates $p<0.05$.

[0117] Figure 12B shows that TGF-β1 induced calcium deposition in MDPC-23 as assessed by alizarin red staining at 3 days; lower panels are low power histological images.

[0118] Figure 12C shows Alizarin Red staining of MDPC23 cells following dose dependent LPL irradiation ($3J/cm^2$) demonstrated increased calcium deposition at 3 days; wells are shown in duplicates.

[0119] Figure 12D shows the quantitation of calcium deposition in these cells, calcium deposition was assessed by Arsenazo dye. LPL irradiation ($3J/cm^2$) demonstrated increased calcium deposition that was inhibited with prior incubation with NAC or TGF-βRI inhibitor, * indicated $p<0.05$.

[0120] Figure 12E shows that TGF-β1 (2.5ng/ml) or hydrogen peroxide (100 μM) treatment of MDPC-23 cells in 3D scaffolds increased DMP-1 expression as determined by immunoblotting at 21days. Right panels show the densitometeric quantitation of immunoblots normalized over actin.

[0121] Figure 12F shows the MDPC-23 cells induce alkaline phosphatase(ALP) activity on treatment with TGF-β1 (2.5ng/ml) and hydrogen peroxide (100 μM) in 3D PLG scaffolds at 21days,* indicates $p<0.05$.

[0122] Figure 12G shows the quantitation of mineral content (calcium, phosphate, oxygen and carbon composition) in PLG scaffolds with media alone or with mineralization supplements at 21days determined by SEM-EDS.

[0123] Figure 12H shows the LPL irradiation ($3J/cm^2$) induced alkaline phosphatase in MDPC-23 cells in 3DPLG scaffolds in the presence of increasing serum concentrations during LPL irradiation representing the increasing serum latent TGF-β1 available for LPL activation, * indicated $p<0.05$.

[0124] Figure 12I shows the LPL irradiation ($3J/cm^2$) increased alkaline phosphatase activity in a dose dependent manner at 21days, * indicated $p<0.05$.

[0125] Figure 12J shows the immunoblotting of MDPC-23 seeded PLG scaffolds at 21days demonstrated the ability of LPL irradiation ($3J/cm^2$) induction of dentin specific ECM that is prevented by inhibitors for ROS and TGF-β1.

[0126] Figure 12K shows the show densitometeric quantitation of immunoblots for individual marker expression normalized over actin.

[0127] Figure 13A shows the release kinetics of TGF-βRI inhibitor from PLG microspheres by LC-MS showed a controlled temporal release over 7days, n=4. Inset shows standard curve used to estimate concentrations.

[0128] Figure 13B shows the stem cell marker expression in dental pulp following implantation of TGF-β1 microspheres showed a down regulation at 7days while use of TGF-β neutralizing antibody (1D11 antibody, R&D systems) prior to LPL irradiation ($3J/cm^2$) was able maintain these marker expression to some extent.

[0129] Figure 13C shows the densitometeric quantitation of immunoblots for individual marker expression normalized over actin.

[0130] Figure 13D shows schematic outline of transgenic mice experiments for reparative dentin.

[0131] Figure 13E shows the increased dentin volume was noted at 8weeks in TGF-βRIIf/f mice following LPL ($3J/cm^2$) irradiation, red line indicates mean, n=2.

[0132] Figure 14 shows a diagrammatic view of a dental treatment system according to one embodiment of the invention.

[0133] Figure 15 shows a diagrammatic view of a handheld dental treatment device according to one embodiment of the invention.

[0134] Figure 16 is a histogram showing the effect of transition metals in mediating LTGF-β activation by laser irradiation ($3J/cm^2$ for 300 seconds). ROS generation in the presence of regular serum or chelexed serum supplemented with lithium, magnesium, barium or all three transition metal salts were assayed by the Amplex assay. Chelexing serum (removal of transition metals) resulted in lack of LTGF-β by both lasers and hydrogen peroxide (ROS source).

DETAILED DESCRIPTION OF THE INVENTION

[0135] Embodiments of the present invention are based on the observation that low power laser (LPL) irradiation can induce stem cells to differentiate. The inventors demonstrated that an infrared (810 nm) low power laser can direct odontgenic differentiation of human dental stem cells; the differentiation was verified by activation of intracellular signaling, extracellular matrix (ECM) production and calcium deposition. Furthermore, the inventors observed that the LPL induces multiple ROS species that, in turn, is capable of activating the latent transforming growth factor β (LTGF-β) complex as a key signaling mediator, e.g., in oral wound healing. These observations indicate the utility of LPL as an alternative, non-invasive clinical tool in dental applications, e.g., dental pulp capping, and also for stem cell differentiation and related applications, e.g., in oral wound healing, general wound healing, healing of bone fractures or bone fusions, and healing of injured nerves and muscles.

[0136] Accordingly, in one embodiment, provided herein is a dental treatment system for inducing dentinogenesis, the system comprising: a source of irradiation, the source being adapted to produce irradiation having a wavelength in the range of 780 nm to 910 nm and a power output in the range of 5 mW to 300 mW; a control element controlling the source of irradiation to turn the source of laser irradiation on and to turn the source of laser irradiation off; and a timer operatively connected to the control element to activate the source of irradiation to produce irradiation for a pre-determined time period and then deactivate the source of irradiation

from producing radiation, resulting in the application of a pre-determined dose of irradiation to a target.

[0137] In one embodiment, the dental treatment system emits irradiation having a wavelength in the range of 780 nm to 840 nm and a power output in the range of 5 mW to 300 mW.

[0138] In one embodiment, the dental treatment system emits irradiation having a wavelength in the range of 800 nm to 820 nm and a power output in the range of 5 mW to 300 mW.

[0139] In one embodiment, the dental treatment system emits irradiation having a wavelength of 810 nm and a power output in the range of 5 mW to 300 mW.

[0140] In some embodiment, the dental treatment system has a power output in the range of 5 mW to 100 mW, 5 mW to 200 mW, 5 mW to 50 mW, 5 mW to 150 mW, 5 mW to 250 mW, 20 mW to 100 mW, 20 mW to 50 mW, 20 mW to 70 mW, 50 mW to 100 mW, 50 mW to 150 mW, 10 mW to 50 mW, 25 mW to 100 mW, and 40 mW to 80 mW, including all the power output having all whole integers between 5-300 mW.

[0141] In other embodiments, the dental treatment system emits irradiation having a wavelength in the range of 780-880 nm, 780-840 nm, 780-820 nm, 780-810 nm, 800-880 nm, 800-840 nm, 800-860 nm, 800-820 nm, 810-835 nm, 800-830 nm, 800-835 nm, 810-840 nm, 810-830 nm, 810-820 nm, 800-910 nm, 810-910 nm, 840-910nm and 810-835 nm, including all the wavelengths having all whole integers between 780-910.

[0142] In one embodiment of the dental treatment system, the pre-determined time period of irradiation ranges from 2 - 8 minutes. In other embodiments, the period of time ranges from 2-7 minutes, 2-6 minutes, 2-5 minutes, 2-4 minutes, 2-3 minutes, 3-7 minutes, 3-6 minutes, 3-5 minutes, 3-4 minutes, 4-7 minutes, 4-6 minutes, 4-5 minutes, 3-8 minutes, 4-8 minutes, 5-8 minutes, 5-7 minutes, 5-6 minutes, 6-8 minutes, 7-8 minutes, 6-7 minutes, 4.5-6.5 minutes, 2.5-7.5 minutes, 3.5-5.5 minutes and 3.5-6.5 minutes, including all the time to two decimal places between 2-8 minutes.

[0143] In one embodiment of the dental treatment system, the irradiation provides a dosage (energy or fluence) that ranges from 0.3-30 joule per cm^2. In other embodiments, the dosage of laser irradiation ranges from 0.5-25 joule per cm^2, 0.5-10 joule per cm^2, 0.5-5 joule

per cm^2, 1-25 joule per cm^2, 1-10 joule per cm^2, 1-5 joule per cm^2, 1.5-25 joule per cm^2, 1.5-10 joule per cm^2, 1.5-5 joule per cm^2, 2-25 joule per cm^2, 2-10 joule per cm^2, 2-5 joule per cm^2, 2.5-25 joule per cm^2, 2.5-10 joule per cm^2, 2.5-5 joule per cm^2, including all the dosage to one decimal places between 0.3-30.

[0144] In one embodiment, the dental treatment system further comprising a collimator coupled to the source of irradiation and collimating the irradiation produced by the source of irradiation.

[0145] In one embodiment, the dental laser treatment system further comprises a switch element operatively connected to and controlling the timer.

[0146] In one embodiment of any aspect of the dental treatment system, the switch element is a foot operated switch.

[0147] In one embodiment of any aspect of the dental treatment system, the timer controls the source of irradiation to apply a dosage of between 1 – 10 J/cm^2 of irradiation to a target location. In one preferred embodiment, the dosage is 3 J/cm^2.

[0148] In one embodiment of any aspect of the dental treatment system, the source of irradiation produces laser irradiation.

[0149] In one embodiment of any aspect of the dental treatment system, the source of irradiation includes a diode laser.

[0150] In one embodiment of any aspect of the dental treatment system, the source of irradiation includes a laser selected from the group including but is not limited to gas lasers, dye lasers, semiconductor lasers and solid state lasers.

[0151] In one embodiment of any aspect of the dental treatment system, the target is a living organism. In one embodiment, the target is a location on or in the organism, i.e., *in vivo*. In another embodiment, the target comprises living tissues that can respond to the irradiation of the dental treatment or laser system. In another embodiment, the target comprises living tissues that comprises a population of stem cells. In one embodiment, the stem cells can respond to the irradiation of the dental treatment or laser system, e.g., differentiate to a cell that is further along the developmental pathway of that original stem cell. For example, the target is an exposed dental pulp having a population of dental stem cells within. Upon the irradiation of the dental treatment or laser system, the dental stem cells are stimulated to differentiate to odontoblasts. In

another embodiment, respond to the irradiation of the dental treatment or laser system comprises an increase in the reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals. Other non-limiting examples of a target include a wound on the skin or an internal organ, a newly sutured laceration on the skin or a newly sutured part of an internal organ, an oral ulcer, a vertebrae implanted with bone fragments or an engineered tissue or scaffold, a muscle sprain, a severed or injured nerve, and a bone fracture.

[0152] In another embodiment, the target is an *ex vivo* material, such as an engineered tissue or scaffold comprising living viable cells. In one embodiment, the living viable cells are stem cells. In another embodiment, the target is an *ex vivo* collection of a population of stem cells.

[0153] In another embodiment, provided herein is a dental laser system for inducing dentinogenesis in a target, the system comprising: a source of laser irradiation, the source being adapted to produce laser irradiation having a wavelength of 810 nm; a control element controlling operation of the source of irradiation to turn the source of laser irradiation on and to turn the source of laser irradiation off; and a timer operatively connected to the control element to turn the source of laser irradiation on for a pre-determined time period and then turn the source of laser irradiation off and wherein the pre-determined time period is determined to apply a dose of 3 J/cm^2 on the target.

[0154] In one embodiment of the dental laser system for inducing dentinogenesis in a target, the pre-determined time period of laser irradiation ranges from 2 - 8 minutes. In other embodiments, the period of time ranges from 2-7 minutes, 2-6 minutes, 2-5 minutes, 2-4 minutes, 2-3 minutes, 3-7 minutes, 3-6 minutes, 3-5 minutes, 3-4 minutes, 4-7 minutes, 4-6 minutes, 4-5 minutes, 3-8 minutes, 4-8 minutes, 5-8 minutes, 5-7 minutes, 5-6 minutes, 6-8 minutes, 7-8 minutes, 6-7 minutes, 4.5-6.5 minutes, 2.5-7.5 minutes, 3.5-5.5 minutes and 3.5-6.5 minutes, including all the minutes to two decimal places between 2-8 minutes.

[0155] In another embodiment, provided herein is a method of stimulating dentinogenesis in a subject, the method comprising exposing a tissue comprising a population of dental pulp stem cells to an irradiation output from the dental treatment system or dental laser system described herein, whereby the dental pulp stem cells is induced to differentiate to odontoblasts.

[0156] In another embodiment, provided herein is a method of dental pulp capping in a subject, the method comprising exposing a tissue comprising an exposed dental pulp to an

irradiation output from the dental treatment system or dental laser system described herein, whereby increased dentin is secreted.

[0157] Figure 14 shows a diagram of a dental treatment system 600 according to one embodiment of the present invention. The system 600 can include an irradiation source 610, a controller 620, a timer 630 and a switch 640. In some embodiments, the system 600 can also include a collimator 612 that collimates the beam 614 of irradiation produced by the irradiation source 610 and applied to the target tissue, in this example, tooth 604. The system 600 can be connected to a power supply, not shown, that can provide electricity to the irradiation source 610, the controller 620, the timer 630 and the switch 640 to support operation of each of the components. In addition, a wave guide and/or a reflector, not shown, can be provided to further direct or focus the irradiation produced by the irradiation source 610 on the target 604.

[0158] The irradiation source 610 can include any component, device or system that can produce a beam of irradiation at the desired wavelength and power level or profile. For example, the output power can be continuous or pulsed at a desired frequency. In accordance with some embodiments of the present invention, the wavelength of the irradiation can be in the range from 780 nm to 840 nm and the output power of the irradiation can be in the range from approximately 1 mW to 300 mW. In other embodiments, the wavelength can range from 800 nm to 820 nm and the output power can be in the range of 5 to 20 mW. In other embodiments, the wavelength can be fixed to 810 nm and the output power can be fixed to 10mW. In accordance with some embodiments of the present invention, the output power of the irradiation can be controlled or limited to control the dosage to a range, for example, from 1 J/cm^2 to 5 J/cm^2. In other embodiments, the dosage can range, for example, from 2 J/cm^2 to 4 J/cm^2. In some embodiments, the dosage can be fixed to 3 J/cm^2. As one of ordinary skill will appreciate, the applied dosage can be determined as a function of the output power of the irradiation source 610, the time duration of the exposure and the area of the beam cross-section applied to the target. Thus, the combination of output power, time duration of exposure and beam area can be selected to provide the desired treatment, with many combinations available to apply the same treatment dosage. In some embodiments of the invention, the power output can be set to about 10 mW and the time duration of the treatment limited to less than 7 minutes. In accordance with one embodiment of the dental treatment system, the irradiation source can be a 300 mW diode laser, limited to 10 mW output power, producing a beam of irradiation at a wavelength of 810 nm for a maximum of 5 minutes. In accordance with one embodiment of the dental treatment

system, the power level of the beam of irradiation can be varied according to the predefined profile during the course of the treatment period.

[0159] In some embodiments of the dental treatment system, the irradiation source 610 can include a light bulb coupled to an appropriate filter to produce the desired wavelength. The light bulb can be coupled to an appropriate reflector, lens system and/or wave guide to concentrate the irradiation on a target area. The reflector can be designed to reflect the light to form a beam having a predefined cross-sectional area to define an area on a surface to be treated. The wave guide can be a fiber optic cable, a bundle of fiber optic cable, an optically transparent tube or similar component that directs the irradiation received from the source along a path to the target. Further, a lens or group of lenses can be used to control the cross-section and intensity of the beam 614. In other embodiments, a fixed or adjustable diaphragm can be used to set the cross-sectional area of the beam 614. The irradiation source 610 can also be coupled to a collimator 612 that collimate the beam 614.

[0160] In some embodiments of the dental treatment system, the irradiation source 610 can include a laser or a source of laser irradiation. The source of laser irradiation can include a solid state laser, a semiconductor laser, such as a diode laser, a gas laser, or a dye laser. In one embodiment of the invention, the source of laser irradiation can include an aluminum gallium arsenide (also gallium aluminum arsenide) semiconductor diode laser.

[0161] The controller 620 can be connected between the irradiation source and the power source and include any device, component or set of components that can be used to control the operation of the irradiation source 610. The controller 620 can control the flow of power to the irradiation source 610 to activate or "turn on" the irradiation source 610 causing it to produce irradiation at a defined wavelength and output power, and deactivate or "turn off" the irradiation source, so it no longer emits irradiation. The controller 620 can include a switch or relay that controls the flow of electricity to the irradiation source 610. The controller 620 can include a voltage regulator that regulates the voltage of the electricity sent to the irradiation source 610. Further, the controller 620 can include circuitry that allows the voltage or other signal (such as a control signal) input to the irradiation source 610 to be adjusted over a range to increase or decrease the power level of the irradiation produced by the irradiation source 610.

[0162] The timer 630 can be connected to the controller 620 and include any electrical or electro-mechanical device that can start and stop the controller 620 and/or the irradiation source 610 according to a predefined or programmable period of time. The timer 630 can include a

real-time clock circuit that activates or closes a switch or a relay to send electricity to the controller 620 and/or the irradiation source 610 to produce a beam 614 of irradiation and, after a pre-determined time period, deactivates or opens the switch or relay to stop the flow of electricity to the controller 620 and/or the irradiation source 610 to extinguish the beam 614 of irradiation. The timer 630 can further include circuitry that allows the time period to be adjusted over a range to increase or decrease the dosage of irradiation applied to the target tissue 604.

[0163] The switch 640 can be connected to the timer 630 to enable a user to control the operation of the timer 630 and initiate or stop the application of a predefined dosage of irradiation. In accordance with one embodiment, the switch 640 can be configured to initiate the application of a treatment dosage of irradiation upon being pressed. Further, the switch can be configure such that if the switch is pressed before the expiration of the timer and application of the pre-determined treatment dose of irradiation, power to controller 620 and/or the irradiation source 610 can be cut off, for example, for safety reasons. In an alternative embodiment, the user can be required to hold the switch closed (pressed) during the duration of the treatment and power is turned off, if the switch is released before the expiration of the timer. In this embodiment, the irradiation source 610 is turned off at the expiration of the timer even though the switch continues to be pressed. Further, the switch can be configured such that a new dose of irradiation cannot be applied until the switch has been released for a predefined period of time.

[0164] In one embodiment, the switch element is a foot operated switch. The foot switch provides a "hands-free" approach for the operator using the laser treatment system, e.g., the operator is a dentist.

[0165] Figure 15 shows diagram of a handheld device 700 in accordance with one embodiment of the invention. The device 700 can be embodied in a handle 702 that encloses some or all of the components of the device 700. The device 700 can include an irradiation source 710, an irradiation guide 716, a controller 720, a timer 730, a switch 740 and a power supply 750. The irradiation source 710, the controller 720, the timer 730 and optionally, the switch 740 can coupled together, for example, on a circuit board and mounted inside the elongated handle 702. The irradiation guide 716 can be coupled to the irradiation source 710 and extend from a first end of the handle 702. The irradiation guide 716 can include a collimator (not shown). At the opposite end of the handle 702, a power cord 752 can extend to the power supply 750. In some embodiments of the invention, the handheld device 700 can be battery operated and recharged between treatments as necessary.

[0166] In accordance with one embodiment of the dental treatment system, the system 600 or device 700 can be preconfigured to apply a predefined dose of irradiation, for example, 3 J/cm^2 of 810 nm irradiation at a predefined distance from the irradiation source or irradiation guide, when the switch is pressed. Further, the system 600 or device 700 can include removable modular component that can be easily removed to configure the performance of the device. For example, different irradiation source 610, 710 modules can be provided to enable treatments using different wavelengths of light, different controller 620, 720 modules can be provided to enable treatments using different irradiation power levels, and different timer 630, 730 can be provided to enable treatments using different treatment time periods. Thus, different treatment dosages can be providing by selecting the appropriate controller and timer modules.

[0167] In an alternative embodiment of the dental treatment system, the system 600 or device 700 can include a computer or micro controller and associated memory that includes software to control the dosage, power and/or time parameters to enable a wide range of irradiation dosages to be applied. In this embodiment, a display, such as an LED readout or LCD display can be included to enable the user to interact with the system 600 or device 700 user interface to select the dosage, power and/or time parameters in order to provide the desired treatment.

[0168] In one embodiment, provided herein is a method for inducing and/or promoting stem cell differentiation, the method comprising: (a) contacting a population of stem cells or a tissue comprising a population of stem cells with an effective amount of at least one metal ion or salt; and (b) exposing the population of stem cells or tissue in step (a) to one or more irradiation, wherein the one or more irradiation are sufficient, individually or collectively, to induce differentiation of the stem cells, wherein each exposure of irradiation is for a pre-determined period of time resulting in the application of a pre-determined dose of irradiation to the tissue or population of stem cells, whereby the irradiation induces the population of stem cells to differentiate. Stem cells that have been induced to differentiate become cells that are further along the developmental pathway of that original stem cell. Such differentiated cells have phenotypes different from that of their original stem cells, e.g., in the expression of proteins such as cell surface markers. Methods of identifying cell surface markers associated with differentiated cell types and/or stem cells are well known in the art and are described in the stem cell section.

[0169] In one embodiment of the method for inducing and/or promoting stem cell differentiation, the pre-determined dose of irradiation is about 0.3 -30 J/cm^2.

[0170] In one embodiment of the method for inducing and/or promoting stem cell differentiation, the pre-determined period of time is about 2-8 minutes.

[0171] In one embodiment of the method for inducing and/or promoting stem cell differentiation, the irradiation has a wavelength of about 780-910 nm.

[0172] In one embodiment, provided herein is a method of inducing differentiation of stem cells, the method comprising exposing a population of stem cells to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of about 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from about 1 to 10 joule per cm^2 within a period of time of between about 2 - 8 minutes, whereby the irradiation induces the population of stem cells to differentiate.

[0173] In some embodiments of any aspects of the method of inducing the differentiation of stem cells, the methods comprise providing or selecting an isolated population of stem cells, a tissue comprising a population of stem cells, isolating the population of stem cells, isolating the tissue comprising a population of stem cells, and/or identifying or selecting the tissue comprising a population of stem cells. In one embodiment, the tissue comprising a population of stem cells can be any tissue in an organism, such as a multicellular organism, e.g., a plant and a mouse. For example, the tissue can be a skin, a liver or an engineered tissue. In some embodiment, the tissue can be *ex vivo* (outside the organism) or *in vivo* (inside the organism).

[0174] In one embodiment, stem cell differentiation is induced, stimulated and/or promoted in a subject in need thereof. In this embodiment, the method comprises identifying or selecting the subject in need of stem cell differentiation. For example, the subject has an oral ulcer, a pressure sore, a bone graft or fusion, a transplanted or engineered tissue or a broken bone. A skilled physician would be able to identify and/or select such a subject.

[0175] In another embodiment, provided herein is a method of inducing dentinogenesis in a subject, the method comprising exposing a tissue comprising a population of dental pulp stem cells to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of about 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from about 1 to 10 joule per cm^2 within a period of time of between about 2 - 8 minutes, whereby the irradiation induces the population of dental pulp stem cells to differentiate to odontoblast.

[0176] In one embodiment, provided herein is a method of dental pulp capping in a subject, the method comprising exposing a tissue comprising an exposed dentine pulp to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of about 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from about 1 to 10 joule per cm^2 for a period of time of between about 2 - 8 minutes, whereby the irradiation induces increase secretion of dentin which covers up the exposed dentine pulp.

[0177] In some embodiments, the methods comprise identifying or selecting the subject in need of dentinogenesis induction or dental pulp capping. For example, a subject with a small tooth cavity wherein the dentine pulp is exposed. A skilled dentist or oral surgeon would be able to identify and/or select such subjects.

[0178] In one embodiment of any aspects of the methods described herein, the irradiation has a wavelength in the range of 780-910 nm. In other embodiments of the methods, the laser irradiation has a wavelength in the range of 780-880 nm, 780-840 nm, 780-820 nm, 780-810 nm, 800-880 nm, 800-840 nm, 800-860 nm, 800-820 nm, 810-820 nm, 800-910 nm, 810-910 nm, 840-910nm and 810-840 nm, including all the wavelengths having a whole integer between 780-910.

[0179] In one preferred embodiment of any aspects of the methods described herein, the irradiation has a wavelength is 810 nm.

[0180] In some embodiments of any aspects of the methods described herein, the irradiation has a wavelength is about 810 nm, about 820 nm, about 830 nm, about 840 nm or about 850 nm.

[0181] In some embodiments of any aspects of the methods described herein, the period of time of each individual dose of irradiation ranges from 2 - 8 minutes. In other embodiments, the period of time ranges from 2-7 minutes, 2-6 minutes, 2-5 minutes, 2-4 minutes, 2-3 minutes, 3-7 minutes, 3-6 minutes, 3-5 minutes, 3-4 minutes, 4-7 minutes, 4-6 minutes, 4-5 minutes, 3-8 minutes, 4-8 minutes, 5-8 minutes, 5-7 minutes, 5-6 minutes, 6-8 minutes, 7-8 minutes, 6-7 minutes, 4.5-6.5 minutes, 2.5-7.5 minutes, 3.5-5.5 minutes and 3.5-6.5 minutes, including all the time to two decimal places between 2-8 minutes.

[0182] In one preferred embodiment of any aspects of the methods described herein, the irradiation is a laser irradiation.

[0183] In one preferred embodiment of any aspects of the methods described herein, the period of time for irradiation is about 5 minutes.

[0184] In some embodiments of any aspects of the methods described herein, the period of time for irradiation is about 1, 2, 3, 4, 6, 7, or 8 minutes.

[0185] In some embodiments of any aspects of the methods described herein, each exposure of irradiation provides a dosage (energy or fluence) that ranges from 0.3-30 joule per cm^2. In other embodiments, the dosage of laser irradiation ranges from 0.5-25 joule per cm^2, 0.5-10 joule per cm^2, 0.5-5 joule per cm^2, 1-25 joule per cm^2, 1-10 joule per cm^2, 1-5 joule per cm^2, 1.5-25 joule per cm^2, 1.5-10 joule per cm^2, 1.5-5 joule per cm^2, 2-25 joule per cm^2, 2-10 joule per cm^2, 2-5 joule per cm^2, 2.5-25 joule per cm^2, 2.5-10 joule per cm^2, 2.5-5 joule per cm^2, including all the dosage to one decimal places between 0.3-30.

[0186] In one preferred embodiment of any aspects of the methods described herein, the dosage of irradiation is 3 J/ cm^2.

[0187] In some embodiments of any aspects of the methods described herein, the dosage of irradiation is 1, 2, 4, 5, 6, 7, 8, 9 or 10 J/ cm^2.

[0188] In another one preferred embodiment of any aspects of the methods described herein, the dosage of irradiation is less than 10 J/ cm^2.

[0189] In another one preferred embodiment of any aspects of the methods described herein, the dosage of irradiation is no more than 10 J/ cm^2, i.e., does not exceed 10 J/cm^2.

[0190] In one embodiment of any aspects of the methods described herein, the one or more irradiation exposures ranges from 1 to 10. It is contemplated that in inducing differentiation of stem cells, inducing dentinogenesis or dental pulp capping, more than one application of irradiation may be need to achieve the desired goal, i.e. differentiation of stem cells, wound healing, bone fusion, dentinogenesis and dental pulp capping etc. In some embodiments, the number of laser irradiation needed ranges from 1-9, 3-9, 6-9, 1-7, 2-7, 2-6, 3-7, 3-8, 4-9, 4-7, 4-9, 4-6, 2-5, and 4-5, including all whole numbers between 1-10. In one embodiment, only one irradiation exposure is needed for inducing the differentiation of stem cells. In one embodiment, only two irradiation exposures are needed. In some embodiments, only three, only four, only five, only six, only seven, only eight, only nine, or only ten irradiation exposures are needed.

[0191] In one embodiment of any aspects of the methods described herein, the population of stem cells is a collection of stem cells isolated from a subject. The collection of stem cells is exposed to LPL *ex vivo.* For example, the red blood cells in the peripheral circulation in a mammal are replaced every three to four weeks. A collection of hematopoietic stem cells from the bone marrow or peripheral circulation can be isolated, exposed to LPL *ex vivo* to induce the stem cells to differentiate to red blood cells, and then transfused back into the mammal. The *ex vivo* population of stem cells can also be expanded *ex vivo* prior to the application of LPL. In one embodiment, the population of stem cells is a cryopreserved collection of stem cells, e.g., mesenchymal stem cells isolated from umbilical cord blood or peripheral circulating blood. These stem cells are then expanded *ex vivo* prior to cryopreservation. When need, they are thawed, exposed to with LPL to induce differentiation, and implant into a subject recipient. Methods of isolation, *ex vivo* expanded, and cryopreservation of various stem cells are well known in the art; see Current protocols in stem cell biology, Eds. Elefanty et al., Wiley Inc.

[0192] In one embodiment of any aspects of the methods described herein, the population of stem cells is found in a tissue in a subject. It is well known that populations of stem cells exist in all sorts of tissues, e.g., in the skin, the bone marrow, the blood, the umbilical cord, in hair follicles, in solid organs such as liver, muscles, and lungs, etc. These stem cells provide the means for the respective tissues to renew and/or repair itself after the tissue has been fully formed when development has been completed.

[0193] In one embodiment of any aspects of the methods described herein, the stem cell includes a progenitor cell. In some embodiment, the stem cells include but are not limited to mesenchymal stem cells, embryonic stem cells, hematopoietic stem, cells dental pulp stem cell, muscle satellite cells, hair follicle stem cell, induced pluripotent stem cells (mouse and human), amniotic stem cells, mammary stem cells, endothelial stem cells, neural stem cells, olfactory adult stem cells, neural crest stem cells, and testicular cells. In other embodiments, the stem cells include but not limited to the stem cells described in U.S. patent Nos: 5,843,780; 6,200,806; and 7,029,913.

[0194] In one embodiment of any aspects of the methods described herein, the tissue is damaged and is in need of repair, e.g., bone fracture, sprained muscle or severed nerve. In one embodiment, the tissue comprises a wound, an exposed dental pulp or a bone fracture. A tissue is damaged and is in need of repair when the structural integrity and/or organization of the tissue in compromised. For example, when there is a wound or laceration on the skin or the oral gums,

when the dental pulp is not fully enclosed by dentin and is separated from the exterior, or when there is a physical break in the continuum of a bone structure.

[0195] In one embodiment, the wound is a skin ulcer. A skin ulcer is an open sore in the skin. Skin ulcers can be caused by a variety of events, such as trauma, exposure to heat or cold (burns and frost bite), problems with blood circulation due to diseases or disorders such as diabetes, chafing, or irritation from exposure to corrosive material. Pressure ulcers, also known as decubitus ulcers or bedsores, are skin ulcers that develop on areas of the body where the blood supply has been reduced because of prolonged pressure; these may occur in people confined to bed or a chair, or in those who must wear a hard brace or plaster cast. Skin ulcers may become infected, with serious health consequences. Other health conditions that can cause skin ulcers include mouth ulcers (canker sores), chronic venous insufficiency, diabetes, infection, and peripheral vascular disease. Skin ulcers are generally maintained by an inflammation, an infection, and/or medical conditions which impede healing, and are often accompanied by the sloughing-off of inflamed tissue. Application of LPL can used to enhance the healing process and reduce the infection. In one embodiment, the application of LPL can be used in conjunction with other methods and/or medication used to treat skin ulcer and/or enhance healing.

[0196] In one embodiment, the wound is an oral wound, such as a mouth ulcer or sore from gingivitis, dentures, canker sore, cold sores etc. In one embodiment, the oral wound is one that resulted from oral surgery. Application of LPL can used to enhance the healing process. In one embodiment, the application of LPL can be used in conjunction with other methods and/or medication used to treat oral wound and/or enhance healing.

[0197] In one embodiment, the tissue comprises an exposed dental pulp. The dental pulp is the part in the center of a tooth made up of living connective tissue and cells such as fibroblasts (the principal cell), odontoblasts, defense cells like histiocytes, macrophage, granulocytes, mast cells and plasma cells, and large nerve trunks and blood vessels. The primary function of the dental pulp is to form dentin (by the odontoblasts); the pulp keeps the organic components of the surrounding mineralized tissue supplied with moisture and nutrients. In addition, the dental pulp also has a sensory function: extremes in temperature, pressure, or trauma to the dentin or pulp are perceived as pain; and a protective function: the formation of reparative or secondary dentin. The dentin is the calcified tissue between the pulp and enamel. It is a dense matrix of minerals, primarily calcium that are secreted by the odontoblasts in the pulp, and it serves to protect the sensitive pulp of the tooth and create a base under the enamel, or

outer coating of the tooth. When there is a cavity in a tooth, bacteria in the cavity can decay the dentin to an extent that the sensitive pulp is exposed. The bacteria can infect the pulp and cause inflammation. An inflammation of a pulp is known as pulpitis. Pulpitis can be extremely painful and in serious cases calls for root canal therapy.

[0198] In one embodiment, the "inside-out" induction of dentine formation in a tooth by the laser-based methods and/or treatment systems described herein served to reduce the sensitivity of the tooth. Accordingly, the treatment systems and methods described herein can be used for tooth desensitization.

[0199] In another embodiment, the "inside-out" induction of dentine formation in a tooth by the laser-based methods and/or treatment systems described herein served to reduce the sensitivity of the dentine of the tooth. Accordingly, the treatment systems and methods described herein can be used for dentine desensitization.

[0200] Applications of LPL can induce the stem cells within the tissue to differentiate to the variety of cells that constitute the tissue for renewal and/or reparative goals. In one embodiment, the methods and laser described herein is used in for wound healing. In another embodiment, the method and laser described herein is used in for oral wound healing. For example, to speed up the closing of a laceration in the skin, LPL can induce the skin stem cells in the epidermis and dermis near of the laceration to differentiate to fibroblasts, myofibroblasts and epidermal cells, and stem cells in circulation to differentiate to endothelial cells and smooth muscle cells. The endothelial cells and smooth muscle cells facilitate angiogenesis at the wound site. The fibroblasts secrete matrix materials such as collagen and fibronectin to seal the aperture of the wound and provide a platform of connective tissues for the new epithelial cells to occupy. In contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges.

[0201] Application of LPL can be used to enhance the dentin repair. In one embodiment, the method and laser described herein are used for dental pulp capping. In one embodiment, the application of LPL induces the differentiation of dental pulp cells to odontoblasts whose function is to secrete the dentin. In one embodiment, the application of LPL induces dentinogenesis in exposed dental pulp by increasing the number of odontoblasts available for dentin formation. In one embodiment, the application of LPL can be used in conjunction with other methods and/or medication used to treat dental pulp capping and/or induce dentinogenesis.

[0202] In one embodiment, the tissue is a transplanted tissue, a graft wherein that the tissue has been removed from its naturally occurring location and moved or grafted to a new location. For example, gum or bone grafts. Grafting is a procedure used to replace / restore missing bone or gum tissue. A gum (gingival) graft is used to replace missing and / or receded gum tissue. Soft tissue grafts are used to replace missing thick tissue (keratinized gingiva), which has worn away from the necks of the teeth for a variety of reasons. The purpose of gum grafting is to minimize and/or arrest the progression of recession.

[0203] Bone grafting is the replacement or augmentation of the bone around the teeth. Bone grafting is performed to reverse the bone loss / destruction caused by periodontal disease, trauma, or ill fitting removable dentures. It is also used to augment bone to permit implant placement, such as augmenting bone in the sinus area for implant placement, or augmenting bone to enhance the fit and comfort of removable prostheses, or to enhance esthetics of a missing tooth site in the smile zone. When one loses a tooth, as in an extraction, the surrounding bone collapses. To preserve this bone for future implant placement or for esthetics, a bone graft is used.

[0204] Bone grafting is also used in spinal fusion surgery. The aim of spinal fusion is to stop motion at a painful vertebral segment, which can decrease back pain. Spinal fusion may be done by itself or in combination with decompression to treat painful symptoms caused by misalignment or instability of the vertebrae, such as spondylolisthesis. Common indications requiring spinal fusion include but are not limited to spinal stenosis, herniated discs, spinal injuries, infection, tumors, and deformities. Bone is taken from the pelvic bone or obtained from a bone bank. The bone is used to make a bridge between adjacent vertebrae. This bone graft stimulates the growth of new bone.

[0205] In the embodiment where the tissue is a transplanted tissue, the application of LPL can induce the stem cells within the transplanted tissue and recipient tissue to differentiate to the variety of cells that constitute the tissue, e.g., bone as in spinal fusion or gum grafting. In one embodiment, the application of LPL can enhance the graft to take hold in the host tissue. The application of LPL occurs during the surgery before the patient is sutured up.

[0206] In one embodiment, the tissue is an engineered tissue comprising scaffold matrix and transplanted cells incorporated within the matrix. In one embodiment, the transplanted cells incorporated within the matrix comprise stem cells, e.g., mesenchymal stem cells or hair follicle stem cells. Such engineered tissues are well known in the art. See WO 2010/030964

(PCT/US2009/056777) for example. In this embodiment, the application of LPL can induces differentiation of the incorporated stem cells to the appropriate cell type of the replacement body part or recipient tissue, e.g., the cartilage and skin forming the replacement part of the exterior of an ear or the replacement heart valve. In one embodiment, the tissue engineering for tissue repair and wound healing can be performed in an autologous individual from whom the stem cells were derived, or in HLA type matched individual, which is HLA typed matched with the donor of the stem cells. In an autologous system, the stem cells should match exactly the recipient patient from whom the stem cells are originally derived. In another embodiment, the stem cells are not used in an autologous system but are HLA typed match to the recipient. For example, the HLA type matched for HLA-A, B, C, and D.

[0207] In one embodiment, the tissue is used for tissue engineering, tissue repair, regenerative medicine and wound healing in humans. Tissue engineering is the use of a combination of cells, engineering and material methods, and suitable biochemical and physiochemical factors to improve or replace biological functions. Tissue engineering aims at developing functional cell, tissue, and organ substitutes to repair, replace or enhance biological function that has been lost due to congenital abnormalities, injury, disease, or aging, or repair fascia in hernias. The tissue that is engineered is used to repair or replace portions of or whole tissues (i.e., bone, cartilage, blood vessels, bladder, etc.). Often, the tissues involved require certain mechanical and structural properties for proper function. Tissue engineering also encompass the efforts to perform specific biochemical functions using cells within an artificially-created support system (e.g. an artificial pancreas, or a bioartificial liver). The term regenerative medicine is often used synonymously with tissue engineering, although those involved in regenerative medicine place more emphasis on the use of stem cells to produce tissues. Tissue regeneration aims to restore and repair tissue function via the interplay of living cells, an extra-cellular matrix and cell communicators.

[0208] Current traditional approaches to treat medical diseases, congenital abnormalities and injury include: drugs, hormones, enzymes, vaccines, prosthetic substitution, surgical reconstruction, and organ transplantation. These methods are all considered essential, but have their limitations. For example, drugs have unwanted side effects, prosthetics are not biologically active and do not integrate or remodel into the body, surgery is invasive, and organ transplantation is limited by donor availability and toxic immunosuppressive cocktails.

[0209] Contrary to traditional approaches, tissue regeneration is an approach in modern medicine that delivers living tissue or cells and stimulates the body's own natural healing

process by activating the body's inherent ability to repair and regenerate. Innovative tissue regeneration therapies are now available that aim to heal or reconstruct diseased tissue and support the regeneration of diseased or injured organs. Doctors use tissue regeneration to speed up healing and to help injuries that will not heal or repair on their own. Tissue regeneration can help heal broken bones, severe burns, chronic wounds, heart damage, nerve damage, and many other diseases.

[0210] In one embodiment, the subject is a multicellular organism. The subject can be a plant, an animal or even a developing embryo. In one embodiment, the subject is a mammal. In one embodiment, the subject is a primate mammal. In another embodiment, the subject is a non-primate mammal. In one embodiment, the subject is a domesticated farm animal. In one embodiment, the subject is a cultivated plant, shrub or tree. Examples of a subject are a human, a cat, a dog, a cow, a horse,

[0211] In one embodiment of the methods of inducing dentinogenesis or dental pulp capping described herein, the methods further comprise contacting the population of stem cells, the exposed dentine pulp of a tooth, or the population of odontoblasts with at least one metal ion prior to exposure to the irradiation. In one embodiment, at least one metal ion and not more than five different metal ions are used in combination for the method.

[0212] In one embodiment, contacting can be in the form of exposing the tissue to a composition comprising at least one metal ion. For example, in a mouth wash solution or a gel.

[0213] In one embodiment, the metal ion is a divalent metal ion.

[0214] In one embodiment, the divalent metal is selected from a group include but are limited to lithium, barium, magnesium, copper, iron, manganese, and zinc. In one embodiment, the divalent metal is a salt. In one embodiment, the divalent metal is in a gel-like composition that can be applied directly to the target, the tissue comprising a population of stem cells, the exposed dentine pulp of a tooth, or the population of odontoblasts. In one embodiment, the divalent metal is in a solution, preferably a physiological isotonic solution that can be used to bathe the target prior to exposure to irradiation. In one embodiment, the physiological isotonic solution comprising the divalent metal is sterile.

[0215] In one embodiment, several different metal ions are used in combination. For example, lithium and zinc; barium, magnesium, and copper; or lithium, manganese, and zinc.

[0216] In one embodiment of the methods of inducing dentinogenesis or dental pulp capping described herein, the methods further comprise contacting the population of stem cells, the exposed dentine pulp of a tooth, or the population of odontoblasts with at least one agent that activates or up-regulates the Wnt pathway, including but not limited to, for example, BIO (6-bromoindirubin-3'-oxime) or LiCl, and soluble wnt ligand, or other compounds that modulate the Wnt pathway at any level of the Wnt signaling cascade. Non-limiting examples of Wnt pathway activators include WAY-316606, (hetero)arylpyrimidines, IQ1, QS11, SB-216763, DCA, 2-amino-4-[3,4-(methylenedioxy)benzyl-amino]-6-(3-methoxyphenyl)pyrimidine, and those described in U.S. Patent Pubilcation No: 20120046242; and 20110008297.

[0217] In one embodiment, contacting can be in the form of exposing the tissue to a composition comprising at least one agent that activates or up-regulates the Wnt pathway. For example, in a mouth wash solution or a gel.

[0218] In one embodiment, the divalent metal or the at least one agent that activates or up-regulates the Wnt pathway is used at a concentration range of 0.001 nM to 0.1 M. In other embodiments, the divalent metal ion or the at least one agent that activates or up-regulates the Wnt pathway is used at a concentration range of 0.001 nM to 0.1 mM, 0.01 nM to 0.1 mM, 1 nM to 0.1m M, 10 nM to 1 mM, 10 nM to 0.1 mM, 1 nM to 0.1 mM, 1 nM to 10 mM, 1 nM to 0.5 mM, 10 nM to 0.5 mM, 0.01 nM to 1 μM, 0. 1 nM to 0.1 μM, 1 nM to 1 μM, 10 nM to 1 μM, or 10 nM to 10 μM including all the integers to the third decimal place between 0.001 nM to 0.1 M.

[0219] In the embodiments where several different metal ions are used in combination or where more than one agent that activates or up-regulates the Wnt pathway are used in combination, the combined concentration of the several different metal ions are used has a range of 0.001 nM to 0.1 M. In other embodiments, the combined concentration has range of 0.001 nM to 0.1 mM, 0.01 nM to 0.1 mM, 1 nM to 0.1m M, 10 nM to 1 mM, 10 nM to 0.1 mM, 1 nM to 0.1 mM, 1 nM to 10 mM, 1 nM to 0.5 mM, 10 nM to 0.5 mM, 0.01 nM to 1 μM, 0. 1 nM to 0.1 μM, 1 nM to 1 μM, 10 nM to 1 μM, or 10 nM to 10 μM including all the integers to the third decimal place between 0.001 nM to 0.1 M.

Dentinogenesis and dental pulp capping

[0220] Dentinogenesis is the formation of dentin, a substance that forms the majority of teeth. Dentinogenesis is performed by odontoblasts, which are a special type of biological cells

on the outside of dental pulps, and it begins at the late bell stage of a developing tooth. The different stages of dentin formation result in different types of dentin: mantle dentin, primary dentin, secondary dentin, and tertiary dentin.

[0221] In a developing tooth, odontoblasts differentiate from cells of the dental papilla. They begin secreting an organic matrix around the area directly adjacent to the inner enamel epithelium, closest to the area of the future cusp of a tooth. The organic matrix contains collagen fibers with large diameters (0.1-0.2 μm in diameter). The secreted collagen forms into a dense matrix of tubes which grow out from the pulp of the tooth. These tubes are known as dentinal tubules, because they are the underlying structure of the dentin area of the tooth. As the tubes grow out towards the surface of the tooth, they become more widely spread apart, but the dentin is still fairly porous, thanks to the multitude of small holes in each tooth. Then the odontoblasts begin to move toward the center of the tooth, forming an extension called the odontoblast process. Thus, dentin formation proceeds toward the inside of the tooth. The odontoblast process is responsible for the secretion of hydroxyapatite crystals and mineralization of the matrix. In addition, dentin also contains mineral rich fluids called dentinal fluids. Dentinal fluids contain proteins, sodium, and calcium, and are concentrated in the dentinal tubules. The majority of dentin is mineralized tissue. However, dentin also contains mineral rich fluids called dentinal fluids, which may be responsible for the mineralization of the dentin as it is secreted by the odontoblasts. Dentinal fluids contain proteins, sodium, and calcium, and are concentrated in the dentinal tubules. This area of mineralization is known as mantle dentin and is a layer usually about 5-30 μm thick.

[0222] Whereas mantle dentin forms from the preexisting ground substance of the dental papilla, primary dentin forms through a different process. Primary dentin is formed in the second stage of dentinogenesis during the development of a tooth. Primary dentin forms the next layer after the mantle dentin and make up most of the tooth structure. Odontoblasts increase in size, eliminating the availability of any extracellular resources to contribute to an organic matrix for mineralization. Additionally, the larger odontoblasts cause collagen to be secreted in smaller amounts, which results in more tightly arranged, heterogeneous nucleation that is used for mineralization. Other materials (such as lipids, phosphoproteins, and phospholipids) are also secreted.

[0223] Secondary dentin is formed approximately after root formation is finished and occurs at a much slower rate. It is not formed at a uniform rate along the tooth, but instead forms

faster along sections closer to the crown of a tooth. This development continues throughout life and accounts for the smaller areas of pulp found in older individuals.

[0224] The next stage of dentinogenesis produces the tertiary dentin which occurs as a tooth response to irritations. Tooth preparation made by a dentist, dentinal caries, attrition, abrasion and/or erosion are the most common irritating factors. The tertiary dentine may also be named according to the quality of the irritation: The tertiary dentin formed as a response to attrition, abrasion or erosion is called "reactional dentin" to separate it from caries and preparation induced "reparative dentin". The tertiary dentinogenesis may be absent even in a fully matured tooth. The quality of the tertiary dentin seems to be dependent on the speed of its formation: the faster it is formed, the more irregular it appears.

[0225] Dentin is the most abundant dental tissue that determines the size and shape of teeth. It also serves to protect the sensitive dental pulp of the tooth and create a base under the enamel, or outer coating of the tooth. Taking care of dentin is extremely important, because although it is dense and hard, it is susceptible to rot and infection, which can lead to oral pain and expensive dental treatments.

[0226] In a carious tooth, the enamel and dentin decays away, and the inner dental pulp gets exposed to bacteria and becomes infected and inflamed, which is “pulpitis". In addition, pain in the tooth can occur when exposure to heat and/or cold, and/or when tapping or biting on the tooth. When the pain lasts more than an instant, e.g., it lasts a few minutes or more, and/or when pain sometimes starts for not apparent reason, these are general indication of "irreversible pulpitis”, where the nerve is irritated beyond its ability to repair itself.

[0227] However, before irreversible pulpitis sets in, when the nerves are just irritated but still alive and healthy enough to repair themselves given a chance to do so, pulp capping can be used to give the pulp a chance to recover. Pulp capping involves getting out the decayed portions of the damaged tooth area as well as one can, followed by stopping the bleeding and placing the right filling material on the exposed pulp, e.g., calcium hydroxide over it, and then a normal filling (usually amalgam).

[0228] Pulp-capping has been done in different ways. This procedure was traditionally carried out using calcium hydroxide formulations that have bactericidal effects. The procedure takes about 5-15 min to place depending on location of the exposed pulp and the skill of dentist. Modern procedures use bonded composite, e.g., adhesive resins such as mineral trioxide aggregate (MTA). However, it has been shown that calcium hydroxide dentinal bridge does not

form a continuous seal and thus may allow bacterial leakage through the tooth. Direct pulp capping with adhesive resins on the exposed pulp indicate that the marginal micro-leakage can be prevented so as to restore the tooth; trials in the monkeys and other primates reveals that adhesive systems and the composite resins are naturally compatible with pulpal tissue when correctly placed on the exposed part after bleeding is well controlled.

[0229] The method and laser system described herein for inducing dentinogenesis and dental pulp capping have advantages over the traditional calcium hydroxide method or the current composite resins based methods. In using laser irradiation to induce inducing dentinogenesis and dental pulp capping, it is possible to regulate and adjust the laser energy and irradiation zone. This is an important factor for controlling the amount and rate of dentin induction. It is also easier to apply the laser irradiation to locations that are hard to reach to implant the calcium hydroxide or composite resins.

[0230] Another advantage is the ability to cap the sensitive pulp via an "inside out" approach, i.e., since the dentin formation is induced from the inside out, dentinogenesis would naturally occur, as opposed to the "outside in" approach when calcium hydroxide or composite resins used. This "inside out" approach virtually eliminates any bacterial leakage through to the tooth's pulp and the possibility of bacterial entrapment therein compared to the calcium hydroxide or composite resins methods when the calcium hydroxide or composite resins solidify from outside in. The laser irradiation method also provides a non-invasive treatment modality compared to the traditional method. Furthermore, the laser irradiation method is non-toxic (local and systemic) natural method, producing less pain in the subject compared to the use of calcium hydroxide, composite resins, and amalgam fillings.

[0231] Reparative dentin produced by the method and laser system described herein has a non-tubular, lamellar or haphazard pattern but has a composition similar to dentin.

Stem cells and stem cell differentiation

[0232] Stem cells are cells that retain the ability to renew them through mitotic cell division and can differentiate into a diverse range of specialized cell types. The two broad types of stem cells are: embryonic stem (ES) cells that are found in blastocysts, and adult stem cells, also known as somatic stem cells, which are found in adult tissues. In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin or intestinal

tissues. Pluripotent stem cells can differentiate into cells derived from any of the three germ layers of a mammal.

[0233] In one embodiment, the term "stem cell" as used herein, refers to an undifferentiated cell which is capable of proliferation and giving rise to more progenitor cells having the ability to generate a large number of mother cells that can in turn give rise to differentiated, or differentiable daughter cells known as precursor cells. The daughter cells themselves can be induced to proliferate and produce progeny that subsequently differentiate into one or more mature cell types, while also retaining one or more cells with parental developmental potential. A committed progenitor cell has typically lost the self-renewal capacity, and upon cell division produces two daughter cells that display a more differentiated (i.e., restricted) phenotype.

[0234] In another embodiment, the term "stem cell" also refers to a subset of progenitors that have the capacity or potential, under particular circumstances, to differentiate to a more specialized or differentiated phenotype, and which retains the capacity, under certain circumstances, to proliferate without substantially differentiating.

[0235] The stem cells can be somatic stem cells or embryonic stem cells. In a preferred embodiment, the stem cells used with the methods and laser system described herein are adult stem cells or somatic stem cells.

[0236] In one embodiment, the stem cells used with the methods and laser system described herein are "c-kit positive" stem cells. Stem cells, progenitor cells and precursor cells are c-kit positive. These c-kit positive cells are mainly negative for markers characteristic of the hematopoietic lineage, mast cell lineage, mesenchymal stromal cell lineage, epithelial lineage and/or endothelial cell lineage such as CD2, CD3, CD6, CD8, CD14, CD16, CD19, CD20, CD24, CD29, CD34, CD44, CD45, CD49d, CD49e,CD66b, CD90, CD105, CD133, glycophorin A, TTF1, p63, pan-cytokeratin, cytokeratin 5, CC10, aquaporin-5, SPC, Est1, vWF1, GATA 6, and alpha-SMA.

[0237] In some embodiments of any aspects of the methods described herein, the somatic stem cells are dental stem cells or dental pulp stem cells.

[0238] In some embodiments of any aspects of the methods described herein, the dental pulp stem cells are substantially positive for at least one of the following cell surface marker proteins: CD44, CD106, and CD117.

[0239] In one embodiment of any aspects of the methods described herein, the dental pulp stem cells are substantially negative for the cell surface marker CD45.

[0240] In some embodiments of any aspects of the methods described herein, the somatic stem cells are mesenchymal stem cells.

[0241] Cellular differentiation is a complex process typically occurring through many cell divisions. A differentiated cell may derive from a multipotent cell which itself is derived from a multipotent cell, and so on. While each of these multipotent cells may be considered stem cells, the range of cell types each can give rise to may vary considerably. Some differentiated cells also have the capacity to give rise to cells of greater developmental potential. Such capacity may be natural or may be induced artificially upon treatment with various factors. In many biological instances, stem cells are also "multipotent" because they can produce progeny of more than one distinct cell type, and is a required as used in this document. Self-renewal is the other classical part of the stem cell definition. In theory, self-renewal can occur by either of two major mechanisms. Stem cells may divide asymmetrically, with one daughter retaining the stem state and the other daughter expressing some distinct other specific function and phenotype. Alternatively, some of the stem cells in a population can divide symmetrically into two stems, thus maintaining some stem cells in the population as a whole, while other cells in the population give rise to differentiated progeny only.

[0242] Stem cells can be differentiate into several cell lineages including but are not limited to osteogenic, myogenic, adipogenic, chondrogenic, neurogenic, hepatogenic, nephrogenic, urogenic, isletogenic, pancreatogenic, gastroenterogenic, epitheliogenic, thyroidogenic, myocardiogenic, pneumogenic, retinogenic, gametogenic, endotheliogenic, or hematopoietic lineages.

[0243] A number of different cell-surface markers have specific expression on specific differentiated cell lineages. These cell-surface markers can be used to determine the differentiation of the stem cells. For example, CD13 and CD33 (expressed on myeloid cells); CD71 (expressed on erythroid cells); CD19 and B220 (expressed on B cells), CD61 (expressed on human megakaryocytic cells); Mac-1 (CD11b/CD18) (expressed on monocytes); Gr-1 (expressed on granulocytes); Ter119 (expressed on erythroid cells); and Il7Ra, CD2, CD3, CD4, CD5, CD8 (expressed on T cells); CD14, CD56, and CD235a; TTF1, p63, pan-cytokeratin, cytokeratin 5, CC10, aquaporin-5 and SPC (for epithelial lineage); Est1, vWF1, GATA 6, and alpha-SMA (for endothelial cell and smooth muscle cell lineage); CD6, CD29,

CD49d, CD49e, CD45 and tryptase (for mast cell lineage); CD44, CD90 and CD105 (for mesenchymal stromal cell lineage); and CD34, CD45, and CD133 (for general hematopoietic lineage).

[0244] Methods of analysis of stem cell differentiation are known, e.g., described in detailed in the osteogenesis differentiation protocol by Millipore which is to be used in conjunction with Millipore's Mesenchymal Stem Cell Osteogenesis Kit; in M. Ahmad, et al., Biomaterials, 1999, 20: 211-20; M.J. Coelho and M.H. Fernandes, Biomaterials, 2000, 21: 1095-102; Paolo De Coppi, et. al., 2007, Nature Biotechnology, 25, 100-106; B. Johnstone, et al., Exp Cell Res, 1998, 238: 265-72; A.M. Mackay, et al., Tissue Eng, 1998, 4: 415-28; M.E. Nuttall, et al., J Bone Miner Res, (1998), 13: 371-82; P.A. Conget and J.J. Minguell, J Cell Physiol, (1999) 181: 67-73; in Current Protocols in Stem Cell Biology (Mick Bhatia, et. al., ed., John Wiley and Sons, Inc.); Björklund & Lindvall, Nat Neurosci, 3: 537 (2000); Björklund & Lindvall, Nature, 405: 892 (2000); Cameron et al., J Neurobiol, 36: 287 (1998); McKay, Nature, 406: 361 (2000); Wachs et al., Lab Invest. 83: 949 (2003); Heng, BC., et. al., J. Gastro, Hepatology, (2005) 20: 975-987; Nat R., et. al., 2007, Glia, 55: 385-99; Schulz TC, et al., Stem Cells (2004) 22:1218-38; Kim D and Dressler GR., J Am Soc Nephrol. (2005) 16:3527-34; Insa S Schroeder, et al., Nature Protocols 1:495-507 (2006); Alejandro Soto-Gutiérrez, Nature Protocols, (2007) 2: in press; Jiang W, et al., 2007, Cell Res.(2007) 17: 333-44; Rivas-Carrillo JD, et. al., 2007, Curr Med Chem. (2007) 14: 1573-8; Invernici G., et. al., Exp Cell Res. 2007, Milne HM, et. al., Biochem Biophys Res Commun. (2005) 328: 399-403; Bruce SJ, et al., (2007) Differentiation, 75: 337-49; Oottamasathien S., et al., (2007), Dev. Biol. 304: 556-66; Wang D., et al., Proc Natl Acad Sci U S A. (2007) 104: 4449-54; McCloskey KE, et. al., Methods Mol Biol. (2006), 330: 287-301; Kang SM, et al., (2007), Stem Cells, 25: 419-24; Arufe MC, et al., 1: Endocrinology (2006) 147: 3007-15; Van Vranken BE, et al., Tissue Eng. (2005)11: 1177-87; Glaser T, et al., PLoS ONE, (2007) 2:e298; in the Current Protocols of Stem Cell Biology, (Mick Bhatia, et. al., ed., John Wiley and Sons, Inc.) and methods described herein. These references are hereby incorporated by reference in their entirety.

[0245] Differentiation assays and kits are also commercially available. They include the Mesenchymal Stem Cell Adipogenesis Kit (Millipore cat. no.SCR020), Pancreatic Islet Cell Characterization Kit (Millipore cat. no. SCR045), Pancreatic Cell Development Pathway Kit (Millipore cat. no. SCR046), Pancreatic Cell DTZ Detection Assay, (Millipore cat. no. SCR047), Mesenchymal Stem Cell Osteogenesis Kit (Millipore cat. no.SCR028) and the Mesenchymal Stem Cell Osteogenesis Kit (Millipore cat. no. SCR028).

[0246] The Mesenchymal Stem Cell Osteogenesis Kit (Millipore cat. no. SCR028) provides a method for differentiating mesenchymal stem cells to an osteoblast phenotype. The kit contains two ECM coating molecules (collagen type I and vitronectin), which have been shown to promote osteogenic differentiation of mesenchymal stem cells (Salasznyk, 2004, J. Biomed. Biotechnol., 2004(1):24-34), and the inducing reagents, dexamethasone, ascorbic acid 2-phosphate and ß-glycerophosphate. Also included is Alizarin Red Solution, a staining solution that is used to detect the presence of calcium in bone.

[0247] The Mesenchymal Stem Cell Adipogenesis Kit (Millipore cat. no. SCR020) contains reagents that readily differentiate mesenchymal stem cells to an adipogenic lineage as assessed with Oil Red O staining of lipid vacuoles in mature adipocytes. These factors include dexamethasone, IBMX, insulin and indomethacin. Along with Oil Red O staining solution, a hematoxylin solution is provided to counterstain the cell nucleus. Using this kit, typically it is possible to obtain > 30% mature adipocytes from the rat bone marrow derived mesenchymal stem cells.

[0248] Pancreatic Islet Cell Characterization Kit (Millipore cat. no. SCR045) provides a convenient set of validated antibodies that allows researchers to reliably identify mature pancreatic islets cells. Along with antibodies generated against discrete hormones secreted by alpha, beta, delta and gamma cells of the pancreatic islets, the kit includes PDX-1 (pancreatic duodenal homeobox gene-1), a master regulator of islet cell development and GLUT-2, a glucose transporter present in beta-islet cells.

[0249] Pancreatic Cell Development Pathway Kit (Millipore cat. no. SCR046) provides a collection of antibodies that are unique to key transition points along the developmental pathway of pancreatic cells. Included in the kit are antibodies to critical transcription factors expressed during the program of development along with two antibodies to hormones secreted by mature islets cells (FoxA2, Hes-1, Pax 6, IDX-1, Glucagon and Pancreatic Polypeptide).

[0250] Pancreatic Cell DTZ Detection Assay Kit (Millipore cat. no. SCR047) provides a simple and quick method to identify insulin-producing beta cells from a mixed cell culture preparation or from pancreatic tissues, by detecting high levels of zinc (typically contained in pancreatic beta cells), with the use of a zinc-chelating agent, DTZ. This kit contains DTZ staining and rinse solutions along with filters and syringes required for live staining reactions.

[0251] In one embodiment of any aspects of the methods described herein, the dental pulp stem cells differentiate to pre-odontoblasts.

[0252] In one embodiment of any aspects of the methods described herein, the dental pulp stem cells differentiate to odontoblasts.

[0253] In one embodiment of any aspects of the methods described herein, the dental pulp stem cells differentiate to cells that exhibit at least one of the following characteristics: increased expression of the dentin matrix markers DMP1, OSAD, and OPN; increased alkaline phosphatase activity; and increased mineral deposition.

[0254] The present invention can be defined in any of the following numbered paragraphs:

[1] A dental treatment system for inducing dentinogenesis, the system comprising:(a) a source of irradiation, the source being adapted to produce irradiation having a wavelength in the range of 780 nm to 840 nm and a power output in the range of 5 mW to 300 mW; (b) a control element controlling the source of irradiation to turn the source of laser irradiation on and to turn the source of laser irradiation off; and (c) a timer operatively connected to the control element to activate the source of irradiation to produce irradiation for a pre-determined time period and then deactivate the source of irradiation from producing radiation.

[2] The dental treatment system of paragraph 1 further comprising a collimator coupled to the source of irradiation and collimating the irradiation produced by the source of irradiation.

[3] The dental treatment system of paragraph 1 or 2 further comprising a switch element operatively connected to and controlling the timer.

[4] The dental treatment system of paragraph 3 wherein the switch element is a foot operated switch.

[5] The dental treatment system of paragraph 1, 2 or 3, wherein the wavelength is between 800 nm to 820 nm.

[6] The dental treatment system of paragraph 5, wherein the wavelength is 810 nm.

[7] The dental treatment system of any one of paragraphs 1-6, wherein the pre-determined time is between 2 to 8 minutes.

[8] The dental treatment system of paragraph 7, wherein the pre-determined time is about 5 minutes.

[9] The dental treatment system of any one of paragraphs 1-8, wherein the timer controls the source of irradiation to apply a dosage of between 1 – 10 J/cm^2 of irradiation to a target location.

[10] The dental treatment system of paragraph 9, wherein the dosage is 3 J/cm^2.

[11] The dental treatment system of any one of paragraphs 1 – 10, wherein the source of irradiation produces laser irradiation

[12] The dental treatment system of any one of paragraphs 1 -10, wherein the source of irradiation includes a diode laser.

[13] The dental treatment system of any one of paragraphs 1-10, wherein the source of irradiation includes a laser selected from the group including gas lasers, dye lasers, semiconductor lasers and solid state lasers.

[14] A dental laser system for inducing dentinogenesis in a target, the system comprising a source of laser irradiation, the source being adapted to produce laser irradiation having a wavelength of 810 nm; a control element controlling operation of the source of irradiation to turn the source of laser irradiation on and to turn the source of laser irradiation off; and a timer operatively connected to the control element to turn the source of laser irradiation on for a pre-determined time period and then turn the source of laser irradiation off and wherein the pre-determined time period is determined to apply a dose of 3 J/cm^2 on the target.

[15] A method of stimulating dentinogenesis in a subject, the method comprising exposing a tissue comprising a population of dental pulp stem cells to an irradiation output from the dental treatment system or dental laser system of any one of paragraphs 1-14, whereby the dental pulp stem cells is induced to differentiate to odontoblasts.

[16] A method of dental pulp capping in a subject, the method comprising exposing a tissue comprising an exposed dental pulp to an irradiation output from the dental treatment system or dental laser system of any one of paragraphs 1-14, whereby increased dentin is secreted.

[17] The method of paragraph 15 further comprising contacting the population of dental pulp stem cells with a metal ion prior to exposure to the irradiation.

[18] The method of paragraph 16 further comprising contacting the exposed dental pulp with a metal ion prior to exposure to the laser irradiation.

[19] The method of paragraph 17 or 18, wherein the metal ion is a divalent metal ion.

[20] The method of paragraph 19, wherein the divalent metal is selected from a group consisting of lithium, barium, magnesium, copper, iron, manganese, and zinc.

[21] A method comprising (a) contacting a population of stem cells with an effective amount of at least one metal ion; and (b) exposing the population of stem cells in step (a) to one or more doses of irradiation sufficient to induced differentiation of stem cells, wherein each dose of irradiation is of a pre-determined period of time resulting in the application of a pre-determined dose of irradiation to the tissue; whereby the irradiation induces the population of stem cells to differentiate.

[22] The methods of paragraph 21, wherein the irradiation is a laser irradiation.

[23] The method of any one of paragraphs 21-22, wherein the population of stem cells is found in a tissue in a subject.

[24] The method of paragraph 23, wherein the tissue is damaged and is in need of repair.

[25] The method of any one of paragraphs 21-24, wherein the tissue comprises a wound, an exposed dental pulp or a bone fracture.

[26] The method of any one of paragraphs 21-25, wherein the at least one metal ion is a divalent metal ion.

[27] The method of paragraph 26, wherein the divalent metal ion is selected from a group consisting of lithium, barium, magnesium, copper, iron, manganese, and zinc.

[28] The method of any one of paragraphs 21-27, wherein the irradiation has a wavelength in the range of 780-910 nm.

[29] The method of paragraph 28, wherein the irradiation has a wavelength is 780 nm to 840 nm.

[30] The method of paragraph 28 or 29, wherein the irradiation has a wavelength is 810 nm.

[31] The method of any one of paragraphs 21-30, wherein the period of time ranges from 2 to 8 minutes.

[32] The method of paragraph 31, wherein the period of time is about 5 minutes.

[33] The method of any one of paragraphs 21-32, wherein the one or more doses of irradiation individually provides a dosage that ranges from 0.3 to 30 joule per cm^2.

[34] The method of paragraph 33, wherein the dosage of irradiation is 3 joule per cm^2.

[35] The method of any one of paragraphs 21-34, wherein the one or more doses of irradiation ranges from 1 to 10.

[36] The method of any one of paragraphs 21-35, wherein the method is used in for wound healing.

[37] The method of any one of paragraphs 21-37, wherein the stem cell is dental pulp stem cell.

[38] The method of paragraph 37, wherein the method is used for dental pulp capping.

[39] A method of inducing differentiation of stem cells, the method comprising exposing a population of stem cells to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from 1 to 10 joule per cm^2 for a period of time of between 2 - 8 minutes, whereby the irradiation induces the population of stem cells to differentiate.

[40] A method of inducing dentinogenesis in a subject, the method comprising exposing a tissue comprising a population of dental pulp stem cells to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from 1 to 10 joule

per cm^2 for a period of time of between 2 - 8 minutes, whereby the irradiation induces the population of dental stem cells to differentiate.

[41] A method of dental pulp capping in a subject, the method comprising exposing a tissue comprising an exposed dentine pulp to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from 1 to 10 joule per cm^2 for a period of time of between 2 - 8 minutes, whereby dentin is secreted.

[42] The methods of any one of paragraphs 38-41, wherein the irradiation is a laser irradiation.

[43] The method of paragraph 38, wherein the population of stem cells is found in a tissue in a subject.

[44] The method of any one of paragraph 38 or 41, wherein the tissue is damaged and is in need of repair.

[45] The method of paragraphs 38, 41 or 42, wherein the tissue comprises a wound, an exposed dental or a bone fracture.

[46] The method of any one of paragraphs 38-44, wherein the method used in for wound healing.

[47] The method of any one of paragraphs 38-44, wherein the stem cell is dental pulp stem cell.

[48] The method of paragraph 39 or 40, wherein the method is used for dental pulp capping.

[49] The method of any one of paragraphs 38-48, wherein the irradiation has a wavelength in the range of 800-820 nm.

[50] The method of paragraph 49, wherein the irradiation has a wavelength is 810 nm.

[51] The method of any one of paragraphs 38-50, wherein the period of time ranges from 2 to 8 minutes.

[52] The method of paragraph 51, wherein the period of time is 5 minutes.

[53] The method of any one of paragraphs 38-52, wherein the dosage of laser irradiation ranges from 1 to 5 joule per cm^2.

[54] The method of paragraph 53, wherein the dosage of laser irradiation is 3 joule per cm^2.

[55] The method of paragraph 38 further comprising contacting the population of stem cells with at least one metal ion prior to exposure to the irradiation.

[56] The method of paragraph 40 further comprising contacting the exposed dentine pulp with at least one metal ion prior to exposure to the irradiation.

[57] The method of paragraph 41 further comprising contacting the population of dental pulp stem cells with at least one metal ion prior to exposure to the irradiation.

[58] The method of any one of paragraphs 55-57, wherein the metal ion is a divalent metal ion.

[59] The method of paragraph 58, wherein the divalent metal is selected from a group consisting of lithium, barium, magnesium, copper, iron, manganese, and zinc.

[60] The method of any one of paragraphs 55-59, wherein a combination of divalent metal ions are used for contacting.

[61] The method of paragraph 60, wherein a combination consists of at least two and not more than five divalent metal ions.

[62] The method of any one of paragraphs 15- 61 further comprising contacting the population of dental pulp stem cells with at least an agent that activates the Wnt pathway prior to exposure to the irradiation.

[63] The method of any one of paragraphs 15-61 further comprising contacting the population of dental pulp stem cells with at least an agent that activates the Wnt pathway after exposure to the irradiation.

[0255] Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Definitions of common terms in molecular biology may be found in Benjamin Lewin, Genes IX, published by Jones & Bartlett Publishing, 2007 (ISBN-13:

9780763740634); Kendrew et al. (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8). Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0256] Unless otherwise stated, the present invention was performed using standard procedures known to one skilled in the art, for example, in Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (1982); Sambrook et al., Molecular Cloning: A Laboratory Manual (2 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (1989); Davis et al., Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (1986); Current Protocols in Molecular Biology (CPMB) (Fred M. Ausubel, et al. ed., John Wiley and Sons, Inc.), Current Protocols in Immunology (CPI) (John E. Coligan, et. al., ed. John Wiley and Sons, Inc.), Current Protocols in Cell Biology (CPCB) (Juan S. Bonifacino et. al. ed., John Wiley and Sons, Inc.), Culture of Animal Cells: A Manual of Basic Technique by R. Ian Freshney, Publisher: Wiley-Liss; 5th edition (2005), Animal Cell Culture Methods (Methods in Cell Biology, Vol. 57, Jennie P. Mather and David Barnes editors, Academic Press, 1st edition, 1998), Methods in Molecular biology, Vol.180, Transgenesis Techniques by Alan R. Clark editor, second edition, 2002, Humana Press, and Methods in Meolcular Biology, Vo. 203, 2003, Transgenic Mouse, editored by Marten H. Hofker and Jan van Deursen, which are all herein incorporated by reference in their entireties.

[0257] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[0258] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages will mean ±1%.

[0259] All patents and publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These

publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[0260] This invention is further illustrated by the following example which should not be construed as limiting. The contents of all references cited throughout this application, as well as the figures and table are incorporated herein by reference.

[0261] Those skilled in the art will recognize, or be able to ascertain using not more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

EXAMPLES

Example 1

Materials and Methods

[0262] An infrared laser (810 nm) was used in all studies, and fluorescent dyes were used to assess generation of reactive oxygen species (ROS). The ability of laser-generated ROS to activate Latent TGF-β1 was assessed using an ELISA and TGF-β responsive (p3TP-luc) reporter assay. The mechanism of activation of LTGF-β1 by ROS was assessed by change in conformation (circular dichroism) and protein modification (mutational analysis and biochemical assays). To assay biological impact, mouse pre-odontoblast cell line (MDPC-23) and primary human dental stem cells were cultured on 2D (plastic) and 3D (PLGA) scaffolds, irradiated with lasers and assessed for dentin induction by biomineralization assays (alkaline phosphatase, alizarin red staining), molecular assays (RTPCR and immunoblotting /staining) and calcium deposition (elemental analysis by SEM-EDAX).

[0263] To assess odontogenic differentiation, we first isolated dental stem cells from extracted teeth specimens, cultured them in 3D scaffold system and irradiated them with lasers. Differentiation to specific odontogenic phenotype was assessed by using biomineralization assays, molecular assays and elemental analysis for mineralization.

Results

[0264] To investigate the efficacy of low power laser (LPL) treatment in wound healing and regeneration and to promote dentinogenesis, the inventors analyzed the activation of latent transforming growth factor-β (TGF-β) by LPL.

[0265] A robust increase in reactive oxygen species (ROS) generation was observed with increasing laser dose (0.3 to 30J/cm^2 for 5min). Laser treatment was capable of activating LTGFβ-1, and the ROS-mediated activation mechanism involved oxidation of a specific methionine residue (m253), resulting in conformational change; this was confirmed by mutational analysis. Laser irradiation of pre-odontoblasts demonstrated activation of intracellular TGF-β signaling (phospho-Smad2), an increase in dentin-specific matrix proteins (Dentin Matrix Protein-1, Osteopontin and Osteomodulin), increased alkaline phosphatase activity, and calcium deposition. Specific inhibitors against ROS or TGF-β in above experiments eliminated these effects, implicating their causal relationship. This work demonstrates a novel molecular mechanism linking ROS generation by lasers and LTGF-β1 activation that is capable of inducing dentinogenesis.

[0266] Further, laser irradiation resulted in differentiation of dental stem cells into a mineralized odontogenic phenotype as assessed by activation of intracellular signaling, extracellular matrix production and calcium deposition.

Example 2

Materials and Methods

[0267] The following materials were procured from SIGMA-ALDRICH® (St Louis, MO): N-acetyl cysteine (NAC, a ROS scavenger), antimycin A, deuterium oxide, potassium oxide, hydrogen peroxide, ferrous perchlorate (II), hydrochloric acid, sodium hydroxide, HEPES, sodium chloride, Alizarin Red (1,2-dihydroxyanthraquinone), 4-methylbelliferyl phosphate (4-MUP) substrate, human placental alkaline phosphatase, poly vinyl alcohol, ethyl acetate, and ethanol, paraformaldehyde. The following materials were procured from the indicated supplier: Chelex-100 Resin (BIORAD®, Hercules, CA); MITOSOX RED™ (2-hydroxy-5-(triphenylphosphonium) hexylethidium), AMPLEX RED™ (N acetyl-3,7,-dihydroxyphenoxazine), CM-H_2DCFDA (chloromethyl-2',7'-dichlorofluorescein diacetate) and proxyl fluorescamine (5-(2-carboxyphenyl)-5-hydroxy-1-((2,2,5,5-tetramethyl-1-oxypyrrolidin-3-yl)methyl)-3-phenyl-2-pyrrolin-4-one), IAEDANS (1-Naphthalenesulfonic acid, 5-((2-((iodoacetyl)amino)ethyl)amino)) (MOLECULAR PROBES®, INVITROGEN™, Carlsbad, CA); Greiss Assay (PROMEGA®, Madison, WI); recombinant latent TGF-β1 and TGF-β1

(R&D SYSTEMS®, Minneapolis, MN); COOMASSIE™ Blue (G250) (Eastman Chemical, Kingsport, TN); TGF-βRI (Alk5) kinase inhibitor IV (SB431542) 4-[4-(3,4-Methylenedioxyphenyl)-5-(2-pyridyl)-1H-imidazol-2-yl]-benzamide hydrate and Smad3 inhibitor (6,7-dimethyl-2-[(2E)-3-(1-methyl-2-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl-prop-2-enoyl) -1,2,3,4-tetrahydro isoquinoline hydrochloride) (SIS3) (CALBIOCHEM®, EMD Chemicals, Gibbstown, NJ).

Cell Culture

[0268] Mv1Lu (Mink Lung Epithelium) cells (kind gift from Dan Rifkin, New York University, Langone Medical Center NYULMC), MDPC-23 (kind gift from Tatiana Bottero, University of Michigan), D1 (Mesenchymal stem cell) and 7F2 (Osteoblast) (ATCC, Manassas, VA) were cultured in complete media composed of 10% FBS, DMEM glutamax, penicillin (100 U/ml) - streptomycin (100 µg/ml) (GIBCO®, INVITROGEN™, Carlsbad, CA) in a 37°C incubator with 5% CO_2. Mouse embryonic fibroblasts (MEF) from TGF-$\beta1^{+/+}$ and TGF-$\beta1^{-/-}$ mice stably transfected with either full length or ROS-insensitive (m253a) latent TGF-β1 (kind gift of Mary-Helen Barcellos Hoff, NYULMC) were maintained in growth media supplemented with G418 (Gibco, Invitrogen, Carlsbad, CA). Human dental stem cells (hDSC) were isolated from the pulp and attached follicle from tooth samples obtained following Institutional Review Board approval (Children's Hospital Boston, Boston) using modifications to previously described protocols (Hao et al., 2002; Ruch et al., 1995). Briefly, tooth specimens were dissected aseptically and incubated with 4 ml of 0.25% Trypsin-EDTA (INVITROGEN™, Carlsbad, CA) at 37°C for 30 min. The solutions were pipetted vigorously to release cells and solution was passed through a cell strainer and cultured in complete media.

PLG scaffold fabrication

[0269] Macroporous poly (lactide-co-glycolide) (PLG) scaffolds were fabricated with a gas foaming - salt leaching technique as described previously (Harris et al., 1998; Mooney et al., 1996). Briefly, PLG (8mg) (85:15, 120 kDa copolymer, ALKERMES®, Waltham, MA) was mixed with the porogen, sodium chloride (150 mg, ground and sieved to particle size between 250 and 425 µm) and compression molded. The resulting disc was allowed to equilibrate within a high-pressure CO_2 environment, and a rapid reduction in pressure causes the polymer particles to expand and fuse into an interconnected structure. Salt was then leached from the scaffolds by immersion in water, yielding scaffolds that were 90 % porous. These were sterilized with 70%

ethanol, serum coated and cell seeded. Encapsulation efficiency and release were assessed with ELISAs or LCMS.

ROS assays (Reagents and probes are outlined in Table 1)

Serum solutions: ROS assays in Cell free system

[0270] Fetal bovine serum (FBS) (GIBCO®, INVITROGEN™, Carlsbad, CA) was diluted in molecular grade water (G BIOSCIENCES®, Maryland Heights, MO) and subjected to LPL irradiation at various fluences or treatment with specific ROS generating reagents (Table 1). The solutions were then immediately incubated with the following fluorescent dyes to assess specific ROS namely; MITOSOX RED™ (5 μM) for superoxide, AMPLEX RED™ (50 μM) for hydrogen peroxide and proxyl fluorescamine (100 μM) for hydroxyl radical. The Amplex assay was performed in the presence of horse-radish peroxidase (0.2U/ml). Fluorescence was assessed with a microplate reader (SYNERGY™ HT, BioTek® Instruments, Winooski, VT) and concentrations were estimated from a standard curve in each assay. In some experiments, pre-incubations with a ROS scavenger, NAC (1mM), for 30 min prior to LPL irradiation was performed. In other experiments, deuterium oxide was used to dilute the serum, in place of molecular grade water, to enhance detection of LPL generated ROS. To eliminate transition metals, FBS and molecular grade water were incubated with chelex 100 resin (5g/100ml) for 24 h on a rotator at 4°C and diluted prior to LPL irradiation as described previously (Barcellos-Hoff and Dix, 1996).

ROS detection in the presence of cells

[0271] Cells were seeded in 8-well chamber slide (NUNC®, Thermo Fisher Scientific, Waltham, MA) in complete media and allowed to attach overnight. The following day, LPL irradiation was performed at varying fluences and cells were probed with fluorescent dyes to assess specific ROS namely; MITOSOX RED™dye (5μM) for superoxide and CM-H_2DCFDA (10μM) for hydrogen peroxide. For both experiments, MITOTRACKER GREEN™ (500 nM) and MITOTRACKER RED™ (500 nM) were used to outline mitochondria respectively. Fluorescence was visualized using a confocal microscope (Olympus IX81, Centerway, PA) and imaging software IP lab (Ver 4.0, Exton, PA). The Greiss assay (PROMEGA®, Madison, WI) was performed as per manufacturers' protocol. Briefly, cells were incubated with 50 μl of sulphanilamide for 10 min followed by N-1-napthylethylenediamine dihydrochloride (NED)

under acidic conditions for another 10 min. Absorbance was read at 520 nm using a microplate reader.

Luciferase Reporter Assay

[0272] Stably transfected MvLu1 cells were kindly provided by Dr. Dan Rifkin. These cells are stably transfected with the plasminogen activator inhibitor promoter tagged to luciferase. These cells are known to be exquisitely sensitive to TGF-β inhibition, therefore, assay cell density and normalization of luciferase activity to total protein was performed.

Reporter assays in 2D

[0273] Mv1Lu cells were plated in a 24-well plate (40,000 cells/well) (NUNC®, Thermo Fisher Scientific, Waltham, MA) in complete media. After 4 hours, LPL irradiation at 3J/cm^2 or recombinant TGF-β1 (2.5 ng/ml) treatment in 0.2%FBS were performed. Some wells were pretreated with NAC (1mM) or SB431542 (10 μM) prior to LPL irradiation. Cells were lysed at 24 h in passive lysis buffer and luciferin substrate (PROMEGA®, Madison, WI) was added to evaluate luciferase activity with a microplate reader (SYNERGY™ HT, BioTek® Instruments, Winooski, VT).

Reporter assays in 3D

[0274] PLG (poly(lactide-co-glycolide)) scaffolds were fabricated as described previously, water leached, sterilized in 70% ethanol and serum coated for 30 min. MvLu1 cells were seeded onto the scaffolds (3 x 10^6 cells /ml) in complete media. Cells were allowed to attach for 30 min and scaffolds were then floated in complete media. After 4 h, LPL irradiation or TGF-β1 treatment were performed. Some scaffolds were pre-incubated with NAC (1mM) prior to LPL irradiation. Scaffolds were replaced in the incubator overnight. The following day, scaffolds were washed briefly in PBS, and coelenterazine (25 μg/ml) (NANOLIGHT™ Technology, Pinetop, AZ) was added to evaluate luciferase activity with a bioluminescence imaging system (Xenogen, Taconic). Further, to assess luciferase expression quantitatively, scaffolds were subsequently washed in PBS, lysed in passive lysis buffer (PROMEGA®, Madison, WI) and assayed in a microplate reader.

Pre-odontoblast 3D cultures

[0275] MDPC-23 (3 x 10^6 cells /ml) (kind gift from Tatiana Bottero, University of Michigan) were seeded in the PLG scaffolds in complete media. Cells were allowed to attach for

30 min and then floated in complete media. Following overnight incubation, LPL irradiation or TGF-β1 treatment was performed. Some scaffolds were pre-incubated with NAC (1 mM) or SB431542 (10 μM) for 30 min prior to LPL irradiation. Following LPL irradiation, the scaffolds were removed from their media and placed in fresh media in a 12-well plate with mineralizing supplements (β-glycerophosphate 10mM, dexamethasone 10nM and ascorbic Acid 20mM). Media was changed every 3 days and cultured for 21days. Scaffolds were analyzed for dentin ALP activity, extracellular matrix (ECM) induction and mineral deposition as described below.

Free Cysteine Screen

[0276] A screen for measuring free cysteines resulting from cleaved disulphide linkages was devised and shown in (Figure 3A). A fluorescent dye, 5-((2-[(iodoacetyl) amino] ethyl) amino) naphthalene-1-sulfonic acid (IAEDANS), binds free thiols producing a spectral shift with a strong fluorescence signal (excitation/emission at 336/490 nm and extinction coefficient 5700) (Johnson et al., 2007). Solutions were incubated with the IAEDANS dye and assessed for increase in fluorescence with a microplate reader. The IAEDANS tagged complexes were separated by gel electrophoresis, under native or reducing conditions, in 1D (based on charge/mass ratio) or 2D format (based on isoelectric points and then, charge/mass ratio). Tagged complexes could be identified either by immunoblotting for suspected candidates or by using a high throughput approach such as mass spectroscopy.

[0277] For the experiments, serum (70%) or latent TGF-β1 (1μg/ml) solutions were LPL irradiated at 3J/cm^2 and incubated with IAEDANS dye (250 μM) at 37°C for 30 min, and fluorescence was assessed with a microplate reader (SYNERGY™ HT and KC4™ software, BioTek® Instruments, Winooski, VT). These solutions were separated on a SDS reducing polyacrylamide gradient gel (4-20% Tris-Glycine PAGE, INVITROGEN™, Carlsbad, CA), visualized on a UV transilluminator (365 nm, HOEFER®, Hollistan, MA) and COOMASSIE™ stained for total protein. Other gels were transferred to a nitrocellulose membrane and subjected to immunoblotting for TGF-β.

Latent TGF-β1 transfected MEFs

[0278] MEFs with either wild type or m253a (ROS insensitive mutant) latent TGF-β1were allowed to become 80% confluent in 10-cm culture dishes (NUNC®, Thermo Fisher Scientific, Waltham, MA) and switched to 0.2% serum to allow them to condition the media (Figure 3G). The media were collected and assessed for total TGF-β1 (chemical activation) and

hydrogen peroxide (100 μM) activation of latent TGF-β1with an ELISA. In addition, LPL irradiation of the plates were performed at 3J/cm^2 and after 15 min, after which the cells were washed with PBS, lysed and subjected to immunoblotting for phospho-Smad2. As controls in these experiments, recombinant (active) TGF-β1 (2.5 ng/ml) was added to both cells to ensure signaling competency. In some cases, conditioned media was removed and treated with hydrogen peroxide (100 μM) before adding back to these cells. The low serum (0.2%) FBS reduces background serum latent and active TGF-β1 and after 24 hours of cell conditioning, the media would consist of secreted wild type or m253a (ROS-insensitive) latent TGF-β1 complexes.

Bradford Assay

[0279] Samples were subjected to a modified Bradford assay to estimate total protein as per manufacturer's instructions (BCA, Thermo Scientific Inc, Rockford, IL). Absorbance was measured at 560 nm with a microplate reader (SYNERGY™ HT and KC4™ software, BioTek® Instruments, Winooski, VT).

Semi-quantitative RT-PCR

[0280] At 7 days, cells were washed with PBS, lysed in TRIZOL® (INVITROGEN™, Carlsbad, CA) and total RNA was extracted with the RNEASY™ Mini kit (QIAGEN®, Valencia, CA) and reverse-transcribed by using SUPERSCRIPT™ III RT-PCR system (INVITROGEN™, Carlsbad, CA) according to the manufacturer's protocol. One microliter of cDNA sample was amplified by PCR gene-specific primers for specific genes as described previously (Table 2).

Immunoblotting

[0281] Cells were lysed in RIPA buffer (SIGMA-ALDRICH®, St Louis, MO) with Complete MINI™ protease inhibitor (ROCHE®, Indianapolis, IN). Lysates from cells in scaffolds and rat pulp tissue were prepared by mincing them with scissors in lysis buffer. This was followed by repeated sonication (Sonics, Newton, CT) in lysis buffer on ice. Lysates were centrifuged at 14000 rpm at 4°C for 20 min and total protein was estimated with a Bradfrod assay (BCA, Thermo Scientific Inc, Rockford, IL). Protein lysates were separated in precast Tris-glycine gels and transferred onto nitrocellulose membranes (INVITROGEN™, Carlsbad, CA). Blots were incubated with various primary antibodies (Table 3) at 4°C overnight. Following washes, blots were incubated with appropriate species-specific secondary antibody

(Jackson Immunoresearch Laboratories, West Groove, PA) and chemiluminescence (Thermo Scientific Inc, Rockford, IL) was detected by films (KODAK® MR, SIGMA-ALDRICH®, St Louis, MO).

ELISA

[0282] A TGF-β1 ELISA was performed as per the manufacturers' instructions (PROMEGA®, Madison, WI). Briefly, solutions were incubated in microplate wells coated with the capture antibody followed by the secondary antibody and colorimetric substrate. Absorbance was read on a microplate reader (SYNERGY™ HT and KC4™ software, BioTek® Instruments, Winooski, VT). To assess total TGF-β1, chemical activation (1N HCl for 10 min followed by 1.2N NaOH with 0.5M HEPES to neutralize for 10 min) was used performed prior to performing the ELISA.

Alizarin Red staining

[0283] Mineral deposits were assessed with Alizarin red staining as described previously (Paul et al., 1983). Briefly, cells were plated in 24-well plates (NUNC®, Thermo Fisher Scientific, Waltham, MA) in complete media and allowed to attach overnight followed by LPL irradiation at various fluences. Media was changed at 3 days. At 7 days, cells were washed with PBS and fixed with 4% paraformaldehyde followed by staining with 0.1% Alizarin Red solution for 10 min and washed thoroughly prior to being photographed.

Alkaline Phosphatase (ALP) Assay

[0284] Cells were lysed in lysis buffer and ALP activity was assessed as described previously (Sodek and Berkman, 1987). Briefly, cells were lysed and 10 μl was incubated with the 4-MUP (SIGMA-ALDRICH®, St Louis, MO) at 37°C for 30 min, fluorescence was assessed with a microplate reader, and the fluorescence was normalized to total protein with bradfords assay.

PLG Microsphere Synthesis and Release

[0285] PLG microspheres containing either SB431542, anti-TGF-β antibody (1D11) or TGF-β1 were prepared by a solvent evaporation method using a double emulsion as described previously (Cohen et al., 1991). Briefly, 100 μl of proteins dissolved molecular grade water were pipetted into 1 ml of 5% PLG in ethyl acetate and immediately sonicated (Sonics, Newtin, CT) for 1 min. The second emulsion composed of 1% polyvinyl acetate and 7% ethyl acetate

was then added and vortexed for 15 sec and finally added to a 1% polyvinyl alcohol solution with continuous stirring for 3 h at room temperature. The solutions were then filtered through a 0.2 micrometer filter (NALGENE®, Rochester, NY), collected by centrifugation 2000 rpm for 10 min (EPPENDORF®, Hauppauge, NY), freeze-dried for 16 h (LABCONCO®, Kansas city, MO) and stored at -20°C. To assess release, microspheres were suspended in PBS with 0.05% sodium lauryl sulfate (SDS) in microfuge tubes (EPPENDORF®, Hauppauge, NY) in a 37°C incubator on a rotator. At given time intervals, solutions were centrifuged and supernatant was analyzed for released substance using either ELISA or LCMS.

LC-MS

[0286] LC-MS studies were carried out with a 1290 Infinity LC interfaced with the 6140 Quadrupole MSD system (AGILENT® Technologies, Santa Clara, CA). The MS instrument was calibrated with NaI/CsI solution infused into the ESI interface using the automated tuning and a built-in calibrant delivery system. Samples were analyzed using the reverse-phase Eclipse Plus C18 RRHD column (1.8u, 2.1 i.d. x 50mm). The solvents were 0.1% ammonium hydroxide in water (solvent A) and 0.1% trifluoroacetic acid (TFA) in acetonitrile (solvent B). At a flow rate of 1 mL/min, 5 % B was held for 0.25 min, followed by a gradient over 4.25 min to 100 % B which was held for 0.44 min and column was returned to initial conditions for 0.06 min at 5% B. Samples were monitored using UV (210 nm and 254 nm), and selective ion monitoring (SIM) of m/z 386 in ES positive mode. Using the SIM signal, a standard curve of serially diluted SB431542 in methanol (HPLC grade, Baxter) was created and used for quantitation. The SIM chromatograms were integrated with LC-MSD Chemstation Software (Rev. B.04.02, AGILENT® Technologies, Santa Clara, CA).

Histological Analyses

[0287] The tooth samples were decalcified and processed routinely for paraffin embedded 4 μ serial sections. The sections were stained with hematoxylin and eosin, Masson-richrome or toluidine blue and examined with routine microscopy or polarizing illumination (NIKON®, Melville, NY).

Rodent model for Dentin repair

[0288] The rodent model for direct pulp capping was used as described previously (Simon et al., 2008). Briefly, animals were anesthetized (80-120 mg/kg ketamine; 5-10 mg/kg xylazine) (ip) and two cavity preparation were made on the occlusal aspect exposing the pulp of

the first maxillary molar tooth with a conventional hand piece (NSK, Savannah, GA), #1 round carbide bur (0.8 mm) (DENTSPLY™ Co, France) and portable dental unit (Dentport Dental Supply, New Smyrna beach, FL) . Low power laser (LPL) was used at a given fluence ($3J/cm^2$) for 5 min by placing the probe directly on the exposed pulpal tissue. Defects were restored with cement (CAVIT™, 3M, St. Paul, MN). Animals were allowed to recover for periods ranging from 1 to 12 weeks. For the loss of function groups, pulp was exposed and filled with TGF-βRI inhibitor microspheres for 12 h, re-instrumented, LPL irradiated and restored with specific microsphere and cement for routine follow up as above.

Laser

[0289] A 810nm GaAlAs laser diode system comprising a driver, a temperature controller, a cooling mount with a fiberoptic delivery system was used. (Newport, Irvine, CA) . Power density (irradiance, W/cm^2) was calibrated with a power meter (Newport, Irvine CA) to achieve various energy densities (fluence, J/cm^2).

Microcomputed tomography (μCT) imaging

[0290] Extracted rodent tooth specimens were individually analyzed for reparative dentin mineralization using a μCT instrument (NIKON® Metrology Inc, Brighton, MI). A transmission source at 90kV, 90 μAmps and a 4 sec exposure time per frame were used. Frames were optimized based on the sample size ranging from 2900 to 3300 frames. A CCD panel detector (Perkin-Elmer, Waltham, MA) with 2000 x 2000 pixels, 7.5 frames per second and pixel size of 200μm was used to capture and reconstruct images with an imaging software (Inspect-X and CT-Pro, NIKON® Metrology Inc, Brighton, MI). Images were analyzed using VGSTUDIO MAX™ version 2.0 (Volume Graphics, Heidelberg, Germany) for newly induced dentin repair as described previously (Paque et al., 2009; Sheng et al., 2010).

Ultra-structural analyses (SEM-EDS and Raman)

[0291] Scaffolds were prepared for ultrastructural analysis with a modified protocol to preserve mineral deposits (Chandler and Battersby, 1976). Briefly, scaffolds were fixed in 4% formaldehyde and 2% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.0) for 2 h followed by staining with 1% osmium tetroxide and 2% potassium pyroantimonate for 1 h at room temperature and subjected to critical point drying (Tousimis, Rockville, MD). Samples were sputter coated with platinum-palladium and assessed with SEM-EDS (EVO®-55, CARL ZEISS®, Thornwood, NY).

[0292] Tooth specimens were processed and the spatial distribution of organic and inorganic components was determined using Raman mapping as described previously (Xu et al., 2008; Lotinun S et al., 2010). Briefly, extracted teeth were cleaned and dehydrated in ethanol, infiltrated, and embedded without demineralization in methyl methacrylate. Undecalcified sections (10 μm thick) were cut with a LEICA® RM 2165 microtome (Heidelberg, Germany). The specimens were further manually polished under water with 600 grit SiC paper. A SENTERRA™ Raman microscope (BRUKER®, Billerica, MA) with a 785 nm laser operating at 50 mW was used to collect Raman spectra and images. It was built on an Olympus BX51 microscope, a motorized XYZ stage with a minimum step width of 100 nm, and a TE-cooled (-70°C) CCD camera with 1024×256 pixels. Raman imaging with the following parameters was used: 400 lines/mm grating, 140-μm confocal hole, 100-μm slit width and a 20X objective and spectra were obtained from 90 to 3500 cm^{-1} and with a 20-second integration time. Without additional spectral smoothing, the individual spectra were adjusted using multiple-point baseline correction (concave rubber band correction) and mapping spectra for imaging were adjusted using polynomial baseline correction. Representative raman spectra from enamel, dentin, cementum and bone were obtained. Characteristic peaks were assigned as described previously (Xu et al., 2008). Raman images based on the ratios of CH at 1450 cm^{-1} to ν1 of phosphate at 960 cm^{-1} were used to measure organic matrix to mineral content (Figure 8). For SEM-EDS, the calcium, phosphorous and oxygen weight percentages were normalized to their carbon content.

Statistical Analyses

[0293] Data was analyzed using EXCEL™ software (MICROSOFT®, Redmond, CA). Mean and standard deviations were calculated and a Student's T test was performed where $p<0.05$ denoted statistical significance.

Results

LPL induces dentin repair *in vivo*

[0294] To confirm low power laser (LPL) can promote dentin regeneration, LPL irradiation of the exposed pulp in the rat maxillary first molar was performed and reparative dentin induction was assessed with a high resolution microcomputed tomographic technique (data not shown). Increased dentin volumes at 12 weeks post-LPL irradiation as compared to non-irradiated controls were observed (Figure 1A). Histological evaluation with biochemical staining demonstrated increased reparative dentin induced by LPL irradiation (data not shown). As positive controls in these experiments, a calcium hydroxide dressing was used to ascertain

reparative dentin induction (data not shown). Reparative dentin is characterized by its distinct mineralized composition and anatomical location. The LPL-induced reparative dentin was assessed with energy dispersive spectroscopy revealing an intermediate mineral content between adjacent normal dentin and pulp matrix (Figure 1B). Further, Raman spectroscopy was performed to characterize the composition of normal tooth mineralized tissues including dentin, cementum, enamel and alveolar bone (Figure 8). Raman analyses demonstrated the lower matrix (CH, 1450cm^{-1}) and higher phosphate (P-O, 970 cm^{-1}) content of LPL-induced reparative dentin compared to adjacent native dentin that was deposited within and along the pulp walls in LPL treated teeth (data not shown).

Specific ROS are generated by LPL

[0295] To begin exploring the LPL photomechanism, ROS generation was first investigated in the present system. Four specific ROS were examined using fluorescent dyes (Table 1). In the presence of Mv1Lu cells, LPL was capable of inducing superoxide and hydrogen peroxide in a dose-dependent manner (data not shown). The use of a ROS scavenger, N-acetylcysteine (NAC), prevented ROS generation following LPL irradiation, demonstrating the specificity of the reporter dyes. Interestingly, previously reported LPL induction of nitric oxide was not observed in the present system (Figure 9A). In order to further delineate the photoabsorbers in this system, PBS diluted solution of serum were subjected to LPL irradiation. Robust generation of superoxide, hydrogen peroxide and hydroxyl radicals were noted in these experiments (Figures 2A-2C). LPL irradiation of dilute serum solutions, without cells, appeared to induce ROS in a linear, non-limiting manner (Figure 9B). However, a significant decrease in ROS generation was noted when cells were present at fluences above 10J/cm^2, probably due to induction of a potent anti-oxidant response. Substitution of water with deuterium, which enhances ROS lifetimes via a kinetic isotope effect, in dilute serum resulted in a significant increase in LPL-induced ROS detection (Figure 2D). Transition metals form core proton donor complexes allowing photon absorption. Depletion of metal ions by chelex resin treatment significantly decreased ROS generation following LPL irradiation (Figure 2E), implicating their role in mediating the photoactivation mechanism.

LPL activates serum latent TGF-β1

[0296] The downstream biological target of LPL generated ROS were next probed. Serum subjected to LPL irradiation demonstrated significant changes in conformation of complexes as assessed by circular dichorism (data not shown). ROS-induced cleavage of

disulphide linkages often changes protein conformation exposing binding or catalytic sites, altering its biological activity. LPL irradiated complexes were subjected to an assay that uses a fluorescent dye, IAEDANs which binds free cysteines (Figure 3A). Increased free cysteines were noted following LPL irradiation of serum (Figures 3B and 10A). These samples were then subjected to gel electrophoresis and demonstrated distinct LPL activated serum complexes on UV illumination (Figure 3C). Depleting or increasing ROS generation by altering serum milieu with chelex resin or deuterium respectively resulted in concurrent changes in levels of free cysteines following LPL irradiation (Figures 10B and 10C). This increased in free cysteines with the IAEDANs assay was also confirmed using the Ellmans assay (data not shown).

[0297] Among the diverse serum complexes amenable to LPL modulation, previous studies have implicated latent TGF-β1. The LPL irradiated samples assayed in the cysteine screen above were further probed by Western blotting. This identified TGF-β as a major LPL-modulated complex (Figure 3C). To confirm this observation, serum and recombinant latent TGF-β1 were subjected to LPL irradiation, and both demonstrated a dose dependent activation (Figures 3D and 10E). Recombinant latent TGF-β1 demonstrated increased free cysteines following LPL irradiation (Figure 3B). It is known that TGF-β activation mediated by a conformational change to the latent complex following integrin binding often results in a physiologically potent ligand and produces significant biological effects. The biological effects of LPL-generated, ROS-mediated activation in the present system was next examined. To assess biological activity, a reporter cell line, MvLu1, was used in the experiments. MvLu1 is stably transfected with a TGF-β early responsive gene, plasminogen activator inhibitor which is tagged to a luciferase gene (p3TP-luc). LPL irradiation increased luciferase activity in Mv1Lu cells indicating activation of the TGF-β transduction pathway (Figure 3E).

TGF-β1 activation of LPL is mediated by ROS

[0298] The p3TP reporter has smad binding elements (SBE) targeted by canonical TGF-β nuclear smad complexes but it also has AP elements amenable to ROS or other growth factor transactivation. To assess the specific role of ROS and TGF-β in p3TP reporter activation, pre-incubation with inhibitors for ROS (NAC) or TGF-βRI (SB431542) was performed. Both treatments diminished the ability of LPL to induce luciferase reporter activity (Figure 3E). Further, as individual ROS generated by LPL irradiation may have different propensities to activate latent TGF-β1, each species identified to be generated by LPL was examined separately. All three species namely, superoxide, hydrogen peroxide and hydroxyl radicals were noted to be capable of activating latent TGF-β complex (Figure 3F).

[0299] The precise mechanism of ROS mediated activation of latent TGF-β1 was then examined. TGF-β1 mammalian isoform, as opposed to TGF-β2 or TGF-β3, has been previously demonstrated to have a critical residue, a methionine at position 253 on the latency associated peptide, which confers ROS sensitivity. MEFs stably transfected with either full length wild type (WT LTGF-β1) or the ROS insensitive mutant (m253a LTGF-β1) were subjected to different treatments (Figure 3G). Cell conditioned media from the two transfected lines had equivalent secretion of latent TGF-β1 as assessed by routine chemical activation and ELISA (Figure 10F). These cells were then subjected to LPL irradiation and assessed for activation of TGF-β signaling by immunoblotting for phospho-smad2. The WT LTGF-β1 MEFs demonstrated a robust increase in phospho-smad2 levels following LPL irradiation while this was markedly lower in m253a LTGF-β1 MEFs (Figure 3H). Addition of recombinant TGF-β1 was able to robustly induce smad2 activation in m253a LTGF-β1 MEFs confirming integrity of TGF-β signal transduction pathway in these cells.

[0300] Hydrogen peroxide treatment of conditioned media from both cell lines demonstrated a similar activation profile with an ELISA and immunoblotting, noting deficient activation in the m253a LTGF-β1 (Figures 10F and 10G). LPL irradiation of TGF-β1-/- MEFs with both constructs (WT and m253a) demonstrated a similar activation profile except for increased baseline phospho-smad2 levels in the m253a LTGF-β1 MEFs, probably due to disrupted basal autocrine feedback (Figure 10H).These observations clearly demonstrate a direct causal link between LPL generated ROS and TGF-β1 activation.

LPL directs human dental stem cells and pre-odontoblast differentiation

[0301] As LPL irradiation was noted to enhance dentin regeneration (Figure 1) and given its key role in craniofacial tissue development, the effects of LPL-activated TGF-β1 on dentin differentiation was investigated. A unique multipotent niche for stem cells resides within the human dental pulp that contributes to its normal pathophysiological roles, specifically in dentin repair and regeneration. These cells possess broad pluripotent potential as demonstrated by their multi-lineage differentiation and characteristic cell surface marker profiles (substantially positive for CD44, CD90, CD106, CD117 and Stro-1, while being substantially CD45 negative). By being substantially positive or substantially negative means the cell are at least 90% positive or at least 90% negative.

[0302] Human dental stem cells (hDSCs) were isolated from extracted tooth specimens and assessed their surface markers representing a pluripotent stem cell state over multiple

passages (Figure 11A). These cells were used in differentiation experiments between 3 to 7 passages as their potency and stability has been shown to vary over passages. These cells were responsive to LPL irradiation and TGF-β1 treatments as indicated by phospho-smad activation and translocation (Figure 4A). Pretreatment with either ROS (NAC) or TGF-βRI (SB431542) inhibitors abrogated smad activation. These cells demonstrated robust ROS generation following LPL irradiation (data not shown). LPL irradiation resulted in a significant down regulation of expression of several cell surface markers: CD44, CD90, CD106, CD117 and Stro-1, indicating their transition from a pluripotent stem cell state (Figure 4B) to a more differentiated developmental state. Interestingly, LPL irradiation was also able to significantly down-regulate CD106, but not CD44 or 117, in a variety of mesenchymal stem cell line that could be prevented by pre-incubation with ROS (NAC) and TGF-βRI (SB431542) inhibitors prior to LPL irradiation (Figure 11B). Along with the loss of stem cell markers, LPL irradiated cells concurrently exhibited an increased expression of dentin differentiation markers, namely alkaline phosphatase (ALP), the dentin matrix markers dentin matrix protein 1 (DMP1), dentin sialoprotein (DSP), and osteopontin (OPN) (Figure 4C). These results indicate that LPL activation of TGF-β1 leads to dental stem cells (DSCs) differentiation to dentin matrix producing cells that could contribute to the dentin repair observed in the *in vivo* pulp capping model.

[0303] LPL induction of cell differentiation to an odontoblastic lineage was then examined using a pre-odontoblast cell line, MDPC-23. Fully differentiated, functional dentin is characterized by its unique extracellular matrix (ECM) composition that is elaborated by the odontoblasts, and is subsequently mineralized. Due to the limited numbers of hDSCs and their stability over higher passages in long term cultures, LPL directed dentin differentiation in two-dimensional (2D) and three-dimensional (3D) culture systems was investigated using MDPC-23. The responsiveness of these cells to LPL activated TGF-β was ascertained by immunoassaying for smad activation and nuclear translocation (Figures 5A and 5B). These cells generated ROS on being subjected to LPL irradiation (data not shown). Treatment with recombinant TGF-β1 induced a mineralizing phenotype, as indicted by increase in alkaline phosphatase (ALP) activity and alizarin red staining for calcium deposition (Figures 12A and 12B). These mineralization markers were also up-regulated following a dose dependent LPL irradiation of MDPC-23 cells (Figures 5C and 12C). Further, these changes could be diminished by the use of specific inhibitors for ROS (NAC) or TGF-βRI (SB431542) (Figures 5D and 12D).

[0304] Morphological differentiation is severely limited in 2D cultures in plastic dishes and many *in vitro* experimental models have been shown to unpredictably fail in translating to relevant *in vivo* mechanisms. To address this, an *in situ* 3D macroporous, polylactide-glycolide scaffolds system was engineered to provide spatio-temporally regulated presentation of soluble and insoluble cues to assess dentin differentiation (data not shown). The ability of LPL activated TGF-β1 to modulate cells in 3D was first examined by assessing their ability to induce Mv1Lu reporter luciferase activity (Figure 5E). MDPC-23 cells were seeded onto scaffolds and LPL irradiation was performed at 3J/cm^2 for 5 min followed by routine culture for 21days with regular media changes. LPL irradiation induced dentin differentiation of MDPC-23 cells in 3D culture as indicated by up-regulation of dentin matrix markers DMP1, OSAD and OPN, ALP activity and mineral deposition (Figures 5F-5H and 12H-K). These effects were abrogated to varying extents when LPL irradiation was performed following pre-incubation of ROS (NAC) or TGF-βRI (SB431542) inhibitors. Further, treatment with recombinant TGF-β1 or hydrogen peroxide directly also demonstrated robust induction of a mineralized dentin phenotype (Figure 12E-12G). These observations with hDSCs and pre-odontoblasts in 2D and 3D culture indicate a causal role of LPL-generated ROS mediated TGF-β1 activation in directing dentin differentiation.

TGF-β mediates LPL induction of dentin repair *in vivo*

[0305] Finally, as *in vitro* studies establish a role for LPL-ROS-TGF-β1 pathway in dentin differentiation, its role in LPL-induced dentin regeneration *in vivo* was examined. Two distinct interventional strategies were chosen to address this question. First, a chemical biology approach was pursued that utilized a controlled delivery of TGF-β reagents in the rat pulp capping model to assess DSC differentiation and mineralized dentin repair induction (Figure 6A). As the *in vitro* studies indicate that LPL irradiation can direct DSC differentiation, DSCs obtained from rat pulp was first examined to determine whether they behave as human DSCs, and are therefore amenable to LPL treatment. It was shown that rat pulp contained DSCs as demonstrated by their cell surface marker positive for CD44, CD106 and CD117 and negative for CD45 (data not shown). LPL irradiation of rat pulp decreased expression of these markers at 7 days consistent with LPL effects on hDSCs *in vitro* (Figure 6B). Controlled release of TGF-β1 directly via PLG microspheres also resulted in a similar decreased expression of these markers (data not shown). Further, sustained release of a small molecule inhibitor against TGF-βRI, SB431542, was achieved with PLG microspheres (Figure 13A). Microspheres were first placed on the exposed pulp overnight to block TGF-β responsiveness in the pulp cell population. In one

group, the pulp was re-exposed, and LPL irradiation was performed followed by reinsertion of these inhibitor microspheres. The presence of the TGF-β inhibitor prevented the down-regulation of DSC surface markers observed with LPL irradiation (Figure 6B). The use of a TGF-β neutralizing polyclonal antibody, 1D11, was able to similarly retain DSC marker expression in the rat pulp DSCs following LPL irradiation (Figures 13B and 13C). As LPL irradiation leads to a robust increase in reparative dentin in the rat pulp DSCs (Figure 1A), the role of TGF-β was also probed using controlled release of the TGF-βRI inhibitor, SB431542. Rat pulp was treated with either SB431542 alone or with LPL irradiation and SB431542, assessed with histology and high resolution microcomputed tomography at 12 weeks. Rat teeth demonstrated minimal reparative dentin induction in both groups (Figure 6C). A slight decrease in LPL and inhibitor group compared to inhibitor alone groups was noted but this was not statistically significant.

[0306] A second approach using transgenic mice was used to confirm the role of TGF-β1 in LPL dentin induction. TGF-β responsiveness of pulp-dentin cells was targeted by generation of a conditional knockout (coKO) mice by crossing a $DSPP^{Cre}$ with $TGF\text{-}\beta RII^{f/f}$ (Figures 6D and 13D). The gene that encodes the dentin sialophosphoprotein (DSPP), the most abundant non-collagenous protein present in dentin, also encoding two distinct matrix proteins, dentin sialoprotein (DSP) and dentin phosphoprotein (DPP). Its key role in dentin mineralization is highlighted by severe defects in mice and human dentin as a result of its mutation or deletions. The TGF-βRII is the specific receptor for TGF-β ligands and has very high affinity for TGF-β isoforms 1, 2 and 3. TGFβ-RII knockout mice have a lethal systemic inflammatory phenotype closely resembling the TGF-β knockout, supporting its key role in normal pathophysiology. The cells of the pulp-dentin complex expressing DSPP include the dental stem cells, pre-odontoblasts and mature odontoblasts that are all capable of potentially responding to LPL mediated reparative dentin induction (data not shown). Hence, the conditional knockout targets most the TGF-β responsive cells in the pulp. Pulp exposure followed by either LPL irradiation and filling or filling alone (control) in these coKO mice demonstrated minimal reparative dentin in both groups (Figure 6E). Similar experiments in $TGF\text{-}\beta RII^{f/f}$ were noted to have the usual LPL-induced reparative dentin response (Figure 13E). Taken together, data from both *in vivo* approaches inhibiting TGF-β signaling indicate its key role in mediating LPL induction of dentin regeneration.

Conclusion

[0307] The results of this study demonstrate a mechanism of LPL generated ROS that, in turn, activates latent TGF-β1. LPL irradiation of dental stem cells and pre-odontoblasts *in vitro* induced dentin differentiation and these effects were mediated via ROS and TGF-β1. Further, LPL irradiation induced dentin repair in vivo in rodent models and this effect was specifically mediated via the TGF-β1 pathway.

[0308] The references cited herein and throughout the specification are incorporated herein by reference in their entirety.

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Paul, H., et al. (1983) Arthritis Rheum., 26, 191-200.

Ruch, J.V., et al. (1995) Int. J. Dev. Biol., 39, 51-68.

Sodek, J., and Berkman, F.A. (1987) Methods Enzymol., 145, 303-324.

Sun, Z.L., et al. (1998) Connect Tissue Res., 37, 251-261.

Table 1

Species	Reagents used to generate	Probe used to detect	Method (Ex/Em or Abs)	Reagent Source
Superoxide	Potassium Oxide (100uM) Antimycin A (500nM)	MitoSOX Red™ (5uM)	510/580 nm	Sigma, Invitrogen
Hydrogen Peroxide	Hydrogen Peroxide (30%w/w)	Amplex UltraRed™ (50uM) CM-H_2DCFDA (10uM)	560/590 nm 490/520 nm	Sigma, Invitrogen
Hydroxyl radical	Ferrous Perchlorate (100uM) & Hydrogen Peroxide (1mM)	Proxyl Fluorescamine (100uM)	390/480 nm	Sigma, Invitrogen
Nitric Oxide	Sodium Nitrite (100mM)	Sulphanilamide (1%) & N-1-napthylethylenediamine dihydrochloride (NED) (0.1%)	520nm	Promega

Table 2. Semi-quantitative PCR

Gene	Forward Primer	Reverse Primer	Method	Reference
DMP1	5′-TCCTTGTGTTCCTTTGGGGG-3′ (SEQ. ID. NO. 1)	5′-GGGGTATCTTGGGCACTGTTTTC-3′, (SEQ. ID. NO. 2)	94 - 45 60 - 45 72 – 45 x 38 cycles = 341bps	(Kobayashi et al., 2006)
DSPP	5′-AACAGCAGTGAGGAAAACGGTG-3′ (SEQ. ID. NO. 3)	5′-GTGTAAGAAGCATCTCCACGGC -3 (SEQ. ID. NO. 4)	94 –30sec 62 – 60sec 72 – 60sec x 36cycles = 875bps	Kobayashi et al., 2006
OPN	5'-TTGCAGCCTTCTCAGCCAA-3' (SEQ. ID. NO. 5)	5'-CAAAAGCAAATCACTGCAATTCTC-3' (SEQ. ID. NO. 6)	94 - 45sec 60 - 45sec 72 - 45sec x 36 cycles = 146bps	(Chen et al., 2007)
ALP	5'-ACGTGGCTAAGAATGTCATC-3' (SEQ. ID. NO. 7)	5'-CTGGTAGGCGATGTCCTTA-3', (SEQ. ID. NO. 8)	94-15 55-15 72-60 x 38cycles = 450bps	(Chen et al., 2007)
GAPDH	5'-ACCACAGTCCATGCCATCAC-3' (SEQ. ID. NO. 9)	5'-TCCACCACCCTGTTGCTGT-3' (SEQ. ID. NO. 10)	94 - 45sec 60 - 45sec 72 - 45sec x 30 cycles = 500bps	(Chen et al., 2007)

Table 3. Antibodies

Antigen	Source	Techniques	Dilution/Comments	Comments
DMP1	Takara	IB	1:1000	37, 57Kda
OSAD (OM)	R&D Systems	IB	1:500	85Kda
OPN	Hybridoma Bank	IB	1:500	60Kda
Actin	Chemicon	IB	1:10000	43KDa
Stro-1	Santa Cruz Biotechnology	IF	1:100	------
CD99	Santa Cruz Biotechnology	IF	1:100	------
CD44	Cell Signaling	IB	1:1000	80Kda
CD45	Cell Signaling	IB	1:1000	180Kda (TrisAcetate gel)
CD106 (Rat)	R&D Systems	IB	1:1000	110Kda
CD106 (Human)	R&D Systems	IB	1:1000	110Kda
CD117	Cell Signaling	IB	1:1000	120/140Kda
PhosphoSmad2	Cell Signaling	IB	1:1000	60Kda
PhosphoSmad2/3	Cell Signaling	IF	1:200	------
TGF-β	R&D Systems	IB	1:500	12.5, 25, 105Kda

What is claimed:

1. A dental treatment system for inducing dentinogenesis, the system comprising:

a source of irradiation, the source being adapted to produce irradiation having a wavelength in the range of 780 nm to 840 nm and a power output in the range of 5 mW to 300 mW;

a control element controlling the source of irradiation to turn the source of laser irradiation on and to turn the source of laser irradiation off; and

a timer operatively connected to the control element to activate the source of irradiation to produce irradiation for a pre-determined time period and then deactivate the source of irradiation from producing radiation.

2. The dental treatment system of claim 1 further comprising a collimator coupled to the source of irradiation and collimating the irradiation produced by the source of irradiation.

3. The dental treatment system of claim 1 or 2 further comprising a switch element operatively connected to and controlling the timer.

4. The dental treatment system of claim 3 wherein the switch element is a foot operated switch.

5. The dental treatment system of claim 1, 2 or 3, wherein the wavelength is between 800 nm to 820 nm.

6. The dental treatment system of claim 5, wherein the wavelength is 810 nm.

7. The dental treatment system of any one of claims 1-6, wherein the pre-determined time is between 2 to 8 minutes.

8. The dental treatment system of claim 7, wherein the pre-determined time is about 5 minutes.

9. The dental treatment system of any one of claims 1-7, wherein the timer controls the source of irradiation to apply a dosage of between 1 – 10 J/cm^2 of irradiation to a target location.

10. The dental treatment system of claim 8, wherein the dosage is 3 J/cm^2.

11. The dental treatment system of any one of claims 1 -10, wherein the source of irradiation produces laser irradiation

12. The dental treatment system of any one of claims 1 -10, wherein the source of irradiation includes a diode laser.

13. The dental treatment system of any one of claims 1 -10, wherein the source of irradiation includes a laser selected from the group including gas lasers, dye lasers, semiconductor lasers and solid state lasers.

14. A dental laser system for inducing dentinogenesis in a target, the system comprising:

a source of laser irradiation, the source being adapted to produce laser irradiation having a wavelength of 810 nm;

a control element controlling operation of the source of irradiation to turn the source of laser irradiation on and to turn the source of laser irradiation off; and

a timer operatively connected to the control element to turn the source of laser irradiation on for a pre-determined time period and then turn the source of laser irradiation off and wherein the pre-determined time period is determined to apply a dose of 3 J/cm^2 on the target.

15. A method of stimulating dentinogenesis in a subject, the method comprising exposing a tissue comprising a population of dental pulp stem cells to an irradiation output from the dental treatment system or dental laser system of any one of claims 1-14, whereby the dental pulp stem cells is induced to differentiate to odontoblasts.

16. A method of dental pulp capping in a subject, the method comprising exposing a tissue comprising an exposed dental pulp to an irradiation output from the dental treatment system or dental laser system of any one of claims 1-14, whereby increased dentin is secreted.

17. The method of claim 15 further comprising contacting the population of dental pulp stem cells with a metal ion prior to exposure to the irradiation.

18. The method of claim 16 further comprising contacting the exposed dental pulp with a metal ion prior to exposure to the laser irradiation.

19. The method of claim 17 or 18, wherein the metal ion is a divalent metal ion.

20. The method of claim 19, wherein the divalent metal is selected from a group consisting of lithium, barium, magnesium, copper, iron, manganese, and zinc.

21. A method comprising:

    a. contacting a population of stem cells with an effective amount of at least one metal ion; and

    b. exposing the population of stem cells in step (a) to one or more doses of irradiation sufficient to induced differentiation of stem cells, wherein each dose of irradiation is of a pre-determined period of time resulting in the application of a pre-determined dose of irradiation to the tissue;

    whereby the irradiation induces the population of stem cells to differentiate.

22. The methods of claim 21, wherein the irradiation is a laser irradiation.

23. The method of any one of claims 21-22, wherein the population of stem cells is found in a tissue in a subject.

24. The method of claim 23, wherein the tissue is damaged and is in need of repair.

25. The method of any one of claims 21-24, wherein the tissue comprises a wound, an exposed dental pulp or a bone fracture.

26. The method of any one of claims 21-25, wherein the at least one metal ion is a divalent metal ion.

27. The method of claim 26, wherein the divalent metal ion is selected from a group consisting of lithium, barium, magnesium, copper, iron, manganese, and zinc.

28. The method of any one of claims 21-27, wherein the irradiation has a wavelength in the range of 780-910 nm.

29. The method of claim 28, wherein the irradiation has a wavelength is 780 nm to 840 nm.

30. The method of claim 28 or 29, wherein the irradiation has a wavelength is 810 nm.

31. The method of claims 21-30, wherein the period of time ranges from 2 to 8 minutes.

32. The method of claim 31, wherein the period of time is about 5 minutes.

33. The method of any one of claims 21-32, wherein the one or more doses of irradiation individually provides a dosage that ranges from 0.3 to 30 joule per cm^2.

34. The method of claim 33, wherein the dosage of irradiation is 3 joule per cm^2.

35. The method of any one of claims 21-34, wherein the one or more doses of irradiation ranges from 1 to 10.

36. The method of any one of claims 21-35, wherein the method is used in for wound healing.

37. The method of any one of claims 21-37, wherein the stem cell is dental pulp stem cell.

38. The method of claim 37, wherein the method is used for dental pulp capping.

39. A method of inducing differentiation of stem cells, the method comprising exposing a population of stem cells to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from 1 to 10 joule per cm^2 for a period of time of between 2 - 8 minutes, whereby the irradiation induces the population of stem cells to differentiate.

40. A method of inducing dentinogenesis in a subject, the method comprising exposing a tissue comprising a population of dental pulp stem cells to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from 1 to 10 joule per cm^2 for a period of time of between 2 - 8 minutes, whereby the irradiation induces the population of dental stem cells to differentiate.

41. A method of dental pulp capping in a subject, the method comprising exposing a tissue comprising an exposed dentine pulp to a dose of irradiation for a period of time, wherein the irradiation has a wavelength in the range of 780 nm to 840 nm ranges, and provides an irradiation dosage ranging from 1 to 10 joule per cm^2 for a period of time of between 2 - 8 minutes, whereby dentin is secreted.

42. The methods of any one of claims 38-41, wherein the irradiation is a laser irradiation.

43. The method of claim 38, wherein the population of stem cells is found in a tissue in a subject.

44. The method of claim 38 or 41, wherein the tissue is damaged and is in need of repair.

45. The method of claims 38, 41 or 42, wherein the tissue comprises a wound, an exposed dental or a bone fracture.

46. The method of any one of claims 38-44, wherein the method used in for wound healing.

47. The method of any one of claims 38-44, wherein the stem cell is dental pulp stem cell.

48. The method of claim 39 or 40, wherein the method is used for dental pulp capping.

49. The method of any one of claims 38-48, wherein the irradiation has a wavelength in the range of 800-820 nm.

50. The method of claim 49, wherein the irradiation has a wavelength is 810 nm.

51. The method of any one of claims 38-50, wherein the period of time ranges from 2 to 8 minutes.

52. The method of claim 51, wherein the period of time is 5 minutes.

53. The method of any one of claims 38-52, wherein the dosage of laser irradiation ranges from 1 to 5 joule per cm^2.

54. The method of claim 53, wherein the dosage of laser irradiation is 3 joule per cm^2.

55. The method of claim 38 further comprising contacting the population of stem cells with at least one metal ion prior to exposure to the irradiation.

56. The method of claim 40 further comprising contacting the exposed dentine pulp with at least one metal ion prior to exposure to the irradiation.

57. The method of claim 41 further comprising contacting the population of dental pulp stem cells with at least one metal ion prior to exposure to the irradiation.

58. The method of any one of claims 55-57, wherein the metal ion is a divalent metal ion.

59. The method of claim 58, wherein the divalent metal is selected from a group consisting of lithium, barium, magnesium, copper, iron, manganese, and zinc.

60. The method of any one of claims 55-59, wherein a combination of divalent metal ions are used for contacting.

61. The method of claim 60, wherein a combination consists of at least two and not more than five divalent metal ions.

62. The method of any one of claim 15-61 further comprising contacting the population of dental pulp stem cells with at least an agent that activates the Wnt pathway prior to exposure to the irradiation.

63. The method of any one of claim 15-61 further comprising contacting the population of dental pulp stem cells with at least an agent that activates the Wnt pathway after exposure to the irradiation.

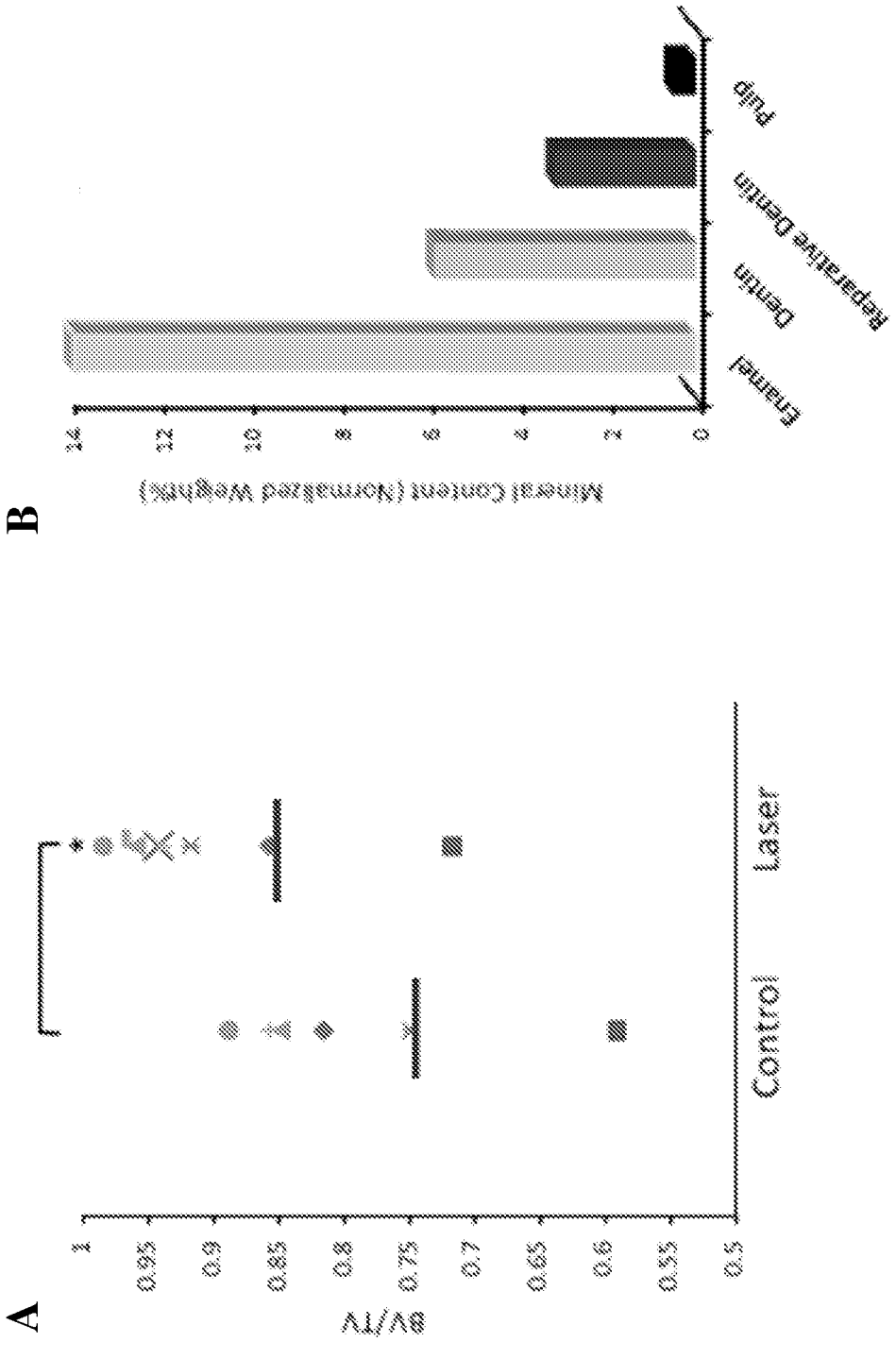

A
BV/TV
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
Control
Laser
B
Mineral Content (Normalized Weights)
14
12
10
8
6
4
2
0
Enamel
Dentin
Reparative Dentin
Pulp

FIG. 1

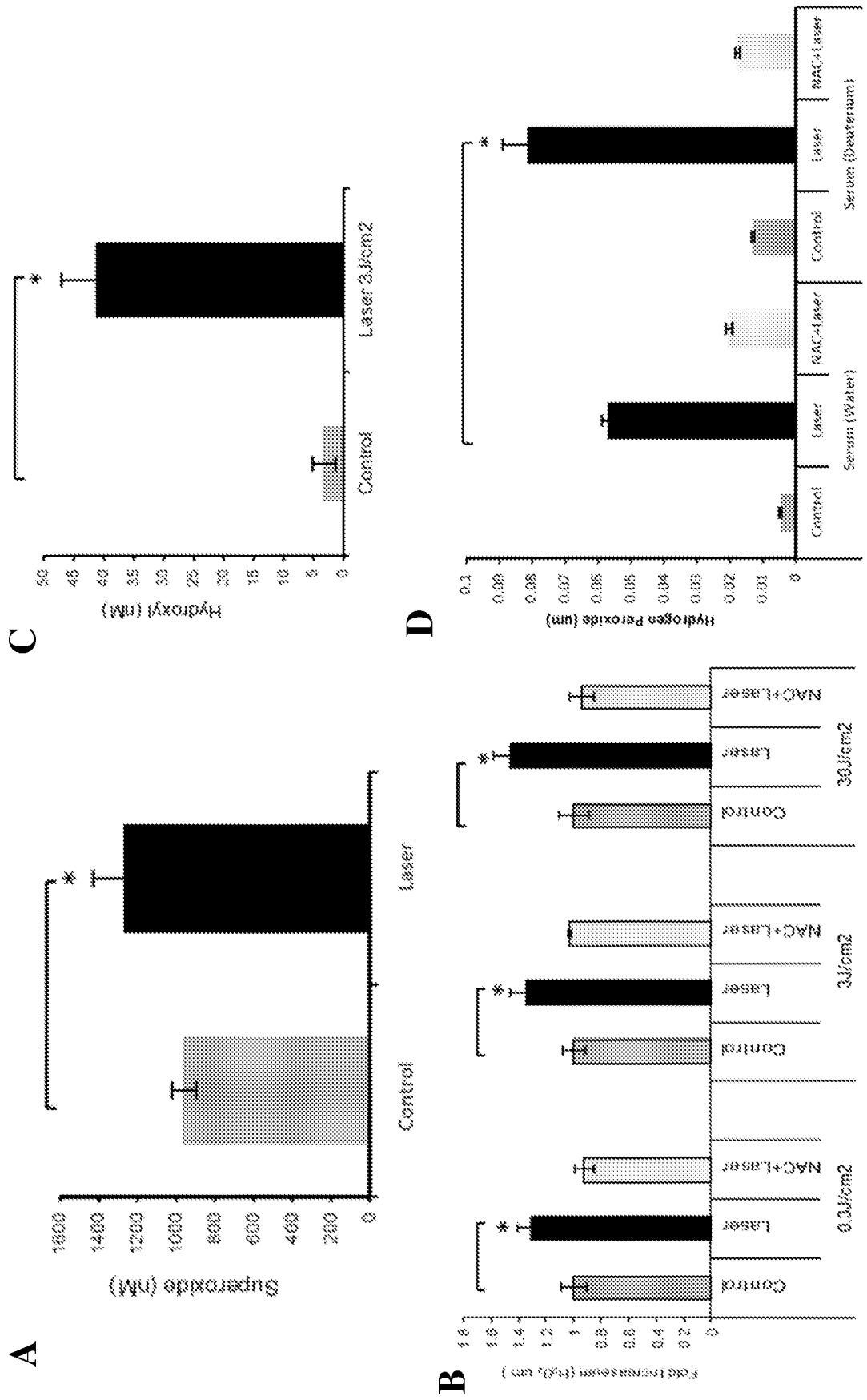
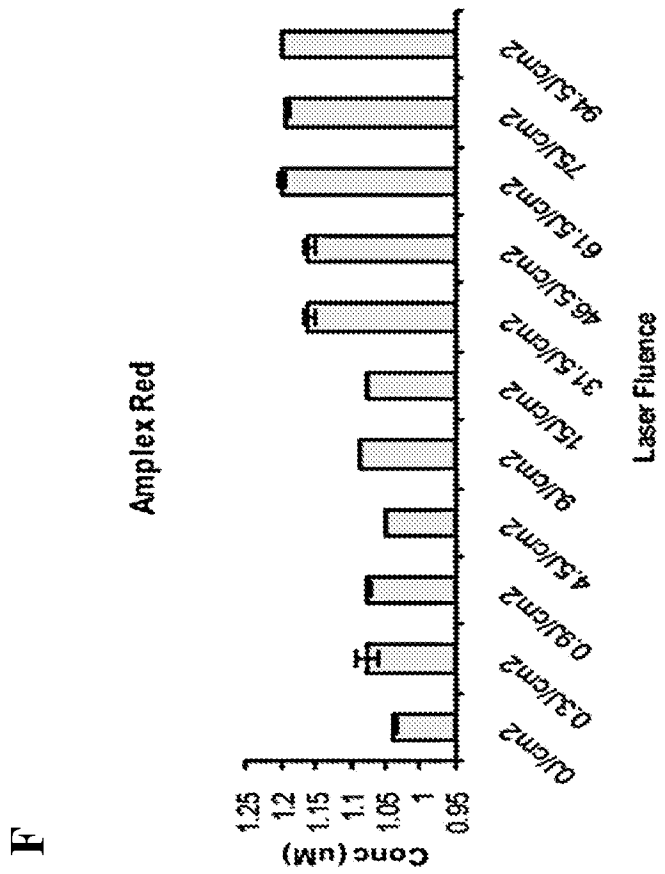
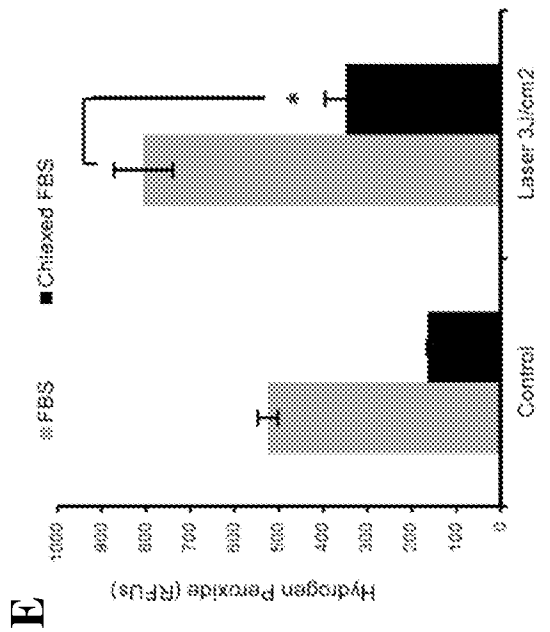

A
Superoxide (nM)
Control
Laser
B
Control
Laser
NAC+Laser
0.3J/cm2
3J/cm2
30J/cm2
C
Hydroxyl (nM)
Control
Laser 3J/cm2
D
Hydrogen Peroxide (um)
Control
Laser
NAC+Laser
Serum (Water)
Serum (Deuterium)

FIG. 2





G

ROS species	Laser
Superoxide	38.06 + 4.07nM
Hydrogen Peroxide	57+ 18uM
Hydroxyl Radical	305.76 + 100.85nM

FIG. 2 continued

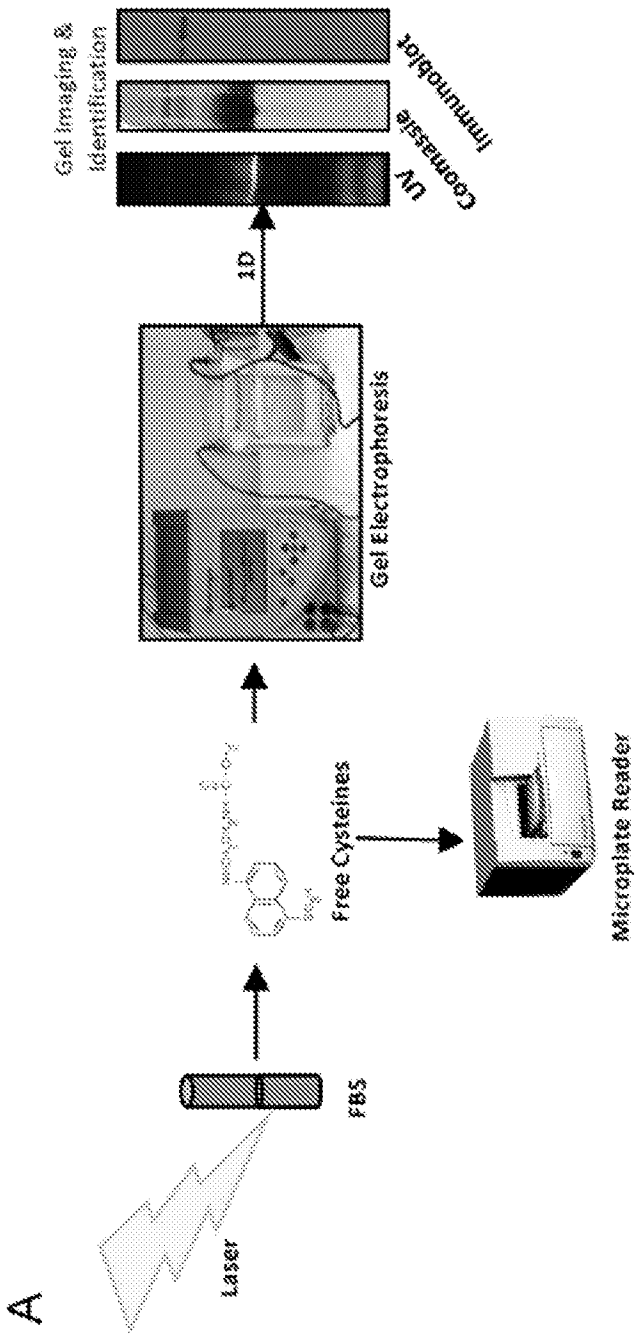

A
Laser
FBS
Free Cysteines
Micropiate Reader
Gel Electrophoresis
1D
UV
Coomassie
Immunoblot
Gel Imaging &
Identification

FIG. 3

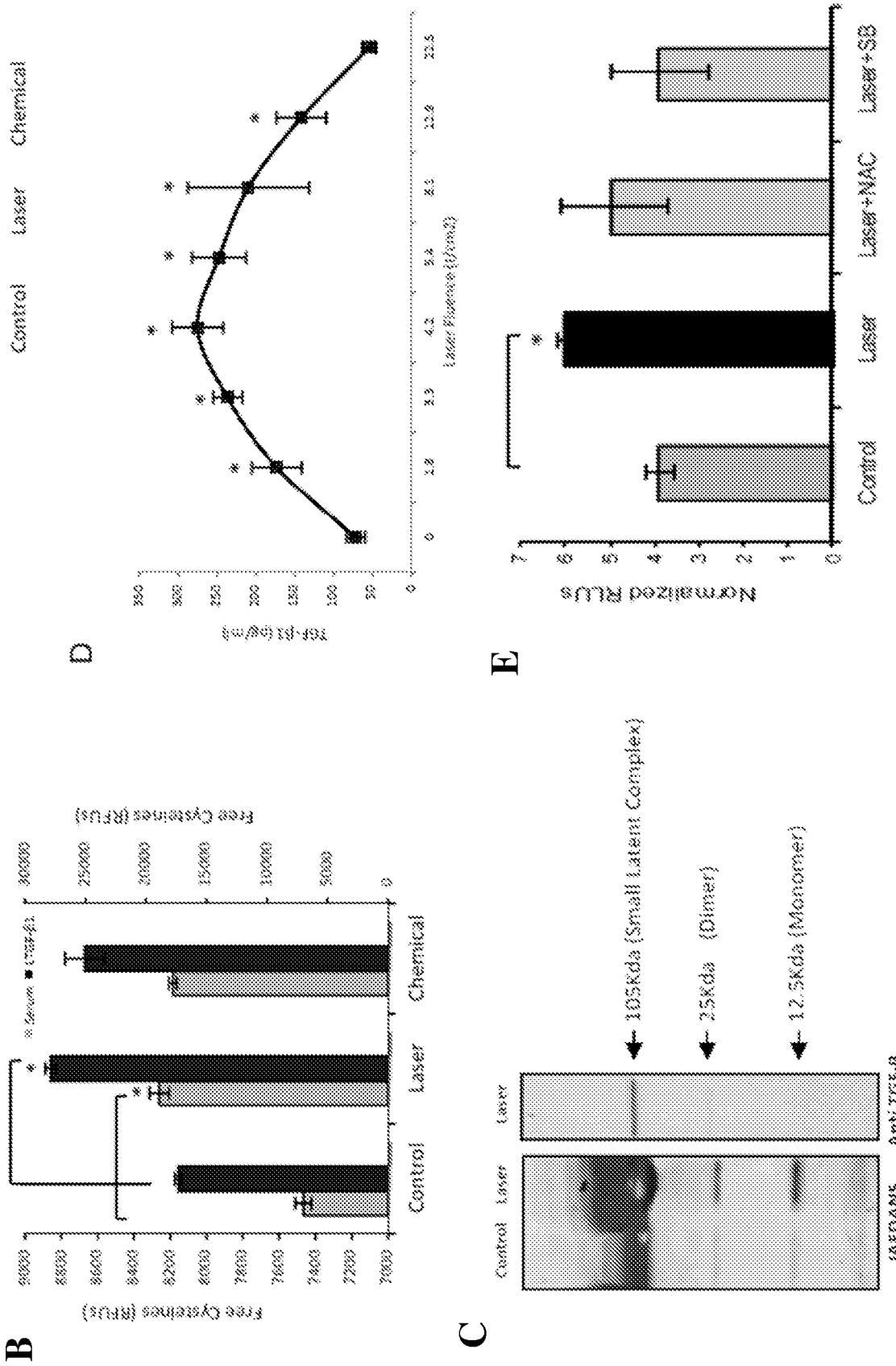


FIG. 3 continued

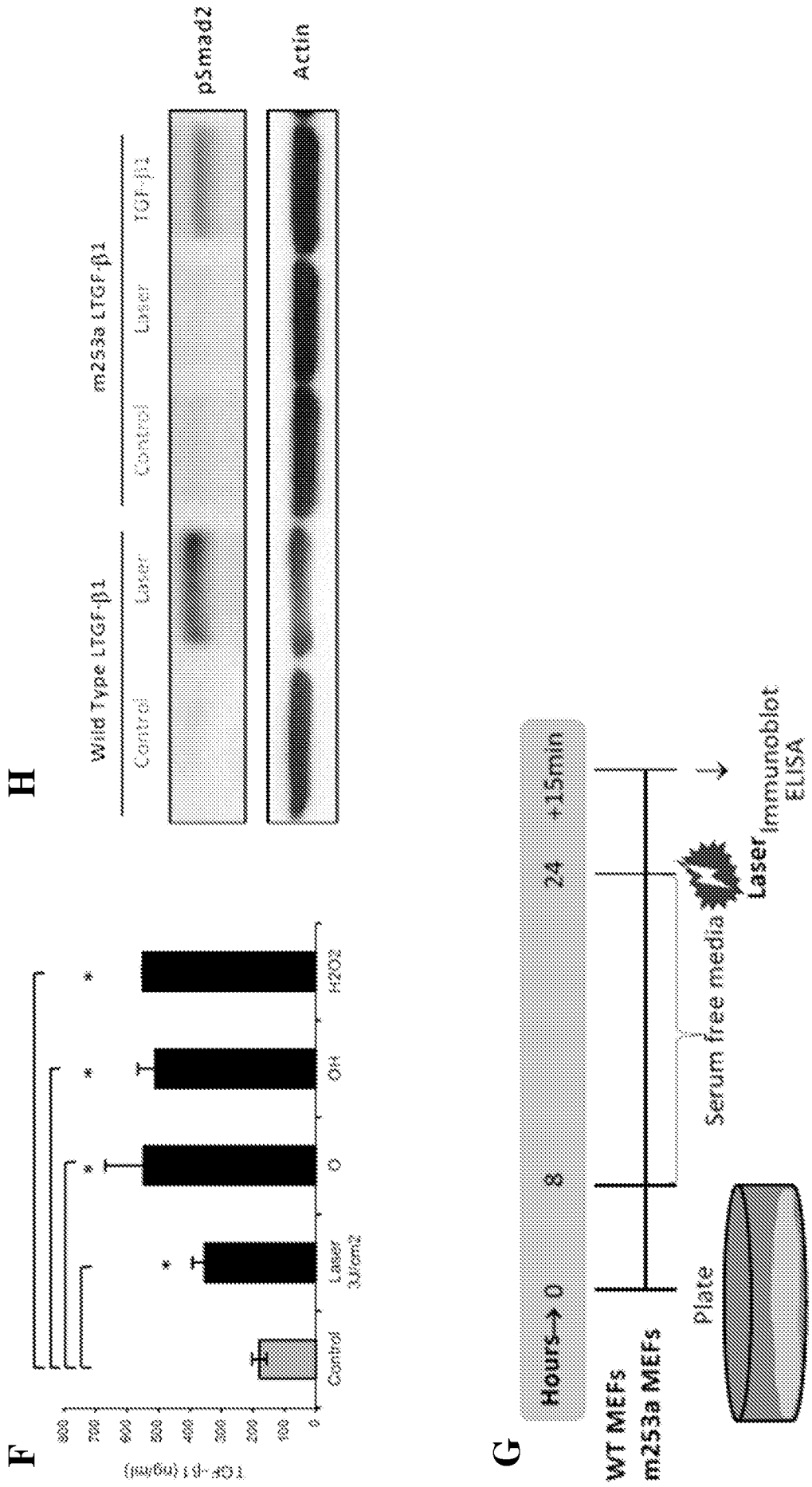
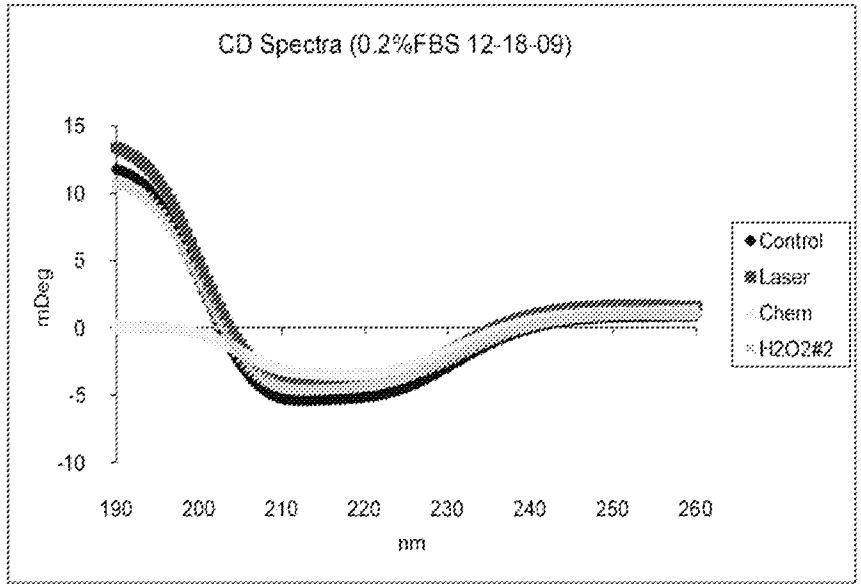
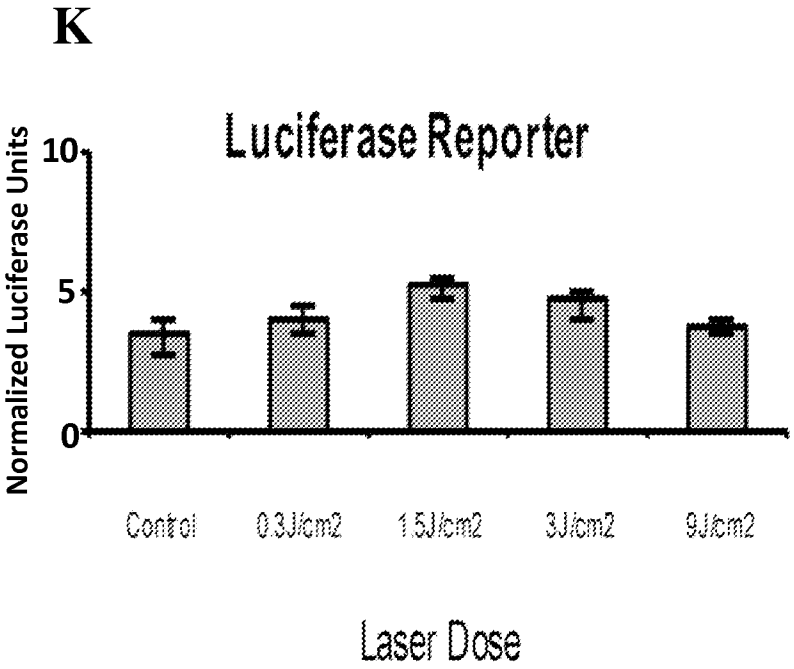


FIG. 3 continued





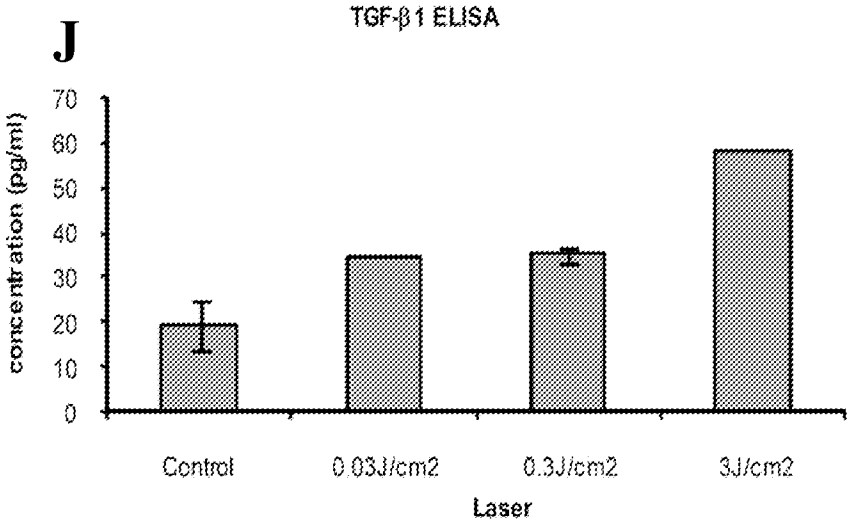


FIG. 3 continued

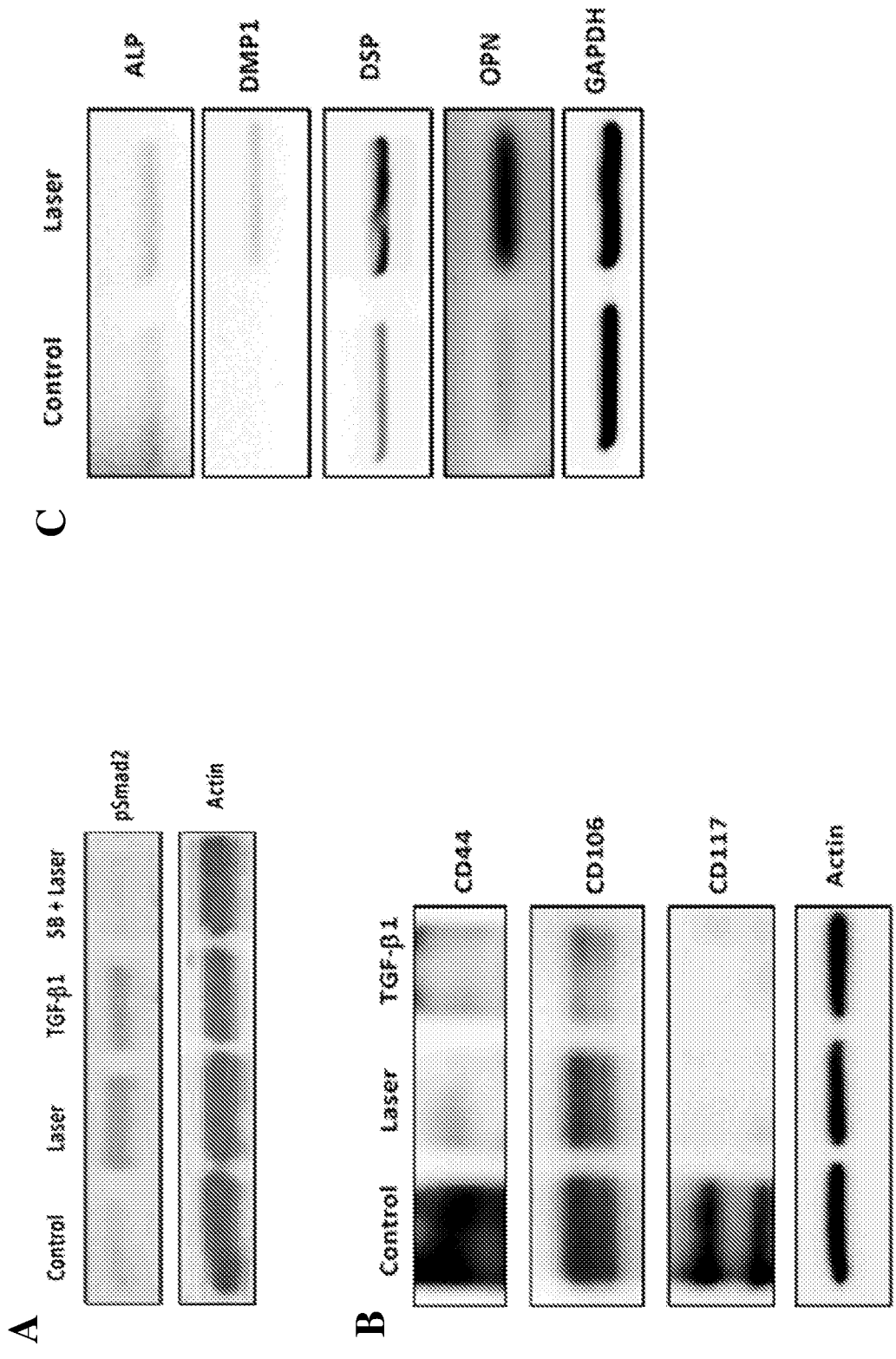

A
Control
Laser
TGF-β1
SB + Laser
pSmad2
Actin
B
Control
Laser
TGF-β1
CD44
CD106
CD117
Actin
C
Control
Laser
ALP
DMP1
DSP
OPN
GAPDH

FIG. 4

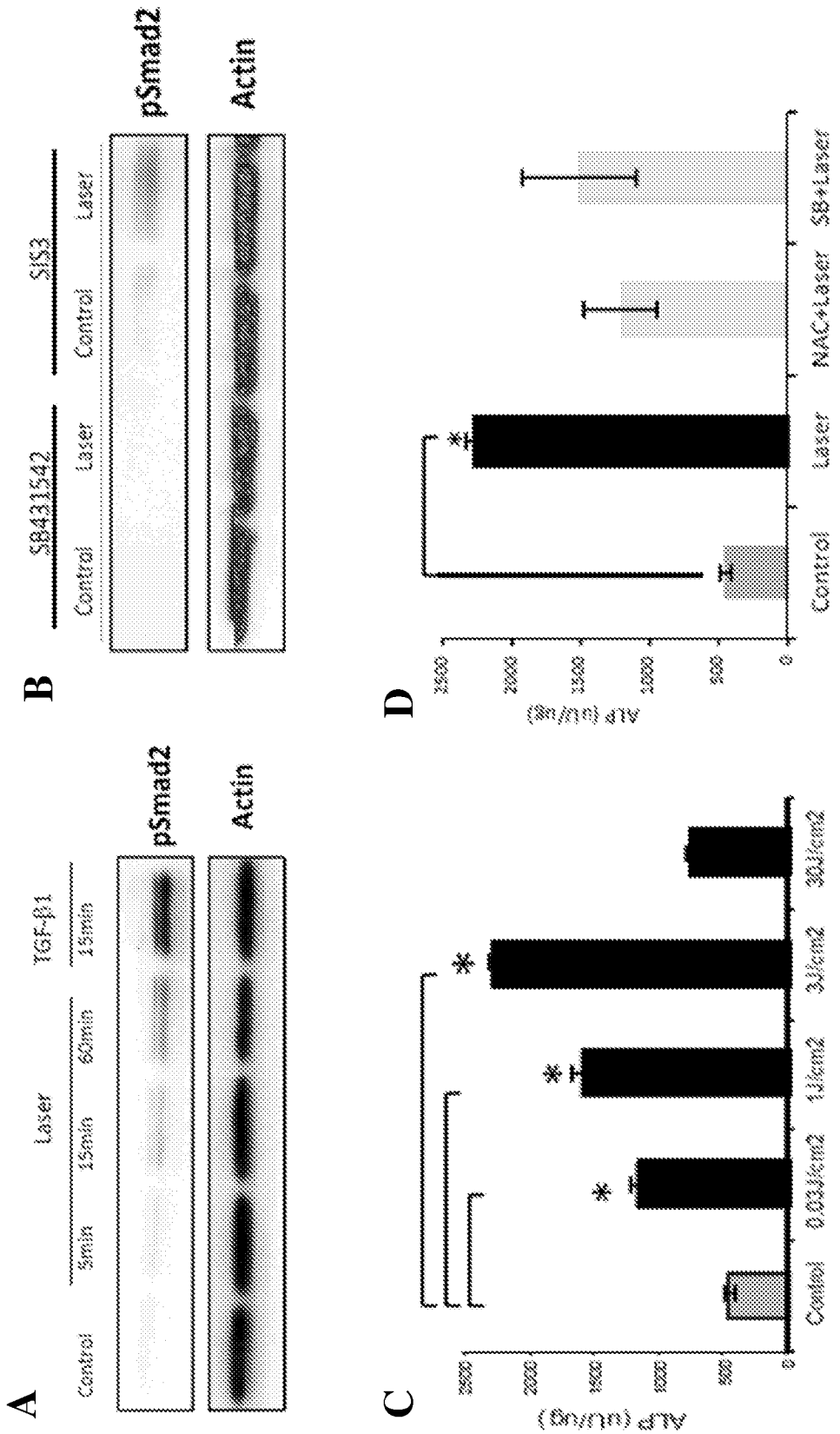

A
Laser
TGF-β1
Control
5min
15min
60min
15min
pSmad2
Actin
B
SB431542
SIS3
Control
Laser
Control
Laser
pSmad2
Actin
C
ALP (u/ug)
2500
2000
1500
1000
500
0
Control
0.03J/cm2
1J/cm2
3J/cm2
30J/cm2
D
ALP (u/ug)
2500
2000
1500
1000
500
0
Control
Laser
NAC+Laser
SB+Laser

FIG. 5

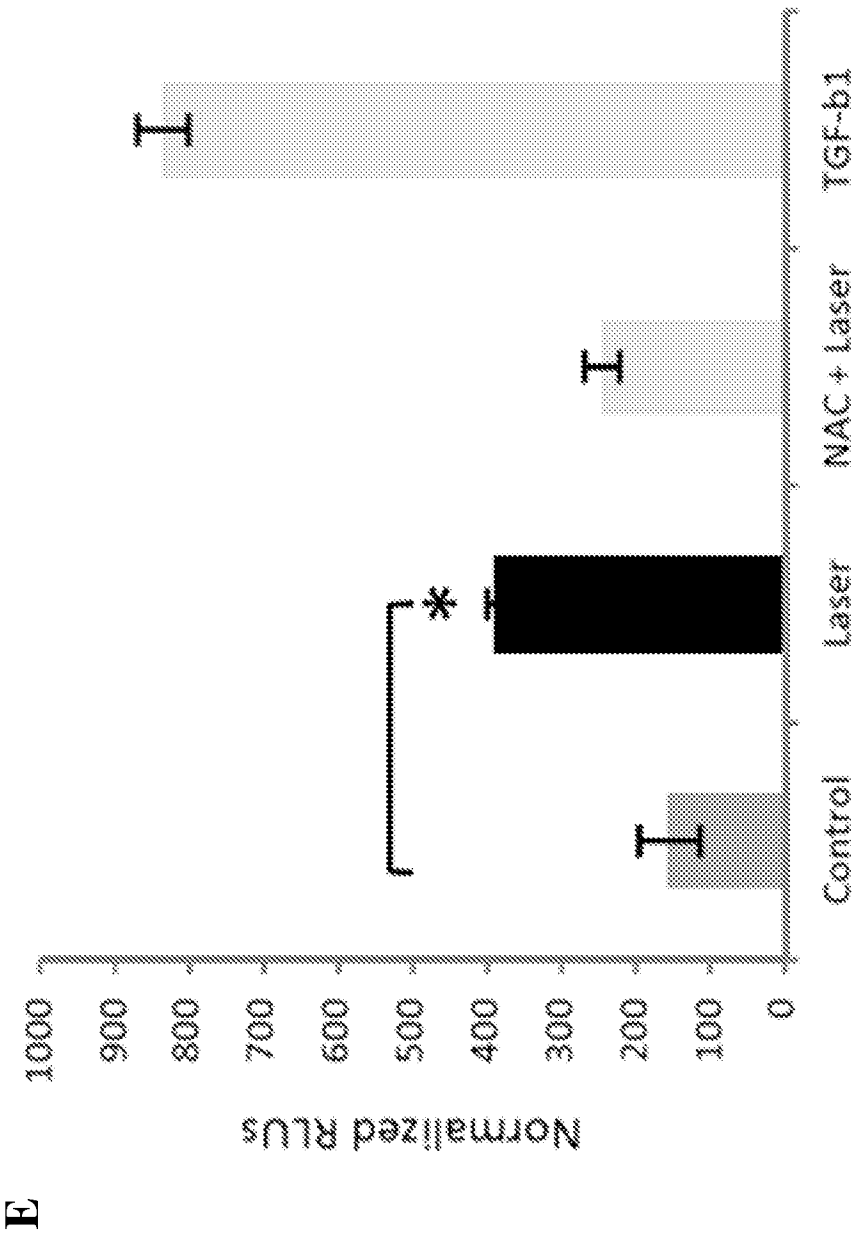


FIG. 5 continued

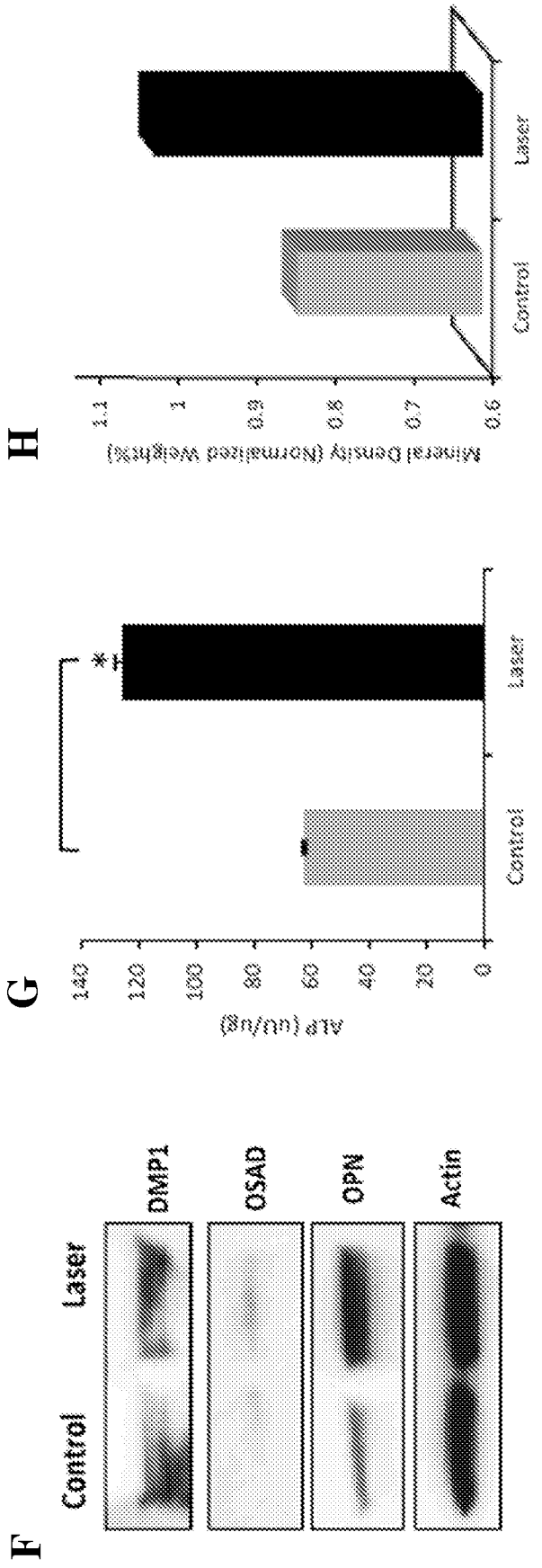


FIG. 5 continued

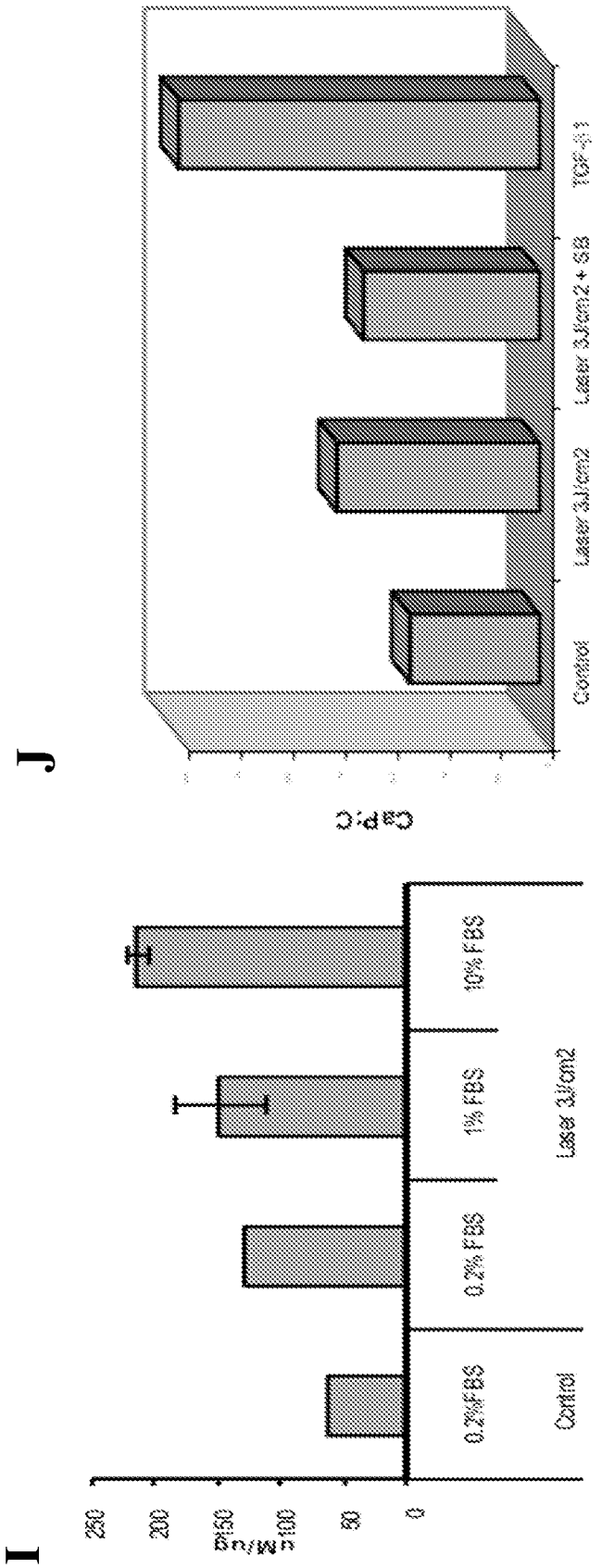


FIG. 5 continued

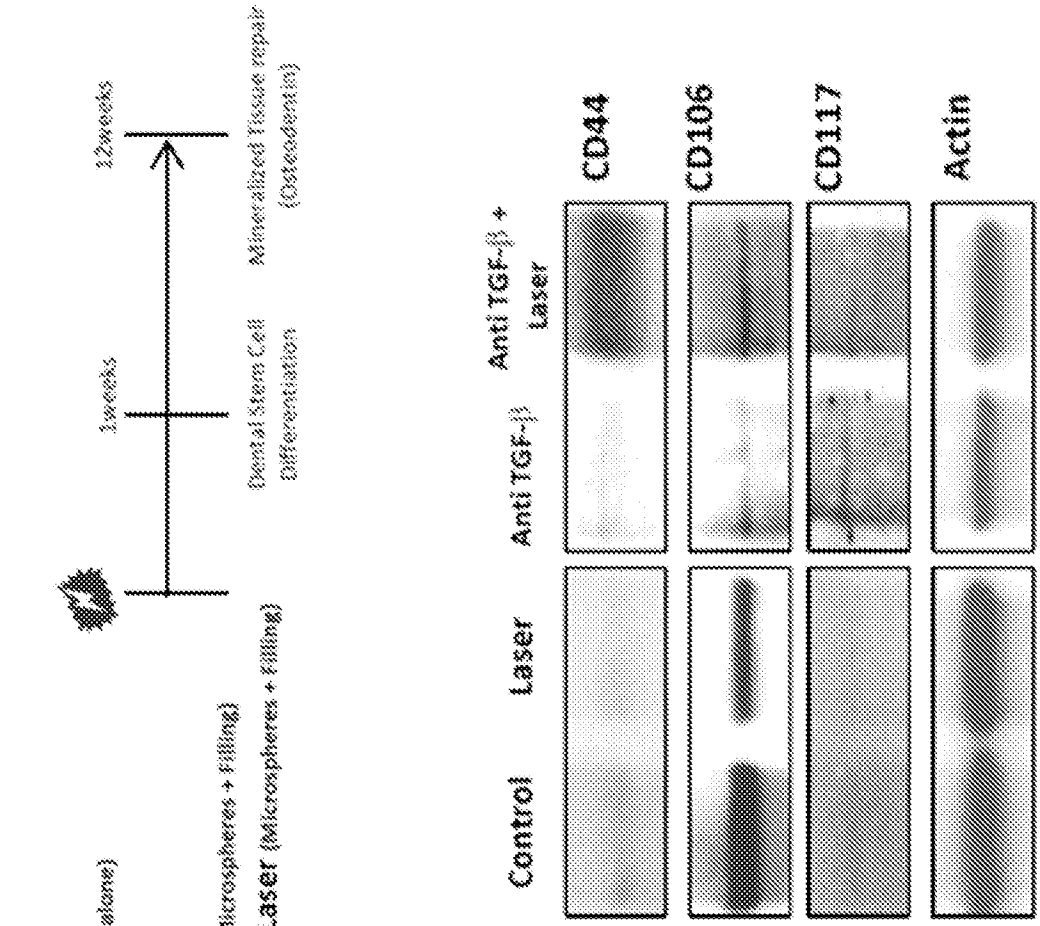

A
Control (Filling alone)
Laser (Filling)
Anti TGF-β (Microspheres + Filling)
Anti TGF-β + Laser (Microspheres + Filling)
1weeks
12weeks
Dental Stem Cell Differentiation
Mineralized Tissue repair (Osteodentin)
B
Control
Laser
Anti TGF-β
Anti TGF-β + Laser
CD44
CD106
CD117
Actin

FIG. 6

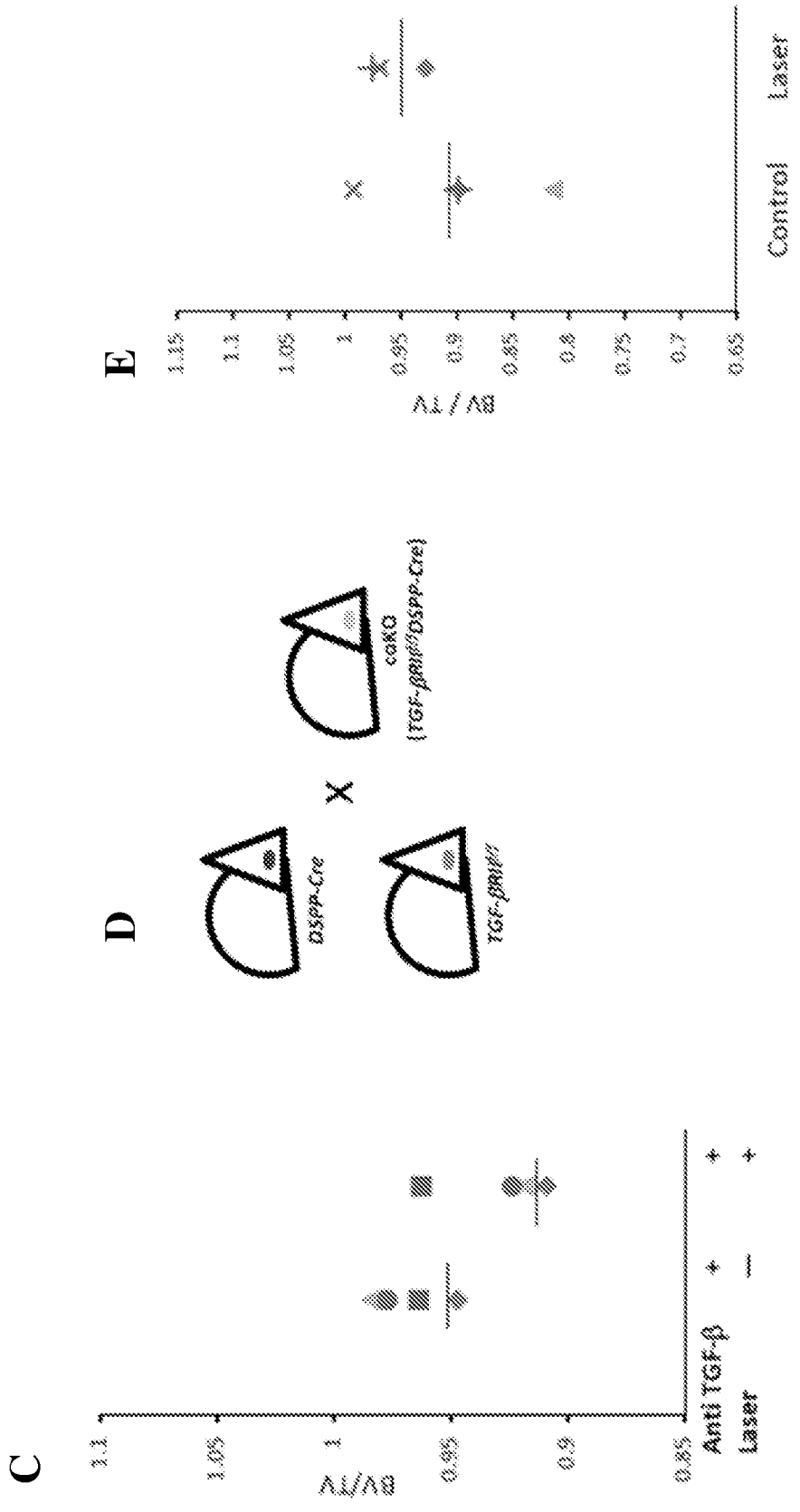


FIG. 6 continued

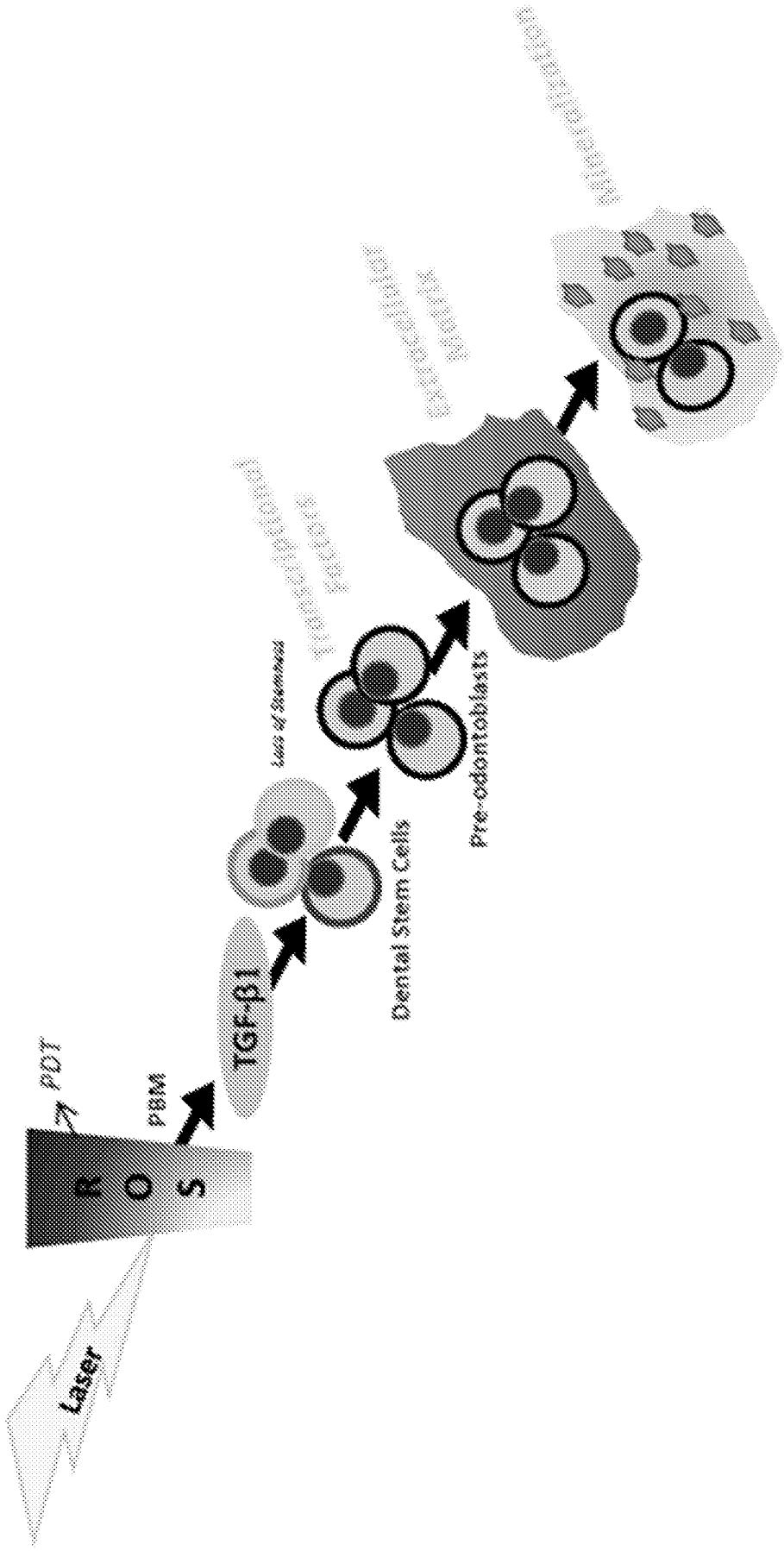


FIG. 7

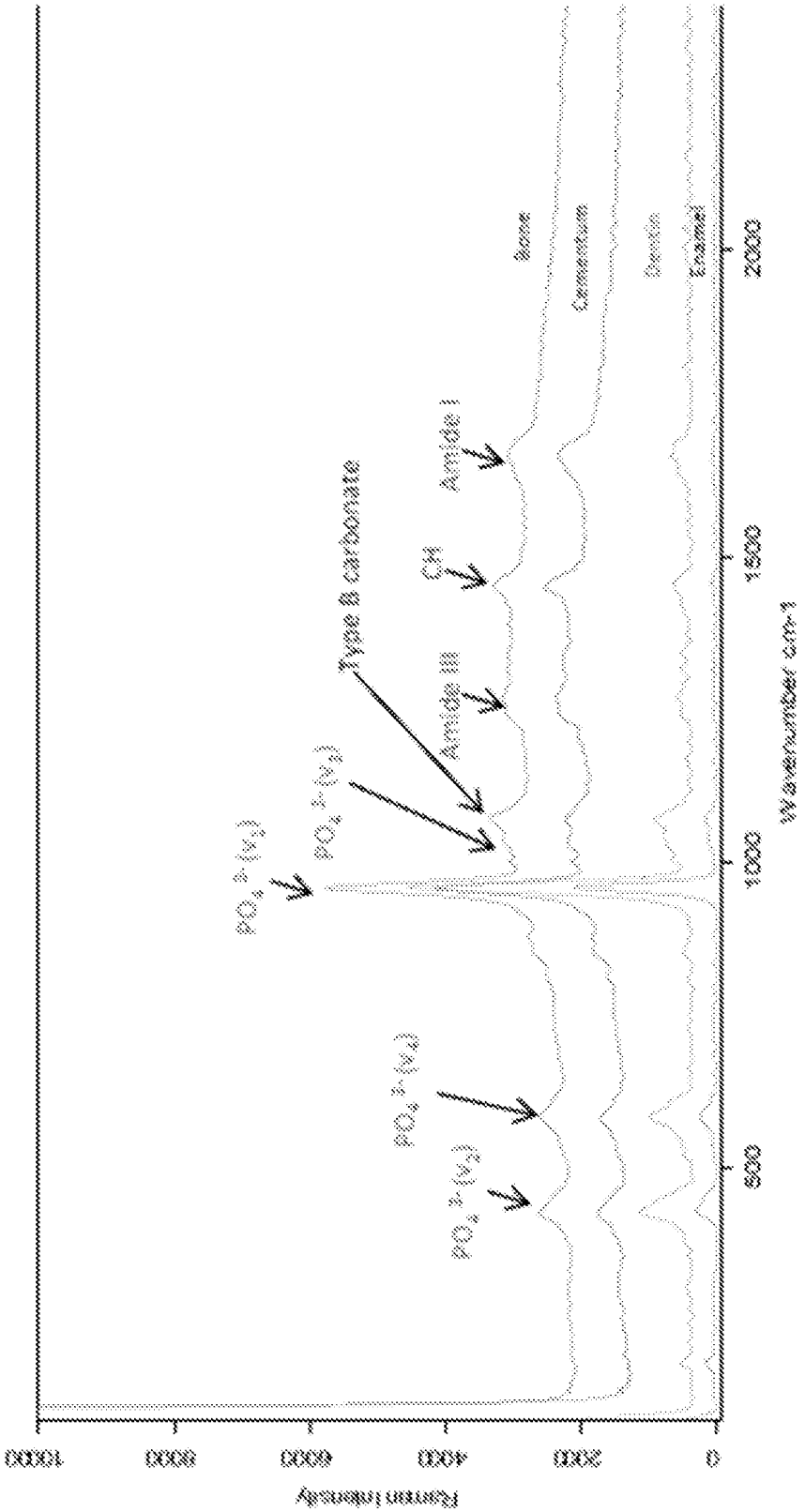

Raman Intensity
0
2000
4000
6000
8000
10000
500
1000
1500
2000
Wavenumber cm-1
Bone
Cementum
Dentin
Enamel
Type B carbonate
Amide III
CH
Amide I
$PO_4^{3-}(\nu_1)$
$PO_4^{3-}(\nu_2)$
$PO_4^{3-}(\nu_4)$
$PO_4^{3-}(\nu_3)$

FIG. 8

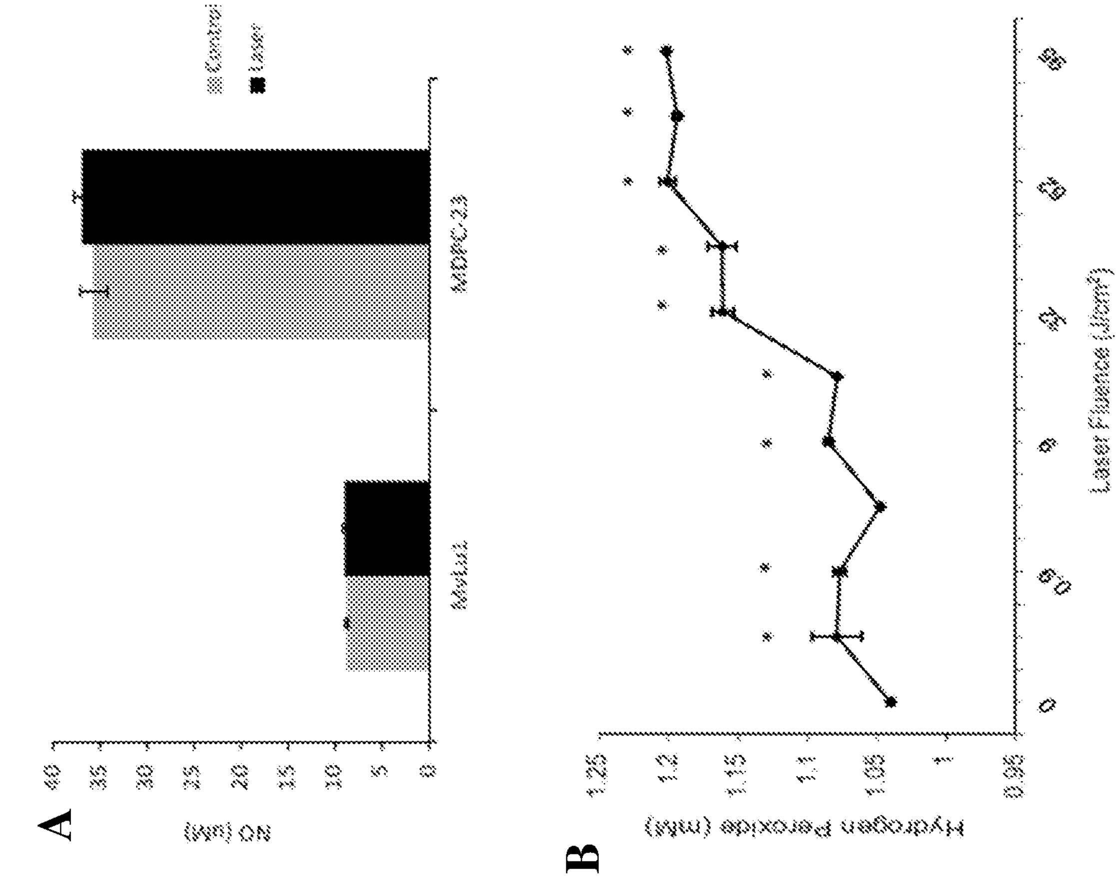

A
NO (uM)
Control
Laser
MDPC-23
B
Hydrogen Peroxide (mM)
Laser Fluence (J/cm²)

FIG. 9

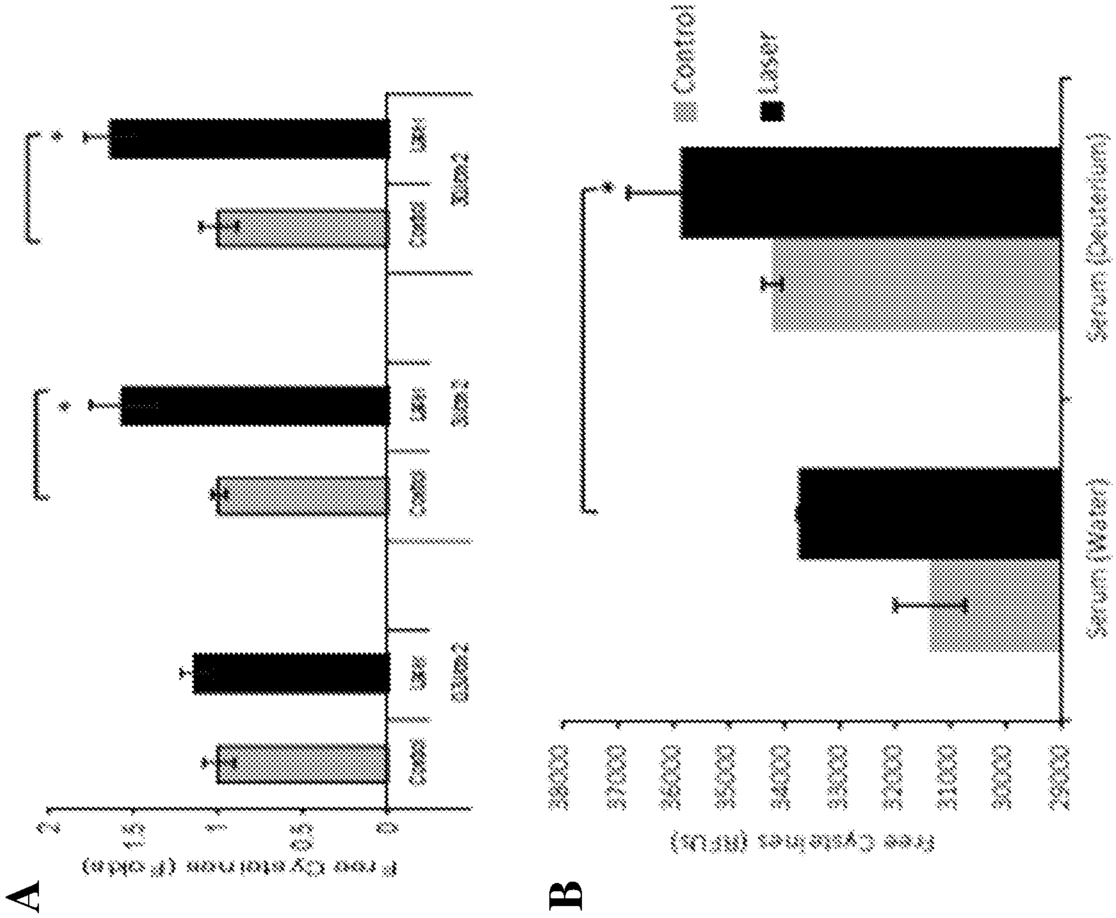

A
Free Cysteines (Folds)
B
Free Cysteines (RFUs)
Serum (Water)
Serum (Deuterium)
Control
Laser

FIG. 10

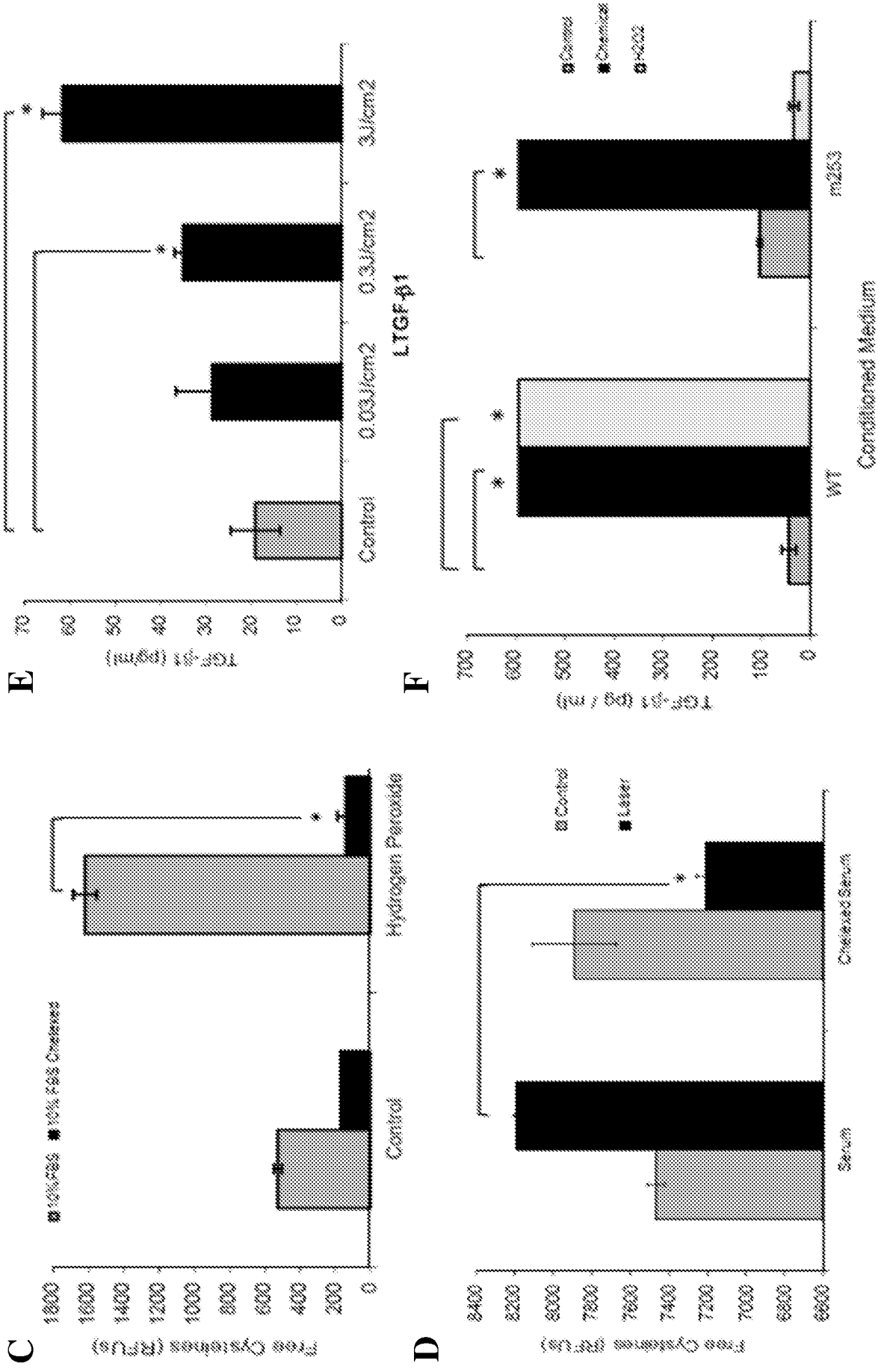


FIG. 10 continued

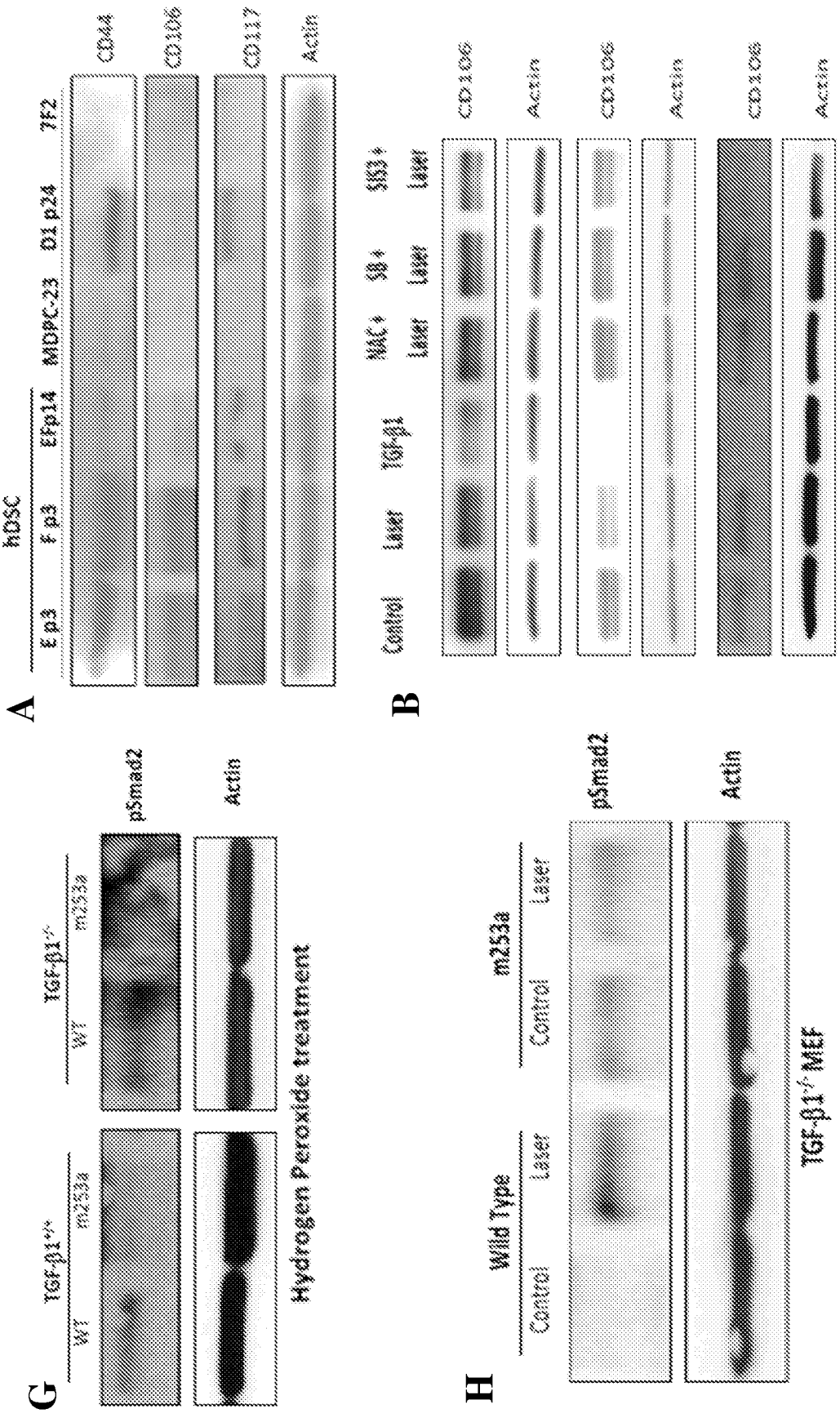


FIG. 10 continued

FIG. 11

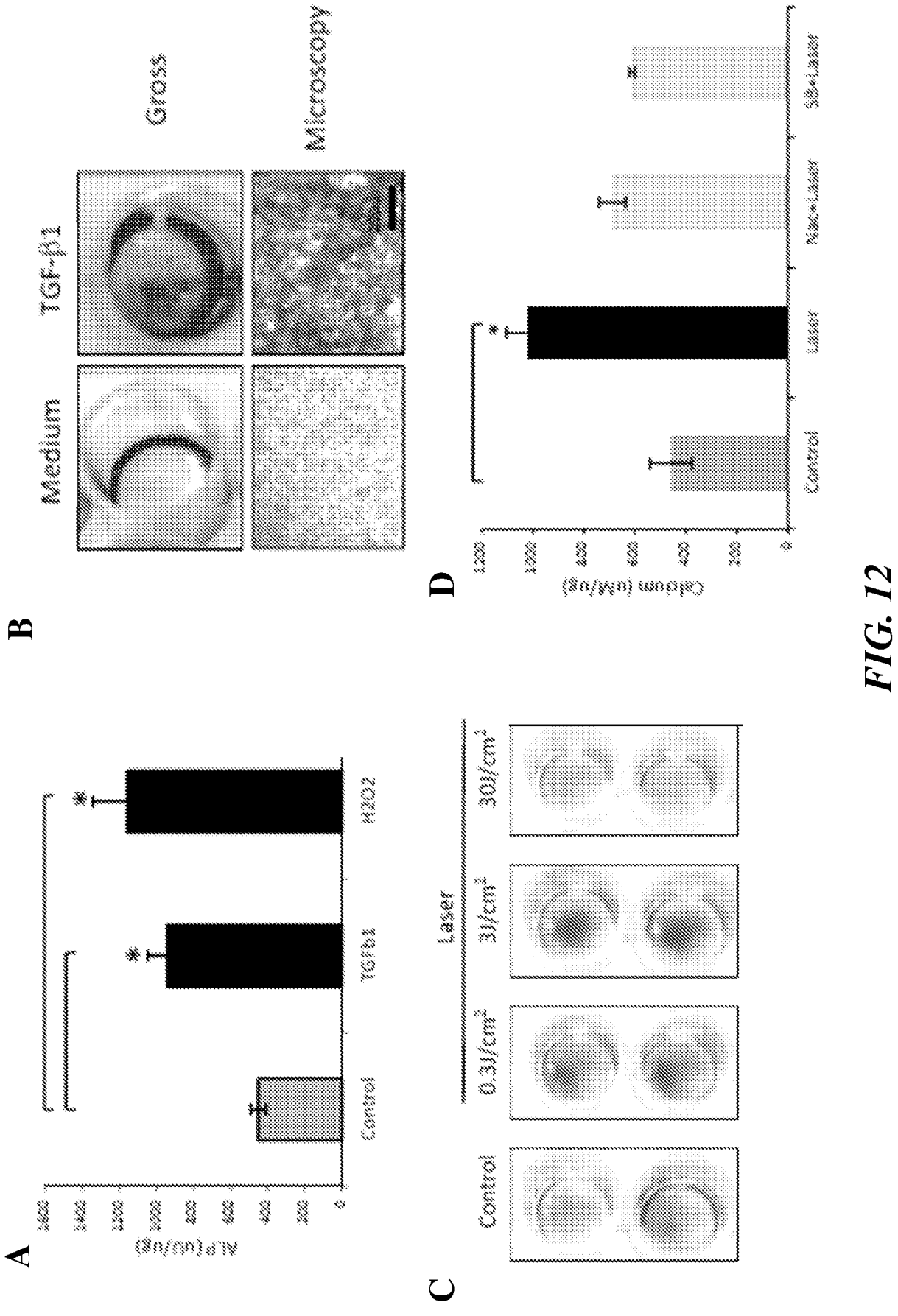

A
ALP(u/l)/ug)
1600
1400
1200
1000
800
600
400
200
0
*
*
Control
TGFb1
H2O2
B
Medium
TGF-β1
Gross
Microscopy
C
Laser
Control
0.3J/cm²
3J/cm²
30J/cm²
D
Calcium (uM)/ug)
1200
1000
800
600
400
200
0
*
*
Control
Laser
Nac+Laser
SB+Laser

FIG. 12

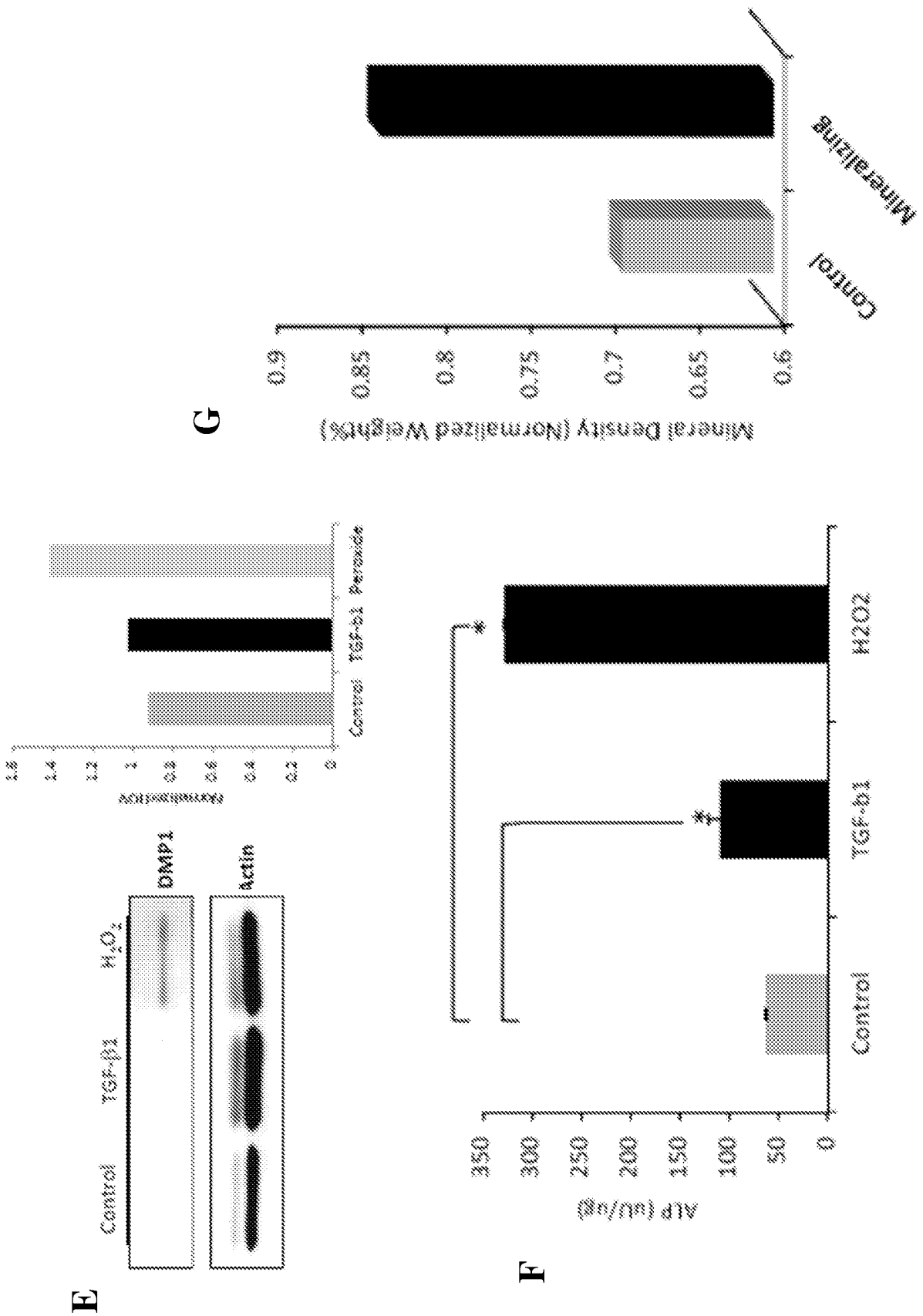


FIG. 12 continued

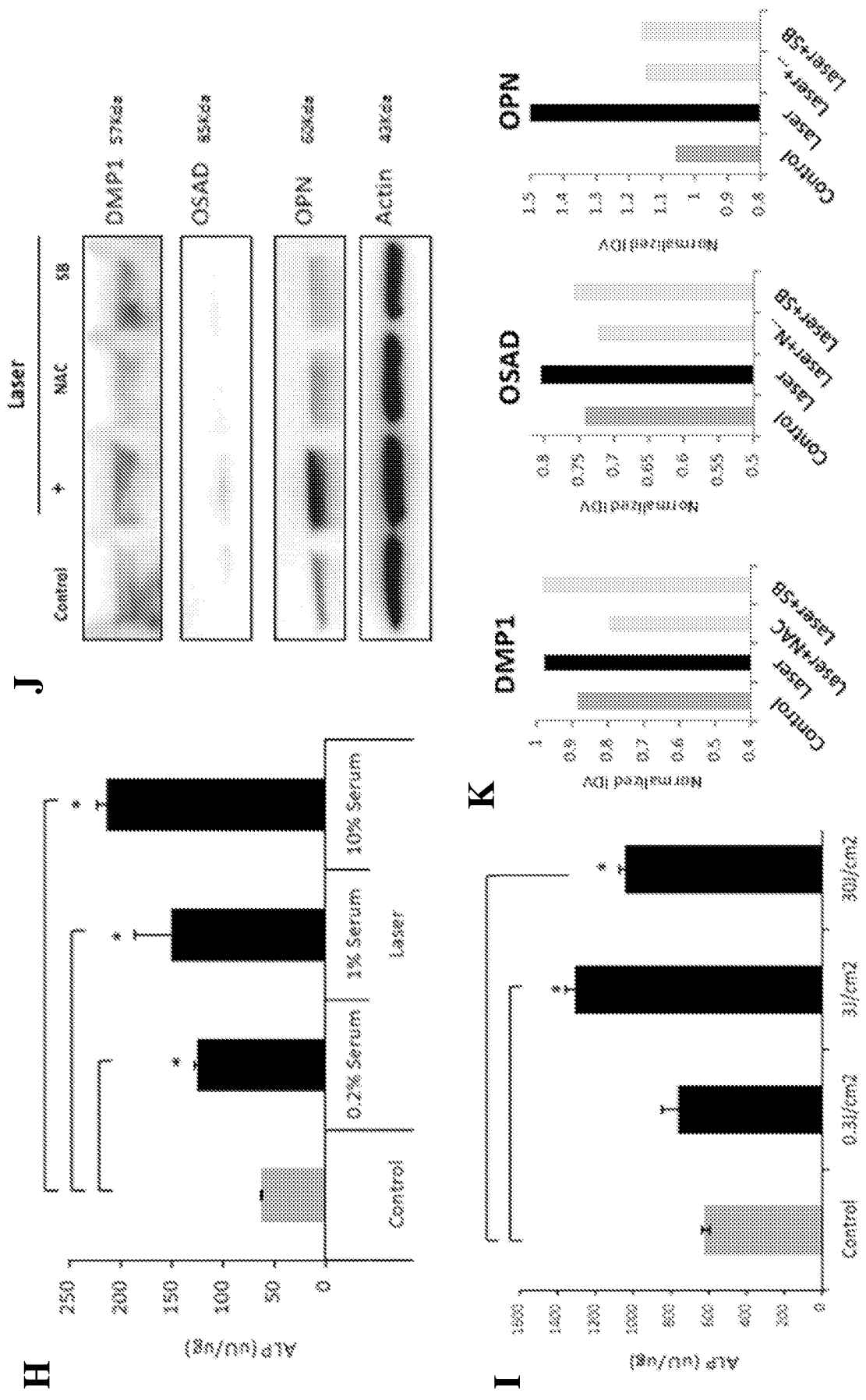


FIG. 12 continued

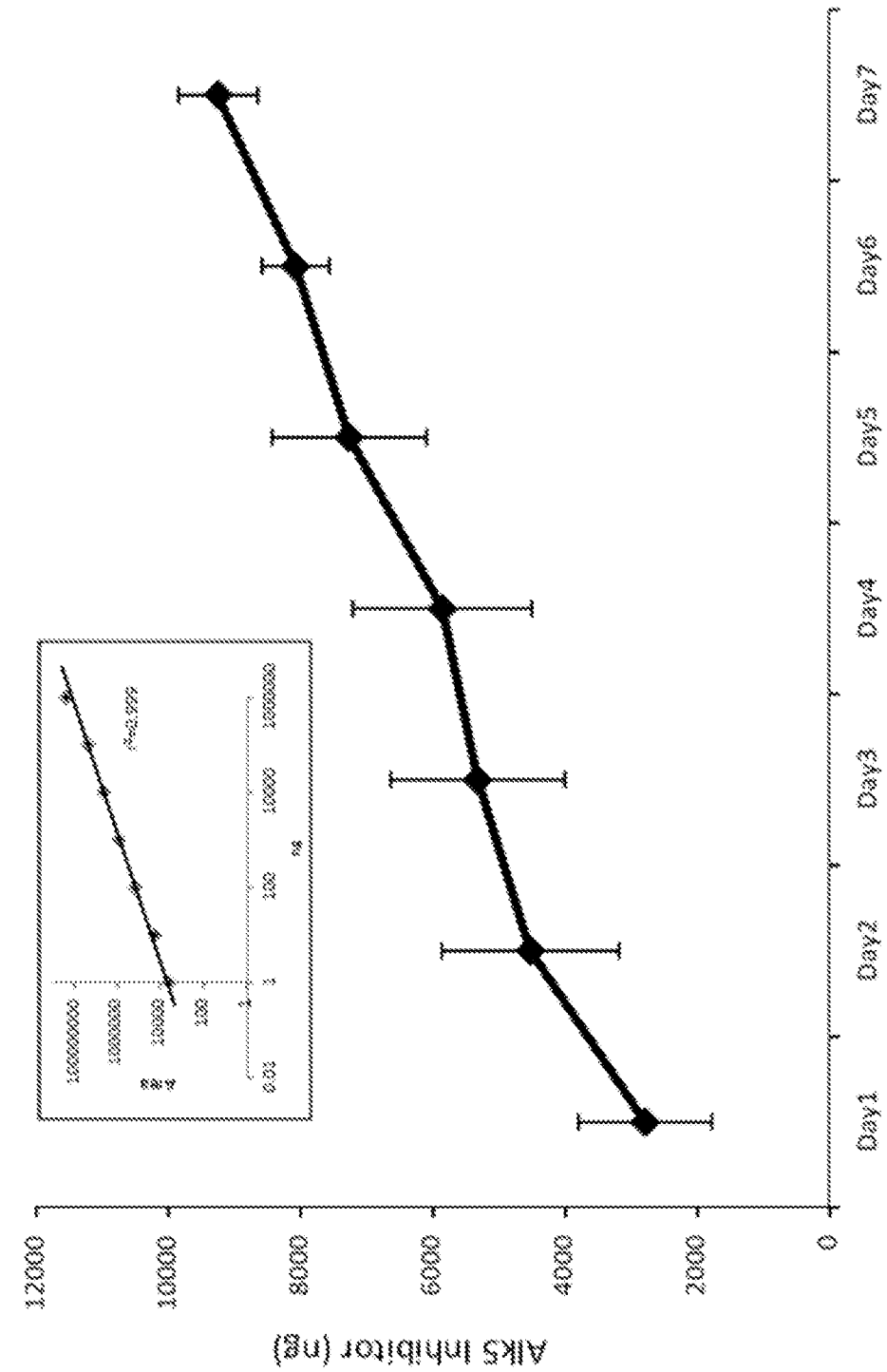

A
Alk5 inhibitor (ng)
12000
10000
8000
6000
4000
2000
0
Day1
Day2
Day3
Day4
Day5
Day6
Day7

FIG. 13

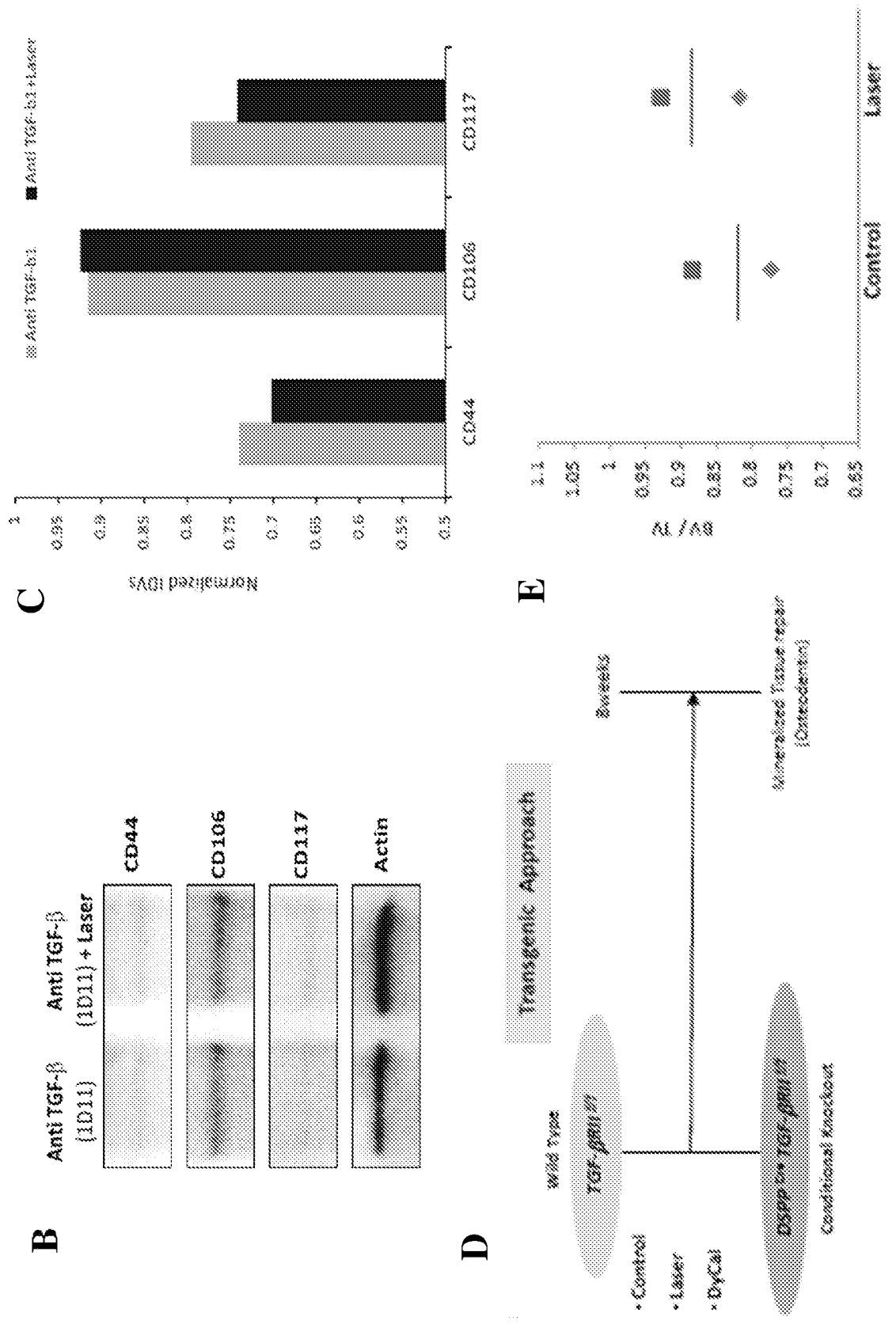


FIG. 13 continued

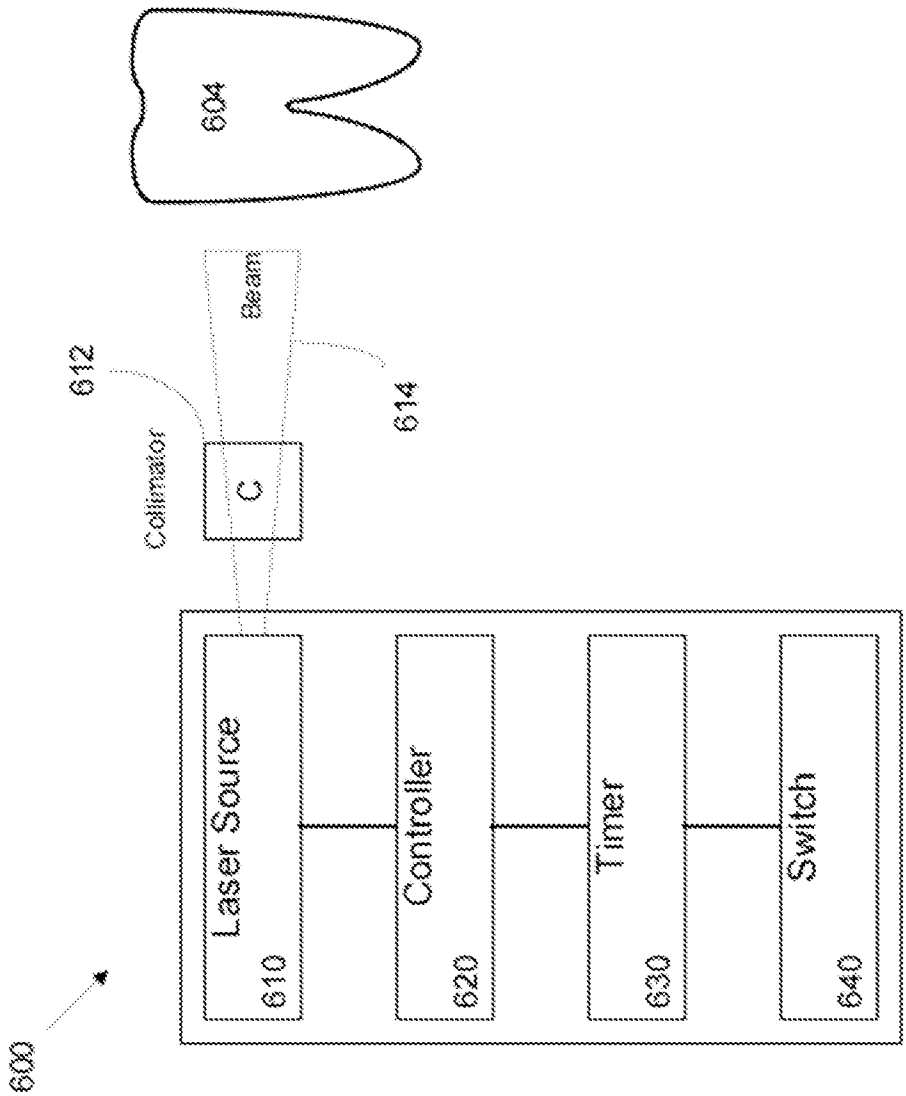

600
Laser Source
610
Controller
620
Timer
630
Switch
640
C
Collimator
612
Beam
614
604

FIG. 14

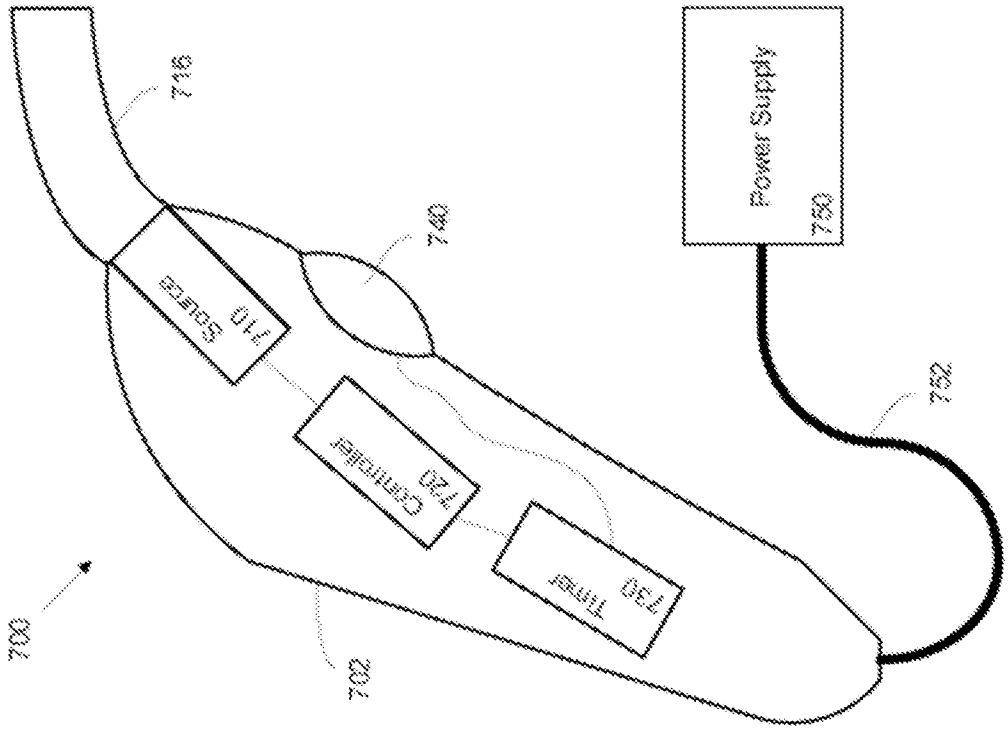

700
716
740
Source
710
Controller
720
Timer
730
702
Power Supply
750
752

FIG. 15

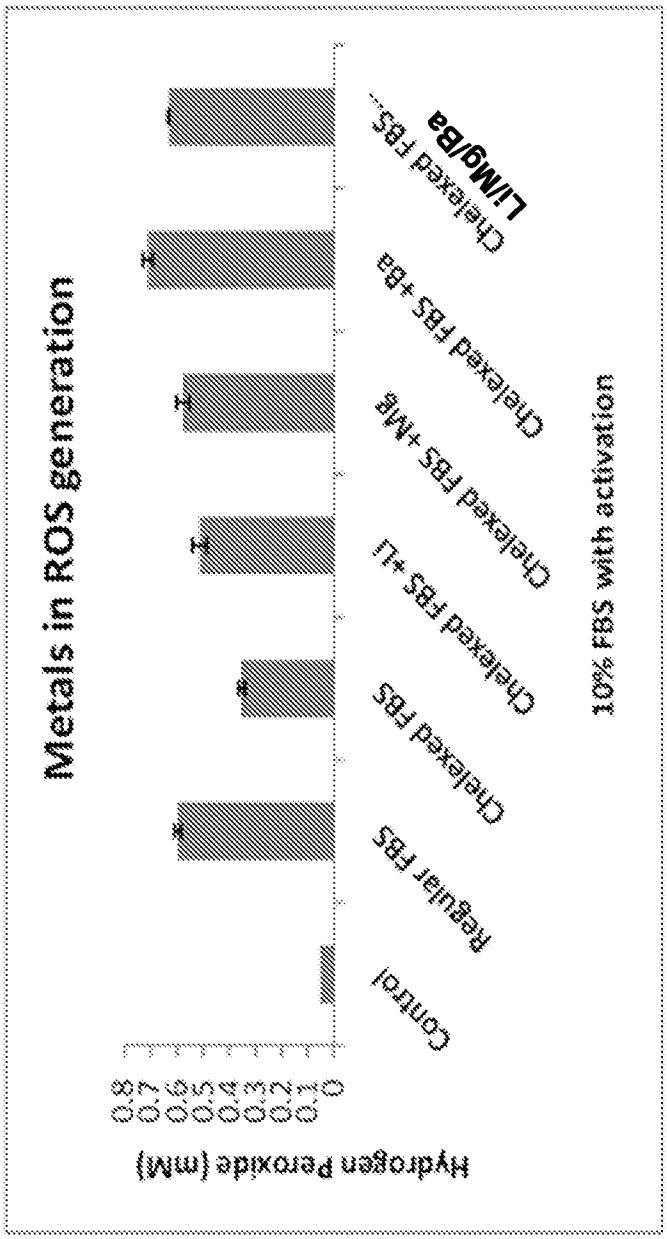

Metals in ROS generation
Hydrogen Peroxide (mM)
0
1
2
3
4
5
6
7
8
Control
Regular FBS
Chelexed FBS
Chelexed FBS +Li
Chelexed FBS +Mg
Chelexed FBS +Ba
Chelexed FBS-
Li/Mg/Ba
10% FBS with activation

FIG. 16