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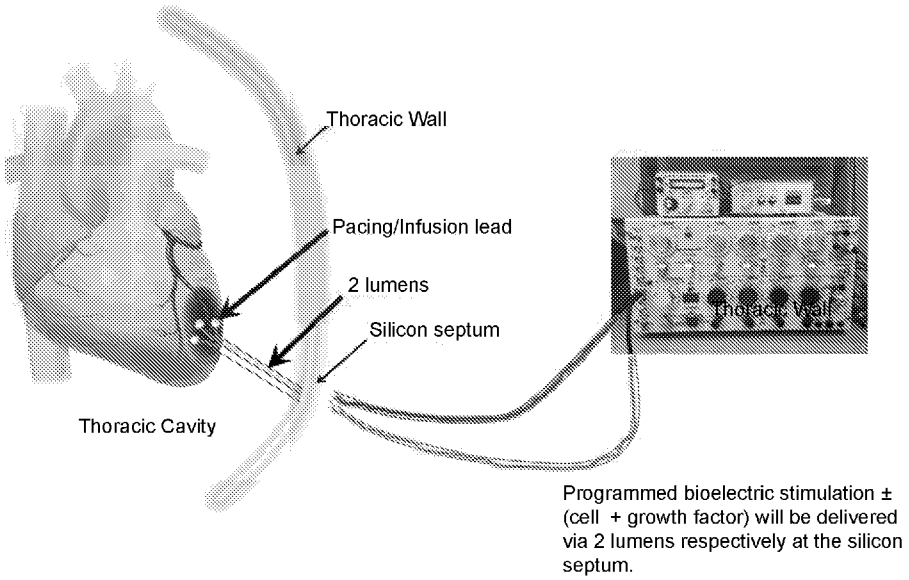


*FIG. 1*

(57) Abstract: Described is a low voltage, pulsed electrical stimulation device for reducing inflammation in a subject, which can be useful in the treatment of concussions, traumatic brain injury, cancer, and endometriosis, and so forth.

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[Continued on next page]

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# SYSTEM AND METHOD FOR TREATING INFLAMMATION

## PRIORITY CLAIM

**[0001]** This application claims the benefit of the filing date of United States Patent Application Serial No. 16/137,467, filed September 20, 2018, the disclosure of each of which is incorporated herein in its entirety by this reference.

## FIELD

**[0002]** This application relates generally to the field of medical devices and associated treatments, and more specifically to precise bioelectrical control of inflammation in a subject. For example, described is that the stimulation of a subject's brain tissue, possibly augmented with the administration of a composition comprising, among other things, stem cells and nutrients, can be useful to stimulate and treat the inflammation associated with, e.g., a concussion.

## BACKGROUND

**[0003]** To date, no effective treatment for a concussion exists. Concussion management thus far has focused on prevention and rest, allowing symptoms to subside naturally before normal activity is resumed.

**[0004]** The use of anti-inflammatory drugs for the treatment of concussion has been examined. Non-steroidal anti-inflammatory drugs (NSAIDs) seemed to be an attractive option. However, evidence exists to suggest that NSAIDs may not be the best pharmacotherapy for managing the neurobiological factors underlying concussive injuries. For example, chronic treatment with ibuprofen worsens the cognitive alterations in rodents exposed to an experimental TBI. Other NSAIDs, such as minocycline, are able to reduce apoptotic damage in several forms of CNS injury, such as spinal cord injury, but do not show any beneficial effects when examining recovery times from mild TBI. Taken together, these data demonstrate that preventing an inflammatory response to a concussion is likely not a viable treatment. Furthermore, the evidence suggests that the concussed brain presents a unique inflammatory signature as opposed to a general inflammatory response that occurs following any CNS injury.

**[0005]** The search for a viable concussion treatment continues. An acute metabolic cascade following a concussion has been characterized in detail. Unfortunately, targeting this metabolic cascade has failed to produce viable treatment options. Treatment strategies focused

on managing the neuroinflammatory responses to concussion may prove more effective. Data exists suggesting that manipulating neuroinflammation can be used as a treatment strategy to manage the long-term deficits produced by a concussive injury. However, general anti-inflammatory drugs will not serve as a "magic bullet." Rather, it seems that a tailored array of pro- and anti-inflammatory compounds given at particular temporal intervals will likely be implemented given the complexity of the inflammatory response to concussion. Treatments will likely differ based on severity of brain injury, age of the patient, and previous brain injury history. Furthermore, treatment strategies require close attention be paid given the fluctuations of inflammatory profiles over time following a concussion.

**[0006]** The failures of NSAIDs and other anti-inflammatory agents in mitigating post-concussive damage in a subject highlight the need for customized approaches for the subject.

## BRIEF SUMMARY

**[0007]** Customized approaches for controlling inflammation in a subject can be provided by controlled protein expression on demand by bioelectric stimulation. Controlled protein expression on demand by bioelectric stimulation can be designed to deliver a very specific protein regimen at a very specific time; often in very specific sequence at a very specific dose.

**[0008]** Described herein is a method of treating inflammation in a subject comprising first reading (or otherwise determining) the levels of a subject's inflammatory cytokines after, e.g., a concussion and then adjusting the subject's levels of inflammatory cytokines to a desired level to improve recovery from the concussion or traumatic brain injury ("TBI"). It is found that the right timing and the right balance in the right sequence of inflammatory cytokines aids in a subject's recovery from stroke, concussion, and/or traumatic brain injury. Electric stimulation should be provided to the subject to control the expression and/or release of SDF-1 (stem cell homing), IGF-1 (DNA repair), HGF, VEGF, PDGF, eNOS, HIF 1 α, IL-6, Activin A+B, Stem cell proliferation signals, and stem cell differentiation control signals. To this may be included, e.g., GDF-10, GDF-11, Neurogenin 3, and FGF.

**[0009]** For recovery from stroke, concussion, or traumatic brain injury, if electric stimulation alone does not provide complete recovery, the patient may then also receive a composition comprising adipose-derived or bone marrow-derived stem cells (MSCs), endothelial progenitor cells, a variety of selected growth factors cocktail, selected exosomes,

selected micro RNAs, selected alkaloids, selected anti-inflammatory agents, nutrient hydrogel, organ specific matrix, and amniotic fluid.

**[0010]** For severe recovery need cases, the method preferably further includes communicating wirelessly with miniature "Bion" implants that are implanted into the damaged organ and/or tissue (e.g., brain) regions that relay and amplify applied protein expression signals.

**[0011]** Described is a device that determines a subject's inflammatory markers following organ injury and then, in response to the determination(s), delivers regenerative and recovery bioelectric protein expression signals to the subject's tissue so as to up-regulate and/or down-regulate selected protein expression thereby, thus helping to establish inflammatory balance for organ healing and recovery in the subject.

**[0012]** Such a device typically comprises: a power source, an input to determine the subject's inflammatory markers, and means for delivering an electrical signal to the subject's tissue. The device utilizes the electrical signal to precisely control protein expression in the tissue on demand. Such proteins are typically selected from the group consisting of insulin-like growth factor 1 ("IGF1"), interleukin 6 ("IL-6"), interleukin 10 ("IL-10"), interleukin-1β ("IL-1β"), transforming growth factor-β ("TGFβ"), tumor necrosis factor alpha ("TNF-α"), and any combination thereof.

**[0013]** Such a device may be utilized, e.g., to treat a subject suffering from a brain concussion, traumatic brain injury ("TBI"), heart failure, etc. In the methods relating to concussion or TBI, the bioelectric protein expressions sequence typically comprises: from about five (5) to about forty (40) minutes, to increase IL-1β following a concussion (precedes the secretion of ciliary neurotrophic factor (CNTF) and nerve growth factor (NGF), both of which promote the growth and survival of neurons and defend against the instigation of apoptotic pathways), wherein the device then determines and adjusts the bioelectric protein expression signals to the subject's tissue for about five (5) minutes of an IL-1β inhibition shut off signal, and then from about five (5) to forty (40) minutes, a rise in TNF-α, wherein the device then determines and adjusts the bioelectric protein expression signals to the subject's tissue for about five (5) minutes of TNF-α inhibition shut off signal, and then from about five (5) to forty (40) minutes rise in TGFβ, and wherein the device then determines and adjusts the bioelectric protein expression signals to the subject's tissue for about five (5) minutes of a TGFβ inhibition shut off signal, and then about three (3) minutes of a rise in IL-10.

**[0014]** In certain cases, the bioelectric stimulator is programmed to produce a bioelectric signal that stimulates target tissue to express and/or release Klotho polypeptide by the target tissue utilizing a bioelectric signal comprising a biphasic square pulse at 20 Hz, 0.1 V (100mV), and a 7.8 ms pulse duration for, e.g., 30 minutes of stimulation. The amount of Klotho expression enhanced by the herein described system (>400% increase) is greater than that seen with generic electrical muscle stimulation or muscle contraction alone.

**[0015]** Such a method may further include separately delivering to the subject (e.g., via a pump and catheter) a "cocktail" of regenerative agents comprising any combination of the following: stem cells, endothelial progenitor cells, selected exosomes, selected alkaloids, selected anti-inflammatory agents, nutrient hydrogel, organ specific matrix, selected growth factors, amniotic fluid, placenta fluid, cord blood, and embryonic sourced growth factors and cells.

**[0016]** The method may be used in a subject to regenerate brain cells. Such a method typically comprises generating electrical signals from the device to control the expression and/or release of a protein, wherein the protein is selected from the group consisting of insulin-like growth factor 1 ("IGF1"), interleukin 6 ("IL-6"), interleukin 10 ("IL-10"), interleukin-1β ("IL-1β"), transforming growth factor-β ("TGFβ"), tumor necrosis factor alpha ("TNF-α"), and any combination thereof. Again the method may further comprise separately delivering to the subject stem cells and/or growth factors comprising any combination of IGF1, IL-6, IL-10, IL-1β, TGFβ, TNF-α, and any combination thereof.

**[0017]** The device may be used to achieve brain regeneration, cognitive function brain improvement, brain stroke, heart recovery, and traumatic injury recovery, eye regeneration, and/or ear hearing regeneration.

**[0018]** The described bioelectric signals appear to have two primary mechanisms of action. First, they provide instructions for the subject's DNA to build protein(s). And, second, they provide instructions for cell membranes to open and close pores and to activate electrical transfers of energy on demand. As further described herein, action potentials management are most often controlled by frequency durations.

**[0019]** While not intending to be bound by theory, the following may help to explain the invention. Inflammation is an immune response of the body that works as a contained fire that is pre-emptively sparked as a defensive process during infections or upon any kind of tissue insult, and that is spontaneously extinguished after elimination or termination of the damage. However, persistent and uncontrolled immune reactions act as a wildfire that

promote chronic inflammation, unresolved tissue damage, and, eventually, chronic diseases. A wide network of soluble mediators, such as endogenous bioactive lipids, governs all immune processes. These mediators are secreted by basically all cells involved in the inflammatory processes and constitute the crucial infrastructure that triggers, coordinates, and confines inflammatory mechanisms. However, these same molecules are also involved in the detrimental transition from acute to chronic inflammation, be it for persistent or excessive action of pro-inflammatory lipids or for the impairment of the functions carried out by resolving ones. For example, bioactive lipids have been linked to several chronic diseases, including rheumatoid arthritis, atherosclerosis, diabetes, cancer, inflammatory bowel disease, systemic lupus erythematosus, and multiple sclerosis.

**[0020]** Provided are a system and methods designed to promote positive inflammation in moderation, which is essential for healing and when the body is ready to turn off the inflammatory cycle loop to avoid the detrimental effects of chronic inflammation.

**[0021]** The invention includes the idea of recording the ideal neuro inflammation bioelectric and biochemical/hormonal responses of a healthy recovery (without chronic neurological inflammation being triggered) and storing this data in a microprocessor database. For example, recording the brain recovery of a toddler with brain injury. The treatment regime for an injured adult is then compared to this ideal (in addition to real time sensing and adjustments of therapy). The treatment regime chosen is a balance between the ideal recovery sequence (recorded in the microprocessor) and real time adjustments made by real time monitoring.

**[0022]** Provided herein (e.g., in the "neuro hormonal closed loop" approach) are systems and methods that, at times, send bioelectric signals to the brain in order to allow the brain to adjust its response(s) to injury. At times, signals are directed to the injured tissue to allow the tissue to release proteins directly, which influences the neuro hormonal loop with the brain, inflammation, and healing. The system and methods may interfere and control messaging up to the brain, back from the brain, or directly in tissues. Such may be used in combined therapy by combining bioelectric signaling control of inflammation, blood pressure, healing, and programmed biologics delivery via a closed loop sensing and customized therapy neuromodulation system.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0023]** FIG. 1 depicts a programmed bioelectric stimulator (with or without cell and growth factor) for delivery to the heart of a human subject via two lumens respectively at a silicon septum.

**[0024]** FIG. 2 depicts a programmed bioelectric stimulator depicted alongside a U.S. quarter.

**[0025]** FIG. 3 depicts an interface for use with the system.

**[0026]** FIG. 4 depicts a micropump for use with the system.

**[0027]** FIG. 5 depicts a pump associated with a subject's heart.

**[0028]** FIG. 6 depicts an image of the signal (voltage and frequency) associated with Activin B at 6.0 mV, pulse width 100 μs, square wave.

**[0029]** FIG. 7 depicts an image of the signal (voltage and frequency) associated with EGF at 10 V/cm (5 V here), 500 Hz, pulse width 180 μs, square wave.

**[0030]** FIG. 8 depicts an image of the signal (voltage and frequency) associated with follistatin at 10 V/cm, 50 Hz, square wave.

**[0031]** FIG. 9 depicts an image of the signal (voltage and frequency) associated with HGF at 3.5 V, 10 second burst every 30 seconds, square wave.

**[0032]** FIG. 10 depicts an image of the signal (voltage and frequency) associated with IGF-1: 3.0 mV, 22 Hz, square wave.

**[0033]** FIG. 11 depicts an image of the signal (voltage and frequency) associated with OPG: 4.0 mV, 2,000 Hz, square wave.

**[0034]** FIG. 12 depicts an image of the signal (voltage and frequency) associated with PDGF 30%: 3 V/cm (100 mV here), 10 Hz, pulse width 200 μs, square wave.

**[0035]** FIG. 13 depicts an image of the signal (voltage and frequency) associated with PDGF 230%: 20 V/cm (7.0 V here), 100 Hz, pulse width 100 μs, square wave.

**[0036]** FIG. 14 depicts an image of the signal (voltage and frequency) associated with proliferation: 15 mV, 70 Hz, square wave.

**[0037]** FIG. 15 depicts an image of the signal (voltage and frequency) associated with proliferation: 2.5-6.0 V (4 V here), 20 Hz, pulse width 200-700 μs, square wave.

**[0038]** FIG. 16 depicts an image of the signal (voltage and frequency) associated with RANKL: 3.0 mV, 2 Hz, square wave.

**[0039]** FIG. 17 depicts an image of the signal (voltage and frequency) associated with SDF-1: 3.5 mV, 30 Hz, square wave.

**[0040]** FIG. 18 depicts an image of the signal (voltage and frequency) associated with tropoelastin: 60 mV, 50 Hz, square wave.

**[0041]** FIG. 19 depicts an image of the signal (voltage and frequency) associated with VEGF: 100 mV, 50 Hz, square wave.

**[0042]** FIG. 20 depicts an image of the signal (voltage and frequency) associated with SDF-1 (2nd part): 0.25 mA (3.0 V shown here), 100 Hz, 100 μs pulse width, square wave.

**[0043]** FIG. 21 depicts a combination bioelectric stimulation and stem cells and growth factors infusion catheter.

**[0044]** FIG. 22 is a close up view of the conductive and infusion cork screw tip for use with the catheter system of FIG. 21.

**[0045]** FIG. 23 depicts a helmet design for use as described herein.

**[0046]** FIG. 24 depicts a helmet design for use as described herein.

## DETAILED DESCRIPTION

**[0047]** Customized inflammation control could also be useful for other disease treatment and management. Preferably, one does not deliver a single drug or single signal or one set of signals for inflammation control. A subject suffering from inflammation benefits from real time monitoring and constant adjustment of signals to achieve inflammation balance. Too much is bad, too little is bad, too much or too little at the wrong time can be bad for the subject. The sequence is preferably correct and constantly adjusted. Multiple inflammatory or anti-inflammatory cytokine levels need to be up or down at just the right time depending on the status of recovery and many other variables. Cytokines such as IL-1, TNF-A or IL-6 at the right time at the right levels can be highly useful for healing of an organ, but at the wrong levels at the wrong time, the very same cytokines can be highly damaging to the organ and/or tissue and can cause rapid deterioration,

**[0048]** Such adjustments include elevating or reducing TNF-A, IL-1, IL-6, and other inflammatory or anti-inflammatory cytokines. Regardless of inflammation control, the organ or tissue should be regenerated back to maximal health. A healthy regenerated organ does not have an inflammation problem, only an unhealthy organ has an inflammation problem. Controlling inflammation only treats the symptom; not the disease. Inflammation is a reaction to an unhealthy organ; not the other way around. A subject's tooth becomes inflamed when, e.g., there is a root infected from trapped bacteria and breakdown. The problem is the source

of the infection and breakdown that is to be treated; not just the inflammation. Inflammation control herein is preferably customized for the subject's condition, and preferably adjusted constantly (e.g., many times a day, sometimes many times a minute, based on real time data).

**[0049]** Described herein is real time, data-based customized bioelectric inflammation management for, e.g., heart, stroke, traumatic brain injury and concussion recovery (see, e.g., FIG. 23 for a preferred "helmet" with nodes particularly useful for real time management when inflammation follows brain injury or stroke).

**[0050]** In certain preferred embodiments, a bioelectric stimulator for reducing inflammation in a subject is configured to generate bioelectric signals responsive to a programmed process, which bioelectric signals comprise a bioelectric signal that exhibits the following characteristics (within 15%): 35 μA, a frequency range of 139-147 Hz + 3 V/cm and/or a bioelectric signal that exhibits the following characteristics (within 15%): 10 Hz, 0.2 ms pulse duration, 3 V.

**[0051]** In other preferred embodiments, bioelectric stimulator for reducing inflammation in a subject is configured to generate bioelectric signals responsive to a programmed process, which bioelectric signals comprise: a bioelectric signal that exhibits the following characteristics (within 15%): 0.1 V applied at a frequency of 50 Hz, square wave, 0.25 mA, 3.0 V, 100 Hz, 100 μs pulse width, square wave, or 3.5 mV, 30 Hz, square wave, and a bioelectric signal that exhibits the following characteristics (within 15%): 20 Hz, 0.1 V, and a 7.8 ms pulse duration.

**[0052]** A bioelectric stimulator may be further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%): 0.1 V applied at a frequency of 50 Hz, square wave.

**[0053]** A bioelectric stimulator may be further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%): 0.25 mA, 3.0 V, 100 Hz, 100 μs pulse width, square wave.

**[0054]** A bioelectric stimulator may be further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%): 3.5 mV, 30 Hz, square wave.

**[0055]** A bioelectric stimulator may be further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%): 3 mV with a frequency of 22 Hz, square wave, and a current of 1 mA, and 3 mV with a frequency of 22 Hz, square wave, and a current of 3 mA.

[0056] A bioelectric stimulator may be further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%): 20 Hz, 0.1 V, and a 7.8 ms pulse duration.

[0057] A bioelectric stimulator may further comprise an electrode for delivering bioelectric signal(s) to the subject and/or a transducer and/or transceiver is configured for delivering bioelectric signal(s) wirelessly to the subject.

[0058] As depicted in FIGS. 23 and 24, such a helmet has, as primary components, wireless means to read real time inflammatory and anti-inflammatory cytokine levels in an organ or blood flow (e.g., in the brain), wireless bioelectric signals directed to specific organ regions to manage the control of inflammation with real time customized delivery of signals for balancing cytokine levels, and bioelectric signals delivered via the nervous and cardiovascular system to manage total body electrical potentials balance, all for inflammation control therapy.

[0059] In certain embodiments, both recording electrodes and stimulating electrodes are appropriately positioned, e.g., about the patient's head with a helmet to measure and sense the inflammatory situation, and then in response stimulating the inflamed tissue appropriately. In some instances, the stimulating electrode can be an external coil, situated above microbeads placed in close proximity to the inflamed tissue. These embodiments can be readily adapted to areas of the body other than the head.

[0060] The device depicted in FIG. 23 has stimulators, EEG hardware (e.g., sensor pads), and stimulator-pump hardware. The legs are relatively thick and follow the form of the subject's head ending in, e.g., EEG sensors. Stimulators are typically placed between the EEG sensors. At the apex of the depicted device, there are hardware storage units for the EEG (e.g., battery and SD storage card) and the stimulator and pump. The stimulator and pump are preferably positioned on the device at the posterior portion of the subject's head.

[0061] Applications of the device include treating a subject for: cerebral stroke recovery, concussion recovery, injury-related brain damage, brain cancer recovery, Parkinson's, Alzheimer's and dementia, cerebral aneurysm repair, depression, brain memory recovery and enhancement, and brain function enhancement.

[0062] In use, the device is focused on brain regeneration utilizing microcurrent signals that home stem cells to the brain and cause new blood vessels to grow. For severe cases, stem cell and growth factor injections are added, and for extremely severe cases, a re-fillable, programmable micro pump regeneration stimulator is incorporated into the system.

**[0063]** The device is designed to stimulate true neurogenesis, i.e., the formation and nurturing of new neuron cells, to regenerate damaged or diseased brain tissue for true brain regeneration. By bioelectric stimulation, the device controls the release of, e.g., brain regeneration promotion cytokines. Such cytokines may include SDF-1 (for stem cell homing factor and recruitment of stem cells from the patient's own bone marrow, fat and circulating blood to the stimulated brain tissue areas). Another signal of opposite polarity for controlling the differentiation of those recruited stem cells into healthy functioning brain tissue. Other cytokines are IGF-1 (for DNA level repair), HGF, EGF, Activin A+B, eNOS, VEGF, follistatin and tropoelastin (all of which contribute to regeneration), eNOS, HGF and VEGF (which help improve blood supply to the treated area).

**[0064]** For severe brain damage recovery and difficult disease cases, the programmable micro infusion pump (fillable daily or weekly) is included, with angiogenic and regeneration compositions comprised of a variety of cell types, growth factors, nutrient hydrogel, exosomes, Micro RNAs, brain matrix and other neurogenesis promoting molecules including harmine and tetrahydroharmine alkaloids and inflammation control agents. The combination of bioelectric regeneration stimulator, micro infusion pump and angiogenic and regeneration compositions are believed to help people recover from brain injuries or brain related diseases better than with previous therapies.

**[0065]** For brain tumor cancer patients, pending tuned bioelectric signals are used to reduce cell division and blood supply to the tumors.

**[0066]** Inflammation is better managed with more than a single drug or single bioelectric signal. The body produces inflammation to promote healing and the right cytokines at the right time in the right sequence greatly aide in healing. The very same cytokines at the wrong time in the wrong sequence and at the wrong levels for the wrong duration can cause detrimental damage to health. The device determines these inflammatory and anti-inflammatory cytokines to deliver multiple cytokine (up- or down-regulation) real time to best attempt to gain the right inflammatory balance.

**[0067]** The depicted device responds to a subject's inflammatory marker levels, delivers bioelectric protein expression signals to the subject's tissue so as to up-regulate and/or down-regulate select protein expression(s) in the subject so as to balance inflammation in the subject. The device comprises: a power source, an input or several inputs to determine the subject's inflammatory markers, and means for delivering bioelectric signals to the subject's

tissue, wherein the device utilizes the electrical signals to precisely control select protein expression(s) in the tissue on demand.

**[0068]** Specifically, anti-inflammatory cytokines serve opposing roles in response to brain injury. Some effects are beneficial while others are detrimental. Balance is essential to optimal recovery.

**[0069]** For example, IL-1β provides neuroprotection following brain injury and therefore might be considered part of the regenerative process. Paradoxically, it has also been shown that chronic inhibition of IL-1β for up to one week following a controlled cortical impact ("CCI") - induced mild/moderate TBI reduces cerebral edema and tissue loss while improving the cognitive outcome by modifying the inflammatory response. These data suggest that prolonged exposure to IL-1β may be associated with neurotoxic effects following a concussion.

**[0070]** TNF-α, in the correct balance, appears to play both neuroprotective and neurotoxic roles following brain injury.

**[0071]** IL-6 acts as both a pro-inflammatory and anti-inflammatory cytokine and is considered to be a key regulator during the acute phase of the inflammatory response to infections and tissue damage. The key is getting the correct IL-6 balance at the right time in the right sequence.

**[0072]** TGF-β confers potential short-term beneficial effects following clinical and experimental TBIs by down-regulating the inflammatory response. However, long-term, TGF-β may be detrimental and may increase the risk of developing other neurological disorders.

**[0073]** The beneficial effects of IL-10 administration are transient and circumstantial. These beneficial effects appear to be dose-specific and site-specific, requiring pre-treatment in order to confer protection.

**[0074]** Lobo-Silva et al. "Balancing the immune response in the brain: IL-10 and its regulation," *Journal of Neuroinflammation*, 13:297 (2016); doi.org/10.1186/s12974-016-0763-8, describes the importance of IL-10 balance in inflammation management. As described therein, manipulating the protective and degenerative neuroinflammation balance is important. Mechanisms exist to avoid exaggerated neuroimmune responses including the production of anti-inflammatory cytokines, such as IL-10. IL-10 binding to its receptor triggers a series of signaling cascades mediated by the Janus kinase signal transducer and activator of transcription (STAT) pathway. Signalling through the IL-10 receptor regulates several steps of the immune response, from decreasing cytokine gene expression to down-regulating the expression of major histocompatibility complex class II and thus antigen presentation to T cells. IL-10 prevents

apoptosis by activating the PI3K/Akt cascade and enhancing the expression of anti-apoptotic factors as Bcl-2 and Bcl-xl, while attenuating caspase-3. IL-10 inhibits the production of pro-inflammatory cytokines by microglia, protecting astrocytes from excessive inflammation. IL-10 also acts on astrocytes and potentiates production of TGF-β. IL-10 is an important mediator of the crosstalk between microglia, astrocytes, and neurons. Several studies directly implicate defective IL-10 production or signaling in patients and animal models of neurological diseases, ranging from neuropathic pain to multiple sclerosis, Alzheimer's disease, or Parkinson's disease.

**[0075]** Prolonged exposure to inflammatory cytokines is typically ultimately harmful, shifting the intrinsic neuroprotective efforts of the immune response to the detrimental effects of neuroinflammation. However, neuroinflammation may contribute to the neuroprotective regenerating efforts of the brain and in its absence the cumulative damage is increased following injury.

**[0076]** In certain embodiments, provided is a system that reads inflammation real time and constantly adjusts bioelectric stimulation and cytokine releases to modulate and manage inflammation in a subject in real time. This is preferably done to manage the cycle of chronic inflammation in the subject. In essence, it activates a "re-start button" on the subject's chronic inflammation cycle.

**[0077]** The microprocessor preferably stores ideal optimal healing cycles. Treatment regimes are adjusted real time between data coming in via real time monitoring of inflammation and ideal optimal healing algorithms stored on the microprocessor of the microstimulator and reader. The ideal optimal healing cycles may be derived from measurements made in healthy subjects having an inflammation causing event, who recover quickly and fully (such as a toddler head injury recovery).

**[0078]** Proper inflammation management is believed to be served best by bioelectric energy management that in turn controls release of the proper cytokines in the correct sequence at the correct time for healthy recovery, especially when combined with proper diet and exercise.

**[0079]** Inflammation has long been a well-known symptom of many infectious diseases, but new molecular and epidemiological research increasingly suggests that it is also intimately linked with a broad range of non-infectious diseases.

**[0080]** Inflammation is a response of the immune system to injury, irritation, or infection caused by invading pathogens, radiation exposure, very high or low temperatures, or

autoimmune processes. Therefore, inflammation is a mechanism for removing damaged cells, irritants, or pathogens. Inflammation is considered to be beneficial when it is short term and under control within the immune system ("acute inflammation"). Inflammation that persists longer is known as chronic inflammation. This inflammation is characterized by the simultaneous destruction and healing of tissue.

**[0081]** The various factors that are known to induce chronic inflammatory responses also cause numerous chronic diseases. These factors include bacterial, viral, and parasitic infections (e.g., Helicobacter pylori, Epstein-Barr virus, human immunodeficiency virus, flukes, schistosomes); chemical irritants (e.g., tumor promoters, such as phorbol ester 12-O-tetradecanoylphorbol-13-acetate, also known as phorbolmyristate acetate); and non-digestible particles (e.g., asbestos and/or silica). Inflammation produces reactive oxygen species and reactive nitrogen species, which cause oxidative damage and further lead to chronic diseases. Inflammation also recruits leukocytes that secrete inflammatory cytokines and angiogenic factors to the site of tissue insult. These cytokines are required for proper wound healing and to stimulate epithelial cell proliferation. However, if uncontrolled, these cytokines can lead to inflammatory disorders. All these inflammatory products have been shown to be regulated by the nuclear transcription factor NF-κB.

**[0082]** A tailored array of pro-inflammatory and anti-inflammatory compounds given at particular temporal intervals is herein implemented. Treatment differs based upon severity of the brain injury, the age of the patient, and a previous history of brain injury. Furthermore, treatment strategies require close attention be paid to when a patient consults a medical professional after brain injury given the fluctuations of inflammatory profiles over time following a concussion.

**[0083]** A customized real time read and then deliver bioelectric protein expression(s) therapy should be able to better strike the balance between acute and delayed actions of cytokines may prove to be appropriate targets for treatment of concussion.

**[0084]** Following the mechanical injury suffered by concussed patients, there is an acute cytokine response. IL-1, a family of 11 cytokines known for their regulation of inflammatory responses, increases rapidly in both human and rodent cases of mild to severe TBI. Within this cytokine superfamily, IL-1α and IL-1β convey a pro-inflammatory response that aids in the defense against infection or injury. In rodent models of concussion, IL-1α and IL-1β are up-regulated within hours following injury. IL-1α shows an acute spike following a

concussion, while IL-1β shows a much more gradual increase which may represent a portion of the delayed cytokine response to CNS injury.

**[0085]** IL-1β levels remain elevated for days following experimental concussions and show significantly higher levels relative to other pro-inflammatory cytokines. The elevation in IL-1β levels seen after brain injury appears to be conditional on the severity of the trauma. As such, experimental injuries lead to contusions, mimicking a more potent TBI than a concussion, and produce more IL-1β mRNA expression in tissues surrounding the contusion, which lasts up to 6 days following the onset of injury.

**[0086]** The rise in IL-1β following a concussion precedes the secretion of ciliary neurotrophic factor (CNTF) and nerve growth factor (NGF), both of which promote the growth and survival of neurons and defend against the instigation of apoptotic pathways. Data suggest that IL-1β provides neuroprotection following brain injury and therefore might be considered part of the regenerative process. Paradoxically, others have shown that chronic inhibition of IL-1β for up to one (1) week following a CCI-induced mild/moderate TBI reduces cerebral edema and tissue loss while improving the cognitive outcome by modifying the inflammatory response. These data suggest that prolonged exposure to IL-1β may be associated with neurotoxic effects following a concussion.

**[0087]** Given that IL-1β is able to stimulate the expression and/or release of other proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), it is not surprising the IL-1β inhibition results in an atypical inflammatory response following fluid percussion injuries. The IL-1β-dependent hypersecretion of other cytokines may produce a toxic inflammatory environment for neurons surrounding the site of injury. Therefore, interrupting IL-1β immediately following the endogenous secretion of neurotrophic factors might prove to be effective in concussion management.

**[0088]** Like the IL-1 family of cytokines, TNF-α shows a rapid response to experimental brain injury and is considered to be an early mediator of CNS damage. Following experimental TBIs, ranging from a mild closed head injury to a more severe lateral fluid percussion injury, TNF-α rises rapidly and peaks within hours, returning to normal levels within 24 hours of the injury. Like IL-1β, TNF-α appears to play both neuroprotective and neurotoxic roles following brain injury.

**[0089]** Acutely, TNF-α alters the permeability of the blood brain barrier (BBB), a well-characterized physiological consequence of concussions. Appropriate alterations to BBB permeability may be necessary to regulate the infiltration of blood-born defense mechanisms

following brain injury. For example, mice lacking complete functional TNF-α signaling show greater tissue damage, increased BBB permeability, and increased recovery times following both moderate and severe CCI injuries, suggesting that TNF-α is necessary for normal recovery. However, inhibiting TNF-α transcription and bioactivity pharmacologically following a mild closed head injury improves neurological outcome and motor function recovery, normalize BBB permeability and decrease edema size, suggesting inhibition of TNF-α activity facilitates recovery. TNF-α signaling may be neurotoxic in the acute stages of TBI. In conjunction with IL-1, TNF-α stimulates the expression and/or release of NGF from astrocytes, which may explain some of its neuroprotective effects. TNF-α may promote proliferation of neurons. Central blockade of TNF-α following a concussion may prove to be beneficial, while prolonged antagonism could be detrimental.

**[0090]** TNF-α stimulates IL-6 expression. IL-6 acts as both a pro- and anti-inflammatory cytokine and is considered to be a key regulator during the acute phase of the inflammatory response to infections and tissue damage. IL-6 increases both mild and moderate/severe TBI in rodents and has been detected at high levels for weeks following severe human brain injuries. In mice lacking IL-6, experimental cortical freeze injuries or cytotoxic brain injuries result in increased oxidative stress, decreased cell survival, and lengthened recovery times compared to WT mice. IL-6 secretion leads to elevated production of NGF in astrocytes and suppresses the production of both TNF-α and IL-1β. Also, IL-6 deficient mice exposed to a closed cortical impact, mimicking a mild TBI, show exaggerated behavioral abnormalities and increased expression of IL-1β one hour following injury.

**[0091]** Overexpression of the IL-6 gene, on the other hand, resulted in shortened recovery times.

**[0092]** TGF-β promotes tissue repair. TGF-β expression is induced by the presence of inflammatory cytokines and forms a negative feedback loop by suppressing the production of pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, and IFN-γ. This negative feedback system ensures that the host is protected from proliferating inflammatory attacks. However, the beneficial effects of TGF-β seem to be dependent on its temporal expression and/or release and concentration. Excessive expression of TGF-β, e.g., hinders the intrinsic repair mechanisms of the brain and confers a predisposition for the development of serious infections.

**[0093]** Expression of TGF-β peaks within 24 hours after TBI in human cases. TGF-β may confer potential short-term beneficial effects following clinical and experimental TBIs

by down-regulating the inflammatory response. Only local administration has been shown to promote tissue repair, while systemic administration results in an immunosuppressive reaction.

**[0094]** IL-10 may be neuroprotective as it decreases levels of reactive oxygenated species, decreases the expression of pro-inflammatory cytokines such as IL-1 and TNF-α, and suppresses further activation of microglia and astrocytes. The beneficial effects of IL-10 administration are transient and circumstantial. These beneficial effects appear to be dose- and site-specific, requiring pre-treatment in order to confer protection, while the type of injury model also seems to influence the use of IL-10.

**[0095]** Provided is a method and system for the controlled up- or down-regulation of CXCL5 expression. CXCL5 is a protein having a role in preventing artery occlusion and cancer tumor growth as well as many other potential useful therapeutic applications including the treatment of arthritis.

**[0096]** Similarly, other CXCLs were induced by electrical stimulation *in vitro*. Using 20 V, 1 HZ with a frequency of 5 ms, a 3-time up-regulation in 15 minutes was attained that decreased to 2.5 in one hour and persisted at around 2.5 - 3 times at 24 hours. These results were obtained on cultured adipocyte-derived mesenchymal stem cells. The results are similar to when the treated cells were bone marrow mesenchymal-derived stem cells. Similar results were found with CXCL1, a chemokine with angiogenic activity, and the potential anti-tumor CXCL3, CXCL 9, and CXCL 10. For CXCL 9, and CXCL 10, the up-regulation was really significant.

**[0097]** The protein encoded by this gene, CXCL5 is a small cytokine belonging to the CXC chemokine family that is also known as epithelial-derived neutrophil-activating peptide 78 (ENA-78). It is produced following stimulation of cells with the inflammatory cytokines interleukin-1 or TNF-α. Expression of CXCL5 has also been observed in eosinophils, and can be inhibited with the type II interferon IFN-γ. This chemokine stimulates the chemotaxis of neutrophils possessing angiogenic properties. It elicits these effects by interacting with the cell surface chemokine receptor CXCR2. The gene for CXCL5 is encoded on four exons and is located on human chromosome 4 amongst several other CXC chemokine genes. CXCL5 has been implicated in connective tissue remodeling. CXCL5 has been also described to regulate neutrophil homeostasis.

**[0098]** CXCL5 plays a role in reducing sensitivity to sunburn pain in some subjects, and is a "potential target which can be utilized to understand more about pain in other inflammatory conditions like arthritis and cystitis." CXCL5 has chemotactic and activating

functions on neutrophils, mainly during acute inflammatory responses. However, CXCL5 expression is also higher in atherosclerosis (a chronic inflammatory condition), but is not associated with neutrophil infiltration. Instead, CXCL5 has a protective role in atherosclerosis by directly controlling macrophage foam cell formation.

5 **[0099]** CXCL5 has potential properties for organ regeneration and recovery including cancer tumor treatment including liver cancer, prostate cancer, atherosclerosis, colorectal cancer, pancreatic cancer, pneumonia, acute respiratory distress syndrome, other lung disorders, arthritis, pain associated with, e.g., sunburn, inflammation, diabetic foot and leg ulcer wound healing, knee stem cell therapy, periodontitis, and neuropathic pain management.

10 **[0100]** The CXCL5 protein was found in previous pre-clinical studies to be a key cytokine in potentially reducing risk of heart attacks, strokes and limb amputations due to atherosclerosis and resulting artery occlusion. Control of CXCL5 expression has also been suggested from data gathered in numerous studies as a possible means to stop cancer tumor growth.

15 **[0101]** CXCL5 is one of the most potent cytokines known to reduce risk of arterial occlusion that is a primary cause of heart attacks and stroke as well as lower limb ischemia. Numerous studies have demonstrated that controlled down-regulation of CXCL5 can be anti-angiogenic and thus help starve cancer tumors of blood supply.

**[0102]** CXCL5 biological processes include positive regulation of leukocyte 20 chemotaxis, positive regulation of cell proliferation, inflammatory response, G-protein coupled receptor signaling pathway, response to lipopolysaccharide, signal transduction, cell-cell signaling, immune response, chemokine-mediated signaling pathway, cell chemotaxis, and neutrophil-mediated immunity.

**[0103]** Described is a bioelectric stimulation device that treats a subject after 25 concussion through real time biosensing and customized bioelectric signal deliveries. Such a device (e.g., FIGS. 23 and 24), which can be in the form of a helmet (see, e.g., CerebraCell from Leonhardt Ventures), preferably non-invasively recruits a subject's stem cells (e.g., via SDF-1 or down-regulation of CXCR4) to damaged brain tissue via at least one bioelectric homing signal. Signals delivered by, e.g., an implantable lead (or micro implant) are also useful, 30 particularly in severe injury cases. Such bioelectric signals can also control the expression and/or release of many proteins that promote new blood vessel growth and brain tissue repair.

**[0104]** The surest way to intended signal purity to a specific region of the brain for a specific treatment however is via controlled protein expression with an implantable lead. A

micro implant may include a multi-site electrode array connected via an output to a VLSI biomimetic model, having an input associated with the subject's hippocampus. An implantable micro stimulator device is already approved for human clinical trials for another deep brain application.

5 **[0105]** Preferably, the device has sensors (or other means) that are able to determine cytokine levels in the subject. For example, nodes on the "cap" or helmet (FIG. 23 or 24) can communicate with implanted micro implants receiving and send signals constantly (sending and receiving signals 24 hours per day). Multiple implants may be wirelessly powered and programmed in one or more region of the brain. For deep brain stimulation, communication 10 exists between the brain cap and deep brain micro implant.

**[0106]** Programmed into the microprocessor are references to cytokine release that occurs (in both balance and sequence) similar to that of when a healthy baby or young child fully recovers from a brain injury as a baseline. The microprocessor adjusts up and down from there, based on real time input from the injured brain. The microprocessor is also pre- 15 programmed in signals and their resulting cytokine release(s) known to contribute to healthy brain development, function and injury recovery.

**[0107]** The device(s) may be used in conjunction with a microinfusion pump to deliver select proteins to the subject. An implantable combination microstimulator and re-fillable micropump with pacing infusion leads may be directly connected with specific brain 20 locations.

**[0108]** With such a device, electrical signals emitted from the device may be used to cause the subject to halt the unchecked flux of ions through regulated channels in membranes, which minimizes brain damage.

**[0109]** Electrical signals emitted from the device may be used to reverse ionic 25 disequilibrium, which minimizes brain damage.

**[0110]** With such a device, electrical signals emitted from the device may be used to cause the subject to reverse neuronal depolarization, which minimizes brain damage. This helps to bring electrical membrane polarizations into healthy balance.

**[0111]** Electrical signals emitted from the device may be used to encourage a healthy 30 balance of Ca2+ levels so as to avoid over accumulation of Ca2+ in the mitochondria, which can hinder metabolism leading to impairments of ATP production.

**[0112]** With such a device, electrical signals emitted from the device may be used to control dependent Na+ and K+ pumps reacting to the concussion.

[0113] With such a device, electrical signals emitted from the device may be used to cause the subject to reduce, stop, or otherwise bring under control indiscriminate release of excitatory neurotransmitters.

[0114] With such a device, electrical signals emitted from the device may be used to stop, slow down, or otherwise bring under balanced control, adenosine triphosphate (ATP).

[0115] With such a device, electrical signals emitted from the device may be used to control glucose metabolism and hyperglycolysis.

[0116] Electrical signals emitted from the device may be used to bring into balance glucose supply and demand, so as to minimize brain damage.

[0117] With such a device, electrical signals emitted from the device may be used to encourage a healthy balance of N-methyl-D-aspartate (NMDA), e.g., by receptor activation. Such action decreases over-activation of the NMDA receptors.

[0118] With such a device, electrical signals emitted from the device may be used to cause the subject to increase cerebral blow flow to healthy levels via controlled release of VEGF, PDGF, eNOS, and HGF, so as to minimize brain damage. IGF-1, growth and differentiation factor 10 ("GDF10"), EGR, activin, and BDNF/TrkB may also be useful. Such bioelectric signals are described herein.

[0119] In summary, bioelectric signals encourage stem cell recruitment to the subject's brain, controlled electrical signals reinforce positive brain pathways, and control expression and/or release of select proteins, thus enhancing memory and recall.

[0120] In a preferred embodiment, an organ regeneration composition hereof comprises adipose-derived stem cells, bone marrow-derived stem cells, muscle-derived stem cells (e.g., when needed for muscle), exosomes, MicroRNAs, nutrient hydrogel, growth factor cocktail, organ specific matrix, selected alkaloids, and/or selected anti-inflammatory agents.

[0121] Referring now to FIG. 1, depicted is a human use stimulator and pump for use with treatment of, *e.g.*, the heart. Preferably, such a device is about the size of two quarters (available from QIG Greatbatch / Greatbatch, Inc. of Frisco, TX, US) (FIG. 2) and is programmable and re-fillable with low cell damage design. Refilling may be by silicon septum ports and reservoir chambers. Depicted particularly in FIG. 1 are the subject's heart, the pacing lead, the infusion lead, the thoracic cavity, two lumens, thoracic wall, silicon septum, and a larger programmed/programmable bioelectric stimulator with composition (e.g., cells and growth factors) for delivery via two lumens via the silica septum. The microinfusion pump for continuous or repeat delivery of a liquid composition, which microinfusion pump includes

silicon septum ports and associated reservoir chambers connected to the bioelectric stimulator microinfusion pump to the tissue with a pacing infusion lead.

**[0122]** The described system is currently being investigated for various applications including heart and cardiovascular (e.g., heart regeneration, aorta regeneration, biological pacemaker regeneration, heart valve regeneration, artery regeneration, limb blood flow improvement and limb salvage, and wireless diabetic foot ulcer treatment), brain (e.g., brain regeneration, stroke, concussion, Parkinson's, Alzheimer's, memory and cognitive function improvement, cerebral aneurysm treatment and cancer, and cognitive function improvement), cosmetic and personal care (e.g., breast regeneration, dental gum regeneration and tooth pulp storage, orthodontics, and skin regeneration), major organ regeneration (e.g., eye, pancreas regeneration, lung, liver regeneration, kidney regeneration, ear hearing, bladder regeneration, whole body regeneration, and sub-gastric mucosa), and associated cancer treatment (e.g., some organ specific technology platforms have integrated cancer tumor stoppage signals).

**[0123]** The described system may be incorporated into, for example, a whole body regeneration chamber that scans and/or analyzes the body for its deficiencies and precisely delivers the right stem cells and proteins to the right location at the right time combined with programmed infusion of whole body regeneration substances. Ultimately, the goal for the technology is whole and complete body regeneration, every organ.

**[0124]** The organ specific matrix is a composition comprising cells of an organ which is to be treated. The organ specific matrix is believed to aid in stem cell differentiation, but in any event is found to be useful in the composition. It has been found that for the multicomponent composition, cells plus selected growth factors are better than just cells alone. *See, e.g.*, Procházka et al. "Therapeutic Potential of Adipose-Derived Therapeutic Factor Concentrate for Treating Critical Limb Ischemia," *Cell Transplantation*, 25(9), pp. 1623-1633(11) (2016) and "Cocktail of Factors from Fat-derived Stem Cells Shows Promise for Critical Limb Ischemia," world wide web at sciencenewsline.com/news/2016012204520017.html (Jan. 22, 2016), the contents of each of which are incorporated herein by this reference.

**[0125]** In case of an advanced disease state, a micro infusion pump (e.g., FIGS. 3 - 5) is used for daily delivery of, e.g., 2 ml of organ regeneration composition (comprised of adipose-derived cells or bone marrow-derived mesenchymal stem cells plus cocktail of growth factors (usually derived from amniotic fluid or placenta), selected Micro RNAs, selected alkaloids,

selected anti-inflammatory agents, nutrient hydrogel, organ specific matrix, selected exosomes). For muscle regeneration, immature myoblasts are included in the composition.

**[0126]** Exosomes represent a specific subset of secreted membrane vesicles, which are relatively homogeneous in size (30–100 nm). Exosomes have been proposed to differ from other membrane vesicles by its size, density, and specific composition of lipids, proteins, and nucleic acids, which reflect its endocytic origin

**[0127]** Exosomes are formed in endosomal vesicles called multivesicular endosomes (MVEs) or multivesicular bodies, which originate by direct budding of the plasma membrane into early endosomes. The generation of exosomes to form MVEs involves the lateral segregation of cargo at the delimiting membrane of an endosome and inward budding and pinching of vesicles into the endosomal lumen. Because exosomes originate by two successive invaginations from the plasma membrane, its membrane orientation is similar to the plasma membrane. Exosomes from many cell types may contain similar surface proteins as the cell from which it is derived. Membrane proteins that are known to cluster into microdomains at the plasma membrane or at endosomes, such as tetraspanins (CD63, CD81, CD82), often are also enriched in EVs. It is also thought that endosomal sorting complex responsible for transport system and tetraspanins, which are highly enriched in MVEs, play a role in exosome production. How cytosolic constituents are recruited into exosomes is unclear but may involve the association of exosomal membrane proteins with chaperones, such as HSC70, that are found in exosomes from most cell types. MVEs are also sites of miRNA-loaded RNA-induced silencing complex accumulation, and the fact that exosome-like vesicles are considerably enriched in GW182 and AGO2 implicates the functional roles of these proteins in RNA sorting to exosomes. Exosomes are released to the extracellular fluid by fusion of MVE to the plasma membrane of a cell, resulting in bursts of exosome secretion. Several Rab GTPases such as Rab 27a and Rab27b, Rab11 and Rab35, all seem to be involved in exosomes release.

**[0128]** Repeat doses of the composition are also preferred. *See, e.g.*, Gavira et al. "Repeated implantation of skeletal myoblast in a swine model of chronic myocardial infarction," *Eur. Heart J.*, 31(8): 1013-1021. doi: 10.1093/eurheartj/ehp342 (2010), the contents of which are incorporated herein by this reference.

**[0129]** For heart muscle regeneration, immature myoblasts and cardiac-derived progenitors cells as well as endothelial progenitor cells (EPCs) may be included in the composition.

**[0130]** Generally, the system hereof involves a bioelectric stimulator controlling expression and/or release of SDF-1, IGF-1, HGF, EGF, VEGF, PDGF, eNOS, follistatin, Activin A and B, and tropoelastin. Optionally and in certain applications, GDF-10, GDF-11, Neurogenin-3 and Relaxin may also be included.

**[0131]** SDF-1 is generally for recruiting stem cells and maturing blood vessels. IGF-1 is for DNA repair. HGF is for tissue regeneration and reduces arrhythmias in the case of heart. EGF grows tissue. VEGF grows blood vessels. PDGF is a second stem cell homing factor and helps tissue regeneration especially heart. eNOS dilates blood vessels. Follistatin promotes muscle growth. Activin A and B regenerates nerve cells and neurons. tropoelastin increases elasticity of all tissues especially arteries, skin, heart, aorta. GDF-10 and GDF-11 promote regeneration especially of nerve cells and neurons. Neurogenin-3 is especially helpful in brain and pancreas regeneration. Relaxin helps heart regeneration.

**[0132]** 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 expression and/or 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.

**[0133]** 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.

**[0134]** The 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.

**[0135]** An infusion and electrode wide area pitch may be constructed by cutting conduction polymer to shape and forming plastic into a flat bag with outlet ports in strategic locations.

**[0136]** Micro stimulators may be purchased or constructed in the same manner heart pacemakers have been made since the 1960's. Micro infusion 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.

**[0137]** Any one of the protein expression signals work well on their own for organ regeneration, but they work better together. SDF-1 is the most powerful regeneration protein followed by IGF-1.

**[0138]** 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.

**[0139]** 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.

**[0140]** FIG. 21 depicts a combination bioelectric stimulation and stem cell and growth factor(s) infusion catheter usable with the described system.

**[0141]** A corkscrew tip may be of a standard type utilized to secure most heart pacemakers in heart tissue. Wireless delivery of the signal or electro-acupuncture needle delivery is included. FIG. 22 is a close up of the conductive and infusion cork screw tip for getting deep into target tissue. The tip include suture tabs for even more secure fixation to the target organ.

**[0142]** Additionally, the micro stimulator and micro pump and regeneration composition and bioelectric signaling programming may be used to generate tissue(s) and/or organ(s).

**[0143]** A preferred composition includes adipose-derived cells (or bone marrow derived MSCs or any pluripotent stem cell, such as iPS cells) and growth factor mix which should include (SDF-1, IGF-1, EGF, HGF, PDGF, VEGF, eNOS, activin A, activin B, follistatin, relaxin, GDF-10, GDF-11 and tropoelastin plus selected exosomes (miR-146a, miR-294, mES-Exo) plus selected alkaloids (harmine and tetrahydroharmine) plus selected anti-inflammatory factors plus nutrient hydrogel (IGF-1, SDF-1, HGF plus FGF) plus organ specific matrix. For regenerating muscle, one includes into the composition skeletal muscle or cardiac muscle-

derived cells. Also, preferably included are amniotic fluid, placenta, or cord blood when available.

**[0144]** For heart treatment/regeneration (e.g., for treating congestive heart failure), the compositions may be modified to include: cardiac tissue biopsy derived cells, adipose tissue-derived cells, skeletal muscle derived cells (immature myoblasts (Tamaki selection process – Tamaki et al. "Cardiomyocyte Formation by Skeletal Muscle-Derived Multi-Myogenic Stem Cells after Transplantation into Infarcted Myocardium," *PLoS ONE* 3(3): e1789. doi:10.1371/journal.pone.0001789 (2008), the contents of which are incorporated herein by this reference)), nutrient hydrogel, selected growth factors (SDF-1, PDGF, HGF, IGF-1, follistatin, relaxin, tropoelastin, eNOS, VEGF, and EGF), exosomes, alkaloids, anti-inflammatory agent(s), cardiac matrix soaked in selected growth factors, and Micro RNAs.

**[0145]** For human use, longer repeat doses are needed and a natural expression and/or release from a patient's own electrically stimulated cells leads to successful human heart regeneration. For example, the described signals for follistatin expression and/or release match more closely with the natural low voltage signals in the human body.

**[0146]** In a booster composition for heart treatment/regeneration, the composition may include: adipose tissue-derived cells, cardiac tissue-derived cells, skeletal muscle derived cells - immature myoblasts (Tamaki selection process - cardiac progenitor – Tamaki et al. supra (2008)), growth factors (SDF-1, PDGF, HGF, Follistatin, and IGF-1), and cardiac matrix. In the basic composition for heart treatment/regeneration, the composition may include: adipose tissue-derived cells and muscle-derived immature myoblast cells (Tamaki process selection - see Tamaki et al. *supra* (2008)) <u>or</u> cardiac derived cells, together with selected growth factors (SDF-1, PDGF, HGF, and Follistatin).

**[0147]** There are three compositions, i.e., a basic composition, an intermediate composition, and an advanced composition. The basic composition includes MSCs or adipose derived cells, amniotic fluid, and myoblasts. The intermediate composition includes the ingredients of the basic composition together with a cocktail of growth factors (Follistatin rich). The advanced composition is adipose-derived or bone marrow-derived stem cells (MSCs), endothelial progenitor cells, selected growth factors cocktail, selected exosomes, selected Micro RNAs, selected alkaloids, selected anti-inflammatory agents, nutrient hydrogel, organ specific matrix, amniotic fluid (240 growth factors), and cardiac derived cells or immature myoblasts.

**[0148]** The concentration of cells in the compositions is preferably about 50,000,000 cells/ml. The amniotic fluid is preferably as described in Pierce et al. "Collection and

characterization of amniotic fluid from scheduled C-section deliveries," *Cell Tissue Bank*, DOI 10.1007/s10561-016-9572-7 (Springer, 2012) and is available from Irvine Scientific.

**[0149]** In certain embodiments, an organ regeneration mixed composition (e.g., a cardio angiogenic and cardio myogenic "cocktail" for heart treatment/regeneration) is loaded into a micro infusion pump (or in the case of limb salvage injected directly in the patient's leg with a needle and syringe). The pump may be refilled, e.g., weekly to achieve a slow, timed infusion delivery of the composition to the heart scar tissue. Administration of the composition(s) is combined with bioelectric stimulation to control the expression and/or release of more than twelve regeneration promoting proteins. Treatment times for assisting the heart may last 36 months.

**[0150]** For treating heart failure, a single (prior art) injection session is insufficient to fully recover a failing organ especially a failing heart. Furthermore, injecting just one cell type alone one time is not enough for full organ recovery. Bioelectric stimulation for controlled expression and/or release of SDF-1 in a subject is powerful to improve organ regeneration results. Bioelectric stimulation controlled expression and/or release of VEGF, eNOS and SDF-1 is powerful in improving blood flow to a failing organ. Nutrient hydrogels and organ specific matrixes can highly improve cell transplantation results. A mix of growth factors provides better organ recovery results than just one growth factor or just one cell type. Bioelectric stimulation controlled expression and/or release of a variety of growth factors offers more improvement than just one. Hepatocyte growth factor not only aides in organ regeneration, but also reduces arrhythmias risk in the heart. Follistatin injected or released via bioelectric stimulation can greatly improve muscle based organ regeneration results. Tropoelastin can improve elasticity of any treated organ, which in itself is valuable and is deemed to be especially valuable in the heart. An implantable micro infusion re-fillable programmable pump designed to reduce cell damage is better than injecting the patient's heart numerous times with separate procedures.

**[0151]** Bioelectric stimulation can be done with the described microstimulator, which has a pacing infusion lead with a corkscrew lead placed/attached at, e.g., the center of heart scar tissue. The microstimulator is actuated and runs through programmed signals to signal the expression and/or release of, e.g., SDF-1 and a differentiation signal. Described is a method of activating a tissue to differentiate a stem cell or to stimulate the tissue to produce a protein. The protein is selected from the group consisting of insulin-like growth factor 1 ("IGF1"), epidermal growth factor ("EGF"), hepatocyte growth factor ("HGF"), platelet-derived growth factor

("PDGF"), endothelial NOS ("eNOS"), vascular endothelial growth factor ("VEGF"), activin A, activin B, receptor activator of nuclear factor kappa-B ligand ("RANKL"), osteoprotegerin ("OPG"), tumor necrosis factor alpha ("TNF α"), follistatin, interleukin 6 ("IL-6"), hypoxia-inducible factor 1-alpha ("HIF-1-α"), and tropoelastin, the method including: stimulating the, e.g., human tissue with an electrical signal appropriate for the protein and tissue.

**[0152]** In such a method, when the electrical signal includes (within 15%): 0.1 V applied at a frequency of about 50 Hz with a duration of about three (3) minutes (wherein the electrical signal is as measured three (3) mm deep into the tissue), the protein produced is VEGF.

**[0153]** In such a method, when the electrical signal includes (within 2%): 200 picoamps for about 10 seconds for about one (1) hour and the pulse has an amplitude of about 5 volts and a width of about 0.5 milliseconds for about one (1) hour, with a duration of about one (1) minute (wherein the electrical signal is as measured three (3) mm deep into the tissue), stem cells differentiate.

**[0154]** In such a method, when the electrical signal includes (within 15%): 10 V at 50 HZ and 100 HZ for about 12 hours each (duration one (1) minute) (wherein the electrical signal is as measured three (3) mm deep into the tissue), the protein produced is follistatin.

**[0155]** In such a method, when the electrical signal includes (within 15%): 3.5 V stimulation in 10 second bursts, one (1) burst every 30 seconds at a frequency of about 50 HZ (duration 5 minutes) (wherein the electrical signal is as measured three (3) mm deep into the tissue), the protein produced is HGF.

**[0156]** In such a method, when the electrical signal includes (within 15%): 3 mv with a frequency of about 22 Hz, and a current of about one (1) mA for about fifteen (15) minutes and 3 ma for about fifteen (15) minutes (duration 5 minutes) (wherein the electrical signal is as measured three (3) mm deep into the tissue), the protein produced is IGF-1.

**[0157]** In such a method, when the electrical signal includes (within 15%): 0.06 V with 50 Z alternating electrical field and a current of about 1 ma for about fifteen (15) minutes and 3 ma for about fifteen (15) minutes (duration 2 minutes) (wherein the electrical signal is as measured three (3) mm deep into the tissue), the protein produced is tropoelastin.

**[0158]** In such a method, when the electrical signal includes (within 15%): alternating high-frequency (HF) and medium-frequency signals (MF), symmetric, biphasic, trapezoid pulses, with 400-μs pulse duration and 1.5/1-s ramp-up/ramp-down duration, respectively (wherein the electrical signal is as measured three (3) mm deep into the tissue), the

protein produced is eNOS. In such a method, when the HF consists of about 75 Hz pulses with six (6) seconds on and 21 seconds off for about fifteen (15) minutes. In such a method, when the MF consists of about 45 Hz pulses with 5 seconds on 12 seconds off for about fifteen (15) minutes followed by stimulation duration set as 20 minutes. In such a method, when the electrical signal includes (within 15%): one (1) Hz stimulation, stimulation applied for about nine (9) seconds, followed by a one (1) second silent period, a total of about 1080 stimulations for about 20 minutes. In such a method, when the electrical signal includes (within 15%): 20 Hz stimulation, stimulation applied for about two (2) seconds, followed by silent period for about 28 seconds, a total of about 1600 stimulations for about 20 minutes (duration 2 minutes).

**[0159]** In such a method, when the electrical signal includes (within 15%): 6 mv at 150 HZ Monophasic square wave pulse 0.1 ms in duration current of fifteen (15) mA for about fifteen (15) minutes (duration two (2) minutes) (wherein the electrical signal is as measured three (3) mm deep into the tissue), the protein produced is Activin B.

**[0160]** In such a method, when the electrical signal includes (within 15%): 10 V/cm, pulse-width 180 μs, 500 Hz (duration nine (9) minutes) (wherein the electrical signal is as measured three (3) mm deep into the tissue), the protein produced is EGF.

**[0161]** In certain embodiments, a bioelectric signal of 40 Hz and 116 Hz alternating (1 mV to 3 mV) can be used for reducing swelling and inflammation in a subject.

**[0162]** For example, up-regulation of RANKL, IGF-1, VEGF, and SDF-1 was achieved in cardiomyoctyes using such signals. Up-regulation of SDF-1 was achieved in pig heart. Up-regulation of VEGF, endothelial NOS ("eNOS"), hypoxia-inducible factor 1-alpha ("HIF-1-α"), and IL-6 was achieved in eye cells. Up-regulation of RANKL and osteoprotegerin ("OPG") was achieved in bone, tooth and gum.

**[0163]** Also described is a method of activating a tissue to produce SDF1, the method including: stimulating the (e.g., human) tissue with an electrical signal, wherein the electrical signal includes (within 15%): 30 pulses per second with a voltage of about 3.5 mV, and successively alternating currents of about 700 to 1500 picoamps for about one minute, and again with 700 to 1500 picoamps for about one minute and stimulated with current of about 0.25 mA, pulse duration of about 40 pulses/s, pulse width of about 100 μs, wherein the electrical signal is as measured three (3) mm deep into the tissue.

**[0164]** Further described is a method of activating a tissue to attract a stem cell, the method including: stimulating the (e.g., human) tissue with an electrical signal, wherein the electrical signal includes (within 2%): fifteen (15) mV and a current of about 500 picoamps at

70 pulses per minute for about three (3) hours and 20 pulses per minute, a pulse amplitude of from about 2.5-6 volts, and a pulse width of from about 0.2-0.7 milliseconds for about three (3) hours for about three (3) minutes, wherein the electrical signal is as measured three (3) mm deep into the tissue.

**[0165]** A combination bioelectric stimulator that controls expression and/or release in the scarred heart of SDF-1, IGF-1, HGF, EGF, eNOS, VEGF, Activin A and B, follistatin, tropoelastin, GDF-10, GDF-11 and Neurogenin 3 combined with repeat delivery of a mixed stem cell and growth factor cardiac matrix composition via an implantable re-fillable micro infusion pump may be advantageously used.

**[0166]** In some cases, SDF-l recruits via a presumed homing signal new reparative stem cells to the damaged organ. VEGF causes new nutrient and oxygen producing blood vessels to grow into the area being treated. IGF-1 repairs damaged cells, tissues and organs. Follistatin repairs damaged muscle. Tropoelastin adds elasticity to treated tissues making them more compliant. HGF aides in all repair processes and in the specific case· of the heart regeneration reduces the risk of arrhythmias. All of these proteins work together to fully regenerate an organ over time.

**[0167]** The healing process can be accelerated with the use of a micro infusion pump that is filled with various types of stem cells and growth factors and in some cases drugs.

**[0168]** In certain embodiments relating to the treatment of cancer and tumors, described is a method of inhibiting the growth of cancer cells in a target region, wherein the method includes treating the cancer cells with an anti-cancer drug; and applying an electric field to the target region for a period of time, wherein the electric field has frequency and field strength characteristics selected to inhibit the growth of cancer cells in the target region. In such a method, in the applying step, the field may be applied in at least two different directions in an alternating sequence.

**[0169]** In such a method, the drug dosage may be less than 20% of a standard dosage for the drug.

**[0170]** In such a method, the period of time is typically at least 24 hours.

**[0171]** In such a method, the field strength is typically at least one (1) V/cm.

**[0172]** In such a method, the drug typically comprises at least one of paclitaxel, doxorubicin cyclophosphamide, and cisplatin. In such a method, the field strength is typically at least one (1) V/cm and the period of time is at least 24 hours.

**[0173]** Also described in certain embodiments is a method of killing or inhibiting the growth of cancer cells in a target region, wherein the method includes applying an electric field to the target region for a period of time while the cancer cells are being treated with an anti-cancer drug, wherein the electric field has a field strength in the target region of at least one (1) V/cm. In such a method, the drug dosage is less than 20% of a standard dosage for the drug. In such a method, the period of time is at least 24 hours. In such a method, the drug comprises at least one of paclitaxel, doxorubicin cyclophosphamide, and cisplatin. In such a method, the field strength is between one (1) V/cm and 5 V/cm and the period of time is at least 24 hours. In such a method, in the applying step, the field is applied in at least two different directions in an alternating sequence. Typically, the drug comprises cyclophosphamide, and typically, the period of time is at least 6 hours.

**[0174]** What follows are preferred signals from the stimulator. For example, described are two PDGF expression control signals, one low voltage and one higher voltage. The test tissue is sheep heart tissue. The test cells are mesenchymal stem cells.

**[0175]** 30% PDGF increase > 3 V/cm, 10 Hz, 2 micro amps (0.000002 amps) and the pulse duration 0.2 ms.

**[0176]** 230% PDGF increase > 20 V/cm 100 Hz, 0.25 mA (2.5e-7 amps) and pulse duration of 40 pulses/s, width of 100 μs.

**[0177]** 40-minute treatment cycles 2 times a week for 4 weeks and then 3 times a week for 12 weeks.

**[0178]** PDGF Signal: 20 V for one (1) minute, 20 MVs for 10 minutes, current of 0.25 mA, pulse duration of 40 pulses/s, pulse width of 100 μs, and frequency of 100 Hz for 5 minutes followed by 528 Hz for 3 minutes and 432 Hz for 3 minutes and 50 Hz for 3 minutes.

**[0179]** VEGF - Blood vessel sprouting growth: 0.1 V applied at a frequency of 50 Hz. Duration 3 minutes.

**[0180]** SDF-1 - Stem cell recruiting signal: 30 pulses per second with a voltage of 3.5 mV, and successively alternating currents of 700 to 1500 picoamps for one minute, and again with 700 to 1500 picoamps for one minute and stimulated with current of 0.25 mA, pulse duration of 40 pulses/s, pulse width of 100 μs, and frequency of 100 Hz - each signal for 40 minutes to 8 hours a day for 2 to 36 months as needed for ideal results. Duration 7 minutes.

**[0181]** Stem cell proliferation signals: 15 mV and a current of 500 picoamps at 70 pulses per minute for 3 hours and 20 pulses per minute, a pulse amplitude of from 2.5-6 volts, and a pulse width of from 0.2-0.7 milliseconds for 3 hours. Duration 3 minutes.

**[0182]** Stem cell differentiation signals to become muscle: 200 picoamps for 10 seconds for one (1) hour and the pulse has an amplitude of 5 volts and a width of 0.5 milliseconds for one (1) hour. Duration one (1) minute.

**[0183]** Another method is to reverse polarity and drop the voltage.

**[0184]** Follistatin - (muscle growth) production signal: 10 V at 50 HZ and 100 HZ 0.25 mA. Duration one (1) minute.

**[0185]** HGF - Hepatocyte growth factor (arrhythmia reduction) signal: 3.5 V stimulation in 10 second bursts, one (1) burst every 30 seconds at frequency 50 HZ. Duration 5 minutes.

**[0186]** IGF-1: 3 mv with electric frequency of 22 Hz, and electric current of one (1) mA for 15 minutes and 3 ma for 15 minutes. Duration 5 minutes.

**[0187]** Tropoelastin: 0.06 V with 50 Z alternating electrical field and electric current of 1 ma for 15 minutes and 3 ma for 15 minutes. Duration 2 minutes.

**[0188]** RANKL/TNF-α, nuclear factor--kappa B (NF-κB) ligand/ TNF-α: 3MV at 2/100 Hz alternating frequency with current of 3 ma followed by 15 Hz, one (1) Gauss EM field, consisting of 5-millisecond bursts with 5-microsecond pulses followed by 200-μs pulse duration at 30 Hz and with current amplitude of 140 mA. (Optional use depending on application.)

**[0189]** eNOS: Alternating high-frequency (HF) and medium-frequency signals (MF): Symmetric, biphasic, trapezoid pulses, with 400-μs pulse duration and 1.5/1-s ramp-up/ramp-down duration, respectively. HF consisted of 75 Hz pulses with 6 second on--21 second off for 15 minutes. MF consisted of 45 Hz pulses with 5 second on--12 second off for 15 minutes. Followed by stimulation duration set as 20 minutes for both one (1) Hz and 20 Hz stimulations. For one (1) Hz stimulation, stimulation is applied for 9 seconds, followed by a one (1) second silent period, a total of 1080 stimulations for 20 min. For 20 Hz stimulation, stimulation is applied for 2 seconds, followed by silent period for 28 seconds, a total of 1600 stimulations for 20 min. Duration 2 minutes.

**[0190]** Activin B: 6 mv at 150 HZ Monophasic square wave pulse 0.1 ms in duration current of 15 mA for 15 minutes. Duration 2 minutes.

**[0191]** EGF - 10 V/cm, pulse-width 180 μs, 500 Hz. Duration 9 minutes.

**[0192]** An exemplary bioelectric signal sequence suggested for heart regeneration in humans split into six phases is as follows.

**[0193]** Phase I - Prepare Scar ("soil prep"): 10 minutes

[0194] – IGF-1 signal 3 minutes

[0195] – PDGF signal 3 minutes

[0196] – HGF signal 2 minutes

[0197] – EGF signal 2 minutes

[0198] Phase II - Grow New Blood Vessels ("lay irrigation system"): 5 minutes

[0199] – VEGF signal - 3 minutes

[0200] – SDF-1 signal - one (1) minute

[0201] – eNOS signal - one (1) minute

[0202] Phase III - Recruit and Inject Stem Cells ("plant"): 15 minutes

[0203] – SDF-1 signal - 10 minutes

[0204] – PDGF-1 signal 5 minutes

[0205] Phase IV - Build Tissue ("grow"): 25 minutes

[0206] – Stem Cell Proliferation Signal - 5 minutes

[0207] – Stem Cell Differentiation Signal - 5 minutes

[0208] – Follistatin Signal - 5 minutes

[0209] – Tropoelastin Signal - 5 minutes

[0210] – GDF-10 - 2 minutes

[0211] – GDF-11 - 3 minutes

[0212] Phase V - Post Tissue Growth Maintenance ("fertilize"): 30 minutes

[0213] – VEGF - 3 minutes

[0214] – EGF - 2 minutes

[0215] – eNOS - 2 minutes

[0216] – HGF - 5 minutes

[0217] – PDGF - 3 minutes

[0218] – Tropoelastin - 5 minutes

[0219] – Relaxin - 5 minutes

[0220] – Follistatin - 5 minutes

[0221] Phase VI - Protect Against Enemies ("pesticides"): 10 minutes

[0222] – Activin A and B - 5 minutes

[0223] – IGF-1 - 5 minutes

[0224] RESULTS OF ELECTRICAL STIMULATION (ES) OF CELLS *IN VITRO*

[0225] IL-1β: mRNA expression was up-regulated from 16 up to more than 400 times when cells were treated with 10 to 20 V between 3 and 20 hours.

**[0226]** IL-6: mRNA expression was up-regulated from 3 times –as soon as 15 minutes- to 10 times.

**[0227]** IL-8: mRNA expression was stimulated by 5 to 50 times.

**[0228]** HGF: mRNA expression was up-regulated by more than 10 times.

**[0229]** TNFα: mRNA expression was up-regulated by 9 to 24 times.

**[0230]** MMP9: mRNA expression was up-regulated 9 to 23 times with 3 and 24 hours of ES, respectively.

**[0231]** CCL2: mRNA expression was up-regulated 15 to 64 times.

**[0232]** CXCL5: mRNA expression up-regulated thousands of times.

**[0233]** CXCL10: mRNA expression up-regulated a thousand times by long term, 24 hour, electrical stimulation *in vitro*. These experiments were performed on adipose derived stem cells (among other cell types).

**[0234]** A week after treatment, samples can be collected for morphometric evaluation by *in-situ* hybridization or RT-PCR.

**[0235]** The PCR machine used herein to detect protein expression was the Applied Biosystems 7900HT.

**[0236]** FIGS. 6-20 are images of the corresponding signals with the name, voltage, and frequency of each signal written on each image. eNOS and differentiation signals were omitted due to of complexity or lack of frequency parameters. The signals are to be further defined in terms of current and frequency, not voltage and frequency as shown. The voltage delivered to the cells will be different for each tissue type, but with current all of the signals can be kept constant regardless of tissue type. The device should have a current driven signal (instead of voltage driven like most other devices).

**[0237]** Specifically, FIG. 6 depicts an image of the signal (voltage and frequency) associated with Activin B at 6.0 mV, pulse width 100 μs, square wave on a TEKTRONIX® TPS 2024 four channel digital storage oscilloscope. FIG. 7 depicts an image of the signal (voltage and frequency) associated with EGF at 10 V/cm (5V here), 500 Hz, pulse width 180 μs, square wave. FIG. 8 depicts an image of the signal (voltage and frequency) associated with follistatin at 10 V/cm, 50 Hz, square wave. FIG. 9 depicts an image of the signal (voltage and frequency) associated with HGF at 3.5 V, 10 second burst every 30 seconds, square wave. FIG. 10 depicts an image of the signal (voltage and frequency) associated with IGF-1: 3.0 mV, 22 Hz, square wave. FIG. 11 depicts an image of the signal (voltage and frequency) associated with OPG: 4.0 mV, 2,000 Hz, square wave. FIG. 12 depicts an image of the signal (voltage and

frequency) associated with PDGF 30%: 3 V/cm (100 mV here), 10 Hz, pulse width 200 μs, square wave. FIG. 13 depicts an image of the signal (voltage and frequency) associated with PDGF 230%: 20 V/cm (7.0 V here), 100 Hz, pulse width 100 μs, square wave. FIG. 14 depicts an image of the signal (voltage and frequency) associated with proliferation: 15 mV, 70 Hz, square wave. FIG. 15 depicts an image of the signal (voltage and frequency) associated with proliferation: 2.5-6.0 V (4 V here), 20 Hz, pulse width 200-700 μs, square wave. FIG. 16 depicts an image of the signal (voltage and frequency) associated with RANKL: 3.0 mV, 2 Hz, square wave. FIG. 17 depicts an image of the signal (voltage and frequency) associated with SDF-1: 3.5 mV, 30 Hz, square wave. FIG. 18 depicts an image of the signal (voltage and frequency) associated with tropoelastin: 60 mV, 50 Hz, square wave. FIG. 19 depicts an image of the signal (voltage and frequency) associated with VEGF: 100 mV, 50 Hz, square wave. FIG. 20 depicts an image of the signal (voltage and frequency) associated with SDF-1 (2nd part): 0.25 mA (3.0 V shown here), 100 Hz, 100 μs pulse width, square wave.

**[0238]** Production of SDF-1 is best under control with two signals in sequence. If ones uses just one signal, the SDF-1 increased expression effect peaks at one (1) hour and then diminishes. If you use two signals, the SDF-1 expression effect does not diminish after one (1) hour.

**[0239]** In certain embodiments, an inflammation management bioelectric signal sequence is generated from a bioelectric stimulator (e.g., a Rigol DG1022Z function generator/stimulator from Rigol US, Beaverton, OR, US modified to deliver microcurrent) applies a direct microcurrent to the subject of about 0.35 μA for from about 10 minutes to about 8.5 hours daily during the inflammation management phase and in an anti-inflammatory frequency range of 139-147 Hz + 3 V/cm, or 10 Hz, 0.2 ms, 3 V, pulse duration for about 4 hours. This bioelectric signal is preferably followed up with a second inflammation management bioelectric signal delivered from a programmed bioelectric stimulator (Rigol) of 10 Hz, 0.2 ms pulse duration, 3 V. See, also, Yuan et al. "Electrical stimulation enhances cell migration and integrative repair in the meniscus" *Scientific Reports* Volume 4, Article number: 3674 (2014), the contents of which are incorporated herein by this reference.

**[0240]** In certain embodiments, a subject's organ(s) and/or tissue(s) are first scanned or analyzed with a device to determine what his or her needs may be before treatment begins. The scanning / analysis can be by, e.g., generating mechanical vibrations at position adjacent the location to be an analyzed as described in, e.g., U.S. 2003/0220556 A1 to Porat et al. (the contents of which are incorporated herein by this reference) and/or by measuring

transmembrane voltage potential of a cell (*see, e.g.*, Chernet and Levin, "Transmembrane voltage potential is an essential cellular parameter for the detection and control of tumor development in a *Xenopus* model," *Dis. Models and Mech.* 6, pp. 595-607 (2013); doi:10.1242/dmm.010835, the contents of which are also incorporated herein by this reference. See, also, Brooks et al. "Bioelectric impedance predicts total body water, blood pressure, and heart rate during hemodialysis in children and adolescents," *J. Ren. Nutr.*, 18(3):304-311 (May 2008); doi: 10.1053/j.jrn.2007.11.008, the contents of which are incorporated herein by this reference, describing the use of bioelectric impedance to evaluate the variability of blood pressure, systolic blood pressure, etc.

**[0241]** As used herein, "scanning" means measuring bioelectrical electrical activity of organs, sometimes by placement of a bion coil reader and transmitter in the organ, and direct that information to a computer. The computer stores the bioelectrical read measurements of diseased organs and healthy organs and makes a comparative exam classifying the organ into one category or another, which is much like a doctor using information to make a diagnosis.

**[0242]** Presently, the best approach for whole body and individual organ scanning is to use a combination of: (a) 3D Body Scannint, (b) Quantum Magnetic Resonance Scanning, (c) Biofeedback scanning, (d) Bioelectric scanning, (e) Bion implant scanning, (f) Nervous system scanning, and (g) Light-activated cell reaction reading.

**[0243]** Scanners such as the Ina`Chi scanner, the Quantum Magnetic Resonance Analyzer (QMRA), the 3D Quantum Health Analyzer Scan whole body organ health 2, BodyScan® scanner, and the "BIONic muscle spindle" are also useful.

**[0244]** *See, also,* P. Collins "Bioelectric Signals Can Be Used to Detect Early Cancer," *Tufts News*, http://now.tufts.edu/news-releases/bioelectric-signals-used-detect-early-cancer (Feb. 1, 2013) reported that scientists had discovered a bioelectric signal that can identify cells likely to develop into tumors, and that they could lower the incidence of cancerous cells by manipulating the electrical charge across cell membranes. After the subject's needs in this regard are determined, then treatment (e.g., enhanced tissue growth or regeneration) may be initiated as needed and/or desired, preferably with the same device.

**[0245]** U.S. Patent 9,032,964 to Schuler, the contents of which are incorporated herein by this reference, entitled "Method and system for processing cancer cell electrical signals for medical therapy" describes a scientific computer system with processor capable of recording, storing, and reprogramming the natural electrical signals of cancer cells as found in tumors of humans and animals. The reprogramming process is designed to create a confounding

electrical signal for retransmission into a malignant tumor to damage or shut-down the cellular internal electrical communication system. Altering the electrical charge on the glycocalyx of the outer cell membrane is also part of the treatment by application of ions. The system causes cancer cell death as a medical treatment using ultra-low voltage and amperage encoded signals which are reprogrammed from cancer cell communication signals.

**[0246]** For example, the subject is positioned for analysis with a device, preferably with a non-invasive testing device for evaluating, e.g., the autonomic nervous system, organ function(s), and risk factors associated with heart disease, diabetes, and stroke. The non-invasive testing device may analyze data from, e.g., the subject's skin galvanic response, skin color, oximeter, blood pressure, and body composition analyzer to determine hardening and thickening of the subject's arteries, the subject's heart health, exercise capacity, thyroid function, neurotransmitter balance, and multiple other markers for health. See, also, Fatemi et al. "Imaging elastic properties of biological tissues by low-frequency harmonic vibration" *Proceedings of the IEEE*, 91(10):1503-1519 (Oct. 2003).

**[0247]** In an alternative embodiment, the analysis conducted by the device comprises (or further includes) detecting minute energy fields around the human body with, e.g., a "SQUID magnetometer" (SQUID is an acronym for "Superconducting Quantum Interference Device"), able to detect biomagnetic fields associated with physiological activities in the subject's body. A quantum resonant magnetic analyzer analyzes such fields. The magnetic frequency and energy of a subject's organ(s) and/or tissue(s) are collected by appropriately positioning the sensor with respect to the portion of the subject's organ(s) and/or tissue(s) to be analyzed, and after amplification of the signal by the instrument, the data are compared with standard quantum resonant spectrum of diseases, nutrition, and other indicators/markers to determine whether the sample waveforms are irregular using a Fourier approach.

**[0248]** Treatment may include, e.g., moving magnets or changing magnetic fields (pulsed electromagnetic fields) about the tissue and/or organ, for example, to reduce inflammation or treat pain or induce tissue growth in the subject.

**[0249]** The subject's body is scanned to detect non-cancerous tissue damage. When non-cancer damage is detected, treatment may be initiated/indicated/scheduled.

**[0250]** The invention is further described with the aid of the following illustrative Examples.

EXAMPLES

[0251] EXAMPLE - Controlling Expression of Follistatin

[0252] Low voltage pulsed electrical stimulation device for controlling expression of follistatin, a muscle formation promotion protein, from tissues.

[0253] Epicardial stimulation is especially useful for heart regeneration.

[0254] In one embodiment, the system stimulates the controlled production/release of follistatin, a known myostatin inhibitor, thus promoting the formation of new muscle and repair of damaged or weakened muscle including heart muscle post heart attack. Follistatin-like 1 (FSTL1) is a protein that encourages the growth of healthy cells, contractile muscle tissue and even blood vessels, helping supply the newly created muscle tissue with oxygen and nutrients. This therapy was originally designed to reduce or eliminate scarring of the heart following a heart attack and reversing heart failure, but may also be applicable to treating other organs suffering from muscle loss or degradation.

[0255] The electrical stimulation device promotes the reliable controlled expression and/or release of follistatin with practical, safe, low voltages. The version of the system described in this Example includes the following components: Micro voltage signal generator (micro-stimulator from QIG Greatbatch); pacing and infusion lead; corkscrew tip; conductive polymer bandage wrap or patch; signal programmer; and external battery charging wand.

[0256] Relationship Between The Components:

[0257] The micro voltage signal generator is attached to the pacing infusion lead with, e.g., a corkscrew tip 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 follistatin producing signal. The device battery may be re-chargeable with an external battery charging wand.

[0258] In use, the signal generator sends a signal to the target tissue organ that causes the genes within the DNA of that tissue to start the follistatin synthesis process on demand. The signal generator sends a signal to the target tissue organ that causes the genes within the DNA of that tissue to start releasing follistatin on demand. The follistatin - (muscle growth) production signal is preferably 10 V at 50 HZ and 100 HZ 0.25 mA alternating back and forth. A 3 V signal is being developed.

[0259] The system not only controls the DNA to build ribosomes and proteins, but also controls the gates of the cell membranes opening and closing correctly to promote regeneration.

[0260] The essential elements are the micro voltage signal generator and the means for delivering the signal to the target tissue.

[0261] A micro infusion pump is included to the system for delivering other supportive substances or even follistatin in greater volume more quickly.

[0262] The signal generator may be external or internal. The transmission of the signal may be wireless, via liquid and/or via wires.

[0263] The tissue contact interface may be a patch or bandage or may be via electrodes or leads.

[0264] The described system produces follistatin under precise dosing control at safe and comfortable low voltages.

[0265] The signal generator programmed with the follistatin expression and/or release signal is directed via a lead, bandage of patch to the target organ tissue in need of muscle repair or build up. As the signal is in stimulation mode the tissue releases follistatin and muscle is built or repaired as needed until full function resumes or the desired enhanced function is reached.

[0266] EXAMPLE - Treatment of the pancreas with bioelectric controlled protein

[0267] Treatment of the pancreas with bioelectric controlled protein expression and micro infusion pump stem cell composition delivery

[0268] A pancreas regeneration system includes three primary components. First, the micro bioelectric regeneration stimulator (micro-stimulator from QIG Greatbatch) that controls expression and/or release of 10 regeneration promoting proteins including SDF-1 a stem cell homing signal, IGF-1, HGF, EGF, activin A and B, eNOS, VEGF, follistatin and tropoelastin. Second, a programmable, re-fillable micro infusion pump. Third, a fifteen component stem cell-based regeneration composition comprising a variety of cell types, growth factors, BMP-7, PDLI-1, HGH, selected alkaloids, micro RNAs, nutrient hydrogel, NADA and pancreatic matrix.

[0269] In use, the stimulator and pump are implanted just below the subject's skin with a re-fillable silicone septum port with pacing infusion lead directed to the pancreas with a total conductive infusion wrap tip that is gentle on the pancreatic tissue. One portion of the pacing infusion lead is directed to the interior portion of the pancreas.

[0270] EXAMPLE - Brain and Organ Regeneration Device based on Bioelectric IGF-l Stimulation

[0271] An organ regeneration device that produces controlled expression and/or release of platelet-derived growth factor by bioelectric stimulation is disclosed. The system provides controlled sustained and repeated expression and/or release of PDGF via a wire conduction lead or wireless signal delivery and may be combined with a micro infusion pump for maximum results in severe organ failure cases.

[0272] A Brain and Organ Regeneration Device based on Bioelectric IGF-l Stimulation is disclosed. The system directs a lead to exactly the right position with the target organ and stimulates controlled expression of IGF-1 in combination with SDF-l, VEGF, HGH, HGF, Follistatin and tropoelastin in the proper sequence to optimize repair and regeneration.

[0273] Damaged aged or cancer stricken organs and tissues are unable to be regenerated back to their original health with prior art therapies. Further, injections wash away and needle pricks are painful and the entry site is too far away from the organ. Other prior art electrical stimulation devices do not: produce the expression IGF-l or other combination useful proteins in the most effective sequence.

[0274] The disclosed system directs a lead to exactly the right position with the target organ and stimulates controlled expression of IGF-l in combination with SDF-1, VEGF, HGH, HGF, follistatin, and tropoelastin in the proper sequence to optimize repair and regeneration.

[0275] IGF-1 can transport raw materials to the cells for repair and renovation. IGF-1 promotes raw material transport to the cells. Meanwhile, nucleic acids are helpful in repairing the damage in the DNA, while stimulating ceil division. IGF-l is able to minimize the DNA and cell stellar damage, but also treat the DNA and the cell. The IGF repair cells and thus tissues and organs, especially when delivered over time in combination with other factors such as SDF-1, VEGF, HGH, HGF, follistatin, and tropoelastin.

[0276] Controlled on demand expression of IGF-l can help repair cells, tissues and organs including brain, muscle, pancreas, lung, skin, kidney and liver.

[0277] IGF-1 injections and infusions do not get enough repair material to the target organ or tissue and cause inflammation, which is counterproductive to regeneration. Thus electrical stimulation is preferred. Prior art electrical stimulation systems failed to express the right regenerative proteins at the right time.

[0278] The system directs a lead to exactly the right position with the target organ and stimulates controlled expression of IGF-1 in combination with SDF-l, VEGF, HGH, HGF,

Follistatin, and tropoelastin in the proper sequence to optimize repair and regeneration. Also, it can produce hearts, kidneys, livers, lungs, brains, pancreas, lung, skin, knees, and elbows, skin, penis, breasts, aorta, arteries, and limbs.

**[0279]** The version of the system discussed for this Example includes the following components: bioelectric regeneration stimulator (micro-stimulator from QIG Greatbatch); signal for causing controlled expression and/or release of IGF-1: applied 20V at one (1) Hz with a frequency of 5 ms for 24 hours; signal for causing controlled expression and/or release of SDF-l; signal for causing controlled expression and/or release of VEGF; signal for causing controlled expression and/or release of HGH; signal for controlled expression and/or release of HGF; signal for controlled expression and/or release of follistatin; signal for controlled expression and/or release of tropoelastin; pacing infusion lead to implant in organ or tissue to be treated; infusion and electrode wide area patch (optional); wireless transmitter for all signals listed above (optional); refillable micro pump (optional); external programmer; and external battery charger.

**[0280]** The regeneration stimulator may be implanted just below the skin of the patient or may be external, especially if the wireless option is chosen. For the implantable model, an infusion conduction lead is directed from the stimulator to the organ or tissue to be repaired. The tip of the lead is lodged into the tissue with a corkscrew or other fixation tip. The regeneration stimulator is programmed by an external programmer. The stimulator is programmed to cause expression and/or release of specific regeneration proteins in a preferred sequence to optimize organ repair starting with VEGF, then SDF-1, then IGF-1, then HGH, then HGF, then follistatin, then tropoelastin. The wireless version is applied externally with the signal pointed to the organ to be regenerated. The signal may be constantly calibrated to adjust for fat, skin, and other obstacles between the signal generator and the organ of Interest to be treated. The device may be recharged with an external charger. In cases of very widespread organ damage, a wide array infusion and electrode patch may be used to cover the damaged organ area more completely. To accelerate the organ regeneration, an implantable, programmable, refillable micro infusion pump may be used to deliver various stem cells, nutrient hydrogels Micro RNA's and growth factors and (in some cases) drugs.

**[0281]** SDF-l recruits via homing signal new reparative stem cells to the damaged organ, VEGF causes new nutrient and oxygen producing blood vessels to grow into the area being treated. IGF-1 repairs damaged cells, tissues and organs. Follistatin repairs damaged muscle. Tropoelastin adds elasticity to treated tissues making them more compliant. HGF aides

in all repair processes and in the specific case of heart regeneration, reduces the risk of arrhythmias. All of these proteins work together to fully regenerate an organ over time. The process am be accelerated with the use of a micro infusion pump that is filled with various types of stem cells and growth factors and in some cases drugs.

5 **[0282]** The construction of electric signal generators, and pacemakers, are known to the art and can be obtained from OEM suppliers as well as their accompanying chargers and programmers. What is unique is the programming of specific signals to use specific protein expressions at precisely the right time for optimal organ regeneration. Pacing infusion leads may be purchased from a variety of OEM vendors. An infusion and electrode wide area pitch 10 may be constructed by cutting conduction polymer to shape and forming plastic into a flat bag with outlet ports in strategic locations.

**[0283]** Any one of the protein expression signals work well on their own for organ regeneration, but they work better together. As previously identified herein, SDF-1 is the most powerful regeneration protein followed by IGF-1.

15 **[0284]** 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.

**[0285]** A bionic neuron ("BION") device (injectable microstimulator) may be adapted to provide the requisite stimulation. Such a device is typically the size of a long grain 20 of rice (2 mm wide by 15 mm long) and comprises an integrated circuit chip sandwiched inside an antenna coil.

**[0286]** The regeneration stimulator lead or wireless signal is directed to the organ to be regenerated and the protein signals are delivered. Again, the most important is SDF-1 which recruits new stem cells to the site and its accompanying reverse polarity signal which triggers 25 differentiation of the recruited stem cells into useful tissues.

**[0287]** The second most important is IGF-1, which is highly potent in cell repair. VE'GF helps grow in blood vessels for feeding the newly created and newly regenerated tissues.

**[0288]** EXAMPLE - PDGF

30 **[0289]** Described herein is the bioelectric controlled expression of platelet derived growth factor (PDGF). PDGF is a powerful organ regeneration protein/cytokine. PDGF is one of the most potent growth factors in promoting cell, tissue and organ repair applicable to a wide variety of uses. It has been demonstrated to be especially useful in heart regeneration.

**[0290]** Described is the precise bioelectric signal for triggering PDGF expression from tissues. PDGF combined with the programmable micro-infusion pump and fifteen component organ regeneration composition is to help patients with degenerating and diseased organs to recover. Both wireless non-invasive and implantable wire lead based means may be utilized to get the regeneration and healing promoting bioelectric signals to organs.

**[0291]** PDGF constitute a family of four gene products (PDGF-A–D) acting by means of two receptor tyrosine kinases, PDGFRα and β. Three of the ligands (PDGF-A, PDGF-B, and PDGF-C) bind to PDGFRα with high affinity. PDGF signaling is essential for epicardial cell proliferation. PDGF signaling plays important roles in coronary vessel formation.

**[0292]** PDGF also induces DNA synthesis in cardiomyocytes. PDGF recruits stem/progenitor cells. PDGF can trigger controlled cell proliferation. PDGF can contribute to cell reprogramming and transformation into induced multipotent stem cells. PDGF downstream effects include regulation of gene expression and the cell cycle. PDGF can be used to create cell-specific antifibrotic compounds including those needed for liver regeneration. PDGFs are required for normal kidney development via recruitment of mesenchymal cells to both glomeruli and the interstitium. PDGF exerts essential roles from the gastrulation period to adult neuronal maintenance by contributing to the regulation of development of preplacodal progenitors, placodal ectoderm, and neural crest cells to adult neural progenitors, in coordinating with other factors. PDGF plays critical roles for maintenance of many specific cell types in the nervous system together with vascular cells through controlling the blood brain barrier homeostasis. PDGF modulates neuronal excitability through adjusting various ion channels, and affecting synaptic plasticity and function. PDGF stimulates survival signals, majorly PI3-K/Akt pathway but also other ways, rescuing cells from apoptosis. PDGF in dendrite spine morphology is critical for memory in the developing brain. PDGF has been found to stimulate regeneration of periodontal tissues and bone. PDGF signaling is essential in regeneration of hearts in animals. PDGF signaling induces DNA synthesis in the cells and is required for cardiomyocyte proliferation during heart regeneration. PDGF was used in biological pacemaker development, and it worked well to help form new sino atrial node cells from atrial myocytes. PDGF has been found useful in regeneration of other organs such as eyes, lungs, kidneys, brains, and aortas.

**[0293]** Described is an organ regeneration device that produces controlled expression and/or release of PDGF by bioelectric stimulation. Failing organs cannot produce enough PDGF to fully regenerate.

[0294] Other devices only provide one time delivery of PDGF, which is insufficient to fully regenerate a failing organ. Infusion systems lose too much therapeutic agent.

[0295] The system provided herein provides controlled sustained and repeated delivery of PDGF via a wire conduction lead or wireless signal delivery and may be combined with a micro infusion pump for maximum results in severe organ failure cases.

[0296] The bioelectric stimulator preferably reads the needs of an organ and produces expression and/or release of PDGF in just needed amounts to enhance organ regeneration. Researchers previously conducted organ regeneration studies of one time injection of PDGF with a needle and syringe. This is impractical and will not work for major organ repair.

[0297] A onetime dose is not enough to fully regenerate an organ. To access the organ with a needle and syringe is very invasive, dangerous and painful. Injected or infused PDGF has a high wash out loss rate.

[0298] The system provides controlled sustained and repeated expression and/or release of PDGF via, e.g., a wire conduction lead or wireless signal delivery and may be combined with a micro infusion pump for maximum results in severe organ failure cases.

[0299] Also, it can produce the device may also be used for organ enhancement instead of just organ repair such as brain function enhancement.

[0300] The version of the system discussed for this Example includes the following components: micro bioelectric signal generator; programming wand; programming computer; pacing infusion lead; micro infusion pump; PDGF bioelectric signal program; PDGF solution; organ reading device and processor; organ reading software program and analysis software; and wireless energy beam transmitter.

[0301] Relationship Between The Components:

[0302] The micro bioelectric stimulator is programmed with the programming wand connected to the programming computer with the PDGF bioelectric signal of 20 V, 50 Hz, and 0.2 amps. The micro stimulator is connected to the pacing infusion lead and the other side of that lead is affixed in the central portion of the damaged or diseased target organ. The programming wand connected to the programming computer can active the micro bioelectric stimulator to become an organ reading device. When programmed with the organ reading and analysis software the organ reader is able to read all the bioelectric activity of the failing organ as well as its phenotype, genotype including genetic defects and variation and chemical and biologically metabolism.

**[0303]** The bioelectric stimulation controlled PDGF expression causing new blood vessels to grow into the failing organ(s) and new healthy organ tissue to form. The reader adjusts the therapeutic dose as needed. The micro infusion pump re-filled daily with a mixed stem cell based composition that includes PDGF and may also include SDF-1, IGF, EGF, HGF, HGH, Activin A and B, eNOS, VEGF, follistatin, tropoelastin. GDF-10, GDF-11 and Neurogenin-3, selected alkaloids, and selected anti-inflammatory factors may be used to supplement the bioelectric stimulation therapy for organ repair in seriously failing organs.

**[0304]** If the organ failure is severe, an added programmable, implantable, re-fillable micro infusion pump may be added to the therapy. The micro pump is refilled daily with about 2 ml of stem cell-based organ regeneration composition that includes PDGF. If it is not easy or desirable to reach the organ to be treated with a wire-based pacing infusion lead, the operator may utilize a wireless energy beam transmitter to deliver the bioelectric regeneration signals wirelessly to the organ.

**[0305]** In this embodiment, the stimulator, lead, and programmer are essential. The micro infusion pump and mixed organ regeneration composition are optional.

**[0306]** The micro stimulator, and if chosen, the micro infusion pump are implanted somewhere below the skin of the patient with the pump silicone septum ports accessible for re-filling just below the skin. The stimulator must be in a location reachable by the programming wand attached to a portable computer. The pacing infusion lead form the stimulator and pump is directed to the central damaged portion of the damaged organ, i.e., heart, kidney, pancreas, liver. The micro stimulator may optionally be non-invasive and external and can deliver its signal to the failing organ via a focalized wireless energy beam. Much like how they focalize radiation to treat cancer tumors, but this energy stimulates organ regeneration.

**[0307]** Additionally: The micro stimulator may be programmed for additional protein expressions. The micro pump may be used a stand-alone device. The sequence of use may be changed.

**[0308]** The device may also be used for organ enhancement instead of just organ repair such as brain function enhancement.

**[0309]** Two PDGF expression control signals. One low voltage and one higher voltage. The test tissue is sheep heart tissue, while the test cells are mesenchymal stem cells. 30% PDGF increase with 3 V/cm, 10 Hz, 2 micro amps (0.000002 amps) and the pulse duration 0.2 ms. 230% PDGF increase with 20 V/cm 100 Hz, 0.25 mA ($2.5e^{-7}$ amps) and pulse duration of 40 pulses/s, width of 100 μs.

**[0310]** EXAMPLE - Treating Cancer Tumors Using Bioelectric Stimulation in Combination with Micro Infusion

**[0311]** Previous cancer treatments failed to address the combination of stopping cell proliferation and blood supply followed by regenerating the damaged tissue or organ.

**[0312]** Cytokine and Chemotherapeutic and regenerative treatment for certain cancers may be combined with low intensity, intermediate frequency alternating electric fields that are tuned to expression and/or release specific beneficial proteins at specific time intervals. More specifically, cell proliferation inhibition and halting blood supply to tumors in the first treatment stage. The bioelectric stimulation treatment may be increased in volume and efficacy by the combination use of an implantable, programmable, re-fillable micro infusion pump that delivers anti-cell proliferation and anti-blood vessel growth proteins as well, if desired, standard cancer treatment drugs such as chemo therapy agents. The second stage of treatment is focused regeneration of cancer damaged tissues back to their most optimal healthy state. The regenerative phase comprises a sequence of recruiting reparative stem cells to the damaged organ by bioelectrically stimulating the expression and/or release of SDF-1 (stem cell homing factor), followed by a controlled proliferation signal, a controlled blood vessel supply signal (VEGF) and if desired and useful expression and/or release of Follistatin, tropoelastin, HGF, IGF-1 and Activin. The stimulation cycle causing expression and/or release of beneficial proteins for regeneration may be upgraded in volume and speed of delivery by the combination use of an implantable, re-fillable, programmable micro infusion pump for delivering a higher quantity of stem cells, nutrient hydrogel, matrix and beneficial tissue and organ regeneration promotion proteins.

**[0313]** Cytokine and Chemotherapeutic and regenerative treatment for certain cancers comprising a combination low intensity, intermediate frequency alternating electric fields that are tuned to expression and/or release particular beneficial proteins in two stages, stage (1) is stopping cancer spread by halting cell proliferation and halting tumor blood supply and stage (2) regenerating the cancer damaged tissue or organ back to optimal health. In many cases, the resulting cell proliferation inhibition is significantly higher than the inhibition obtained by drug-only regimens of treatment.

**[0314]** A method of killing or inhibiting the growth of cancer cells in a target region followed by regenerating the tissue or organ back to optimal health, the method comprising the steps of:

[0315] Stage 1 = Stop cancer growth by:

[0316] Applying, to the target region, a series of bioelectric signals that damages the cancer cells or inhibits the growth of the cancer cells via stopping cell proliferation and halting blood supply temporarily, but leaves normal cells in the target region substantially unharmed; and

[0317] Treating the cancer cells with another anti-cancer regimen via programmable micro pump infusion, wherein the applying step and the treating step are performed simultaneously.

[0318] Stage 2 = Regeneration of post cancer tissue or organ by:

[0319] Treating the target region with a series of bioelectric signals to recruit stem cells, grow healthy blood vessels and re-grow healthy functional tissues in the previous cancer damaged region

[0320] In such a method, in the applying step, the field may be applied in at least two different directions in an alternating sequence to halt cell proliferation and to stop blood supply to the tumor.

[0321] In such a method, the other anti-cancer regimen may comprise treating the cancer cells with an anti-cancer drug. In this method, the drug may comprise at least one drug selected from the group consisting of paclitaxel, doxorubicin cyclophosphamide, and cisplatin. In such a case, the drug dosage may be less than 20% of a standard dosage for the drug.

[0322] In such a method, the bioelectric stimulation may expression and/or release any one of these regeneration of tissue and organ beneficial proteins SDF-1, IGF-1, Activin, HGF, VEGF, Follistatin or tropoelastin and in specific sequences for optimal organ health.

[0323] In such a method, all bioelectric regeneration signal may be delivered wirelessly and/or non-invasively.

[0324] In such a method, the target cancer may be breast cancer and the target regenerative organ may be breast reconstruction.

[0325] In such a method, the target cancer may be brain cancer and the target regenerative organ is brain.

[0326] In such a method, the target cancer may be prostate cancer and the target regenerative organ may be the prostate.

[0327] In such a method, the target cancer may be colon cancer and the target regenerative organ may be the colon.

**[0328]** In such a method, the target cancer may be throat or esophageal cancer and the target regenerative organ may be throat or esophagus.

**[0329]** In such a method, the target cancer may be pancreas cancer and the target regenerative organ may be the pancreas with improved insulin production.

**[0330]** In such a method, the target cancer may be lung cancer and the target regenerative organ may be lung(s).

**[0331]** In such a method, the target cancer may be eye cancer and the target regenerative organ may be the eye.

**[0332]** EXAMPLE - A combination protein expression stimulator, micro infusion pump, and fifteen (15) component stem cell-based composition for saving brain function in a subject following stroke or injury.

**[0333]** Brain function is lost when a stroke or brain injury occurs in a subject due to lack of oxygen and nutrients reaching a particular portion of the brain. Prior art therapies are typically drugs that do nothing to regenerate lost brain tissue. Chemical drugs do not do anything to affect neurogenesis (the growth of new brain tissue to replace damaged brain tissue). For example, the most popular simply dissolves blood clots, stopping further damage, but doing nothing to recover brain tissue already lost.

**[0334]** Prior art electrical stimulation devices do not have the correct signals for homing stem cells or for regenerating brain tissue. Existing electrical stimulation devices deliver one signal and that signal does not promote regeneration of lost brain tissue. Burst electrical pulses of old-type stimulators do nothing to affect neurogenesis.

**[0335]** Prior art one-time stem cell injections of one type of stem cell or modified stem cell have achieved some success, but this therapy is limited and incomplete. One-time needle injection cell therapies are too limited to recover major lost brain function. One-time injection of stem cells on a stand-alone basis mostly die out without a support system and cannot affect major neurogenesis.

**[0336]** The herein described combination of bioelectric stimulation of ten (10) key regeneration proteins via bioelectric signals, 24 hours a day for seven days a week, combined with daily or weekly infusions of the herein described fifteen component compositions provides much more complete repair, recovery, and regeneration of lost brain function.

**[0337]** The herein described device, method, and system practice all forms of "good farming" to grow a "new crop" of functional brain tissue in the skulls of post-stroke

and post-injury subjects.

**[0338]** The herein described system rapidly and easily delivers ten (10) brain regeneration promoting bioelectric signals to the subject within minutes, combined with a micro infusion pump that delivers fifteen (15) component angiogenic and regeneration compositions rapidly and safely. This, in combination, can fully restore brain functionality back to normal.

**[0339]** The ten (10) key regeneration proteins are SDF-1 (stem cell homing signal), IGF-(1 DNA repair and brain regeneration signal), HGF, EGF, Activin A and B, eNOS, VEGF, follistatin, and tropoelastin signal as described herein.

**[0340]** The system discussed in this Example preferably includes: the bioelectric signal generator, a programmable, re-fillable micro infusion pump, a brain saving helmet with electroacupuncture needles built in, micro infusion leads stereotaxic directed to deep brain regions, a fifteen component angiogenic composition, a fifteen component regeneration composition, human placenta, fetal serum, a cell proliferation signal, and a cell controlled differentiation signal.

**[0341]** In use, the bioelectric signal generator and the micro infusion pump are both attached to the brain saving helmet with electroacupuncture needles (not shown). The helmet is placed on the head of the patient. If the brain saving helmet with electroacupuncture needles is not used, one may use "off the shelf" standard, readily available electro-acupuncture needles. The bioelectric signal generator stimulator is activated and the micro infusion pump is filled with first the fifteen component angiogenic composition to increase blood flow and then the next day with the fifteen component regeneration composition.

**[0342]** The bioelectric stimulator cycles through the SDF-1 signal for stem cell homing, then IGF-1 for DNA repair, then HGF, EGF, Activin A and B, eNOS, VEGF, follistatin, tropoelastin, cell proliferation, and cell differentiation. The micro infusion pump may be re-loaded with fetal serum and placenta in severe cases to enhance results. Anti-inflammatory agents may also be used. The bioelectric signal generator stimulator recruits stem cells, causes expression and/or release of regeneration support factors, and multiples cells, and then controls their differentiation into healthy full functioning brain tissue.

**[0343]** The micro infusion pump is filled daily or week with the fifteen component angiogenic and regeneration compositions designed to facilitate neurogenesis. The fifteen component angiogenic and regenerative compositions provide much more complete repair, recovery, and regeneration of lost brain function.

[0344] If electrical stimulation alone does not work, the micro pump is filled with angiogenic and regeneration compositions for daily delivery. If those compositions do not work, then fetal serum and placenta may be added.

[0345] A bioelectric signal generator can be as described otherwise herein. For some signals, a drop down resistor in the pacing infusion lead may be necessary to drop the lowest voltage and current from the standard pacemakers down to a natural micro voltage level (the same level of natural electricity in a human body). A micro infusion pump can be as described otherwise herein and may be sourced from various drug delivery pump manufacturers and adapted by taking any filters out. The compositions for angiogenic and regeneration purposes are comprised of mixing together components that can be obtained from a person's own body as described herein further processed in a standard cell culturing laboratory (many contract manufacturers are available) or from reliable known suppliers.

[0346] The bioelectric signal generator is essential. All other components may be optional. The micro infusion pump, compositions, fetal serum, placenta, and anti-inflammatory agents are only necessary if the bioelectric stimulation on its own has not restored complete function or (e.g., in emergency recovery cases) where time is of the essence such as in an acute stroke situation.

[0347] One could use the compositions on their own injected by needle syringe. One could use the micro infusion pump on its own filled with other mixes of stem cells or drugs. One could use the bioelectric stimulator on its own running only one or a few signal programs instead of all of them, or one could program the bioelectric stimulator for entirely different signaling.

[0348] Upon arrival to the location of an acute stroke patient, a rapid assessment is made including video phone examination of the patient. A clot dissolving drug is first administered. Then, the brain-saving helmet (e.g., FIG. 23) is placed on the patient's head, and the bioelectric signal generator is turned on running though all ten (10) regeneration signals and the micro infusion pump is loaded first with an angiogenic composition followed immediately thereafter with a regeneration composition. If normal brain function is not restored in the subject with the above steps, the micro infusion pump may be re-filled with fetal serum, placenta, and anti-inflammatory agents, which are then administered.

[0349] EXAMPLE

[0350] In bioelectric stimulation tissue studies, a 2000% and increase in IL-6 was achieved.

[0351] IL-6 is a key promoter of regeneration. With respect to IL-6, Mosteiro et al. (2016) shows that tissue damage is a relevant factor for cells to go back to an embryonic state. Nobel Prize winner Shinya Yamanaka opened the door to regenerative medicine by cell reprogramming, based on introducing a combination of four genes known as OSKM (for genes, OCT4, SOX2, KLF4, and MYC), which reverts adult cells to an embryonic-like state, and transforms these cells into pluripotent cells. Cell reprogramming was later achieved within a living organism (i.e., a mouse) in 2013.

[0352] Mosteiro et al. (2016) analyzes what happens in living tissues when reprogramming is induced using OSKM. OSKM was found to be inefficient at inducing reprogramming or pluripotency in the highly specialized cells that constitute adult tissues. Tissue damage plays a critical role by complementing the activity of the OSKM genes.

[0353] This relationship between damage and reprogramming is mediated by the proinflammatory molecule, interleukin-6 (IL-6). Without IL-6 being present, the OSKM genes are far less efficient at inducing the reprogramming process. These findings suggest the following sequence of events: the expression of the OSKM genes results in damage to the cells; accordingly, they secrete IL-6; the presence of this molecule induces the reprogramming of some neighboring cells.

[0354] 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.

[0355] The controlled expression of Hypoxia Inducible Factor 1 ("HIF-1α") for, e.g., promoting organ regeneration (particularly liver regeneration) is also described herein. HIF-1α is a powerful organ regeneration protein. A more than 286% increase of HIF-1α on demand in test article tissues was achieved with a specific, optimized bioelectric signal. In other experiments, a 2300% increase in expression of HIF-1α was achieved.

[0356] Hypoxia has been proven as a critical element in the organ regeneration process. HIF-1α is a master regulator of the adaptive response to hypoxia. HIF-1α over expression in cells mimics the mechanisms triggered by hypoxia in injured or diseased tissues and increases their therapeutic potential without direct hypoxia stimulation.

[0357] Potential useful properties of HIF-1α for organ regeneration include: HIF-1α signaling promotes heart regeneration, HIF-1α signaling reduces infarction size and attenuates cardiac dysfunction, HIF-1α induces coronary collateral vessel formation, HIF-1α is a tumor suppressor, HIF-1α has been reported a gateway controller of cancer, HIF-1α promotes liver regeneration, HIF-1α promotes lung regeneration via alveolar development, HIF-1α promotes brain saving following traumatic brain injury or stroke, HIF-1α promotes retinal eye regeneration, HIF-1α management seems to be important to healthy kidney function and can protect against kidney injury, HIF-1α helps promote muscle regeneration, HIF-1α helps promote wound healing, HIF-1α promotes extracellular matrix, HIF-1α has a critical role in bone development and healing, HIF-1α may be important to stabilize teeth positions after accelerated tooth movement, and HIF-1α is an essential regulator of inflammation.

[0358] **EXAMPLE:** Endometriosis

[0359] A 34 year old, female patient was presenting with endometriosis with MR. She had symptoms for more than 5 years including chronic pelvic pain and deep dyspareunia.

[0360] A bioelectric stimulator programmed to produce bioelectric signals for SDF-1 and Klotho was used to treat the patient intravaginally. Ten (10) sessions of bioelectric stimulation ("BES") (Klotho and SDF-1) with an intravaginal probe for 15 minutes per session.

[0361] Evaluation of the patient before BES was Deep Dyspareunia Scale (DDS) of 3, and assessment of pelvic pain (Visual analogue scale or "VAS") was 6

[0362] Evaluation after the ten session of BES was DDS = 1, and VAS = 2, a notable improvement for the patient.

[0363] Deep Dyspareunia Scale (DDS)

| | Score | Description |
|---|---|---|
| [0364] | | |
| [0365] | 0 | No pain during intercourse |
| [0366] | 1 | Mild pain, which does not require interruption of intercourse |
| [0367] | 2 | Mild pain not requiring interruption of intercourse, but difficult |
| [0368] | 3 | Intense pain, which requires interruption of the intercourse |

**[0369]** An EMG was conducted on the patient both before and after. The goal of the EMG is that the reading stays within a designated range. And this was only possible after the BES – meaning the relaxation of pelvic floor muscles leading to less pelvic pain and dyspareunia.

**[0370]** The patient's endometriosis symptoms had improved significantly after treatment with BES as described herein.

## REFERENCES

(The contents of the entirety of each of which is incorporated herein by this reference.)

**[0371]** Corrigan et al. "Neurogenic inflammation after traumatic brain injury and its potentiation of classical inflammation", *Journal of Neuroinflammation*, 2016, 13:264; doi://doi.org/10.1186/s12974-016-0738-9.

**[0372]** Liesz et al. "Editorial: Mechanisms of neuroinflammation and inflammatory neurodegeneration in acute brain injury" *Front. Cell. Neurosci.*, 2015. doi://doi.org/10.3389/fncel.2015.00300.

**[0373]** Schimmel et al. "Neuroinflammation in traumatic brain injury: A chronic response to an acute injury" *Brain Circ*, 2017, 3(3):135-142.

**[0374]** Xiong et al. "Current understanding of neuroinflammation after traumatic brain injury and cell-based therapeutic opportunities" *Chin J Traumatol.* 2018 Jun; 21(3): 137–151. doi: 10.1016/j.cjtee.2018.02.003.

**[0375]** Thattaliyath et al. "Modified Skeletal Myoblast Therapy For Cardiac Failure Using AAV SDF1," *Proc. Intl. Soc. Mag. Reson. Med.* 16, page 579 (2008).

**[0376]** Prochazka et al. "Cocktail of Factors from Fat-derived Stem Cells Shows Promise for Critical Limb Ischemia," http://www.sciencenewsline.com/news/2016012204520017. html (January 21, 2016).

**[0377]** Wei et al. "Epicardial FSTL1 reconstitution regenerates the adult mammalian heart," *Nature* 525: 479–485 (24 September 2015).

**[0378]** "Hearts build new muscle with this simple protein patch," jacobsschool.ucsd.edu/news/news_releases/release.sfe?id=1813 (Sept. 16, 2015).

**[0379]** Salcedo et al. "Low current electrical stimulation up-regulates cytokine expression in the anal sphincter," *Int. J. Colorectal Dis.*, 2012 Feb;27(2):221-5. doi: 10.1007/s00384-011-1324-3. Epub (Oct 2011).

**[0380]** Marie Ellis "Cure for baldness? Stem cells bring hope," http://www.medicalnewstoday.com/articles/271898.php.

**[0381]** Robert Ferris "Battle against baldness turns to stem cells," http://www.cnbc.com/2015/01/29/studies-indicate-its-possible-to-use-stem-cells-to-cure-baldness.html (Jan. 29, 2015).

**[0382]** Thattaliyath et al. "Modified Skeletal Myoblast Therapy For Cardiac Failure Using AAV SDF1," *Proc. Intl. Soc. Mag. Reson. Med. 16*, p. 579 (2008).

**[0383]** "Electrical brain stimulation could support stroke recovery,"

https://www.sciencedaily.com/releases/2016/03/160316151108.htm (March 16, 2016).

**[0384]** "Electric Tumor Treatment Fields," No. 0827 Policy, http://www.aetna.com/cpb/medical/data/800_899/0827.html (Nov. 18, 2016).

**[0385]** D. Grady "Electrical Scalp Device Can Slow Progression of Deadly Brain Tumors," *New York Times*, https://www.nytimes.com/2014/11/16/health/electrical-scalp-device-can-slow-progression-of-deadly-brain-tumors.html?_r=0 (Nov. 15, 2014).

**[0386]** B. Borgobello "FDA approves the treatment of brain tumors with electrical fields," *New Atlas*, http://newatlas.com/treatment-of-brain-tumors-with-electrical-fields/21433/ (Feb. 13, 2012).

**[0387]** Hopkins Medicine "Overview of Pacemakers and Implantable Cardioverter Defibrillators (ICDs)," http://www.hopkinsmedicine.org/healthlibrary/conditions/cardiovascular_diseases/overview_of_pacemakers_and_implantable_cardioverter_defibrillators_icds_85,P00234/.

**[0388]** Medtronic "Cardiac Resynchronization Therapy (CRT) Devices For Heart Failure," http://www.medtronic.com/us-en/patients/treatments-therapies/crt-devices.html.

**[0389]** Columbia "Implant Procedure Concepts - Pacemaker, ICD and CRT Overview," http://www.columbia.edu/itc/hs/medical/hickey/docs/Pacemaker,%20ICD%20and%20CRT%20Overview%20022007.pdf.

**[0390]** "FDA Approves Algovita Spinal Cord Stimulation System from Greatbatch," http://www.odtmag.com/contents/view_breaking-news/2015-12-02/fda-approves-algovita-spinal-cord-stimulation-system-from-greatbatch (Dec. 2, 2015).

**[0391]** Mass Device "Greatbatch wins FDA PMA for Algovita SCS," http://www.massdevice.com/greatbatch-wins-fda-pma-for-algovita-scs/ (Dec. 1, 2015).

**[0392]** P. Banerjee "Electrical muscle stimulation for chronic heart failure: an alternative tool for exercise training?" *Curr. Heart Fail. Rep.*, 7(2):52-8. doi: 10.1007/s11897-010-0013-9 (June 2010).

**[0393]** HN Sabbah "Electrical vagus nerve stimulation for the treatment of chronic heart failure," *Cleve. Clin. J. Med.*, 78 Suppl. 1:S24-9. doi: 10.3949/ccjm.78.s1.04 (Aug 2011).

**[0394]** Bio-Leonhardt "Micro Stimulator," http://www.bioleonhardt.com/micro-stimulator/.

**[0395]** HU Klein "Vagus Nerve Stimulation: A new approach to reduce heart failure," *Cardiology Journal* (2010).

**[0396]** "Israeli innovation uses nerve stimulation to treat heart failure," https://www.israel21c.org/israeli-innovation-uses-nerve-stimulation-to-treat-heart-failure/ (Feb. 11, 2007).

**[0397]** Sahoo and Losordo "Exosomes and Cardiac Repair After Myocardial Infarction," *Circulation Research*, 114:333-344 (Jan. 16, 2014).

**[0398]** Tamaki et al. "Cardiomyocyte Formation by Skeletal Muscle-Derived Multi-Myogenic Stem Cells after Transplantation into Infarcted Myocardium," *PLoS ONE* 3(3): e1789. doi:10.1371/journal.pone.0001789 (March 2008).

**[0399]** W. Hoffmann "Regeneration of the gastric mucosa and its glands from stem cells," *Curr. Med. Chem*, 15(29):3133-44 (2008).

**[0400]** Cerrada et al. "Hypoxia-Inducible Factor 1 Alpha Contributes to Cardiac Healing in Mesenchymal Stem Cells-Mediated Cardiac Repair," *Stem Cells and Development*, 22(3): 501-511 (2013).

**[0401]** Fatemi et al. "Imaging elastic properties of biological tissues by low-frequency harmonic vibration," *Proceedings of the IEEE*, 91(10):1503-1519 (Oct. 2003) DOI: 10.1109/JPROC.2003.817865.

**[0402]** Guimarães-Camboa and Evans "Redox Paradox: Can Hypoxia Heal Ischemic Hearts?" *Cell*, 39(4):392–394, (21 Nov. 2016) DOI: http://dx.doi.org/10.1016/j.devcel.2016.11.007.

**[0403]** Huang et al. "Myocardial transfection of hypoxia-inducible factor-1α and co-transplantation of mesenchymal stem cells enhance cardiac repair in rats with experimental myocardial infarction," *Stem Cell Research and Therapy* 5:22 (2014) DOI: 10.1186/scrt410.

**[0404]** Kido et al. "Hypoxia-Inducible Factor 1-Alpha Reduces Infarction and Attenuates Progression of Cardiac Dysfunction after Myocardial Infarction in the Mouse," *JACC*, Volume 46, Issue 11, 6 December 2005, Pages 2116-2124. https://doi.org/10.1016/j.jacc.2005.08.045.

**[0405]** Mosteiro et al. "Tissue damage and senescence provide critical signals for cellular reprogramming *in vivo*." *Science*, 2016; 354 (6315): aaf4445 DOI: 10.1126/science.aaf4445.

**[0406]** Tajima et al. "HIF-1α is necessary to support gluconeogenesis during liver regeneration," *Biochem. Biophys. Res. Commun.* 2009 Oct 2; 387(4):789-94. doi: 10.1016/j.bbrc.2009.07.115. Epub 2009 Jul 28.

**[0407]** Tamaki et al. "Cardiomyocyte Formation by Skeletal Muscle-Derived Multi-Myogenic Stem Cells after Transplantation into Infarcted Myocardium," *PLoS ONE* 3(3): e1789. doi:10.1371/journal.pone.0001789 (2008).

**[0408]** Paulus, "Cytokines and heart failure," *Heart Fail. Monit.* 2000; 1(2):50-6.

**[0409]** Gullestad et al. "Inflammatory cytokines in heart failure: mediators and markers," *Cardiology.* 2012;122(1):23-35. doi: 10.1159/000338166. Epub 2012 Jun 12.

**[0410]** Mann, "Innate Immunity and the Failing Heart: The Cytokine Hypothesis Revisited," *Circ. Res.* 2015 Mar 27; 116(7): 1254–1268.

**[0411]** Deswai et al. "Cytokines and Cytokine Receptors in Advanced Heart Failure An Analysis of the Cytokine Database from the Vesnarinone Trial (VEST)," *Circulation.* 2001;103:2055-2059; ://doi.org/10.1161/01.CIR.103.16.2055.

**[0412]** Matsumori, "Cytokines and Heart Failure: Pathophysiological Roles and Therapeutic Implications," *Heart Failure*, Springer, Tokyo; doi.org/10.1007/978-4-431-68331-5_3.Blum and Miller "Role of cytokines in heart failure," *American Heart Journal*, Volume 135, Issue 2, February 1998, Pages 181-186; doi.org/10.1016/S0002-8703(98)70080-8.Ueland et al. "Inflammatory cytokines as biomarkers in heart failure," *Clinica Chimica Acta*, Volume 443, 30 March 2015, Pages 71-77; doi.org/10.1016/j.cca.2014.09.001.

# CLAIMS

What is claimed is:

1. A bioelectric stimulator for reducing inflammation in a subject, wherein the bioelectric stimulator is configured to generate bioelectric signals responsive to a programmed process, which bioelectric signals comprise:
a bioelectric signal that exhibits the following characteristics (within 15%):
35 μA, a frequency range of 139-147 Hz + 3 V/cm.

2. A bioelectric stimulator for reducing inflammation in a subject, wherein the bioelectric stimulator is configured to generate bioelectric signals responsive to a programmed process, which bioelectric signals comprise:
a bioelectric signal that exhibits the following characteristics (within 15%):
10 Hz, 0.2 ms pulse duration, 3 V.

3. The bioelectric stimulator of claim 1, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):
0.1 V applied at a frequency of 50 Hz, square wave.

4. The bioelectric stimulator of claim 2, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):
0.1 V applied at a frequency of 50 Hz, square wave.

5. The bioelectric stimulator of claim 1, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):
0.25 mA, 3.0 V, 100 Hz, 100 μs pulse width, square wave.

6. The bioelectric stimulator of claim 2, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):
0.25 mA, 3.0 V, 100 Hz, 100 μs pulse width, square wave.

7. The bioelectric stimulator of claim 1, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3.5 mV, 30 Hz, square wave.

8. The bioelectric stimulator of claim 2, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3.5 mV, 30 Hz, square wave.

9. The bioelectric stimulator of claim 1, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3 mV with a frequency of 22 Hz, square wave, and a current of 1 mA, and

3 mV with a frequency of 22 Hz, square wave, and a current of 3 mA.

10. The bioelectric stimulator of claim 2, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3 mV with a frequency of 22 Hz, square wave, and a current of 1 mA, and

3 mV with a frequency of 22 Hz, square wave, and a current of 3 mA.

11. The bioelectric stimulator of claim 3, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3 mV with a frequency of 22 Hz, square wave, and a current of 1 mA, and

3 mV with a frequency of 22 Hz, square wave, and a current of 3 mA.

12. The bioelectric stimulator of claim 4, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3 mV with a frequency of 22 Hz, square wave, and a current of 1 mA, and

3 mV with a frequency of 22 Hz, square wave, and a current of 3 mA.

13. The bioelectric stimulator of claim 5, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3 mV with a frequency of 22 Hz, square wave, and a current of 1 mA, and

3 mV with a frequency of 22 Hz, square wave, and a current of 3 mA.

14. The bioelectric stimulator of claim 6, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3 mV with a frequency of 22 Hz, square wave, and a current of 1 mA, and

3 mV with a frequency of 22 Hz, square wave, and a current of 3 mA.

15. The bioelectric stimulator of claim 7, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3 mV with a frequency of 22 Hz, square wave, and a current of 1 mA, and

3 mV with a frequency of 22 Hz, square wave, and a current of 3 mA.

16. The bioelectric stimulator of claim 8, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

3 mV with a frequency of 22 Hz, square wave, and a current of 1 mA, and

3 mV with a frequency of 22 Hz, square wave, and a current of 3 mA.

17. The bioelectric stimulator of any one of the preceding claims, which has been further configured to generate a bioelectric signal that exhibits the following characteristics (within 15%):

20 Hz, 0.1 V, and a 7.8 ms pulse duration.

18. The bioelectric stimulator of any one of the preceding claims, further comprising an electrode for delivering bioelectric signal(s) to the subject.

19. The bioelectric stimulator of any one of the preceding claims, further comprising a transducer and/or transceiver, wherein the transducer and/or transceiver is configured for delivering bioelectric signal(s) wirelessly to the subject.

20. A bioelectric stimulator for reducing inflammation in a subject, wherein the bioelectric stimulator is configured to generate bioelectric signals responsive to a programmed process, which bioelectric signals comprise:

a bioelectric signal that exhibits the following characteristics (within 15%):

0.1 V applied at a frequency of 50 Hz, square wave,

3.0 V, 100 Hz, 0.25 mA, 100 μs pulse width, square wave, or

3.5 mV, 30 Hz, square wave, and

a bioelectric signal that exhibits the following characteristics (within 15%):

20 Hz, 0.1 V, and a 7.8 ms pulse duration.

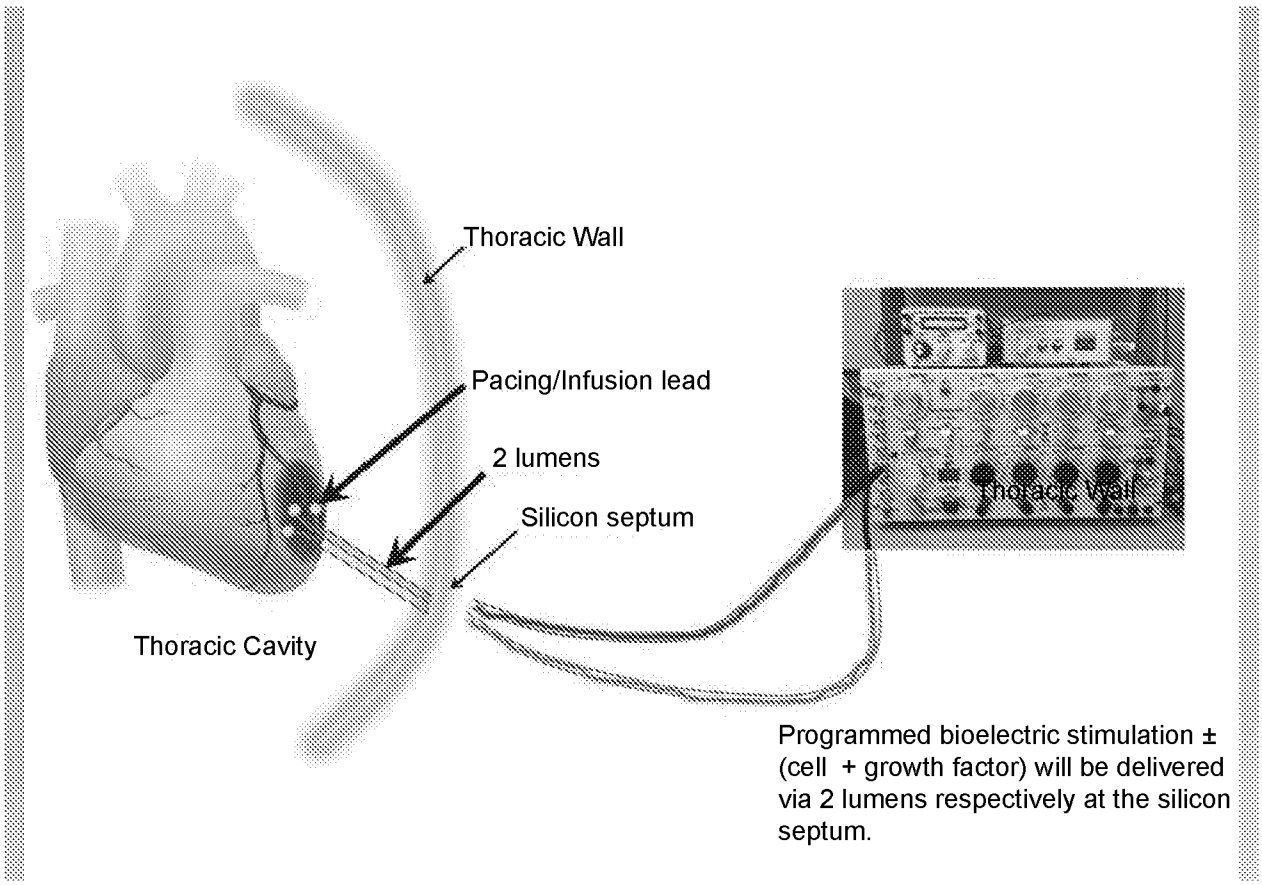
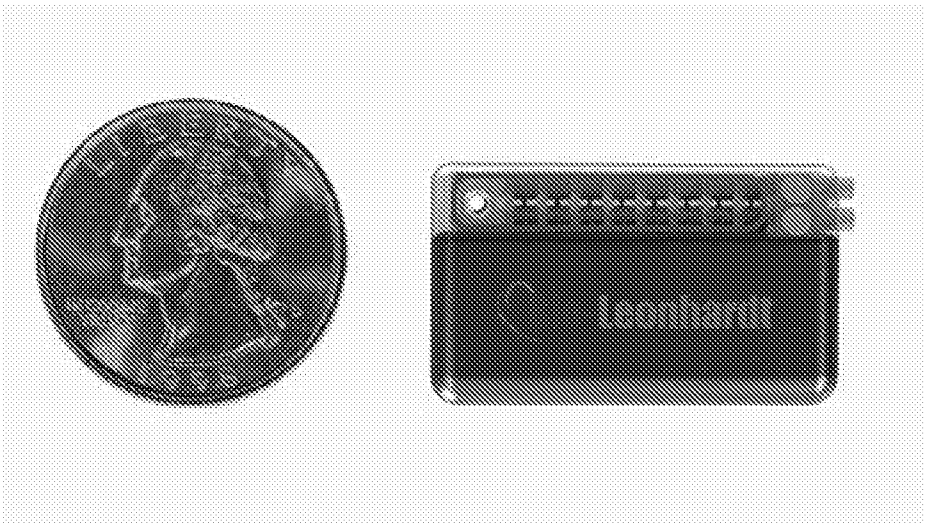

Thoracic Wall
Pacing/Infusion lead
2 lumens
Silicon septum
Thoracic Wall
Thoracic Cavity
Programmed bioelectric stimulation ± (cell + growth factor) will be delivered via 2 lumens respectively at the silicon septum.

FIG. 1



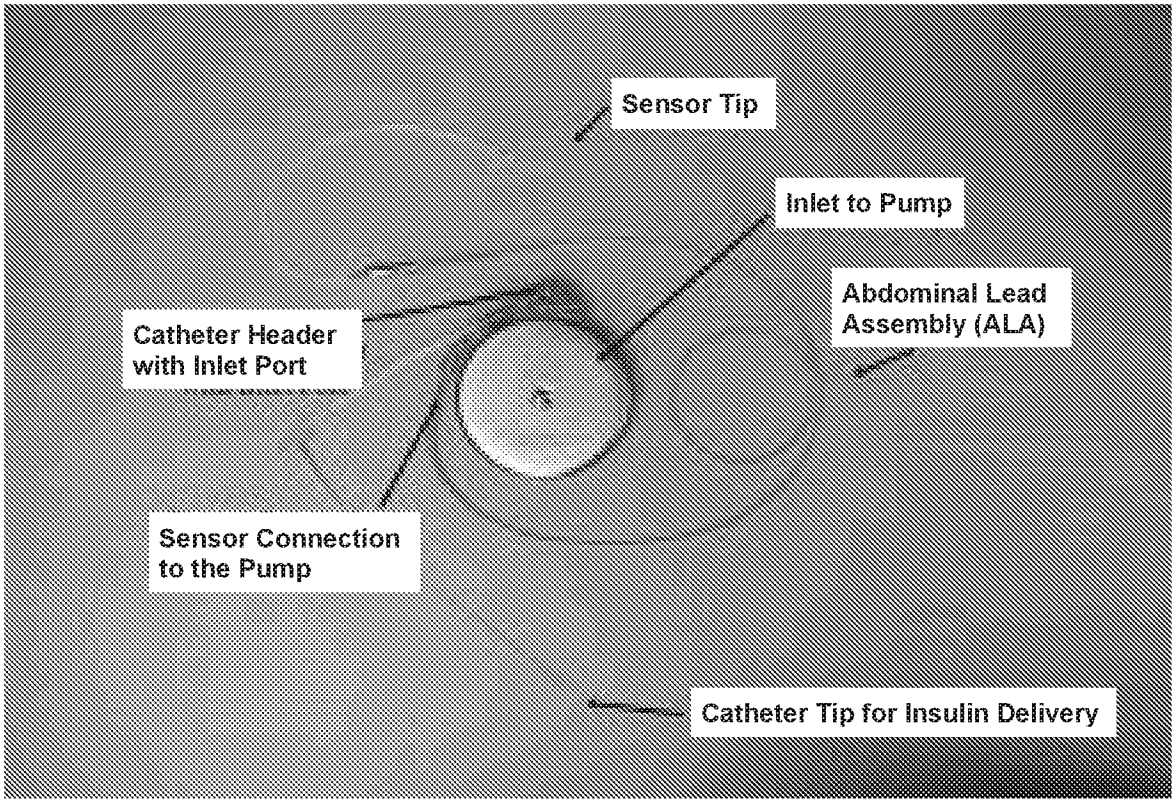
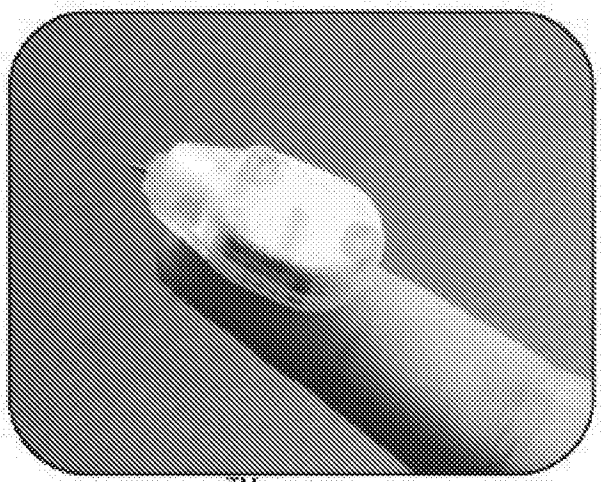



*FIG. 3*



FluidSync™ M200 micropump

*FIG. 4*

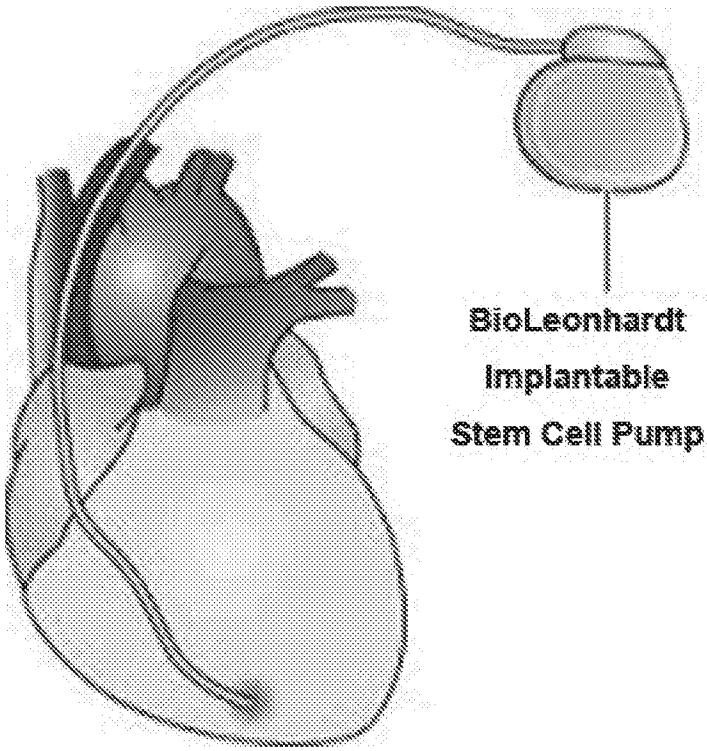



FIG. 5

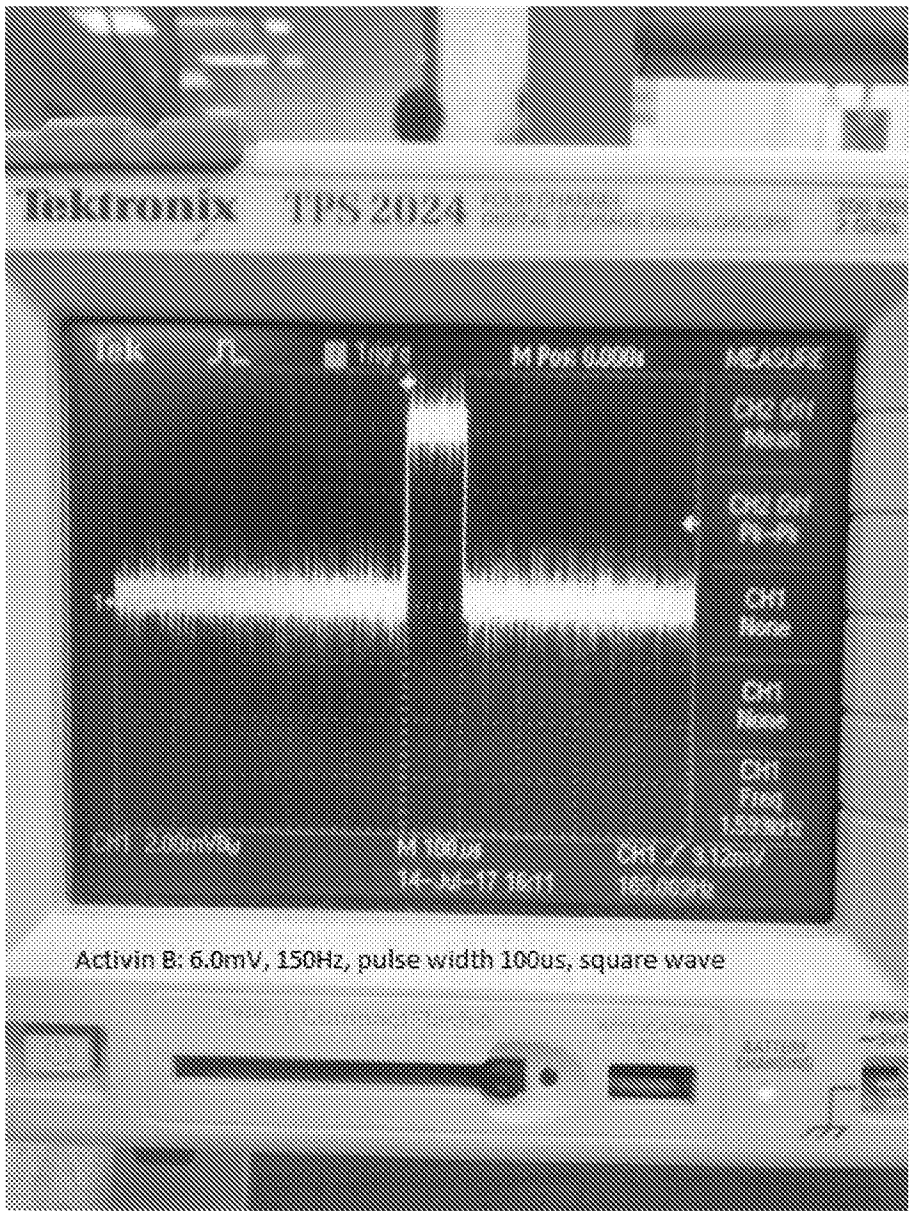

Tektronix TPS 2024
Activin B: 6.0mV, 150Hz, pulse width 100us, square wave

FIG. 6

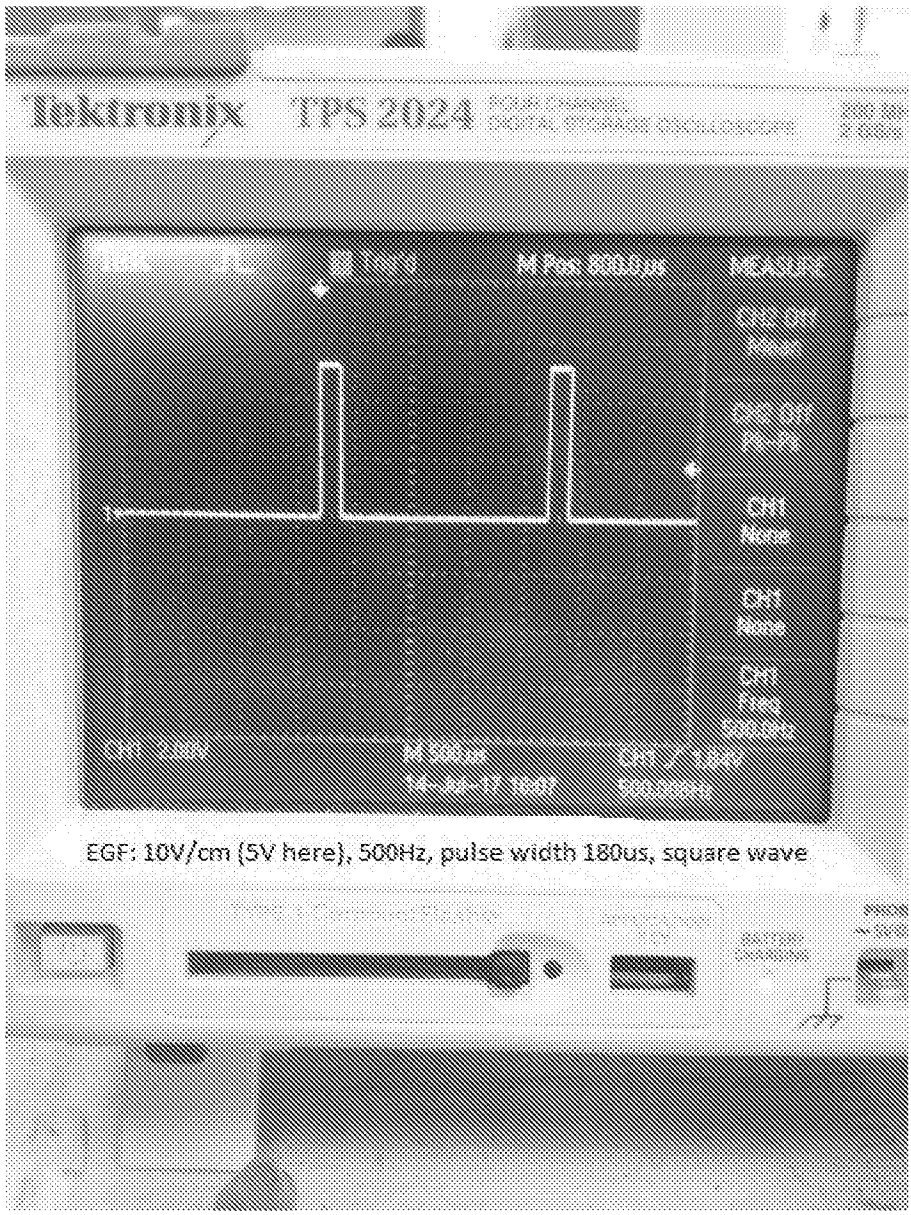

Tektronix TPS 2024
M Pos: 800us
EGF: 10V/cm (5V here), 500Hz, pulse width 180us, square wave

FIG. 7

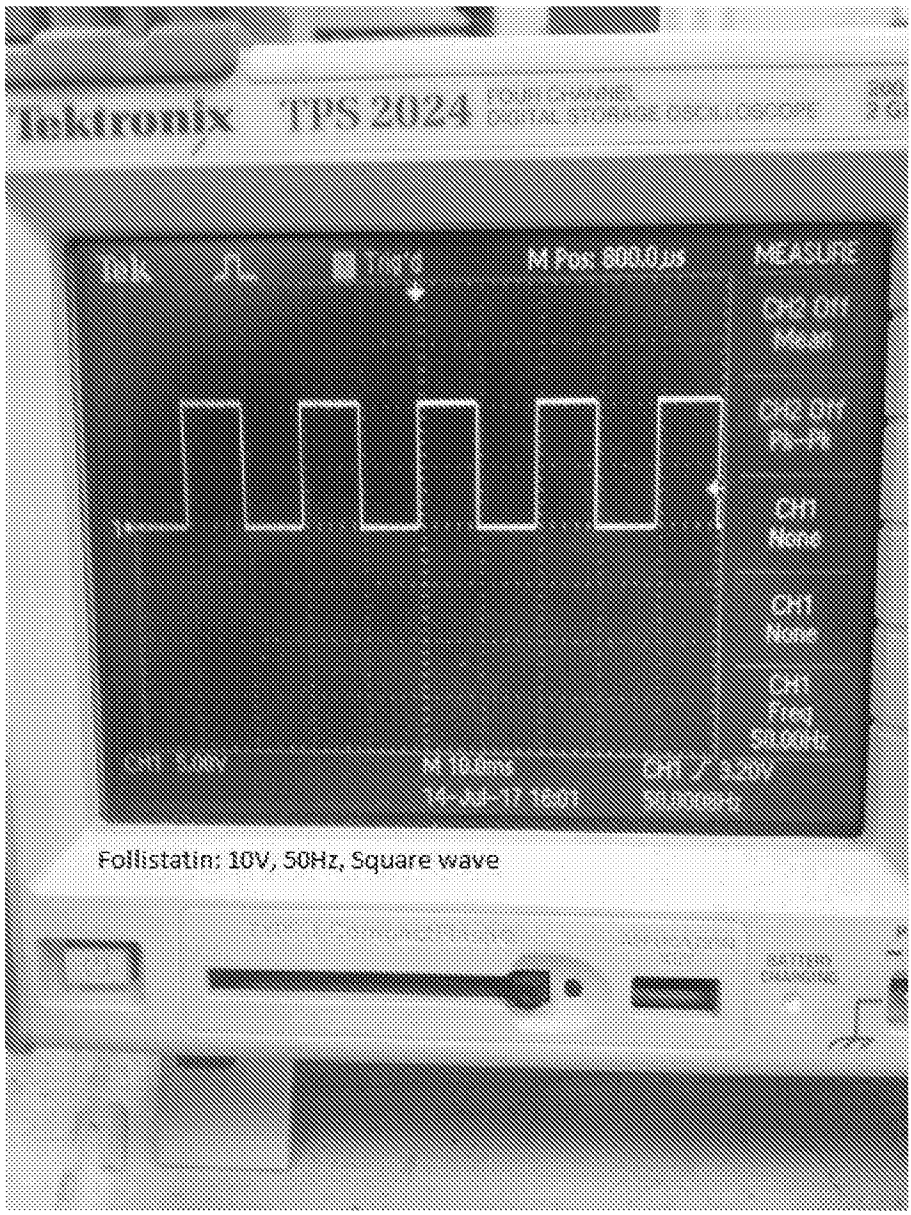
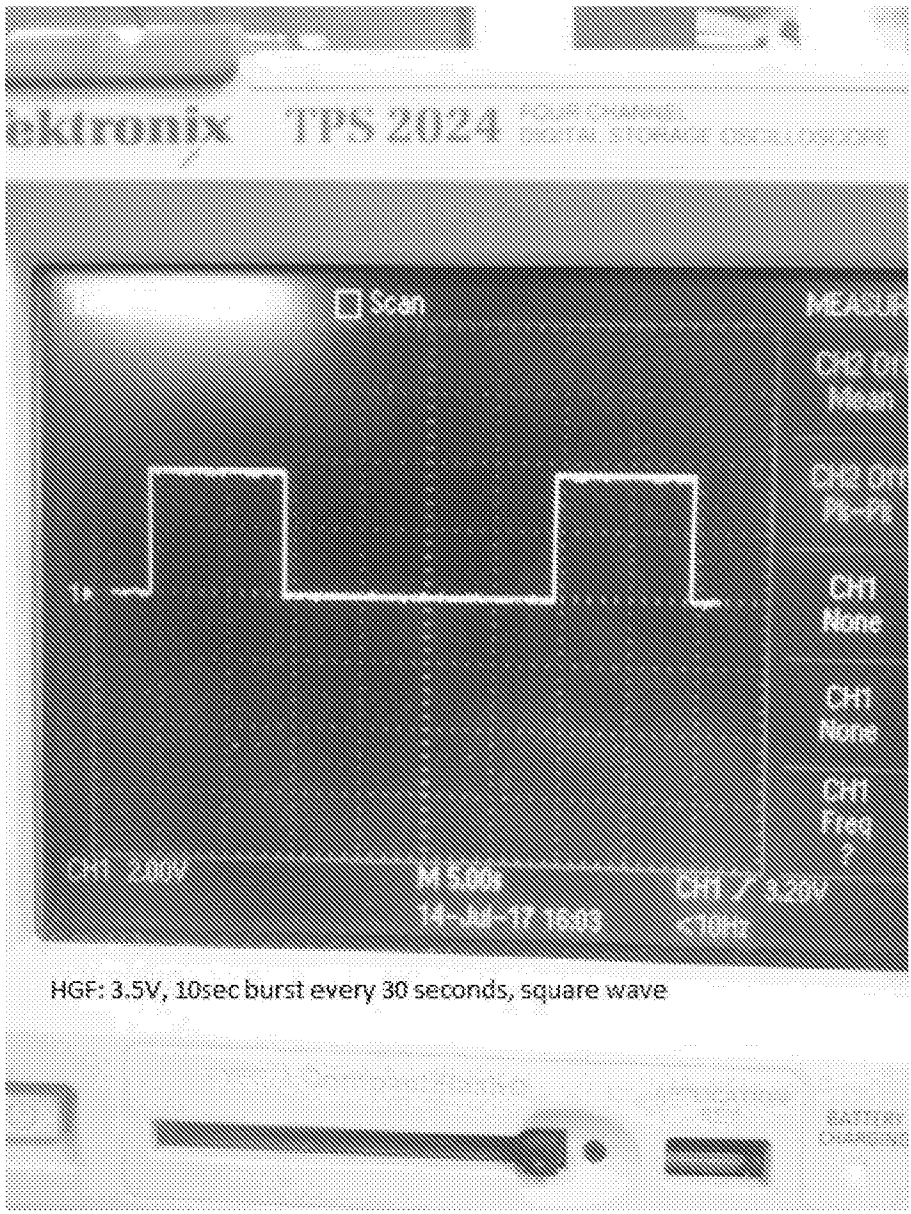

Tektronix
TPS 2024
MEASURE
CH1
None
CH1
None
CH1
Freq
Follistatin: 10V, 50Hz, Square wave

FIG. 8




HGF: 3.5V, 10sec burst every 30 seconds, square wave

*FIG. 9*

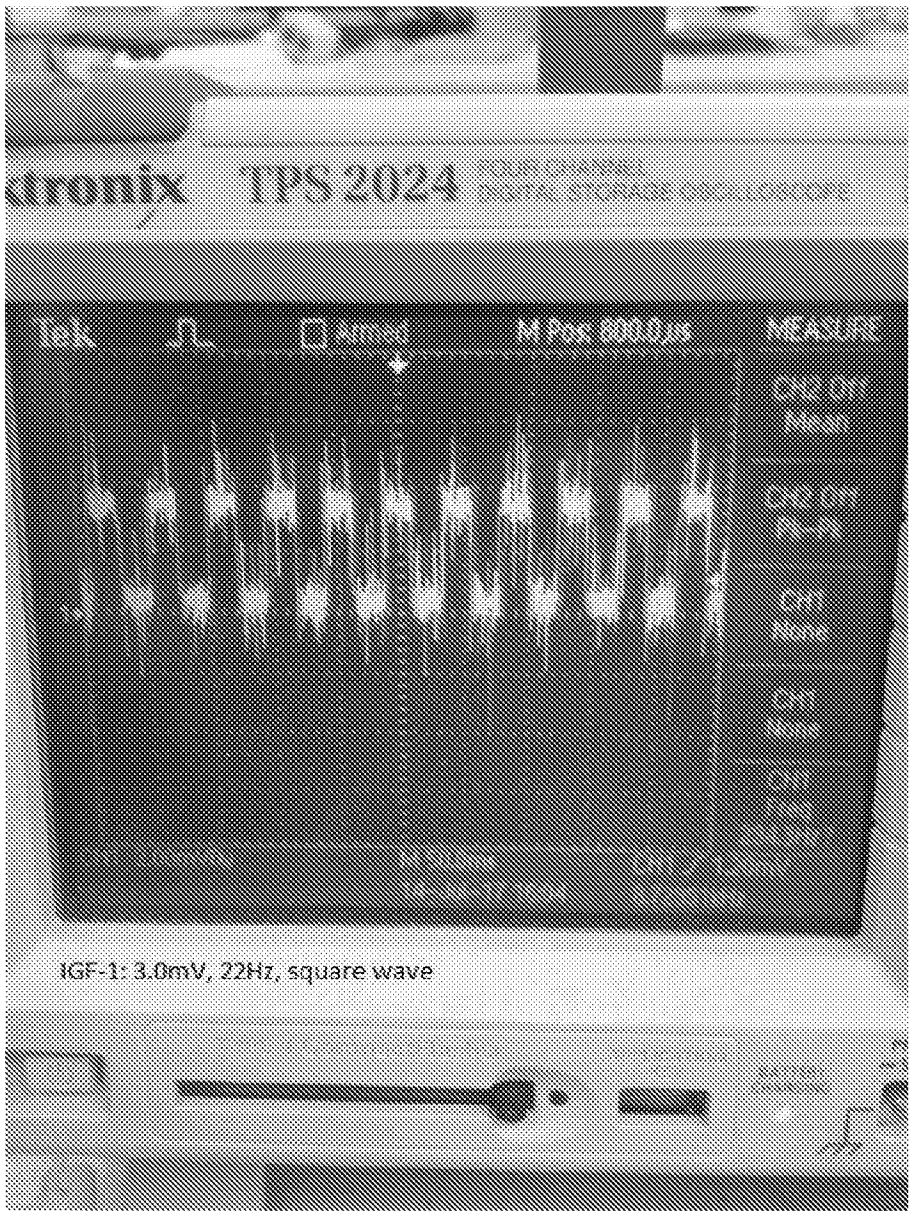



FIG. 10

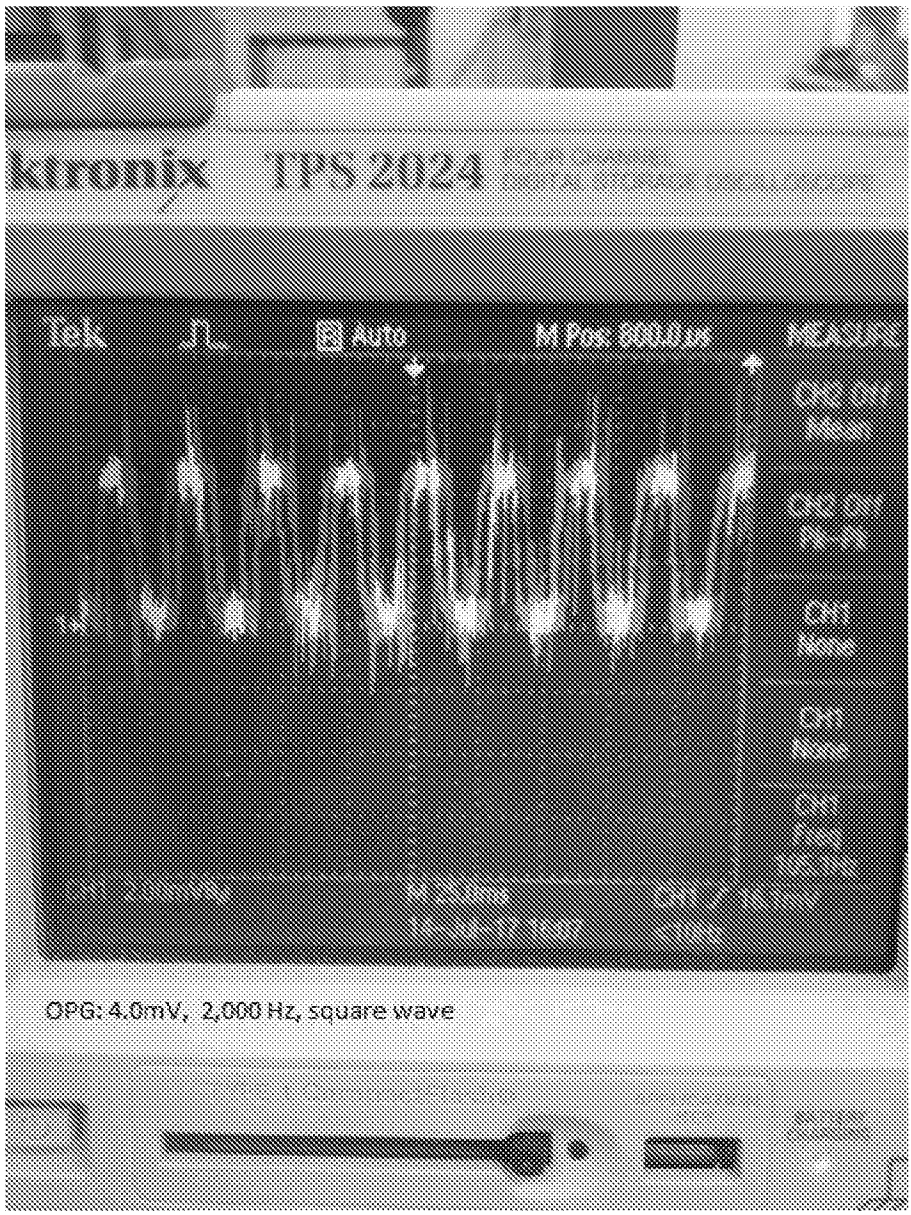



FIG. 11

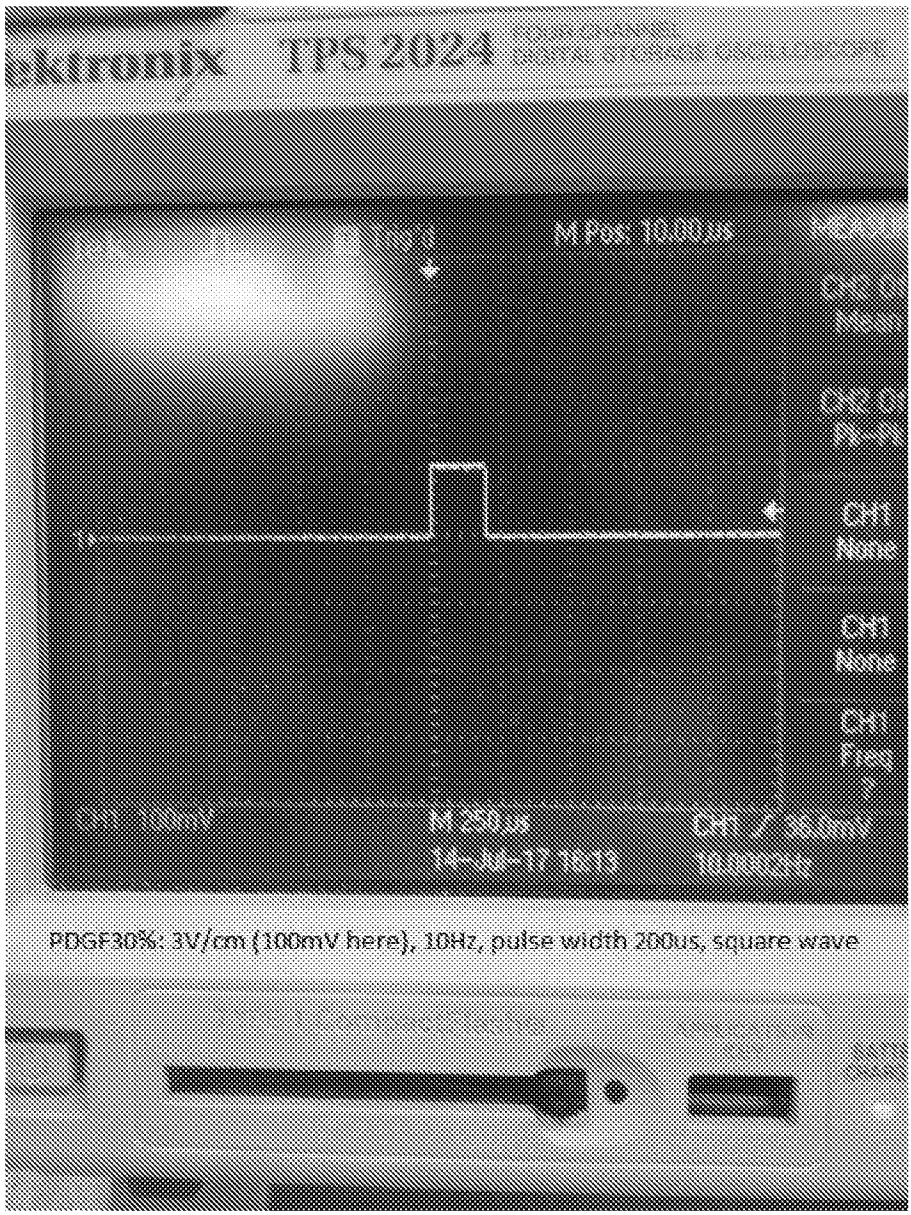

ektronix
TPS 2024
M Pos: 10.00µs
CH1
None
CH1
None
CH1
Freq
M 250µs
14-Jul-17 16:13
PDGF30%: 3V/cm (100mV here), 10Hz, pulse width 200us, square wave

FIG. 12

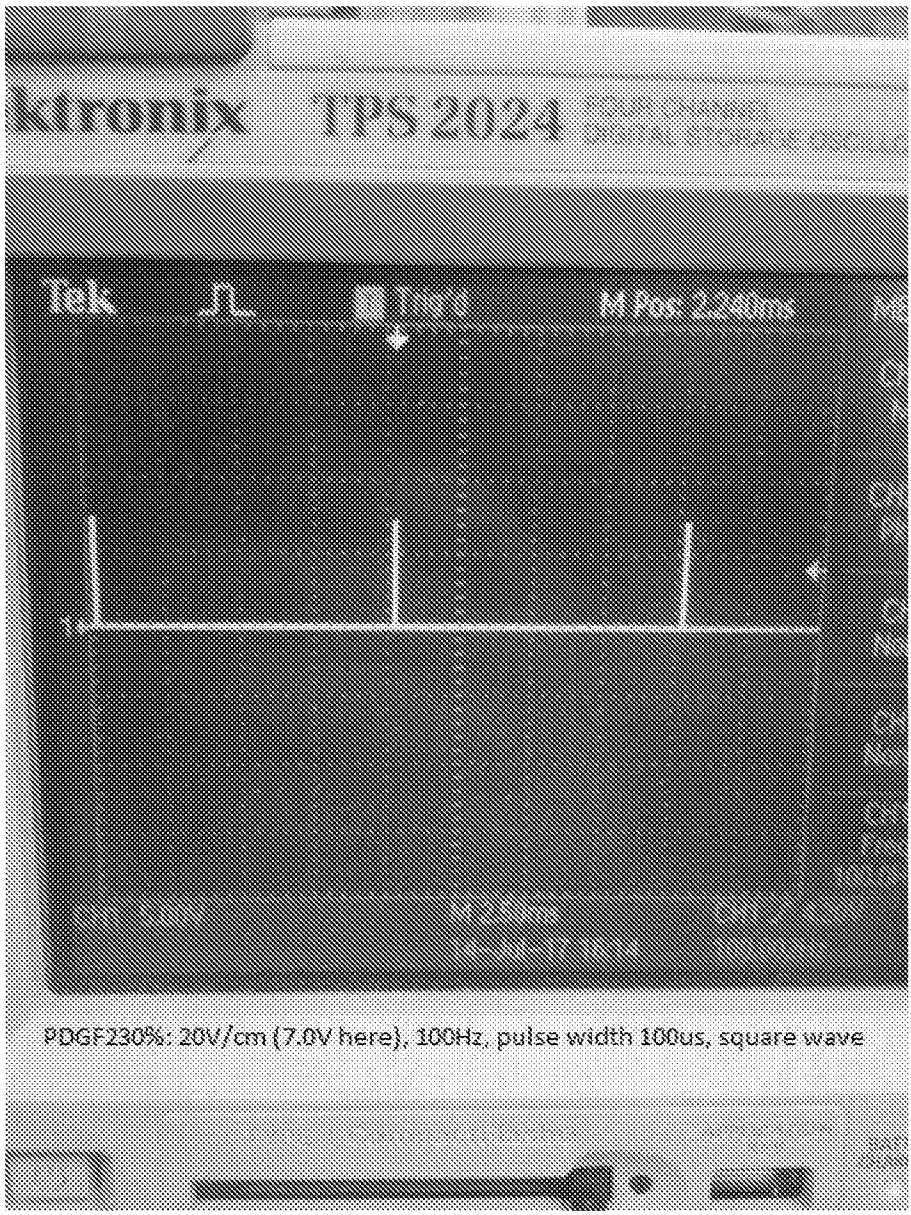

TPS 2024
Tek
M Pos: 2.40ms
PDGF230%: 20V/cm (7.0V here), 100Hz, pulse width 100us, square wave

FIG. 13

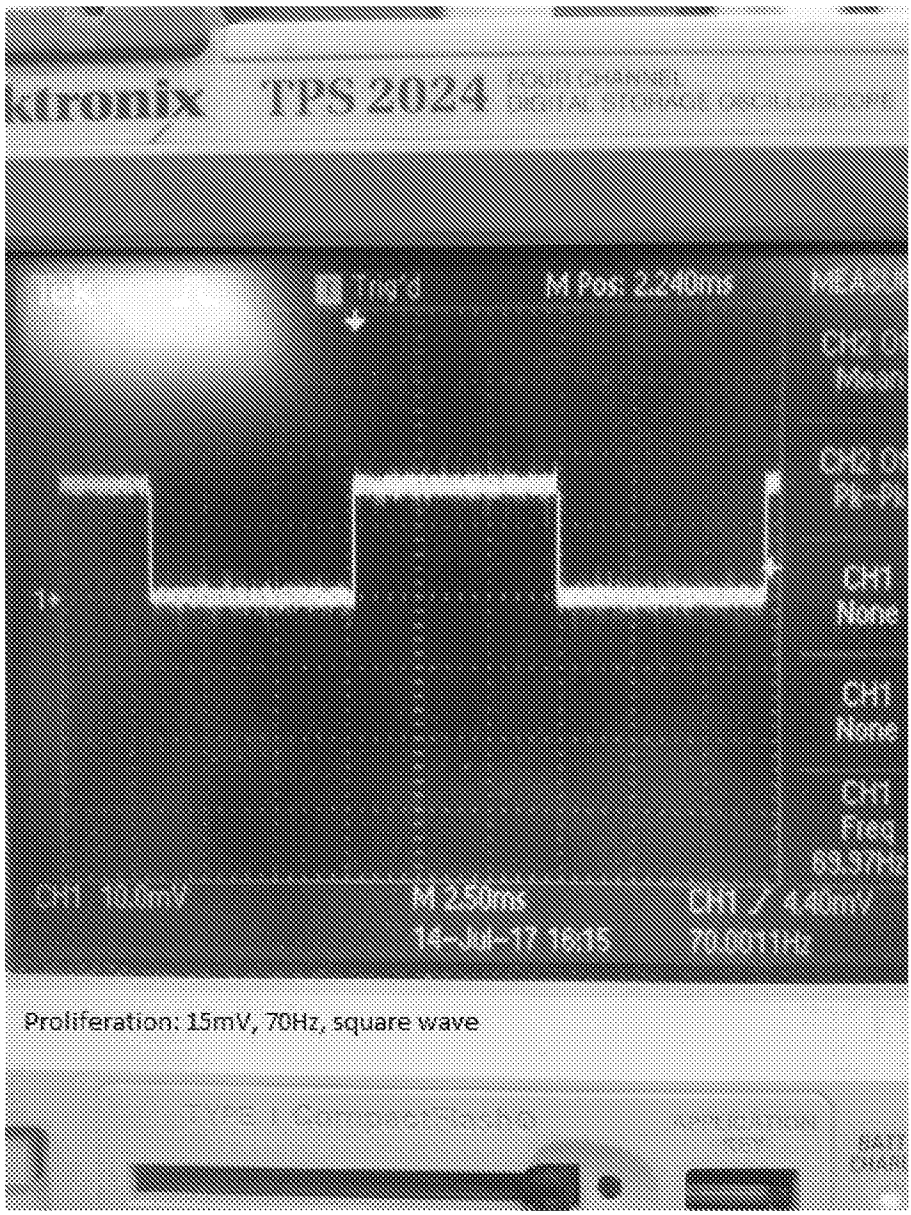

ktronix
TPS 2024
M Pos: 2.240ms
CH1
None
CH1
None
CH1
Freq
M 2.50ms
14-Jul-17 16:15
Proliferation: 15mV, 70Hz, square wave

FIG. 14

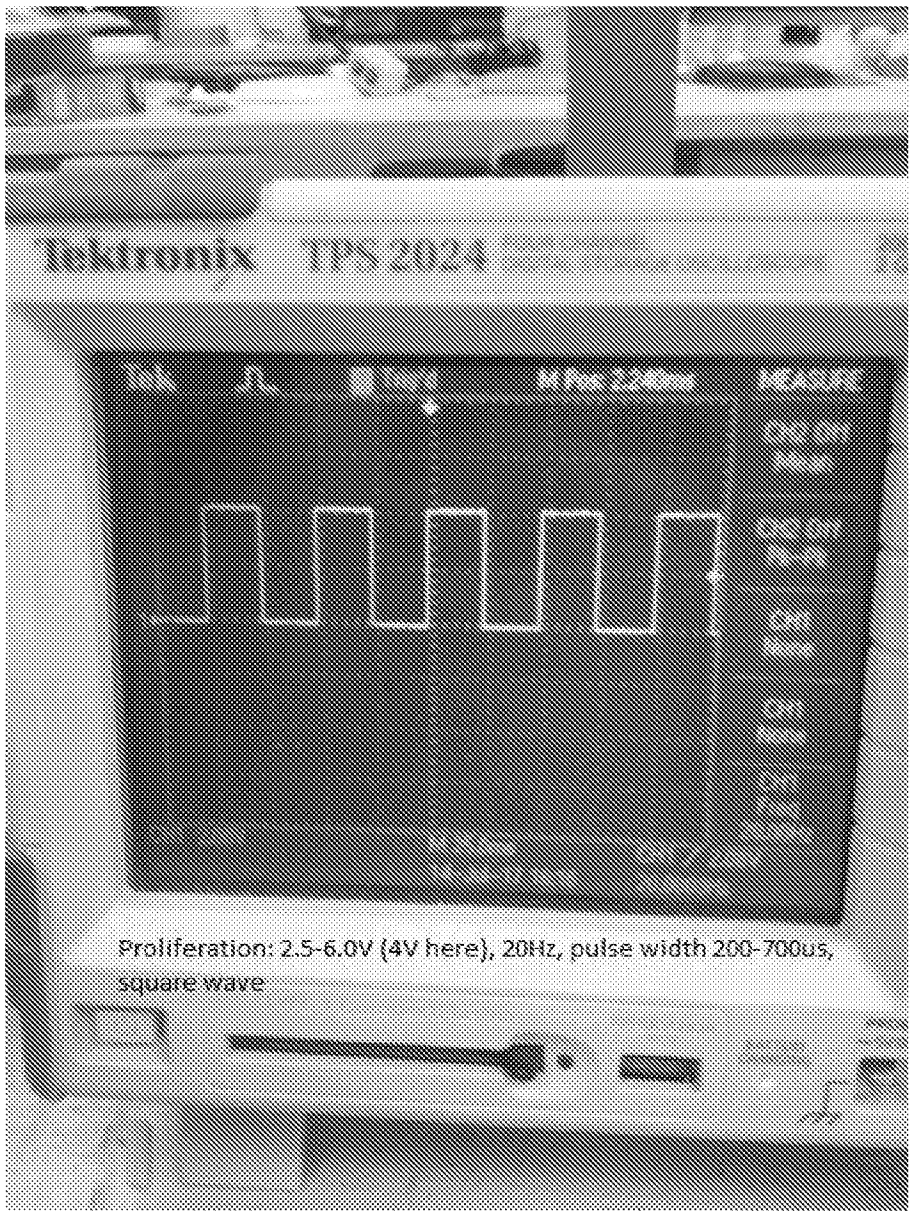

Tektronix TPS2024
Proliferation: 2.5-6.0V (4V here), 20Hz, pulse width 200-700us, square wave

FIG. 15

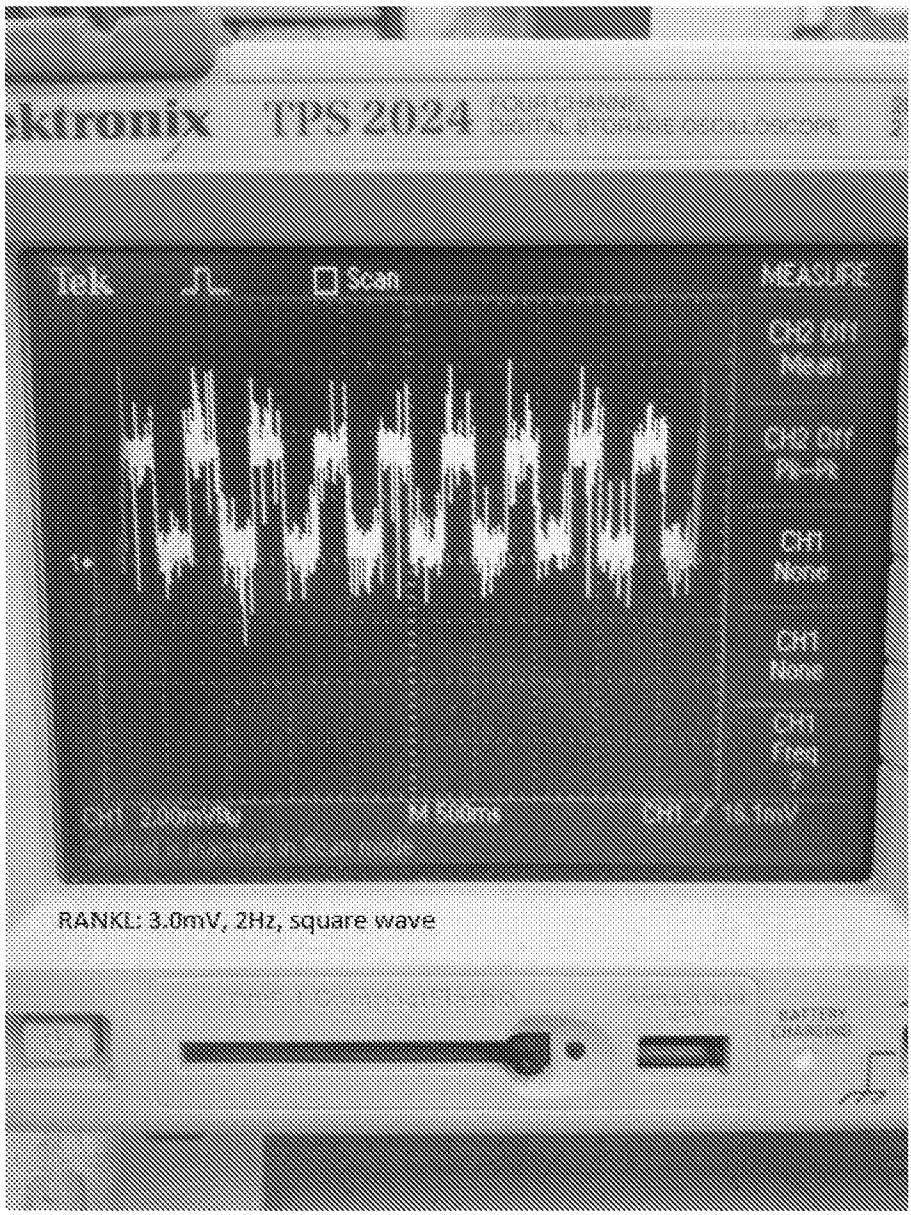



FIG. 16

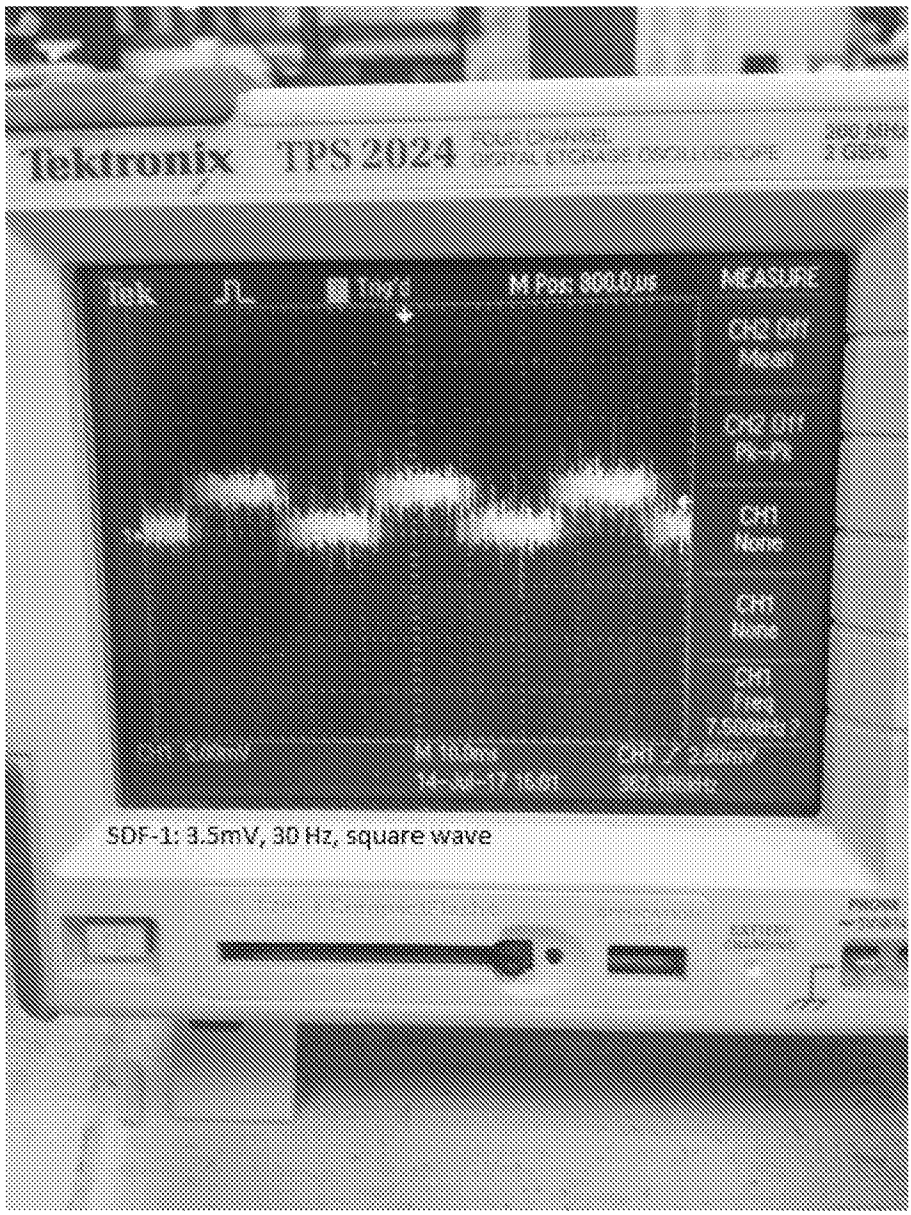



FIG. 17

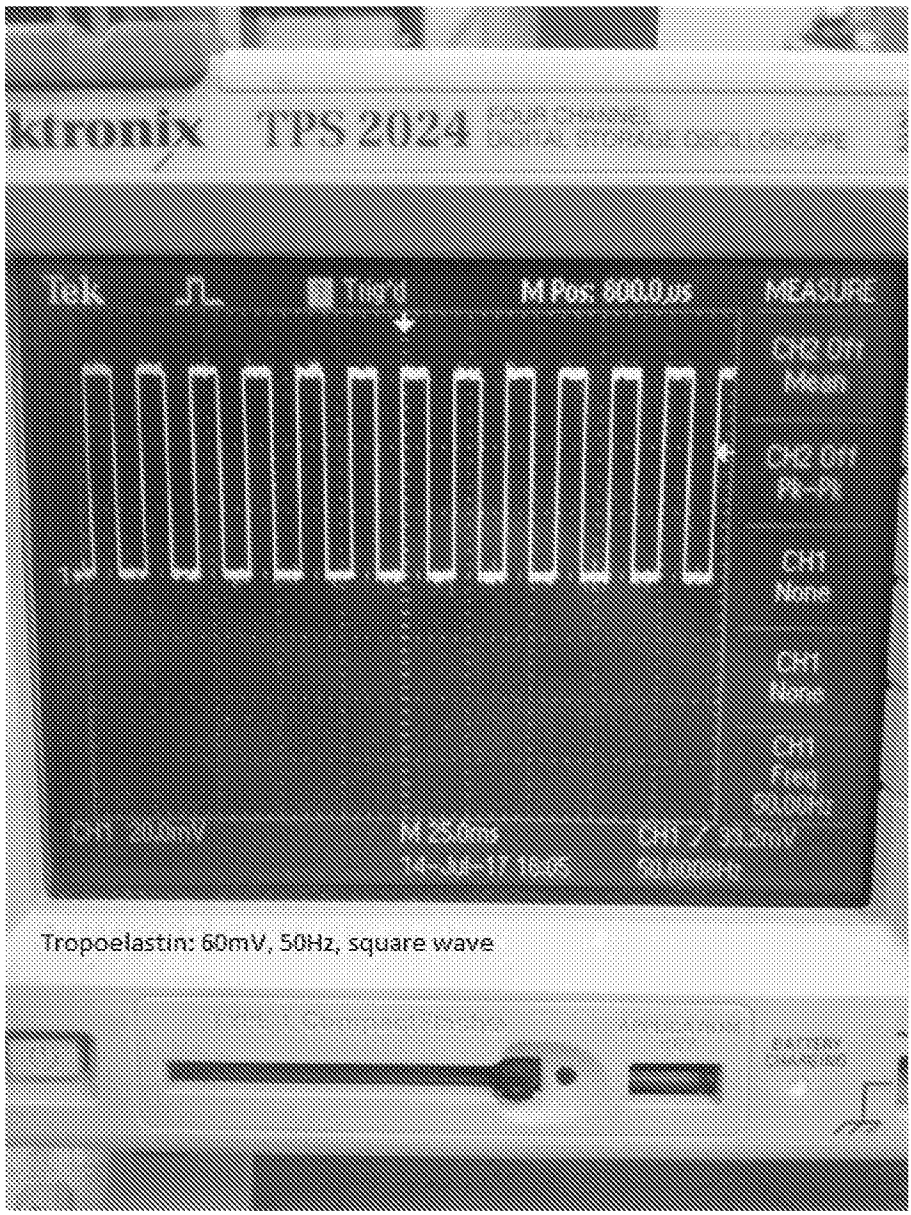



FIG. 18

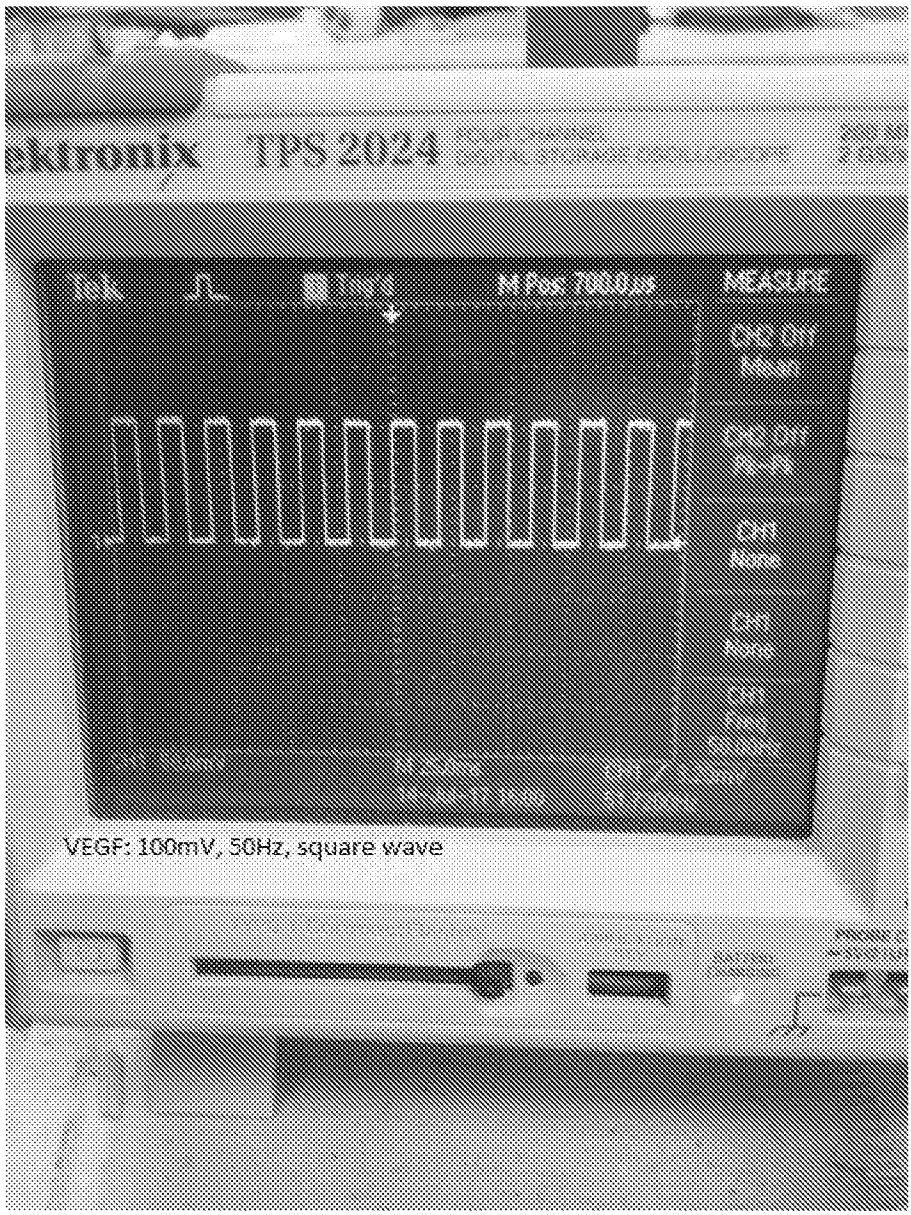



FIG. 19

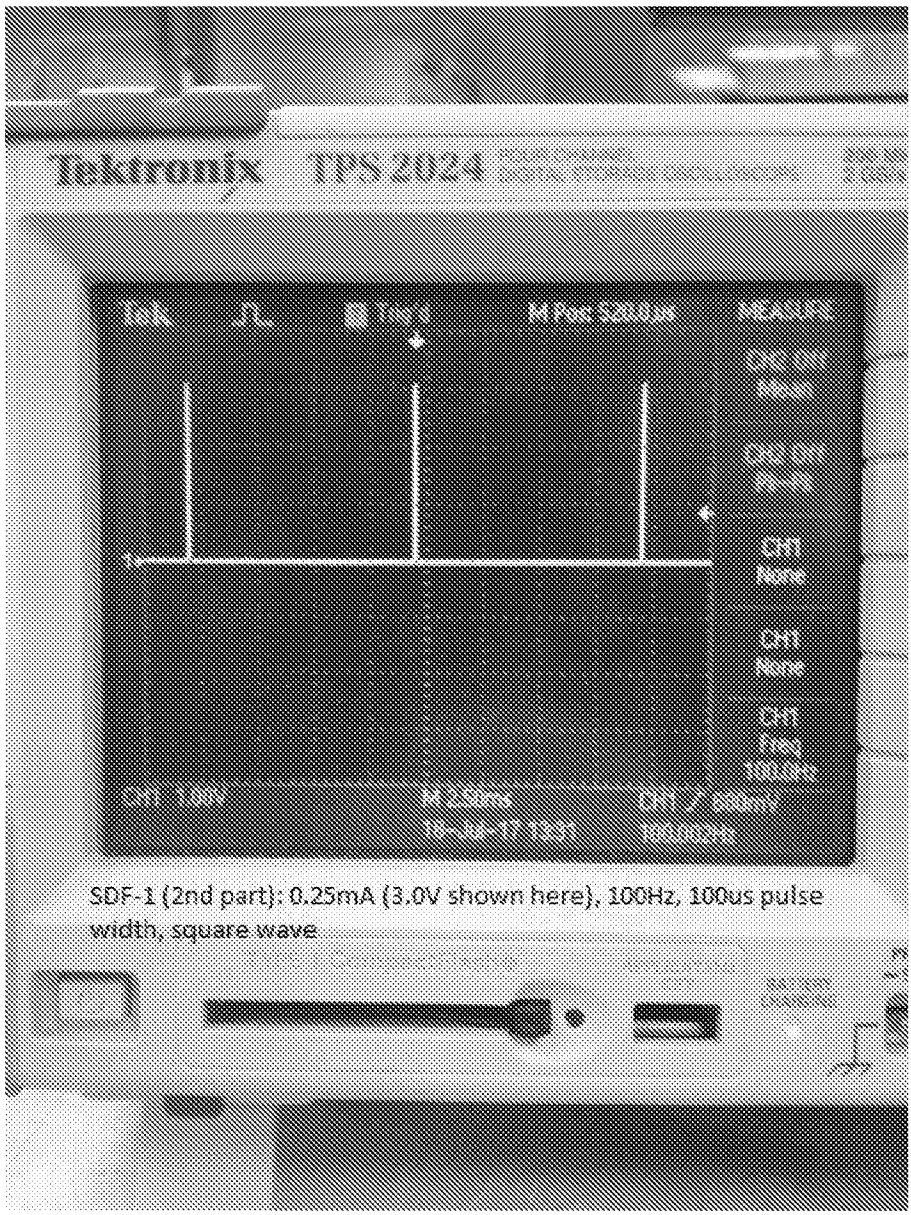

Tektronix TPS2024
SDF-1 (2nd part): 0.25mA (3.0V shown here), 100Hz, 100us pulse width, square wave

FIG. 20

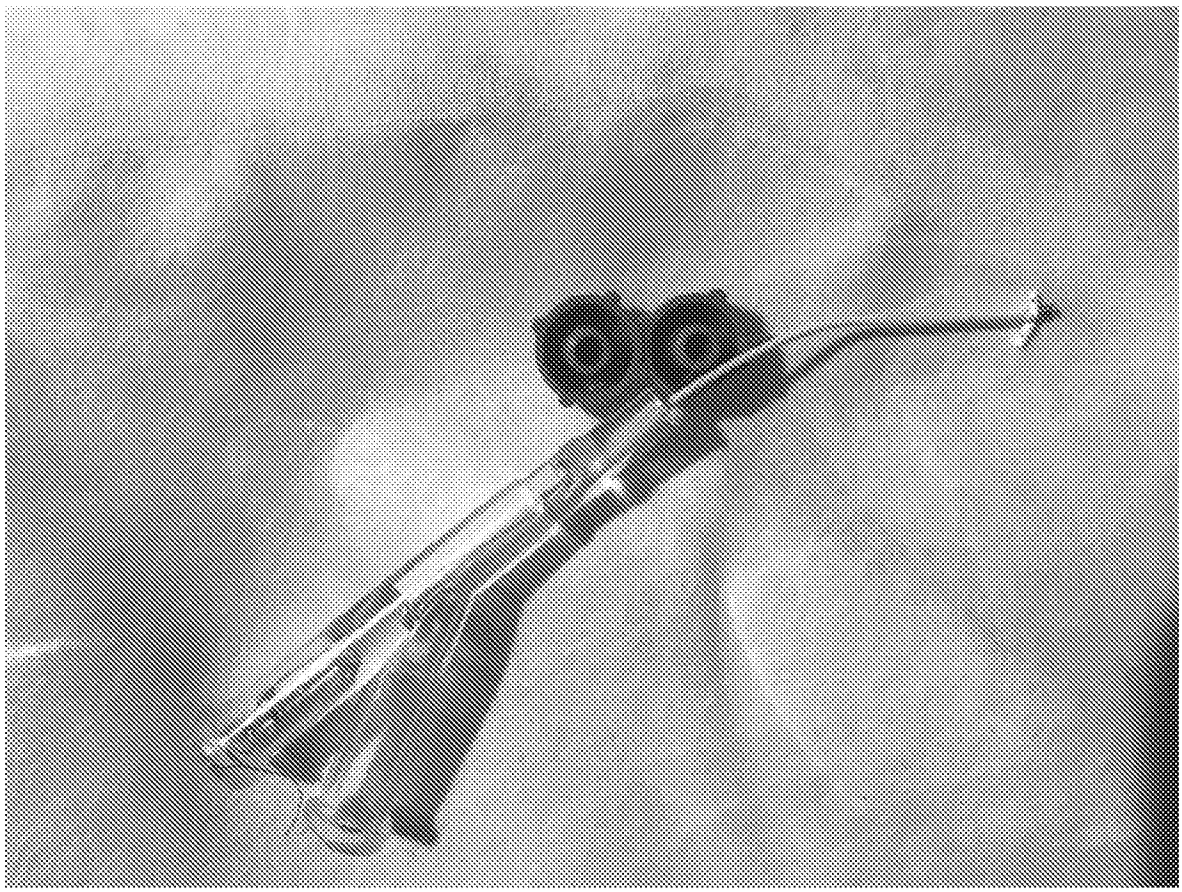


FIG. 21

FIG. 22

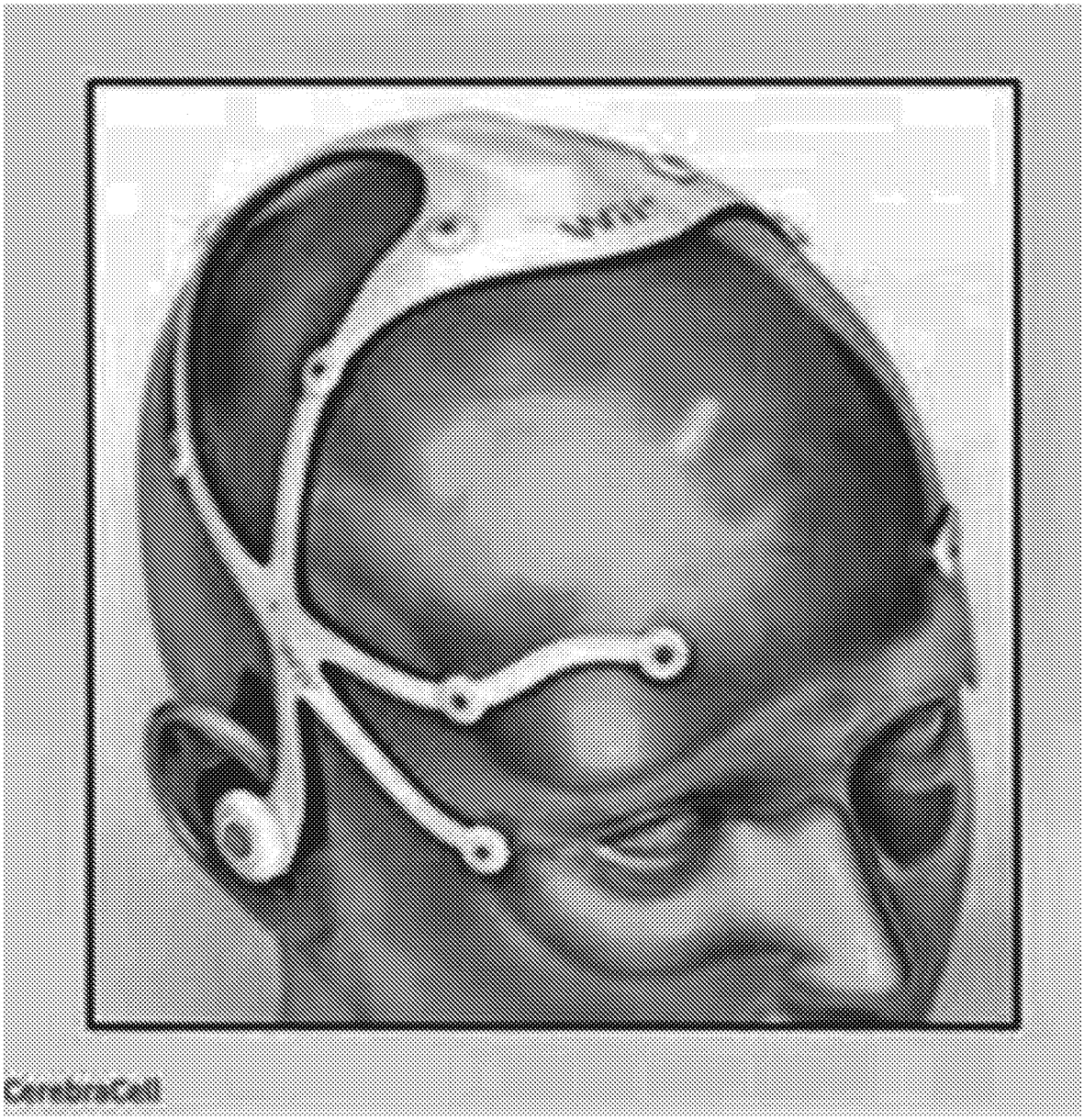



FIG. 23

FIG. 24

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/US2019/052278**

**A. CLASSIFICATION OF SUBJECT MATTER**

**A61N 1/36(2006.01)i, A61M 5/142(2006.01)i, A61N 1/362(2006.01)i, A61H 39/00(2006.01)i, A61K 35/28(2006.01)i, A61K 38/19(2006.01)i, A61K 38/18(2006.01)i, A61K 38/20(2006.01)i**

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)
A61N 1/36; A61B 5/00; A61B 5/04; A61M 5/14; A61N 1/00; A61N 1/18; A61M 5/142; A61N 1/362; A61H 39/00; A61K 35/28; A61K 38/19; A61K 38/18; A61K 38/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & keywords: bioelectric, stimulator, brain injury, inflammation

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 2017-142948 A1 (NALU MEDICAL, INC.) 24 August 2017<br>claims 1, 54, 57, 60, 77 | 1-17,20 |
| A | US 2007-0156179 A1 (S.E., K.) 05 July 2007<br>the whole document | 1-17,20 |
| A | US 2012-0053508 A1 (WU, J. et al.) 01 March 2012<br>the whole document | 1-17,20 |
| A | US 7117034 B2 (KRONBERG, J. W.) 03 October 2006<br>the whole document | 1-17,20 |
| A | US 2018-0153467 A1 (LICHTENSTEIN, Y. et al.) 07 June 2018<br>the whole document | 1-17,20 |

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

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"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 |
|---|---|
| 10 January 2020 (10.01.2020) | **10 January 2020 (10.01.2020)** |


Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-481-8578

Authorized officer
HAN, Inho
Telephone No. +82-42-481-3362

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/US2019/052278**

**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.: 18, 19
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required addtional 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 an additional fees, this Authority did not invite payment of any additional fees.

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

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

**Remark on Protest**

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

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/US2019/052278**

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2017-142948 A1 | 24/08/2017 | AU 2018-221321 A1 | 30/08/2018 |
| | | CA 3014317 A1 | 24/08/2017 |
| | | EP 3416719 A1 | 26/12/2018 |
| | | US 2019-0001139 A1 | 03/01/2019 |
| US 2007-0156179 A1 | 05/07/2007 | WO 2004-078252 A2 | 16/09/2004 |
| | | WO 2004-078252 A3 | 11/11/2004 |
| US 2012-0053508 A1 | 01/03/2012 | US 9211411 B2 | 15/12/2015 |
| | | WO 2012-027286 A1 | 01/03/2012 |
| US 7117034 B2 | 03/10/2006 | CN 100842356 A | 04/10/2006 |
| | | CN 100842356 B | 04/05/2011 |
| | | EP 1648553 A2 | 26/04/2006 |
| | | HK 1096882 A1 | 16/12/2011 |
| | | JP 2007-524459 A | 30/08/2007 |
| | | JP 4980713 B2 | 18/07/2012 |
| | | KR 10-1134242 B1 | 09/04/2012 |
| | | KR 10-2006-0033737 A | 19/04/2006 |
| | | US 2004-0267333 A1 | 30/12/2004 |
| | | WO 2005-002663 A2 | 13/01/2005 |
| | | WO 2005-002663 A3 | 18/08/2005 |
| US 2018-0153467 A1 | 07/06/2018 | EP 3302244 A2 | 11/04/2018 |
| | | WO 2016-193929 A2 | 08/12/2016 |
| | | WO 2016-193929 A3 | 12/01/2017 |