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(54) Title: ENGINEERED CELL TRANSFECTED BY ALL-IN-ONE VECTOR FOR THERAPEUTIC AGENTS PRODUCTION AND DELIVERY AND APPLICATIONS THEREOF

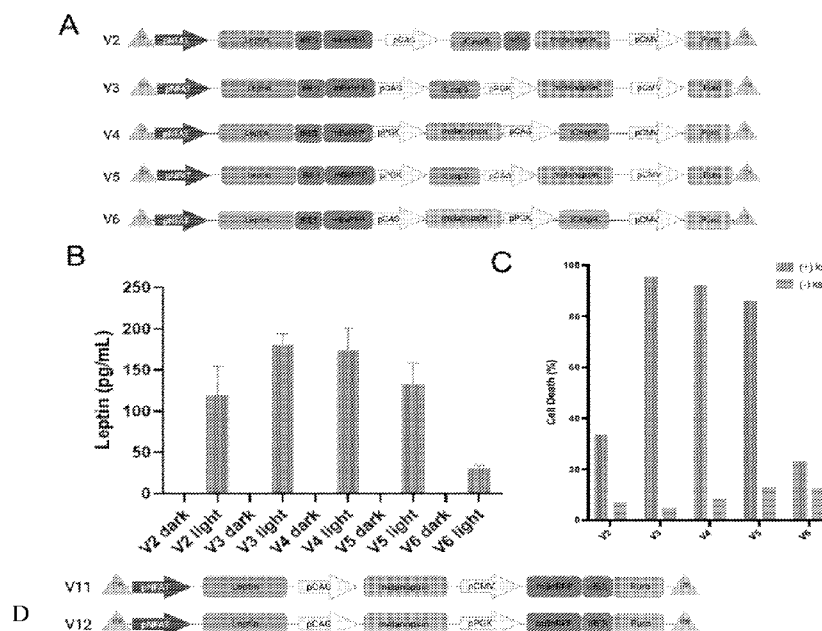


Fig. 24

(57) Abstract: A vector transfecting a cell for producing a therapeutic agent. The vector comprises at least one promoter; a therapeutic agent encoding sequence; a reporter encoding sequence; and an optical controller encoding sequence, wherein the vector comprises a DNA sequence.

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**ENGINEERED CELL TRANSFECTED BY ALL-IN-ONE VECTOR FOR
THERAPEUTIC AGENTS PRODUCTION AND DELIVERY AND APPLICATIONS
THEREOF**

STATEMENT AS TO RIGHTS UNDER FEDERALLY-SPONSORED RESEARCH

[0001] This invention was made with government support under FA8650-21-2-7119 awarded by the Air Force Research Laboratory. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0002] This application claims priority to and the benefit of U.S. Provisional Patent Application Serial No. 63/545,996, filed October 27, 2023, which is incorporated herein in its entirety by reference.

[0003] This application is also a continuation-in-part application of PCT application No. PCT/US23/36276, filed October 30, 2023, which itself claims priority to and the benefit of U.S. Provisional Patent Application Serial No. 63/420,219, filed October 28, 2022, which is incorporated herein in its entirety by reference.

[0004] This application is also a continuation-in-part application of U.S. Patent Application Serial No. 18/287,671, filed October 20, 2023, which is a U.S. national stage application of PCT application No. PCT/US2022/025686, filed April 21, 2022, which itself claims priority to and the benefit of U.S. Provisional Patent Application Serial No. 63/177,806, filed April 21, 2021, which is incorporated herein in its entirety by reference.

[0005] This application is also a continuation-in-part application of U.S. Patent Application Serial No. 18/287,684, filed October 20, 2023, which is a U.S. national stage application of PCT application No. PCT/US2022/025706, filed April 21, 2022, which itself claims priority to and the benefit of U.S. Provisional Patent Application Serial No. 63/177,806, filed April 21, 2021, which is incorporated herein in its entirety by reference.

[0006] This application is also a continuation-in-part application of U.S. Patent Application Serial No. 18/287,709, filed October 20, 2023, which is a U.S. national stage application of PCT application No. PCT/US2022/025724, filed April 21, 2022, which itself claims priority to and the benefit of U.S. Provisional Patent Application Serial No. 63/177,806, filed April 21, 2021, which is incorporated herein in its entirety by reference.

SEQUENCE LISTING

[0007] A Sequence Listing conforming to the rules of WIPO Standard ST.26 is hereby incorporated by reference. Said Sequence Listing has been filed as an electronic document via PatentCenter encoded as XML in UTF-8 text. The electronic document, created on October 24, 2024, is entitled “0116936.276WO22.xml”, and is 88,657 bytes in size.

FIELD OF THE INVENTION

[0008] The present disclosure relates generally to the field of biotechnology, and more particularly to optically controlled engineered cell systems transfected by all-in-one vector for delivery of therapeutic agents into an individual's body, and applications of the same.

BACKGROUND OF THE INVENTION

[0009] The background description provided herein is for the purpose of generally presenting the context of the present invention. The subject matter discussed in the background of the invention section should not be assumed to be prior art merely as a result of its mention in the background of the invention section. Similarly, a problem mentioned in the background of the invention section or associated with the subject matter of the background of the invention section should not be assumed to have been previously recognized in the prior art. The subject matter in the background of the invention section merely represents different approaches, which in and of themselves may also be inventions.

[0010] The function of implanted cells, tissues, and devices depends on numerous factors, including their ability to provide a product, e.g., therapeutic agents, and the biological immune response pathway of the recipient (Anderson et al., Semin Immunol (2008) 20:86-100; Langer, Adv Mater (2009) 21:3235-3236). Efficient therapeutic agents production and precise control over the production in the engineered cells are essential for implanted cells, tissues, and devices to effectively deliver therapeutic agents to the recipient.

[0011] Therefore, there remains an imperative need for engineered cells having stable and effective production of desired therapeutic agents with a controlling system providing precise control of the functionality of the engineered cells.

SUMMARY OF THE INVENTION

[0012] In light of the foregoing, this invention discloses a vector transfecting a cell for producing a therapeutic agent. The vector comprises a first promoter controlling a transcription of a therapeutic agent encoding sequence and a reporter sequence; a second promoter controlling a transcription of an optical controller sequence encoding a light sensitive protein; a third promoter controlling a transcription of a kill switch encoding sequence; and a fourth promoter controlling a transcription of a selectable marker, wherein the first promoter is a NFAT promoter, each of the second promoter and the third promoter is one of a CAG promoter and a PGK promoter, and the second promoter is different from the third promoter; wherein the light sensitive protein comprises a melanopsin, the kill switch encoding sequence encodes iCasp9; wherein the therapeutic agent comprises at least one of IL-1ra, IL-1 β , IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IL-23, IFN- γ , IFN-g-inducible protein-10, ISG15, CXCL1, TIMPs, PTUPB, TLR4, TNF- α , TGF- β , HIF-1 α , VEGF, NOS inhibitor (L-NAME), TRPM2 channel blockers (ACA and 2-APB), leptin, ACTH, insulin, GLP-1, and any adjuvant, antibody, agonist, or antagonist thereof.

[0013] In another aspect of the invention, the present invention discloses a vector transfecting a cell for producing a therapeutic agent. The vector comprises at least one promoter; a therapeutic agent encoding sequence; a reporter sequence; and an optical controller sequence, wherein the vector comprises a DNA sequence.

[0014] In one embodiment, the at least one promoter comprises a first promoter and a second promoter, wherein the first promoter is different from the second promoter.

[0015] In one embodiment, the first promoter controls a transcription of the therapeutic agent encoding sequence and the reporter sequence.

[0016] In one embodiment, the second promoter controls a transcription of the optical controller sequence.

[0017] In one embodiment, the optical controller sequence encodes a light sensitivity protein.

[0018] In one embodiment, the light sensitivity protein participates in an activation of the first promoter.

[0019] In one embodiment, the vector further comprises a kill switch encoding sequence.

[0020] In one embodiment, the second promoter controls a transcription of the kill switch encoding sequence.

[0021] In one embodiment, the at least one promoter further comprises a third promoter different from the first and the second promoters, and the third promoter controls a transcription of the kill switch encoding sequence.

[0022] In one embodiment, the light sensitivity protein is melanopsin.

[0023] In one embodiment, the light sensitivity protein is reporter sequence encodes a fluorescent protein.

[0024] In one embodiment, the kill switch encoding sequence encodes iCasp9.

[0025] In one embodiment, the therapeutic agent comprises at least one of IL-1ra, IL-1 β , IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IL-23, IFN- γ , IFN-g-inducible protein-10, ISG15, CXCL1, TIMPs, PTUPB, TLR4, TNF- α , TGF- β , HIF-1 α , VEGF, NOS inhibitor (L-NAME), TRPM2 channel blockers (ACA and 2-APB), leptin, ACTH, insulin, GLP-1, and any adjuvant, antibody, agonist, or antagonist thereof.

[0026] In one embodiment, the first promoter comprises a NFAT promoter.

[0027] In one embodiment, each of the second and the third promoters comprises one of a CAG promoter, a PGK promoter, and a CMV promoter.

[0028] In one embodiment, the at least one promoter comprises a fourth promoter, wherein the fourth promoter is different from the first, second and third promoters, and the fourth promoter controls a transcription of a selectable marker.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] The accompanying drawings illustrate one or more embodiments of the invention and together with the written description, serve to explain the principles of the invention. Wherever possible, the same reference numbers are used throughout the drawings to refer to the same or like elements of an embodiment.

[0030] Fig. 1 depicts phases of peripheral and central clocks in response to an 8hr shift, for normal entrainment (left), providing therapy affecting only the central clock (middle), and the proposed NTRAIN approach (right) with therapy targeting both central and peripheral clocks in accordance with an illustrative embodiment.

[0031] Fig. 2 is a table that depicts the rationale for using optical induction to perform control and feedback in accordance with an illustrative embodiment.

[0032] Figs. 3A-3D provide different embodiments of hybrid bioelectronic device. Fig. 3A

illustrates an implantable embodiment having a single cell housing containing engineered cells. Fig. 3B illustrates an implantable embodiment having plurality of cell housings containing same or different engineered cells. Fig. 3C illustrates an implantable embodiment having one or more cell housings integrated with power transduction management system, optoelectronics and other accessory systems e.g., O₂ generation system. Fig. 3D illustrates a wearable embodiment having cell culture media cartridge independent from the cell housing.

[0033] Fig. 4 depicts operations performed to implement the proposed NTRAIN system in accordance with an illustrative embodiment.

[0034] Fig. 5 is a graphical depiction of proposed synthetic biology circuit for optogenetic control of production of the therapeutic agents, e.g., Orexin A, in accordance with an illustrative embodiment.

[0035] Fig. 6 depicts preliminary data showing that ARPE-19 cells can be made to express luciferase with high on/off ratio in response to blue light using an EL222 optogenetic system in accordance with an illustrative embodiment.

[0036] Fig. 7 shows a biohybrid precision control scheme based on a process of co-production of therapeutic peptide and proxy reporter fluorophore (GFP*) in accordance with an illustrative embodiment.

[0037] Fig. 8 shows a comparison of traditional optogenetic control strategies that use constant illumination to activate ion channels with the proposed step-function opsin control strategy that utilizes a blue LED to open light-gated channels and an orange LED to close the channels in accordance with an illustrative embodiment.

[0038] Figs. 9A-B show wired and wireless prototype devices in accordance with an illustrative embodiment. Fig. 9A shows a design of the top side of the circuit board with the LEDs and photodiodes. Fig. 9B shows a wired prototype device with its wires extending out from the right side of the device.

[0039] Fig. 10 shows charts regarding utilization of blue LEDs and photodiodes to measure green fluorescence of cells.

[0040] Fig. 11 shows a top view of blue and orange LEDs on an encapsulated circuit board at the end of a soak test.

[0041] Figs. 12A-C show a filter and its application to isolate a photodiode from the blue LED. Fig. 12A shows a chart of optical density of a Wratten 12 filter. Fig. 12B shows the laser

cut optical filter which is bent into a box shape before being glued to the photodiode in accordance with an illustrative embodiment. Fig. 12C shows a chart of photovoltages obtained with and without the optical filter.

[0042] Figs. 13A-B show device molds in accordance with two illustrative embodiments. Fig. 13A shows an aluminum device mold in accordance with an illustrative embodiment. Fig. 13B shows a PDMS device molded by the aluminum mold in accordance with another illustrative embodiment.

[0043] Figs. 14A-D show power transmission elements and their relevant output power through a porcine tissue. Fig. 14A shows a battery-powered transmitter in accordance with an illustrative embodiment. Fig. 14B shows a ME film being used as a receiver. Fig. 14C shows a setup for testing power transmission. Fig. 14D shows a chart of output power transferred through 2cm porcine tissue.

[0044] Figs. 15A-B show a synthesis reaction for RZA15 in accordance with an illustrative embodiment. Fig. 15A shows a process for RZA15 synthesis. Fig. 15B shows NMR and ES MS characterization of the resulting RZA15 product.

[0045] Figs. 16A-B show a synthesis reaction for RZA15 UPVLVG in accordance with an illustrative embodiment. Fig. 16A shows a synthesis process; Fig. 16B shows NMR and elemental characterization of the product.

[0046] Fig. 17 shows an aid device for loading the bioelectric device with the engineered cells.

[0047] Fig. 18 is a block diagram of a computing system to perform operations described herein in accordance with an illustrative embodiment.

[0048] Fig. 19 shows another systemic review of the bioelectric device in association with a user.

[0049] Fig. 20 shows a chart reflecting leptin production from repeated on/off cycles of engineered APRE-19 cells under an optical control system.

[0050] Fig. 21 shows a chart reflecting linear relationship between mBeRFP concentration and detected signal.

[0051] Fig. 22 shows a chart reflecting sensitivity of mBeRFP sensor with an improved filter set.

[0052] Fig. 23 shows vector design and testing chart of mBeRFP-containing all-in-one

vector.

[0053] Fig. 24 shows ARPE-19 cells engineered to produce light inducible leptin and a small molecule inducible kill switch. Panel (A) shows 5 versions of vector design containing all the necessary components to produce leptin in response to light and the small molecule inducible kill switch; Panel (B) shows light inducible leptin produced from cells engineered with the plasmids depicted in panel A; Panel (C) shows cell death induced in engineered ARPE-19 cells with the plasmids depicted in panel A, 6 hours after administration of the small molecule inducer.

[0054] Fig. 25 shows ACTH production from repeated on/off cycles of ARPE-19 engineered cells.

[0055] Fig. 26 shows a chart reflecting kill switch performance in mBeRFP all-in-one cell line.

[0056] Fig. 27 shows leptin production from an implantable scale device. Panel (A) shows an embodiment of the implantable scale device measures 10 mm x 20 mm x 5 mm; Panel (B) shows images of engineered cells on the PCL scaffold and the in vitro setup for testing the devices; Panel (C) shows leptin production from cells in the implantable devices after 8 hours of light exposure.

[0057] Fig. 28 shows an embodiment of implantable device for in vivo experimentation; Panel (A) shows LEDs and light to digital converter viewed through the side of the cell well; Panel (B) shows side view of implantable device showing the components stackup (from top to bottom: circuit board, battery, NFC coil).

[0058] Fig. 29 shows a chart reflecting fluorescence readings using photodiodes.

DETAILED DESCRIPTION OF THE INVENTION

[0059] The invention will now be described more fully hereinafter with reference to the accompanying drawings, in which exemplary embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this invention will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art. Like reference numerals refer to like elements throughout.

[0060] The terms used in this specification generally have their ordinary meanings in the art, within the context of the invention, and in the specific context where each term is used. Certain

terms that are used to describe the invention are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner regarding the description of the invention. For convenience, certain terms may be highlighted, for example using italics and/or quotation marks. The use of highlighting has no influence on the scope and meaning of a term; the scope and meaning of a term is the same, in the same context, whether or not it is highlighted. It will be appreciated that same thing can be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein, nor is any special significance to be placed upon whether or not a term is elaborated or discussed herein. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of other synonyms. The use of examples anywhere in this specification including examples of any terms discussed herein is illustrative only, and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to various embodiments given in this specification.

[0061] One of ordinary skill in the art will appreciate that starting materials, biological materials, reagents, synthetic methods, purification methods, analytical methods, assay methods, and biological methods other than those specifically exemplified can be employed in the practice of the invention without resort to undue experimentation. All art-known functional equivalents, of any such materials and methods are intended to be included in this invention. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0062] Whenever a range is given in the specification, for example, a temperature range, a time range, or a composition or concentration range, all intermediate ranges and subranges, as well as all individual values included in the ranges given are intended to be included in the invention. It will be understood that any subranges or individual values in a range or subrange that are included in the description herein can be excluded from the claims herein.

[0063] It will be understood that, as used in the description herein and throughout the claims that follow, the meaning of “a”, “an”, and “the” includes plural reference unless the context clearly dictates otherwise. Thus, for example, reference to “a cell” includes a plurality of such cells and equivalents thereof known to those skilled in the art. As well, the terms “a” (or “an”), “one or more” and “at least one” can be used interchangeably herein. It is also to be noted that the terms “comprising”, “including”, and “having” can be used interchangeably.

[0064] It will be understood that when an element is referred to as being “on”, “attached” to, “connected” to, “coupled” with, “contacting”, etc., another element, it can be directly on, attached to, connected to, coupled with or contacting the other element or intervening elements may also be present. In contrast, when an element is referred to as being, for example, “directly on”, “directly attached” to, “directly connected” to, “directly coupled” with or “directly contacting” another element, there are no intervening elements present. It will also be appreciated by those of skill in the art that references to a structure or feature that is disposed “adjacent” another feature may have portions that overlap or underlie the adjacent feature.

[0065] It will be understood that, although the terms first, second, third etc. may be used herein to describe various elements, components, regions, layers and/or sections, these elements, components, regions, layers and/or sections should not be limited by these terms. These terms are only used to distinguish one element, component, region, layer or section from another element, component, region, layer or section. Thus, a first element, component, region, layer or section discussed below could be termed a second element, component, region, layer or section without departing from the teachings of the invention.

[0066] Furthermore, relative terms, such as “lower” or “bottom” and “upper” or “top,” may be used herein to describe one element’s relationship to another element as illustrated in the figures. It will be understood that relative terms are intended to encompass different orientations of the device in addition to the orientation depicted in the figures. For example, if the device in one of the figures is turned over, elements described as being on the “lower” side of other elements would then be oriented on “upper” sides of the other elements. The exemplary term “lower”, can therefore, encompass both an orientation of “lower” and “upper,” depending of the particular orientation of the figure. Similarly, if the device in one of the figures is turned over, elements described as “below” or “beneath” other elements would then be oriented “above” the other elements. The exemplary terms “below” or “beneath” can, therefore, encompass both

an orientation of above and below.

[0067] It will be further understood that the terms “comprises” and/or “comprising”, or “includes” and/or “including”, or “has” and/or “having”, or “carry” and/or “carrying”, or “contain” and/or “containing”, or “involve” and/or “involving”, “characterized by”, and the like are to be open-ended, i.e., to mean including but not limited to. When used in this disclosure, they specify the presence of stated features, regions, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, regions, integers, steps, operations, elements, components, and/or groups thereof.

[0068] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art and the invention, and will not be interpreted in an idealized or overly formal sense unless expressly so defined herein.

[0069] As used in the disclosure, “around”, “about”, “approximately” or “substantially” shall generally mean within 20 percent, preferably within 10 percent, and more preferably within 5 percent of a given value or range. Numerical quantities given herein are approximate, meaning that the term “around”, “about”, “approximately” or “substantially” can be inferred if not expressly stated.

[0070] As used in the disclosure, the phrase “at least one of A, B, and C” should be construed to mean a logical (A or B or C), using a non-exclusive logical OR. As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0071] As used in the disclosure, the term “implantable” refers to an ability of a device to be positioned at a location within a body of a user, such as subcutaneously, within a body cavity, or etc. Furthermore, the terms “implantation” and “implanted” refer to the positioning of a device at a location within a body of a user, such as subcutaneously, within a body cavity, or etc.

[0072] As used in the disclosure, the term “wearable” refers to articles, adornments or items designed to be worn by a user, incorporated into another item worn by a user, act as an orthosis for the user, or interfacing with the contours of a user's body.

[0073] As used in the disclosure, “biocompatible” material is a material that is compatible with living tissue or a living system by not being toxic or injurious and not causing

immunological rejection.

[0074] As used in the disclosure, “therapeutic agent” refers to any substance that provides therapeutic effects to a disease or symptom related thereto. In certain embodiments, a therapeutic agent refers to a substance that provides therapeutic effects to any diseases or biological or physiological responses to the diseases.

[0075] As used in the disclosure, the term “therapy” refers to any protocol, method, and/or agent that can be used in the management, treatment, and/or amelioration of a given disease, or a symptom related thereto. In certain embodiments, the terms “therapies” and “therapy” refer to a biological therapy, supportive therapy, and/or other therapies known to one of skill in the art, such as medical personnel, useful in the management or treatment of a given disease, or symptom related thereto.

[0076] As used in the disclosure, “treat”, “treatment”, and “treating” refer to the reduction or amelioration of the progression, severity, and/or duration of a given disease resulting from the administration of one or more therapies (including, but not limited to, the administration of microspheres disclosed herein). In certain embodiments, the terms refer to the reduction of pain associated with one or more diseases or conditions.

[0077] As used in the disclosure, “engineered cell(s)” refers herein to cells having been engineered, e.g., by the introduction of an exogenous nucleic acid sequence or specific alteration of an endogenous gene sequence. An exogenous nucleic acid sequence that is introduced may comprise a wild type sequence of any species that may be modified. An engineered cell may comprise genetic modifications such as one or more mutations, insertions and/or deletions in an endogenous gene and/or insertion of an exogenous nucleic acid (e.g., a genetic construct) in the genome. An engineered cell may refer to a cell in isolation or in culture. Engineered cells may be “transduced cells” wherein the cells have been infected with e.g., an engineered virus. For example, a retroviral vector may be used, such as described in the examples, but other suitable viral vectors may also be contemplated such as lentiviruses. Non-viral methods may also be used, such as transfections or electroporation of DNA vectors. DNA vectors that may be used are transposon vectors. Engineered cells may thus also be “stably transfected cells” or “transiently transfected cells”. Transfection refers to non-viral methods to transfer DNA (or RNA) to cells such that a gene is expressed. Transfection methods are widely known in the art, such as calcium phosphate transfection, PEG transfection, and liposomal or lipoplex transfection of nucleic acids.

Such a transfection may be transient, but may also be a stable transfection wherein cells can be selected that have the gene construct integrated in their genome.

[0078] Present system described herein features an implantable device housing engineered cells, e.g., ARPE-19 cells, that produce or are capable of producing one or more therapeutic agents. The therapeutic agent may be a biological substance, such as a nucleic acid (e.g., a nucleotide, DNA, or RNA), a polypeptide, a lipid, a sugar (e.g., a monosaccharide, disaccharide, oligosaccharide, or polysaccharide), a small molecule, etc. In some embodiments, the therapeutic agent is a polypeptide. Each engineered cell comprises a promoter operably linked to a nucleotide sequence encoding the polypeptide. In such an implementation, the promoter can essentially be a nucleotide sequence. In some embodiments, the therapeutic agent is a replacement therapy or a replacement protein, e.g., useful for the treatment of a blood clotting disorder or a lysosomal storage disease in a subject.

[0079] In some embodiments, the implantable device includes one or more engineered cells, which can be provided as a cluster or disposed in a microcarrier. In some embodiments, the engineered cells produce or release a therapeutic agent (e.g., a polypeptide) for at least 0.5 day, 1 day, 10 days, or more, when implanted into a subject. In one embodiment, more than one therapeutic agent are produced by the engineered cells. In one embodiment, the implantable device may include one or more types of engineered cells, one type of the engineered cells may produce a therapeutic agent which is different from the therapeutic agent produced by other types of the engineered cells.

[0080] In another aspect, the present disclosure features a method of treating a subject comprising administering to the subject an implantable device housing the engineered cells producing at least one therapeutic agents.

[0081] In one illustrative embodiment, the subject is a human and the engineered active cell is a human cell. Alternatively, the subject may be a dog, cat, or other animal. In some embodiments, the therapeutic agent produced by the engineered cell(s) is a replacement therapy or a replacement protein, e.g., useful for the treatment of metabolic diseases. In some embodiments, the implantable device is formulated for implantation or injection into a subject.

[0082] The produced therapeutic agents can be evaluated by an art-recognized reference method, e.g., polymerase chain reaction or in situ hybridization for nucleic acids; mass spectroscopy for lipid, sugar and small molecules; microscopy and other imaging techniques for

agents modified with a fluorescent or luminescent tag, and ELISA or Western blotting for polypeptides. In some embodiments, the implantable device comprises an encapsulating component (e.g., formed in situ on or surrounding the engineered cells, or preformed prior to combination with the engineered cells). In other embodiments, the implantable device is chemically modified, as described herein.

[0083] Thus, described herein is a hybrid bioelectronics/engineered cells pharmacy that enables production of therapeutic agents within a subject. The therapeutic agents can be used to control pain, treat metabolic disorders, treat immune system disorders, treat psychiatric disorders, improve fertility, and any other medical or health conditions requiring a frequent and/or precise administration of therapeutic agents.

[0084] The present invention provides a therapy having a timing and dosing control which far exceeds the existing therapies and/or bioelectronics. The proposed system is able to achieve 1) specific biological activation/inhibition on select target receptors or molecules that cannot be accomplished with current bioelectronics, and 2) precise control of timing and dosage that cannot be accomplished with current synthetic biology.

[0085] In one embodiment, the living pharmacy includes engineered cells that produce therapeutic agents, e.g., peptides, with a timing and dose profile that is precisely and tightly controlled by optical triggers from an implanted bioelectronic carrier device (i.e., implant device). Together with a subcutaneous bioelectronic carrier, the proposed system overcomes the major challenges facing hybrid bioelectronic devices, including: 1) selective activity on biological targets, 2) precise control of therapeutic agents production, 3) high dose to load volume ratio, 4) protection from the host's immune response, and 5) wireless data and power transfer through biological tissue. In alternative implementations, the system may have fewer, additional, and/or different features.

[0086] In one embodiment, the biohybrid system of the present invention provides a general platform for precise drug delivery and regulation that can be implanted in a subject for long-term treatment of either short term or long term diseases, physical and/or mental health conditions, as well as improvement of the subject's health and performance, without the need to carry pharmaceuticals. In one exemplary embodiment discussed below, the proposed system minimizes the adverse health consequences of circadian misalignment by achieving at least a 50% reduction in entrainment time using an implanted living biohybrid pharmacy that remains

functional for an extended period of time (e.g., 30 days, 60 days, 90 days, etc.). In other embodiments, the disclosed invention provides treatments to diseases and/or physiological conditions including metabolic diseases, e.g., obesity and diabetes (e.g., Type 1, Type 2) by producing metabolically active molecules, e.g., leptin, ACTH, insulin, and GLP-1; cancers by producing therapeutic cytokines e.g., IL-2, IL-12, IL-15, GCSF; autoimmune diseases by producing regulated molecules e.g., IL-10, IL-35, treatment resistant depression and pains by producing neuropeptides e.g., GLYX-13, rapastinel, and ziconotide; osteoporosis or hypoparathyroidism by producing parathyroid hormone (PTH); infertility by producing gonadotropin releasing hormone GnRH or gonadotropin hormones (LH, FSH); and etc.

[0087] To achieve these metrics, the system focuses on five main innovations to overcome barriers of current bioelectronic and synthetic biology technologies, as well as an innovative approach to accelerating entrainment. These innovations, which are described in detail below, include performing selective activity on biological targets using natural peptides, precisely controlling biomolecule production, obtaining a high dose to load volume ratio, providing protection from the host's immune response, and wirelessly transmitting data and power through biological tissue.

[0088] With respect to selective activity on biological targets using natural peptides, the present invention has proposed using engineered allogeneic cells to produce select peptides that are otherwise naturally produced by the body to control pain, fight disease, regulate sleep cycles, treat metabolic related conditions, and etc. It is to be understood that in other applications the system can be used to produce other types of therapeutic agents. The body naturally produces these native peptides which are structurally similar to their recombinant counterparts. However, the native peptides diverge in potency and bioactivity. Significantly, it is noted that native peptides have not been commercialized due to their instability. However, the inventors have determined that a cell delivery platform which supports on demand in situ production use of native peptides as therapeutics is feasible. These naturally produced molecules are excellent candidates to regulate the specific activity of central and peripheral circadian clocks because they act selectively on these biological targets and do not elicit the immune responses that are shortcomings of recombinant peptides or exogenous drugs (i.e., antidrug antibodies). Furthermore, these peptides feature short metabolic half-lives, and are useful for relatively fast cessation of drug production. These traits make the proposed cell platform uniquely suited to

deliver such biologics on demand. Additionally, it has been demonstrated that allogeneic cells encapsulated and implanted in vivo can survive for greater than 130 days in non-human primates without immunosuppression, suggesting that the proposed solution can enable living engineered cells-based devices with lifetimes that can extend for several months, or even years.

[0089] The novel system is also able to perform precision dosing with closed-loop bioelectronic control. A key challenge for biological production of therapeutic agents is controlling the production levels, which can vary due to cell health, temperature, and metabolism. To overcome this challenge, the proposed system includes a state-of-the art bioelectronic feedback control system incorporating optogenetically controlled therapy production and fluorescent tracking of therapy production levels. In alternative embodiments, the feedback control system may not be used. Cells engineered with optogenetic systems start protein production in response to exposure to specific activating light signals. By controlling light exposure, production of therapeutic agents can be controlled.

[0090] Another innovation of the proposed system is a bioelectronic feedback loop based on fluorescent tracking of the production levels. To create this feedback control loop, the cells are engineered to produce a fluorescent protein at a fixed ratio relative to the therapeutic agents. Using this fluorescence measurement as the feedback signal, the system is able to regulate the on time of the engineered cells to maintain a stable fixed point of therapeutic agents' production with precision that exceeds synthetic biological feedback loops.

[0091] In some embodiments, in addition to the fluorescent signal provided by the fluorescent proteins, the bioelectronic feedback loop is based on biochemical signals which can be electronically detected. In addition to the fluorescent signal, the biochemical signals may include bioluminescence signal, impedance signal, pigment signal, and free radical signal.

[0092] In some embodiments, the proposed system also provides high-dose to load volume with on-chip life support and engineered cells. To support a higher concentration of therapeutic agents produced by the implanted device, one could increase the density of the engineered cells inside the chassis. However, the maximum cell density is currently limited by the amount of diffuse oxygen available in the subcutaneous space. To reach higher cell densities, the carrier is engineered to produce local O₂ with the bioelectronic carrier. Furthermore, using synthetic biology tools, the system amplifies transcription of the therapeutic peptides and programs cells to be resilient to senescence and cell death.

[0093] In another aspect of the invention, the proposed system also provides protection from the host immune response using a small molecule coating. Specifically, engineered cells are encapsulated within a life support system that protects them from the immune system of the host and that supports cell viability and productivity. Hydrogels and permeable or semi-permeable membrane biomaterials can be used to block cells from the body's immune system via their physical, hierarchical pore structure and biochemical functionalization. The hydrogels and permeable or semi-permeable membrane biomaterials further promote vascularization near the device/tissue interface to boost oxygenation from the body's circulatory system.

[0094] In some embodiments, the proposed system also performs efficient wireless data and power transfer through tissue using magnetoelectrics. Traditional wireless power delivery by electromagnetic or ultrasound waves has to overcome absorption by tissue and impedance mismatches between air, bone, and tissue, and such techniques often struggle to provide large powers to small bioelectronic devices. In contrast, magnetic fields are not affected by tissue-absorption or differences in interfacial impedances. The proposed approach uses recently developed magnetoelectric (ME) technology and custom low power application specific integrated circuits (ASICs) to enable compact, reliable, wireless transmission of power and data, providing superior power densities and alignment tolerance. This approach for wireless power can enable ultra-miniature versions of the implantable device that can be injectable.

Alternatively, the proposed system can use other sources of power and data transfer such as inductive coupling, photovoltaic data and power control, radio frequency (RF) data and power control, inductive data and power control, ultrasound data and power control, direct current (DC) coupled data and power control, etc. In alternative implementations, battery power or energy harvesting from the body could reduce or eliminate the need for wireless power. The system can be used for delivery of single or multiple therapeutics. In one embodiment, the engineered cells produce one or more therapeutic agents. In another embodiment, there are more than one type of engineered cells housed either in one cell housing, or each type of the engineered cells is housed in separated housings. In one exemplary embodiment of multiple therapeutic delivery, multi-clock targeting with precision timing for circadian rhythm regulation can be performed by the proposed system, as discussed below.

[0095] Unlike bioelectronic or gated biofluidic systems that feature pre-filled (or even refillable) reservoirs of drugs, the proposed system delivers naturally-occurring peptides

throughout its functional lifetime without the need to stock, carry, or refill therapies that are vulnerable to loss, degradation, or that add to the already burdensome load carried by the user. The developed technology will serve as a platform whereby the optical control and feedback to achieve precision therapies can be applied to delivery of a broad swath of naturally occurring peptides/proteins by following the procedures and protocols described herein.

[0096] Thus, the disclosed system provides a hybrid bioelectronics platform and forms the basis and components for a number of bioelectronic and biohybrid tools to address or alleviate dysfunction and injury, to enhance readiness and performance, to treat pain, to treat disease, improves metabolism, and etc. The rationale behind the proposed system, along with details of its implementation, use, and testing are described in more detail below.

Biohybrid System For On Demand Therapy

[0097] Engineering a cell-based hybrid bioelectronic system for on demand therapy is a challenge involving a careful balance between cellular and bioelectronic device engineering. Several factors have to be taken into consideration to balance the strengths of each pillar and to minimize their drawbacks and deficiencies. Cells possess their own natural machinery to synthesize and release specific biomolecules. Furthermore, their machinery can be hacked to externally induce such production, but without the level of timing, control, and user interfacing that is possible with bioelectronics. On the other hand, optoelectronic components have inherent chemical and mechanical mismatches with cells and tissue that can limit their lifetime (degradation, rejection), or make them otherwise incompatible with reliable use in vivo. Striking the proper balance between the two is important.

[0098] Key design decisions that permeate the proposed system focus on (i) promoting long-term viability/efficiency and (ii) controlling and creating feedback-loop of therapeutic agent production. To promote viability of engineered cells, the proposed system both genetically engineers cells to be more resilient, and in some embodiments can also produce O₂ to support them. For control and feedback-loop, optical induction can be used. Compared to other cellular control mechanisms, optical induction methods enable fast response, tunable, localized induction properties (wavelength control), and are readily integrated into the platform with minimal power and size demands. Furthermore, optoelectronic cell interfacing enables innovative precision low-power dosing control. Fig. 2 is a table that depicts the rationale for using optical induction to

perform control and feedback in accordance with an illustrative embodiment.

Technological Components of the System

[0099] In an illustrative embodiment, the proposed system includes an implantable device, (e.g., subcutaneous implanted) featuring individually controlled cell housing, and an external wearable hub (extHub) (hardware and software) for power, user interface, and sensing. In alternative implementations, only the implantable device may be used. It should be noted that, in the application, the term “cell house”, “cell housing” and “cell well” are used interchangeably.

[00100] Fig. 3A provides an illustrative diagram showing the general structure of a unit of the bioelectronics device. As shown in the lower panel, the bioelectronics may comprise a unit of the implantable device 10 inside the body of a user, and an external hub 20 located outside the body of the user. The external hub 20 is in communication with the implantable device 10 for power charging and data exchanging/transmission. As shown in the upper panel, the implantable device 10 comprising a cell housing 11 for containing engineered cells 1000 which produces the therapeutic agents 1010 and a reporter agent/molecule 1020. A stimulator 17 for triggering the production of the therapeutic agents 1010 and the reporter agent/molecule 1020 by the engineered cells 1000 locates in the cell housing 11. A sensor 21 for sensing the reporter agent/molecule 1020 also locates in the cell housing 11. Both the stimulator and the sensor locate in vicinity to the engineered cells 1000 such that they effectively stimulate the production of the therapeutic agents 1010 and the reporter agent/molecule 1020 and sensing the production of the reporter agent/molecule 1020. At least one side of the implantable device 10 are coated or encapsulated with a permeable material/membrane 23 which shield the implantable device 10 from the immune system/cells of the user.

[00101] Fig. 3B provides an illustration diagram of another embodiment of the implantable device 30 which has multiple cell wells/housings 31. The implantable device has more than one cell well/housing 31 attached to an electronic layer 40. Each of the cell well/housing 31 has an individual stimulator 37 and an individual sensor 41. In one embodiment, the stimulators 37 and sensor 41 locate in vicinity to the engineered cells 1000 such that they effectively stimulate the production of the therapeutic agents 1010 and the reporter agent/molecule 1020 and sensing the production of the reporter agent/molecule 1020. In one embodiment, cell wells/housings 31 contain engineered cells producing same therapeutic agent 1010. In another embodiment,

different types of engineered cells 1000 producing different therapeutic agents 1010 may be each contained in a separate cell well/housing 31, such that the each type of engineered cells may be individually controlled by the stimulator and the sensor in each cell well/housing 31, so as to produce a particular therapeutic agents 1010 for a specific amount and/or at a specific time different from that of in the other cells/housings.

[00102] Fig. 3C depicts an alternative embodiment showing a subcutaneous NTRAIN device 110, including a cross-section that depicts method of operation and associated tasks for engineered components in accordance with an illustrative embodiment. The implanted subcutaneous device includes (i) genetically engineered allogeneic mammalian cells programmed to deliver peptide therapeutics in accordance with an optical trigger 117, (ii) hybrid synthetic biology/bioelectronic feedback control to provide precision dosing 121, (iii) O₂ generation capabilities/device 115, (iv) immune-isolating materials for enhanced cell viability and protection 123, (v) a custom application-specific integrated circuit (ASIC) 125 for low-power feedback control, temperature sensing, and power management, (vi) mm-scale magnetoelectric transducers 126 for wireless power and data/controls downlink, and (vii) a near field communication (NFC) coil 127 for wireless data uplink. In alternative implementations, the device can have fewer, additional, and/or different features.

[00103] In one embodiment, the implanted device is approximately 0.8 cm x 3 cm, with a thickness of about 2-3 mm, and bendable over a 1-centimeter radius of curvature. In alternative embodiments, different dimensions and/or radius of curvature may be used. Each cell well/housing 111 can include one or more isolated compartments (or enclosures). In one embodiment, each cell well/housing houses about 240 k cells, 2 x 2 x 1 mm in size. Alternatively, a different number of cells and/or a different compartment size may be used. At the base of the compartment is a bioelectronic carrier, on which control LEDs (stimulator) 117 initiate and stop peptide production. Specifically, an LED/photodiode pair (sensor) 121 is used to probe production of destabilized fluorescent proteins which are produced as a proxy for the delivered peptide, providing optical feedback of production levels, for closed loop dosage control. The compartment also contains O₂ generating particles or an O₂ generating electrochemical device 115 in one embodiment, which allows the system to have increased density of engineered cells within the chassis. In an illustrative embodiment, the housings that form the cell compartments can be made opaque by using opaque PDMS walls 113 between the

compartments 111 to minimize crosstalk of the optical control signals between cell compartments.

[00104] In one embodiment, the implantable device can be implanted subcutaneously, pericardially, intracranially, or intraperitoneally for delivery of the therapeutic agents, so as to customized to the subject's needs. In one embodiment, the implantable device can be implanted in a proper location for delivering the therapeutic agents either locally or systematically.

[00105] Fig. 3D illustrates another embodiment of the invention. Instead of an implantable device, this embodiment is directed to a wearable external device housing and controlling the engineered cells for production of therapeutic agents. The external wearable device 50 may include (i) a cell housing/cartridge 51 containing engineered cells, (ii) a replaceable cell media cartridge 52, (iii) a pump 53 pumping the media from the media cartridge 52 to the cell housing/cartridge 51, (iv) a cannula 58 extending from the cell housing/cartridge 51 and providing the therapeutic agents to a user, (v) a filter between the cell housing/cartridge 51 and the outlet of the cannula 58 for removing unwanted agents and compositions, (vi) a stimulating system 57 providing light source to the engineered cells housed in the cell housing/cartridge 51, (vii) a sensing system 61 detecting the fluorescent signals produced by the engineered cells for feedback control, (viii) a control unit 63 controlling the stimulation system 57 and sensing system 61, and (ix) a battery unit 65 providing power supply.

[00106] In this embodiment, the engineered cells are housed and supported in the wearable external device 50, which delivers the in situ synthesized and excreted therapeutic agents in a regulated manner via the cannula 58 into the body, e.g., subcutaneously, intraperitoneally, and etc.

[00107] In one embodiment, the cell housing/cartridge 51 can be a separate, replaceable modular chamber, so as to flexibly customize the production of the therapeutic agents. In one embodiment, the cell housing/cartridge 51 includes microcarriers.

[00108] In one embodiment, the stimulating system 57 providing light source(s) of one or more wavelength is disposed in vicinity to the cell housing/cartridge 51. The stimulating system 57 and the cell housing/cartridge 51 are aligned in a manner maximizing the engineered cells' exposure to the light source, e.g., substantially parallel with each other.

[00109] In one embodiment, fresh media can be exchanged in the cell housing/cartridge 51, and an on-board pump 54 circulated fresh media through the cell housing/cartridge 51 and

carries the excreted therapeutic agents through the filter 56 and into the body through the cannula 58. In one embodiment, the replaceable cell media cartridge 52 is replaceable and detachable modular, and the media inside the cell media cartridge 52 can be refilled or replaced.

[00110] In another embodiment, a user's interstitial fluid can be collected into the device through a different cannula, circulated through the cell housing/cartridge 51 and then through the filter, before being transferred back into the user's body through the cannula 58 or a separate delivery cannula attached to the cell housing/cartridge 51.

[00111] The pump system 54 and the control unit 63 are housed in the wearable external device 50. The battery 65 provides power supply to the wearable external device 50. The battery is replaceable and/or rechargeable.

[00112] The wearable external device can be worn by a user or can be attached to the user's skin with adhesive.

[00113] The engineered cells housed in the wearable external device 50 have optogenetic system which controls the production of one or more therapeutic agents upon receiving the light signal from the stimulating system 57. The coordination between the optogenetic system in the engineered cells and the stimulating system 57 and sensing system 61 is the same as described for the implantable device in Figs. 3A-3C.

[00114] In this embodiment, the wearable external device relieves the demand for the immune-isolating barrier and the external hub, which may be necessary for certain embodiments of the implantable device. In addition, the wearable external device permits a more flexible size and design choice for the cell housing/cartridge 51.

[00115] Fig. 19 illustrates another embodiment of the bioelectronics device system. As shown in Fig. 19, the system includes an implantable device 1902 implanted inside the body of the user, as described above. In addition, the system also includes a control system 1903, and one or more biometric sensors 1901. In one embodiment, the biometric sensor 1901 is an individual component. In another embodiment, the biometric sensor 1901 can be integrated into the implantable device 1902.

[00116] In one embodiment, the biometric sensor 1901 is implanted inside the user's body, either individually or integrated into the implantable device 1902. In one embodiment, the biometric sensor 1901 is a wearable device which is wore by the user external to the body. In one embodiment, the biometric sensor 1901 is a remote device in a remote location relative to the

user, e.g., a camera or a mobile device. In one embodiment, the biometric sensor 1901 is a combination of an implantable device, a wearable device and a remote device.

[00117] The biometric sensors 1901, as discussed above, collects the biometrics of the user, including temperatures, heart rate, blood pressure, chemical levels in blood such as blood oxygen, blood sugar, hormones, and etc. The collected biometrics are used to determine the appropriate therapeutic dose of the medications provided to the user by the implantable device 1902.

[00118] In one embodiment, the control system 1903 is integrated into the implantable device 1902. In one embodiment, the control system 1903 locates external to the user's body. In one embodiment, the biometrics collected by the biometric sensors 1901 are transmitted to the control system, which then determines the therapeutic dose for the medications and then controls the implantable device 1901 to provide medications to the user accordingly.

[00119] In one embodiment, the control system 1903 controls the implantable devices and biometric sensors according to a control algorithm. In one embodiment, the control algorithm can be trained based on a model for a particular indication, for example, jet lag or other therapies that are tied to circadian rhythms or therapies that are tied to reproductive cycles like fertility treatments. In one embodiment, the control system used to determine the therapeutic dose can be based on a model for the physiological response. In one embodiment, the model is developed using machine learning algorithms from collected and/or aggregate patient data, including biometrics collected by the biometric sensors, as well as patient's information input into the system, biophysical models of the dose response, or from individual patient data making the controller specific for each patient.

[00120] In one embodiment, the bioelectronics device/system of the present invention accommodates a patient's tolerability and/or response to the therapeutic agents delivered by the bioelectronics device/systems. In one embodiment, the present invention achieves this by gradually titrating up or down of dosages of the therapeutic agents being administered to the patient. In one embodiment, the bioelectronics device/system of the present invention accommodates the patient tolerability and/or response via a control algorithm. In another embodiment, the bioelectronics device/system of the present invention accommodates the patient tolerability and/or response via manual control by the patient or medical practitioners.

Implementation and Operation: Implantation, Implementation, and Life Cycle

[00121] In an illustrative embodiment, implementation of the proposed system involves implantation of the subcutaneous device in a subject. The subcutaneous device can be implanted via an outpatient procedure at approximately 2 cm or less below the skin in the abdomen. This implantation location can vary, and depends on the balance of comfort/adoption and systemic delivery efficacy. Additionally, in alternative embodiments, a different implantation location may be used such as fat, muscle, brain, heart, skin, hips, joints, etc. During use, the implanted device can be secured in a subcutaneous pocket to prevent movement. In some embodiments, the user is outfitted with an external hub in a harness and provided startup operation instructions via an application running on a user device in one embodiment. These instructions guide the user in how to place the external hub by monitoring a power coupling between the implant and the external hub.

[00122] In some embodiments of the device the current state of the patient would be evaluated before therapy in order to improve therapeutic timing and dosing. Generally, parameters for determining the dose, timing and etc. of a therapeutic agent delivery schedule are either detected by the external hub via sensing the relevant parameters, e.g., heart rate, blood pressure, core temperature activity status, locations of the user, and etc., or entered manually by the user or another via the external hub. Based on these parameters, a customized therapeutic agent delivery schedule is determined by the control unit of the implantable device or the external hub. Therefore, the delivery schedule can be precisely customized to the user's situation. Once the delivery schedule is determined, the user would be asked to initiate the schedule in the app or directly in the external hub, and confirm its execution by engaging a button on the external hub. The therapeutic schedule is then stored on board the external hub, which triggers the therapy at the appropriate times. Cancellation can be done by the user at any time through external hub or the app.

[00123] In one illustrative example regarding circadian rhythm control, before first therapeutic activation of a system designed to control circadian rhythm, the user undergoes a baseline period of approximately 3 days (typically at least 1 day) to establish circadian phase with respect to light/dark cycle. When requesting therapy, the user enters a value for a magnitude of an upcoming or recently experienced clock shift in terms of number of time zones, numbers of hours, etc. The magnitude value can be entered in an application in communication with the

external hub and/or subcutaneous device, or it can be entered directly into the external hub. The ideal therapeutic schedule (dose, duration, and timing of both peptide therapeutics) is determined by the device based on the magnitude of the clock shift. The user is asked to initiate the schedule in the app or directly in the external hub, and confirm its execution by engaging a button on the external hub. The therapeutic schedule is then stored on board the external hub, which triggers the therapy at the appropriate times. This procedure allows the therapy to be scheduled for delivery at times that may be inconvenient for the user to initiate. In an illustrative embodiment, the user is able to cancel a set schedule at any time using either the external hub controls or the application. Measurements from sensors confirm the progress of entrainment. Additional instructions related to suggested behavior can be implemented at the application or external hub level as appropriate (e.g., suggested use of sunglasses). At any time, the user can input a new shift, for example, return home, and initiate a new therapeutic schedule. Fig. 4 depicts operations performed to implement the proposed hybrid bioelectronics system in accordance with an illustrative embodiment. It is to be understood that other procedures, schedules, and user interaction can be used to treat other conditions.

[00124] In an ideal use case, the subcutaneous device can be implanted for a needed duration of time (e.g., length of a deployment, length of a project or job, etc.) and explanted via outpatient procedure once the duration of time ends. Depending on the materials used and the implementation, the proposed system can have a 60 day lifetime, a 130 day lifetime, a lifetime measured in years, etc. For example, it is anticipated that, using the technology described herein, the system could last for years and that repeated administration would be possible. In one embodiment, the engineered cells can be developed to include a genetically inducible safety kill switch to ensure that the cell therapy can be terminated should there be an untoward event during patient use. In the event that the device needs to be rendered non-functional, kill switch activation is initiated by an FDA-approved small molecule biologic. In an illustrative embodiment, viability of cells can be tracked optically to confirm efficacy of the kill switch.

Engineering Cell-Based Drug Factories

[00125] In an illustrative embodiment of a system used for circadian rhythm control, ARPE-19 cells are engineered to produce high levels of the desired therapeutic proteins (e.g., GLP-1 and Orexin A) on an optical trigger. In one embodiment, a melanopsin based optogenetic system

can be used. In other embodiments, a step-function opsin or dimerizable transcription factor (e.g., EL222) or split transcription factor (e.g., PhyB-TAD, DBD-PIF6) can be used. Additionally, the cells can be engineered to co-express a fluorescent reporter protein, for example, a destabilized GFP (GFP*) in a fixed ratio with GLP-1 and Orexin A, such as 1:1, allowing the system to observe the expression of GLP-1 and Orexin A in real time by using the easily readable destabilized GFP* fluorescence as a proxy. Additionally, a small-molecule-inducible kill switch can be engineered into the cells to allow for easy termination of the cells, rendering the device inactive. Fig. 5 is a graphical depiction of the proposed synthetic biology circuit for optogenetic control of the peptide therapeutic Orexin A in accordance with an illustrative embodiment. Preliminary data demonstrates the utility and feasibility of this architecture.

Optical Induction of Production

[00126] Each of the engineered cells have an optogenetic system. Using engineered cells enables the use of an optogenetic control system to control and produce the desired therapies. Using optogenetic systems, dosing can be controlled by modulating the amount of time that the cells are in the on state of LEDs. Cells are activated to the “ON” state by exposure to light from LEDs of the stimulating system housed within the bioelectric device. Cells in this “ON” state actively transcribe the therapeutic agents needed to produce the therapeutic.

Achieving Enhanced Dosing for Therapy

[00127] In order to engineer the cells to reach therapeutic dosing, and produce higher quantities of GLP-1 and Orexin A, a catalytically dead version of a CRISPR/Cas9 system (termed dCas9) can be used. The dCas9 system binds to a DNA site-specifically, but does not make any cuts or double-strand breaks. In an illustrative embodiment, the dCas9 can be deployed to recruit transcription activation domains to inserted copies of the NFAT promoter. This will allow amplification of the therapeutic protein and destabilized GFP (GFP*) in a stoichiometrically equal manner amenable to high throughput screening of activation levels and quantification of kinetics. Using this system enables target-agnostic gene activation in a highly specific manner and provides a toolbox of validated synthetic biology tools to tailor activation and kinetics to ideal levels, such as synthetic promoters (NFAT or others), protein degradation

tags, and 3'UTR variants among others to facilitate gene amplification only when desired.

Fluorescent Reporter

[00128] Since some embodiments of system utilizes the GFP*, co-expressed with the therapeutic, one can observe the production of the therapeutic in real time by observing the fluorescence from the GFP*. Since this protein has a half-life of approximately 7 minutes, it provides an accurate real-time indicator of production levels. This real-time observation of protein production enables feedback for precision dosing control, which can be quantified.

Resilience to Apoptosis

[00129] In an illustrative embodiment, the engineered cells are designed to be durable to apoptosis and senescence, which is important for prolonged and durable expression over the course of usage. To do this, parallel genetic screening is conducted to find genetic modifications that confer resistance to apoptosis and senescence, but that retain the ability for robust kill switch operation. By applying a selective pressure that elicits these phenotypes in the engineered cells (e.g., ARPE-19 cells) and then sequencing them, the system will enrich for cells harboring genotypes robust to these conditions. These genotypes are then recapitulated in an engineered cell line to be encapsulated as a living drug factory.

Kill Switch

[00130] An important consideration with cell-based therapeutics is that the body may reject the cells, leading to a harmful immune response. Additionally, the user may want to render the system inactive. To address this issue, a kill switch is engineered into the cells. Because it has been used in multiple clinical trials and has shown to be safe, the small molecule inducible kill switch iCaspase 9 (iCasp9) can be used in one embodiment. This will allow for the controlled apoptosis of the implanted cells by administering the small molecule AP1903. The molecule can be administered orally or intravenously in some embodiments. Alternatively, in in one embodiment, the system can feature a small on-board payload of the molecule to be released electronically. In other alternative embodiments, a different type of kill switch may be used.

[00131] As shown in Fig. 5, in an illustrative embodiment, plasmids are designed for therapeutic protein expression. In one embodiment, 4 plasmids can be used as follows: plasmid

(1), codes for a optogenetic system driven by a CAG promoter, to enable constitutive expression of the optogenetic system, e.g., production of opsin SOUL; plasmid (2) codes for therapeutic protein (i.e., GLP-1 or Orexin A) linked with GFP* via a linker such as P2A, all driven by pNFAT (activated by NFAT), plasmid (3) codes for dCas9 modification of protein expression levels and can include a unique pNFAT driving transcription of a dCas9 coding region fused to copies of the transcription activation domains p65 or HSF1, or to the human p300 acetyltransferase (p300), plasmid (4) codes for iCaspase 9 being driven by a CAG promoter.

[00132] The components of plasmid (3) can be non-virally-derived domains found in human proteins that activate gene expression and will be modulated in copy number to elicit desired amounts of expression. Downstream of plasmid (4) is a synthetic 3'UTR and a U6 promoter driving transcription of the gRNA to target the therapeutic gene promoter for activation. Each plasmid can have a different selection marker (e.g., puromycin, neomycin, blasticidin, and zeocin) and be engineered to have the backbone to allow for lipofectamine transfection with PiggyBac transposase genomic integration.

[00133] For cell engineering, in one embodiment, an allogenic human cell line, ARPE-19 (retinal pigment epithelium, or RPE), was chosen because it is non-tumorigenic, displays contact inhibited growth characteristics, is amenable to genetic modification, and has been shown safe in previous human trials. Genetic components can be introduced using the standard piggyBac transposase system to the engineered cells. Other transfection method commonly known in the art can also be used.

[00134] In vitro validation and optimization is also performed via fluorescence output and kinetics. For example, the system can measure GFP* after stimulation by blue light and orange light via a live-cell plate reader over the duration of expression. Using this as the basis for further engineering, expression is tuned to be stronger by modifying the dCas9 system as follows: 1) adding more copies of transactivation domains; 2) using stronger activators (e.g., p300); 3) adding more NFAT binding sites to the promoter region; 4) and/or tuning the Kozak sequence. In one embodiment, synthetic 3'UTR variants and degradation tags are used to control stability of the mRNA transcript and protein, respectively.

[00135] In vitro validation and optimization of therapeutic outputs is also performed. Therapeutic outputs can be monitored via qPCR, RNA-seq, ELISA, and Western blot across fixed intervals following stimulation by varying durations of blue light and orange light. GFP*

production can also be determined via fluorescence reading and compared to GLP-1 and Orexin A production by way of ELISA measurements to confirm a 1:1 stoichiometric ratio. Small molecule kill switch validation can also be performed. To show that the kill switch functions as expected, cells can be cultured with AP1903 (the trigger molecule), and cell viability can be assayed via live-dead staining at various time points after culturing. To determine apoptotic and senescence resistance, the system can also screen for senescence and apoptosis resistant cells using CRISPR guide RNA (gRNA) knockout libraries in combination with doxorubicin, cisplatin, and/or DMSO challenge for a total of 4 different screens (using DMSO as a control). Cells harboring resistance genotyped and iCaspase9 are administered to ensure that the kill switch retains function. Cell fitness, proliferation, viability, and expression levels can be validated through morphological evaluation, BrdU incorporation, MTT assay, and ELISA, respectively.

[00136] In alternative embodiments, an optogenetic system other than the above-discussed systems to perform cell activation may be used. Other optogenetic system that can be used include melanopsin, EL222 and PhyB/PIF6, which, while they do not have the trigger benefit, but are more established and are shown to work in multiple situations. Fig. 6 depicts preliminary data showing that ARPE-19 cells can be made to express luciferase with high on/off ratio in response to blue light using an EL222 optogenetic system in accordance with an illustrative embodiment.

Precision Control of Dosing based on Optical Feedback

[00137] To enable precise and controllable drug production levels despite changes in cell health, stress, and metabolism, a hybrid bioelectronic feedback control system can be created and used. This control system exploits synthetic biology to produce bioactive peptide therapies, and a bioelectronic layer for precise feedback control of production levels. Fig. 7 shows a biohybrid precision control scheme based on co-production of therapeutic peptide and proxy reporter fluorophore (for example, GFP*) in accordance with an illustrative embodiment. As shown, optoelectronics such as photodiode are used to sense and adjust optical stimulation periods to maintain a given setpoint for delivery of therapeutic agents.

[00138] To implement this hybrid feedback control system, light source of stimulation system (LEDs) can be integrated into the implantable device to drive optogenetic channels which

regulate therapeutic agent production in the engineered cells. To provide this control signal with minimal power consumption, step-function opsins that are activated and inactivated by different color LEDs are used. Specifically, below each cell housing/well in the implantable device are bonded Individual Cree UltraThin blue LED and Rohm semiconductor PicoLED series orange LEDs. In alternative embodiments, different types and/or wavelengths of light sources may be used. The blue LEDs provide the optical “ON” signal (e.g., 2s pulse) that turns on the step-function opsin, e.g., SOUL, leading to the elevated calcium levels in the engineered cells, as illustrates in plasmid (1) of Fig. 5, which in turn lead to the production of the therapeutic agents by the engineered cell, as illustrated in plasmid (2) of Fig. 2. The orange LED will provide an optical “OFF” signal (e.g., 2s pulse) that closes the step-function opsin. By tuning the interval between the ON and OFF signal (Δt), one can control the intracellular Ca^{++} levels and thus the therapeutic dose with a power savings of approximately 50X compared to traditional optogenetic control, as shown in Fig. 8. To make the dose levels precise, optoelectronics are integrated in the carrier and used to track the fluorescent reporters associated with each therapy. In an illustrative embodiment, the same blue LED used for the ON signal can be used as the excitation light source to track GFP* fluorescence.

[00139] Fig. 8 shows a comparison of traditional optogenetic control strategies that use constant illumination to activate the ion channels for the proposed step-function opsin control strategy that utilizes a blue LED to open the light-gated channels and an orange LED to close the channels in accordance with an illustrative embodiment. As discussed, by varying the interval Δt between the ON and OFF, one can control the intracellular Ca^{++} levels, which in turn determine production levels. As a result, the proposed techniques significantly reduce the average power consumption from $> 5 \text{ mW}$ to 0.1 mW .

[00140] In another illustrative embodiment, fluorescence measurements can be made by integrating a green emission light collected by the photodiode over the blue light stimulation block. The LED and photodiode performance can be measured in vitro by comparing fluorometry data to ground truth microscopy data that will measure LED timing, intensity, and fluorescence. In one implementation, lifetime testing can include soaking the encapsulated LEDs in phosphate buffered saline at 37°C for two months. For in vivo experiments to test photometry, fluorescent microspheres are encapsulated in the chassis and the fluorescence levels from the carrier implanted subcutaneously can be measured.

[00141] The GFP* emission is not expected to interfere with the optogenetic system activation state since the emission light is approximately 10^6 times weaker than the LEDs. Additionally, the feedback controller will account for any non-idealities by adjusting Δt to maintain a desired production setpoint. In an alternative embodiment, an alternative destabilized fluorescent protein such as DsRed-Express that can be excited using the orange “OFF” LED is used, such that any issues regarding cross-talk between the fluorophore and the control signals can be solved.

[00142] Figs. 9A-9B show an alternative embodiment of the optical system for the implantable device. Fig. 9A shows the design of the top side of a circuit board with the LEDs and photodiodes. In this embodiment, the light source of the stimulating system and the photodiode of the sensing system are integrated. Fig. 9B shows the LEDs integrating the blue and orange light into one wired prototype LED device with the wires extending out the right side of the LED device.

[00143] In Fig. 10, the LEDs device as shown in Fig. 9 were used in hybrid bioelectronic device having one or more cell wells/housings 31, as shown in Fig. 3A-D. One cell well/housing 31 contains an alginate capsule filled with non-GFP producing ARPE cells, while the other cell wells/housings contains an alginate capsule filled with GFP producing cells. In this embodiment of the device, the LEDs color was alternated between blue and orange during the “ON” period. As shown in Fig. 10 below, this alternating color between blue and orange showed that when the blue LEDs were illuminated, there was a clear difference in the photodiode readings that was not seen when the orange LEDs were on, as seen in panel B of Fig. 10. This difference is expected to be from the green fluorescent light that is present in the cell well/housing containing GFP producing cell. To further support this claim, a control test was performed by filling the housings with PBS only. As seen in panel A of Fig. 10 reflecting the control test, there was nearly no difference between the photodiode readings of the two cell housings even when the blue LEDs were on. This illustrates the notion that the difference in the readings was caused by the green fluorescence produced by the engineered cells.

[00144] Fig. 11 shows the testing of the integrity of the encapsulation of LEDs device and to ensure that the LEDs and photodiodes would be able to withstand conditions similar to the body. Specifically, a carrier containing the electrical components was placed into a saline solution for 14 days during which time it was continuously powered. If any of the saline solution were to reach the circuitry, the board would short and the LEDs would turn off. The board was visually

inspected daily to make sure that the LEDs were still illuminated.

[00145] The photodiode performance cannot be directly inspected by the visual inspection of LED light. However, if the saline solution penetrating the carrier, the photodiodes would be shorted due to the saline solution, and thus the LEDs would have turned off. Since the LEDs stayed lit throughout the experiment, it was assumed that the photodiodes did not fail during testing.

[00146] The results of the test reflects that the optical source of the stimulating system is capable for proving long term use after being implanted into the body cavity of a user, and the board showed no decrease in performance during or after the 14-day test, as the picture in Fig. 11 was taken after the 14-days period. No images of the board at the beginning of the test are provided since they look the exact same as the images in Fig. 11. This test not only proved that the encapsulation method was successful, but that the LEDs and photodiodes were able to work after encapsulation and even when submerged into a saline solution.

[00147] In one embodiment of the invention, similarly sized photodiodes and LEDs were selected for the device so that the LED light would not be blocked by the photodiode. Additionally, the photodiodes are in series with a 100 k Ω resistor to create a measurable voltage. It should be noted that placing the LEDs directly next to the photodiode did lead to noise in the fluorescent photovoltage readings. When the blue LEDs were on, the photovoltage readings averaged approximately 960 mV. The orange LEDs generated a small photovoltage, but since these LEDs will not be illuminated during fluorescent readings, the photovoltage is inconsequential.

[00148] In order to prevent the blue light from reaching the photodiode, the LED light is filtered. The chosen filter was the Wratten 12 filter by Kodak. The Wratten 12 filter is a thin photography filter that acts as a long pass filter with a cut-on wavelength of approximately 500 nm. According to the absorption spectra, the filter should block 99.8% of the blue excitation light. As shown in Fig. 12A, based on the measured absorption curve transmission of the GFP emission light was expected to be 20%. In other embodiment, other filters having approximately same cut-on wavelength may be used.

[00149] In one embodiment, the thin film filter was bent into a box shape using a 3D printed mold. The filter was first laser cut into a cross shape and then the flaps were bent with the mold to form the box. This box was then laid on top of the photodiode and adhered to the photodiode

using optical adhesive (NOA 84). This procedure is shown in Fig. 12B. In other embodiment, the filter can be formed into other shapes, and cutting and adhering of the filter can be achieved with any known tools in the field of arts.

[00150] Photovoltage measurements were taken again after the addition of the filter and the results shows that the filter effectively block the blue light. In particular, as shown in Fig. 12C, comparing to the blue light source without being encapsulated in the filter, the blue light encapsulated in the filter no longer generates any photovoltage, while the orange light still generated the same low photovoltage as before.

Device Mold and Materials

[00151] In one embodiment, the structure of the prototype is formed by pouring liquid PDMS into a mold and then letting it cure. Many iterations of mold designs were tested, and once the best design was found, the mold was machined out of aluminum, as shown in Fig. 13A. This was necessary to prevent the carrier from sticking to the mold which was seen with all 3D printed molds.

[00152] The shape of the mold allowed for bubbles to become trapped at the top, which would form voids in the device. These voids are points of failure for the device and therefore needed to be removed. A large vacuum glove box was purchased so that the PDMS could be poured into the mold under vacuum. When the mold is returned to atmospheric pressure, many of the bubbles pop and any that do not rise to the surface where they are manually popped with a heat gun. This molding technique has led to bubble-free devices and has expedited the molding process.

[00153] The mold design was made so that the circuit board designed in that subtask would fit into the mold and be encapsulated inside of the PDMS. Encapsulation of the circuitry protects the board during use in a saline solution and thus in the user's body. The mold design also took into account the need for cell housings/wells that will be located in the implantable device. The cell wells/housings are formed during molding by placing a comb, similar to electrophoresis gel combs, into the liquid PDMS. The cell wells/housings were successfully formed and are located directly over the photodiodes on the circuit board as shown in Fig. 13B.

Protecting Cells from Immune Response

[00154] The implantable device is also designed to protect the engineered cells from the detection and destruction by the host's immune system. Furthermore, when cells are isolated, they may not receive the oxygen and nutrient supply needed to stay alive or productive, which can limit total cell loading, density, and/or efficacy. The present invention balances between protection against the user's immune response and encapsulated cell viability. In one embodiment, the implantable device uses permeable or semi-permeable membranes, such as track-etched polycarbonate or polytetrafluoroethylene (PTFE) have small pores, but at relatively low density, which can limit diffusion of nutrients, oxygen, or produced drug across the membrane, while protect the engineered cells from the user's immune response.

[00155] In one embodiment, a multi-layer membrane can be implemented, with hierarchical or layered pore structures and/or (bio) material coatings to both isolate the engineered cells from the immune system and promote vascularization near the membrane interface to promote oxygenation. To mitigate the foreign body response (FBR) of the host, the membrane contains sub-micron pores which do not allow for immune cells to transit the membrane, whereas micropores on the tissue side of the membrane will facilitate vascularization. The pore structure can be hierarchically controlled throughout the membrane in a layered manner. In another illustrative embodiment, the membrane surface can be functionalized with either heparin to bind endogenous growth factors or antifouling molecules. Increasing vascularization at the interface facilitates oxygen and nutrient diffusion, hence boosting cell metabolism.

[00156] Pore size is characterized by SEM in one embodiment. Additionally, heparin can be further functionalized on the membrane to bind endogenous growth factors, thus, promoting vascularization. Oxygen diffusivity and glucose permeability of the membrane are tested using an oxygen probe and glucose assays to ensure that the requirements for immune-isolating membranes are met (e.g., oxygen diffusivity of 4×10^{-3} cm/s and glucose permeability of 150 $\mu\text{g/h}$). Both in vitro human fibroblast (HDF) and endothelial cell (HUVEC) viability and proliferation are also be evaluated. Biocompatibility/stability of the membrane and the ability of the membrane to be combined with cells and in vivo cell viability can further be investigated by histology in both the normal (C57BL/6J mice) and immunodeficient (NU/J mice) mice model. The proposed advances enable enhancement in cell viability and drug production, well over 30 days in vivo.

[00157] In another embodiment, commercially available TheraCyte (ePTFE) membranes is

modified for enhanced vascularization with nanoporous polymer layers and heparin coating. In one embodiment, to enhance the protection of the engineered cells from the immune system/cells of the user, immune-evasive small molecule coatings is added to the fabrication process for the membrane. It has been shown that a handful of molecules can convey immune-evasive properties to material once coated on the surface. In one embodiment, these small molecule coatings can be added to the material to bolster its ability to mitigate the immune response.

Wireless Power and Communications and ASIC Development

[00158] The present invention also significantly improves the ability to provide efficient, safe, and reliable wireless power, communication, and control for the implantable device through biological tissue. Existing technologies, based on radio frequency (RF) electromagnetics, magnetic induction, or ultrasound, have severe limitations in providing the anticipated 1 mW average power to the implantable device if it is implanted approximatively 1 cm beneath the skin. Electromagnetics (EM) power transfer faces a fundamental tradeoff between antenna size and maximum allowable energy, because tissue absorbs higher EM energy at smaller wavelengths, which are required for small antennas. Ultrasound provides an alternative for wireless power. However, ultrasound suffers from impedance mismatches between air and tissue, and thus ultrasonic gel is typically required, which would be cumbersome during extended use by a system user.

[00159] To overcome this challenge, it has been shown that magnetoelectric (ME) materials enable wireless power delivery more than 8 centimeters beneath bone and tissue, which is possible because low-frequency magnetic fields are not absorbed or reflected by the body. It has further been demonstrated that ME materials can be integrated with CMOS chips to create regulated power supplies and transfer data to implants. Importantly, this technology allows one to effectively deliver power and commands to miniature implantable devices without the need for any impedance matching gels or liquids.

[00160] Results also suggest that ME transducers are inherently less sensitive to misalignments and angle changes with the external transmitter than inductive coupling, which ensures that the minimum required power can be delivered even under movements of a user. In one embodiment, a near-field communication (NFC) scheme by inductive backscattering can be used for uplink data transmission. The sensitive NFC protocol can be used for the uplink because

compared to the power and data downlink, the uplink is less sensitive to loss and alignment errors. The larger antenna and stronger computational and filtering capabilities of the external device allows it to extract the uplink data from noisy backscattered signals.

[00161] Fig. 14A-D illustrate one embodiment of the ME system. To demonstrate the effectiveness of the magnetoelectric technology (ME) for wireless power transmission through porcine tissue, a ME film was fabricated using a 30 μm -thick layer of Metglas 2605SA1 (Metglas Inc) attached using epoxy to a 270 μm -thick layer of PZT-5A4E (Piezo Inc.). The sheet is cut using a laser cutter to a miniaturized 7*2.4 mm films, and Fig. 14B shows the size and thickness as compared to a penny coin. For such films, the mechanical resonance frequency is found to be 240 kHz.

[00162] The ME films is powered using a low magnetic field that can be generated using a wearable, battery-powered transmitter. To design such a transmitter, rechargeable lithium-ion batteries, H-bridge, microcontroller, Bluetooth module, DC magnet, resonance coil of a circular spiral coil and capacitor bank are integrated as shown in Fig. 14A. To test the proposed system performance, the ME film and the transmitter coil are separated by a 2 cm thick porcine tissue as shown in Fig. 14C. The AC field generated by the coil =1.1 mT whereas the measured open-circuit voltage at the ME film terminal =7 Vp-p.

[00163] The output power generated by the ME film depends on the connected load, hence, different resistive loads are connected to the film terminal and the load voltage is measured to compute the output power as shown in Fig. 14D. As can be seen, the output power is greater than 2 mW for the different loading conditions.

[00164] One of the main implementation challenges arises from fluctuations of ME induced signals caused by body movements, the stringent power budget, and heating limits of the carrier. One way to mitigate the challenges is to make the system adaptive to external conditions and workloads, which increases robustness and reduces testing/calibration efforts over the conventional design methodology using pre-defined specifications and tolerance. In one embodiment, critical information (system clocks, data decoding thresholds, and transitions between different modes) can be extracted from the real-time ME induced signals. The single-input, multi-output power management unit is adaptive to workload changes and spends minimum power to provide regulated supplies that accurately meet the diverse needs (voltage, ripple, load current) of each module. The goal is to minimize power waste and unnecessary

heating due to excessive headroom and quality of outputs.

[00165] Moreover, the power management unit is able to monitor and report the received power and produced voltage to enable efficient control of the external hub's transmitter output power, and to provide a measure of the relative distance between the implant and the external hub, which enables simple tracking of the implant. In addition to system-level optimizations, ultra-low-power and highly digital circuit techniques have been developed to achieve comparable performance as conventional high-precision analog circuits at much lower power and complexity. Minimizing the power consumption of all chip components effectively increases the end-to-end efficiency from power source to bioelectronics, leading to reduced heating and better tolerance to body movements, and longer battery lifetime of the external hub.

[00166] In certain embodiments, the hybrid bioelectronics system, instead of a wireless power charging system, may have a battery on board. In one embodiment, the battery is replaceable and/or rechargeable.

External Sensing and Communications (External Hub)

[00167] As discussed herein, the external hub is a battery powered wearable that provides power, communication, processing, storage, and a user interface to monitor and control the implant. In some embodiments the external hub is not used. In one embodiment, the external hub clicks securely into a socket in a comfortable, adjustable harness positioned on the abdomen or other area on the host that is proximate to the subcutaneous implant. Alternatively, a different mounting technique may be used. The external hub serves as a gateway for all data and control, and includes a multi modal sensor suite designed to understand human behavior— such as sleep rhythms. The external hub provides controls and a display on the hub itself, in a protected pocket, or by connecting to a phone application. The phone application, beyond providing a mechanism for user input and control, also provides a way for the user to localize the implant and perform initial setup and system configuration. In alternative embodiments, a phone application may not be used, and all control and functionality can be performed through the user interface of the external hub, which can include a touchscreen display, one or more buttons or other controls, etc. The external hub is designed to enable long wear time, provide bio-sensing ability, enable intuitive controls, and be reconfigurable for diverse applications, including supporting the proposed entrainment therapies and future therapies.

[00168] In one embodiment, the external hub can be built iteratively, verifying and testing novel functionality as more advanced prototypes are designed. As one example, a desktop, wall powered system with essential components can be designed, so that low level software development can begin at speed. A portable prototype can be developed for testing and validating sensors to monitor circadian rhythms, where the portable prototype includes only the external sensors, and a data acquisition unit/ microcontroller with built-in telemetry functions for data offload to a nearby desktop, thereby facilitating circadian rhythm sensing for NHPs. Concurrently, power circuitry and battery lifetime management circuitry can be handled in a more portable prototype holding all functionality beyond just sensing. This also enables harness and enclosure design to begin, and for the external hub to be further miniaturized until the final device with all functionality and user controls is delivered. This sequence of hardware designs enable the handling of various device challenges, which are described below.

[00169] In commercial off the shelf wearables, battery lifetime can vary dramatically based on the actions of the user, the software running, the signals environment, and the internal components that are activated. While running out of battery is merely an inconvenience for the average person, for a user of the proposed system it may severely hamper mission/work readiness. Reliability and predictability of battery lifetime for complex cyber-physical systems like the external hub is critical but challenging because of the intersection of software, hardware, and user non-determinism. Therefore, a robust energy model is embedded in the firmware of the external hub that is circadian rhythm and environment aware, enabling prediction of external hub lifetime with high accuracy, such that completion of therapies and the mission/work is ensured. The energy modeling can include complex cyber and physical components, including the implant operation, a physical signals environment, sensing algorithms, user interaction modeling, and therapy delivery. This static model can be augmented by in-situ power measurements and execution traces such that the static model is continuously refined. This energy model is a core portion of the external hub operating system, and can leverage embedded energy models to enable ultra-long (e.g., nine month plus) battery lifetime.

[00170] In an illustrative embodiment, the external hub uses non-contact COTS sensors to measure and track diurnal variations in body temperature, heart rate and variability, and physical activity, which are inputs to a model of circadian control that estimates the circadian phase of the wearer. Leveraging the sensing capability of the external hub for circadian phase monitoring,

instead of sensing in the implant, allows for a smaller sized, more comfortable, and lower power implant. The system can also use well studied sensors for sleep and activity monitoring, such as ballistocardiography based heart rate sensing and accelerometer based actigraphy.

Ballistocardiography (BCG) is a measure of ballistic forces generated by the heart, enabling measurement of R-R interval. Accelerometers can measure these forces even if not directly above the heart, or even attached to the body (for example, R-R interval was captured when an accelerometer was placed under the mattress of a sleeping person). As the system will continuously sense circadian phase, estimates of heart rate will occur even when the wearer is active. As such, a 9-axis inertial measurement unit is used that allows for removal of gravity effects and motion artifacts, and provides orientation of the wearer (from the magnetic sensor), to understand posture. Actigraphy methods can be used to separate sleep activities from confounding activities such as exercise, eating, or walking. Infrared based skin temperature sensors are also included to validate/calibrate internal temperature from the implant, and provide a coarse estimate of heat flux based on known values of thermal conductivity of human skin, which can be used to estimate core body temperature. The external hub captures all raw signals from these sensors, cleans the signals, and extracts relevant biomarkers for sensing circadian phase. Software machine learned models that reduce noise are developed for each sensor.

[00171] The user of the proposed system needs mechanisms to control therapies no matter the situation (before a mission, when traveling, while working, in the field, etc.). Physical controls of the system are designed with visual feedback on the external hub to program, stop, and start therapies in the field. In an illustrative embodiment, the controls lie in a protected compartment in the enclosure to ensure no accidental actions. The controls can be mirrored on a smartphone application with the same capabilities in one embodiment. This provides seamless control no matter what situation the user is in. The smartphone application connects with the external hub using an encrypted Bluetooth LE channel in one embodiment, and allows for richer visualization of entrainment progress. As a result, the wearer can understand the effect of designed therapies in real time. This innovative, mirrored, multi-context interaction approach provides a new way to think about and visualize on the go applications for users in high stress, highly mobile environments.

[00172] In one embodiment, the enclosure for the external hub protects the circuitry in a slim profile watertight package. In one embodiment, controls are placed in a protected pocket to

prevent accidental button presses. The enclosure is designed iteratively, in a large functional form factor, then miniaturized, hardened, and waterproofed. In one embodiment, on first use, the external hub is placed in the harness, and the localization procedure is initiated from the smartphone app (or alternatively on a user interface of the external hub). The application (or external hub itself) guides the user on which direction to move the external hub for optimal power based on measuring received signal strength from the implant NFC uplink. Once the location is set, the harness is tightened to secure the placement. The harness is co-designed along with the external hub based on existing abdomen harnesses that secure items like radio transmitters, etc.

[00173] Security of communication, and security of operation are critical for delivery of entrainment therapies. This problem is addressed by the proposed design. Specifically, ME communication is highly localized, making it difficult to emulate control commands sent from the external hub to the implant. A highly sophisticated attacker would need to be uncomfortably close to the implant location to interfere with operation. In an illustrative embodiment, the implant will confirm commands it has received via the NFC uplink with the external hub to ensure the command originated from the external hub. In another embodiment, a co-processor model is used that involves processors operating in tandem to continuously verify software operation. The smartphone application, if used, can connect with the external using an encrypted Bluetooth LE channel, reducing possibility of malicious data exfiltration. Alternatively, a different secure communication channel may be used to perform communication between the user device (e.g., smartphone, tablet, laptop, etc.) upon which the application is located and the external hub. Security checks can also be performed at each layer of the software/hardware stack in the external hub, which will further reduce the possibility of tampering and data exfiltration.

[00174] In the event of issues with battery lifetime being shorter than anticipated due to ME power costs, the size and/or shape of the external hub can be adjusted to support a larger battery. Further, if it is determined that the non-contact sensor selection is not sensing heart rate accurately enough for the circadian phase sensing algorithm, electrodes for EKG can be placed on the harness itself, utilizing skin contact.

Buffer/Nutrient Solution for Cell Wells/Housings

[00175] In one embodiment, RZA 15 is used as a suspension solution to suspend engineered cells in the wells/housings. RZA15 is a molecule that will help protect the cells from fibrosis in vivo promoting cell viability. RZA15 was synthesized in the following manner as shown in Fig. 15A.

[00176] In particular, in one embodiment for synthesizing the RZA15, 4-Propargylthiomorpholine 1,1-Dioxide (1 eq.) was added to a 250 mL round bottom flask and dissolved in methanol: water mixture (5:1). Consequently, Tris[(1-benzyl-1H-1,2,3-triazol-4-yl) methylamine (0.25 eq.), Triethylamine (0.25 eq.), and copper iodide (0.1 eq.) was added. The reaction mixture was purged with argon for 15 mins and cooled to 0°C following which 11-azido-3,6,9-trioxaundecan-1-amine (1 eq., 6.30 g, 28.86 mmol) was added. The reaction mixture was stirred at room temperature for 15 mins and afterward heated to 55°C for overnight. The reaction was cooled to room temperature and filtered through celite to remove any insoluble part. The filtrate was dried using rotavap under reduced pressure with silica. The crude reaction was then purified by liquid chromatography with dichloromethane: ultra (22% MeOH in DCM with 3% NH₄OH) mixture 0% to 40% on a 120 gm ISCO silica column. The final product was further characterized with ESI mass and NMR mass spectroscopy according to Fig. 15B.

[00177] Once the RZA5 is synthesized, it is conjugated to UPVLVG alginate to be used as the hydrogel to suspend cells in the carrier. The conjugation was then carried out in the following manner as shown in Fig. 16A.

[00178] In one embodiment for conjugation, in a round bottom flask, 2g (1eq) of UPVLVG (BP-1903-04; Novamatrix) was dissolved in water (75 mL). Then RZA15 small molecule (3.99 g, 10.20 mmol, 1eq) was dissolved in water by vortexing and the pH was adjusted to 7.4 using HCl. Then RZA15 aqueous solution was slowly added to the UPVLVG while stirring. Subsequently a solution of (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride) (DMTMM, 0.5 eq.) was added dropwise to the mixture of UPVLVG and RZA15. The reaction was heated to 55°C and stirred overnight. The solution was filtered through a cyano-silica and dialyzed in a 40cm long 10-12K pretreated dialysis tubing in a beaker using saline (2 days) and mili-Q water (3 days). The dialyzed solution was frozen at -80 degrees and lyophilized until dry. The final product was then characterized by NMR and elemental analysis with elemental analysis showing a 16% modification of the alginate material. NMR and elemental characterization of the conjugates is shown in Fig. 16B.

Supporting and Controlling Cells in a Bioelectronic Carrier

[00179] One key challenge for implementing a feedback control on the bioelectronic carrier is to engineer sufficient energy efficiency to operate within the desired wireless power budget. In one embodiment, a step-function control strategy is used to reduce the power needs. In one embodiment, the step-function control strategy reduces the power needs by approximately 50X. In addition to reducing the average power consumption, the inventors also designed the system to minimize the peak instantaneous power requirements, to avoid the need for large energy storage elements on the device. To reduce the need for large peak power, LED control sequences can be interleaved within 10-minute blocks so that only one LED will be active in any cell housing/well at any one time. Additionally, the seconds scale activation and inactivation pulses required to turn on and off the optogenetic system channels can be converted into high frequency (50 Hz) pulse trains with the same total energy. In one embodiment, these two strategies offer an approximately 10X reduction in peak power that allows for miniaturization of the charge storage elements. Light-leakage and optical cross talk between wells can be reduced in some embodiments by doping the PDMS with gold NPs that will render the walls opaque without compromising the permeability and elastomeric properties of the material. To ensure that the LED and photodiode remain functional inside the body, Parylene C, Parylene N, SiC (Silicon Carbide), and Medical grade epoxies can be used for effective bioencapsulation. In addition, the photodiode can be coated with a combination dielectric and absorption filter which has been shown to be effective for on-chip fluorescent imaging.

[00180] To enable the proposed high-density cell loading, oxygen can be supplied to the encapsulated cell well/housing. This can be accomplished via electrolysis of water at adjacent microelectrodes in one embodiment. In another embodiment, O₂ gradients are tailored and maintained to optimize the performance of encapsulated cell well/housing by precisely tuning the platform's electrode size, spacing, and power supply. The use of selective polymer membranes can be used to minimize reactive byproducts and protect the device from bio-fouling. A primary goal is to generate enhanced oxygen concentration on a small device footprint and with a low overall power budget. In alternative embodiments, depending on the type of cell(s) being used or the cell density, O₂ may be supplied with oxygen producing CaO nanoparticles, or O₂ may not be supplied to the cell well/housing at all.

Cell-Carrier Integration and Validation

[00181] Another challenge in creating the proposed system is integration of the bioelectronic cell housing with the living engineered cells. To overcome this challenge, the inventors developed a biohybrid co-fabrication strategy using an established program for non-traditional fabrication of bioelectronics and the developed cell encapsulation strategies. In one embodiment, the carrier and cell chassis are fabricated separately and combined using a biocompatible epoxy-based glue. The system can be sterilized using EtOH and placed in a double sterile sealed container. Immediately prior to implantation, the system can be unsealed and cells can be injected through injection ports in the carrier and sealed with the FDA-approved biocompatible epoxy glue by the clinician at point of application. The system was designed in this manner to account for ease of commercial manufacturing and shipping, and in accordance with regulatory guidance.

Cells Loading Aid Device

[00182] Fig. 17 shows a cell-loading aid device 900 responsible for loading the engineered cells into the cell wells/housings of the hybrid bioelectronic device. The aid device 900 has a base 910 and a guide 940 attached to one longitudinal side of the base 910. The base 910 has a niche 920 for receiving the hybrid bioelectronic device. In the guide 940, there exists at least one hole 930 through which the engineered cells are injected into the cell wells/housings.

Computing System

[00183] In an illustrative embodiment, components of the proposed system such as the subcutaneous implant device, external hub, and/or associated user device can include and/or be in communication with one or more computing systems that include a memory, processor, user interface, transceiver, and any other computing components. Additionally, any of the operations described herein may be performed by the computing system(s) of these components. The operations can be stored as computer-readable instructions on a computer-readable medium such as the computer memory. Upon execution by the processor, the computer-readable instructions are executed as described herein. As an example, Fig. 18 is a block diagram of a computing system to perform operations described herein in accordance with an illustrative embodiment.

[00184] Specifically, Fig. 18 depicts one embodiment of a computing device 1400 (e.g., an external hub) in direct or indirect communication with a network 1435, one or more user devices 1440, and a subcutaneous implant device 1445. The user device(s) 1440 can include a smartphone, tablet, laptop, smartwatch, activity tracker, or other user device that is in communication with the computing device 1400. As discussed, the user device(s) 1440 can include an application that interfaces with and controls the computing device 1400.

[00185] In this illustrative embodiment the computing device 1400 includes a processor 1405, an operating system 1410, a memory 1415, an input/output (I/O) system 1420, a network interface 1425, and power/sensing hardware and software 1430. In alternative embodiments, the computing device 1400 may include fewer, additional, and/or different components. The components of the computing device 1400 communicate with one another via one or more buses or any other interconnect system. Although not depicted in Fig. 18, it is to be understood that the subcutaneous implant device 1445 and the user device(s) 1440 can similarly include computing components such as a processor, an operating system, a memory, an input/output (I/O) system, a network interface, power/sensing hardware and software 1430, and/or any of the other computing components described herein.

[00186] The processor 1405 of the computing device 1400 can be in electrical communication with and used to control any of the external hub components described herein. The processor 1405 can be any type of computer processor known in the art, and can include a plurality of processors and/or a plurality of processing cores. The processor 1405 can include a controller, a microcontroller, an audio processor, a graphics processing unit, a hardware accelerator, a digital signal processor, etc. Additionally, the processor 1405 may be implemented as a complex instruction set computer processor, a reduced instruction set computer processor, an x86 instruction set computer processor, etc. The processor 1405 is used to run the operating system 1410, which, as discussed herein, can be a custom operating system specific to the requirements of the external hub.

[00187] The operating system 1410 is stored in the memory 1415, which is also used to store programs, sensed patient data, algorithms, network and communications data, peripheral component data, and other operating instructions. The memory 1415 can be one or more memory systems that include various types of computer memory such as flash memory, random access memory (RAM), dynamic (RAM), static (RAM), a universal serial bus (USB) drive, an optical

disk drive, a tape drive, an internal storage device, a non-volatile storage device, a hard disk drive (HDD), a volatile storage device, etc.

[00188] The I/O system 1420, or user interface, is the framework which enables users and peripheral devices to interact with the computing device 1400. The I/O system 1420 can include one or more keys or a keyboard, one or more buttons, one or more displays, a speaker, a microphone, etc. that allow the user to interact with and control the computing device 1400. The I/O system 1420 also includes circuitry and a bus structure to interface with peripheral computing components such as power sources, sensors, etc.

[00189] The network interface 1425 includes transceiver circuitry that allows the computing device 1400 to transmit and receive data to/from other devices such as the subcutaneous implant device 1445, the user device(s) 1440, remote computing systems, servers, websites, etc. The network interface 1425 enables communication through the network 1435, which can be one or more communication networks. The network 1435 can include a cable network, a fiber network, a cellular network, a Wi-Fi network, a landline telephone network, a microwave network, a satellite network, etc. The network interface 1425 also includes circuitry to allow device-to-device communication such as near field communication (NFC), Bluetooth® communication, etc.

[00190] The power/sensing hardware and software 1430 can include hardware, software, and algorithms (e.g., in the form of computer-readable instructions) which, upon activation or execution by the processor 1405, performs any of the various operations described herein such as sensing data, receiving sensed data, performing analyses of sensed data, generating control signals, generating power and controlling power usage, etc. The power/sensing hardware and software 1430 can utilize the processor 1405 and/or the memory 1415 as discussed above.

[00191] In an illustrative embodiment, the subcutaneous implant device 1445 can be any of the implant devices described herein, and can include any of the functionality/components described herein. In one implementation, the subcutaneous implant device 1445 can include an electronic layer that can include one or more actuators to control cell production, one or more sensors, an ASIC, a processor, a memory, a battery, a transceiver, etc. Attached to the electronic layer is a biological cell layer that includes engineered cells. In an illustrative embodiment, the engineered cells are within a hydrogel that forms at least a portion of the biological cell layer. The hydrogel can be within a chamber that is accessible to the sensor(s) and/or actuator (e.g., a

transparent bottom of the chamber can be used if the cells are actuated via optoelectronics). In another embodiment, the implant device 1445 can be separated from the body by a membrane that allows diffusion of small molecules but blocks cells (i.e., does not allow immune cells in, or engineered cells out).

Circadian Rhythm Control

[00192] In one exemplary implementation, the proposed system can be used to help control the circadian rhythm of the subject in which the system is implanted. For example, the system can be used to accelerate human adaptation to a new time zone or work schedule by synergistically shifting central and peripheral circadian clocks. While various examples and implementation details are provided herein with respect to control of circadian rhythm, it is to be understood that the proposed system is not limited to circadian rhythm applications. Rather, as discussed herein, the proposed implantable cell generation system can be used to provide pain relief, fight diseases, cure disorders, provide immune response control, treat infertility, etc.

[00193] It has been shown that desynchrony between multiple circadian clocks and the light/dark cycle can result in decrements in mental performance and health and act to hinder entrainment. Traditional methods to accelerate adaptation, or entrainment, focus on light therapies or fixed-dose, single-target medications that may not be ideally timed or efficacious. These approaches focus largely on central clock-driven rhythms such as the sleep/wake cycle, without considering adverse effects of misaligned internal clocks that entrain more slowly than central hypothalamic clocks. To most effectively accelerate entrainment, the proposed system engages the entire network of a host's central and peripheral clocks with precise control of the timing and dose of peptide therapies. This level of precision exceeds what is possible with current therapies. Namely, the proposed system is able to achieve 1) specific biological action on select target receptors or molecules that cannot be accomplished with current bioelectronics, and 2) precise control of timing and dosage that cannot be accomplished with current synthetic biology.

Sensing Circadian Phase

[00194] In an illustrative embodiment, a multi-sensor fusion strategy is used to accurately measure the phases of the multiple 24-hour rhythms that are disrupted by long-distance travel

and late-night operations. Specifically, biophysical, physiological, and behavioral markers are measured to track multiple rhythms and overcome confounding factors (like physical activity) that could mask a CR measurement based on any singular sensing modality. Real-time assessment of circadian phases of both central and peripheral clocks enables precise timing of therapeutics. Existing, robust sensor technologies, including internal and skin surface temperature, 9-axis inertial measurement units, and heart rate sensing techniques are used. These parameters exhibit robust circadian rhythms and are can be used to access the circadian timing. The timing of these rhythms are regulated by distinct SCN output pathways as well as by different local tissue physiology and environmental timing cues, thus together depicting the status of the hierarchical circadian system as a whole. In alternative embodiments in which the cell factory is used to treat pain, manage diseases, etc., sensor data may not be used. In other embodiments, different types of data may be sensed, specific to the non-circadian application.

[00195] The sensor data is used as inputs to a well-established model of circadian control to produce an estimate of circadian phase and predict phase shifts in response to stimuli and delivery of therapies. The most common approach is to model circadian control as a limit cycle oscillator. These models simulate the rate of change in state variables (e.g., core body temperature, activity, heart rate) as functions of the current status of state variables plus “drives” from external stimulus (e.g., entraining agents such as light or therapeutic peptides). Using such models, the current internal timing of the animal (i.e., circadian phase) can be resolved from the values of the state variables, and predictions of phase-shifts induced by light and/or therapeutic peptides can be made at any phase of the cycle.

[00196] Since diurnal variations in each of the selected biomarkers reflect different facets of circadian control, a unified measure of circadian phase is generated that incorporates all 3 biomarkers as state variables in a single “limit sphere” model. Each state variable can have a different driving function in response to light or peptides to represent their different rates of entrainment. In this way, the model can also detect misalignment between the phase reference points of different state variables during re-entrainment. Estimates of circadian phase are measured as a percent error relative to reference measures obtained with a fully implanted sensor system that is the standard used in most sleep studies. In addition to data collected for model development and testing, data of peptide-induced phase shifts in mice can also be used to facilitate the selection of model framework. The model can be fine-tuned to direct daily peptide

treatment schedule to achieve accelerated entrainment.

[00197] Testing can be performed in subjects instrumented with a standard suite of sensors used in sleep research, including electroencephalography (EEG), electrooculography (EOG, eye-movements), and electromyography (EMG, neck muscles) that will provide gold-standard reference measures of circadian phase for comparison with the NTRAIN sensor suite. Data can be collected continuously around the clock via COTS implantable telemetry devices that integrate all of the required sensing functions in a fully implantable, battery-powered package that can transmit data continuously for long periods of time. An initial prototype of the NTRAIN sensor set (external hub) will be implanted to verify sensors in vivo prior to full integration of the external hub. In addition to performing continuous monitoring of physical and physiological biomarkers, established behavioral assays of cognitive function (e.g., working memory, attention) are also implemented to measure changes in cognitive performance throughout the circadian cycle. These tests can be incorporated into the daily enrichment schedule for the test subjects, which minimizes stress and improves psychological well-being. The enrichment schedule includes social interaction, physical activity, sensory stimulation, food, and cognitive/occupational activities. Thus, the cognitive testing protocols provide enrichment in all five categories, and the testing data generates operationally-relevant, performance-based measures for evaluating the effects of CR-entrainment therapies.

[00198] Circadian phase-sensing is used for determining the type, timing, and dose of therapies to deliver. Successful completion of this task depends on 3 key factors of low to moderate risk and are discussed in order of decreasing risk. First, obtaining reliable measures of circadian biomarkers is moderately risky, particularly in large animals. An established COTS system (DSI telemetry) that has been used for similar long-term monitoring studies in many species, including non-human primates, can be used. Second, the circadian control model is essential for interpreting the biomarker data. Accurate phase predictions are essential and there is a low risk that the algorithm will not generalize across all conditions. However, this risk is thought to be low to very low.

Circadian Rhythm Therapeutics Delivery

[00199] A range of conditions are classified as circadian rhythm sleep-wake disorders. Many such conditions are comorbid with neurodegenerative conditions, or may arise as a consequence

of schedule constraints such as in jet lag or shift work. The adverse health consequences of disruptions to circadian rhythms and sleep are numerous. The chronic or recurring nature of these conditions adds to the challenges in effective treatment and the safety concerns associated with long-term use of therapeutics. Existing therapeutic approaches have relied primarily upon oral administration of compounds. These have included compounds that target benzodiazepine, histamine, melatonin, and orexin receptors, as well as other sedative and stimulant compounds.

[00200] For efficacy in circadian rhythm sleep-wake disorder, the timing of administration is critical. Injection of some compounds may be used for acute administration. Non-biochemical therapeutic approaches have relied primarily on light therapy. While this offers considerable safety and efficacy advantages, control of environmental light exposure is not always feasible.

[00201] In one exemplary embodiment of multiple therapeutic delivery, multi-clock targeting with precision timing for circadian rhythm regulation can be performed by the proposed system. For example, in the circadian rhythm example, by targeting both the central and peripheral clocks, the hybrid bioelectronics system of the present invention provides synergistic effects towards enhanced entrainment, as shown in Fig. 1. However, because the same therapy applied during different phases of a circadian rhythm can have both phase-advancing or phase-delaying effects, it is important to validate therapeutic efficacy in terms of its administration window. The system uses phase response curves (i.e., the phase shift induced by therapy as a function of the phase of delivery), combined with real time sensing of internal body temperature and/or commercial off the shelf (COTS) wearable sensors to inform actuation-timing for most effective delivery of therapies. Fig. 1 depicts phases of peripheral and central clocks in response to an 8 hr shift, for normal entrainment (left), providing therapy affecting only the central clock (middle), and the approach of the hybrid bioelectronics system of the present invention (right) with therapy targeting both central and peripheral clocks in accordance with an illustrative embodiment. In Fig. 1, the fill color green represents normal phase relationship, and red represents misaligned phases. The system of the present invention rises above the current state-of-the-art in circadian rhythm management because it delivers a personalized therapy with precision dosing and timing for maximum efficacy. This is not possible with single-dose approaches that act only on sleep/wake rhythms.

[00202] In the illustrative embodiment for the circadian rhythm application, the therapeutics targeted for production and delivery by the engineered cells are GLP-1 and Orexin A. In

alternative embodiments, different types of therapeutics may be produced. Production of such peptides presents an inherent advantage, especially in the application of circadian management. The peptides are produced by mammalian cells and thus are native, non-recombinant peptides. Unlike recombinant variants, GLP-1 and Orexin A have short metabolic half-lives (GLP-1, 4.6–7.1 min; Orexin A, 27 min), making their use for entrainment more effective. Such half-lives are long enough to reach target tissues, short enough to have a precisely timed phase-shifting action, and are known to readily cross the blood-brain barrier, exhibiting potent actions on the brain when peripherally administered.

[00203] Precision timing and dosing is paramount to the therapeutic approach. As such, the external hub can be used to determine current state of the patient. In one embodiment the relevant state is the patient's circadian phase, and with knowledge of target shift magnitude (e.g., how many time zones will the user traverse) and ideal timing of both therapies (from phase response curves), will determine the optimal dose/timing schedule, which may be initiated by the user. As such, each therapy will be administered at most once per day in one embodiment. This routine can be repeated daily, per suggestion of the system, until the entrainment is achieved. Delivery of each therapy is expected to occur within seconds of illumination of the light source, and actively regulated to a fixed level and duration by the dosing control system. Therapy production is expected to stop within about 2 minutes of turning off the light source, and presence in the blood stream is dictated by the metabolic half-life of the peptide (5-30 min). The daily timing and dosing schedule can be generated and stored in the external hub, and initiated by the user via pressing a button, voice command, etc.

[00204] In one embodiment, the therapeutics used in association with the bioelectronic device disclosed above for affecting the timing of circadian rhythms includes AVP, VIP, Ghrelin, Leptin, Orexin and Melanin Concentrating Hormone, PACAP, ACTH, GLP-1, and any adjuvant of the aforementioned.

EMBODIMENT 1: Therapeutic agents for various diseases produced by the engineered cells

Treatment of Cancers

[00205] In one embodiment, the bioelectronics device/system of the present invention can be used for treatment of peritoneal cancers. In one embodiment, the present invention provides treatment of peritoneal cancers by mitigating factors that contribute to peritoneal cancers.

[00206] Peritoneal cancers such as ovarian, colorectal and pancreatic cancers are particularly challenging to address with traditional therapeutic approaches. One of the overarching goal of the present invention is to leverage a combination of synthetic biology, biomaterial design, immunoengineering, and mathematical modeling to develop a clinically translatable immunotherapeutic approach for the treatment of ovarian cancer. The pro-inflammatory cytokine, Interleukin 2 (IL-2), triggers the expansion and activation of cytotoxic T and natural killer (NK) cells for cancer immunotherapy and recombinant IL-2 has been FDA approved for use in melanoma and renal cancers. However, effective tumor reduction requires intravenous administration, high-dose infusion regimens. Inevitable downsides of such regimens are the associated toxicity side effects such as vascular leak syndrome that leads to fluid accumulation and tissue damage in major organs. The severity of such side effects often forces patients to be taken off infusions and/or off clinical trials. Thus, effective IL-2 immunotherapy requires programmable spatial and temporal kinetics of IL-2 delivery with tunable dosage and therapeutic window to maintain immunostimulatory effect while avoiding the toxicity. Synthetic biology approaches to reprogram cells for tunable, feedback-responsive IL-2 production offer a unique opportunity to address these issues and develop effective strategies for cancer immunotherapy.

[00207] However, constitutive IL-2 production and relatively short therapeutic window limits clinical versatility of this initial platform design. Optimal therapeutic outcome requires exquisite control of the dosage and the duration of the therapeutic window to achieve robust local activation of effector T and NK cells while preventing accumulation of IL-2 levels that lead to negative side effects. An effective strategy for IL-2 mediated immunotherapy thus requires smart, cell-based devices that adjust IL-2 production dynamics in response to sensitive detection of immunotherapy biomarkers.

[00208] To address the limitations of current IL-2 based immunotherapy, the present invention proposes use of IL-2 as therapeutics delivered by the bioelectronics device as described above. In particular, the bioelectronics device houses a clinically translatable cell lines engineered for regulated production of IL-2 and encapsulated into an immunomodulatory hydrogel. The present invention allows for long-term, continuous local (tumor adjacent) delivery of high concentrations of IL-2 that elicit immunostimulatory effects without causing toxicity typically observed with systemic administration. Additionally, in one embodiment, to assess and properly account for the natural heterogeneity of the host pharmacokinetics (PK) and

pharmacodynamics (PD), the present invention integrates cells that are engineered to sense and report extracellular levels of Interferon Gamma (IFN γ , biomarker of tumor treatment efficacy). To further improve therapeutic and safety profiles of the treatment, in one embodiment, the present invention combines validated PK and PD models with newly engineered cell lines that sense IL-2 and IFN γ and, in response, adjust the rate and the temporal profile of IL-2 production for the desired therapeutic effect.

[00209] In one embodiment, the bioelectronic device of the present invention houses any cells capable of delivering IL-2 or other therapeutic molecules capable of stalling, stopping, or reversing peritoneal cancers. In one embodiment, the bioelectronic device of the present invention houses any cell type able to be engineered to deliver IL-2 or sense IFN γ .

[00210] In one embodiment, the bioelectronics device is implanted in an intraperitoneal space within vicinity of cancer Sub-q space near to cancer or capable of delivering the therapeutic to the space containing the cancer.

[00211] IL-2 producing cells leveraging a clinically translatable human retinal pigmented epithelial (RPE) cell line engineered with an inducible kill switch for safe therapy termination. In one embodiment, these cells are encapsulated using immunomodulatory alginate-hydrogels to enable use of allogenic cells in the clinic with long-term (months) production. In addition, the engineer RPE cells sense levels of IFN γ to be encapsulated for in vivo kinetic and dosing studies. IFN γ levels provide an effective prognostic marker of immunotherapy efficacy in immunotherapy clinical trials. As described above, regulating IL-2 production based on detection of IFN γ response in the tumor microenvironment enables effective control of the duration of IL-2 therapeutic window that accounts for the natural variability of the host immune response.

[00212] In one embodiment, the cellular control system encoding a negative feedback loop for regulating IL-2 production based on the signals associated with activation of intermediate-affinity IL-2 receptors enables continuously adjusted production of therapeutic doses of IL-2.

[00213] In one embodiment, the present invention can be used to provide delivery of therapeutics for treatment of bone marrow disorders. The therapeutic agent may include IL-2, pain therapeutic, chemotherapeutic agent, TIMPs, PTUPB, IL-6, TGF- β , HIF-1 α , IL-1 β , VEGF, IL-8, IL-10 and any antibody, agonist, or antagonist of the aforementioned.

Treatment of Infertility and Ovulatory Induction

[00214] *Hypogonadotropic hypogonadism* (aka Kallman Syndrome; hypothalamic amenorrhea in women) incidence is about 1-10/100,000 births, and about 3,000-30,000 in US. Lack of hypothalamic signals gonadotropin-releasing hormone (GnRH), which is needed to drive pituitary to produce gonadotropins (LH/FSH), is one major cause of the Hypogonadotropic hypogonadism. Complications of hypogonadism include infertility, low sex drive, and osteoporosis in both untreated males and females, in addition to menstrual irregularities and early menopause in untreated females, and erectile dysfunction and decreased muscle mass and body hair in untreated males. Patients will typically respond via hormone replacement therapy, including exogenous FSH or LH, or can be treated with pulsatile delivery of GnRH.

[00215] There are several options available in existing treatments, including, for *females*, estroprogestins therapy (estradiol, progestin regimen via 14d of **oral ingestion**, and or **application of patch of gel**); generally considered exploratory: pulsatile GnRH (**IV pump**: 75 ng/kg per pulse every 90 min, adjusted based on response; **Subcutaneous pump**: 15ug per pulse every 90 min, dose adapted based on response up to 30ug per pulse); Placental human chorionic gonadotropin (hCG) 1500U every 3 days 3 times. **SC Injection** hMG (FSH + LH) 75 to 150 IU SC daily, dose adapted based on follicular growth; Progesterone 200 mg **intravaginally** daily.

[00216] Options for males includes T enanthate, hCG **injections** periodically to induce puberty. To treat Hypogonadism: T enanthate 250 mg **intramuscular injection** every 2-4 weeks (self-injection), T undecanoate 100 mg IM every 10-14 weeks, **Topical gel**. Treatment of infertility: Generally considered exploratory: pulsatile GnRH **SC pump** 25 ng/kg per pulse every 120 min, dose adjusted, up to 600ng/kg/pulse. hCG 500-1500 IU **SC injection** three times weekly, dose adjusted. rFSH: 75-150 IU **SC injection** three times weekly, dose adjusted.

[00217] In one embodiment, the GnRH is used as the therapeutic produced by engineered cells which are housed by the bioelectronic device described above for medication delivery. In one embodiment, GnRH pump application is used for females for ovulation induction treatment. The ovulation induction frequently occurs with pulsatile GnRH 75 ng/kg iv per pulse – pulse frequency of 60-120 min (potentially initiate every 90 min – then increase to every 60 min per Martin 1990 protocol). Either iv or SC works. In one embodiment, lower dose of 25 ng/kg is applied. Single dominant follicle is the desired and most common outcome. In one embodiment, 75+% will achieve conception at/after 3 sequential monthly cycles.

[00218] In one embodiment, the present invention avoids multiple gestations; and is convenience for the user over a 3 month duration.

[00219] In one embodiment, for male's lacking of sperm production, the present invention provides better response to pulsatile GnRH (in 6 month), as compared to exogenous gonadotropins (mean 18 month).

[00220] The most frequent underlying cause of female infertility is polycystic ovarian syndrome (PCOS). Patients usually respond to a regimen of Clomiphene or Aromatase inhibitor to induce endogenous LH surge from pituitary. Potentially about 20% of the patients do not respond to this approach and require exogenous gonadotropin Rx, which consists of daily FSH IM for 8-10 days – with gradually escalating doses until ovulation occurs. There is also evidence for pulsatile GnRH pumps for PCOS.

[00221] In one embodiment, the therapeutic agents used in association with the bioelectronics device are Gonadotropins FSH/LH.

[00222] In one embodiment, the bioelectronics device for delivery of GnGH is implanted in a location which is nor reachable by the user. In one embodiment, the bioelectronics device for delivery of GnGH is implanted for local delivery intra-vaginally.

Treatment of Addictive Personality Disorders

[00223] In one embodiment, the bioelectronics device/system of the present invention is used for treatment of addictive personality disorders, e.g., addictions to alcohol, cocaine, morphine, or opioids. In one embodiment, the present invention provides treatment of addictive personality disorders by reducing factors that contribute to abuse cravings.

[00224] It is readily appreciated that the addictive disorders are widely spread problems existing in all branches of medicines. For example, specific inflammatory cytokines have been associated with more than one form of addictive disorders. In addition, antibiotics have been shown to be beneficial in many medical clinical settings. However, frequent use of the antibiotics should be limited because overuse or abuse of antibiotics would lead to superbugs, strains of bacteria, viruses, parasites, and fungi, that are resistant to most of the antibiotics and other medications commonly used to treat the infections they cause. In addition, increasing cAMP concentration by using Ibudilast has been shown to reduce the inflammations and result in reduced NF- κ B activation.

[00225] Therefore, the present invention can be used to provide delivery of therapeutics for treatment of addictive personality disorders. The therapeutics may include IL-1ra, IL-10, IL-6, TLR4, IL-8, IL-1 β , TNF- α , IL-10, NOS inhibitor (L-NAME), TRPM2 channel blockers (ACA and 2-APB, and any antibody, agonist, or antagonist of the aforementioned.

[00226] In one embodiment, the antibody of IL-6 is used as therapeutics for treatment of alcohol addiction, depression, and anxiety. In one embodiment, IL-10 is used as therapeutics for treatment of morphine addictions.

Treatment of Inflammatory Autoimmune Disorders

[00227] In one embodiment, the bioelectronics device/system of the present invention can be used for treatment of inflammatory autoimmune disorders. In one embodiment, the present invention provides treatment of inflammatory autoimmune disorders by mitigating factors that contribute to inflammatory autoimmune disorders.

[00228] In one embodiment, the present invention is used to provide delivery of therapeutic agents for treatment of inflammatory autoimmune disorders. The therapeutic agents may include one or more of IFN γ , TNF- α , IL-6, IL-1 β , IL-10, CXCL1, IL-12, IL-13, IL-17, IL-23, IFN-g-inducible protein-10, ISG15 and any antibody, agonist, or antagonist of the aforementioned.

[00229] In one embodiment, the present invention is used to treat Crohn's disease by providing therapeutic agents which selectively intervene and inhibit inflammatory processes caused by pro-inflammatory mediators like IL17 and IL23. The anti-IL12p40 which targets the common subunit of IL-12 and IL-23, or anti-TNF- α is a therapeutic used with the bioelectronics device of the present invention for treating Crohn's disease. In another embodiment, therapeutic agent targeting IL-23p19 or signaling mediators downstream of multiple cytokines can be used with the bioelectronics device of the present invention for treating Crohn's disease.

Treatment of Bone Marrow Disorders

[00230] In one embodiment, the bioelectronics device/system of the present invention can be used for treatment of bone marrow disorders. In one embodiment, the present invention provides treatment of bone marrow disorders by mitigating factors that contribute to bone marrow disorders.

[00231] In one embodiment, the present invention can be used to provide delivery of

therapeutic agents for treatment of bone marrow disorders. The therapeutic agents may include IL-1ra, IL-10, IL-6, TLR4, IL-8, IL-1 β , TNF- α , and any antibody, agonist, or antagonist of the aforementioned.

Treatment of Diabetes

[00232] In one embodiment, the bioelectronics device/system of the present invention can be used for treatment of diabetes. In one embodiment, the present invention provides treatment of diabetes by mitigating factors that contribute to diabetes.

[00233] Diabetes is a global epidemic afflicting more than 285 million people worldwide. While the current standard of care for those suffering from type I diabetes (T1D) consists of a rigorous regimen of blood glucose monitoring together with daily administrations of exogenous insulin and diabetic diet, these individuals still are challenged with untoward side effects due to complications of the disease in part the result of daily compliance issues. Additionally, the process by which the beta cells of the pancreatic islets of Langerhans secrete insulin in response to blood glucose fluctuations is imperfectly simulated by periodic insulin injections. The ‘holy grail’ of diabetes treatment research thus is achieving insulin independence for T1D patients.

[00234] In one embodiment, the present invention can be used to provide delivery of therapeutic agents for treatment of diabetics. The therapeutic agents may include native insulin or insulin analogs and other insulin functional replacement.

[00235] In one embodiment, to ensure free diffusion capacity of nutrients and oxygen, the bioelectronic device is implanted in a location preventing from provoking inflammation, and free of pericapsular fibrotic overgrowth after implantation. In one embodiment, the bioelectronics device is implanted intraperitoneal or sub-q.

[00236] In one embodiment, the engineered cells used for producing therapeutic agent includes one or more of pancreatic islet cells, β -cells, endothelial cells, and engineered iPSC, MSCs, and etc.

Treatment of Neuro Disorders

[00237] In one embodiment, the bioelectronics device/system of the present invention can be used for treatment of neuro disorders. In one embodiment, the present invention provides treatment of neuro disorders by mitigating factors that contribute to neuro disorders.

[00238] In one embodiment, the present invention can be used to provide delivery of therapeutic agents for treatment of neuro disorders. The therapeutic agents may include IL-4, IL-6, IL-2R and IL-8, TNF- α , IL-17, IL-1 β , CRP, IL-17A, and any antibody, agonist, or antagonist of the aforementioned.

[00239] In one embodiment, the present invention is used to treat neuro disorders by providing therapeutic agents which inhibits IL-6. In particular, IL-6 is elevated in major depressive disorders. In one embodiment, therapeutic agents reducing the IL-6 level such as any antibody, agonist, or antagonist of IL-6 are used in association with the bioelectronics device for treatment of neuro disorders. In one embodiment, the present invention is used to treat neuro disorders by providing therapeutics which inhibits IL-17A. In particular, IL-17A is correlated with symptoms of major depressive disorders. In one embodiment, therapeutic agents reducing the IL-17A level such as any antibody, agonist, or antagonist of IL-17A is used in association with the bioelectronics device for treatment of neuro disorders.

[00240] In addition to the aforementioned disorders and diseases, the bioelectronics device/system of the present invention can be used for treatment of gastrointestinal disorders, cardiovascular disorders, psychiatric disorders, neurological disorders, neurodegenerative disorders, muscle disorders, kidney disorders, liver disorders, retinal/visual disorders, sensory disorders, motor disorders, skin disorders, arthritis, Lupus, and infectious diseases.

[00241] In one embodiment, the bioelectronics device/system of the present invention can be used in animals. For example, the bioelectronics device/system can be implanted into livestock or animals for food production, e.g., chickens, so as to increase and/or control the growth and development of the animals.

[00242] In one embodiment, the therapeutic agents delivered by the bioelectronics device/system of the present invention are artificially engineered molecules, such as GLP1 analogs or bifunctional proteins, e.g., a protein having functions of both GLP1 and GIP. In one embodiment, the therapeutic agents are produced by genetically engineered cells.

EMBODIMENT 2: Optical control system of engineered cells for therapeutics production

[00243] In certain embodiments, the present invention discloses engineered cells with an integrated vector system producing one or more desired therapeutic agents, reporters, and a kill switch.

Optical Control Feed Back System – Expression of mBeRFP

[00244] Fig. 20 shows an embodiment of engineered cells subject to LEDs light cycle for production of desired therapeutics. In particular, APRE-19 cells are engineered to express leptin were exposed to repeated cycles of 8h of blue light at 16h of dark for 6 days. The protein produced at the end of each 8 hour day was collected and quantified by ELISA. As shown in Fig. 20, after an initial acclimation day, the protein production for each day fell within 50-200% of the mean productivity of 1775pg/mL, with dotted lines represent 50-200% variability. In one embodiment, to mitigate the variability of the therapeutics produced by the engineered cells, e.g., APRE-19, the optical feedback system is used to change the light activation to regulate production from the engineered cells.

[00245] Fig. 21 shows an embodiment of engineered cells' production of a molecule for optical feedback control. In one embodiment, mBeRFP was used as a molecule for optical feedback control. mBeRFP is a large stokes shift protein where the excitation and emission wavelengths do not overlap, which allows the excitation wavelength to be more easily filtered out while detecting the fluorescent signal. The engineered ARPE-19 cells were encapsulated in alginate at varying concentrations with unengineered ARPE-19 cells to demonstrate the fluorescence signal from increasing concentrations of mBeRFP in a constant cell concentration. A spectrophotometer was used to read the fluorescent signal. Fig. 21 demonstrates a linear relationship between mBeRFP concentration and detected optical signal.

[00246] In one embodiment, when the excitation light from the implantable device or engineered cells covers a wider range of wavelengths and is dimmer than the commercial spectrophotometer; a selective filter is added to the optical signal detector of the implantable device. In another embodiment, a detector with tunable sensitivity may be used on the implantable device so that the sensitivity can be increased as needed.

[00247] As shown in Fig. 22, an improved filter set on the fluorescence sensor enables detection of as few as 1.2×10^4 mBeRFP positive cells per milliliter. In particular, in certain embodiments, the present invention designed and assembled devices with improved filter sets for detecting mBeRFP while eliminating background signal from the blue LEDs used to excite the fluorophore. These devices were then tested by reading the fluorescent signal from decreasing concentrations mBeRFP cells suspended in alginate. The new filtered sensors can detect

fluorescence from as few as 1.2×10^5 cells mBeRFP positive cells per milliliter which equates to 0.2% of the total 60×10^6 cells/mL that will be loaded into the device.

Single vector integrating optogenetic system

[00248] Fig. 23 shows vector design and testing chart of mBeRFP-containing all-in-one vector. Panel (A) shows three different plasmid designs for producing Leptin, melanopsin, and mBeRFP. V1 is a multiple plasmid system and V(2.2) are all-in-one systems containing mBeRFP. Panel (B) shows comparison of leptin production by the previous best leptin producing engineered ARPE-19 cells (V1) and all-in-one engineered ARPE-19 cells (V2.2 +/- CL1 degon), all of which were exposed to blue light or left in the dark for 8h. Protein production from the cells was then quantified by ELISA. Panel (C) shows that all-in-one vector (V2.2) production. In particular, leptin were produced with varying ratios of transposase to transposon and antibiotic selection conditions. Selected, engineered ARPE-19 cells were exposed to blue light for 8 h. Protein production from the cells was then quantified by ELISA. 1:1 and 2:1 transposase to transposon ratio cells treated under antibiotic ramp up to 4 ug/mL were carried forward for subsequent monoclonal selection. Cells engineered using the 2:1 abx ramp condition secrete 184 pg/mL. Panel (D) shows single cell monoclonal line testing of V2.2 optimized ARPE-19 cells. In particular, ARPE-19 cells engineered to express leptin were exposed to blue light for 8h. Protein production from the cells was then quantified by ELISA and cell number was normalized by performing Alamar Blue cell viability assay at end of 8h light exposure. Clones 3, 13, and 20 were then expanded for subsequent validation.

[00249] As shown, ARPE-19 cells expressing light-inducible leptin and mBeRFP with and without the degon were engineered in an all-in-one vector format, improving leptin production by ~80% from previous engineered ARPE-19 cells V1. These constructs were optimized further by altering the antibiotic selection and ratio of DNA to helper plasmid to achieve a 2.4-fold increase in productivity over previous cell engineering techniques.

[00250] Fig. 24 shows ARPE-19 cells engineered to produce light inducible leptin and a small molecule inducible kill switch.

[00251] In certain embodiments, multiple plasmid designed and each of them is designed to express all of the optogenetic system and kill switch components in a single vector. The multiple different constructs to test that alter the ordering and promoters used to express individual components of the system. These constructs were stably integrated into ARPE-19 cells using the

PiggyBac transposase system and selected to ensure usage of pure population of optogenetically inducible cells. These engineered cells were then assayed for leptin production as described previously. Briefly, we plated cells and 24 hours later, exchanged the media and exposed to blue light for 8 h. At this time point, supernatant was harvested and assayed for leptin production.

[00252] In particular, as shown in Panel (A), five plasmid designs V2-V6, are designed for engineer cells to express both leptin under light inducible control and the small molecule inducible kill switch iCasp9.

[00253] In certain embodiments, ARPE-19 cells were also engineered with each of these constructs and assayed for leptin productivity in response to light and cell death in response to the small molecule inducer. Panel (B) shows light inducible leptin produced from cells engineered with the plasmids V2-V6, with V3 shows the best leptin production performance. Panel (C) shows cell death induced in engineered ARPE-19 cells with the plasmids V2-V6, 6 hours after administration of the small molecule inducer. The V3 plasmid produced both the most leptin, as shown in Panel (B), and the highest levels of cell death, as shown in Panel (C). Panel (D) shows another two plasmid designs V11 and V12, which are designed to remove the need for separate sentinel cells and express both the mBeRFP and the light inducible leptin from the same cell.

[00254] In one embodiment, ARPE-19 cells engineered with the V3 plasmid were encapsulated in alginate and implanted subcutaneously in mice. The small molecule inducer was administered and then 6 hours later the cells were explanted and assayed for viability using a trypan blue assay. 6 hours after treatment with the small molecule inducer cells were >98% dead.

[00255] In certain embodiments, when the optogenetically regulated engineered cells express lower levels of mBeRFP than the mBeRFP engineered cells used in testing, the present invention uses a small percentage of bright mBeRFP engineered cells as sentinel cells reporting on cell health and viability.

[00256] In certain embodiments, the kill switch performance in new vector designs may be suboptimal. As a compensate, the present invention selects optimal balance of leptin secretion and kill switch performance and selects monoclonal lines of improve activity across both aspects.

[00257] Fig. 25 shows ACTH production from repeated on/off cycles of engineered cells. APRE-19 cells engineered to express ACTH were exposed repeated cycles of blue light and dark

for 4 days. At the end of each light period the amount of ACTH produced was quantified by ELISA. The dotted lines represent 50-200% variability. ACTH production on all days fell between 50-200% of the mean protein expression level.

Table 1. Sequences for vectors V2-V6 and elements thereof

Sequence Name	SEQ ID
pNFAT	SEQ ID No: 1
pCAG	SEQ ID No: 2
pPGK	SEQ ID No: 3
pCMV	SEQ ID No: 4
Leptin	SEQ ID No: 5
IRES	SEQ ID No: 6
mBeRFP	SEQ ID No: 7
iCasp9	SEQ ID No: 8
Melanopsin	SEQ ID No: 9
Vector V2	SEQ ID No: 10
Vector V3	SEQ ID No: 11
Vector V4	SEQ ID No: 12
Vector V5	SEQ ID No: 13
Vector V6	SEQ ID No: 14

Kill Switch Performance

[00258] Fig. 26 shows kill switch performance in mBeRFP all-in-one cell line. In particular, panel (A) shows all-in-one vector (V2.2) producing leptin were produced and reengineered to contain kill switch components, an inducible Caspase 9 kill switch molecule that induces apoptosis in response to exposure to the small molecule, AP-1903. Engineered ARPE-19 cells were exposed to AP-1903 for 6 h. After 6 hours, cells and supernatant were harvested and underwent cell death quantification and propidium iodide. 100% of cells treated with the kill switcher inducer molecule underwent programmed cell death.

[00259] Panel (B) shows kill switch performance in vivo. In particular, as shown in Fig. 24, five plasmids are designed to express both leptin under light inducible control and the small molecule inducible kill switch iCasp9. ARPE-19 cells were engineered with each of these

constructs and assayed for leptin productivity in response to light and cell death in response to the small molecule inducer. The V3 plasmid produced both the most leptin and the highest levels of cell death.

[00260] ARPE-19 cells engineered with the V3 plasmid were encapsulated in alginate and implanted subcutaneously in mice. The kill switch inducer molecule was injected and 6 hours later the engineered cells were explanted and assayed for viability. >98% of cells were dead 6 hours post kill switch induction.

In Vivo Testing of Inducible Therapeutics

[00261] Fig. 27 shows leptin production from an implantable scale device according to one embodiment of the invention.

[00262] In particular, panel (A) shows a top view of an embodiment of the implantable scale device measures 10 mm x 20 mm x 5 mm; and Panel (B) shows images of engineered cells on the PCL scaffold in the upper row, and the in vitro setup for testing the devices in the lower row.

[00263] In one embodiment, printed PCL scaffolds is tested as an alternative substrate to 3 dimensionally grow cells with increased productivity and potency. APRE-19 cells engineered to produce leptin in response to light were grown on scaffolds with various pore sizes and then were activated with blue light for 8 hours alongside an alginate encapsulated and two-dimensional tissue culture plate control. After 8 hours leptin concentration was measured via ELISA and cell numbers were counted by dissociating the cell from the scaffold and counting with a trypan blue viability assay. Cells grown on the 250 μ M scaffold were 4x more productive than the alginate encapsulated control. Cells grown on these 250 μ M scaffolds were put in a bioelectric device measuring 10 mm x 20 mm x 5 mm, small enough to be implanted subcutaneously in a mouse. These devices were turned on for 8 hours, illuminating and activating the engineered cells. After 8 hours media was collected and an ELISA was run to measure leptin production. Engineered cells in the implantable device produced 50pg of leptin. Panel (C) shows leptin production from cells in the implantable devices after 8 hours of light exposure.

Integrated Implantable Device

[00264] Fig. 28 shows an embodiment of implantable device for in vivo experimentation. As shown in Panel (A), in one embodiment, the LEDs and LCDs are placed directly next to each other on the walls of the implantable device. They are overmolded with silicone to prevent water

ingress and to extend the lifetime of the implantable device. In one embodiment, the silicone is transparent, such that the implantable device has a negligible amount of light lost due to this overmolding. Panel (b) shows a side view of the implantable device according to one embodiment of the invention, reflecting the stackup structure of the component, from top to bottom: circuit board 2401, battery 2402, NFC coil 2403. The large silver layer is the battery 2402 that powers the device during short term in vivo experiments. This eliminates the need for an external power transmitter.

[00265] In one embodiment, the collected data from the fluorescence monitoring system is stored in the NFC memory 2403 on board the implant. This data can be read by an external NFC reader so the data can be processed. The NFC memory also contains the settings that control the frequency that fluorescent readings are taken. This allows us to repeatedly test different settings without needing to exchange implants or reprogram the device.

[00266] In one embodiment, the circuit board containing the microcontroller and other controllers of the implantable device were merged with the LED/PD walls. In one embodiment, the LED/PD walls are attached vertically to the main circuit board and spaced far enough apart for the cell well to fit between the walls. In one embodiment, the oxygenators are situated below the cell well and between the LED/PD walls. In one embodiment, the oxygenators are wire-bonded to the main circuit board to form the electrical connection with the main board to allow for control of oxygen production.

[00267] In one embodiment, the LED intensity can be modulated in the implantable device. If the LED intensity is too low, the cells will not be activated. However, if the LED intensity is too high, the cells would die. In one embodiment, in order to allow for testing different LED intensity settings, the NFC memory contains a byte that sets the intensity. This memory can be overwritten with an external NFC reader which allows us to adjust the LED intensity as needed even after implantation.

[00268] In one embodiment, the photodiodes (PDs) are located directly next to the LEDs for use in the fluorescence monitoring system. In one embodiment, the fluorescent intensity data can be collected at any time during implantation. In another embodiment, the fluorescent intensity data can be collected at regular intervals. This data is then stored in the NFC memory which can then be read using an external NFC reader. The PDs are filtered to increase the SNR of the fluorescent data. Fig. 29 shows a chart of collected fluorescence data. In particular, the PDs

produced a linear response as the fluorescent cell fraction was adjusted. The overall number of engineered cells remained constant, while only the fraction of these cells was changed. The y-axis value is the reported value from the ADC.

[00269] It is important to validate engineered cells in vivo to verify that they display or execute to the proper engineered functions. In one implementation, an experimental group (e.g., mice) has encapsulated engineered cells implanted via an incision/suture procedure, and subsequently have the engineered cells turned on/off using the optogenetic system. Control groups include a first group implanted with encapsulated engineered cells with no optogenetic activation, a second group implanted with just the materials with no cells, and a third group implanted with triazole-thiomorpholine dioxide (TMTD) modified alginate.

[00270] When compared to the experimental group, the group implanted with engineered cells with no optogenetic activation allows one to verify the ability of the system to trigger production of the therapeutic protein. Also, when compared to the experimental group and first control group, the second group implanted with just the material allows one to determine whether the optogenetic system is “leaking” and producing the therapeutic agents without being triggered. TMTD modified alginate is a material that is known to not evoke an immune response in mice, and can thus be used as a negative control when looking at the immune response that the material would evoke.

[00271] The above-discussed procedure is used to validate the ability of the proposed system to controllably deliver therapeutics, e.g., GLP-1 and Orexin-A, in vivo. Specifically, engineered cells are encapsulated in an immune-protective material and implanted in the subcutaneous space of the test subjects and the skin sutured shut. In vivo light exposure and assaying for protein production is performed. The therapeutics (e.g., GLP-1 and Orexin A in one embodiment) are assayed for via ELISA, while GFP* is assayed via microscopic imaging (e.g., simple fluorescence microscopy, or in vivo imaging system (IVIS)). Implanted engineered cells are exposed to activating light through the skin of the test subject in varying patterns to demonstrate control over expression patterns. Various time points after light exposure are taken to determine the rate that the therapeutics are secreted once the cells are turned on. At each time point, blood samples were taken, along with IVIS fluorescence images. Blood samples are assayed for therapeutics, and the IVIS images are used to quantify GFP* production. ELISA and fluorescence data is compared to calibrate how much fluorescence correlates to a quantity of

therapeutic produced. Immune response to implanted encapsulated cells is also measured. Specifically, immune response to the implanted material can be determined by simple microscopy after explant (fibrosis will appear as a layer of biological deposition on the implant if it evoked an immune response). Additionally, immune cell phenotyping can be performed at the implant site to identify any immune cells that are present.

[00272] The foregoing description of illustrative embodiments of the invention has been presented for purposes of illustration and of description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed, and modifications and variations are possible in light of the above teachings or may be acquired from practice of the invention. The embodiments were chosen and described in order to explain the principles of the invention and as practical applications of the invention to enable one skilled in the art to utilize the invention in various embodiments and with various modifications as suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto and their equivalents.

CLAIMS

What is claimed is:

1. A vector transfecting a cell for producing a therapeutic agent, the vector comprising:
 - a first promoter controlling a transcription of a therapeutic agent encoding sequence and a reporter sequence;
 - a second promoter controlling a transcription of an optical controller sequence, wherein the optical controller sequence encodes a light sensitive protein;
 - a third promoter controlling a transcription of a kill switch encoding sequence; and
 - a fourth promoter controlling a transcription of a selectable marker sequence,wherein the first promoter is a NFAT promoter, each of the second promoter and the third promoter is one of a CAG promoter and a PGK promoter, and the second promoter is different from the third promoter;
 - wherein the light sensitive protein comprises a melanopsin, the kill switch encoding sequence encodes iCasp9;
 - wherein the therapeutic agent comprises at least one of IL-1ra, IL-1 β , IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IL-23, IFN- γ , IFN-g-inducible protein-10, ISG15, CXCL1, TIMPs, PTUPB, TLR4, TNF- α , TGF- β , HIF-1 α , VEGF, NOS inhibitor (L-NAME), TRPM2 channel blockers (ACA and 2-APB), leptin, ACTH, insulin, GLP-1, and any adjuvant, antibody, agonist, or antagonist thereof.
2. A vector transfecting a cell for producing a therapeutic agent, the vector comprising:
 - at least one promoter;
 - a therapeutic agent encoding sequence;
 - a reporter encoding sequence; and
 - an optical controller encoding sequence,wherein the vector comprises a DNA sequence.
3. The vector according to claim 2, wherein
 - the at least one promoter comprises a first promoter and a second promoter, wherein the first promoter is different from the second promoter.

4. The vector according to claim 3, wherein the first promoter controls a transcription of the therapeutic agent encoding sequence and the reporter encoding sequence.
5. The vector according to claim 4, wherein the second promoter controls a transcription of the optical controller encoding sequence.
6. The vector according to claim 5, wherein the optical controller encoding sequence encodes a light sensitivity protein.
7. The vector according to claim 6, wherein the light sensitivity protein participates in an activation of the first promoter.
8. The vector according to claim 7 further comprising a kill switch encoding sequence.
9. The vector according to claim 8, wherein the second promoter controls a transcription of the kill switch encoding sequence.
10. The vector according to claim 8, wherein the at least one promoter further comprises a third promoter different from the first and the second promoters, and the third promoter controls a transcription of the kill switch encoding sequence.
11. The vector according to claim 8, wherein the light sensitivity protein is melanopsin.
12. The vector according to claim 8, wherein the reporter encoding sequence encodes a fluorescent protein.
13. The vector according to claim 8, wherein the kill switch encoding sequence encodes iCasp9.
14. The vector according to claim 8, wherein the therapeutic agent comprises at least one of IL-1ra, IL-1 β , IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IL-23, IFN- γ , IFN-g-inducible protein-10,

ISG15, CXCL1, TIMPs, PTUPB, TLR4, TNF- α , TGF- β , HIF-1 α , VEGF, NOS inhibitor (L-NAME), TRPM2 channel blockers (ACA and 2-APB), leptin, ACTH, insulin, GLP-1, and any adjuvant, antibody, agonist, or antagonist thereof.

15. The vector according to claim 8, wherein the first promoter comprises a NFAT promoter having the SEQ ID No: 1.

16. The vector according to claim 10, wherein each of the second and the third promoters comprises one of a CAG promoter having the SEQ ID No: 2, a PGK promoter having the SEQ ID No: 3, and a CMV promoter having the SEQ ID No: 4.

17. The vector according to claim 10, wherein the at least one promoter comprises a fourth promoter, wherein the fourth promoter is different from the first, second and third promoters, and the fourth promoter controls a transcription of a selectable marker.

18. A vector transfecting a cell for producing a therapeutic agent, the vector comprising the nucleotide sequence selected from the group consisting of SEQ ID No: 10-14.

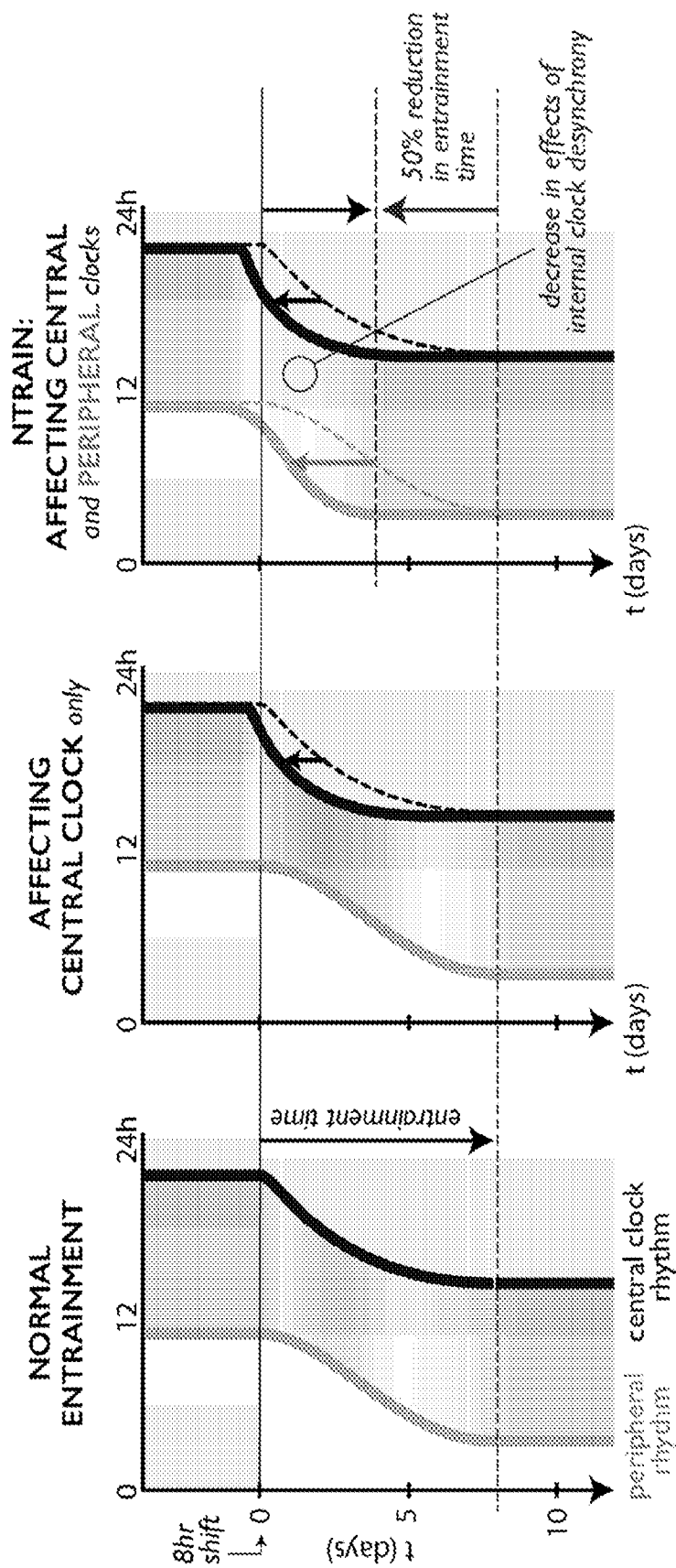


Fig. 1

mode property	chemical	mechanical	magnetic	thermal	electrical	optical
specificifcty	++	+	+	+	+	++
time to activation	—	+	+	+	++	++
induction signal localization	—	—	—	—	++	++
ability to tune induction signal (incl. multiplexing)	++	—	—	—	—	++
ease of integration (power, size, etc)	—	—	—	+	++	++

Fig. 2

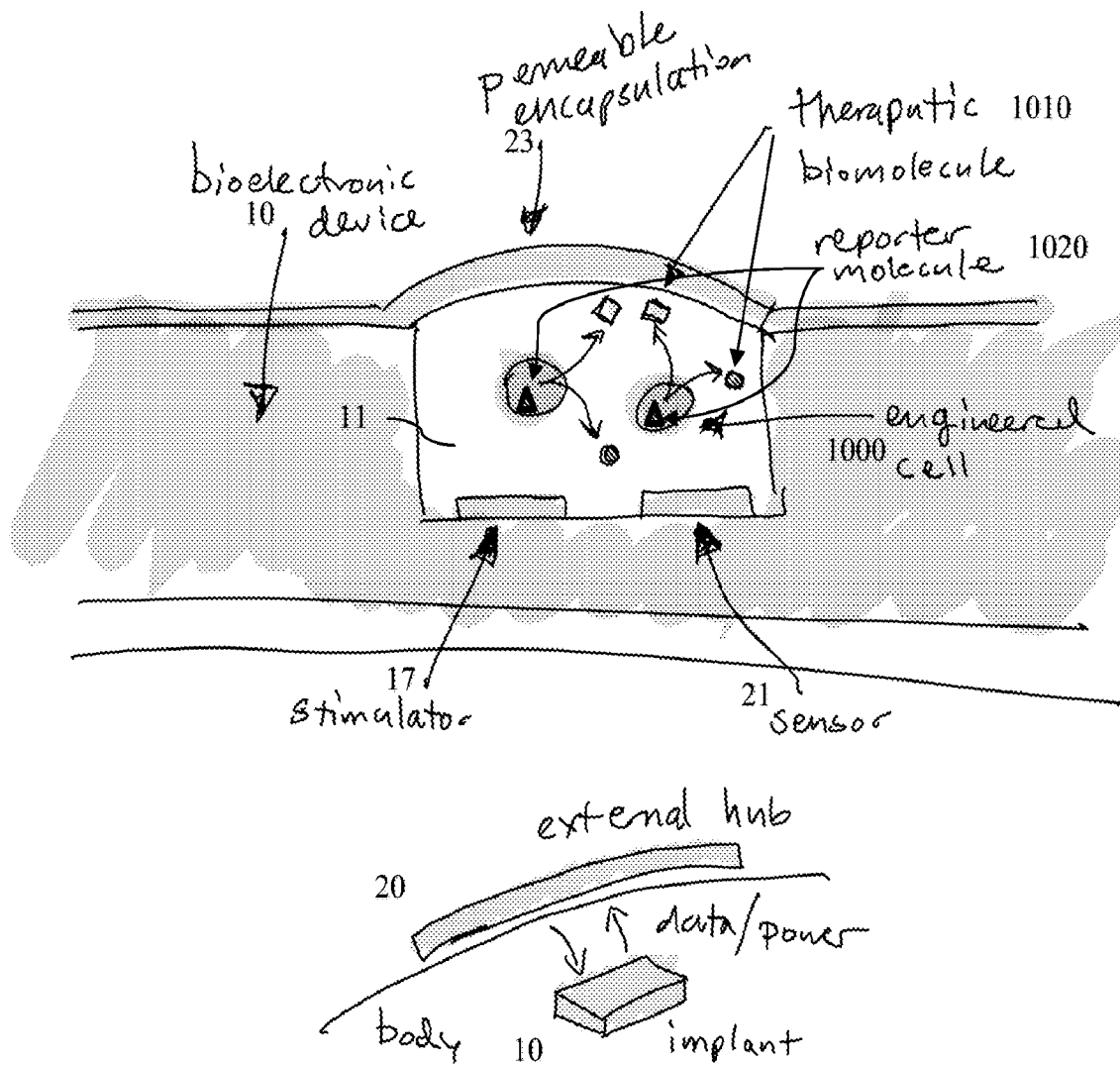


Fig. 3A

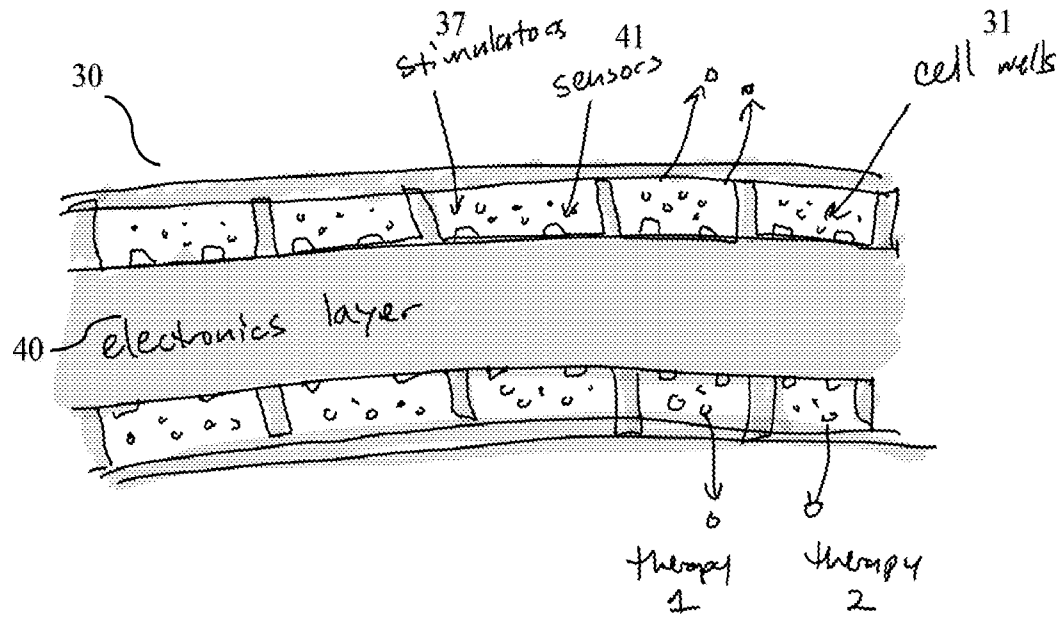


Fig. 3B

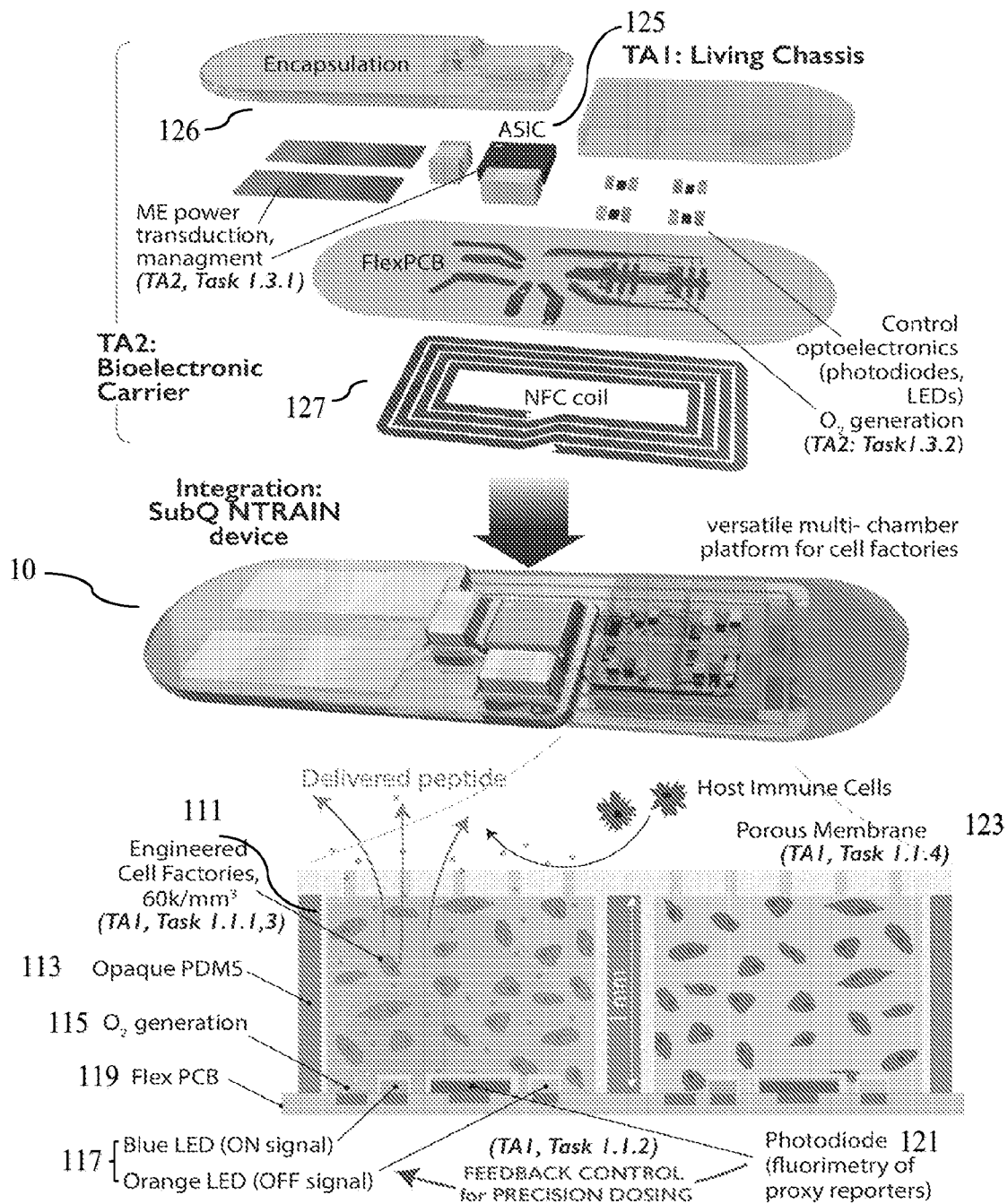


Fig. 3C

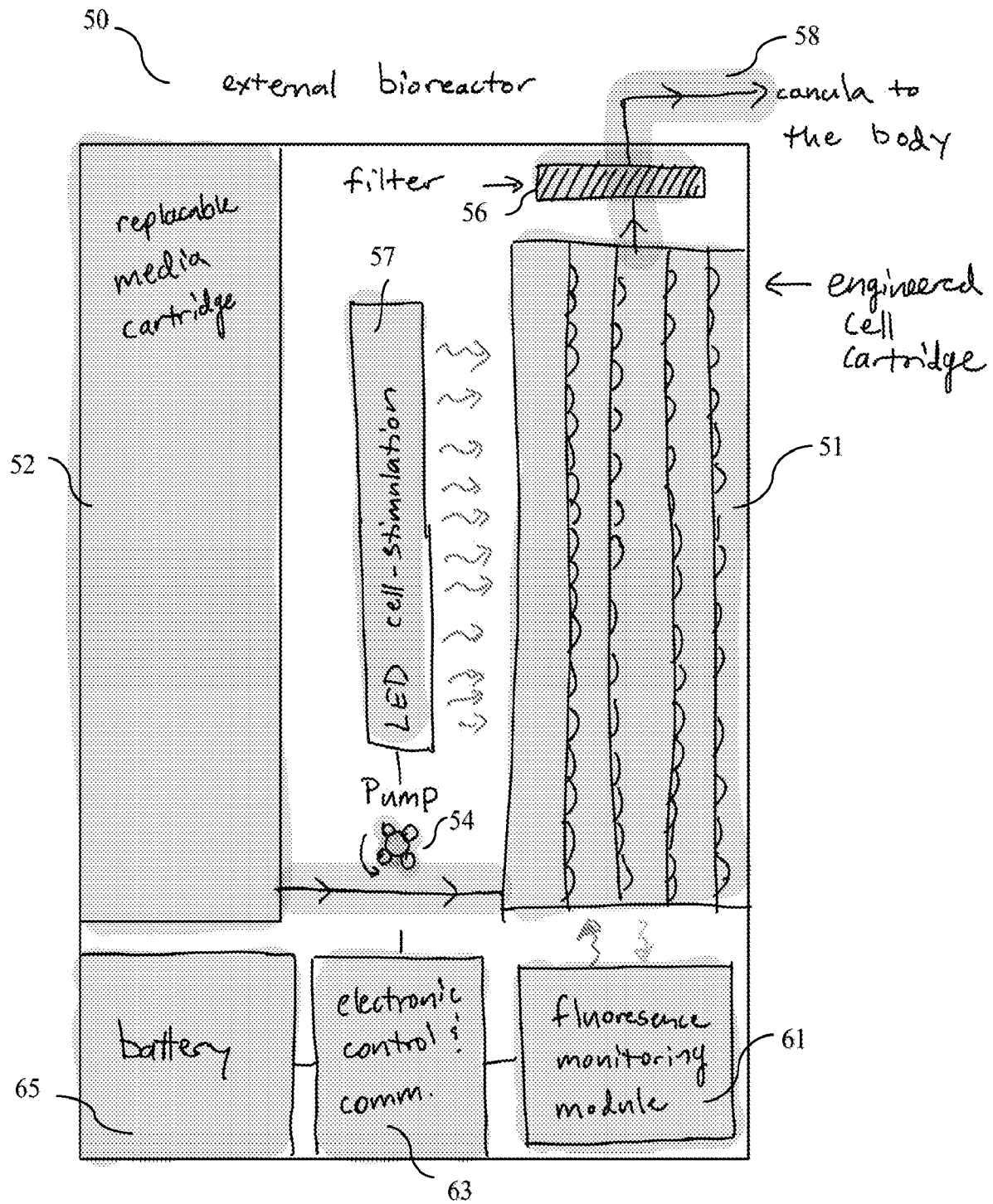


Fig. 3D

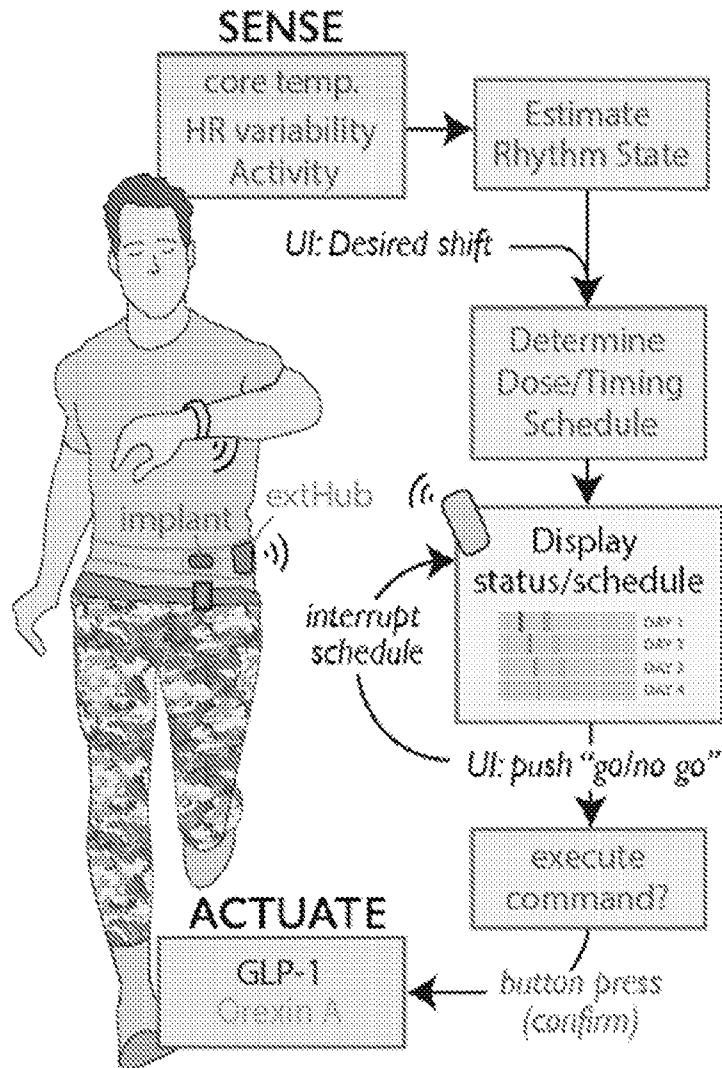


Fig. 4

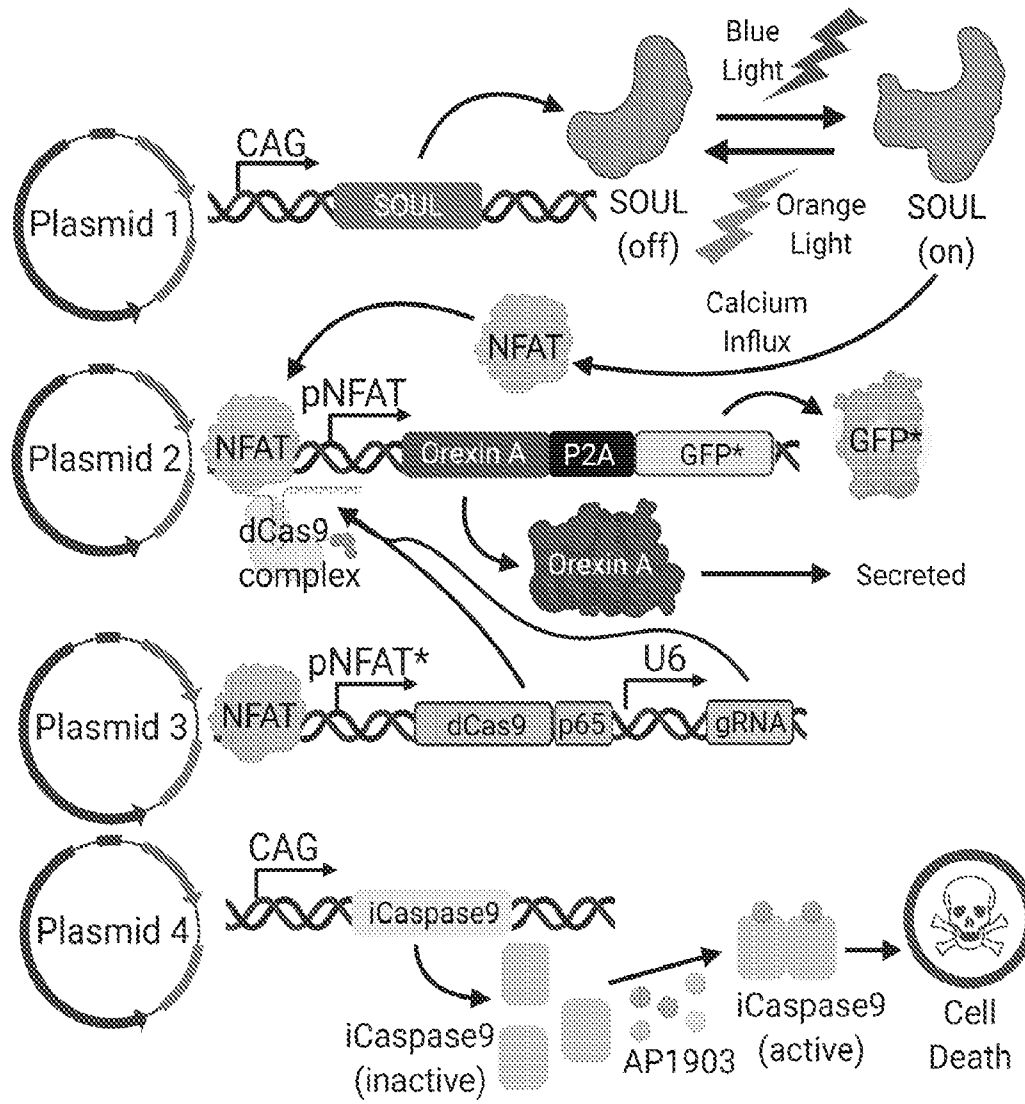


Fig. 5

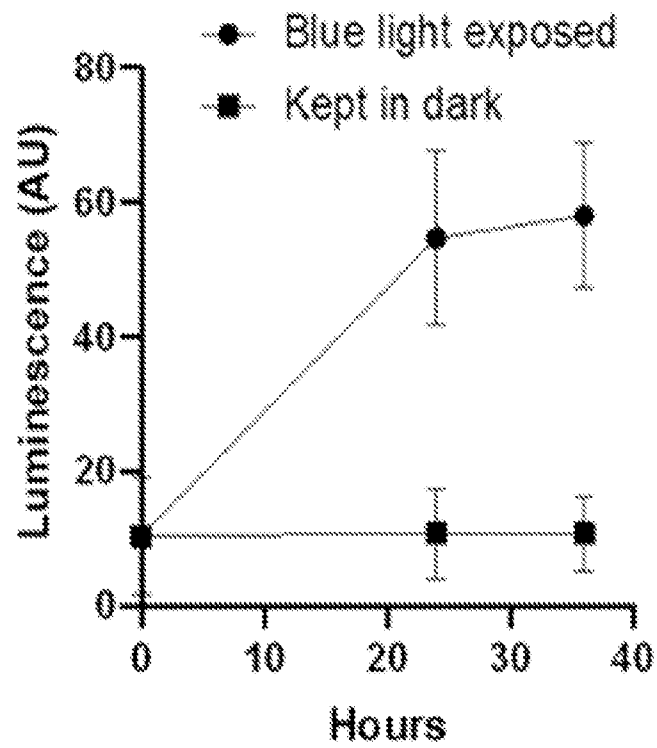


Fig. 6

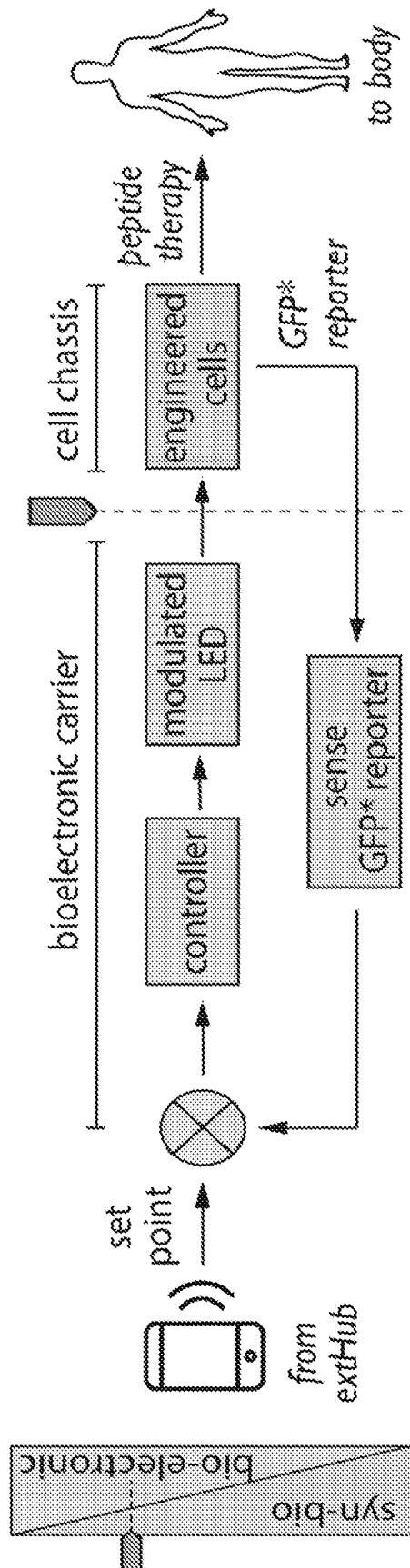


Fig. 7

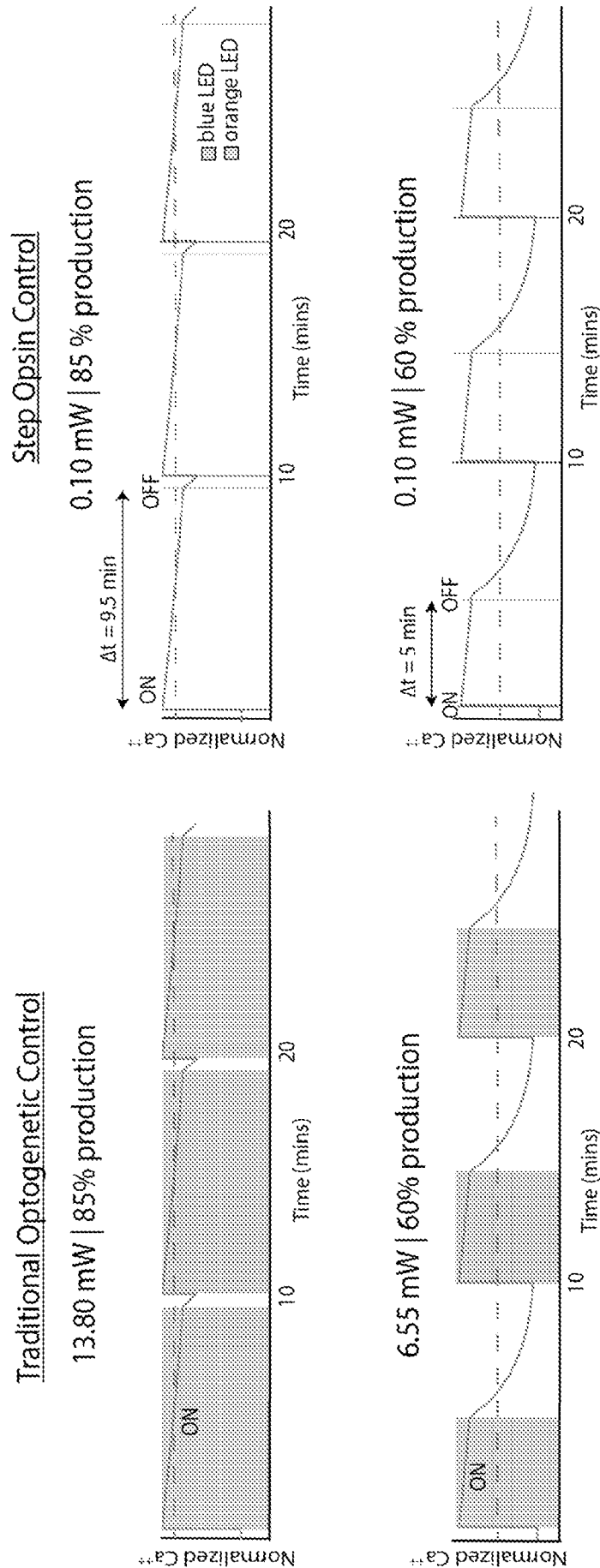


Fig. 8

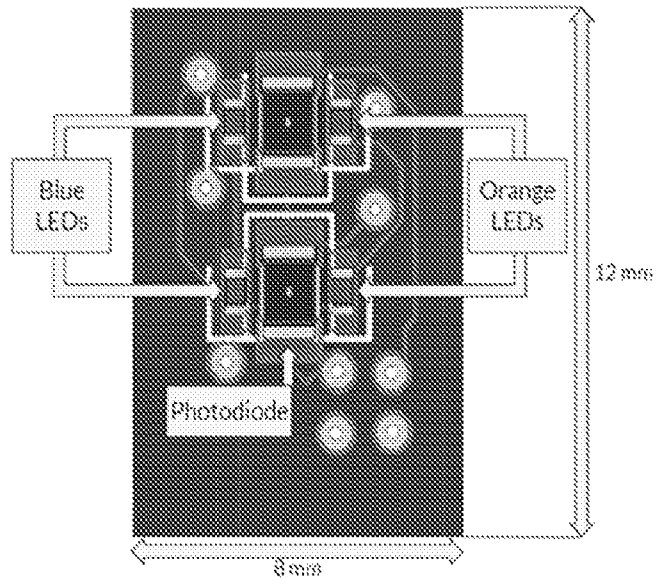


Fig. 9A

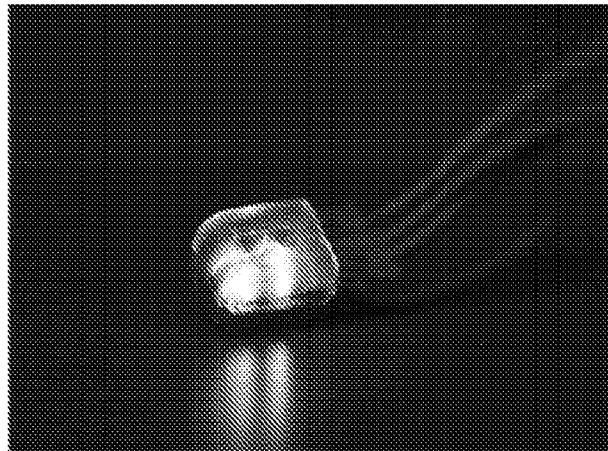


Fig. 9B

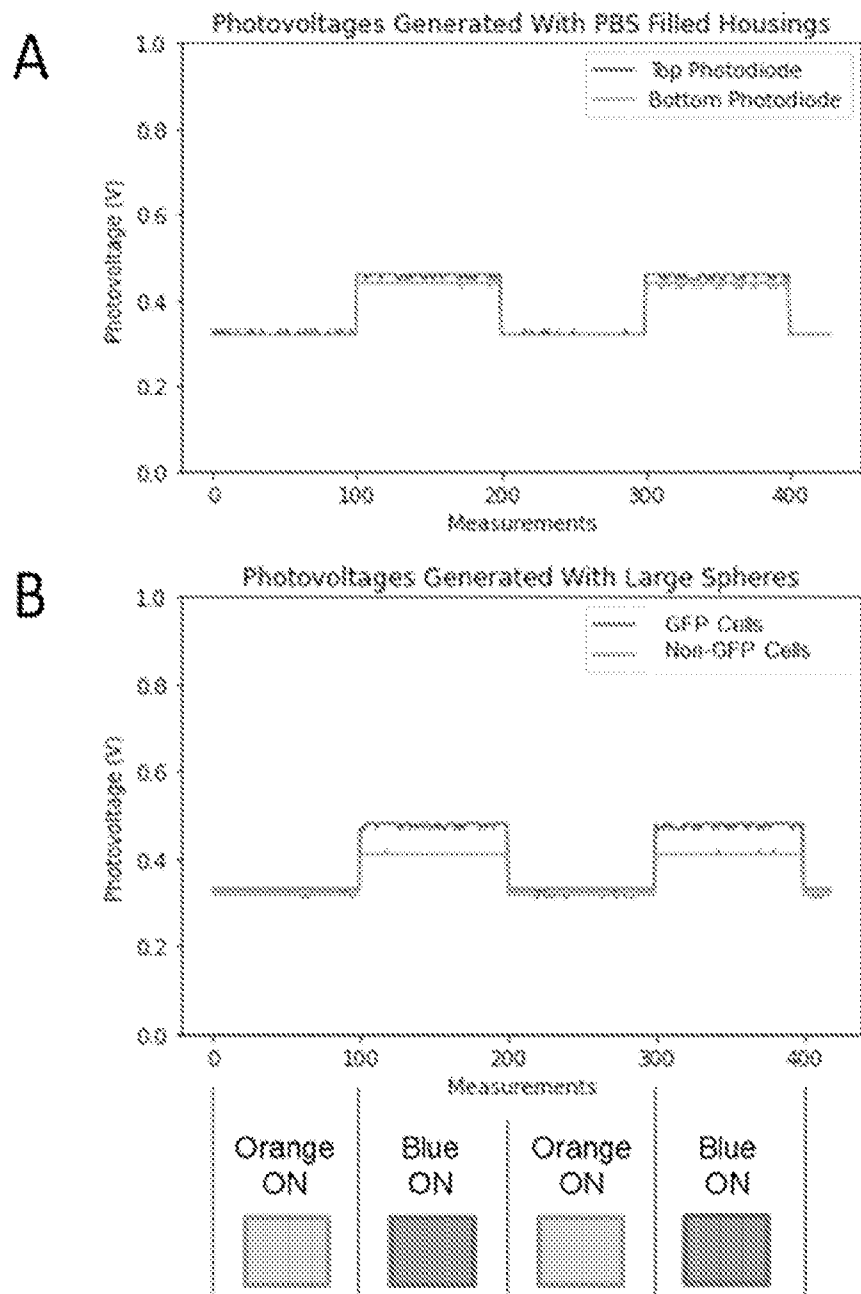


Fig. 10

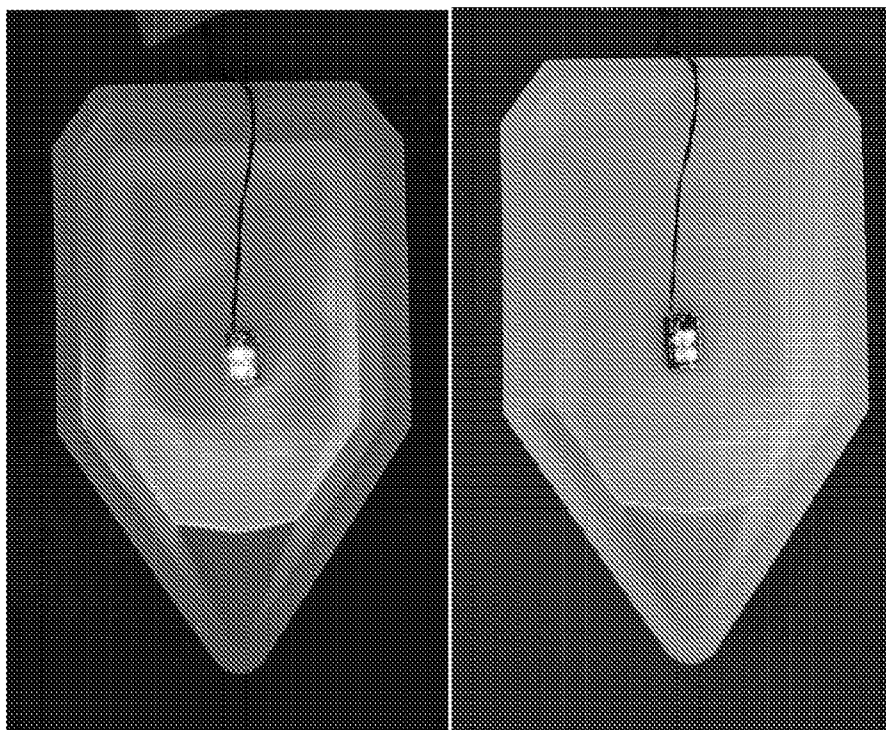


Fig. 11

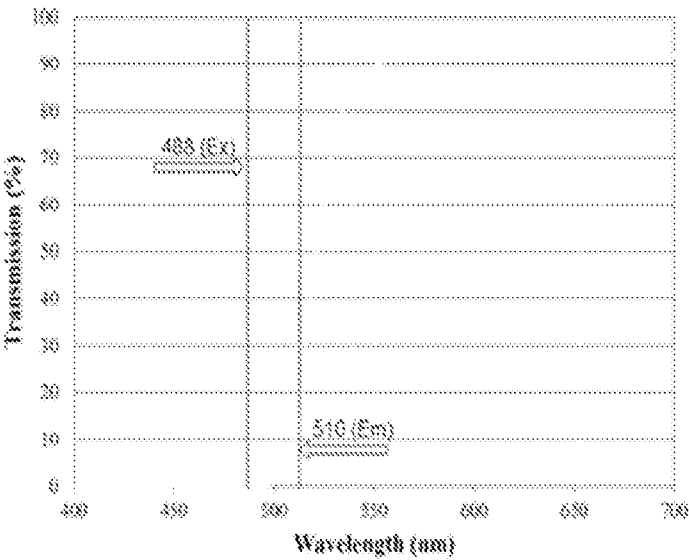


Fig. 12A

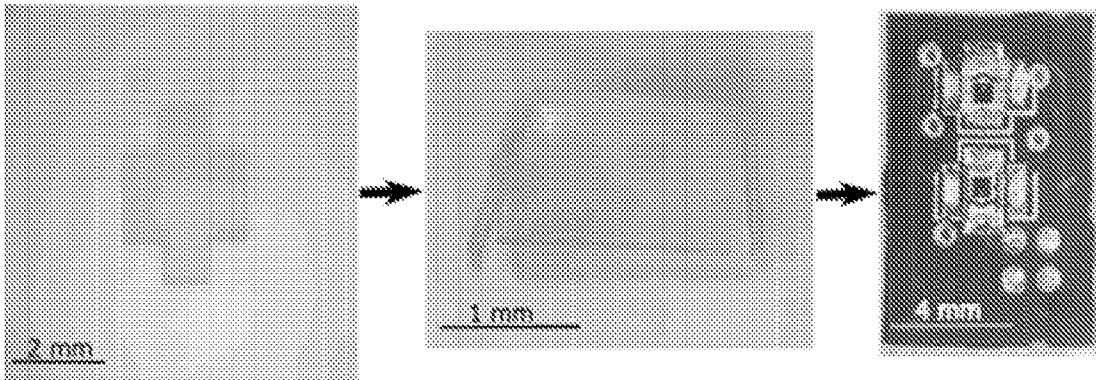


Fig. 12B

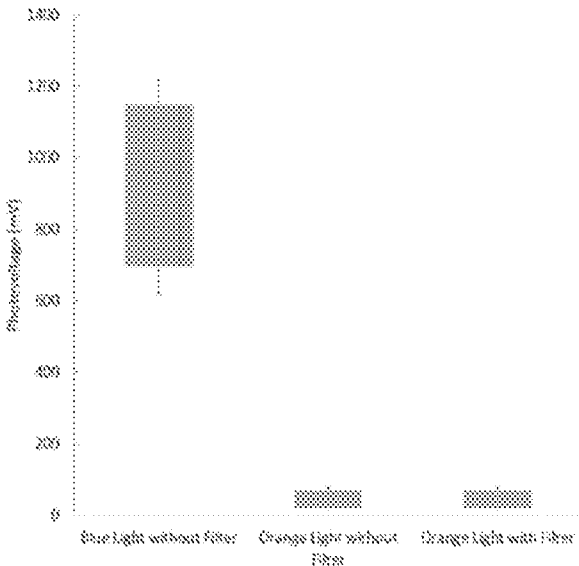


Fig. 12C

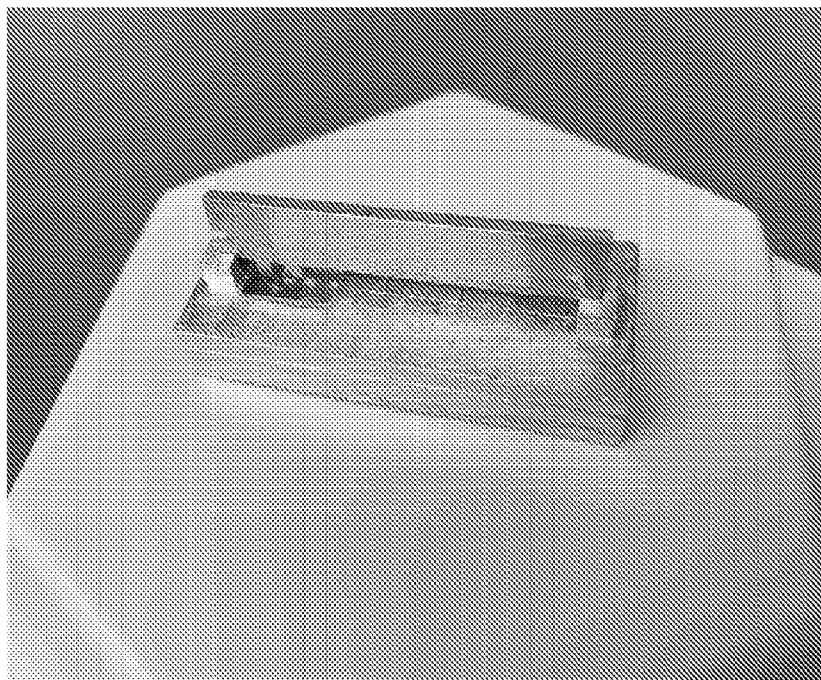


Fig. 13A

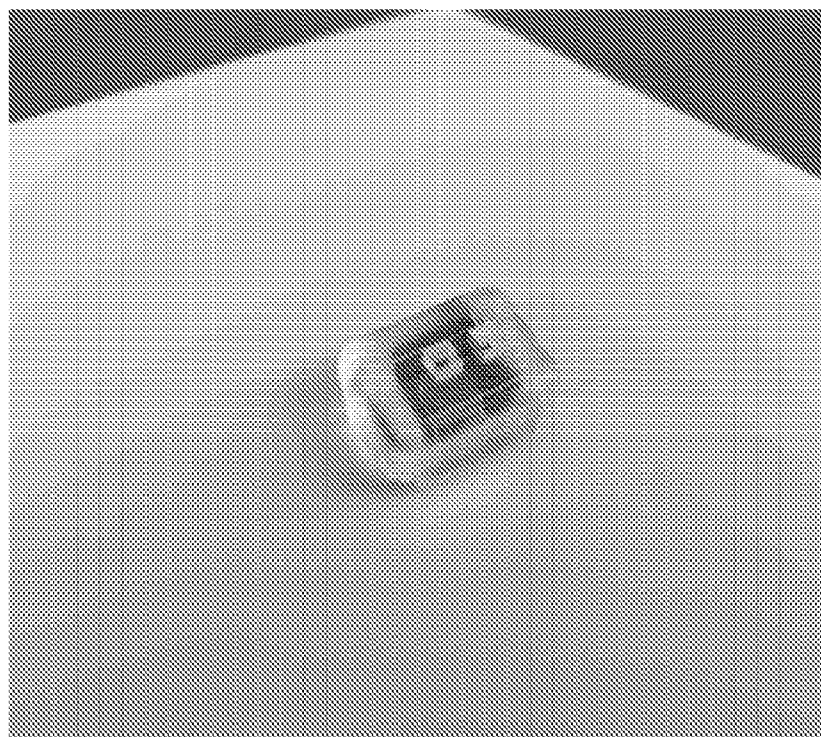


Fig. 13B

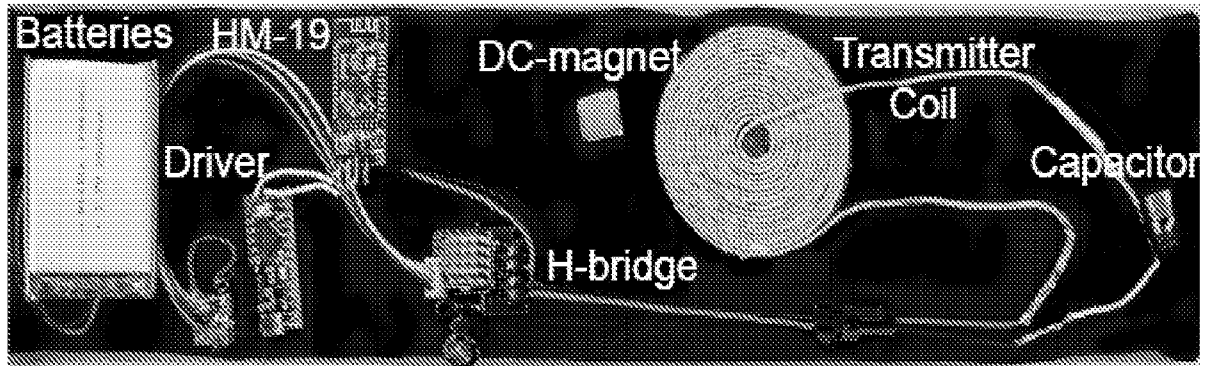


Fig. 14A

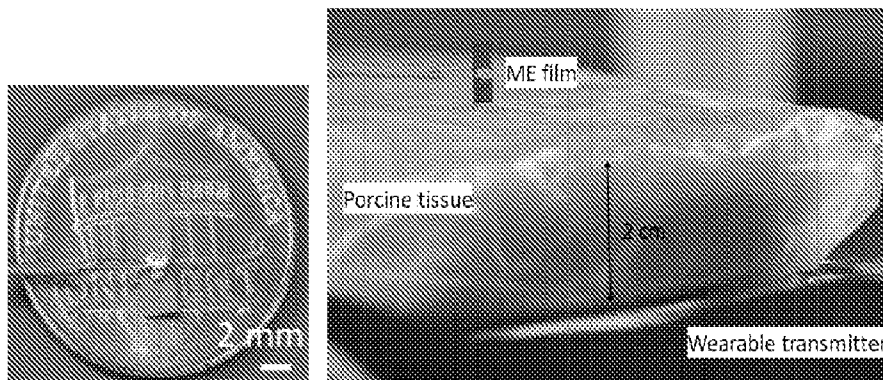


Fig. 14B

Fig. 14C

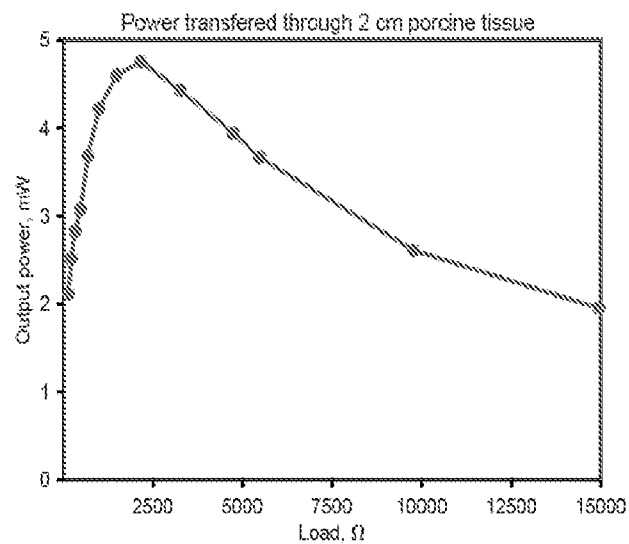


Fig. 14D

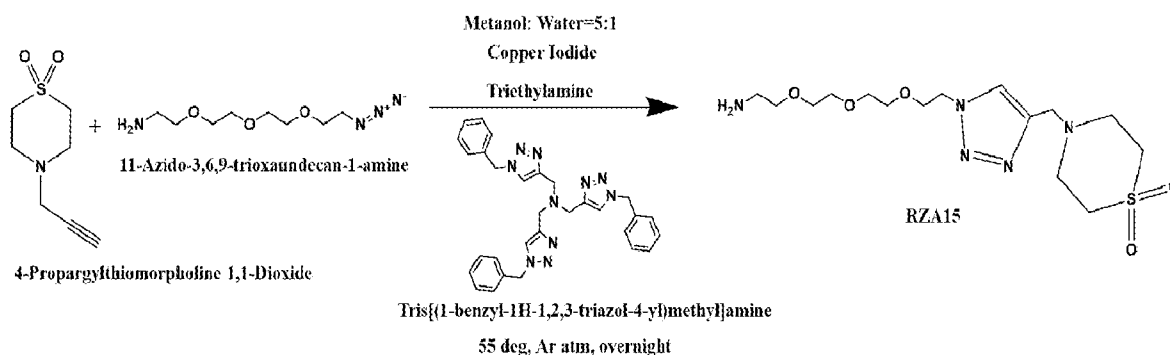


Fig. 15A

NMR: ^1H (600 MHz; CDCl_3): 2.87 (2H, s, $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-O}$), 3.05 (8H, m, $\text{N-CH}_2\text{-CH}_2\text{-S}$), 3.51 (2H, t, $J=5.2\text{Hz}$, $\text{NH}_2\text{-CH}_2$), 3.61 (8H, m, PEG) 3.81 (2H, s, Thiomorpholine- CH_2 -Triazole), 3.89 (2H, t, $J=5.2\text{Hz}$, $\text{N-CH}_2\text{-CH}_2\text{-O}$), 4.55 (t, 2H, $J=5.2$, $\text{N-CH}_2\text{-CH}_2\text{-O}$), 7.69 (1H, s, triazole)

^{13}C (600 MHz; CDCl_3): 41.74 ($\text{NH}_2\text{-CH}_2$), 50.42 (N-CH_2), 50.56 (N-CH_2 Thiomorpholine) 51.52 (S-CH_2 Thiomorpholine) 52.2 (Thiomorpholine- CH_2 -Triazole), 69.54-73.14 (m, PEG), 124.10 (CH triazole), 143.32 (C triazole)

ESI MS: $[\text{M}+\text{H}^+] = 392.1939$

Fig. 15B

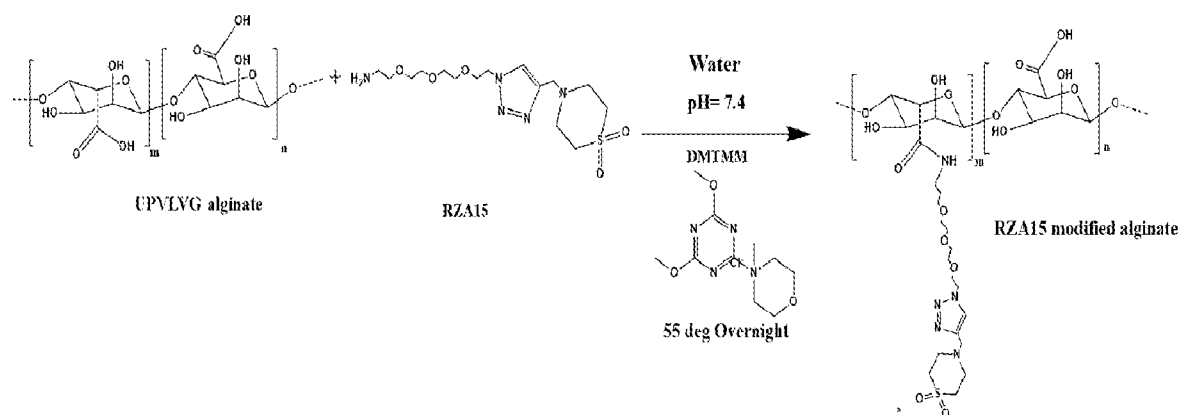


Fig. 16A

NMR: ^1H (600 MHz; D_2O): 3.07 (4H, s, N-CH₂-CH₂-S), 3.17-3.40 (m, alginate protons), 3.46 (4H, s, N-CH₂-CH₂-S), 3.50-3.70 (16H, m, ethoxy), 3.7-5.2 (m, alginate protons), 8.08 (1H, s, triazole)

Elemental: C: 35.67%, H: 4.34%, N: 5.08%, O: 33.50%.

Fig. 16B

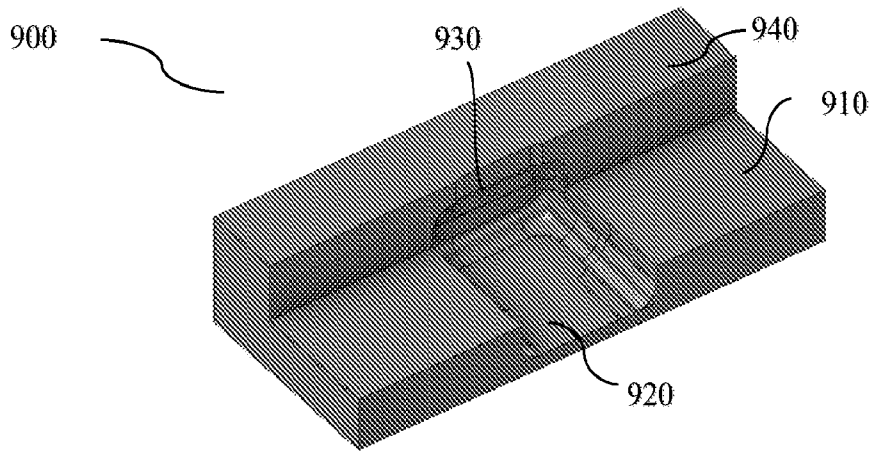


Fig. 17

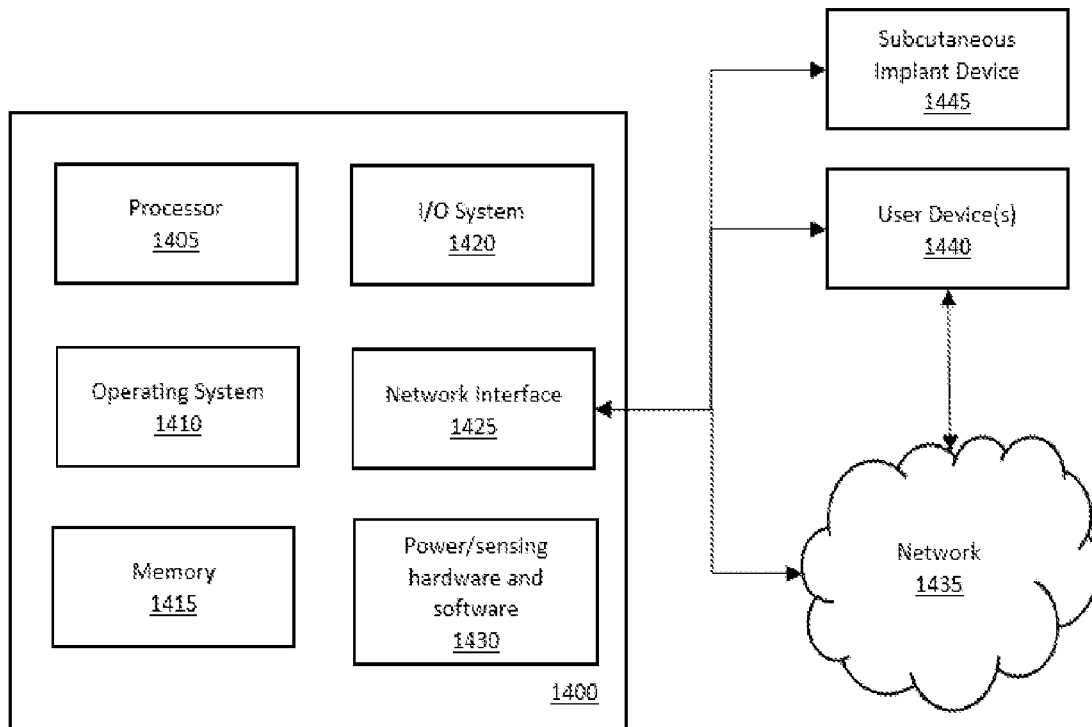


Fig. 18

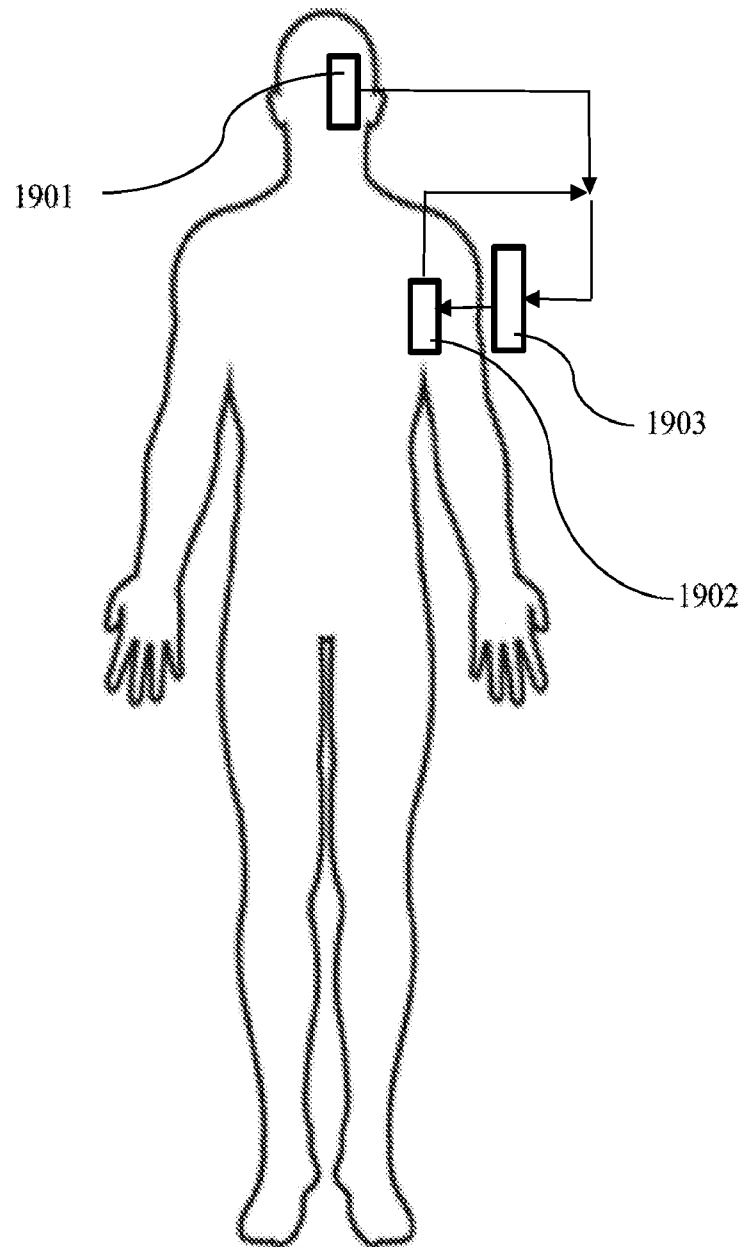


Fig. 19

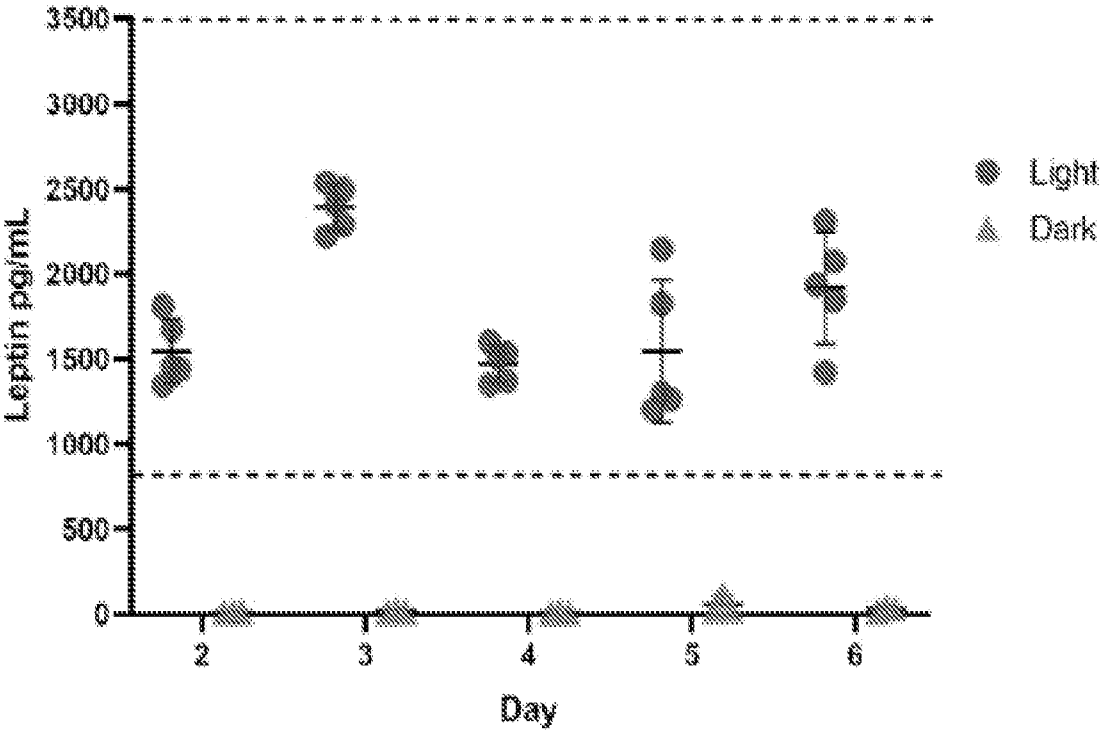


Fig. 20

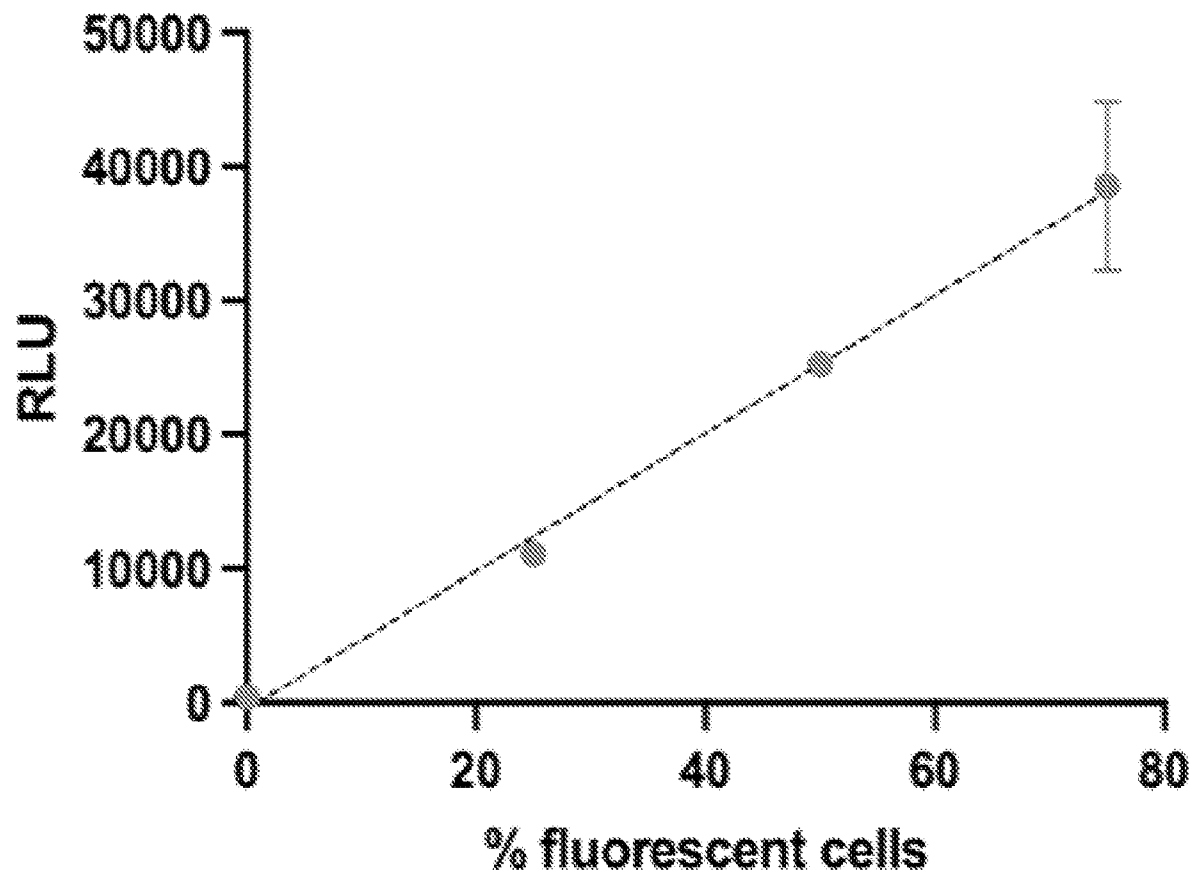


Fig. 21

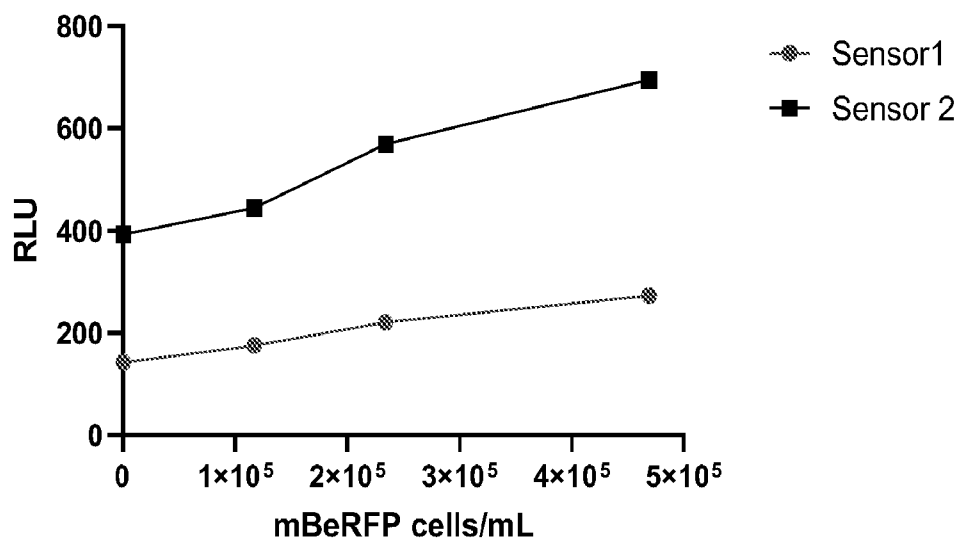
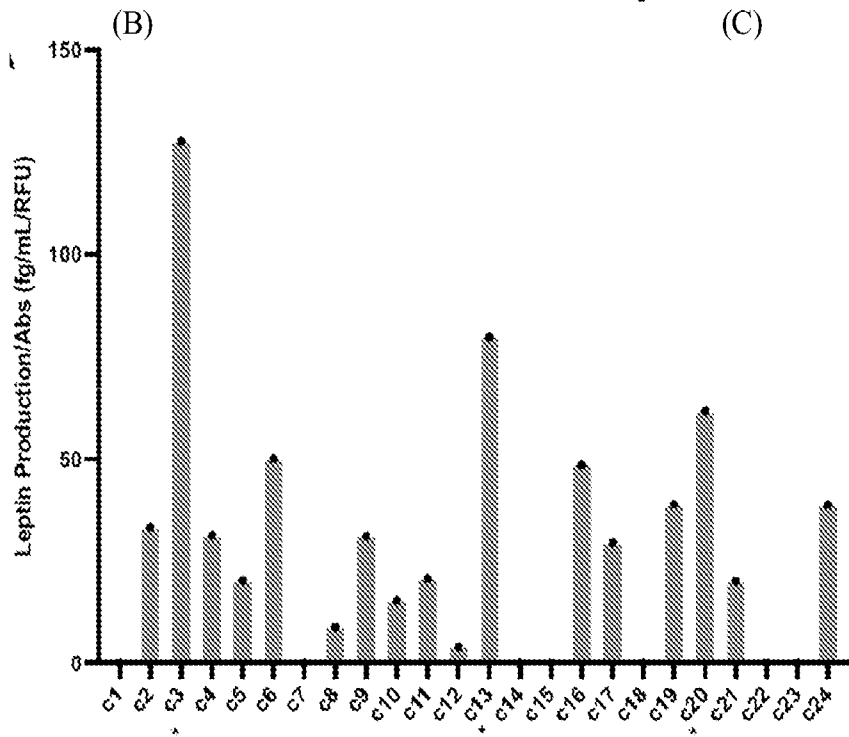
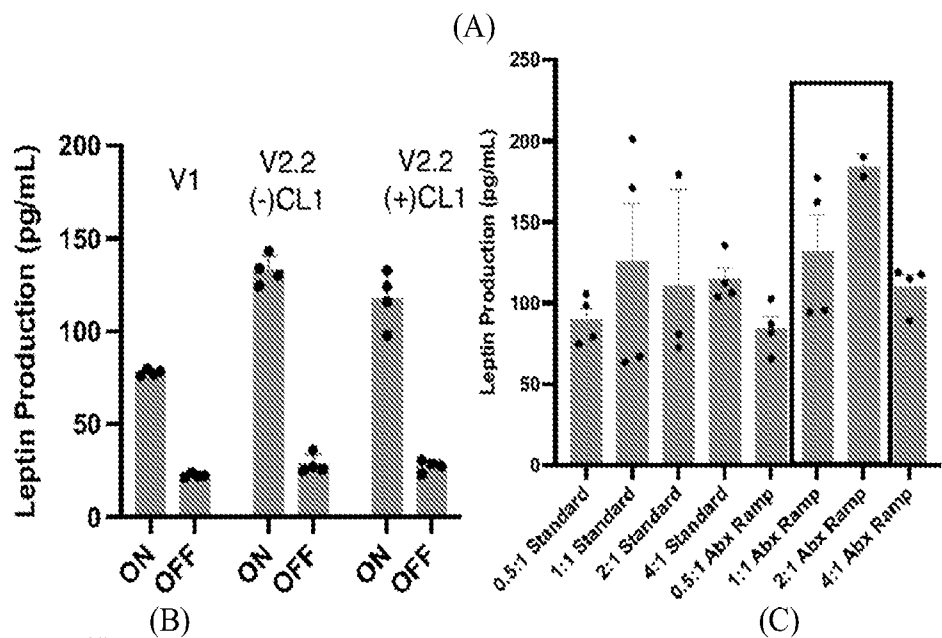
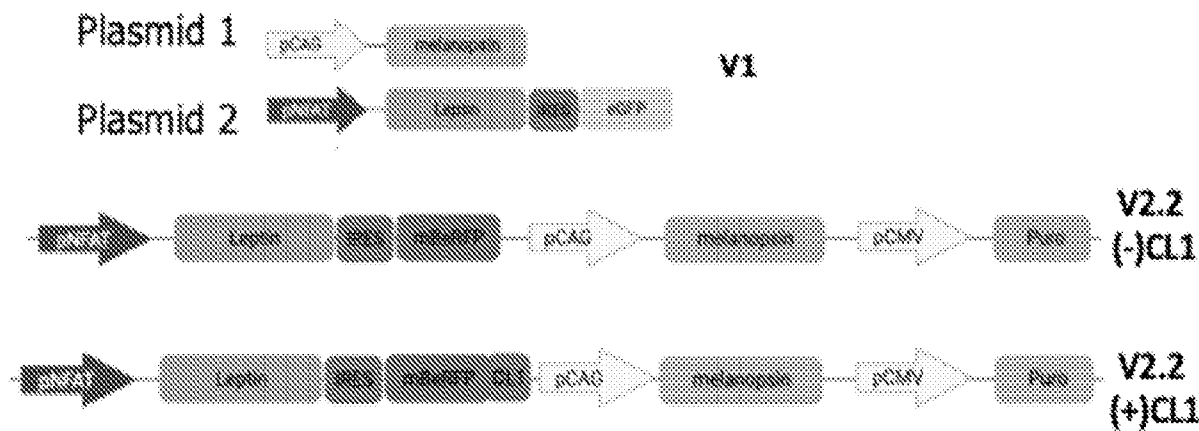


Fig. 22



(D)

Fig. 23

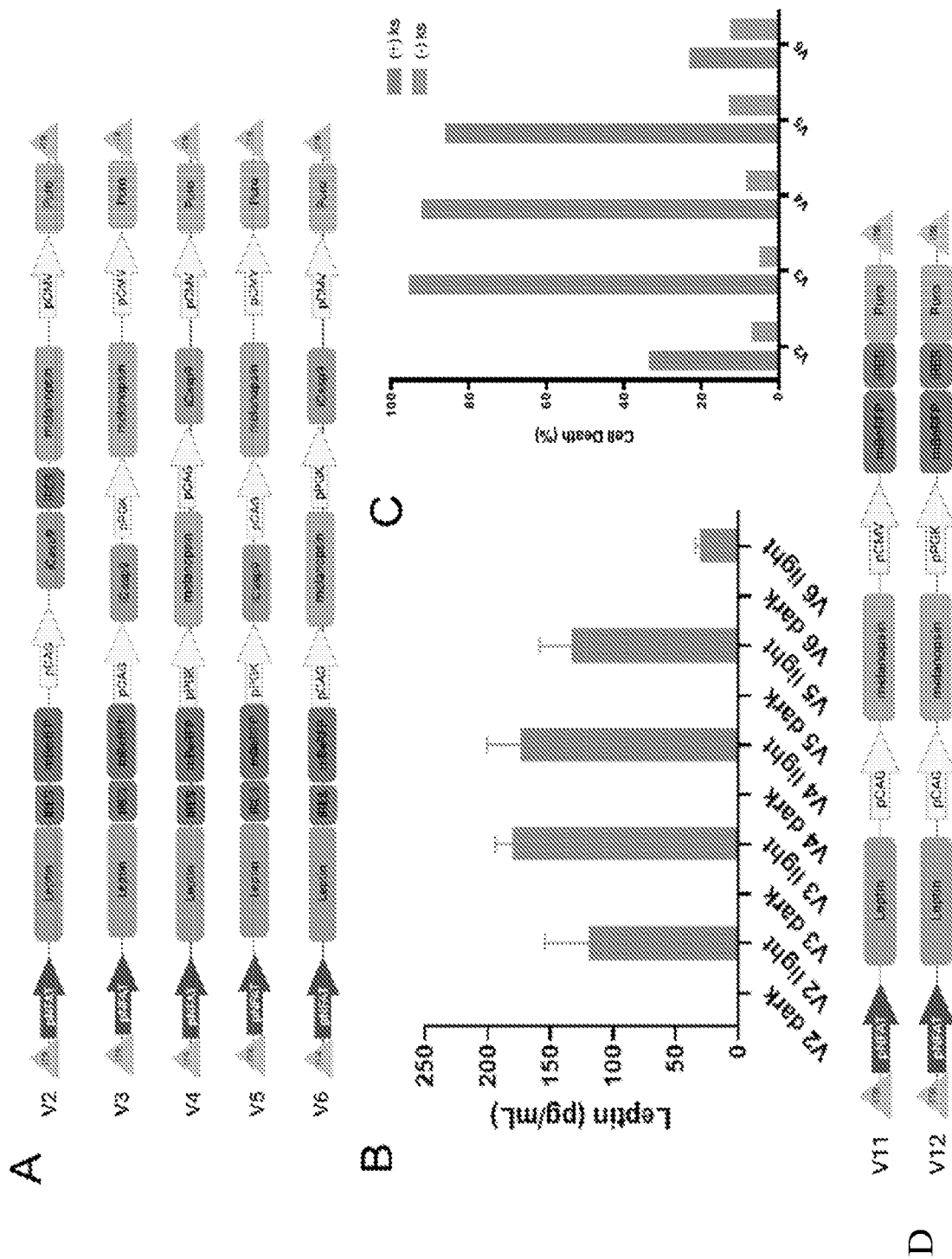


Fig. 24

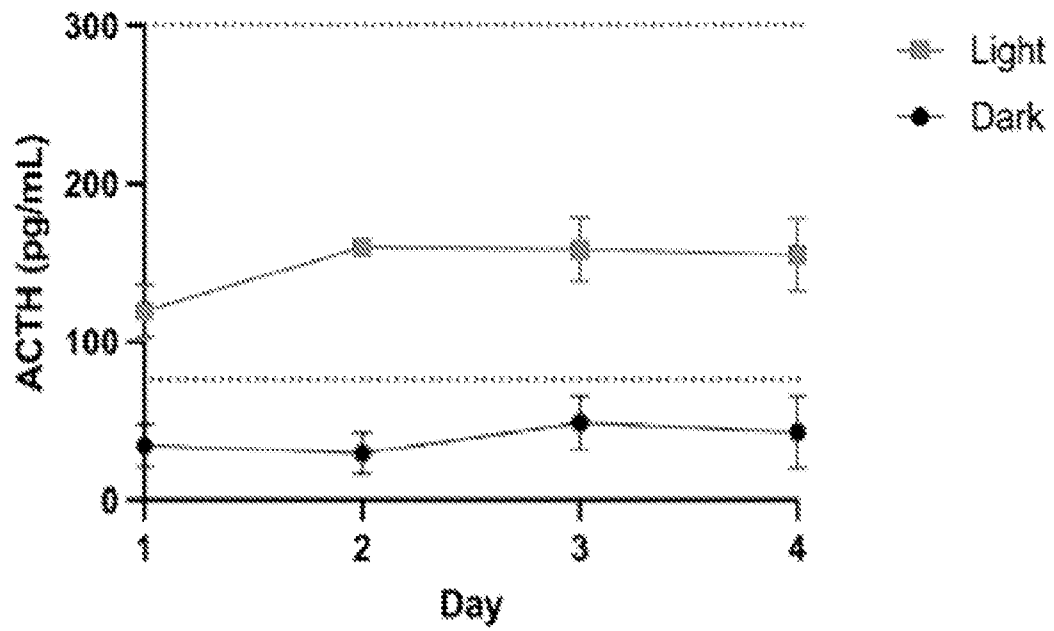
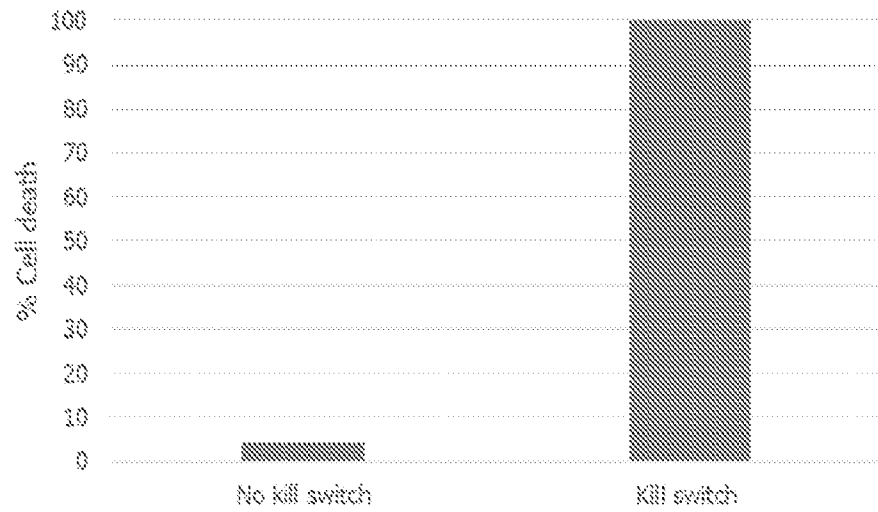
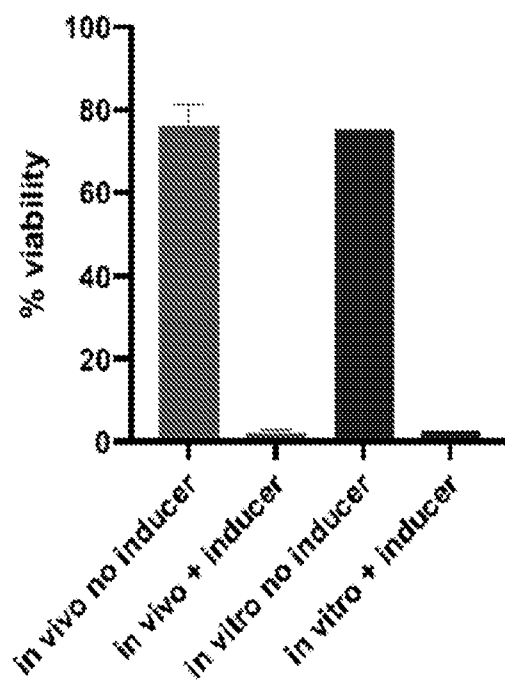


Fig. 25



(A)



(B)

Fig. 26

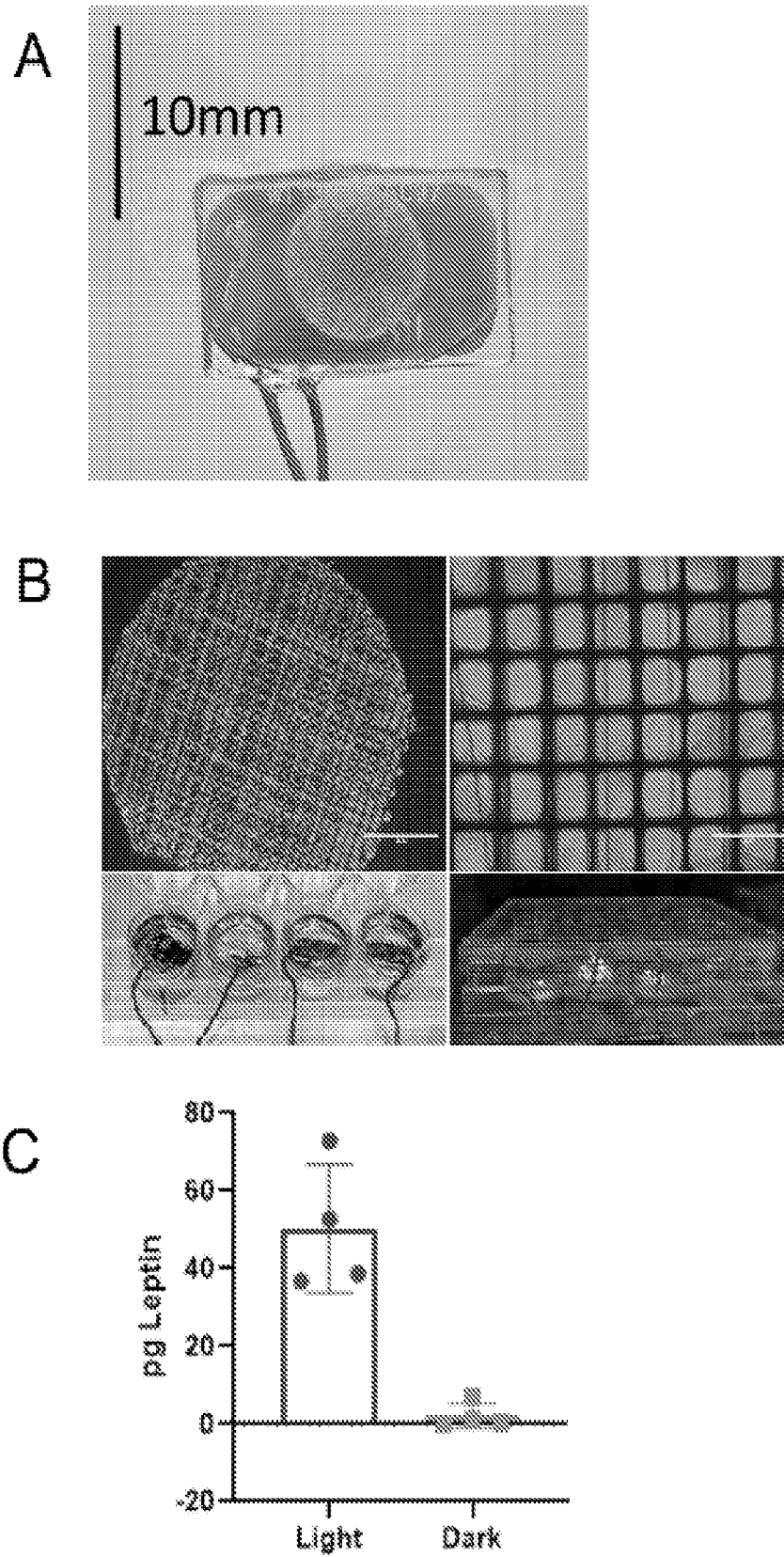


Fig. 27

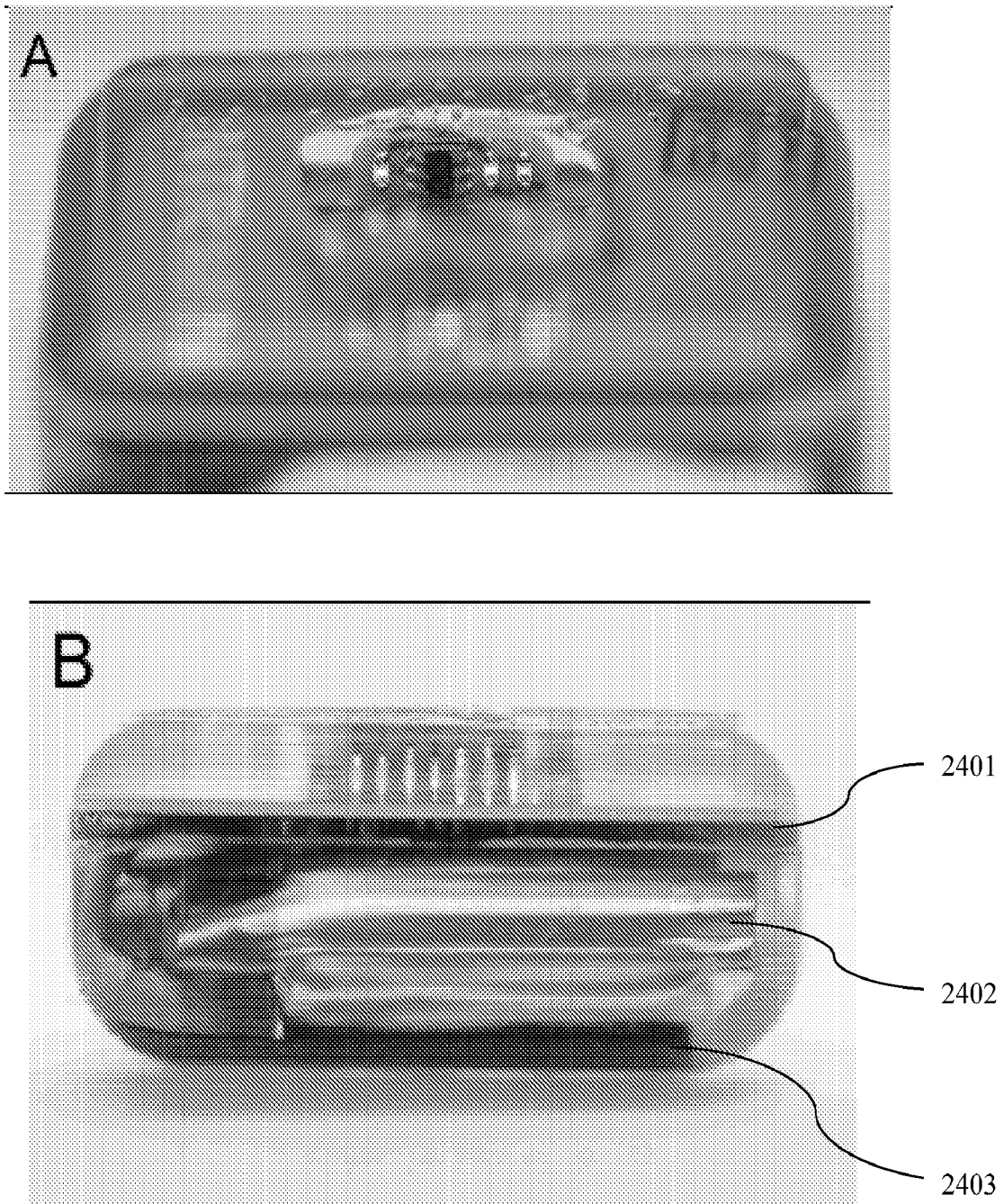


Fig. 28

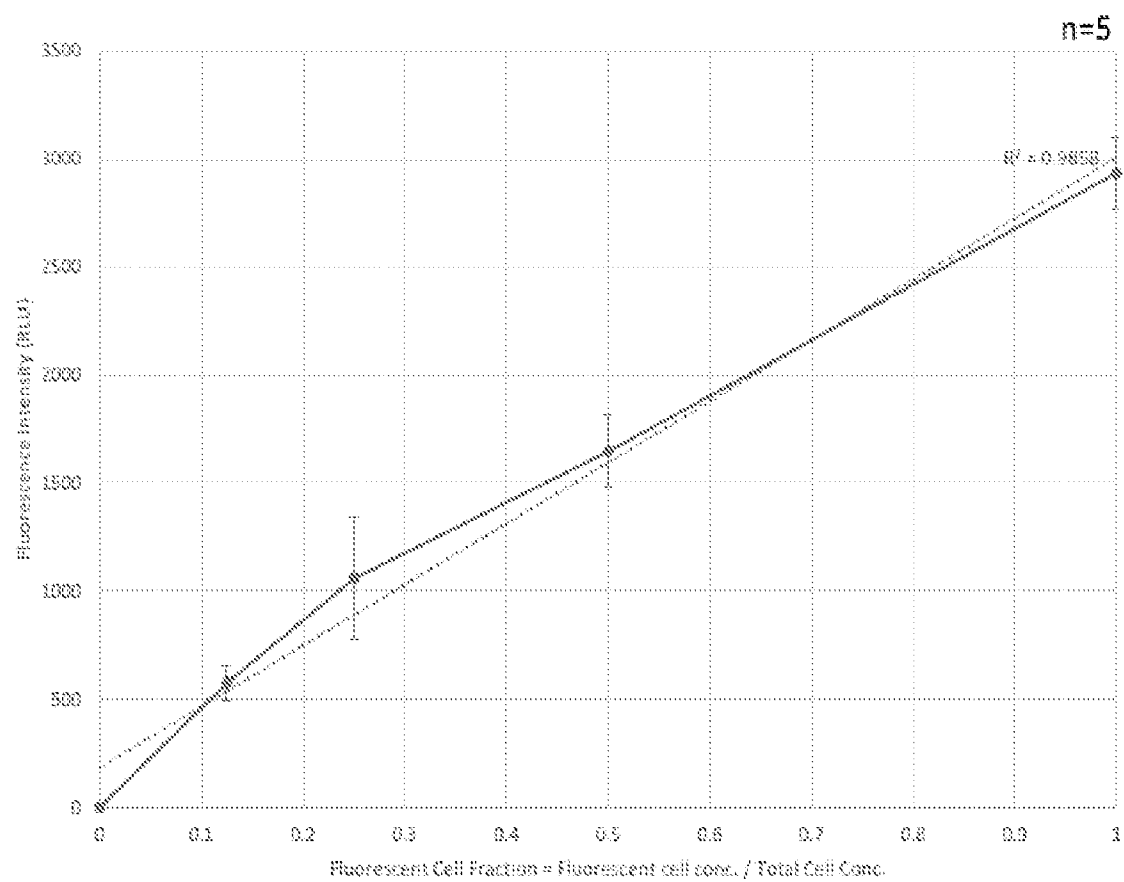


Fig. 29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/053241

A. CLASSIFICATION OF SUBJECT MATTER C12N 15/63(2006.01)i; C12N 9/64(2006.01)i; C07K 14/54(2006.01)i; C07K 14/555(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) C12N 15/63(2006.01); A61B 5/06(2006.01); A61K 35/30(2006.01); A61K 39/395(2006.01); A61K 9/70(2006.01); A61M 5/00(2006.01); C07K 14/705(2006.01); C07K 16/28(2006.01) 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: therapeutic agent, reporter, optical controller, light sensitive protein, kill switch protein, NFAT promoter, CAG promoter, PGK promoter, melanopsin, iCasp9, GLP-1		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
DX	WO 2022-260764 A2 (NORTHWESTERN UNIVERSITY et al.) 15 December 2022 (2022-12-15) paragraphs [0008], [0056], [0091], [00152], [00158], [00187], [00188]; claims 1, 2, 11, 12, 32; figures 2, 5A-5B; SEQ ID No: 1, 2, 23	2-15,17
DY		1,16
DA		18
Y	WO 2016-115482 A1 (NOVARTIS PHARMA AG et al.) 21 July 2016 (2016-07-21) claims 1, 5; SEQ ID NO: 2-5	1,16
A	KR 10-2020-0132957 A (CEDARS-SINAI MEDICAL CENTER) 25 November 2020 (2020-11-25) paragraph [0028]	1-18
A	KR 10-2020-0130468 A (BIOGEN MA INC.) 18 November 2020 (2020-11-18) paragraph [0301]	1-18
☒ 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 10 February 2025		Date of mailing of the international search report 10 February 2025
Name and mailing address of the ISA/KR Korean Intellectual Property Office 189 Cheongsa-ro, Seo-gu, Daejeon 35208, Republic of Korea Facsimile No. +82-42-481-8578		Authorized officer HEO, Joo Hyung Telephone No. +82-42-481-5373

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/053241

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2023-159186 A2 (WILLIAM MARSH RICE UNIVERSITY et al.) 24 August 2023 (2023-08-24) claims 1-17	1-18
A	WO 2021-247623 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 09 December 2021 (2021-12-09) claims 1, 11	1-18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/053241

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US2024/053241

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
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				US	2024-207508	A1	27 June 2024
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				WO	2022-226157	A1	27 October 2022
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				EP	3765058	A4	22 December 2021
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				IL	238804	B	31 August 2021
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				US	2018-362652	A1	20 December 2018
				WO	2014-093396	A1	19 June 2014
				ZA	201503881	B	28 November 2018
WO	2023-159186	A2	24 August 2023	WO	2023-159186	A3	30 November 2023
WO	2021-247623	A1	09 December 2021	None			