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for

NEURODEGENARATIVE DISORDER TREATMENT

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TITLE

NEURODEGENARATIVE DISORDER TREATMENT

FIELD

[0001] This patent application relates generally to the field of medical devices and associated treatments, and more specifically to precise bioelectrical stimulation of a subject's tissue, possibly augmented with the administration of a composition comprising, among other things, stem cells and nutrients, useful to stimulate and treat the subject, the subject's tissue(s), the subject's organ(s), and/or the subject's cells. More specifically in particular embodiments, the application relates to a device, programmed bioelectric signaling sequences, and associated methods for the controlled expression of, for example, excitatory amino acid transporter 2 ("EAAT2" or GLT-1 (glutamate transporter-1)) via precise bioelectrical signaling sequences, particularly in the treatment of neurodegenerative disorders.

BACKGROUND

[0002] Essential tremor is a relatively common movement disorder in the United States, affecting about 10 million Americans. ET causes involuntary, rhythmic trembling, usually in the hands, and is exacerbated during activities such as eating, drinking, dressing, and using utensils or tools. Although essential tremor is not life-threatening, it severely impacts a patient's quality of life.

[0003] As noted by Lee et al. (2014) *infra* "Essential tremor (ET) is a prevalent neurological disease marked by a persistent 4 - 12 Hz action tremor in the arms. ET has a strong genetic component, as ET patients often have a family history of tremor, and twins with ET are highly concordant for disease status. Recently, polymorphisms in the *solute carrier family 1 (glial high affinity glutamate transporter), member 2* (*SLC1A2*) gene have emerged as a potential genetic risk factor for ET in a genome-wide association study in Europe. While this association was subsequently confirmed in two Asian cohorts, another study did not show an association, and a meta-analysis revealed conflicting results. Given this evolving picture, the *SLC1A2* gene remains of considerable interest. The *SLC1A2* gene encodes (EAAT2), which is a protein that is critical for maintaining glutamate levels in the synaptic cleft in the adult brain." Lee et al. (2014) went on

to find that "a significant reduction in cerebellar cortical EAAT2 protein levels in essential tremor".

[0004] Current pharmacological treatment of ET includes beta blockers such as propranolol; anti-seizure medications, such as primidone, gabapentin, and topiramate; tranquilizers, such as clonazepam; and OnabotulinumtoxinA ("Botox") injections.

[0005] Other treatments include "deep brain stimulation," where a long, thin electrical probe is inserted into, e.g., the thalamus, and a wire from the probe runs under the patient's skin to a pacemaker-like device (neurostimulator, available, e.g., from Medtronic, Minneapolis, MN, US as the Medtronic DBS System or Activa™ Neurostimulator). This device transmits painless electrical pulses to interrupt signals from your thalamus that may be causing tremors.

[0006] Heo et al. (2015) *infra* described the use of two commercially available electrical stimulators (Walking man 2, Cybermedic, Korea) to stimulate four muscles (Flexor Carpi Radialis, Extensor Carpi Radialis, Biceps Brachii, and Triceps Brachii), associated with the movements of finger, wrist, and elbow in ET patients. Electrodes of 50 mm×50 mm size were attached on the motor points of target muscles. Constant-current, monopolar, and rectangular stimulation were applied with the frequency of 100 Hz and pulse-width of 300 μs. The current intensity was increased from 0 mA in 0.2 mA step, until the subjects felt discomfort or the muscle contraction occurred. Then, the optimal intensity was defined as the maximum current in this range for each muscle in each subject. Heo et al. concluded that sensory stimulation may be an effective clinical method to treat essential tremor.

[0007] More recently, Pahwa et al. (2019) *infra* described stimulation of ET patients' wrists with a series of charge balanced biphasic pulses, 300 μs biphasic pulses, with a 50 μs interpulse period between pulses, delivered at a frequency of 150 Hz. The stimulation alternated between the median and radial nerve at a frequency equal to tremor frequency as measured by on-board accelerometers (e.g., for a measured 5 Hz tremor frequency, stimulation was applied over the median nerve for 100 msec, and then was applied over the radial nerve for 100 msec). Both treatment and sham subjects were exposed to the frequency calibration procedure and to stimulation during an amplitude calibration period, during which study personnel increased the stimulation level by 0.25 mA steps until the subject reported first perceived sensation in the hand or finger area corresponding to distributions of the palmar digital branches of the median nerve and the superficial branch of the radial nerve. Final stimulation amplitude was the highest level

of tolerable stimulation level (always below muscle contraction) that the subject found comfortable (mean: 5.4 mA +/- 2.9). Once final stimulation amplitude was identified, treatment subjects received stimulation at that level during a 40-minute stimulation session, while sham subjects received no stimulation. Subjects were blinded to whether they were randomized to receive treatment stimulation or sham stimulation. The subjects who had received peripheral nerve stimulation did not show significantly larger improvement in the Archimedes spiral task compared to sham, but did show significantly greater improvement in upper limb TETRAS tremor scores ($p = 0.017$) compared to sham. Subject-rated improvements in ADLs were significantly greater with treatment (49% reduction) than with sham (27% reduction; $p = 0.001$). A greater percentage of ET patients (88%) reported improvement in the stimulation group as compared to the sham group (62%) according to CGI-I ratings ($p = 0.019$).

[0008] As identified by Takahashi et al. (2015), glutamate is the predominant excitatory neurotransmitter in the central nervous system. EAAT2 is responsible for clearing extracellular glutamate to prevent neuronal excitotoxicity and hyperexcitability, and EAAT2 plays a critical role in regulation of synaptic activity and plasticity. Besides ET, EAAT2 has been implicated in the pathogenesis of many central nervous system disorders, such as stroke, Parkinson's disease, epilepsy, amyotrophic lateral sclerosis, Alzheimer's disease, major depressive disorder, and addiction.

BRIEF SUMMARY

[0009] Described herein is a bioelectric stimulator particularly configured to activate expression and/or release of a protein such as EAAT2 in cellular tissue, such as the thalamus or caudal zona incerta of a subject, and/or the limb(s), arm(s), lower arm(s), wrist(s), hand(s), and/or finger(s), particularly a subject suffering from (or at a risk of suffering from) a neurodegenerative disorder such as ET, Parkinson’s disease (“PD”), stroke, epilepsy, amyotrophic lateral sclerosis, Alzheimer's disease, major depressive disorder, and/or addiction.

[0010] Also described is a bioelectric stimulator including: a power source (*e.g.*, battery, capacitor, or other suitable source of electricity), and means for delivering an electrical signal to a subject’s tissue (e.g., via electrode(s) or wirelessly). The bioelectric stimulator utilizes the electrical signal to precisely control protein expression in the tissue on demand.

[0011] In certain cases, the bioelectric stimulator is programmed to produce a bioelectric signal that stimulates target tissue to express and/or release EAAT2 polypeptide by the target tissue by utilizing a bioelectric.

[0012] In certain cases, the upregulation of EAAT2 bioelectric protein expression is used for controlling, curing, or reducing essential tremors in a subject.

[0013] In certain embodiments (particularly when the subject is suffering from neurodegenerative disorders such as multiple sclerosis (MS), Parkinson's disease or essential tremor), Lingo-1 is further regulated (downregulated to produce less LINGO1) in the subject to assist in treating the subject. This may be accomplished with, e.g., RNA interference (- encoding Lingo-1 short hairpin RNA (LV/Lingo-1-shRNA) constructed to inhibit Lingo-1 expression). See, e.g., Wang et al. "Lingo-1 inhibited by RNA interference promotes functional recovery of experimental autoimmune encephalomyelitis" *Anat Rec (Hoboken)*. 2014 Dec; 297(12):2356-63. doi: 10.1002/ar.22988. Epub 2014 Jul 17, which describes the effect of RNA interference on Lingo-1 expression, and the impact of Lingo-1 suppression on functional recovery and myelination/remyelination in experimental autoimmune encephalomyelitis mice.

[0014] A preferred system includes: a bioelectric stimulator that controls/stimulates the release/production of EAAT2 by a target cell or tissue. The stimulator may be associated with (*e.g.*, connected to) the organ or tissue to be treated with a pacing infusion lead (available from Nanoscribe of Eggenstein-Leopoldshafen, Germany) or wirelessly. In certain cases, the interface with the subject's tissue may be by a conductive soft wrap (FIG. 1).

[0015] The bioelectric stimulator can be designed to externally deliver all regeneration promoting signals wirelessly to the subject's organ(s), tissue(s), and/or cells. In certain embodiments, a micro infusion pump may be included in the system to deliver other supportive substances (e.g., RNAi directed against Lingo-1) in greater volume more quickly.

[0016] While not intending to be bound by theory, the described system utilizes precise bioelectric signaling sequences that appear to communicate with DNA and cell membranes within stimulated tissues of the subject to cause the cells to produce high volumes of the EAAT2 protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 depicts a bioelectric stimulator for delivery to a subject including a display panel, program control dials, switch, and conductive soft electrodes electrically associated with the bioelectric stimulator

[0018] FIG. 2 depicts an alternative bioelectric stimulator (Mettler Electronics Corp. of Anaheim, CA, US) for delivery of a programmed bioelectric signal to a subject (depicted alongside a pen.)

[0019] FIG. 3 depicts an uncased bioelectric stimulator associated with a glove (Prizm Medical, Inc. of Buford, GA, US) and electrodes for delivery of the selected bioelectric signal(s) to the subject's hand or fingers.

[0020] FIG. 4 depicts a bioelectric stimulator associated with a glove and electrodes for delivery of the selected bioelectric signal(s) to the subject's hand, fingers, or wrist-area.

[0021] FIG. 5 depicts a prototype "helmet" or stimulator cap with electrodes for association with a bioelectric stimulator.

DETAILED DESCRIPTION

[0022] Referring now to FIGs. 1 and 2, depicted are bioelectric stimulators for use in treating a subject, such as a human. The depicted devices are typically about the size of a pen and are programmable and rechargeable. In FIG. 1, the bioelectric stimulator is depicted with exemplary leads for application of the bioelectric signal to the patient suffering from essential tremor (e.g., at the wrist(s), hand(s), and/or ankle(s) of the subject).

[0023] The micro voltage signal generator may be produced utilizing the same techniques to produce a standard heart pacemaker well known to a person of ordinary skill in the art. An exemplary microvoltage generator is available (for experimental purposes from Cal-X Stars Business Accelerator, Inc. DBA Leonhardt's Launchpads or Leonhardt Vineyards LLC DBA Leonhardt Ventures of Salt Lake City, UT, US). The primary difference is the special electrical stimulation signals needed to control, *e.g.*, precise follistatin release on demand (which signals are described later herein). The leading pacemaker manufacturers are Medtronic, Boston Scientific Guidant, Abbott St. Jude, BioTronik and Sorin Biomedica.

[0024] Construction of the electric signal generators and pacemakers, are known in the art and can be obtained from OEM suppliers as well as their accompanying chargers and programmers. The electric signal generators are programmed to produce specific signals to lead to specific protein expressions at precisely the right time for, e.g., optimal organ treatment or regeneration.

[0025] Methods of inducing stem cell proliferation and the upregulation in the expression of various proteins (e.g., Activin B, EGF, follistatin, HGF, IGF-1, PDGF, OPG, RANKL, SDF-1, VEGF, and tropoelastin) by specific bioelectric signals are described in US Patent Application Publication US20180064935A1 to Leonhardt et al. (March 8, 2018), the contents of which are incorporated herein by this reference.

[0026] In certain embodiments, a neurostimulator (e.g., ActivaTM PC, Activa SC, or Activa RC neurostimulator from Medtronic or similar device from PINS Medical (Beijing, China, and SceneRay (Suzhou, China)) is programmed to specifically produce a bioelectric signal that up regulates the expression of EAAT2 in a cell.

[0027] A pacing infusion lead may be constructed or purchased from the same suppliers that build standard heart pacemaker leads. Pacing infusion leads may be purchased from a variety of OEM vendors. The pacing infusion lead may, for example, be a standard one currently used in heart failure pacing studies in combination with drug delivery.

[0028] An infusion and electrode wide area patch may be constructed by cutting conduction polymer to shape, and forming plastic into a flat bag with outlet ports in strategic locations.

[0029] Micro stimulators may be purchased or constructed in the same manner heart pacemakers have been made since the 1960's. When used with a micro infusion pump, such pumps can be purchased or produced similar to how they have been produced for drug, insulin, and pain medication delivery since the 1970's. The programming computer can be standard laptop computer. The programming wand customary to wireless programming wands may be used to program heart pacers.

[0030] In certain embodiments, the system includes real time reading of promotors of essential tremors and real time bioelectric signaling to control. Ren et al. (2016) *infra.*

[0031] In certain embodiments, the system is preferably miniaturized and wireless, and takes the form of a comfortable bioelectric wristwatch (not shown) or glove (FIG. 3) for the

hand/wrist and small bioelectric micro stimulators placed on a patient's upper neck lower base of brain or behind the ears so as not to be generally visible.

[0032] In certain embodiments, described herein is a combination bioelectric hand and brain cap stimulator (FIG. 5) to reduce uncontrolled essential tremors. The bioelectric signaling device precisely regulates control of specific protein expressions or suppressions, which help reduce essential tremor movements and duration.

[0033] Both wireless non-invasive and/or implantable wire lead ("electrode") based means may be used to deliver the regeneration and healing promoting bioelectric signals to target organs.

[0034] A wireless, single lumen infusion pacing lead or infusion conduction wide array patch may all be used to deliver the regeneration signals and substances to the organ of interest to be treated or they may be used in combination.

[0035] In certain embodiments, the lead is associated with the subject's wrist(s) and/or ankle(s) to apply a bioelectric signal to upregulate the expression of EAAT2 protein by the subject's cells and tissue and thus treat and/or control essential tremors. In certain embodiments, a bioelectric signal to upregulate the expression of EAAT2 protein by the subject's cells and tissue is applied wirelessly to the subject's wrist(s) and/or ankle(s).

[0036] A re-charging wand for use herein is preferably similar to the pacemaker re-charging wand developed by Alfred Mann in the early 1970's for recharging externally implantable pacemakers.

[0037] Bioelectric stimulation can be done with the described microstimulator, which can have a pacing infusion lead with, e.g., a corkscrew lead placed/attached at, e.g., the center of the tissue to be stimulated and/or treated.

[0038] The microstimulator is actuated and runs through programmed signals to signal the release of, *e.g.*, EAAT2.

[0039] The device should have a current driven signal (instead of voltage driven like most other devices).

[0040] Relationship Between The Components:

[0041] The micro voltage signal generator is attached to the pacing infusion lead with, e.g., a corkscrew tip, deep vein stimulation lead (Medtronic) (e.g., for bioelectric stimulation of the brain), or conductive polymer bandage or patch to the tissue or organ to be treated. An external

signal programmer may be used to program the micro voltage signal generator with the proper signals for treatment including the EAAT2 upregulating signal. The device battery may be re-chargeable with an external battery charging wand.

[0042] The essential elements are the micro voltage signal generator and the means for delivering the signal to the target tissue(s).

[0043] The signal generator may be external or internal. The transmission of the signal may be wireless, via liquid and/or via wires.

[0044] The tissue contact interface may be, e.g., a patch or bandage or may be via electrodes or leads. FDA-cleared gel tape electrodes (Mettler) may be used for skin delivery. Electro acupuncture needles may be used to ensure the signals positively reach target tissues under the skin. See, e.g., Chen et al. (2016) *infra,* Ren et al. (2016) *infra*, Rajabi et al. (2016) *infra*, Yu et al. (2009) *infra*, O'Mahony et al. (2016) *infra*, US Patent 8,588,884 to Hegde et al. (Nov. 19, 2013), and UCLA Samueli Newsroom (2020) *infra*.

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CLAIMS

What is claimed is:

1. A method of treating a subject suffering from a neurodegenerative disorder, the method comprising:

applying a bioelectric signal to the subject that stimulates the expression and/or release of polypeptide(s) useful in the treatment of the neurodegenerative disorder.

2. The method according to claim 1, wherein the neurodegenerative disorder is selected from the group consisting of essential tremor, intentional tremor, Parkinson’s disease, stroke, epilepsy, amyotrophic lateral sclerosis, Alzheimer's disease, major depressive disorder, addiction, and any combination thereof.

3. A bioelectric stimulator programmed to produce a bioelectric signal that stimulates target tissue to upregulate expression by the target tissue of polypeptide(s) useful in the treatment of a neurodegenerative disorder.

4. A method of using the bioelectric stimulator of claim 3 to stimulate tissue of a subject, the method comprising:

connecting the bioelectric stimulator to the target tissue of the subject, and

actuating the bioelectric stimulator to produce the programmed bioelectric signal.

5. The method according to claim 4, wherein the target tissue comprises tissue selected from the group consisting of thalamus, brain, nerve, and caudal zona incerta.

6. The method according to claim 4 or claim 5, wherein the target tissue comprises the ankle(s), the hand(s), and/or the wrist(s) of the subject.

7. The method according to any one of claims 4 to 6, wherein the subject is suffering from or is at a risk of suffering from essential tremor, intention tremor, Parkinson’s disease, stroke, epilepsy, amyotrophic lateral sclerosis, Alzheimer's disease, major depressive disorder, and/or addiction.

8. The method according to claim 7, wherein Lingo-1 is further down-regulated in the subject.

9. A method of treating a subject suffering from essential tremor, the method comprising:

applying a bioelectric signal to the cell that stimulates the cell to express and/or release EAAT2 polypeptide.

10. The method according to claim 9, further comprising down-regulating Lingo-1 in the subject.

11. A method of treating a subject suffering from or at a threat of suffering from Parkinson's disease, the method comprising:

applying a bioelectric signal to a cell that stimulates the cell to express and/or release EAAT2 polypeptide.

11. The method according to claim 11, wherein Lingo-1 is further down-regulated in the subject.

ABSTRACT

Described is a low voltage, pulsed electrical stimulation device for controlling expression of a useful protein to treat a patient's tissues, particularly a patient suffering from a neurodegenerative disorder. Also described are methods of enhancing expression of EAAT2 in cells. Upregulation of EAAT2 expression has implications in the treatment and/or prevention of essential tremor, Parkinson's disease, stroke, epilepsy, amyotrophic lateral sclerosis, Alzheimer's disease, major depressive disorder, and/or addiction.