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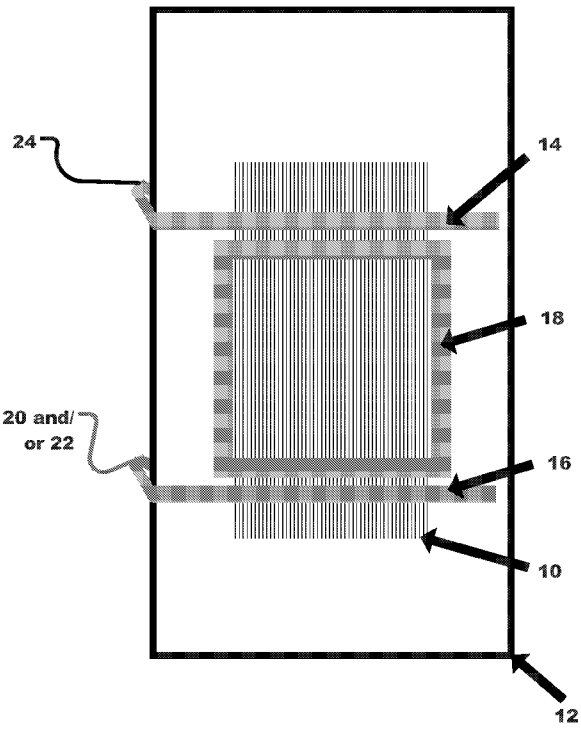

Fig. 1

(57) Abstract: Embodiments of the present disclosure provide electroactive scaffolds, method of making the electroactive scaffold, method of using the electroactive scaffold, and the like.

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ELECTROACTIVE SCAFFOLD, METHOD OF MAKING THE ELECTROACTIVE SCAFFOLD, AND METHOD OF USING THE ELECTROACTIVE SCAFFOLD

CLAIM OF PRIORITY TO RELATED APPLICATION

This application claims priority to co-pending U.S. application entitled "ELECTROACTIVE POLYMERIC SCAFFOLDS AND METHODS FOR DELIVERING NERVE GROWTH FACTOR TO NERVE TISSUE" having Serial No. 14/491,686, filed on September 19, 2014, which is entirely incorporated herein by reference.

BACKGROUND

When a peripheral nerve has a defect of more than two centimeters, a biomaterial is needed to repair the defect. This is conventionally done using either an autologous nerve graft (i.e. by taking a functioning section of a nerve from elsewhere in the patient's body) or using an allograft (i.e. by using a section of a nerve that has been removed from another living individual or from a cadaver). An autologous graft causes a loss of function from the site where the nerve section was removed, and an allograft is expensive. It would therefore be advantageous to provide a biomaterial having properties similar to an autologous nerve graft or a nerve allograft, so that the biomaterial could be used to repair peripheral nerve defects.

SUMMARY

Embodiments of the present disclosure provide electroactive scaffolds, methods of making the electroactive scaffold, method of using the electroactive scaffold, and the like.

An embodiment of the present disclosure provides for an electroactive scaffold, among others, that includes: a matrix and a polymerizable unit yielding an electrochemically responsive polymer attached to the matrix and anchored thereto by polymerization *(e.g.,* covalently, non-covalently, or as an interpenetrating network). In an embodiment, the matrix can include: polycaprolactone, polyesters, polyamides, PCL, PLLA, PLGA, proteins, polysaccharides, lignins, polyalanine, oligoalanine, collagen, silk, cellulose, chitin, chitosan, or a combination thereof. In an

embodiment, the polymerizable unit can include: an aniline; an aniline derivative; a furan; a furan derivative; a thiophene; a thiophene derivative; ferrocene; a ferrocene derivative; a porphyrin; a porphyrin derivative; or a combination thereof.

An embodiment of the present disclosure provides for a method of stimulating cells, among others, that includes: providing an electroactive tissue scaffold having a matrix and a polymerizable unit yielding an electrochemically responsive polymer attached to the matrix and anchored thereto by polymerization *(e.g.,* covalently, non-covalently, or as an interpenetrating network); introducing cells to the scaffold, wherein the scaffold and the cells are cultured in a medium; and periodically providing electrical stimulation to the cells.

An embodiment of the present disclosure provides for a method of delivering nerve growth factor to nerve tissue, among others, that includes: culturing Schwann cells on an electroactive tissue scaffold having a matrix and a polymerizable unit yielding an electrochemically responsive polymer attached to the matrix and anchored thereto by polymerization *(e.g.,* covalently, non-covalently, or as an interpenetrating network); implanting the scaffold and cultured Schwann cells into the peripheral nerve tissue; and electrically stimulating the Schwann cells by placing a voltage across the scaffold.

An embodiment of the present disclosure provides for a method of manufacturing an electroactive tissue scaffold, among others, that includes: obtaining a matrix; obtaining a polymerizable unit that yields an electrochemically responsive polymer; and initiating a polymerization of the matrix and the unit in the presence of a solvent *(e.g.,* where the polycaprolactone is insoluble in the solvent).

BRIEF DESCRIPTION OF THE DRAWINGS

Many aspects of the present disclosure can be better understood with reference to the following drawing.

Fig. 1 shows the experimental setup used to electrically stimulate rat Schwann cells which increases the production of nerve growth factor by the Schwann cells.

DISCUSSION

This disclosure is not limited to particular embodiments described, and as such may, of course, vary. The terminology used herein serves the purpose of describing

particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

Where a range of values is provided, each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method may be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of organic chemistry, biochemistry, microbiology, molecular biology, pharmacology, medicine, and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

Prior to describing the various embodiments, the following definitions are provided and should be used unless otherwise indicated.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art of microbiology, molecular biology, medicinal chemistry, and/or organic chemistry. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described herein.

As used in the specification and the appended claims, the singular forms "a," "an," and "the" may include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a support" includes a plurality of supports. In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings unless a contrary intention is apparent.

Discussion:

Embodiments of the present disclosure provide electroactive scaffolds, methods of making the electroactive scaffold, methods of using the electroactive scaffold, and the like. In an embodiment, the electroactive scaffold has electroactive characteristics so that an electrical stimulation can be periodically applied to the electroactive scaffold. In a particular embodiment, a cell and/or tissue can be incubated with the electroactive scaffold and cultured in an appropriate medium and optionally with an appropriate tissue so that the cells are stimulated.

Embodiments of the present disclosure include a polymerizable electrically responsive unit *(e.g.,* pyrrole) that is attached by polymerization *(e.g.,* covalently, non-covalently, or as an interpenetrating network) within a polymer matrix *(e.g.,* polycaprolactone matrix) in order to form an electroactive scaffold upon which cells can be cultured. In an embodiment, the micro- and nano-topological features *(e.g.,* grooves, pores, bumps or other features) of the matrix can be preserved during polymerization with the unit. In an embodiment, scaffolds of the present disclosure can be used to deliver agents such as drugs, antibacterial agents, and antifungal agents. They can also be used as electroactive actuators capable of mechanotransduction of cells such as stem cells.

Generally, embodiments of the disclosure provide for methods of stimulating cells or tissue. An embodiment of the present disclosure includes introducing cells to the scaffold, where the scaffold (and optionally a tissue or other cells) and the cells are cultured in an appropriate medium. Subsequently, electrical stimulation can be periodically applied to the cells to cause a desired outcome.

Electrical stimulation can include direct contact of the material with a power source via a wire, wireless energy transfer, magnetic force, and the like. The term "periodically" refers to applying the electrical stimulation at established time frames that may be at regular or irregular time intervals on the time frames of seconds, hours, days, weeks, or months *(e.g.,* about 1 s to 2 months, about 1 hour to 1 day, about 1 day to 1 month, or other the like) depending upon the specific circumstances. In an embodiment, the impulses of the electrical stimulation can last on the time frame of seconds, hours, or days *(e.g.,* about 1 second to 1 day, about 10 seconds to 1 hour, about 1 minute to 12 hours, about 1 hour to 1 day, or the like) depending upon the specific circumstances. In an embodiment, the electrical stimulation can be in the range of millivolts to volts *(e.g.,* about 10 mV to 10 volts, about 1 mV to 100 mV, or

the like). The time frame, duration of electrical stimulation, and intensity of the electrical stimulation can be designed based on particular circumstances and requirements of a specific situation.

Fig. 1 illustrates an embodiment of an experimental setup that can be used to electrically stimulate cells. The embodiment includes a support structure 12, such as a glass slide. The electroactive scaffold 10 having aligned pores is disposed on the support structure 12. One or more support contacts 14 and 16 *(e.g.,* copper contact) can be in contact with the electroactive scaffold 10 and electrodes, such as the working electrode 24, the counter electrode 20, and the reference electrode 22. A well 18 can be positioned on a portion of the electroactive scaffold 10 and appropriate material *(e.g.,* cells, tissue, reagents such as culturing mediums, and the like) can be disposed in the well 18.

As shown in Example 1, electrical stimulation of the electroactive scaffold in the presence of Schwann cells produced enhanced nerve growth factor (NGF) production. In particular, the present disclosure relates to delivery of NGF to nerve tissue, and to implantable electroactive scaffolds used for such delivery. In an embodiment, when the unit is anchored within a polymer matrix, cells such as Schwann cells can be cultured on the scaffold. Schwann cells are known to increase the production of nerve growth factor when electrically stimulated, and nerve growth factor has been demonstrated to promote regeneration of nerve tissue. In this regard, nerve growth factor can be delivered to nerve tissue by implanting in the tissue in a scaffold upon which Schwann cells have been cultured, and electrically stimulating the Schwann cells.

An embodiment of the electroactive scaffold includes a matrix and a polymerizable unit yielding an electrochemically responsive polymer located throughout the matrix and anchored thereto by polymerization (either via covalent bonds or non-covalent physical interactions *(e.g.,* chain entanglement)) yielding an interpenetrating network of matrix and electrochemically-responsive polymer. In other words, the electrochemically responsive polymer can be used to provide electrical stimulation to cells, tissue, and the like disposed on the electroactive polymer.

The matrix is embodied in any of a variety of material morphologies *(e.g.,* fibers, films, foams, gels, particulates etc.), optionally with micrometer or nanometer-scale features *(e.g.,* grooves, bumps, pores, where the grooves and pores can be an

indention into the surface and/or extend through the matrix). In an embodiment, the matrix can include polymer fibers such as synthetic polymers *(e.g.,* polycaprolactone, polyesters, polyamides, PCL, PLLA, PLGA, etc.) and natural polymers *(e.g.* proteins, polysaccharides, lignins, polyalanine, oligoalanine, collagen, silk, cellulose, chitin, chitosan, and the like), or a combination thereof. In a particular embodiment, the matrix is a polycaprolactone matrix. In an embodiment, the matrix can include a mixture of different types of polymer *(e.g.,* a portion of polycaprolactone fibers and polyester fibers).

In an embodiment, the pores of the matrix can include depressions in the matrix and/or holes through the entire thickness of the matrix. In an embodiment, the pores can be randomly located or non-randomly positioned in patterns or aligned in a desired manner. In an embodiment, the pores can have a spherical cross section or a semi-spherical cross section, or the pores can appear as long and narrow rows along the length and/or width of the scaffold that may be substantially parallel to one another or crisscross one another.

As mentioned above, the polymerizable unit *(e.g.,* the conducting component of the matrix) is bonded to the matrix. In particular, the polymerizable unit is bonded to the matrix either via covalent bonds, non-covalent bonds, and/or non-covalent physical interactions *(e.g.,* chain entanglement) yielding an interpenetrating network of matrix and electrochemically-responsive polymer. In an embodiment where the matrix is biodegradable and the electrochemically-responsive polymer is water soluble, this facilitates degradation of the entirety of the material. An initiator can be used to bond the polymerizable unit to the matrix. In an embodiment, an initiator can include ferric chloride, ammonium persulfate, and peroxide.

In an embodiment, the polymerizable unit can be an aromatic compound or a compound including an aromatic functional group. The aromatic compound or functional group can be non-heterocyclic or heterocyclic. In an embodiment, the polymerizable unit can a pyrrole, an aniline, an aniline derivative, a furan, a furan derivative, athiophene, athiophene derivative *(e.g.,* poly(3,4-ethylenedioxythiophene)), ferrocene, a ferrocene derivative, a porphyrin, a porphyrin derivative, a fluorine, a fluorene derivative, the polymerizable unit include a phenylene, a phenylene derivative, a pyrene, a pyrene derivative, an azulene, an azulene derivative, a naphthalene, a napthalene derivative, an indole, an indole derivative, an azepinesp-phenylene, an azepinesp-phenylene derivative, a sulfide p-

phenylene vinylene, a sulfide p-phenylene vinylene derivative, and a combination thereof. In a particular embodiment, the polymerizable unit can be pyrrole, or more particularly, the polymerizable unit can be 3,4-ethylenedioxythiophene; and a derivative of 3,4-ethylenedioxythiophene. In an embodiment, there are biodegradable versions, in which there are block of conducting units within a polymer chain containing biodegradable bonds *(e.g.* esters and amides), that can also be used as the polymerizable unit. The amount of the polymerizable unit in the matrix can be about 0.1 to 99 wt %.

As stated above, embodiments of the present disclosure provide for a polymerizable electrically responsive unit is anchored by polymerization within a polymer matrix in order to form an electroactive scaffold. In addition, cells and the differentiated products of the cells may be present or disposed within the scaffold. In an embodiment, the scaffold can include one or more agents *(e.g.,* a chemical or biological agent), where the agent can be disposed indirectly or directly on the scaffold. In an embodiment, the agent can include, but is not limited to, a drug, a therapeutic agent, a radiological agent, a small molecule drug, a biological agent *(e.g.,* polypeptides *(e.g.,* proteins such as, but not limited to, antibodies (monoclonal or polyclonal)), antigens, nucleic acids (both monomeric and oligomeric), polysaccharides, haptens, sugars, fatty acids, steroids, purines, pyrimidines, ligands, and aptamers) and combinations thereof, that can be used to image, detect, study, monitor, evaluate, and the like, the differentiation of the stem cells. In an embodiment, the agent is included in an effective amount to accomplish its purpose, where such factors to accomplish the purpose are well known in the medical arts.

In general, the agent can be bound to the scaffold by a physical, biological, biochemical, and/or chemical association directly or indirectly by a suitable means. The term "bound" can include, but is not limited to, chemically bonded *(e.g.,* covalently or ionically), biologically bonded, biochemically bonded, and/or otherwise associated with the material. In an embodiment, being bound can include, but is not limited to, a covalent bond, a non-covalent bond, an ionic bond, a chelated bond, as well as being bound through interactions such as, but not limited to, hydrophobic interactions, hydrophilic interactions, charge-charge interactions, π-π stacking interactions, combinations thereof, and like interactions.

In an embodiment, the electroactive tissue scaffold can be made by introducing the polymerizable unit to the matrix and then initiating a polymerization

reaction of the matrix and the unit in the presence of a solvent. In an embodiment, the polycaprolactone is insoluble in the solvent. The polymerizable unit bond to the matrix to form the scaffold. In an embodiment, the matrix is insoluble in the solvent *(e.g.,* water). Additional details are provided in the Example.

As briefly mentioned above, the scaffold can be used to stimulate cells or tissue. In an embodiment, the cells or tissue can be stimulated by initially providing an electroactive tissue scaffold and then introducing cells to the scaffold. The cells and the scaffold can be cultured in an appropriate medium. The cells can be periodically exposed to electrical stimulation for a time frame to accomplish the desired stimulation and/or other desired result. In an embodiment, the cells can include: stem cells, human dermal fibroblasts, myoblasts, osteoblasts, osteoclasts, neurons, Schwann cells, pluripotent stem cells, and the like.

In an embodiment, the cells can be human mesenchymal stem cells, human dermal fibroblasts, myoblasts, osteoblasts, osteoclasts, neurons, Schwann cells, pluripotent stem cells, or the like. In an embodiment the osteogenic medium is based on standard cell culture medium with the optional addition of other components such as serum, non-essential amino acids, bone morphogenetic protein 2 (BMP-2), dexamethasone, β-glycerophosphate, ascorbic acid, ascorbic acid-2-phosphate, heparin, retinoic acid, and 1,25-dihydroxycholecalciferol (for example: high glucose Dulbecco's Modified Eagle Medium (DMEM, 425 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), dexamethasone (100 nM), β-glycerol phosphate (10 mM) and ascorbic acid (50 μM)). The volume of medium used should be in line with the recommended guidelines of the manufacturer of the cell culture dishes.

In a particular embodiment, the scaffold can be used to deliver nerve growth factor to nerve tissue. The method includes culturing Schwann cells on an electroactive scaffold and implanting the scaffold and cultured Schwann cells into the peripheral nerve tissue. Subsequently, the Schwann cells are electrically stimulated by placing a voltage across the scaffold. The result is increased production of NGF relative to a method that does not include electrical stimulation. Additional details are provided in the Example.

While embodiments of the present disclosure are described in connection with the Examples and the corresponding text and figures, there is no intent to limit the disclosure to the embodiments in these descriptions. On the contrary, the intent is to

cover all alternatives, modifications, and equivalents included within the spirit and scope of embodiments of the present disclosure.

EXAMPLE:

Production of Electroactive Polycaprolactone-Based Scaffolds

Initially, pyrrole was purified by passage over basic alumina. A polycaprolactone matrix with aligned pores was placed in a solution of the pyrrole (291 μL, [84 mM], 1 eq.) and polystyrenesulfonate (0.799 g, [84 mM], 1 eq., Mn 70 kDa) in a solvent of distilled water (50 mL). Samples were sonicated for five minutes and cooled to 4 °C for one hour. Thereafter, ferric chloride (1.848 g, [228 mM], 2.7 eq.) was added as an initiator, the mixture was shaken to assure dissolution of the ferric chloride, and the shaken mixture was then incubated for a further twenty four hours at 4 °C. Electroactive scaffolds with an interpenetrating network of polypyrrole and polystyrenesulfonate) were removed from the reaction mixture, placed in fresh distilled water, sonicated for five minutes, and then exhaustively washed (to remove monomers, oligomers and initiators) with deionized water until the water was clear, colorless and the pH was neutral (approximately forty-eight hours). The scaffolds were then dried under high vacuum at 21 °C.

Electroactive polycaprolactone-based scaffolds so prepared have their microscale and nanoscale topological properties preserved. This is advantageous because clinical evidence suggests that micro- and nano-topographical features incorporated into scaffolds is beneficial for the restoration of nerve tissue.

Preparation and Sterilization of Electroactive-Coated Polycaprolactone-Based Scaffolds

The dried scaffolds with aligned pores were incubated in an aqueous solution of poly-D-lysine (PDL, 50 μg/mL) for one hour and then washed thoroughly with sterile water to remove any weakly adsorbed poly-D-lysine (exchanging the water every ten minutes for one hour). Samples were inserted in untreated polystyrene tissue culture plates and sterilized by incubation in 70% ethanol solution, followed by exposure to UV for sixty minutes.

In Vitro Culture of Human Dermal Fibroblasts

Growth medium for human dermal fibroblasts was composed of: high glucose Dulbecco's Modified Eagle Medium (DMEM, 440 mL); fetal bovine serum (50 mL); antibiotic-antimycotic (5 mL); non-essential amino acids (5 mL), and 2 ng/mL basic

fibroblast growth factor. The sterilized scaffolds were incubated for thirty minutes under three millimeters of this growth medium. The growth medium was aspirated and replaced before seeding with human dermal fibroblasts at 5,000 cells/cm^2 under three millimeters of medium, and incubated at 37 °C, 95% humidity, and a CO_2 content of 5%. Cell viability before starting the experiment was determined by the Trypan Blue (Sigma, USA) exclusion method, and the measured viability exceeded 95% in all cases. After two days the medium was aspirated, the scaffolds were washed gently with phosphate-buffered saline, the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline for fifteen minutes, and the scaffolds were washed again with phosphate-buffered saline (3 x 1 mL).

The human dermal fibroblasts were observed to adhere to the scaffolds.

In Vitro Culture of Schwann Cells

Schwann cell growth medium was composed of: 25.5 mL of low glucose Dulbecco's Modified Eagle Medium (DMEM); 8.5 mL of GIBCO® Ham's F-12 Nutrient Mixture; 350 µL Penicillin Streptomycin (1 % of the final volume); 350 µL N2 supplement (2 % of the final volume); Forskolin [5 µM]; and Neuregulin- 1β (50 ng/mL). The sterilized scaffolds were incubated for thirty minutes under three millimeters of this growth medium. The growth medium was aspirated and replaced before seeding with rat Schwann cells at 5,000 cells/cm^2 under three millimeters of medium, and incubated at 37 °C, 95% humidity, and a CO_2 content of 5%. Cell viability before starting the experiment was determined by the Trypan Blue (Sigma, USA) exclusion method, and the measured viability exceeded 95% in all cases. After two days the medium was aspirated, the scaffolds were washed gently with phosphate-buffered saline, the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline for fifteen minutes, and the scaffolds were washed again with phosphate-buffered saline (3 x 1 mL).

The Schwann cells were observed to adhere to the scaffolds.

Electrical Stimulation of Schwann Cells

Non-conductive glass slides, polycarbonate wells (2.5 cm square polycarbonate blocks 1 cm thick, sides of 2.5 cm, with 0.9 cm square holes cut out of them), Dow Corning® high vacuum grease, and medium binder clips were sterilized by autoclave. Polystyrene petri dishes (diameter of 10 cm) had holes drilled in their sides and were sterilized by exposure to ultraviolet radiation for sixty minutes. Adhesive-backed copper tape (5 mm width, Ted Pella, Inc.), waterproof Kapton®

tape (1 cm width, Fisher Scientific, Waltham, MA), wires and alligator clips were sterilized by exposure to ultraviolet radiation for sixty minutes.

Electroactive polycaprolactone-based sterilized scaffolds with aligned pores 10 were placed on sterilized glass slides 12 and secured in position with two thin strips 14, 16 of sterilized adhesive-backed copper tape that were attached to the scaffolds 10, parallel to one another, separated by a distance of approximately four centimeters. A polycarbonate well 18 for holding the Schwann cell growth medium was mounted watertight to the slide 12. The counter and reference electrodes (20 and 22 respectively) were connected together to the strip 16, and the working electrode 24 was clipped to the strip 14. Schwann cells were plated and cultured as described above. A potential step of + 50 mV/mm was placed across the substrate for the duration of 1 hour, after which the wires were disconnected and the substrates cultured as normal.

The amount of nerve growth factor expressed by rat Schwann cells under electrical stimulation was measured and compared with the amount of nerve growth factor expressed by rat Schwann cells that were not electrically stimulated and that served as controls. The electrically stimulated cells in the electroactive PCL-based tissue scaffolds with aligned pores 10 were exposed to a potential difference of 50 mV/mm.

The concentration of NGF in the medium (in pg/mL) was determined (using a Rat NGF ELISA Kit, Insight Genomics, Falls Church, VA) immediately after electrical stimulation and thereafter in intervals of twelve hours for three days. There were no significant differences in NGF production by Schwann cells in any of the non-stimulated controls over the three day study, whereas, after forty eight hours in culture the rat Schwann cells responded to the electrical stimulation by increased production of NGF, a trend that was markedly more apparent during the following twenty four hours to approximately three times the amount produced by an equivalent number of rat Schwann cells cultured without electrical stimulation. Such increases in nerve growth factor production have been shown to encourage neurite outgrowth from neurons in a number of studies.

In-Vitro Degradation of Electroactive Polycaprolactone-Based Scaffolds

To demonstrate that enzymatic/hydrolytic degradation of the electroactive polycaprolactone-based scaffolds is possible, the scaffolds were incubated in phosphate-buffered saline in the absence or presence of a high concentration (4

units/mL) of cholesterol esterase, an enzyme known to hydrolyze ester bonds in polyesters. When incubated in phosphate-buffered saline for twelve days the mass of the scaffolds did not change significantly because hydrolysis of phosphate-buffered saline occurs very slowly. The presence of the esterase increased the rate of hydrolysis, resulting in an approximately 50% mass loss over 12 days. The presence of the electroactive polyelectrolyte complex of polypyrrole and polystyrenesulfonate appears to increase the hydrophilicity of the scaffolds allowing the enzyme to more easily access the polycaprolactone chains. The scaffolds are likely to degrade slowly if administered in vivo (over the period of several months) in line with other polycaprolone-based materials, leaving behind the residual water insoluble polyelectrolyte complex of polypyrrole and polystyrenesulfonate that preclinical trials have shown to be relatively non-immunogenic. Indeed, histological analyses of tissue in the vicinity of polypyrrole-based tissue scaffolds implanted subcutaneously or intramuscularly in rats, showed immune cell infiltration comparable to FDA-approved poly(lactic acid-co-glycolic acid) [Schmidt CE, Shastri VR, Vacanti JP, Langer R: Stimulation of neurite outgrowth using an electrically conducting polymer. Proc Natl Acad Sci U S A 1997, 94:8948-8953] or poly(D,L-lactide-co-glycolide). Wang Z, Roberge C, Dao LH, Wan Y, Shi G, Rouabhia M, Guidoin R, Zhang Z: In vivo evaluation of a novel electrically conductive polypyrrole/poly(D,L-lactide) composite and polypyrrole-coated poly(D,L-lactide-co-glycolide) membranes. J Biomed Mater Res A 2004, 70:28-38 Similarly, there was no significant inflammation in the vicinity of polypyrrole-based materials implanted in the coronary artery of rats after 5 weeks, [Mihardja SS, Sievers RE, Lee RL: The effect of polypyrrole on arteriogenesis in an acute rat infarct model. Biomaterials 2008, 29:4205-4210] sciatic nerve guidance channels implanted in rats after 8 weeks, [Durgam H, Sapp S, Deister C, Khaing Z, Chang E, Luebben S, Schmidt CE: Novel degradable co-polymers of polypyrrole support cell proliferation and enhance neurite out-growth with electrical stimulation. J Biomater Sci Polym Ed 2010, 21: 1265-1282] or electrodes in rat brains after 3 or 6 weeks. [George PM, Lyckman AW, LaVan DA, Hegde A, Leung Y, Avasare R, Testa C, Alexander PM, Langer R, Sur M: Fabrication and biocompatibility of polypyrrole implants suitable for neural prosthetics. Biomaterials 2005, 26:3511-3519

Nerve Tissue Therapy

As stated above, Schwann cells are known to produce nerve growth factor when electrically stimulated. In accordance with the invention, Schwann cells are cultured on the above-described scaffolds and the scaffolds with the Schwann cells cultured on them are implanted into the peripheral nerve tissue to be repaired. Delivery of nerve growth factor to the nerve tissue is accomplished by applying a voltage across the scaffolds and thereby electrically stimulating the Schwann cells. The nerve growth factor thereby produced promotes regeneration of nerve tissue.

It should be noted that ratios, concentrations, amounts, and other numerical data may be expressed herein in a range format. It is to be understood that such a range format is used for convenience and brevity, and thus, should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. To illustrate, a concentration range of "about 0.1% to about 5%" should be interpreted to include not only the explicitly recited concentration of about 0.1 wt% to about 5 wt%, but also include individual concentrations *(e.g.,* 1%, 2%, 3%, and 4%) and the sub-ranges *(e.g.,* 0.5%, 1.1%, 2.2%, 3.3%, and 4.4%) within the indicated range. In an embodiment, the term "about" can include traditional rounding according to significant figures of the numerical value. In addition, the phrase "about 'x' to 'y'" includes "about 'x' to about 'y'"\

Many variations and modifications may be made to the above-described embodiments. All such modifications and variations are intended to be included herein within the scope of this disclosure and protected by the following claims

CLAIMS

We claim:

1. An electroactive scaffold, comprising:

a matrix; and

a polymerizable unit yielding an electrochemically responsive polymer attached to the matrix by polymerization.

2. The scaffold of claim 1, wherein the matrix is selected from the group consisting of: polycaprolactone, polyesters, polyamides, PCL, PLLA, PLGA, proteins, polysaccharides, lignins, polyalanine, oligoalanine, collagen, silk, cellulose, chitin, chitosan, and a combination thereof.

3. The scaffold of claim 1, wherein the matrix is a polycaprolactone matrix.

4. The scaffold of claim 1, wherein the polymerizable unit is an aromatic compound.

5. The scaffold of claim 4, wherein the aromatic compound is heterocyclic.

6. The scaffold of claim 5, wherein the heterocyclic aromatic compound is a pyrrole.

7. The scaffold of claim 1, wherein the polymerizable unit is selected from a group consisting of: an aniline; an aniline derivative; a furan; a furan derivative; a thiophene; a thiophene derivative; ferrocene; a ferrocene derivative; a porphyrin; a porphyrin derivative; and a combination thereof.

8. The scaffold of claim 1, wherein the polymerizable unit is selected from a group consisting of: 3,4-ethylenedioxythiophene; and a derivative of 3,4-ethylenedioxythiophene.

9. A method of stimulating cells,

providing an electroactive tissue scaffold having a matrix and

a polymerizable unit yielding an electrochemically responsive polymer attached to the matrix by polymerization ;

introducing cells to the scaffold, wherein the scaffold and the cells are cultured in a medium; and

periodically providing electrical stimulation to the cells.

10. The method of claim 9, wherein the cells are selected from the group consisting of: stem cells, human dermal fibroblasts, myoblasts, osteoblasts, osteoclasts, neurons, Schwann cells, pluripotent stem cells, and the like.

11. A method of delivering nerve growth factor to nerve tissue, comprising:

culturing Schwann cells on an electroactive tissue scaffold having a matrix and a polymerizable unit yielding an electrochemically responsive polymer attached to the polymer matrix by polymerization;

implanting the scaffold and cultured Schwann cells into the peripheral nerve tissue; and

electrically stimulating the Schwann cells by placing a voltage across the scaffold.

12. The scaffold of claim 11, wherein the matrix is selected from the group consisting of: polycaprolactone, polyesters, polyamides, PCL, PLLA, PLGA, proteins, polysaccharides, lignins, polyalanine, oligoalanine, collagen, silk, cellulose, chitin, chitosan, and a combination thereof.

13. The scaffold of claim 11, wherein the polymerizable unit is selected from a group consisting of: an aniline; an aniline derivative; a furan; a furan derivative; a thiophene; a thiophene derivative; ferrocene; a ferrocene derivative; a porphyrin; a porphyrin derivative; and a combination thereof.

14. A method of manufacturing an electroactive tissue scaffold, comprising:

obtaining a matrix;

obtaining a polymerizable unit that yields an electrochemically responsive polymer; and

initiating a polymerization of the matrix and the unit in the presence of a solvent.

15. The method of claim 14, wherein polycaprolactone is insoluble in the solvent.

16. The scaffold of claim 14, wherein the matrix is selected from the group consisting of: polycaprolactone, polyesters, polyamides, PCL, PLLA, PLGA, proteins, polysaccharides, lignins, polyalanine, oligoalanine, collagen, silk, cellulose, chitin, chitosan, and a combination thereof.

17. The scaffold of claim 14, wherein the polymerizable unit is selected from a group consisting of: an aniline; an aniline derivative; a furan; a furan derivative; a thiophene; a thiophene derivative; ferrocene; a ferrocene derivative; a porphyrin; a porphyrin derivative; and a combination thereof.

18. The method of claim 14, wherein the matrix is polycaprolactone, the unit is pyrrole, and the solvent is water.

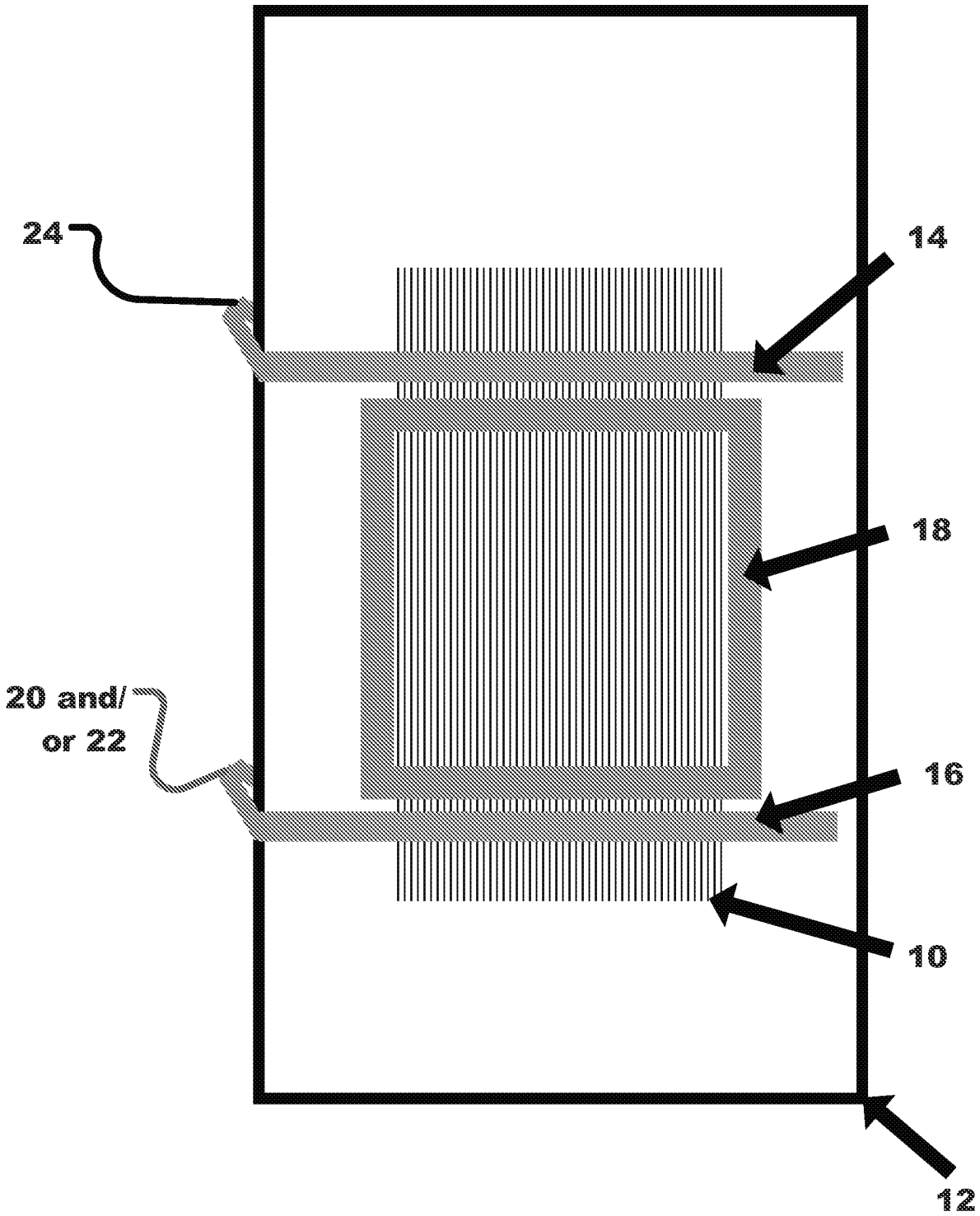

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/50594

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 13/00, B29C 47/00, A61P 37/00, C12N 5/071 (2015.01)
CPC - C12N1 1/08, C12N2529/00, C12N2533/30, A61K35/28, C12N5/0068
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 - C12N1 1/08, C12N2529/00, C12N2533/30, A61K35/28, C12N5/0068
IPC(8) - C12N 13/00, B29C 47/00, A61 P 37/00, C12N 5/071 (2015.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/93.7, 264/465, 435/395, 435/173.1

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: eiectroactive electrochemical conductive responsive scaffold matrix polymer polymerizable polymerization deposit attach polycaprolactone

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 6,569,654 B2 (SHASTRI et al.) 27 May 2003 (27.05.2003), abstract; col 5, In 55-col 6, In 5; col 10, In 40-col 11, In 12; claims 9, 14	1-2, 4-8, 14, 16-17 --- 3, 15, 18
Y	US 2013/0195955 A1 (REICHERT et al.) 01 August 2013 (01 .08.2013), abstract	3, 15,18

☐ 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
12 January 2016 (12.01 .2016)	02FEB 2016

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/50594

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

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

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

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

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

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept undor PCT Rule 13.1 . In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-8 and 14-18, directed to an electroactive scaffold and the method of manufacturing an electroactive tissue scaffold

Group II: Claims 9-13 directed to a method of stimulating cells and a method of delivering nerve growth factor to nerve tissue

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

-----continued on supplemental sheet — -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-8, 14-18

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

No protest accompanied the payment of additional search fees.