17 Mar 2016

Glass Derived Nanoparticles for Nerve Tissue Repair

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 March 2016 (17.03.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/040481 Al

(51) International Patent Classification:
A61K 9/14 (2006.01)

(21) International Application Number:
PCT/US20 15/049 184

(22) International Filing Date:
9 September 2015 (09.09.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/048,146 9 September 2014 (09.09.2014) US
62/048,148 9 September 2014 (09.09.2014) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,

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(54) Title: GLASS DERIVED NANOPARTICLES FOR NERVE TISSUE REPAIR

(57) Abstract: A biocompatible, biodegradable composite material, and method of using nanoparticles formed within the composite material for nerve repair are disclosed. The nanoparticles may not be formed until the glass degrades upon contact with a fluid *in vivo* or *in vitro.*

FIG. 10A FIG. 10B

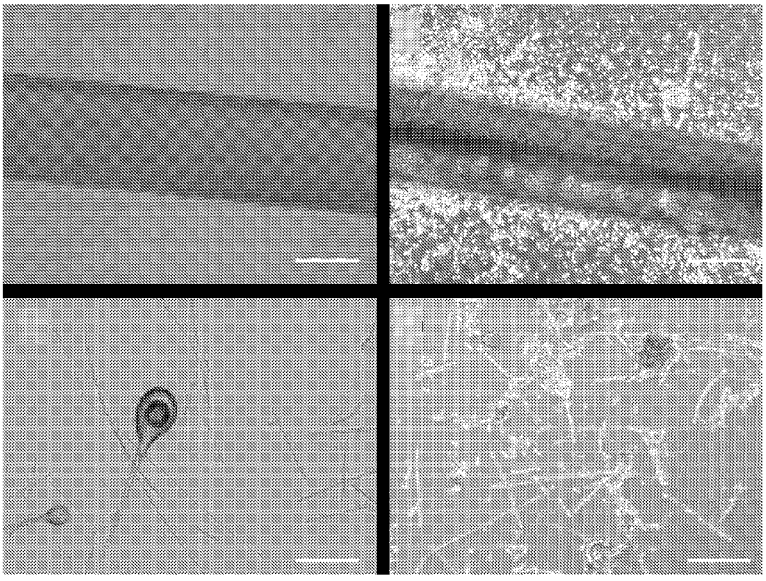

FIG. 10C FIG. 10D

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SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

GLASS DERIVED NANOPARTICLES FOR NERVE TISSUE REPAIR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/048,146 filed September 9, 2014 and U.S. Provisional Patent Application No. 62/048,148 filed September 9, 2014, the entire disclosures of which are both incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to doped biodegradable glass compositions and methods of treating nerve damage using doped biodegradable glass to deliver nanoparticles.

BACKGROU ND

[0003] Ideal nerve repair biomaterials or scaffolds should be biocompatible and noninflammatory, yet flexible with adequate tensile strength to prevent nerve compression. The materials should be biodegradable with a porosity and permeability to supply adequate oxygen and nutrients. Nerve autografts remain the gold standard in nerve repair and regeneration because of their performance. However, autografts require additional surgery, donor site morbidity, and the loss of nerve function; all reasons that alternative materials in nerve repair and regeneration are needed.

[0004] Bioresorbable synthetic natural polymers such as type I collagen (for example the NeuraGen™ collagen tube available from Integra LifeSciences of Plainsboro, NJ) have received attention over the last decade due to ease of production and controlled degradation. Type I collagen supports cell attachment, unidirectional proliferation, and growth ***in vivo.*** The disadvantages to synthetic natural polymers include irreproducibility of collagen type I and the need for immunosuppressant drugs to reduce host rejection. Resorbable synthetic

polymers have been used extensively to repair peripheral nerves due to lower cost, simple fabrication, and proven efficacy. Compared to an autograft, these synthetic materials eliminate the shortcomings of the autograft.

[0005] Over the past **50** years many methods have been developed to produce different types of inorganic materials with different sizes, shapes and properties. Most of these methods use a soluble salt such as nitrate or carbonate to provide the desired ions in a solution and then use other chemicals or processes to precipitate or reduce from an ionized to neutral form. However, small materials such as nanomaterials can be formed through other routes like Sol-Gel processing, Microemulsion, Hydrothermal/Solvothermal processing, Templated synthesis, and Biomimetic Synthesis.

[0006] Therefore, a need exists for methods for creating and delivering nanoparticles of different chemical composition, size, and shape formed within a biodegradable, biocompatible inorganic glass, to improve nerve regeneration and improve the quality of life following injury to the spinal cord or peripheral nerves.

SUMMARY

[0007] Provided herein is a biocompatible, biodegradable composite material for soft tissue repair, such as repair of nerve or nerve cells, and related methods of use. A biocompatible, biodegradable composite material as described herein includes a matrix material and a parent glass suspended within the matrix material, the parent glass including a glass and a dopant. The parent glass may release a plurality of dopant-based nanoparticles upon contact and reaction with a body fluid or a simulated body fluid. The glass may comprise, for example, a borate glass such as sodium tetraborate, or a borosilicate glass. The dopant may be selected from a metal ion, a transition metal ion, an oxide, a rare earth oxide, a halide, carbonate, a compound containing a cation, and any combination thereof. The dopant may be selected from CeO_2, Ce_2O_3, Y_2O_3, and ZrO_2 and mixtures of any two or more thereof. The dopant may be selected from Co, Ni, Cu, Ag, Au, Pt, Fe, Ru, Si, V, Cr, Mn, Fe, Ni, Zn, Sn, Sb, Zn, Ti, Y, Zr, W,

La, Ce, Pr, Nd, Sm, Eu, Lu, Yb, Er, Ba, Ga, I, N, S, Si, and any combination thereof. The dopant may be selected from the combinations I/Ce, I/Y, I/Ce/P, and Cu/Zn/Sr/Fe. The parent glass may include $Na_2O.2B_2O_3.xCeO_2$ and x ranges from about 0.001 to about 0.30 moles. The biocompatible, biodegradable composite material may be degradable *in vivo.* The biocompatible, biodegradable composite material may further include a therapeutic agent. The matrix material of the biocompatible, biodegradable composite material may comprise a material selected from a polymer, a ceramic, and any combination thereof. The matrix material may for example be selected from collagen, laminin, fibrin, PCL, PLA, PLLA, PEG, PGA, PLGA and any combination thereof. The parent glass may have a conformation selected from irregular particles, microspheres, fibers, rods, ribbons and any combination thereof. Any of the foregoing conformations of a biocompatible, biodegradable composite material may be combined with a second matrix material, which may comprise any scaffold material such as collagen, laminin, fibrin and any combination thereof. A combination of any conformation of a biocompatible, biodegradable composite material and a second matrix material can be formed in any shape, such as for example a generally cylindrical conduit form.

[0008] Further provided herein are methods of delivering nanoparticles to a region of interest, including placing any biocompatible, biodegradable composite material as described herein, in or in contact with a body fluid or simulated body fluid; and allowing the parent glass to react and/or degrade to form and release the nanoparticles. It should be understood that the body fluid or simulated body fluid may be combined or associated with a region of interest in the body, such as a body tissue. The region of interest may be for example any suitable location relative to nerve cells, which may be in a tissue or in a body, such as but not limited to a peripheral nerve, or the spine and/or spinal cord.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The following figures illustrate various aspects of the disclosure.

[001 0] **FIG. 1** is an x-ray diffraction pattern, showing the electron diffraction pattern for the as-made borate glass with the molar composition $Na_2O.2B_2O_3.0.01CeO_2$.

[001 1] **FIGS. 2A-B** are TEM images of nanoparticles formed from the borate glass with the composition $Na_2O.2B_2O3.0.01\ CeO_2$ reacted in Dl-water at room temperature. **FIG. 2A** is a low magnification TEM image of nanoparticle clusters. **FIG. 2B** is a high magnification TEM image of nanoparticles (boundaries of two individual nanoparticles selected randomly identified with outline).

[001 2] **FIGS. 3A-B** are TEM images of nanoparticles formed from the borate glass with the molar composition $Na_2O.2B_2O3.0.01\ CeO_2$ reacted in SBF at 37°C. **FIG. 3A** is a low magnification TEM image of nanoparticle clusters. **FIG. 3B** is a high magnification TEM image of the nanoparticles.

[001 3] **FIGS. 4A-B** are images of nanoparticles (agglomerated) formed with the borate glass with the composition of $Na_2O.2B_2O3.0.01\ CeO_2$ doped with 1 wt.% Y_2O_3 **(FIG. 4A)** and 1 wt.% ZrO_2 **(FIG. 4B),** which was reacted in DI water at 37°C. The scale mark is 20 nm.

[0014] **FIGS. 5A-C** are images of live cells for control **(FIG. 5A),** Ce1 **(FIG. 5B),** and Y 1 **(FIG. 5C).**

[001 5] **FIG. 6** is a graph depicting the mean +/- standard error for the mean percent (%) number of live neurons for the 4 different sets of wells imaged per condition at day 7 and day 10. Statistical analysis was performed with a two sample t-test with data considered significantly different than control for the same days in culture ($^{*}p<0.05$; $^{**}p<0.01$).

[001 6] **FIGS. 7A-C** are photographic images of the forms of fabricated glass. **FIG. 7A** shows small glass frit doped with cobalt (blue); polymer sheets with punches made, "cotton balls" of glass microfibers, and the glass rods. **FIG. 7B** shows polymer/glass composite sheets can be rolled and punched. **FIG. 7C**

shows long glass rods can be formed or broken into smaller lengths, shown as added to cells in culture.

[001 7] **FIG. 8** is scanning electron micrographs of biodegradable 1393-B3 glass (left images) compared to the same magnification of Y-containing B3 glass (right images).

[001 8] **FIGS. 9A-B** show scanning electron micrographs of biodegradable glass microfibers that show the ultrafine cotton ball fiber-like structure at two magnifications.

[001 9] **FIGS. 10A-D** are images of rods **(A, C)** and microfibers **(B, D)** taken at 1 week and 9 weeks in media showing the reaction and degradation of biodegradable 1393-B3 glass rods and microfibers with cell culture over time. Scale bar = 100 μιτι.

[0020] **FIGS. 11A-C** are optical images of the air-side **(A)** and the glass side **(B)** of the glass (1393-B3)/polymer composite sheet. After immersion for 14 days in SBF, images show the changes in the air-side of a 100% PCL sheet **(C)** compared to the air-side of the 1393-B3 glass **(D).** Inset shows higher magnification of the islands of agglomerated Hydroxyapatite (HA).

[0021] **FIG. 12A-B** are scanning electron micrographs of HA microspheres on the surface and within the cross-section **(A)** after immersion of glass/polymer composite (Blend Composite: 25wt% 1393-B3, 25wt% 45S5, 50wt% PCL) in SBF for 14 days. An expanded image of the section shown in the box **(B)** shows the HA microspheres.

[0022] **FIGS. 13A-D** are graphs showing the weight loss **(A)** and ion release of Boron ions **(B),** Calcium ions **(C),** and Phosphorus ions **(D)** over time for a composite composed of 50 wt. % PCL/50 wt. % B3 (squares) compared to 100% PCL (triangles). Splines are only for guidance.

[0023] **FIG. 14** is a schematic of the sciatic peripheral nerve model with a biodegradable glass/polymer rolled or cylindrical conduit, with glass rods or microfibers comprising a biocompatible, biodegradable composite material as

disclosed herein, embedded within second matrix comprising a collagen (CN) gel, either with or without crosslinking to growth factors and/or drugs.

[0024] **FIG. 15** is a schematic of repair of a spinal cord hemi-section. The doped BG rods or microfibers can be embedded into collagen and injected at the site of injury.

[0025] **FIGS. 16A-C** are graphs of mechanical properties for peak stress **(A),** strain at break **(B),** and elastic modulus **(C)** for the composites that are either unreacted or reacted for 3 or 6 weeks in media. For each graph, asterisks denote statistical significance at $p<0.05$ for either the B3, Blend, or 45S5 for each condition in **(A)** and **(B),** or B3 compared to PCL **(C).** Significance is indicated in **(C)** for 45S5 greater than B3(#).

[0026] **FIG. 17** is a photograph of sciatic nerves sutured to rolled PCL/B3 glass composite which has an average suture retention strength of 0.35 ± 0.07 N.

[0027] **FIG. 18** shows images of live, dead, and the merge of live plus dead cells of a dish of dissociated neurons not exposed to any glass. Scale bar is 100 μιτι.

[0028] **FIG. 19A** is a graph of the average number of neurons and **FIG. 19B** is a graph of the average number of support cells comprised of fibroblasts and glia for 3, 7, and 10 days for each of the 13-93 B3 biodegradable glass doped with the element shown along the x axis. For each of the conditions, the cells were treated with about 14 mg/mL of the degradable glass. The Ce2 and Y2 glasses both contain trace amounts of iodine, and the CZSF glass contains a mixture of Cu, Zn, Sr, and Fe ions.

[0029] **FIG. 20A** is a graph of the average neurons normalized to the total number of cells (Live plus Dead) and **FIG. 20B** is a graph of the average neurons normalized to the total live cells (neurons plus support cells). The graphs show normalized average number of neurons for each of the types of B3 biodegradable glass doped with the element shown along the x axis. For each of the conditions, the cells were treated with about 14 mg/mL of the degradable

glass. The Ce2 and Y2 both contain trace amounts of iodine, and the CZSF glass consists of Cu, Zn, Sr, and Fe ions.

[0030] **FIGS. 21A-D** are graphs of a few selected biodegradable borate based glass to compare the effects of adding more glass to increase the concentration of released ions as the glass reacts in the cell media. Results are shown for only the percent of live neurons/total live cells for 1, 2, 4, and 8 pieces of 1 cm long glass rods, that is approximately 7, 14, 28, and 56 mg/mL of biodegradable glass per ml_ of media, respectively.

[0031] **FIG. 22** shows whole DRG extended neurites on bioactive borate glass composite sheets. Images of whole DRG stained with Calcein AM show the composites support outgrowth of neurites from the body of the DRG. Scale bars = 100 μιτι. The average neurite outgrowth from the DRG body is shown for each of the dopants tested, and compared to the control 13-93 B3 glass. Fe supports greater outgrowth than Ce1 , Y 1, and Y2 (*p<0.05); Zn supports greater outgrowth than Y 1 (#p<0.05).

[0032] **FIG. 23** is a graph of whole DRGs whose neurites had measured extension lengths on the composites of undoped biodegradable glasses when either pre-reacted for 6 weeks or unreacted. Unreacted 45S5 composite exhibited greater outgrowth than any other unreacted PCL or polymer sheet (*p<0.05). After pre-reaction, B3 exhibited greater outgrowth than the blend composite (#p<0.05), but not any differences with the 100% PCL or the 45S5 composite.

[0033] **FIG. 24** is a bar graph of the single longest neurite outgrowth measurement per DRG after three (3) days in culture, for each dopant tested.

[0034] **FIG. 25** shows measurement of directed neurite outgrowth on aligned 13-93 B3 glass rod/fibrin scaffolds. **FIG. 25A** shows a representative fluorescent image of DRG stained with Calcein AM. Scale bar is 200 μιτι. **FIG. 25B** is a graph of neurite and neurite bundle outgrowth from DRG aligned with rods, in which 0 and 180 degrees is considered aligned. **FIG. 25C** is a schematic

that show the orientation of the glass rods with the angle of extension measured by outgrowth from the DRG body.

[0035] Corresponding reference characters and labels indicate corresponding elements among the views of the drawings. The headings used in the figures should not be interpreted to limit the scope of the claims.

DETAILED DESCRIPTION

[0036] Provided herein are materials and methods of nerve repair in which a biocompatible, biodegradable composite material is used to produce and deliver a nanomaterial. In various aspects, the biocompatible, biodegradable composite material comprises a glass, which is biocompatible and biodegradable, suspended in a matrix. The glass contains inorganic dopants which are released as ions when the composite comes into contact with a body fluid or simulated body fluid. A biocompatible, biodegradable composite material as disclosed herein may be used to produce and deliver nanomaterials to cells or tissues *in vivo* or *in vitro.*

[0037] As used herein, the term "subject" refers to an animal, including but not limited to a mammal including a human and a non-human primate (for example, a monkey or great ape), a cow, a pig, a cat, a dog, a rat, a mouse, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig).

[0038] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms as used herein and in the claims shall include pluralities and plural terms shall include the singular.

[0039] The use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

[0040] The term "body fluid" as used herein refers to a liquid that originates from inside the organism of a subject, including for example blood, interstitial fluid, cerebrospinal fluid, or lymph. The term "simulated body fluid" as used herein refers to a liquid having an ionic composition comparable to that of a body fluid. In non-limiting example, a simulated body fluid can be a buffered saline solution.

[0041] Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, and chemistry described herein are well known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Any chemical, enzymatic or staining reactions, or purification techniques are performed according to manufacturer's specifications and protocols, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are also well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, delivery, diagnosis and treatment of all subjects, human and animal.

[0042] In one aspect, a biocompatible, biodegradable composite material comprises a glass comprising a nanomaterial. The nanomaterial is for example a

nanoparticle. The size, shape, and chemical composition of a given type of nanoparticle can be controlled by the synthesis process. For example, chemical dopants can be added to the parent glass composition to contain the desired percent composition of compounds. By way of non-limiting example, dopants can include an oxide, halide, carbonate or any other type of compound that may contain or produce a desired cation. The parent glass for making the nanoparticles may be formed by melting the doped glass under controlled conditions (melting atmosphere, temperature and time) and cooling the glass in a controlled manner. The parent glass may then be degraded, *in vivo* or *in vitro,* forming the nanoparticles in the process.

[0043] Two primary types of biomaterials are available for use in connection with the present disclosure: biodegradable materials that degrade with time, or bio-inert materials that do not degrade. The bio-inert materials may be unreactive to provide long-term structural support. These may be used predominantly as scaffolding in bone and hard tissues for regeneration. In contrast, biodegradable materials react and degrade with time in the body, or when subjected to simulated body fluids (SBF) or cell culture media. These biodegradable materials may be better suited to soft tissue regeneration as with time, as the tissues regrow and infiltrate the damaged regions, the biodegradable material will react with simulated body fluids and be absorbed by the body as the scaffold is no longer required, thus leaving only the regenerated tissue in its place. Provided herein are biodegradable materials optimized for reacting and degrading with appropriate time for soft tissue repair, and while doing so, provide the needed structural scaffold and delivery system for advantageous chemical dopants and molecules. It may also have the ability to chemically cross-link and deliver drugs and growth factors. Optimization of this system may provide a better-suited soft tissue regenerative system than what currently exists for soft tissue regeneration and repair.

[0044] In an aspect, borate based biodegradable glass with added chemical dopants may be fabricated in many different forms such as

nanoparticles, irregular shaped particles (frit), rods, spheres, and microfibers. This biodegradable glass may be used for soft tissue repair and nerve regeneration. The addition of chemical dopants such as Ce or Y has been shown to be neuroprotective to neurons *in vitro,* as well as prevent overproliferation of the scar forming fibroblasts. In various aspects, the forms of glass may be used as composites or mixed materials in scaffolds and nerve guide conduits to promote regeneration of peripheral and central nerves that have been damaged through trauma or disease, as well as promote the regrowth of nearly any type of soft tissue.

I. Nanomaterials

[0045] A biocompatible, biodegradable glass composite material as disclosed herein may include a parent glass that contains or releases desired cations, and/or creates nanoparticles. The parent glass may be semi-crystalline or non-crystalline/amorphous, and the nanoparticles may be crystalline or non-crystalline/amorphous. In one aspect, the parent glass reacts or degrades when exposed to fluid(s), for example a body fluid or a simulated body fluid (SBF) *in vivo* or *in vitro,* and may generate non-crystalline/amorphous nanoparticles. The parent glass may be, for example, biocompatible and non-toxic.

[0046] The materials synthesized may be formulated by methods to deliver inorganic nanomaterials *in situ* using biocompatible, biodegradable glasses. In one aspect, the nanomaterials are nanoparticles. The nanomaterials may be produced in two different ways. Methods of making nanoparticles include a solid-state (glassy-state) method to produce nanoparticles using a degradable glass as disclosed herein. In this method, nanoparticles may be produced in two different ways. In a first method, the desired nanoparticle base material (metal ions, transition metal ions, rare earth oxide, etc.) may be dissolved in a glass melt to form the parent glass. In one aspect, the parent glass may be doped with an oxide, halide, or other compound containing a desired cation. Then the parent glass may be reacted or degraded in a desired fluid in a laboratory *(e.g.,*

in vitro) or inside the body of subject *(in vivo).* As the glass reacts or degrades the desired ions may be released to form nanoparticles of a desired composition/size. A second method may be to dissolve the desired material in the molten glass and then form the nanomaterials inside the glass using techniques such as controlled heat treatments, radiation, etc., as will be understood by those of skill in the art. The glass may then be reacted or degraded *in vitro* or *in vivo* to release the nanoparticles. In this second method, the glass network may react when in contact with a body fluid or simulated body fluid, while the insoluble nanoparticles remain in the fluid, at least temporarily. The nanoparticles may for example remain in solution *in vivo* until they are removed or sequestered by the body.

[0047] The glass and other desired compounds may be mixed as powdered raw materials. In one aspect, the glass raw material may be a borate glass such as sodium tetraborate, or a borosilicate glass. In another aspect, the nanoparticle base material may be a dopant. The size, shape, and chemical composition of a given type of nanoparticle can be controlled by doping the parent glass with the desired amount of a chosen oxide, halide *{e.g.,* F, Cl, I), carbonate, or other type of compound *(e.g.,* sulfate, oxalate, or nitrate) that contains the desired cation, *(e.g.,* Ce, Y, Zr, Gd) and melting the doped glass under (a) controlled conditions (melting atmosphere, temperature and time), and (b) cooling the glass in a controlled manner. In an aspect, powdered sodium tetraborate and CeO_2 may be mixed together to form a parent glass mixture of sodium borate glass.

[0048] In one aspect, the parent glass may have the molar composition of $Na2O.2B_2O3.xCeO2$, where x ranges from about 0.001 to about 0.30 moles. In another aspect, the parent glass may have the molar composition $xNa_2O.2xB_2O3.(1-3x)Al_2O3$, where x ranges from about 0 to about 0.2 moles. In an aspect, the parent glass may have the molar composition $xR_2O.yR'O.(1-x-y)B_2O3$, where R may be alkali ions such as Li, Na, K, etc. and R' may be alkaline earth ions such as Mg, Ca, Sr, etc. and where x ranges from about 0 to about 0.5

moles and y ranges from about 0 to about 0.5 moles. In yet another aspect, the parent glass may be $xR_2O.yR'O.$ $zR_2"O3.$ m R2'"O5 .(1 -x-y-z)B_2O3, where R may be alkali ions such as Li, Na, K, etc., R' may be alkaline earth ions such as Mg, Ca, Sr, etc., R" may be modifiers that form a R'’203 oxide such as Al, Fe, etc., and R'" may be modifiers that form a $R'"_2O_5$ oxide such as phosphorus (P) and x and y range from about 0 to about 0.5 moles, and z and m range from about 0 to about 0.2 moles.

[0049] Non-limiting examples of nanoparticle base material dopants for producing metallic ionic nanomaterials include Co, Ni, Cu, Ag, Au, Pt, Fe, and Ru. Non-limiting examples of nanoparticle base material dopants for producing oxides, phosphate, and borate nanoparticles may include Si, V, Cr, Mn, Fe, Ni, Zn, Sn, Sb, Zn, Ti, Y, Zr, W, La, Ce, Pr, Nd, Sm, Eu, Lu, Yb, and Er. Dopants that may be used for soft tissue regeneration include, but are not limited to Ag, Ba, Ce, Co, Cu, Fe, Ga, I, Mn, N, S, Si, Sr, Ti, Y, and Zn, and a mixture of elements such as: l/Ce (Ce2), l/Y(Y2), l/Ce/P, and Cu/Zn/Sr/Fe (CZSF). Two or more of these dopant elements may be combined within the nanoparticles. In an aspect, Y or La doped CeO_2 nanoparticles may be produced (co-doped cerium oxide-yttrium nanoparticles). In another aspect, the parent glass may contain up to about 4 dopants.

[0050] The parent glass mixture may be heated from about 600°C to about 1000°C, forming a parent glass melt. In an aspect, the parent glass mixture may be heated to about 1000°C for about 1 hour. The parent glass melt may be cooled to from about 20°C to about 25°C in various aspects. In an aspect, the parent glass melt may be cooled to room temperature to form the parent glass. In this aspect, the nanoparticles may form during the dissolution of the glass in aqueous solution.

[0051] In one aspect, a solid amorphous (glass) or semi-crystalline (glassceramic) material may be used to make nanoparticles. For example, nanoparticles can be formed by giving the glass a prescribed heat treatment such that the desired nanoparticles are formed within the melted glass matrix,

which is then cooled and solidified. The resulting solid glass matrix containing a controlled and specified amount of nanoparticles can then be delivered to a desired site in humans or animals in several ways.

[0052] The heat treatment of the glass may include cooling the glass melt to a certain temperature and holding it for a chosen time before cooling to room temperature. In another aspect, the heat treatment may include cooling the glass melt to room temperature and then reheating to a certain temperature and holding for a chosen time before re-cooling to room temperature. In yet another aspect, the glass melt may be bubbled with gas to form the nanoparticles within the parent glass. The bubbling gas may be reducing (forming gas, CO/CO2 mixture), neutral (N_2, Ar, He), or oxidizing (pure oxygen). The gas flow rate may be from about 0.1 cm^3/min to about 1000 cm^3/min depending upon the size/volume of the melt. The bubbling time may range from about 5 minutes to about 72 hours depending up on the melt temperature and composition, dopants in the melt, and melt size/volume. The bubbling temperature may be any temperature where the viscosity of the melt is low enough to permit the gas to escape from the melt. In one aspect, the viscosity may be lower than 10 poise.

[0053] The nanoparticles may be made and/or released by reacting the glass in contact with a desired fluid under certain reaction conditions *{e.g.,* of temperature, pressure, pH, etc.). Physiological conditions can be used. Alternatively the temperature for reacting the glass *in vitro* may range from about -20 to about 120°C under ambient pressure or up to about 500°C under higher pressures. The pH of the fluid for reacting the glass *in vitro* may range from about 2 to about 12. In one aspect, the glass composite materials can be designed to react or degrade over the span of minutes to years. Additionally, it will be understood that by changing various conditions such as temperature or solution compositions, it may be possible to form a variety of nanomaterials with special/different properties.

[0054] In another aspect, the body fluid or simulated body fluid may contain added organic or inorganic stabilizers or surfactants to stabilize the

nanopartides and prevent them from agglomerating. Non-limiting examples of such stabilizers include tetrahydrofuran (THF), ethylene glycol (EG), hexadecylamine (HDA), mercaptosuccinic acid (MSA), poly(vinylpyrrolidone) (PVP), CTAB, and Polyvinyl Alcohol (PVA).

[0055] In any of the foregoing aspects, the nanopartides may be less than 10 nm. In any aspect, the nanopartides created from the parent glass may range in size from about 2 nm to about 60 nm. In any aspect, the nanopartides may range in diameter from about 2 nm to about 20 nm, from about 10 nm to about 30 nm, from about 20 nm to about 40 nm, from about 30 nm to about 50 nm, and from about 40 nm to about 60 nm. In any aspect, it will be understood that the size of the nanopartides may be affected by the composition of the parent glass, temperature, and pH.

II. Biomaterials from the Nanopartides and Biodegradable Glass

[0056] A biodegradable glass composite material as disclosed herein includes for example a parent glass containing or capable of forming nanopartides, and a matrix material. Nanopartides can be formed in various ways. Glass raw materials may be mixed with nanoparticle base materials to form a parent glass mixture. The parent glass mixture is heated to a first temperature, forming a parent glass melt. The parent glass melt is cooled to a second temperature to solidify the parent glass melt. In so doing, the parent glass may be formed into a desired shape using standard shaping techniques as known in the art, which may then in use be degraded or otherwise react upon contact with a body fluid or simulated body fluid, wherein the nanopartides are created as the parent glass reacts or degrades. For example, a borate glass composite containing cerium may in use produce ceria nanopartides. Methods of making nanopartides include the solid-state (glassy-state) method and other methods as described in detail above in section I. Methods of using a biodegradable glass composite material as disclosed herein are described in further detail below in section III.

[0057] The parent glass or matrix material may further include one or more therapeutic agent. This may include chemotherapeutics, growth factors, angiogenic compounds, or any other substance that may be beneficial to the region of interest. The therapeutic agent may diffuse out of the parent glass faster than the nanoparticles are formed. In an aspect, the therapeutic agent may provide a synergistic effect with the nanoparticles. The therapeutic agent may, for example, be copper, iron, zinc or strontium.

[0058] The nanoparticles and parent glass may be incorporated into many forms such as, for example, small irregularly shaped glass particles (frit), microfibers, microspheres, thin flexible polymer sheets, and micron diameter rods. **FIG.** 7 shows photographs of various forms of biodegradable glass that may be fabricated and used towards soft tissue repair. The biodegradable glass may be fabricated in many different forms, such as, but not limited to irregular particles, microspheres, fibers, rods, to ribbons in different sizes, and may be mixed with, or coated, on a polymer, metal, ceramic, or composite of desired composition to provide a delivery system. The size of the biodegradable glass material may be on the micron scale. In an aspect, the biodegradable glass rod may be about 0.2 μιτι to about 400 μιτι in diameter.

[0059] Delivery of nanoparticles created from the biodegradable glass may include injection of the nanoparticles alone, or as a suspension within a matrix material of soluble gels that will degrade with time (hours to weeks), insertion by surgical intervention, or placement at desired locations such that the linked or unlinked nanoparticles can be released over time into the body fluids at the site as the glass degrades in the body fluids. In an aspect, the nanoparticles may be suspended in a matrix material. In another aspect, the parent glass may be suspended in the matrix material. In yet another aspect, the parent glass may be on the surface of the matrix material.

[0060] In one aspect, the matrix material may be, but is not limited to collagen, laminin, fibrin, polycaprolactone (PCL), polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), poly-L-lactide (PLLA), polyethylene glycol

(PEG), any other biodegradable polymer matrix material, and combinations thereof. The matrix material and parent glass may be present in a mixture ratio of matrix material to parent glass from about 20:80 to about 40:60, from about 30:70 to about 50:50, from about 40:60 to about 60:40, from about 50:50 to about 70:30, and from about 80:20 to about 60:40. The parent glass may be present within the matrix material as a gradient from high dopant concentration to low dopant concentration. In another aspect, the parent glass may be evenly distributed throughout the matrix material. Multiple dopants with varying release rates and/or solubility may be included within a matrix material to provide a delayed release or gradient.

[0061] In another aspect, a biocompatible, biodegradable composite material may formed initially as a flat sheet which is then rolled in the form of a generally cylindrical conduit. Such a conduit may be any size, and in one example can be usefully formed to be about 10mm to about 20mm long, about 3 mm to about 5 mm in outer diameter, and have a thickness of about 3 to about 4 rolls of the polymer sheet, wherein the thickness ranges from about 25 μιτι to 250 μιτι. The conduit may be porous in one aspect. In another aspect, as described in further detail below, a biocompatible, biodegradable composite material in various conformations may be combined with a second matrix material. For example, a matrix material sheet containing a biodegradable composite material may be rolled into a conduit shape which may be filled with another matrix material containing a biodegradable composite material with the same or a different dopant. In one of many possible examples, a biodegradable composite material comprising PCL as the matrix and formed as a sheet may then be formed into a conduit which contains, or surrounds a gel-like scaffold material such as collagen or fibrin, in which a second biodegradable composite material is suspended or contained.

[0062] Because of their biodegradability, poly ε-caprolactone (PCL) and its copolymers have been used for soft tissue regeneration applications including peripheral nerves. PCL slowly degrades *in vivo* and its degradation can take

several years depending upon its molecular weight. Furthermore, the degradation rate of PCL can be altered by polymerization with other polymers such as poly-lactic acid (PLA). Other copolymers may be used in place of PCL. Addition of inorganic materials like biodegradable glass to a biodegradable polymer improves the mechanical strength and enhances wetting properties of certain polymers, which can improve cell adhesion. Together these properties of copolymers provide the structural scaffold to develop a mixed biomaterial consisting of biodegradable glasses to help heal and repair wounds, regenerate nerves and repair other soft tissues such as blood vessels, and soft tissues such as muscle, liver, and kidney.

[0063] In one aspect, biodegradable glass composites may be fabricated in PCL or another such polymer as shown in **FIG.** 7 (polymer sheets). The polymer sheet may then be rolled to form a cylindrical conduit. About 1.14g of PCL (molecular weight about 70,000) may be dissolved in about 15 ml of chloroform. The desired amount of glass particles may then be added to the PCL/chloroform solution, stirred for about 30 min, and ultrasonicated several times at about 1 min intervals. The glass/PCL mixture may be poured onto a polished glass plate and a film of the mixture may be tape-casted using a blade set at a thickness of about 600μηη. In an aspect, the glass plate may be about 8 cm wide and about 50 cm in length. The composite film may be dried at room temperature for about 30 min, removed from the glass plate, and stored in a desiccator.

[0064] The thickness of the dried films, measured with a micrometer at several locations, may be about 60 ± 10 μιτι. In various aspects, the thickness of the dried films may range from about 45 μιτι to about 55 μιτι, from about 50 μιτι to about 60 μιτι, from about 55 μιτι to about 65 μιτι, from about 60 μιτι to about 70 μιτι, and about 65 μιτι to about 75 μιτι. In one aspect, the composition of the 13-93 B3 borate glass (in wt.%) may be: 53% B_2O_3, 20% CaO, 12% K_2O, 6% Na_2O, 5% MgO, and 4% P_2O.

[0065] Bioglass composites may be fabricated consisting of varying percentages of PCL and 13-93 B3 glass chemically doped with varying types and quantities of dopants. Additionally, in place of PCL, other types of polymers that are inert and degrade with time may be substituted with many other polymer materials used commonly in conduits including, but not limited to poly-lactic acid (PLA), poly-L-lactic acid (PLLA), and poly-lactic-co-glycolic acid (PLGA).

III. Methods of Using Nanoparticles and Biodegradable Glass for Soft Tissue Repair

[0066] In one aspect, the biocompatible, biodegradable composite material is placed in contact with a body fluid or simulated body fluid. This may be at or near or in association with a region of interest in body of a mammal such that the nanoparticles are released into the body fluid(s) at the region of interest as the glass degrades upon contact with a body fluid(s), such as blood, interstitial fluid(s), cerebrospinal fluid, or lymph. A region of interest may for example be a peripheral nerve, or the spine and/or spinal cord. Alternatively, a biocompatible, biodegradable composite material is placed in contact with a body fluid or simulated body fluid in *vitro.*

[0067] For example, chemical dopants such as cerium oxide (CeO_2-x) nanoparticles have been shown to prevent the death of HT22 nerve cells, protect rat endothelial cells, increase the life span of brain cells, and provide benefits for the survival of damaged spinal cord cells in rats. Cerium oxide nanoparticles may also be used to treat, perhaps prevent, Alzheimer's, Parkinson and Huntington's diseases, and multiple sclerosis. Other elements such as Y, Zn, and Ag may also be neuroprotective, anti-oxidative, and overall provide improved health of cells. Therefore, a number of types of chemical dopants may be added to the glass composition at low weight %.

[0068] As described above and shown for example in **FIG.** 7, biocompatible, biodegradable glass composite materials as disclosed herein can

be formed into various shapes and sizes. Different forms may also be usefully combined with other biocompatible materials to create structures especially useful for use in soft tissue repair, including any repair of any organ or soft tissue, for example for wound healing or injured muscle repair. In one aspect, for example, the composite materials disclosed herein may be used for nerve regeneration. For example, a common repair mechanism for damaged or severed nerves involves suturing a nerve guide conduit to opposing ends of a severed peripheral nerve, to guide growth of the nerve into and through the conduit for repair. **FIG. 14** provides schematic illustrations showing how different forms of a biodegradable glass composite material as disclosed herein may be used in such a scheme. Any biodegradable glass composite material as disclosed herein may be formed for example as a conduit from a rolled polymer sheet, one or more glass rods, or may be formed as microfibers. **FIG. 14** schematically illustrates various structures in the context of the sciatic peripheral nerve model. As shown at left, a biodegradable glass composite material can be formed as a cylindrical tube (conduit), for example from a rolled sheet of the biodegradable glass composite material. Glass rods formed from the biodegradable glass composite material can be used to build a generally cylindrical scaffold, either alone or in association with a conduit of the biodegradable glass composite material, or in association with another matrix or scaffold material such as a collagen (CN), laminin or other similar gel. Alternatively, microfibers of the biodegradable glass composite material can be formed, and these can be embedded in a gel-like scaffold material such as a collagen gel, fibrin, or collagen+laminin, which may optionally contain or may be crosslinked to one or more growth factors or therapeutic agents. Such structures may be usefully implanted at a site of tissue damage to promote repair. For example, nerves can be easily sutured to a rolled polymer conduit.

[0069] It should be understood that many and various comparable structural iterations are contemplated by the fact that the biodegradable glass composite material is amenable to formation into many and various shapes and

sizes for building such structures The resulting structure can be used in the aforementioned method to support suturing tissue repair, for example, to support the nerve endings for peripheral repair and/or for embedding the mixed materials for nerve regrowth. Multiple different biodegradable glass composite materials, each with a different dopant could be used in combination. The nanoparticle dopants themselves may be released as the glass reacts with a body fluid or simulated body fluid, providing a timed release of dopants, growth factors, and/or therapeutic agents to promote tissue repair. Further, for example, structures embedded into an injectable matrix such as a collagen gel can be injected at the site of injury.

[0070] In addition, similar concepts may be utilized for central nervous tissue repair as in a spinal cord injury **(FIG. 15).** For instance, a collagen gel may be formed by containing aligned biodegradable glass rods or microfibers, or both. For a rod-containing gel, the gel may be formed outside of the site of injury in a mold that would form a similar shape as the rods cannot be injected; however the microfibers or frit may be readily suspended and may easily be injected while the collagen may be a gel. Then, as it forms a gel scaffold structure upon warming to body temperature, the glass may be embedded and the gel may take the shape of the injury site. Furthermore, as in the sciatic nerve injury model, the glasses used may be chemically doped, and either cross-linked to contain releasable drugs and/or growth factors with time as the glass reacts with the bodily fluids. Similar concepts may be utilized for any soft tissue injury repair, either injection at the site or forming a molded gel structure from collagen, fibrin or other types of gel matrix materials.

[0071] The results described below illustrate the individual aspects for the feasibility and optimization of this system. Together, they will help to determine how the biodegradable glass types and various forms could function, and with particular dopants/factors/drugs to include for the greatest benefit towards regeneration for the particular tissue. For instance, in the case of tissues that are

highly susceptible to oxidative stress, like nerves, including anti-oxidant dopants would be beneficial.

[0072] Biodegradable and chemically doped glasses may provide multiple means to aid and promote neuronal regeneration. Many of these examples of biodegradable glass may be used in other tissues for tissue regeneration.

EXAMPLES

[0073] The following examples are included to demonstrate the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the following examples represent techniques discovered by the inventors to function well in the practice of the disclosure. Those of skill in the art should, however, in light of the present disclosure, appreciate that many changes could be made in the disclosure and still obtain a like or similar result without departing from the spirit and scope of the disclosure, therefore all matter set forth is to be interpreted as illustrative and not in a limiting sense.

Example 1: ***Preparation of Parent Glass and Nanoparticles***

[0074] A sodium borate glass, with the molar composition of Na2O.2B_2O_3.xCeO2 (0.001 <x<0.06), was prepared using sodium tetraborate (Alfa Aesar Ward Hill MA, USA) and cerium oxide (CeO_2) (Alfa Aesar Ward Hill, MA, USA). The powdered raw materials were mixed, the homogeneous mixture was melted in a platinum crucible at 1000˚C for 1 hour, and stirred several times. The melt was poured on a cold steel plate and cooled to room temperature, forming a glass. **FIG. 1** shows the XRD pattern of the as-made glass. The absence of diffraction peaks in the pattern in **FIG. 1** indicates the material was non-crystalline with no identifiable nanoparticles.

[0075] The glass was crushed to particles of less than 150 μιτι in diameter and 200 mg of the glass particles were reacted in 50 ml of DI water and simulated body fluid (SBF) at 37˚C for 12 hours. As the glass particles degraded the solution became cloudy due to formation of ceria nanoparticles. The solution

was centrifuged to separate the nanoparticles from the solution, the residue was washed with fresh DI water, sonicated for 5 minutes, and then centrifuged for a second time. This procedure was repeated several times to insure that any boron or sodium that had dissolved from the glass was removed.

[0076] Finally, a drop of the solution containing the nanoparticles was placed on a 400 mesh copper TEM grid coated with a thin-holed carbon, dried at 50˚C, and then placed in the TEM. **FIG. 2** shows low and high magnification TEM images of the CeO_2-x nanoparticles released from the borate glass as it degraded in DI water. The parallel patterns visible in **FIG. 2B** are lattice fringes (Planes of atoms in a certain crystal structure) of cerium oxide crystals in a particular crustal structure. Each nanopartide was identified when the direction of these patterns changes because each nanoparticle/crystal has parallel planes (Boundaries of two individual nanoparticles identified by solid lines). The cerium oxide nanoparticles were crystalline (as indicated by the lattice fringes) and had an average size of 2-3 nm.

[0077] **FIG.** 3 shows the nanoparticles formed in Simulated Body Fluid (SBF) solution. Simulated body fluid is a buffered solution with a pH of 7.4 and has a composition very close to human blood plasma. The SBF was composed of the following (in mM): 137.5 NaCl, 3 KCl, 2 $MgCl_2$, 2.6 $CaCl_2$, 4.2 $NaHCO_3$, 1 $K2HPO_4$, 0.5 Na_2SO_4, and 50.6 Tris-Cl, pH 7.4 at 37°C. Table 1 shows the ion concentration of SBF and human blood plasma.

Table 1.

Ion	Concentration ($mmol/dm^3$)	
	Simulated body fluid (SBF)	Blood Plasma
Na^+	142.0	142
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	148.8	103
HCO_3^-	4.2	27

HPO_4^{2-}	1.0	1
SO_4^{2-}	0.5	0.5

[0078] Nanoparticles formed in SBF were about 50 ± 10 nm in diameter and had an amorphous (non-crystalline) structure with no lattice fringes/ parallel lines or patterns. Nanoparticles of yttrium and zirconium oxide may be formed in DI water using the same procedure **(FIG. 4).**

[0079] Table 2 provides examples of parent glass compositions, in mol % that have been melted.

Table 2.

	BX-A	BX-AM	BX-AMK	BX-MKL1	BX-MKL2	BN-MKL	B3-0Ca	B3	B2.5	B2
B_2O_3	59	57	57	57	52	60	53	50.2	42	33
Na_2O	29	28	28	28	26	14	12	6.5	6	6
CeO2	2	2	2	2	2	2	2	0.4	0	0
Al2O3	10	7	4	0	0	0	0	0	0	0
MgO	0	6	5	7	15	8	15	8.3	8	8
K_2O	0	0	4	4	4	14	15	8.6	8	8
Li2O	0	0	0	2	2	2	0	0	0	0
P2O5	0	0	0	0	0	0	3	1.9	2	2
SiO2	0	0	0	0	0	0	0	0	10	19
CaO	0	0	0	0	0	0	0	24	24	23

Example 2: *Cell growth with nanoparticles*

[0080] Embryonic (E10-11) chick dorsal root ganglia (DRG) were acutely dissociated and incubated with doped glass in the form of small pieces of glass (frit). The DRG are comprised of 3 types of cells, neurons and two support cells, fibroblasts and glia cells. Control cells were grown in the absence of glass. B3 glass refers to the biodegradable borate glass. Additional chemical elements

added to the B3 biodegradable borate glasses are referred to by the chemical abbreviation. For example, Ce is B3 glass doped with Cerium.

[0081] In the initial experiments, dissociated cells grew as well as, if not better than control cultures based on qualitative visualization only, for up to 10 days in culture with the following doped glasses: Ce1 , Ce2, Fe, Ga, Zn, and B3. These glasses were used further for quantification with Live/Dead assay and counting cells with glass in the form of rods, microfibers and polymer/glass composite sheets. The doped glass that supported some neuronal or cellular growth by qualitative visualization includes: Y 1, Y2, Ag, I_2, Mn, N_2, S, and Sr.

[0082] Quantitative analysis was performed on B3, Ce1 , Ce2, Y 1, and Y2 doped glasses compared to control (without any glass added). The cells were dissociated, seeded onto wells with the glass added and incubated for 10 days. Wells were treated with Live/Dead Assay (Molecular Probes) as per the instructions, and fluorescent images were acquired for each of the wells (4 per condition) at both 7 and 10 days. Green fluorescence indicates the live cells (calcein) and red fluorescence indicates the dead cells (ethidium bromide). Images were acquired individually, merged with Image J, and live and dead cells were counted. Neurons were distinguished based on their physical aspects of the cells: larger cells bodies with long and thin processes growing from the cell body. Fibroblasts are wide and flat, and glia are small round bodies without any processes. The total number of cells from both live and dead conditions per merged images was calculated and the fraction of live neurons/total cells for percent live neurons was determined. The images in **FIG.** 5 are representative live images for control cells (without glass added), Ce1 and Y 1. **FIG.** 6 is a graph that depicts the mean +/- standard error of the mean for the 4 different sets of wells imaged per condition at day 7 and day 10. Statistical analysis was performed with a two sample t-test with data considered significantly different than control for the same days in culture ($^{*}p<0.05$; $^{**}p<0.01$). Furthermore, both Ce and Y incubated for 7 days improved the neuronal survival and growth compared to either B3 glass without added chemicals ($+p<0.01$). Preliminary

results show that doped glass with Ce and Y improved the survival over that of control and B3 only conditions.

Example 3: *Fabrication of biodegradable glass composites*

[0083] To fabricate the PCL/biodegradable glass composites, or another such polymer, as shown in **FIG.** 7 (polymer sheets), 1.14g of PCL (molecular weight ~70,000) was dissolved in 15 ml of chloroform. The desired amount of glass particles was then added to the PCL/chloroform solution, stirred for 30 min and ultrasonicated several times at 1 min intervals. The glass/PCL mixture was poured onto a polished glass plate and a film of the mixture 8 cm wide and 50 cm in length was tape-casted using a Dr. Blade set at a thickness of ΘΟΟμιτι. The composite film was dried at room temperature for 30 min, removed from the glass plate, and stored in a desiccator. The thickness of the dried films, measured with a micrometer at several locations, was 60 ± 10 μιτι. The composition of the 13-93 B3 borate glass (in wt.%) was: 53 B_2O_3, 20 CaO, 12 K_2O, 6 Na_2O, 5 MgO, and 4 P_2O.

Example 4: *Degradation in Solution*

[0084] Noticeable changes in the microstructure and morphology of the rods and composite polymer sheets occurred after immersion in SBF or cell culture media. The rods react and degrade with exposure time in the cell culture media **(FIG. 10).** When the polymer sheets were exposed to SBF, both sides of the composite sheet became rougher, and were covered with regions of submicron crystals of hydroxyapatite (HA, see below). Using scanning electron microscopy, the fabricated 100% PCL sheet is shown in **FIG. 11** compared to that of 13-93 B3 without any dopants. The micrographs show that the glass particulates agglomerate during the drying process to form islands, see **FIG. 11.** These agglomerates were likely responsible for the rougher surfaces. The agglomerate islands of the 13-93 B3 composites were nearly covered with

regions composed of hydroxyapatite crystals (inset higher magnification) that were identified by X-ray diffraction, see FIG. 5. Cross-section scanning electron microscopy images show the sub-micron HA microspheres (FIG. 12). Spherical shapes composed of small HA crystals were also present within the cross section of the composite film. This image indicates that the PCL-composite sheets were permeable to the SBF solution since the HA crystals could only have formed if the 13-93 B3 glass particles had been in contact with the SBF solution.

[0085] Degradation of the 100% PCL and the 13-93 B3 composite in SBF was determined in two ways: weight loss measurements and analysis of the SBF solution with inductively coupled plasma (ICP) for elemental analysis of B, Ca, and P, as a function of time. The weight loss degradation profile for each composite is shown in FIG. 13A for 14 days of SBF reaction. Weight loss for the 13-93 B3 composite rapidly increased for the first 3 days and reached a maximum for the duration of the time period compared to the weight loss for the 100% PCL film which was negligible over the 14 day period. Ion release was measured for the composites for 14 days in SBF. The concentration of B, Ca, and P released from the 13-93 B3 polymer composite was compared to 100% PCL (FIG. 13B-D). The average concentration of B released from the 13-93 B3 reached its highest value in 3 days. For Ca release, most of the change occurred in the first 24 hours, although it was small compared to the nominal 100 ppm Ca concentration in the starting SBF media. The increase in Ca concentration for the 13-93 B3 composite indicates that this fast reacting glass was releasing Ca, which temporarily increases the overall super-saturation of the SBF. Eventually, this leads to the precipitation of the insoluble HA material and accounts for the slightly lower Ca concentration in the SBF at longer times. The P concentration in the composites decreased with time. This reduction in P concentration was consistent to form HA until either the Ca or P was consumed. Similar experiments can be performed to show the weight loss and ion release effects with different chemically doped glasses.

Example 5: *Mechanical Property Testing*

[0086] Uniaxial tensile testing was performed to assess the mechanical properties of the composites to confirm that the composites were strong enough for an initial repair, but degrade within an appropriate time. Unreacted composites were compared to composites reacted with media for 3 and 6 weeks. For this experiment, glass composites consisted of 50% 13-93 B3:50% PCL (B3), 50% 45S5 (Bioglass®): 50% PCL (45S5), and 25% 13-93 B3:25% 45S5:50% PCL (blend) were compared to 100% PCL. Peak stress, strain at break, and elastic modulus were calculated for all composite samples **(FIG. 16).** Compared to unreacted composites, peak stress was only significantly affected after 6 weeks in cell culture media for 100% PCL polymer sheets. PCL alone resulted in higher peak stresses than any of the biocompatible, biodegradable composite material sheets at any time point. The blend and 45S5 composites did not show any significant change in peak stress or strain at break. The elastic moduli also indicate stiffer properties of PCL and 45S5 sheets compared to the B3 composite sheets. As expected, the blend of 45S5 and B3 glasses yielded moduli between the B3 and 45S5 polymer sheets. In addition, PCL and 45S5 polymers trended toward stiffer moduli with increased time in media, though these results were not significant. Polymers with B3 did not vary in stiffness with incubation time.

Example 6: *Suture and mechanical strength of conduits*

[0087] The biodegradable glass/PCL polymer sheets can be readily rolled and formed into a conduit. Conduits were formed by rolling the polymer sheet (single layer ~ 0.06 mm) about 5x, gluing with collagen to form a nerve guidance conduit that was 15-16 mm long, 4.3 mm outer diameter and ~0.3 mm inner thickness (although these dimensions can be easily modified). We sutured cadaver rat sciatic nerve with 9-0 sutures to one side of each end of the rolled 13-93 B3 conduits **(FIG. 17)** and performed mechanical strength testing on the nerves. Each of the strength test resulted in the nerve suture being pulled from

the conduit or the nerve pulling apart from the suture, but not the conduit itself failing.

Example 7: *Neuronal survival and outgrowth*

[0088] To test the effects of the chemically doped glasses on neurons, two main experiments were conducted to test whether the dopants were a) promoting or inhibiting survival, and b) promoting or inhibiting neuronal outgrowth as a model for nerve regeneration. To accomplish this, embryonic (E10-11) chick dorsal root ganglia (DRG) were dissociated. The "whole" DRGs are comprised of 3 types of cells: neurons and two support cell types called fibroblasts and glia. When dissociated, the types of cells can be distinguished from one another morphologically, neurons have brightly round and clustered cells bodies interconnected by neurite processes, fibroblasts are flattened broad cells, and glia are small, polarized cells. In addition, Live cells were stained with Calcein AM and for Dead cells with eithidium bromide (Live/Dead Assay; Molecular Probes). Cells were then counted as live neurons, live support cells, or dead cells. Percentage of live neurons compared to all live cells or total cells were calculated. **FIG. 18** shows a representative image of neurons in culture, stained for Live/Dead and merged.

[0089] These types of experiments were repeated with biodegradable borate-based glass without dopants (B3, control) or with chemical dopants. The cells were dissociated, and cultured for 3 days prior to adding any biodegradable glasses. For this experiment, all of the cells were exposed to two, 1 cm pieces of glass that approximated ~ 14 mg/mL of glass reacting in cell culture media. After 3, 7, or 10 days in culture with the biodegradable glass, cells were counted and analyzed for survival. Figure 19 shows the average number of neurons alive **(FIG. 19 A)** and the average number of support cells alive **(FIG. 19 B)** for the doped glass conditions. Each of the dopants is shown labeled below the graph for three (3) days (medium gray), seven (7) days (dark gray) and ten (10) days (light gray) of incubation with the glass types. Statistical comparisons are shown

for each of the dopants compared to the control BGG without dopant for the same day, where the averages that are significantly less than the control are shown as the pound (#) sign, and the averages significantly greater are shown as an asterisk (*), $p<0.05$. Figure 20 shows the averaged and normalized percent of neurons/total live cells **(FIG. 20 A)** and averaged and normalized support cells/total live cells **(FIG. 20 B)** for the dopant glass conditions at 3, 7, and 10 days of exposure. The dead cells were not taken into consideration. Note that the difference between the two different Ce and Y (1 and 2) glasses is that the Ce2 and Y2 are both formulated with a trace amount of iodine, and thus iodine was tested alone. In addition, the effects of adding 1, 2, 4 and 8 pieces of 1 cm (7mg/mL) biodegradable glass **(FIG. 21)** were tested.

[0090] The response of DRG neurons to the biodegradable glass B3/PCL polymer sheets was determined by measuring the length of neurite outgrowth from whole DRGs after culture for 3 days on each doped polymer. Representative images of whole DRGs are shown, and demonstrate that the neurites survive and extend after culture on the doped polymer composites compared to the 13-93 B3 **(FIG. 22).** PCL supports neurite outgrowth very well. Addition of 13-93 B3 and/or dopant glass particles to PCL show that some of the dopants improve outgrowth on the composite polymer sheet compared to 13-93 B3 glass without dopant. For example, Zn exhibited increased outgrowth compared to Ce1 , Ce2, and Y 1 (*$p<0.05$) and Fe exhibited increased outgrowth compared to Y 1 (#$p<0.05$). Overall, the mix of Cu/Zn/Sr/Fe, Fe, and Zn improved outgrowth over that of 13-93 B3 alone. However, none of the dopants exhibited any significant decrease compared to the undoped B3 glass. Because the composite polymer sheets may be used to form a conduit for neurons to grow within, it was primarily important that none of the dopants would inhibit the regrowth. When the 13-93 B3 glass was pre-reacted, 13-93 B3 composites showed a significant increase in neurite outgrowth compared to other undoped biodegradable glasses of 45S5 and the blend of 45S5/B3/PCL **(FIG. 23).** These

experiments may be repeated for the pre-reacted doped biodegradable B3 polymer sheets.

[0091] Further data was obtained by measuring the length of the single longest neurite to grow out from the whole dorsal root ganglia, when measured from the center of the ganglia after 3 days in culture. The whole ganglia were placed on top of a poly-L-caprolactone (PCL) polymer sheet of bioactive glass (50% polymer, 50% doped borate glass). As shown in **FIG. 24,** compared to control 13-93B3 polymer glass without dopant, the Fe-, Ga-, and Zn-containing polymer sheets significantly improve neurite outgrowth. Copper is also tested in the same way. With iodine, neuronal survival was quite poor over 10 days as shown in **FIGS. 19A and 20A,** which indicates that dopant inclusion alone is not supportive of neuronal growth, but that specific release of particular dopants are important for neuronal regrowth.

[0092] Interestingly, the chemical dopants that improve the neuronal survival of single dissociated cells as shown in **FIG. 20** are not always the same dopants that support outgrowth of neurites from a whole ganglion growing on the surface of the polymer glass composites as shown in **FIG. 24.** Although it is not yet known why one dopant supports growth better than the others, ion release experiments were performed to determine the rate of release of the ions from the glass fibers used in the experiments for **FIGS. 19 and 20,** as compared to the release of ions from the composite PCL polymer sheets on which the whole dorsal root ganglia are grown, as shown in **FIG. 24.**

Example 8: *Aligned glass cause aligned growth*

[0093] Whole chick dorsal root ganglia (DRG) were seeded onto aligned 13-93 B3 rods "glued" onto thin fibrin or collagen scaffolds to form a "raft" of biodegradable glass rods. The whole DRG were imaged growing on the aligned 13-93 B3 glass rods as shown in **FIG. 25.** When the number of neurites were counted that were growing within each 45 degree angle from 0 to 360 degrees, and taking the center of the whole DRG as the apex of the angle, the neurites

aligned with the axis of the glass rods as shown in the bar histogram of **FIG. 25.** The schematic that shows the angles and neurites were oriented with the aligned glass fibers. Together, the DRG experiments show that the DRG grow on biodegradable glass, and when the glass was aligned, neurites extend from the DRG in an oriented direction.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the present disclosure pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. A biocompatible, biodegradable composite material for soft tissue repair, comprising:

a matrix material; and

a parent glass suspended within the matrix material, the parent glass comprising one or more dopants;

wherein the parent glass releases a plurality of dopant based nanoparticles when in contact with a body fluid or simulated body fluid.

2. The biodegradable glass composition of claim **1**, wherein the parent glass comprises sodium tetraborate.

3. The biodegradable glass composition of claim **1**, wherein the dopant is selected from a metal ion, a transition metal ion, an oxide, a rare earth oxide, a halide, carbonate, a compound containing a cation, and any combination thereof.

4. The biocompatible, biodegradable composite material of claim **3,** wherein the dopant is selected from CeO2, Ce2O3 Y2O3, and ZrO2 and mixtures thereof.

5. The biocompatible, biodegradable composite material of claim **1**, wherein the dopant is selected from cations of Co, Ni, Cu, Ag, Au, Pt, Fe, Ru, Si, V, Cr, Mn, Fe, Ni, Zn, Sn, Sb, Zn, Ti, Y, Zr, **W,** La, Ce, Pr, Nd, Sm, Eu, Lu, Yb, Er, Ba, Ga, I, N, S, Si, and any combination thereof.

6. The biocompatible, biodegradable composite material of claim **5,** wherein the dopant is selected from the combinations I/Ce, I/Y, I/Ce/P, and Cu/Zn/Sr/Fe.

7. The biocompatible, biodegradable composite material of claim 1, wherein the parent glass comprises $Na_2O.2B_2O_3.xCeO_2$ and x ranges from about 0.001 to about 0.30 moles.

8. The biocompatible, biodegradable composite material of claim 1, wherein the material is degradable *in vivo.*

9. The biocompatible, biodegradable composite material of claim 1, further comprising a therapeutic agent.

10. The biocompatible, biodegradable composite material of claim 1, wherein matrix material is selected from a polymer, a ceramic, and any combination thereof.

11. The biocompatible, biodegradable composite material of claim 10, wherein the matrix material is selected from collagen, laminin, fibrin, PCL, PLA, PLLA, PEG, PGA, PLGA and any combination thereof.

12. The biocompatible, biodegradable composite material of claim 1, wherein the parent glass comprises at least one conformation selected from irregular particles, microspheres, fibers, rods, ribbons and any combination thereof..

13. The biocompatible, biodegradable composite material of claim 12, in which the conformation is combined with a second matrix material.

14. The combination of claim 13, wherein the second matrix material comprises a scaffold material selected from collagen, laminin, fibrin and any combination thereof.

15. The combination of claim 13, wherein the combination has a generally cylindrical conduit form.

16. A method of delivering nanoparticles to a region of interest, comprising:

contacting a biocompatible, biodegradable composite material with a body fluid or simulated body fluid, the biocompatible, biodegradable composite material comprising:

a matrix material; and

a parent glass suspended within the matrix material, the parent glass comprising a dopant; and

allowing the parent glass to react or degrade to form and release the nanoparticles.

17. The method of claim 16, wherein the glass comprises sodium tetraborate.

18. The method of claim 16, wherein the dopant is selected from a metal ion, a transition metal ion, an oxide, a rare earth oxide, a halide, carbonate, a compound containing a cation, and any combination thereof.

19. The method of claim 18, wherein the dopant is selected from CeO_2, Y2O3, and ZrO_2.

20. The method of claim 16, wherein the dopant is selected from Co, Ni, Cu, Ag, Au, Pt, Fe, Ru, Si, V, Cr, Mn, Fe, Ni, Zn, Sn, Sb, Zn, Ti, Y, Zr, W, La, Ce, Pr, Nd, Sm, Eu, Lu, Yb, Er, Ba, Ga, I, N, S, Si, and any combination thereof.

21. The method of claim 20, wherein the dopant is selected from the combinations l/Ce, l/Y, l/Ce/P, and Cu/Zn/Sr/Fe.

22. The method of claim 16, wherein the parent glass comprises Na2O.2B_2O3.xCeO2 and x ranges from about 0.001 to about 0.30 moles.

23. The method of claim 16, wherein the biocompatible, biodegradable composite material further comprises a therapeutic agent.

24. The method of claim 16, wherein the matrix material is selected from a polymer, ceramic, and any combination thereof.

25. The method of claim 24, wherein the matrix material is a polymer selected from collagen, laminin, fibrin, PCL, PLA, PLLA, PEG, PGA, PLGA, and any combination thereof.

26. The method of claim 16, wherein the parent glass comprises at least one conformation selected from irregular particles, microspheres, fibers, rods, ribbons, and any combination thereof.

27. The method of claim 16, in which the conformation is combined with a second matrix material.

28. The method of claim 27, wherein the second matrix material comprises a scaffold material selected from collagen, laminin, fibrin and any combination thereof.

29. The method of claim 27, wherein the combination has a generally cylindrical conduit form.

30. The method of claim 16, wherein the body fluid is associated with a region of interest.

31. The method of claim 30, wherein the region of interest is selected from a peripheral nerve and the spinal cord.

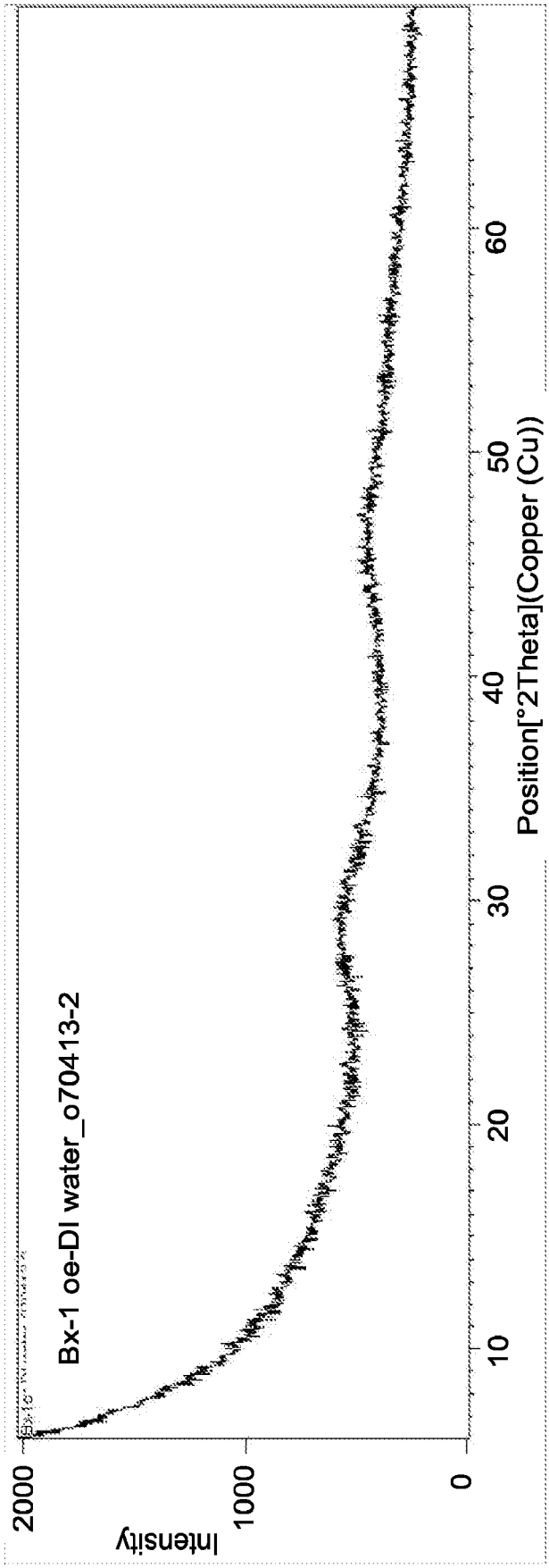

Bx-1 oe-DI water_o70413-2
Intensity
2000
1000
0
10
20
30
40
50
60
Position[°2Theta](Copper (Cu))

FIG. 1

FIG. 2A

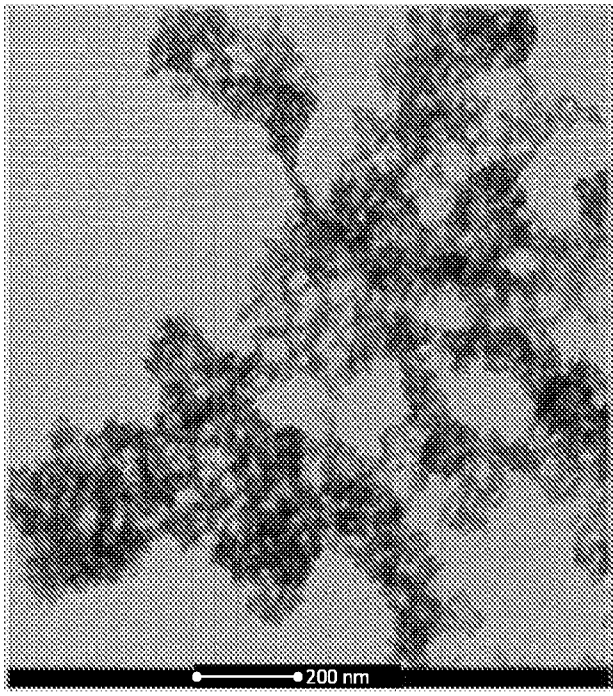


FIG. 2B

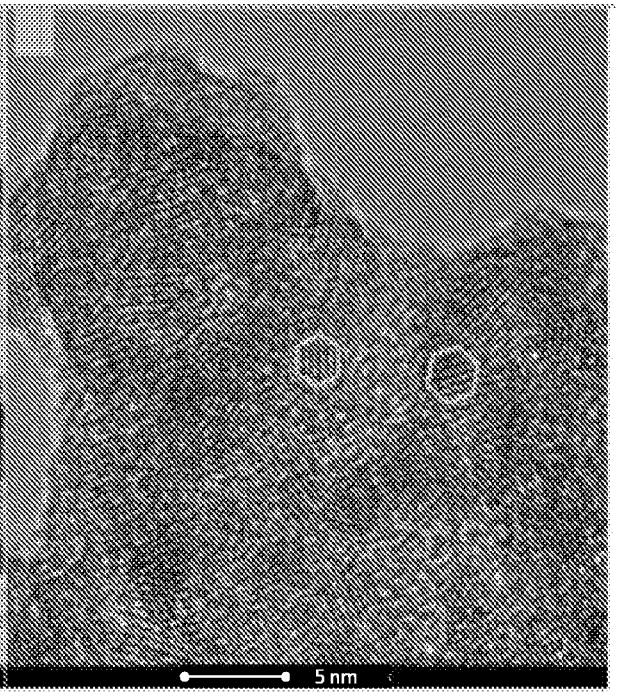


FIG. 3A

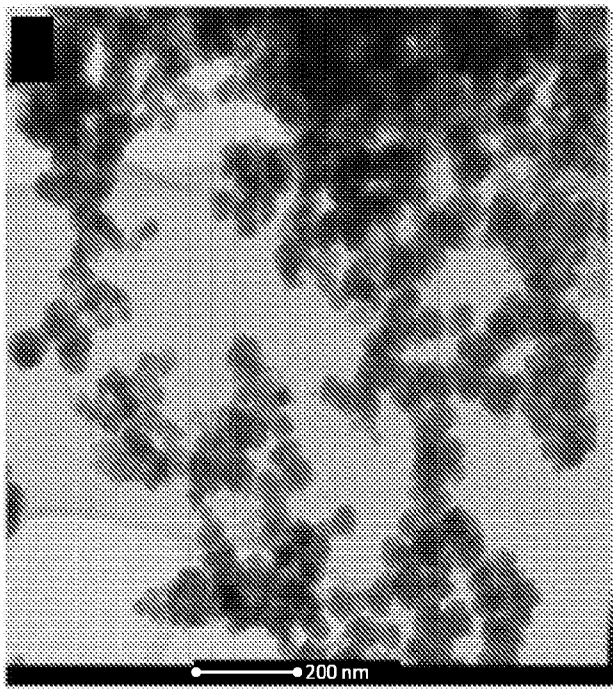


FIG. 3B

FIG. 4A

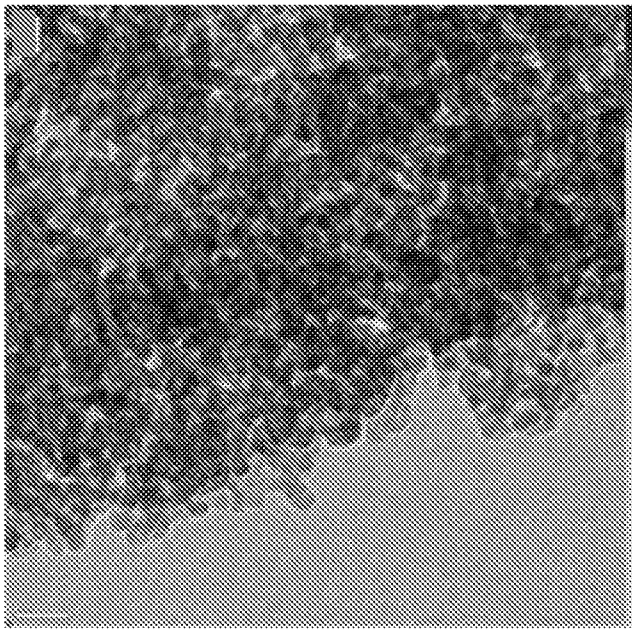


FIG. 4B

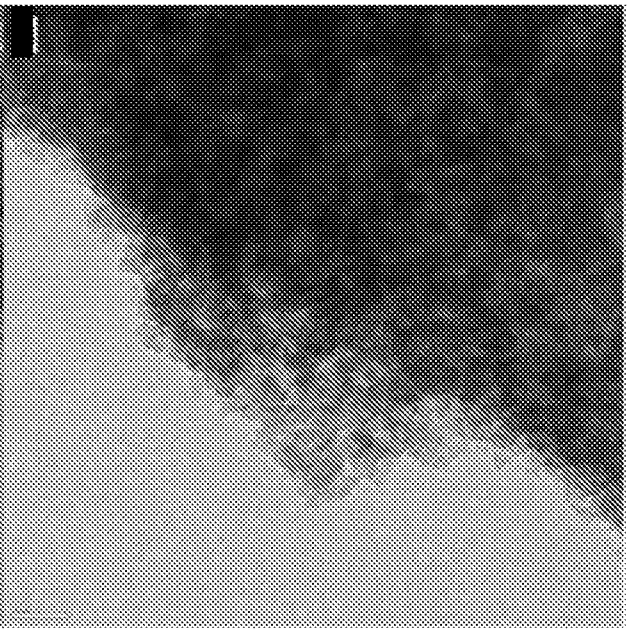
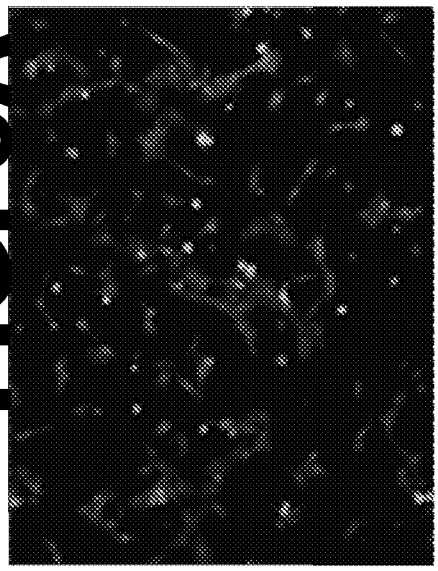
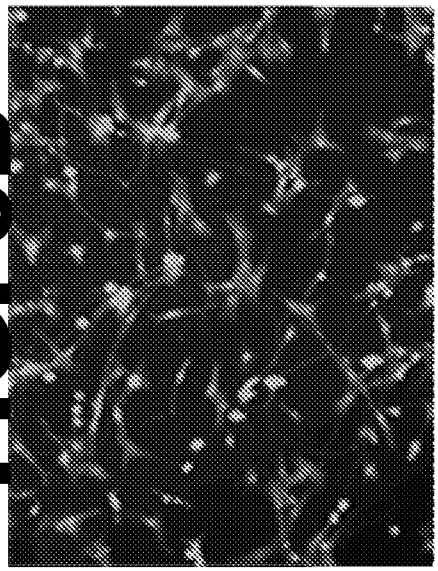


FIG. 5A



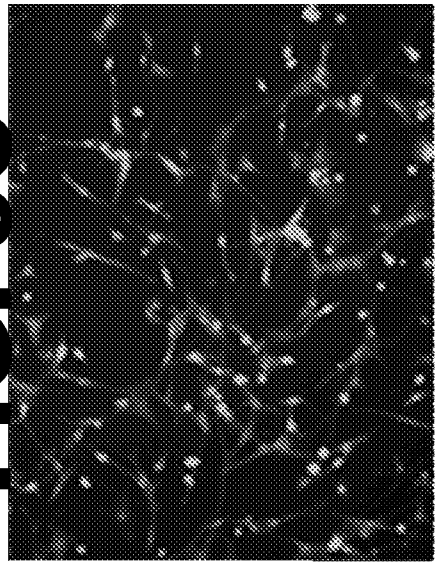
Control

FIG. 5B



Ce1

FIG. 5C



Yt1

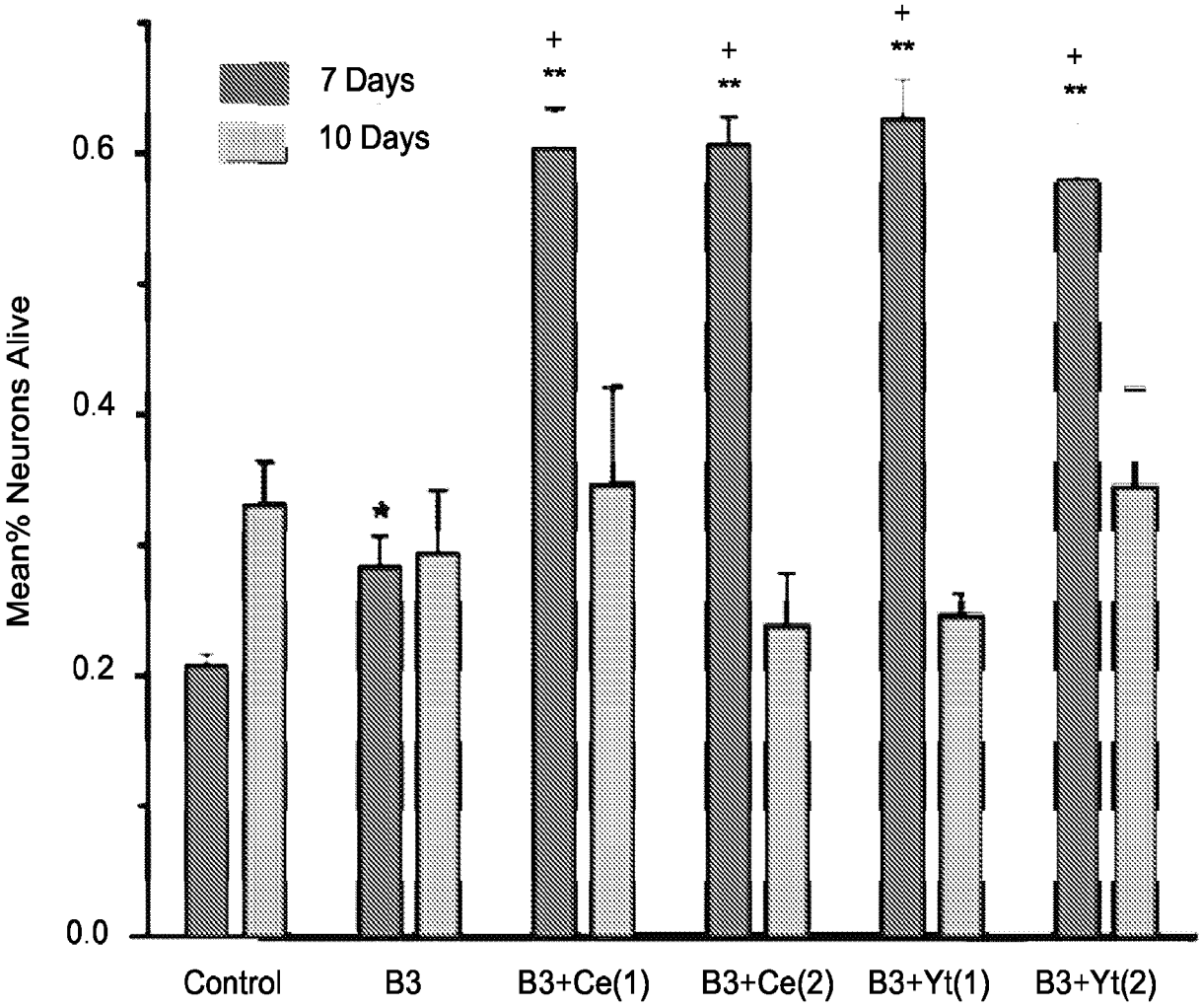

7 Days
10 Days
Mean% Neurons Alive
0.0
0.2
0.4
0.6
Control
B3
B3+Ce(1)
B3+Ce(2)
B3+Yt(1)
B3+Yt(2)

FIG. 6

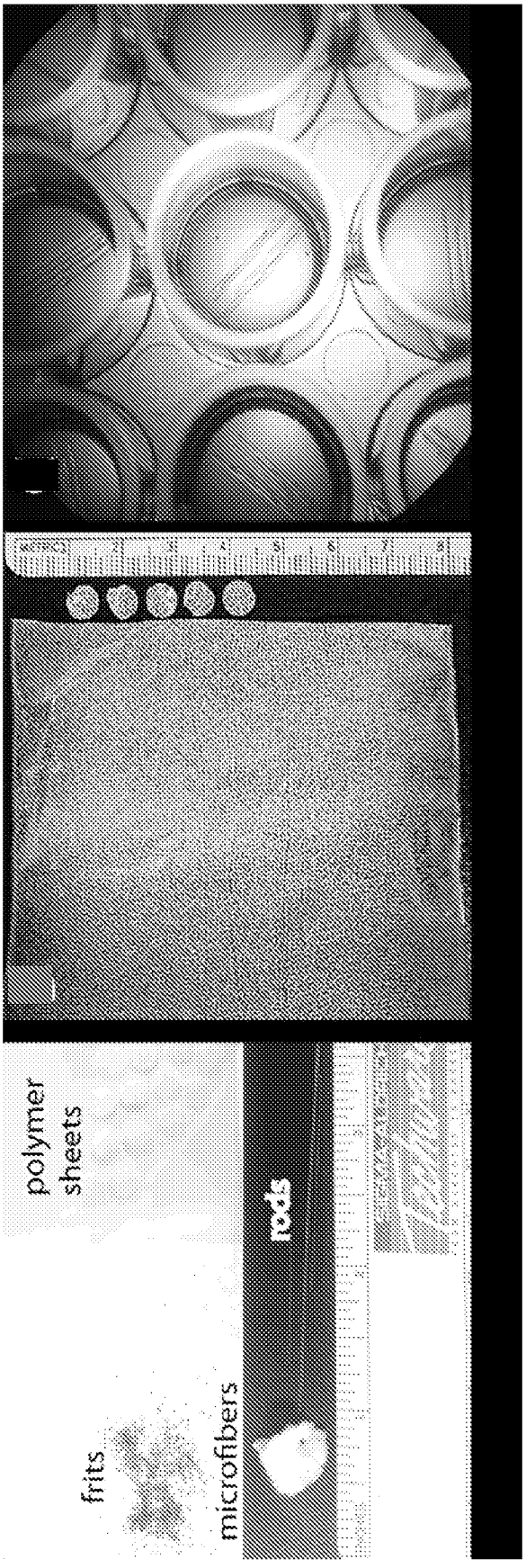


FIG. 7A FIG. 7B FIG. 7C

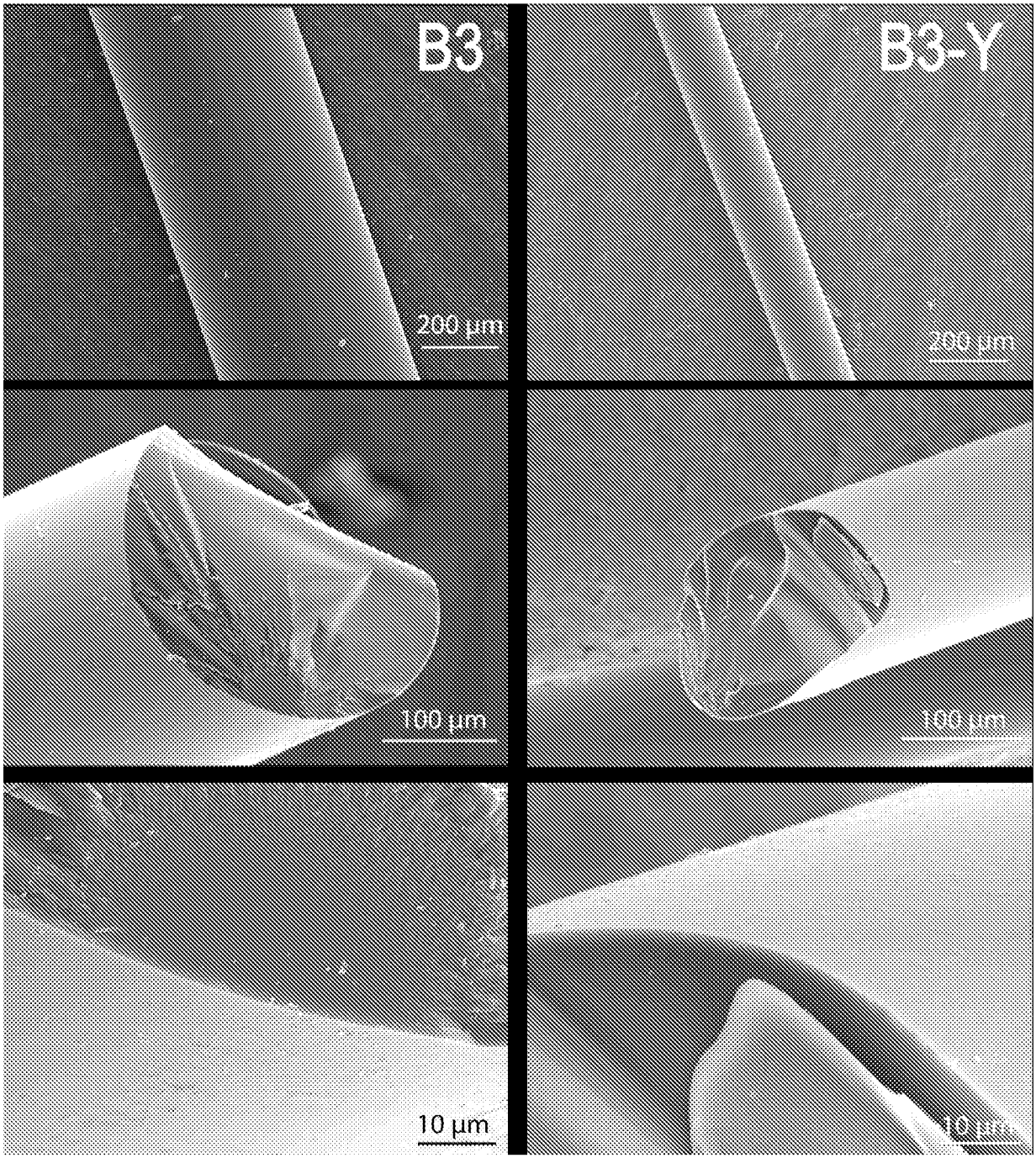


FIG. 8

FIG. 9A FIG. 9B

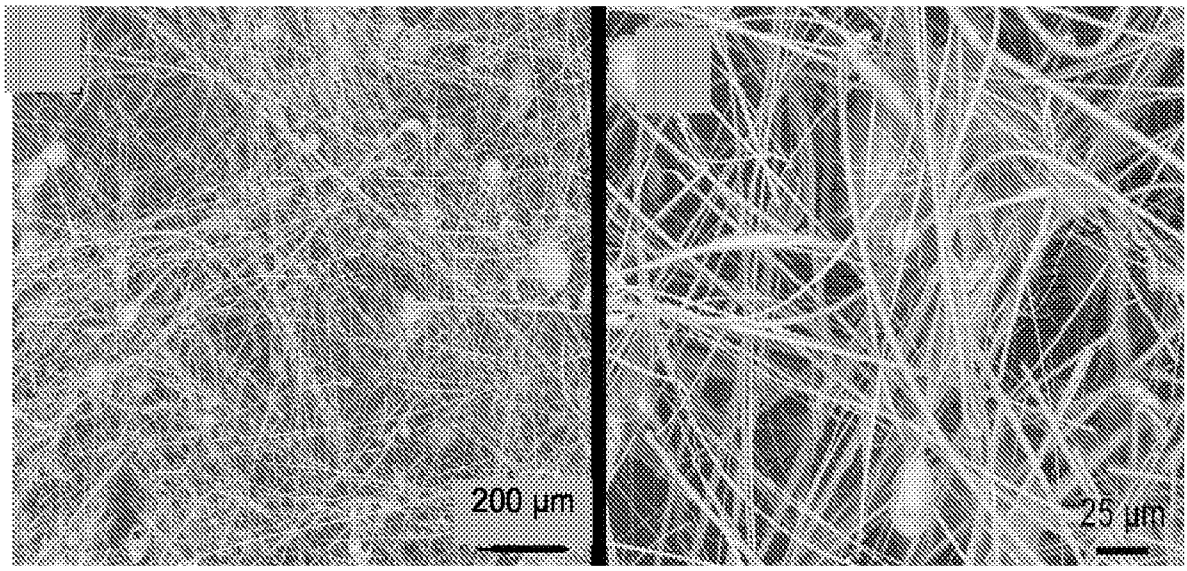


FIG. 10A

FIG. 10B

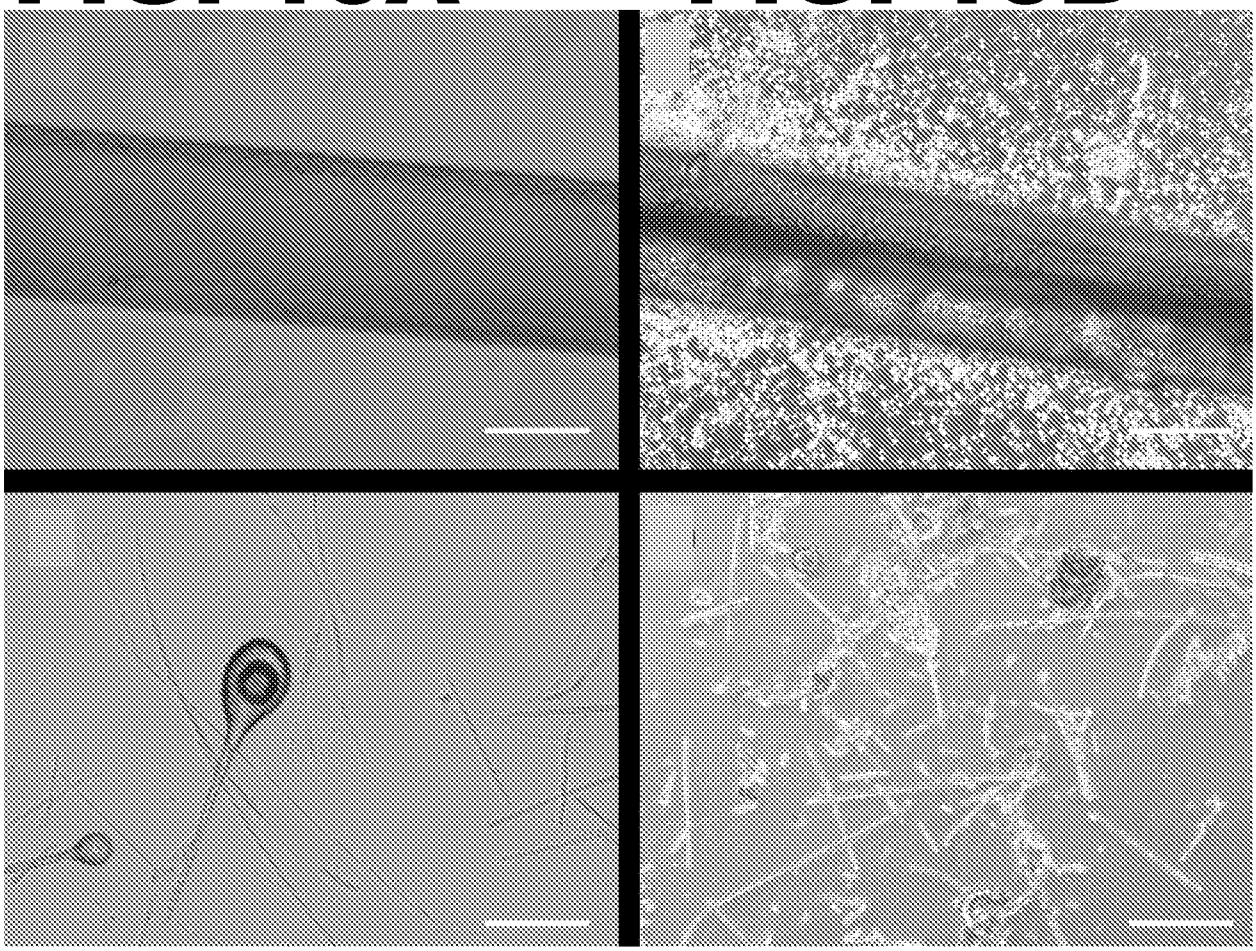


FIG. 10C

FIG. 10D

FIG. 11A FIG. 11B

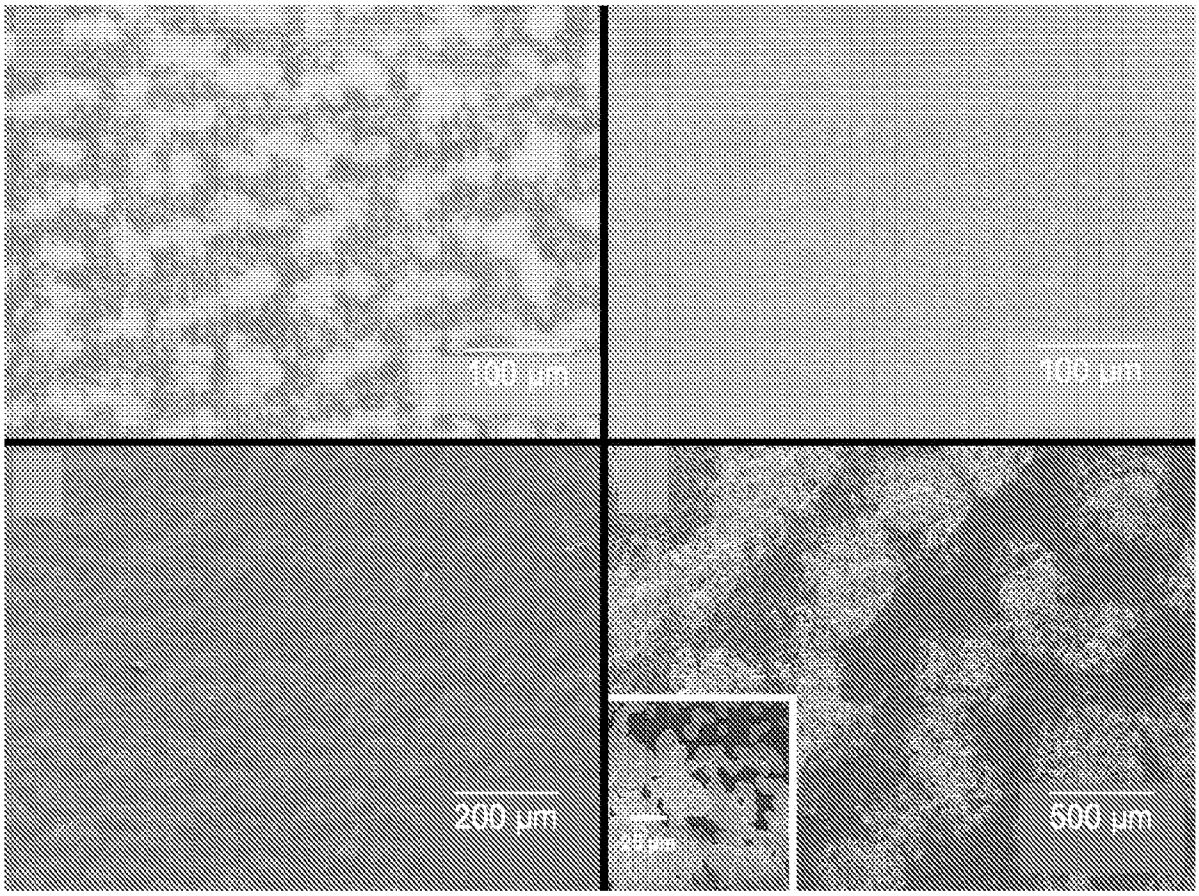


FIG. 11C FIG. 11D

FIG. 11

FIG. 12A

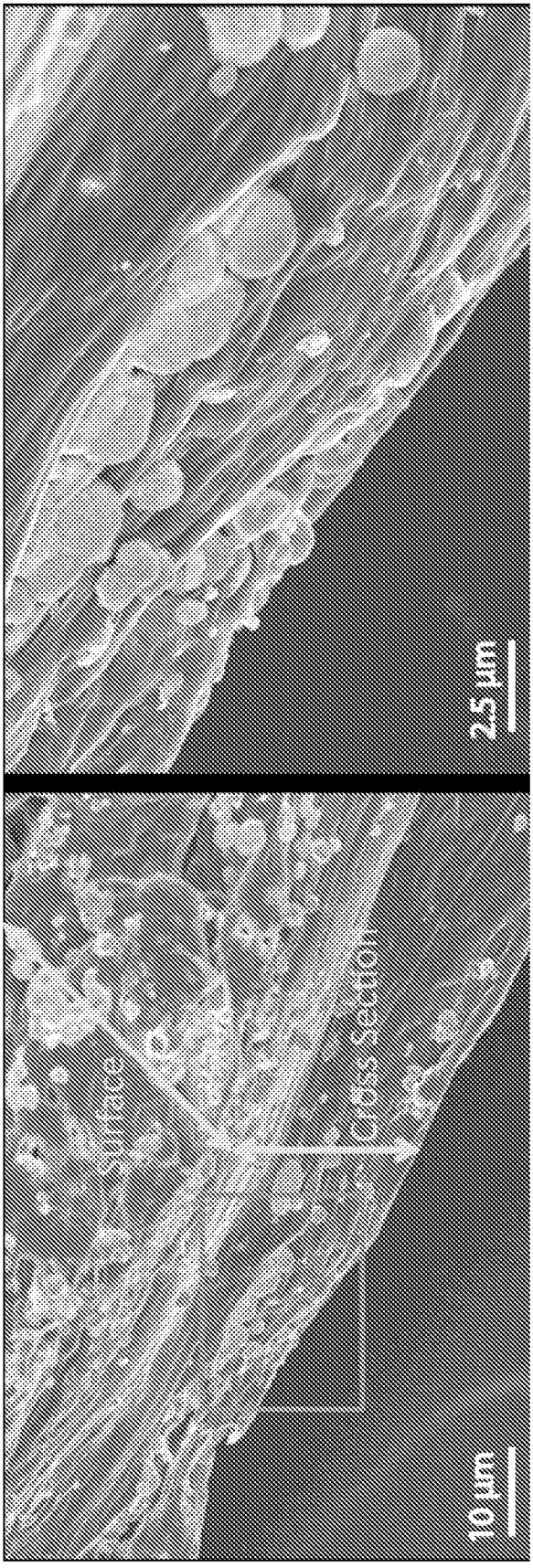


FIG. 12B

FIG. 13A

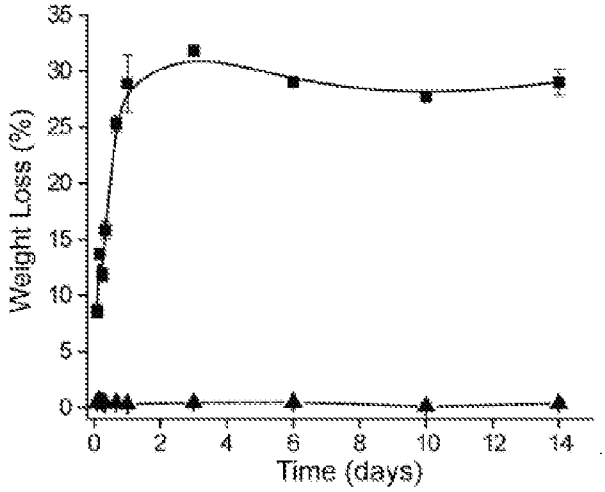
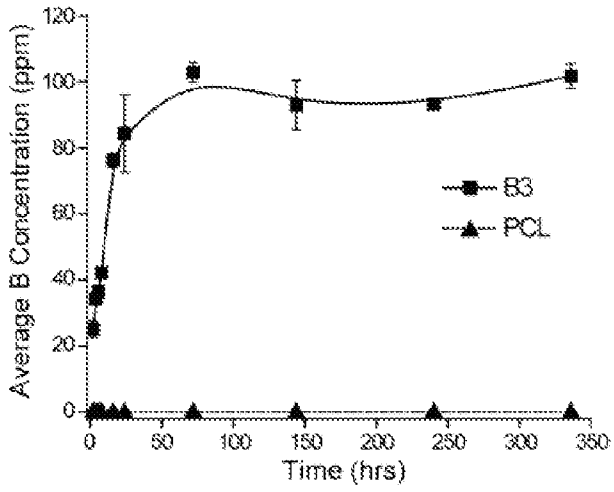


FIG. 13B



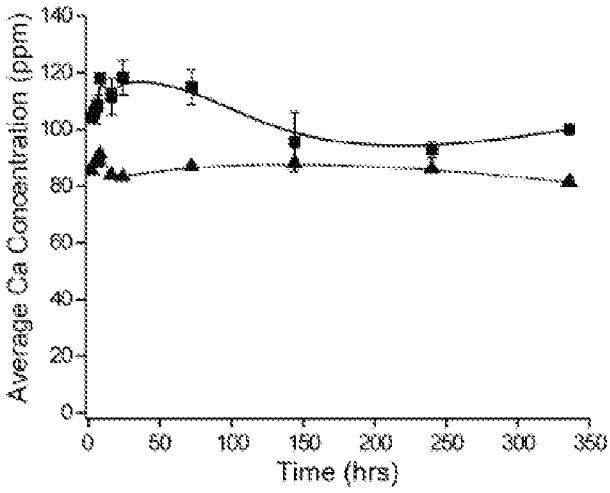


FIG. 13C

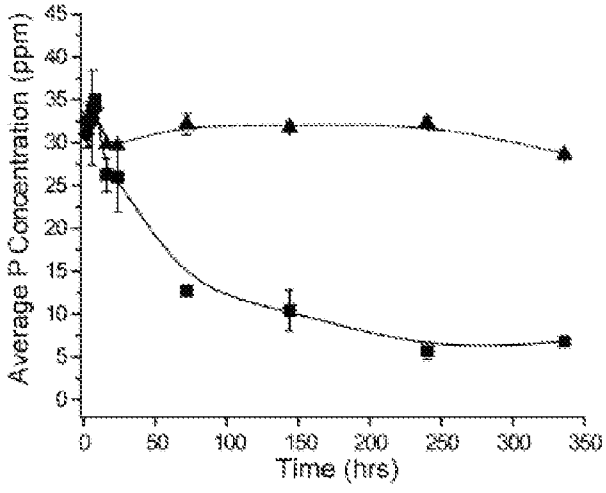


FIG. 13D

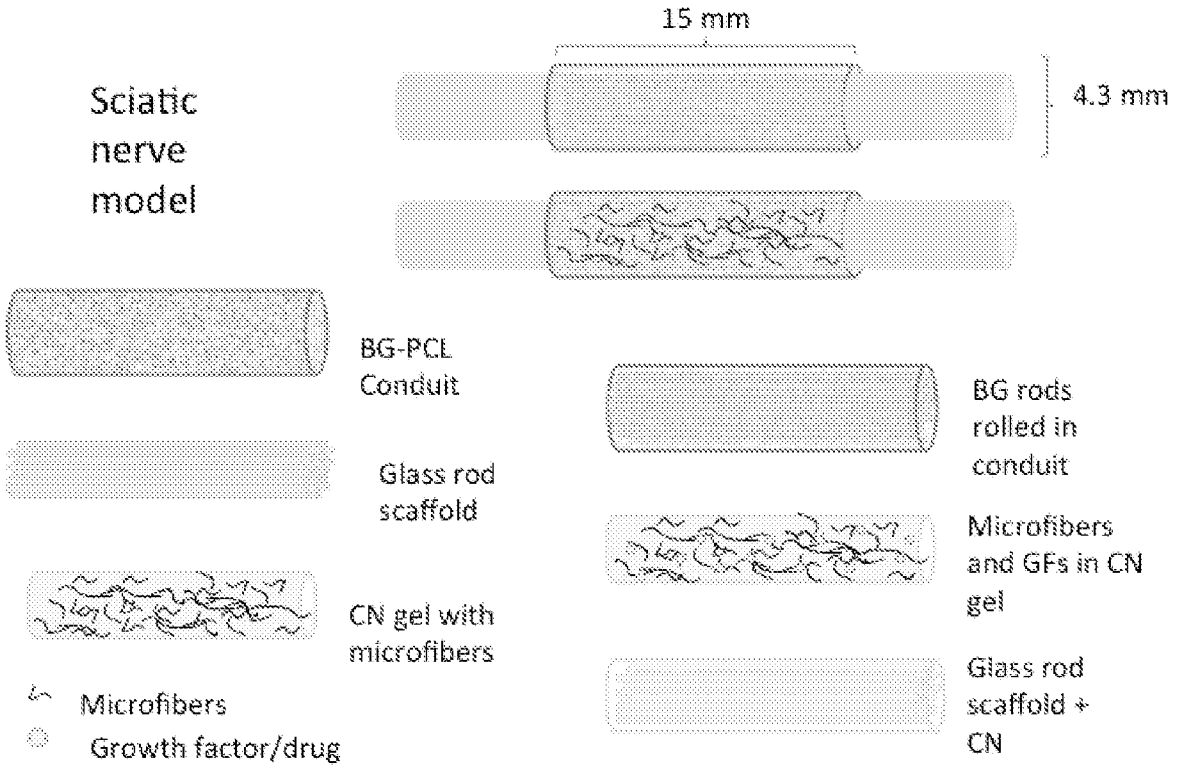

15 mm
4.3 mm
Sciatic nerve model
BG-PCL Conduit
Glass rod scaffold
CN gel with microfibers
Microfibers
Growth factor/drug
BG rods rolled in conduit
Microfibers and GFs in CN gel
Glass rod scaffold + CN

FIG. 14

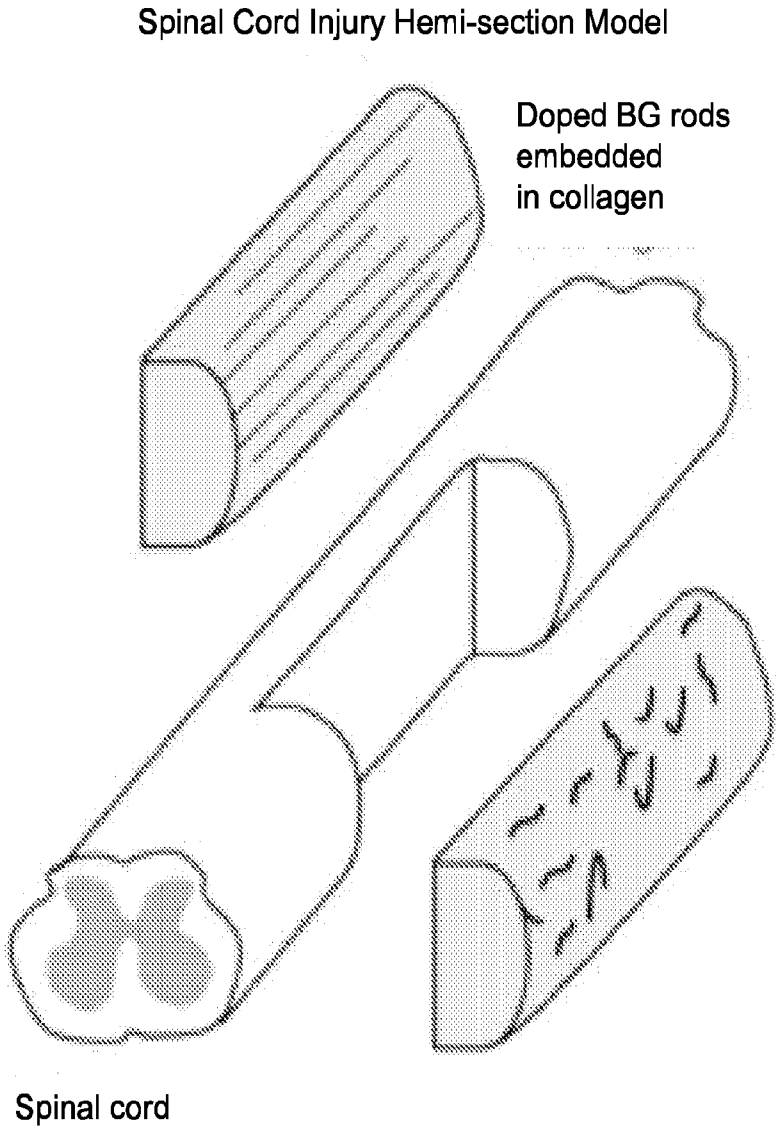

Spinal Cord Injury Hemi-section Model
Doped BG rods
embedded
in collagen
Spinal cord

FIG. 15

FIG. 16A

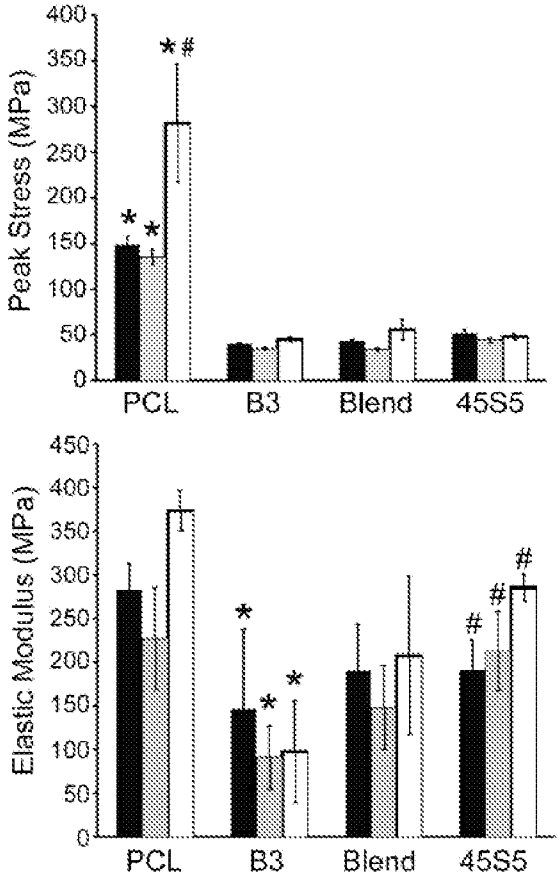


FIG. 16B

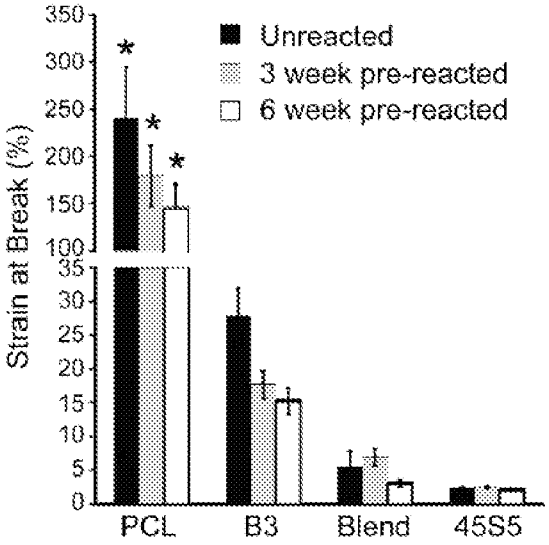


FIG. 16C

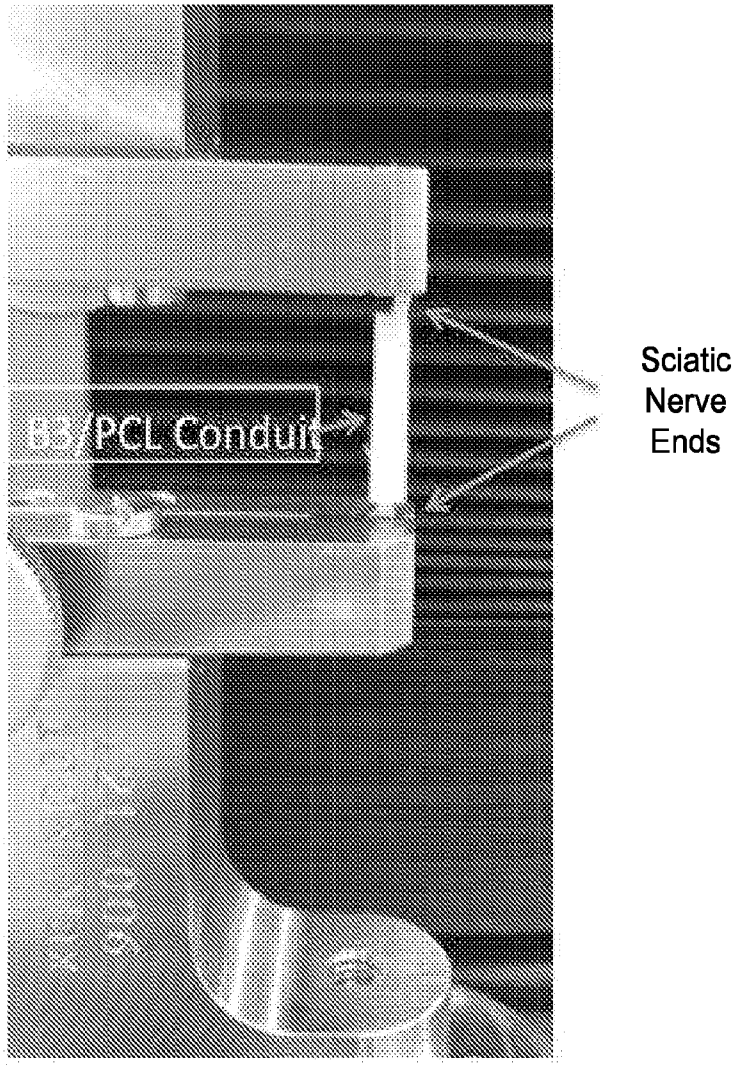


FIG. 17

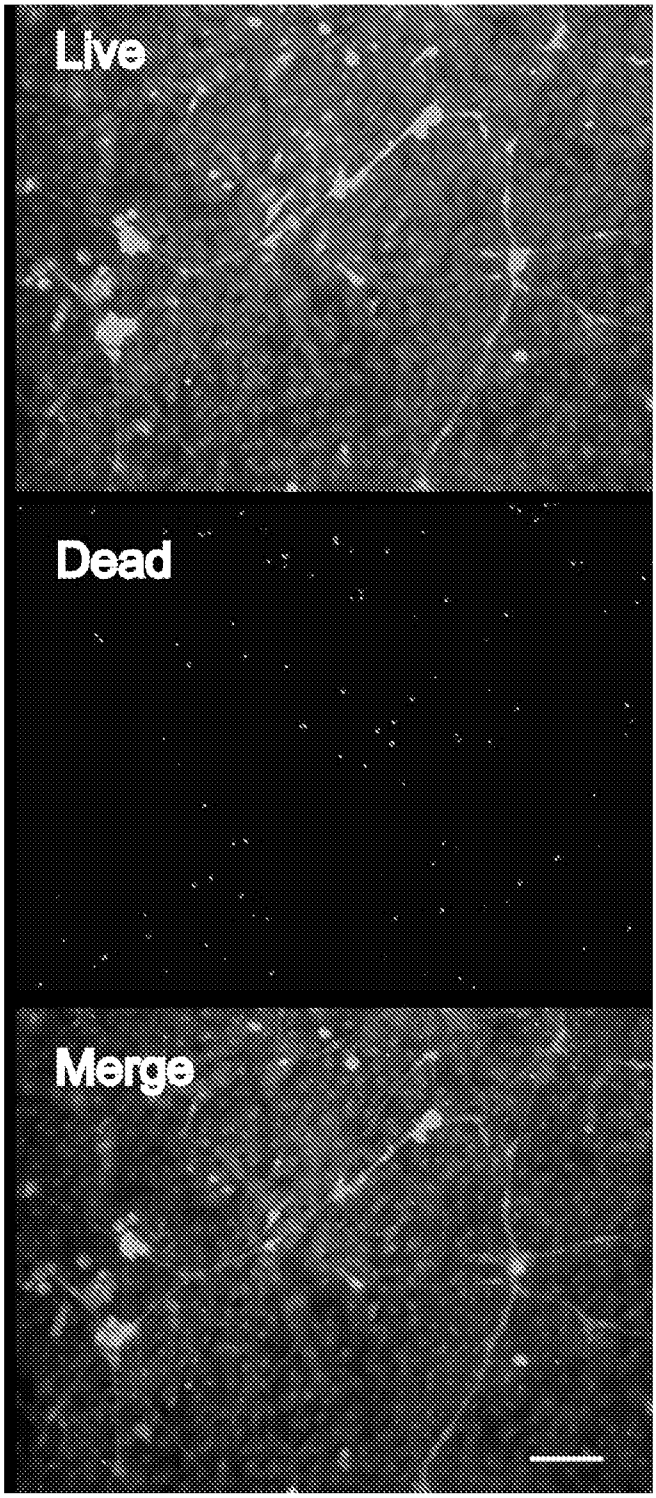
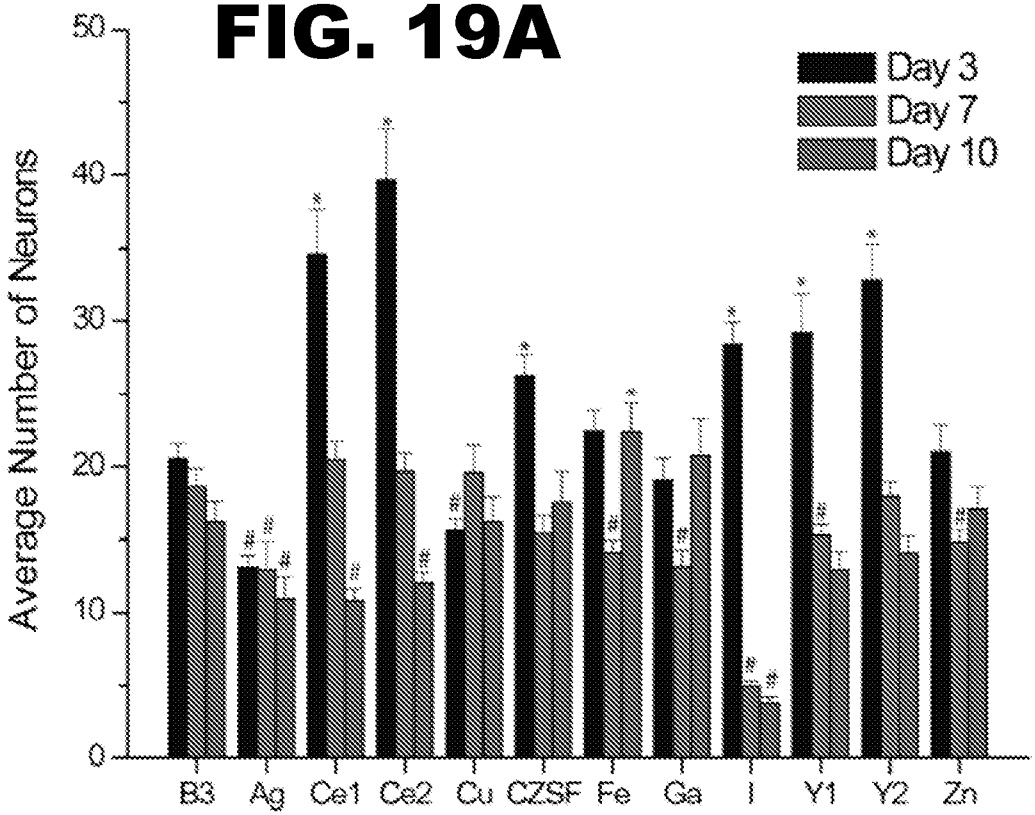


FIG. 18

FIG. 19A



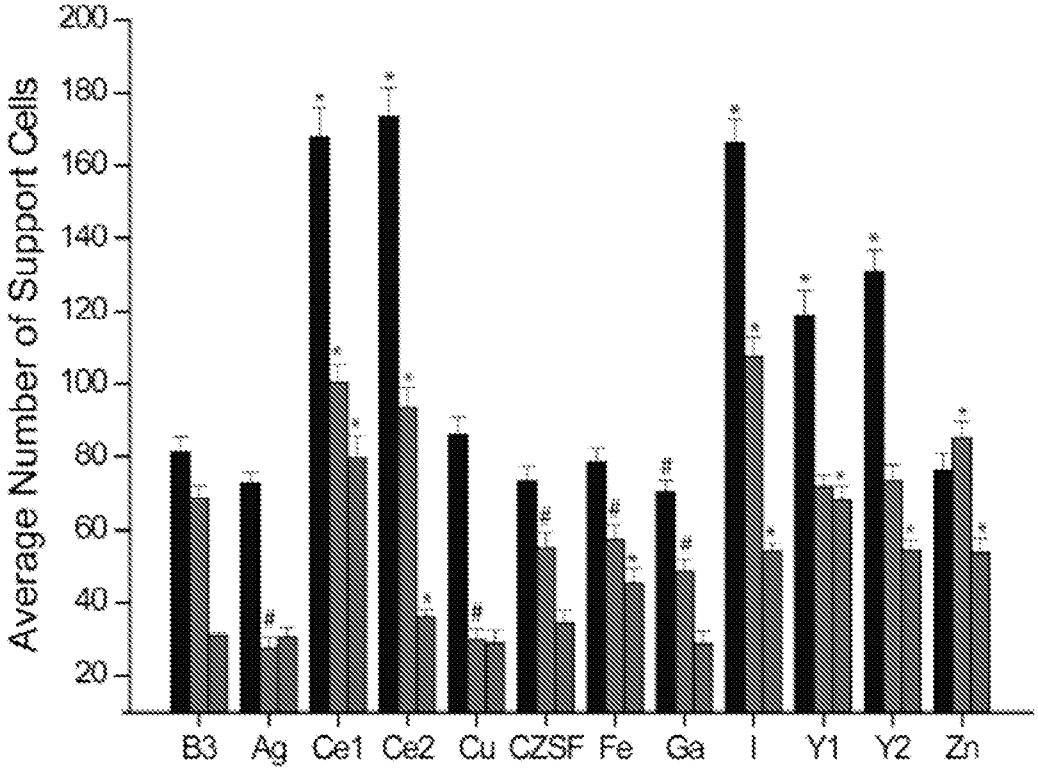
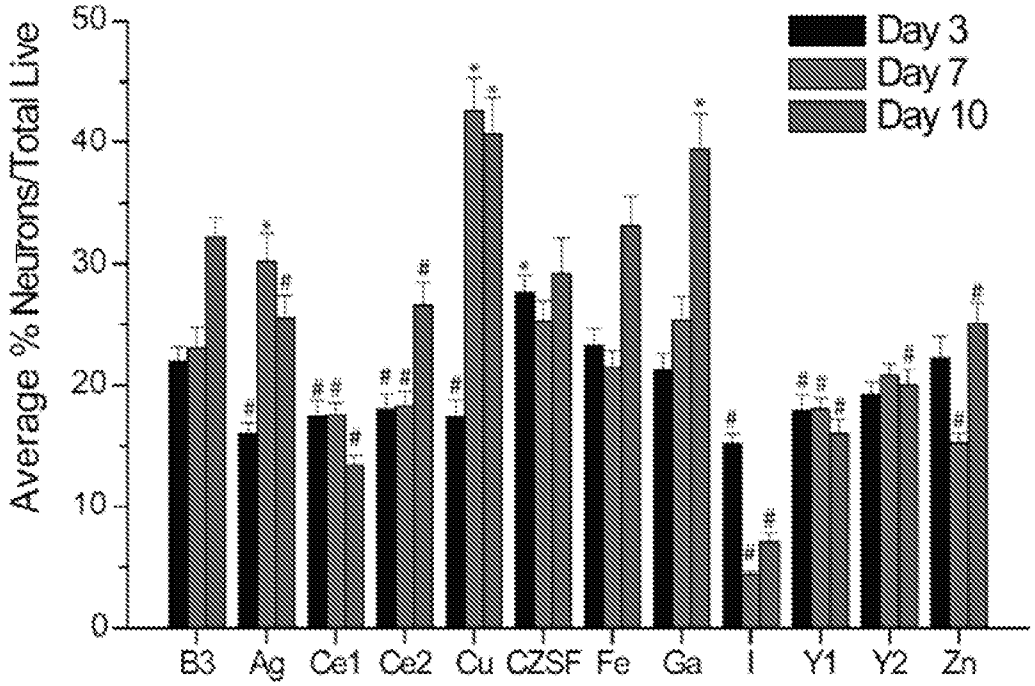


FIG. 19B

FIG. 20A



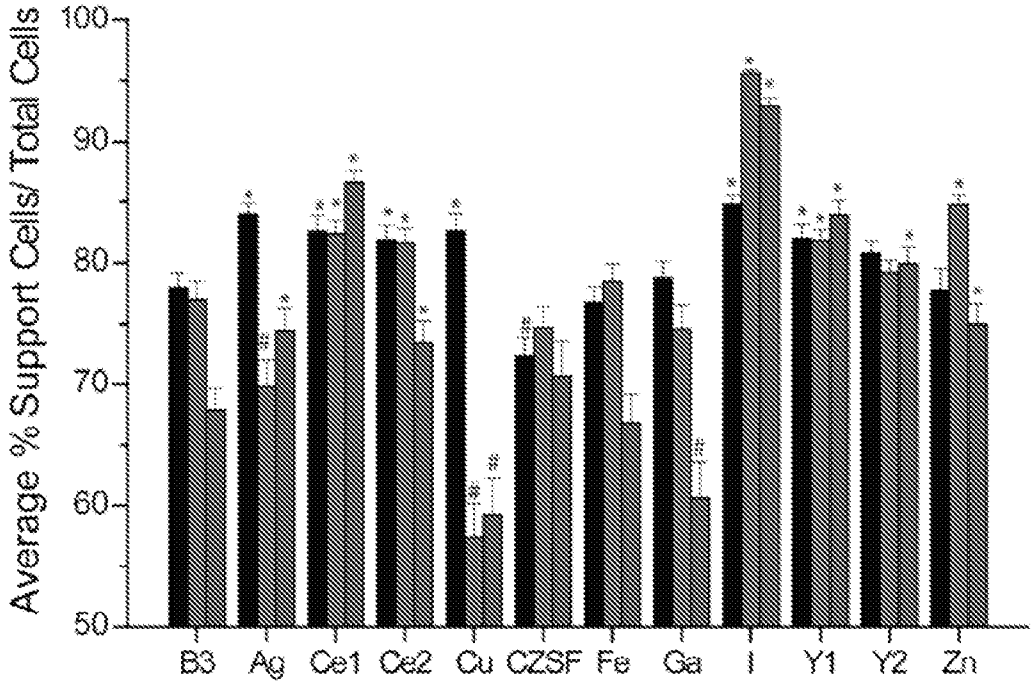


FIG. 20B

FIG. 21A

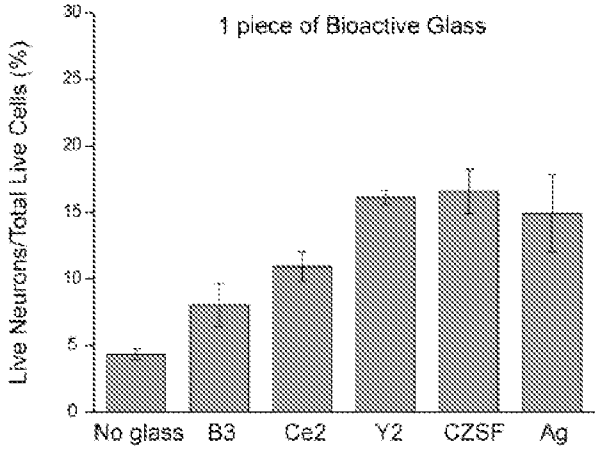


FIG. 21B

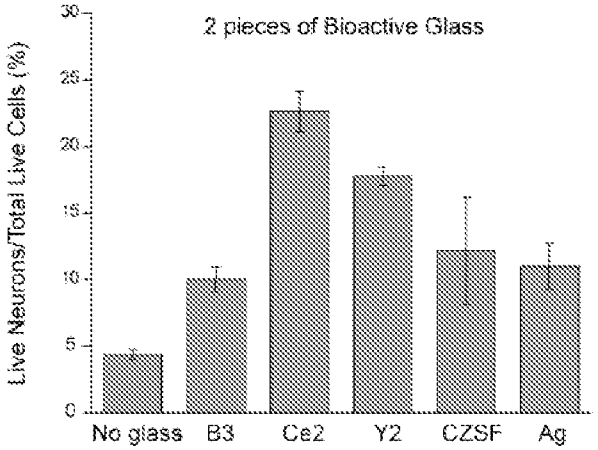


FIG. 21C

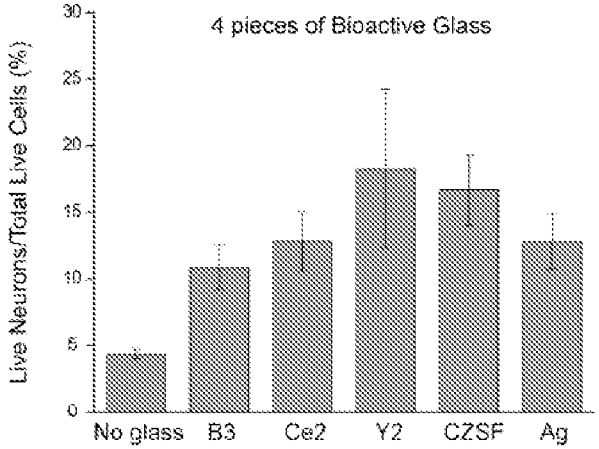
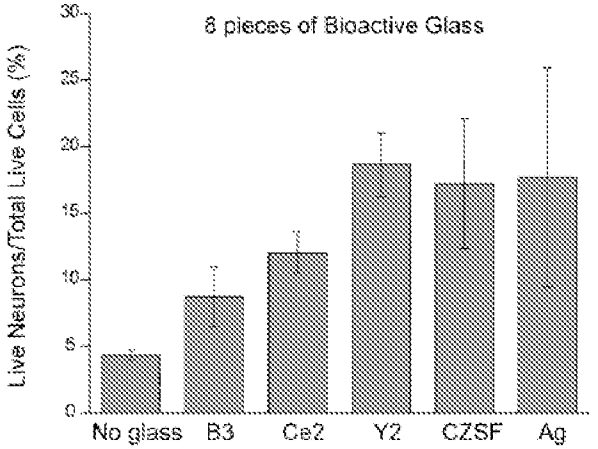


FIG. 21D



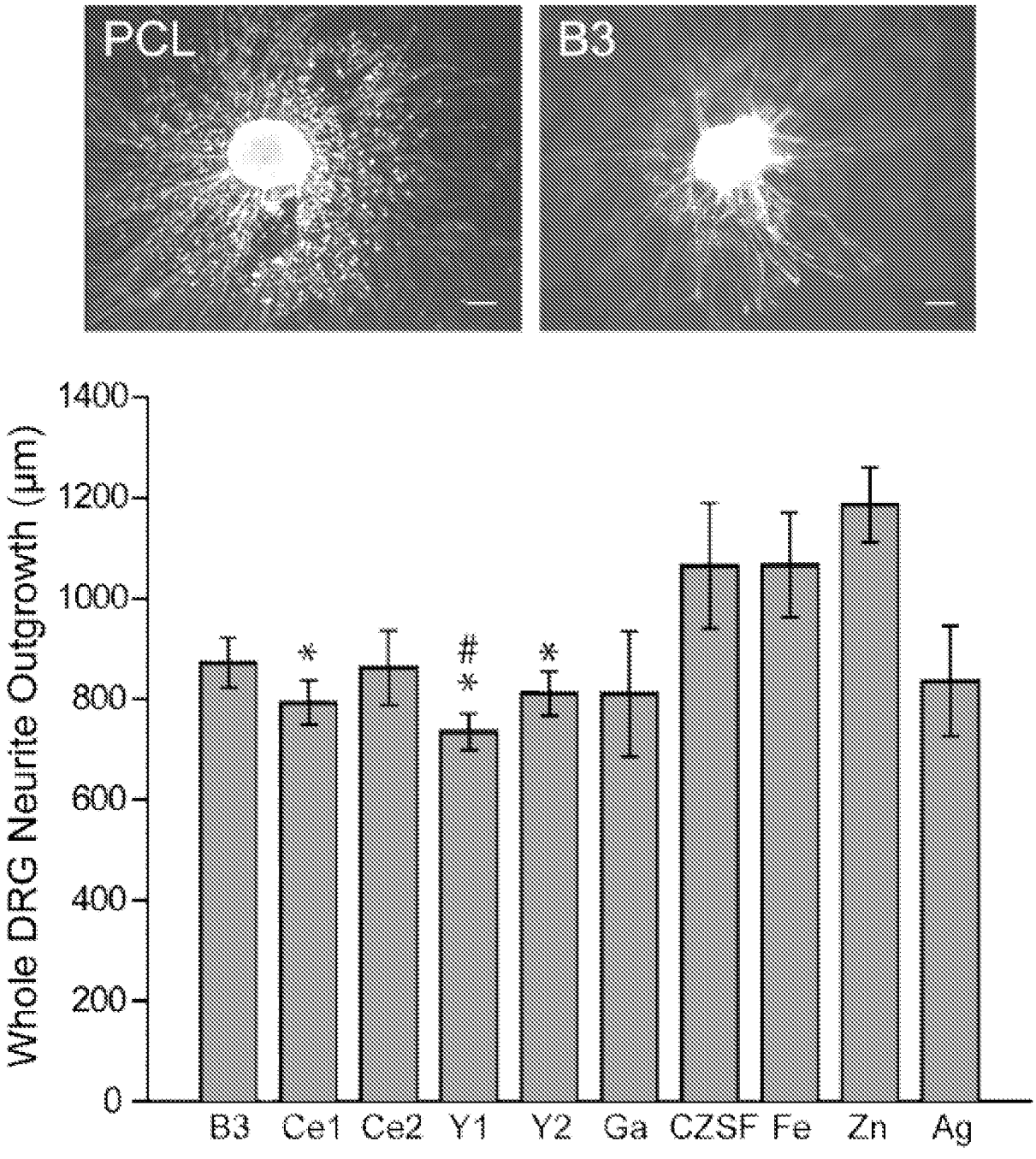

PCL
B3
Whole DRG Neurite Outgrowth (μm)
1400
1200
1000
800
600
400
200
0
*
#
*
*
B3
Ce1
Ce2
Y1
Y2
Ga
CZSF
Fe
Zn
Ag

FIG. 22

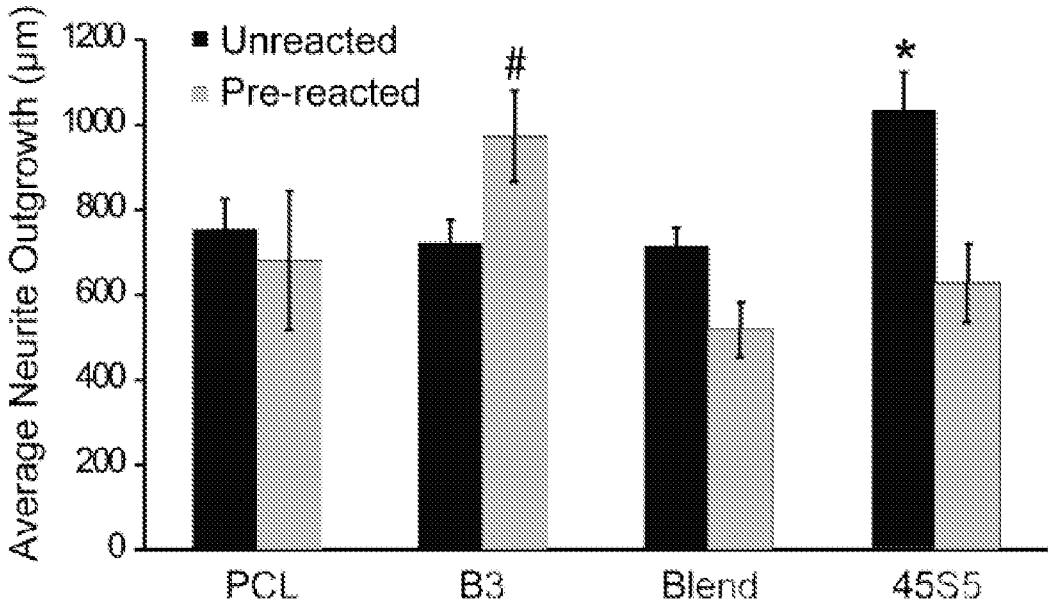

Average Neurite Outgrowth (µm)
1200
1000
800
600
400
200
0
Unreacted
Pre-reacted
#
*
PCL
B3
Blend
45S5

FIG. 23

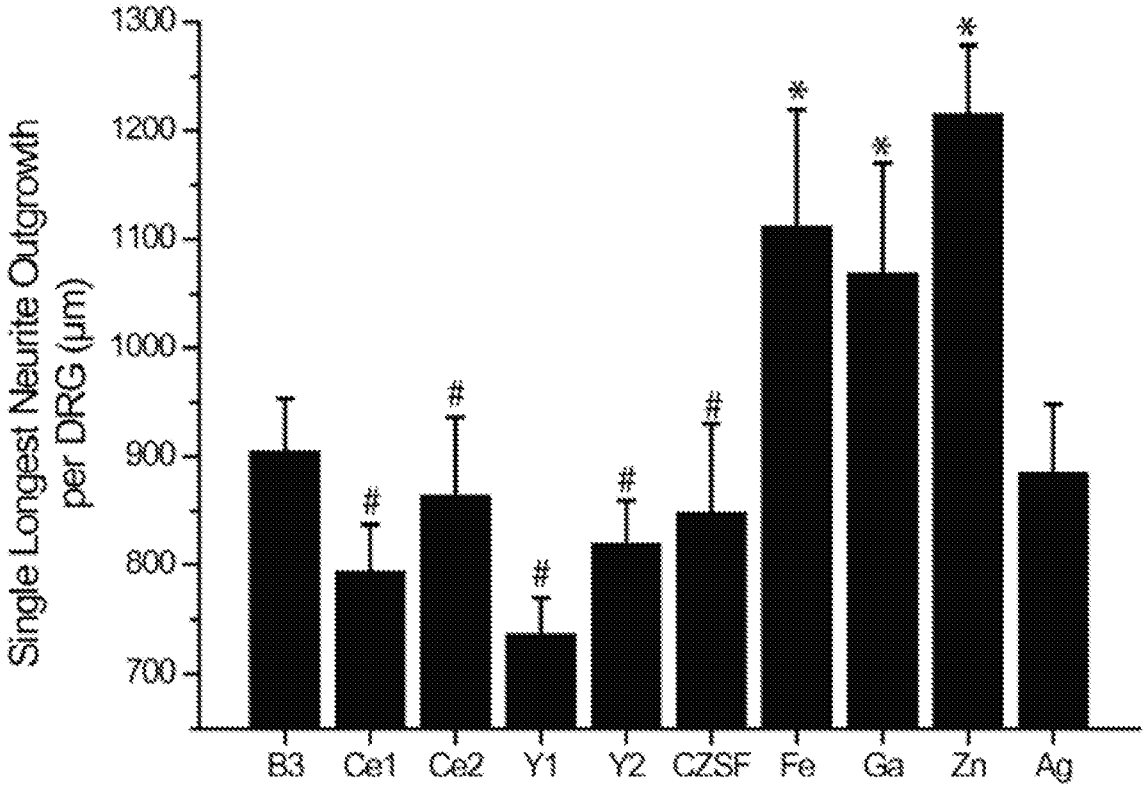

Single Longest Neurite Outgrowth per DRG (µm)
1300
1200
1100
1000
900
800
700
#
*
B3
Ce1
Ce2
Y1
Y2
CZSF
Fe
Ga
Zn
Ag

FIG. 24

FIG. 25A

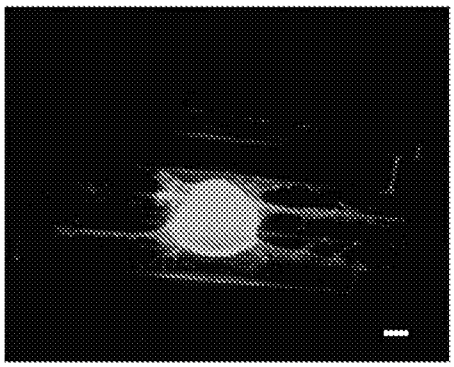
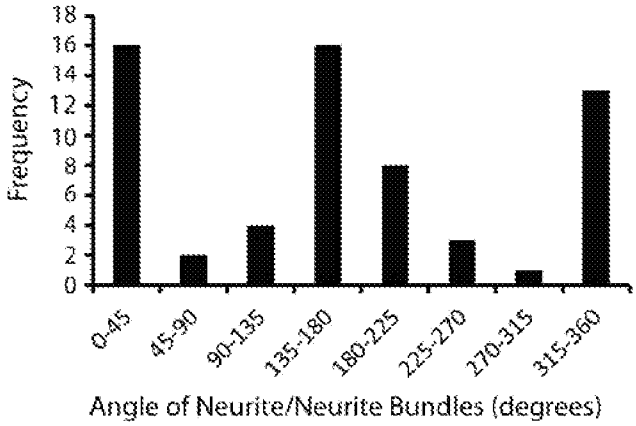


FIG. 25B



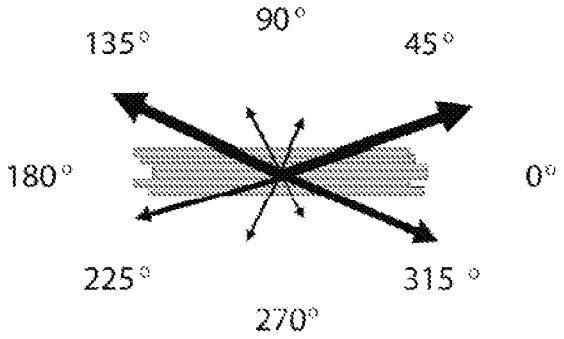


FIG. 25C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/49184

A. CLASSIFICATION OF SUBJECT MATTER
IPC (8) - A61K 9/14 (2015.01)
CPC - A61K 9/0024; A61K 9/0056; A61K 9/0014
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
CPC - A61K 9/0024; A61K 9/0056; A61K 9/0014
IPC(8) - A61K 9/14 (2015.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 9/0024; A61K 9/0056; A61K 9/0014; IPC(8) - A61K 9/14 (2015.01); USPC - 424/484,486

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Google Scholar. Search Terms: tissue nerve repair release antioxidant nanoparlicie sodium tetraborate biodegradable glass combined dopant cerium yttrium oxide cation scaffold polymer fibrin laminin collagen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MARQUARDT et al., Effects of borate-based bioactive glass on neuron viability and neurite extension, J Biomed Mater Res Part A 2014:102A:2767?2775, published online 30 September 2013, abstract; p. 2770, col 1, para 1; p. 2770, col 2, para 1; p.2773, col 2, para 2; p. 2774, col 1, para 2	1-31
Y	US 2010/0098768 A1 (ANDREESCU et al.) 22 October 2010 (22.10.2010), abstract; paras [0018]-[0019], [0022H0023]	1-31
Y	US 5,250,355 A (NEWMAN et al.) 05 October 1993 (05.10.1993), col 4, In 14-16	2, 17
Y	US 2013/034151 1 A1 (SHAH et al.) 26 December 2013 (26.12.2013), para [0048]	6, 21
Y	US 2012/0126172 A1 (ZHOU et al.) 24 May 2012 (24.05.2012), para [0045]	7,22
Y	US 2010/02331 15 A1 (PATEL et al.) 16 September 2010 (16.09.2010), paras [0002], [0122]	13-14, 27-29

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 2015 (20.10.2015)	14 DEC 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCTOSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)