

Charging Ahead: Examining the Future Therapeutic Potential of Electroceuticals

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Electroceuticals derived from electronic and pharmaceuticals is a term coined to describe the therapeutic manipulation of neuronal signaling. In recent years, the complexity and range of applications of electroceuticals have grown considerably. Therefore, here a revised ontology and framework for defining electroceuticals that broadens the concept beyond neuron-targeted interventions is suggested. This more inclusive framework aims to provide greater coherence and bring together different stakeholders to accelerate progress in this field. It is suggested that electroceuticals can be categorized according to the level of physiology for which they act: cellular, tissue, organ, and systemic. Emerging developments for each category of electroceuticals and future directions are discussed from a pharmaceutical industry perspective. Potential challenges are highlighted for translation of electroceuticals, such as a lack of clinical biomarkers and incomplete understanding of mechanisms of action and offer solutions for stimulating progress in this exciting field.

1. Introduction

Electroceuticals originating from the combination of electronic and pharmaceuticals describes the manipulation of neuronal

signaling using devices for therapeutic effect.^[1] Since its inception, the field has undergone substantial evolution.

The nervous system plays a key role in regulating functional and homeostatic processes in the body; therefore, its modulation via electroceuticals has powerful potential for treating diseases (Figure 1). In particular, the development of new genetic, biochemical, and bioinformatics technologies have supported an improved understanding of the cellular mechanisms underpinning neuronal communication. In parallel, the emergence of the research field of developmental bioelectricity has revealed that this phenomenon is an important mediator of the phenotypes and functions of cells and tissues at multiple levels. This recent research has also extended the role of ion-driven electrical communication beyond neurons to various cell types. This

opens possibilities for intervention in diverse areas such as birth defects, oncology, neurodegeneration, and metabolic diseases.^[2-4]

Despite possessing high therapeutic potential and garnering significant initial industry interest,^[7,8] pharmaceutical companies have only explored electroceuticals to a limited extent. Pharma involvement could be beneficial in many ways for helping electroceuticals reach their therapeutic potential, including providing expertise in clinical trial design/know-how, designing combination strategies with pharmaceuticals, and scaling validation and distribution of innovative therapies.

In this review, we suggest a revised ontology and framework for defining electroceuticals that reflects a broadening of the concept beyond devices and neural targets, and incorporates recent developments in bioelectricity research, with the aim of bringing greater coherence and inclusivity to the field. By exploring the exciting scientific progress in electroceuticals and outlining some of the outstanding challenges, we aim to bolster their application to a wider set of disease areas and encourage diverse stakeholders, including both academia and industry, to collaborate in maximizing the therapeutic potential of electroceuticals.

2. Electroceuticals: The Need for a Revised Ontology and Framework

Electroceuticals encompasses well-established therapeutic interventions such as pacemakers, cochlear, and retinal implants.

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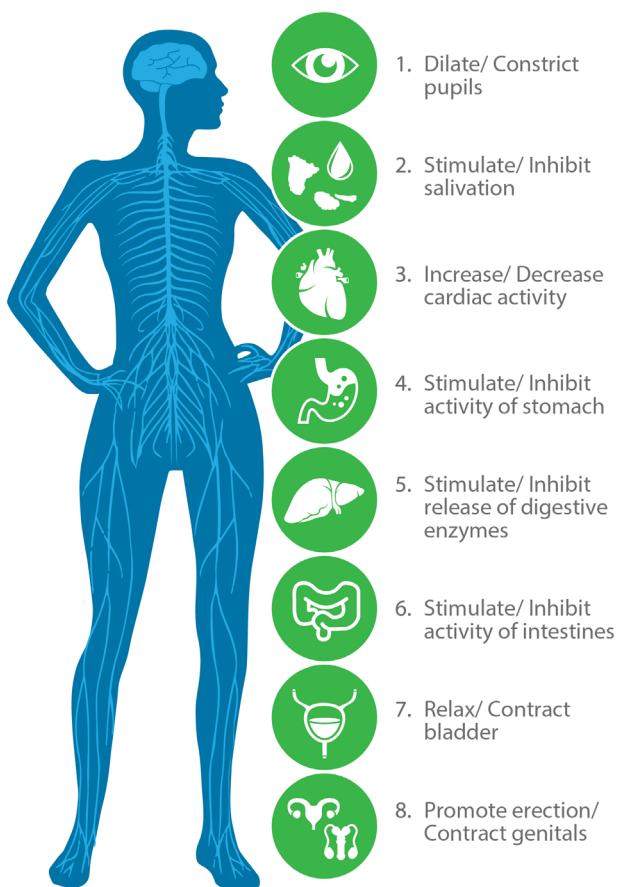


Figure 1. Anatomy showing nervous system control of key organs. The nervous system is central to controlling the function of all major organs and homeostatic processes in the body and mediated through electric signaling. Some of the body functions controlled by the human nervous system include food intake, dilation of airways, cardiac activity, pancreatic activity, brain activity, bladder control, liver, kidney, and spleen functions, and inflammation. Studies in other model systems have shown that the central nervous system also controls some aspects of embryogenesis^[5] and regeneration. Adapted with permission.^[6] Copyright 2012. [Correction added on 17 April 2024, after first online publication: Figure 1 was exchanged.]

Over 20 years ago, Tracey and Tak and coworkers' groundbreaking research characterizing the vagal inflammatory reflex presented the initial evidence of the neural control of immune system. This revelation underscored the potential for more targeted electrical therapies, grounded in a comprehensive understanding of the underlying biology.^[9,10] This potential has now been illustrated by the emergence of therapies including CVRx's Barostim Baroreflex activation therapy, which electronically stimulates the autonomic nervous system to treat symptoms of heart failure,^[11] and SetPoint medical's implant, which uses targeted electric pulses to trigger the intrinsic inflammatory reflex and treat rheumatoid arthritis (RA), is currently undergoing clinical evaluation.^[12]

Given the growth in complexity, and application of these technologies, as well as the need to highlight and reconcile overlap of electroceuticals with the bioelectricity field, we have revisited the

classic definition of electroceuticals to suggest a new ontology. This ontology could offer the following benefits:

- 1) *Aligning Terminology and Definitions:* The term "electroceuticals" has been interchangeably used with bioelectronic medicine (BEM), electrical nerve stimulation, and neuromodulation,^[13] grouping together technologies with a range of specificities and modes of application, and creating ambiguity within the field. A revised ontology that subdivides applications would help more effectively discriminate the mechanisms and anticipated impact of different interventions.
- 2) *Broadening beyond Neuron-Targeted Devices:* Electroceuticals is a term often used to describe devices that target electrical signaling in nerves, reflecting the focus for scientific development and commercialization. However, electrical signaling governs cellular, tissue, organ, and system-wide processes beyond neuronal tissues, necessitating recognition of the broader applications of electroceuticals.^[14]

In addition, because non-neuronal cellular interfaces for bioelectric circuits within the body consists of ion channels and electrical synapses, ion-channel-targeting compounds (many of which are already approved for other purposes) represent a powerful class of electroceuticals.^[2,15] Similarly, recently developed techniques yet to show clinical success should also be considered, such as optogenetic approaches that use light to electrically control tumorigenesis^[16] or induce regenerative repair.^[17]

We therefore believe that a clearer and more inclusive framework with consistent terminology to describe electroceuticals will be helpful for understanding and appreciating the full range of their potential applications, providing more coherence and accelerating progress in the field. It is to be noted that EndoNovo Therapeutics Inc. has trademarked the term "electroceuticals" for specific commercial purposes. We use the term "electroceuticals" in the paper to describe the field and as such used for information purposes only.

3. Re-Defining Electroceuticals

Based on their therapeutic potential, and the needs listed above, we propose a revised definition and ontology for electroceuticals: the use of a therapeutic intervention to treat or prevent disease through the alteration of electrical signals in excitable or nonexcitable cells.

We have used the broad term "therapeutic intervention" to encompass both the major products available today; drugs and devices, as well as new modalities that could be utilized in the future. Similarly, the applications of electroceuticals are intentionally broad, to cover a range of potential therapeutic benefits, from symptomatic to disease-modifying interventions, or even prevention and repair of birth defects, injuries, and other disease states. We have widened the targets of electroceuticals beyond neurons to include excitable and nonexcitable cells. In addition, "alteration" covers the gamut of potential modifications: stimulatory, inhibitory, normalizing, etc. and can be achieved via manipulation of inherent electrical signals or by introducing external electrical stimuli.

Building on this definition we also suggest a potential ontology for bioelectric research according to the physiological level that they target (**Figure 2**):

At the systems level, electrical signalling has been shown to regulate and be able to induce regeneration, organ shape and patterning in a range of contexts including repair of birth defects, induction of appendage growth, and cancer normalization.

At the organ level, electrical signals like those conducted through the vagus nerve (a key component of the parasympathetic nervous system) act as a modulator of the brain-gut axis and regulate immune response, digestion, heart rate, mood and healthy functioning of various organs.

At the tissue level, endogenous transepithelial electric currents have been studied in the context of wound healing and other diseases.

At the cellular level, electrical signalling across the plasma membrane regulates cell behavior and gene expression, including controlling differentiation of stem cells.

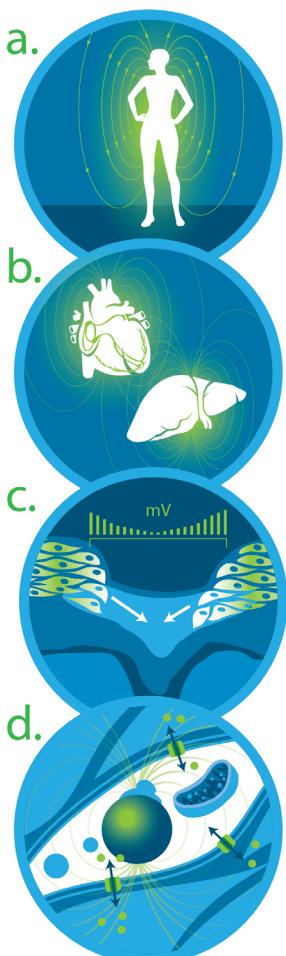


Figure 2. Bioelectric signaling operates at four different levels within the body. A progressively zoomed-in view and definitions of electrical signaling at a) system, b) organ, c) tissue, and d) cellular levels of the body. [Correction added on 17 April 2024, after first online publication: Figure 2 was exchanged.]

- 1) Cellular level bioelectric signaling
- 2) Tissue level bioelectric signaling
- 3) Organ level bioelectric signaling
- 4) Systems level bioelectric signaling

At each physiological level, nondevice-driven therapeutic approaches manipulating bioelectric signaling through cellular or molecular biochemistry would be known as bioelectroceuticals while the application of exogenous electric stimuli via devices would be known as bioelectronic medicine or BEM (**Table 1**).

In the following sections, we explore each of these product classes, outlining the applications and challenges for each product class, and highlighting examples to illustrate the application of this novel framework.

3.1. Cellular Level Bioelectric Signaling

Cellular level bioelectric signaling examines inter- and intracellular electrical signaling mediated by changes in resting mem-

Table 1. Physiologic levels at which bioelectric signaling is leveraged to treat a disease and the modalities (biochemical or physical) used to administer the intervention.

Terminology	Physiologic level at which effect is seen	Cellular/molecular biochemistry	External electrical stimuli using devices
Cellular bioelectroceutical	Cellular	Yes	No
Cellular level BEM		No	Yes
Tissue bioelectroceutical	Tissue	Yes	No
Tissue level BEM		No	Yes
Organ bioelectroceutical	Organ	Yes	No
Organ level BEM		No	Yes
Systems bioelectroceutical	Systems	Yes	No
Systems level BEM		No	Yes

brane potential (V_{mem}) in cells, which is governed by ion (K^+ , Na^+ , Ca^{2+} , and Cl^-) flow across the plasma membrane.^[15,18] Bioelectrical signals produced by ion channels are transduced into secondary messenger responses, altering key aspects of cell behavior such as proliferation, differentiation, apoptosis, and migration.

An interesting subcategory of cellular level bioelectric signaling occurs at the organelle and molecular level. This electric signaling is mediated by intracellular signaling cascades and is essential for homeostasis in organelles such as the nuclear envelope, mitochondria, endoplasmic reticulum, phagosomes, and lysosomes.^[19] For instance, in human dermal stem cells and mouse neural stem cells, two subpopulations of mitochondria with varying mitochondrial membrane potential ($\Delta\psi_m$) have been identified.^[20] Cells with low $\Delta\psi_m$ are dormant stem cells required to sustain a neuronal precursor pool whereas high $\Delta\psi_m$ stem cells are prone to neuronal differentiation. This could provide an alternative approach to current protocols for neuronal differentiation by simply modulating mitochondrial potential. Organelle level bioelectric signaling is a nascent research area, partly owing to the lack of tools to measure voltage gradient across organelle membranes, which is an active area of method development.^[21] At the molecular level, alternating electric field has been shown to degrade magnetite-bound protein aggregates in Alzheimer's disease via an electro-Fenton effect generated by electric-field-sensitized magnetite.^[22,23] In another study, electrical stimulation (ESTim) administered via gold nanoparticles functionalized with redox active molecules was shown to initiate quantum biological tunneling for electron transfer and triggered selective apoptosis of cancer cells.^[24]

3.1.1. Applications

The applications of cellular level bioelectric signaling are multi-fold and include embryogenesis, metabolism, quorum sensing, and biofilm formation in bacteria^[25] and cancer.^[26] Herein, we discuss two of the applications where there has been much recent progress:

1) Stem Cells: Recent studies examining the role of bioelectric signaling in stem cells have demonstrated a functional role for endogenous electrical signaling in maintaining proliferative

states and inducing differentiation.^[27–29] For instance, studies in *Drosophila* gut identified conserved pathways (such as mGluR) that link bioelectric regulators of Ca^{2+} , Na^+ , and Cl^- with intestinal stem cell proliferation.^[30,31] In both human mesenchymal and cardiac stem cells, the big potassium (BK) channel has been shown to be a key regulator of proliferation, self-renewal, and hyperpolarization-induced differentiation.^[32] The role of mechanosensitive ion-channel-triggered Ca^{2+} in stem cell proliferation, differentiation, and migration has also only been recently realized.^[33–35] V_{mem} has been shown to drive the exit from pluripotency and the onset of germ layer differentiation (cell fate commitment) during embryonic development via calcium and mammalian target of rapamycin (mTOR) signaling pathways.^[36] Under the new terminology, therapeutics manipulating these endogenous cellular bioelectric signaling fall under cellular bioelectroceuticals.

Exogenous EStim has also been shown to influence stem cell fate and is relevant in both in vitro and in vivo culture settings (Figure 3a).^[37–39] EStim of human-induced pluripotent stem cells (iPSCs) cultured in a conductive polymer system could induce multilineage differentiation and a robust neuronal fate.^[40] In another study, EStim of stem cells transplanted in a conductive polymer system has been shown to improve functional stroke recovery in rodents.^[41] Under the new terminology, these examples fall under cellular BEMs. Overall, manipulation of cellular level bioelectric signaling provides a novel and alternative way to control stem cell behavior and could have an impact in regenerative medicine.

2) *Cancer:* Misregulation of ion channels has long been linked to cancer.^[43] However, more recently the role of resting membrane potential has been explored, with changes in V_{mem} being associated with increased cell proliferation, invasion, and metastasis^[44] (and in general, loss of cooperativity toward normal morphostasis).^[45] The bioelectrical properties of individual cancer cells differ vastly from their normal counterparts (Figure 3b); their V_{mem} values are more depolarized (-5 to -40 mV vs -40 to -90 mV in normal epithelial cells) and they have dysregulated ion-channel expression and activity.^[42] This work is now being extended to human glioblastoma (GBM)^[46] and breast cancer.^[47]

Classic research by Cone has shown that mitosis and V_{mem} are correlated in somatic cells, with proliferation being induced by sustained depolarization in terminally differentiated cells.^[48] More recently, *Xenopus* studies have shown that when messenger RNA (mRNA) of a hyperpolarizing potassium channel (Kir2.1) is co-injected with an oncogene, tumor incidence is significantly reduced, while depolarization resulted in increased tumor incidence.^[49] The *Xenopus* work has also informed applications in human cells; for example, repurposed ion-channel drugs have recently been used to target bioelectric properties in GBM cells, suppressing tumor growth and driving the cells toward a differentiated and senescent phenotype.^[46] The Djambazov laboratory proposed the “Celex (cellular excitability) hypothesis” of metastasis, which suggests that electrical excitability makes cancer cells hyperactive, disruptive, invasive, and ultimately metastatic.^[50–52] In parallel, Hancock and co-workers have uncovered an electric control mechanism in cancer growth, showing that V_{mem} modulates plasma membrane phospholipid dynamics and K-Ras signaling, which are associated with cell division.^[53] These and other

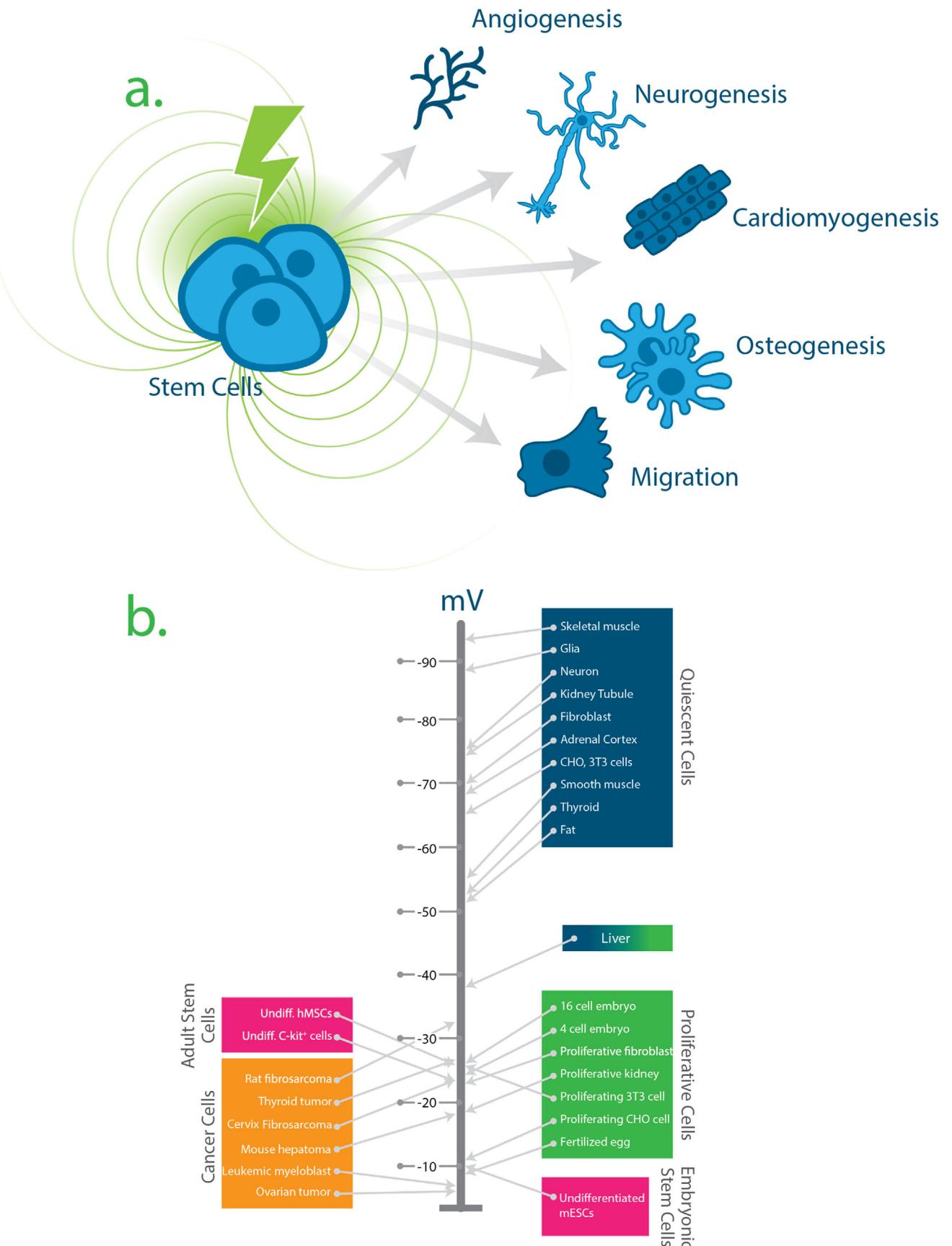
studies^[54] have provided data indicating that ion-channel modulators, cellular bioelectroceuticals, could be an important emerging class of cancer drugs.^[55–57]

3.1.2. Challenges

Despite the growing number of academic researchers working in cellular level bioelectric signaling, there are inconsistencies in data and conflicting results from different groups.^[58] The main challenges in translating this concept into the clinic are as follows:

- 1) *Incomplete Biological Understanding:* Several questions remain before we understand the molecular and cellular mechanisms that underpin bioelectric signaling. For example, how does bioelectricity influence the fate of different cell types? Which genes and pathways are involved? Does this vary from cell to cell, and disease to disease? Cancer cells and immune cells behave in fundamentally different but complementary ways to changes in ionic levels and V_{mem} .^[59,60]
- 2) *Lack of High-Throughput Screens and Relevant Models:* How do we study and screen for compounds that alter cellular level bioelectric signaling in vitro? Patch clamp is the gold standard for measuring V_{mem} , but is labor intensive and low throughput. Use of fluorescent dyes such as bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DIBAC₄(3)) are an alternative, but the fluorescent gradient is small, sensitive to differences in normalization and only semiquantitative. Clustered regularly interspaced short palindromic repeats (CRISPR) screens have been helpful in elucidating the functions of various proteins but do not provide information on the functional (i.e., open or closed) state of ion channels and how this influences V_{mem} . Simple in vivo systems such as *Xenopus* tadpoles and zebrafish have been useful to study organism level responses to bioelectricity. However, translation remains a considerable challenge when considering the scale and complexity of the gaps between these model organisms and humans. The emergence of new and robust methods for studying cellular bioelectroceuticals,^[57] and screening for compounds that alter V_{mem} , could help to accelerate progress from concept to the clinic.
- 3) *Locally Targeted Modulation of Bioelectricity to Bring about a Desired Therapeutic Effect:* How can we selectively alter or restore the V_{mem} of diseased cells or stem cells? Which ion channel or combination thereof provides the desired change in V_{mem} ? Should we drive cells toward a hyperpolarized or depolarized state, and how will this influence cell fate? How much change in V_{mem} is needed to achieve the desired cellular effect? What would be the effects of cellular resistance to an electrical perturbation? Some of these questions are beginning to be addressed as computational tools such as machine learning and bioelectric simulators are being used for predictive control of bioelectric states.^[61,62]

Addressing these challenges will collectively improve our understanding of the mechanisms of cellular level bioelectric signaling and equip us with the tools to screen and develop approaches for therapeutic modulation.



3.2. Tissue Level Bioelectric Signaling

Electrical signals are inherent to most tissues and vary depending on the specific state of the tissue: development, injury, diseased, or healthy. Electric currents have been measured, for example, in tissue development, wound healing,^[63] bacterial infection of the gut epithelium,^[64] and tumor tissue.^[65] Consequently, introducing external electric stimuli into tissues can alter the course of development^[66] and healing.^[67] We discuss three of the most advanced applications in this category in further detail.

3.2.1. Applications

1) *Wound Healing*: In wounds, an endogenous electrical field is generated across the injured tissue,^[68] the strength of which is dependent on the tissue type.^[69] The transepithelial membrane potential collapses at the site of the wound to zero compared with the unwounded epidermis,^[63] creating an electrical gradient that provides the cue for directed cell migration into the wound (electrotaxis; Figure 4a).^[70] The importance of this bioelectric cue is highlighted by its ability to override other directional cues for cell migration in wound healing.^[71] Endogenous electric fields in wounds were first observed 150 years ago,^[68] but it was not until 2006 that the first genes, Phosphoinositide 3-kinase/Phosphatase and tensin homolog (PI3 kinase/PTEN), that control electrotaxis were identified.^[72] Vomaris utilizes this principle of electrotaxis in their moisture-activated bioelectric dressing (Procellera), comprised of elemental silver and elemental zinc embedded in a substrate.^[73] When applied to the injury, microcurrents similar to the skin's physiological electric field are generated wirelessly via a redox reaction, promoting wound healing and exerting an antimicrobial effect.^[74,75]

2) *Tumor Tissue*: A recent addition to cancer treatment is tumor-treating electrical fields (TTFields), which are locally applied to tumor tissue to disrupt proliferation and stimulate apoptosis of cancer cells (Figure 4b).^[76,77] TTFields are low intensity ($1\text{--}3\text{ V cm}^{-1}$), intermediate frequency (100–300 kHz) alternating electric fields ($100\ 000\text{--}300\ 000\text{ times s}^{-1}$) tuned specifically to target cancer cells, while leaving quiescent cells intact.

Two specific mechanisms for the effects of TTFields on cancer cells have been proposed.^[78,79] In the first, TTFields disrupt the positioning of polar cell proteins such as tubulin and septin, thus interfering with the formation of a functional mitotic spindle during metaphase. In the second, TTFields elicit a nonuniform intracellular electric field during cytokinesis, which acts on polarizable structures such as microtubules, organelles, ions, proteins, and DNA. This leads to structural disruption and impaired cell division.

Novocure has been developing TTFields since 2000, and the U.S. Food and Drug Administration (FDA) has approved them for recurrent GBM, newly diagnosed GBM and mesothelioma (sold as Optune and Optune Lua). Novocure is currently

evaluating TTFields in combination with the anti-PD-1 (anti-Programmed Death-1) therapy KEYTRUDA (pembrolizumab) for the treatment of non-small cell lung cancer (KEYNOTE-B36 trial)^[80] and newly diagnosed GBM (EF-41/KEYNOTE D58)^[81] in a collaboration with MSD (tradename of Merck & Co., Inc., Rahway, NJ, USA).

3) *Engineered Tissue*: Electrical signaling can also be employed to improve tissue engineering.^[66,82] In matured cardiac tissue, electrical signal activation is regulated by exchange of ions via voltage-gated sodium or potassium channels and propagated via conduction of these signals through gap junctions. To reproduce the maturity and function in an engineered tissue, researchers have utilized EStim and mechanical stimulation (MStim) of cardiomyocytes.^[83,84] For example, applying pulsed electrical signals at 3 V cm^{-1} amplitude and 3 Hz frequency resulted in significant structural organization (as measured by cell elongation, tissue compactness, and protein levels) and contractile behavior (as measured by excitation threshold, maximum capture rate, and amplitude of contraction) *in vitro*.^[85] Such effects were also observed *in vivo* using a tissue engineering chamber to culture and electrically stimulate human iPSC-derived cardiomyocytes, resulting in a compact and vascularized cardiac tissue.^[86] In the case of bone defect repair, piezoelectric biomaterials have also been shown to induce vascularized bone regeneration through microcurrent stimulation and Zn^{2+} enrichment.^[87]

3.2.2. Challenges

The effects of bioelectric signaling at the tissue level have been explored in limited contexts by only a few companies and their academic collaborators. While the clinical data from these groups look promising and have led to FDA approval and commercial use of Procellera and Optune, additional exploration by independent groups is warranted to increase adoption. Clinical readouts from the TTFields/PD-1 combination trials should provide support for drug-device combinations in this space. For the optimized application of EStim, the underlying mechanisms of cellular responses require a better understanding, and there is a need to standardize and improve therapeutic EStim protocols and devices.^[88]

3.3. Organ Level Bioelectric Signaling

Communication at the organ level is mediated by electrical signaling through peripheral nerves, which connect organs to both the central nervous system and to other organs.^[89] Organ level BEMs aim to address dysfunction in such electric signaling and nerve circuitry by utilizing electronic devices (e.g., implants or wearables) to deliver corrective electric doses or fields targeting specific molecular mechanisms.^[90] In reality, BEMs lie on a continuum in terms of the specificity of their actions on target pathways, which is constantly evolving as the mechanisms of diseases

Figure 3. Cellular level bioelectric signaling. a) Electric field stimulation has been shown to modulate stem cell proliferation, migration, and differentiation in regenerative processes such as osteogenesis, neurogenesis, cardiomyogenesis, and angiogenesis. Stem cell behavior is also controlled by bioelectric states of nearby somatic cells. Reproduced with permission.^[37] Copyright 2018, Elsevier. b) A sample of membrane potential of various cell types (V_{mem} scale). Cancer cells (and other proliferative cell types such as stem cells, embryonic cells, and liver cells) are more depolarized compared to their healthy counterparts (quiescent, terminally differentiated cells). Reproduced with permission.^[42] Copyright 2012, John Wiley and Sons.

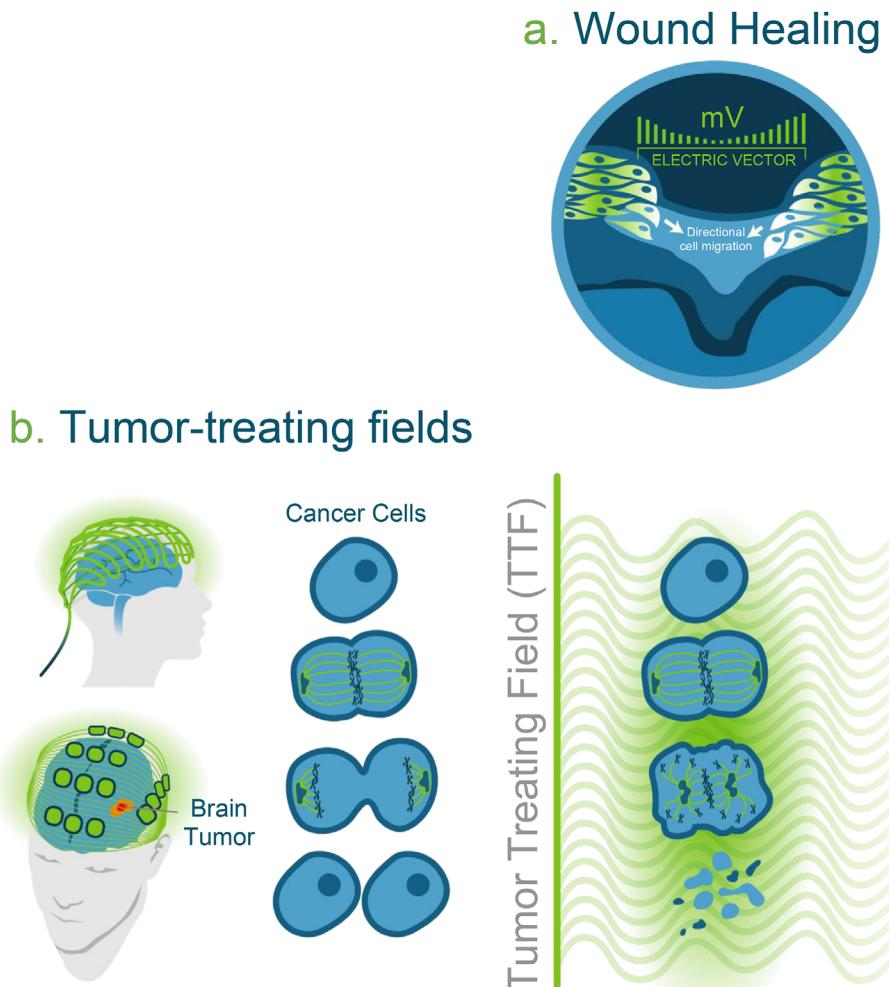


Figure 4. Tissue level bioelectric signaling. a) Electrical signals direct cell migration in wound healing. Wounding induces endogenous, lateral electric fields directed toward the wound center (white arrow), by collapsing the local transepithelial membrane potential from + 25 to 40 to 0 mV. b) Tumor-treating fields are alternating electric fields applied to tumor tissue via a wearable device to disrupt proliferation and stimulate apoptosis of cancer cells.

are being deciphered. However, we propose that they can be classified into two groups, “broad” and “precise,” based on whether the intervention alleviates the symptoms of the disease in a non-specific manner or by specifically targeting the neural-molecular pathways causing disease.

3.3.1. Organ Level Broad BEMs

Broad BEMs include devices that deliver nontargeted electrical pulses to alter nerve signaling and manage symptoms in chronic conditions such as pain and tremor.^[91] Broad BEMs have been marketed for decades and have interchangeably been referred to as neuromodulatory, neurostimulatory, or nerve stimulation devices, reflecting their historic use to modulate neural function.

In many cases, broad BEMs block or stimulate with constant, simple waveforms rather than modulating dynamically on the millisecond scale, which is important for generating precise therapeutic effects. Furthermore, for many of these interventions, the

mechanism of action (MoA) is unknown,^[92] with mixed evidence on effectiveness.^[91] This subclass can be further divided based on the disease impact and the amount of evidence needed for approval and commercial use.

Examples of broad BEMs include, but are not limited to (**Figure 5a**) transcutaneous electrical nerve stimulation (TENS) and spinal cord stimulation (SCS), which use low-voltage electrical currents for pain relief by applying electric fields across whole nerve areas; and deep brain stimulation (DBS) which applies mild pulses of electricity across whole brain regions to regulate abnormal impulses and alleviate symptoms of Parkinson’s disease and essential tremor.^[91,93] These approaches activate nerves that contain anatomically overlapping cell populations related to multiple mechanisms and hence produce non-selective effects.^[89,93] In the USA, TENS devices are sold directly to consumers and are cleared through the 510(k) pathway. This requires only a comparable legally marketed class II device for demonstration of “substantial equivalence.” SCS and DBS devices are approved via the PreMarket Approval pathway, which

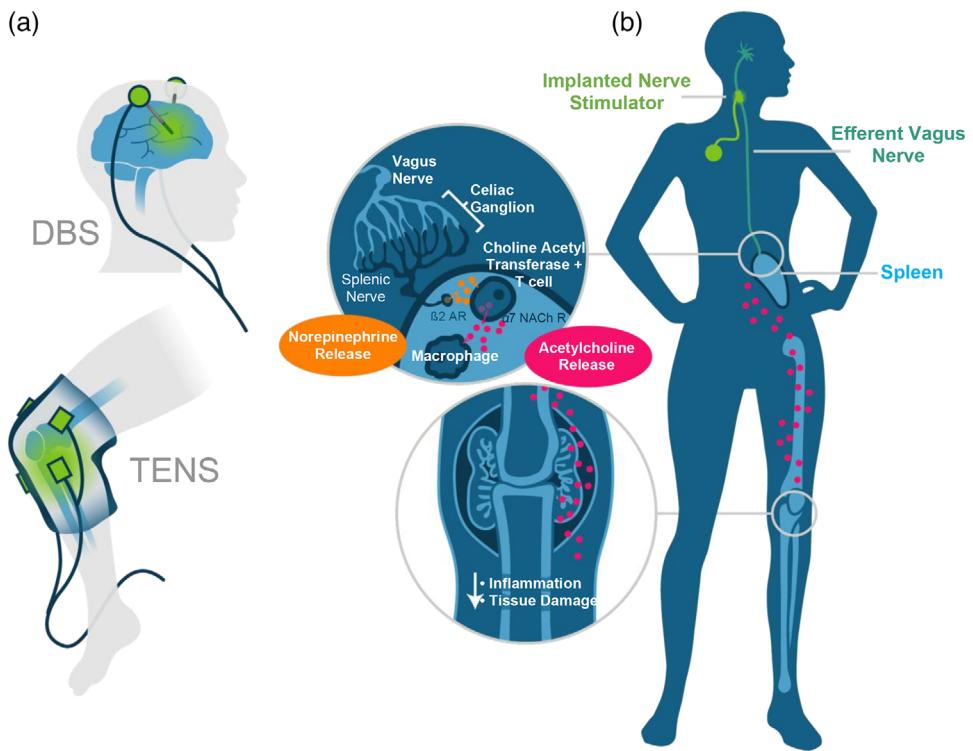


Figure 5. Organ level bioelectric signaling. a) Broad bioelectronic medicine: examples include deep brain stimulation (DBS) and transcutaneous electrical nerve stimulation (TENS). DBS involves implanting electrodes within certain areas of the brain to produce electrical impulses that regulate abnormal signaling in neurological conditions such as Parkinson's disease, epilepsy, obsessive-compulsive disorder, etc. TENS therapy involves the use of low-voltage electric currents to ameliorate pain. b) Precise bioelectronic medicine: schematic representation of a bioelectronic approach targeting the Vagus nerve to control inflammation, a key driver in many chronic diseases. AR, adrenergic receptor; NACH R, nicotinic acetylcholine receptors. Reproduced with permission.^[95], Copyright 2014, Elsevier.

requires clinical data to demonstrate a reasonable assurance of safety and effectiveness.^[94]

Cutting-edge developments in broad BEMs include “closed-loop therapies” such as Saluda Medical’s Evoke, which is the first commercially available SCS system capable of instantaneously reading, recording, and responding to evoked compound action potentials to provide precise, continually optimized therapy for chronic intractable pain of trunk and/or limbs;^[96] and “applications to new diseases,” such as Abbott receiving FDA Breakthrough Device designation to explore use of DBS to manage severe depression.^[97]

The selective targeting of many broad BEMs is being actively explored; however, this is often limited by disease mechanism understanding and access to biomarkers of neural activity to inform targeting and titration of therapy dosage. In the case of DBS, placement of electrodes at specific sites in the brain and current injection methods have been explored as ways to target specific neuronal populations but more evidence is needed to support the precision of this approach.

3.3.2. Organ Level Precise BEMs

Precise BEMs include devices that deliver “targeted” electrical pulses to trigger specific biological mechanisms. This creates a precision therapeutic effect and potentially minimizes side ef-

fects because of the localized nature of the intervention. As an example, the inflammatory reflex, where neural information is transmitted via the Vagus nerve, detects and responds to cytokine production and inflammation to maintain immune system homeostasis (Figure 5b).^[95] Delivering targeted electrical pulses along the Vagus nerve at specific locations modulates this neural reflex, suppresses inflammatory cytokines, and restores immune homeostasis.^[98] Precisely targeting the inflammatory reflex is expected to be less immunosuppressive and safer than systemic antiinflammatory molecules.^[99] Precise BEMs for inflammatory indications target various (auricular, cervical, splenic, or pancreatic) branches of the Vagus nerve, which underpin biological circuits that control inflammation and cytokine expression in chronic autoimmune diseases. For example, SetPoint Medical Inc. is conducting a stage 2 pilot study in RA and a proof of concept (POC) study in Crohn’s disease under an FDA breakthrough designation^[12,100] and Galvani bioelectronics are carrying out a POC study in RA targeting the splenic nerve.^[101]

Precise BEMs can also be used to electrically stimulate cardiovascular reflexes. Examples include targeting the blood-pressure-sensitive baroreflex to treat hypertension (Kyushu University)^[102] and heart failure (CVRx).^[11,103]

In parallel to generating data supporting the effectiveness of precise BEMs, manufacturers are innovating to improve and expand the application of these devices. Three of these innovations include the following:

- 1) *Device Form and Function:* Continuous optimization in miniaturization (micro- or nano- scale devices), power source, invasiveness (minimally or noninvasive such as wearables), and durability (newer biomaterials to improve longevity of device) is underway.^[104] Companies are also looking at new ways of targeting nerves (optogenetics; ultrasound-mediated control of neurons) and artificial intelligence (AI)-driven algorithms for optimizing electrical dosing. For example, Parasymp and CardiaCare are investigating wearable electroceuticals stimulating either the auricular branch of the Vagus, or secondary projections to the Vagus-innervated brain regions via the median nerve, respectively, to make therapy more convenient for patients.^[103]
- 2) *Sense and Respond Mechanisms:* Most organ level BEMs stimulate only a single nerve or are open-loop therapies where they stimulate specific targets with no direct feedback loop.^[105] In the medium term, we expect bimodal neuromodulation stimulating two neural pathways simultaneously to be more widely adopted in diseases where it is expected to be beneficial, such as tinnitus.^[106] In addition, the emergence of sense-and-response devices, such as closed-loop neuromodulators, is expected to circumvent the limitations of fixed-dose therapies.^[107]
- 3) *New Targets:* As our understanding of the nervous system evolves via initiatives such as National Institutes of Health's (NIH) Stimulating Peripheral Activity to Relieve Conditions (SPARC), which is developing peripheral nerve maps,^[108] we expect electroceuticals to be applied beyond inflammatory and cardiovascular diseases to challenging diseases such as chronic cough, nephrotic syndrome, and endometriosis. Companies such as BIOS Health may help accelerate this progress by finding neural biomarkers allowing us to understand and correct aberrant signals in real time.^[109]

3.3.3. Challenges

Organ level BEMs are sufficiently advanced to have received some investment and partnering interest from pharmaceutical companies,^[7] but the level of activity is limited in comparison to other therapeutic modalities. We suggest that the following translational and clinical challenges, particularly those for precise BEMs should be addressed:

- 1) *Incomplete Understanding of Disease Biology:* Current applications for organ level BEMs are limited by our understanding of neuronal control of diseases. For instance, in neuropathic cough, it is unclear which nerve fibers are responsible for the initiation versus suppression of cough. Alliances such as SPARC are developing detailed anatomical and functional maps for how peripheral nerves control organ function.^[108] Once we have a better understanding of neural anatomy, signaling patterns in healthy versus diseased states, functional mechanisms, and translation between animal models and humans, we can use this information to effectively target more diseases using electroceuticals.
- 2) *Incomplete Understanding of Mechanism of Action:* The underlying biology of how broad BEMs work is unclear,^[92] although we have a better understanding of the MoA of some precise BEMs (e.g., stimulating the Vagus inflammatory reflex), as they

tend to rely on well-characterized innate autonomous or inflammatory reflexes. A more detailed understanding of neural MoA is important to establish target validation and translational evidence, which forms a large part of the preclinical packages required for clinical progression. Building a greater mechanistic understanding of electroceuticals may increase the attractiveness of BEMs (and electroceuticals more broadly) and invite further research into synergistic combinations of devices and drugs.

- 3) *Evidence Requirements and Controls:* Organ-level BEMs are the most advanced electroceutical category, with multiple precise BEMs entering the clinic. To determine the true treatment effect of electroceuticals, clinical results need to be rigorously compared between experimental and control groups (as opposed to a literature control). Otherwise, it becomes difficult to interpret if the improvement in disease is due to natural remission, the Hawthorne effect or electrical stimulation; Hawthorne effect is the alteration of behavior by the subjects of a study due to their awareness of being observed. For wearable devices, trial designs are further complicated by improper positioning of the device by patients and the inability to effectively control the trials, as patients can discern if they are in the treatment group by the differential placement of the wearable device compared to the control group. Therefore, trial designs need to acknowledge these limitations and carefully refined to produce high-quality data that could be used to show efficacy.
- 4) *Distinguishing Therapeutic Effect and Safety Concerns:* Variation in responses among patients receiving treatment is expected due to differences in patient subpopulations and the amount of tailoring of stimulation parameters needed for efficacy. For instance, initial clinical trials using Vagus nerve stimulation for heart failure with reduced ejection fraction has had mixed results.^[110] Ardell et al. indicated that the difference in clinical outcomes can be attributed to incorrect dosing protocols of stimulation parameters (such as bipolar electrode orientation, stimulation frequency, pulse width, and intensity) in these studies.^[111] This underscores the importance of better understanding and optimizing stimulation protocols as we move from preclinical models to humans.^[107,112] Furthermore, variations in stimulation parameters such as electrode position, voltage, frequency, and duration of electroceutical devices could have a major impact on neuronal action potential and cause damage to both neuronal and non-neuronal cells.^[113] Therefore, these parameters should be carefully assessed to mitigate any safety concerns in the clinic. Key questions still to be answered include the following: how big of a treatment effect can we expect from precise BEMs and in which subsegments of patients? Are there predictive biomarkers of neural therapies that can distinguish responders versus nonresponders? How do we optimize treatment parameters and understand the potential for tachyphylaxis or desensitization over time? How do we set the parameters for safe administration of bioelectroceuticals? Could further development of closed-loop systems and personalized digital twin enable better optimization of input stimulation and output effect?^[114]

In anticipation, there is optimism that if SetPoint's RESET-RA clinical study replicates their prior successful studies^[12] and if Galvan's splenic stimulation study for RA has a favorable

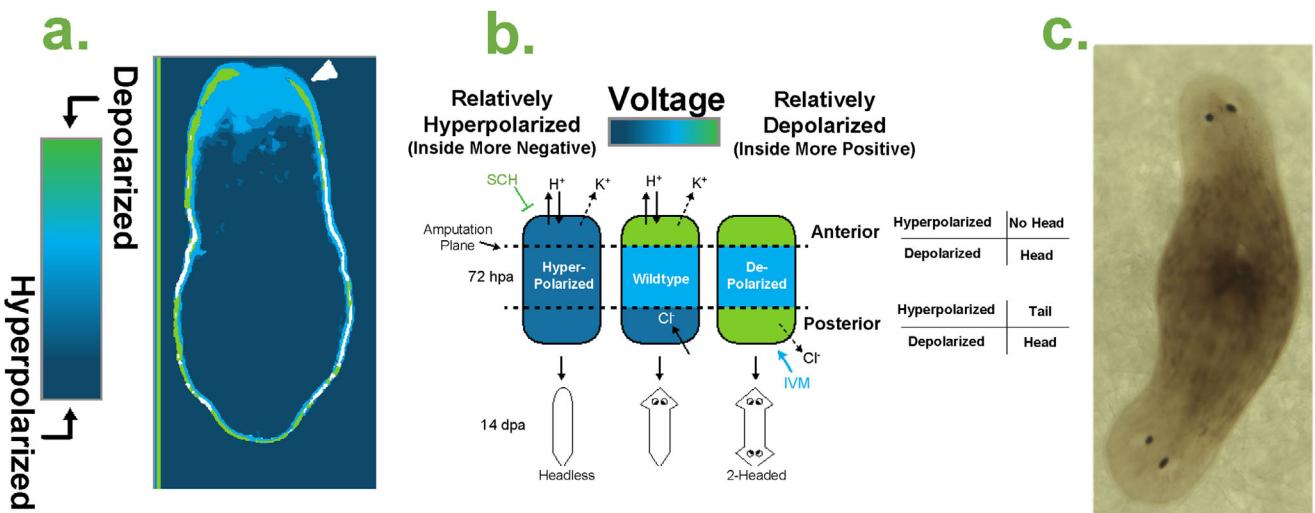


Figure 6. Systems level bioelectric signaling. Bioelectrical signaling sets anatomical structure at the whole organism level, enabling ion-channel-targeting compounds (systems bioelectroceuticals) to trigger large-scale morphological changes. a) A voltage reporter dye, DIBAC4(3) reveals gradients of V_{mem} across the anterior–posterior axis of planarian flatworms; this enables the visualization of the bioelectrical pattern memory that sets the number and location of heads in the regenerating organism. Reproduced with permission.^[121] Copyright 1987, Company of Biologists. b) Model showing that in amputated worms, a circuit composed of ionic conductance sets the V_{mem} states at each blastema, which in turn determines the polarity (i.e., head vs tail anatomical identity) of each end of a regenerating fragment. Reproduced with permission.^[122] Copyright 2011, Elsevier. c) Manipulating this circuit in amputated planaria using pharmacological techniques that target ion flux results in the creation of two-headed planaria shown here; note that ectopic second head is properly shaped, scaled, and positioned with respect to the body despite a simple inducing stimulus. This demonstrates that V_{mem} regulates axial polarity and downstream blastema properties during planarian regeneration. Reproduced with permission. 2017, Photo by Junji Morukuma, Levin lab.

outcome, this will offer essential clinical validation for precise BEMs.^[115,116]

The examples provided here focus on BEMs applied at the organ level. However, there may be other bioelectroceuticals that manifest at the organ level that are not explored here.

3.4. Systems Level Bioelectric Signaling

At the organism level, voltage gradients specify information such as axial polarity, patterning, organ identity (shape and size)^[117] and play a vital role in embryonic development, remodeling, and regeneration.^[118,119] It has been argued that these system level patterning outcomes are determined by a morphogenetic code that encodes information at levels above the genetic and molecular codes and provides top-down instructions by regulating gene expression and individual cell behaviors toward specific organ level outcomes.^[120] Bioelectricity is one layer of this complex morphogenetic field and is uniquely tractable as a control mechanism for large-scale changes because it underlies the medium in which collective cell behaviors process information.

Foundational studies in planaria showed that the information for cells to regenerate after damage is stored in a stable bioelectric pattern, which can be pharmacologically rewritten to produce animals that permanently regenerate with two heads (despite their normal genome; **Figure 6**).^[121,122] Similarly, exploiting the native bioelectric pattern that normally regulates the pattern of the vertebrate face^[123,124] enables the induction of eyes in other tissues, including regions that are not normally competent to form eyes (such as the gut and lateral mesoderm).^[125] Bioelectric properties also regulate proper tissue scaling and size in zebrafish fins

via potassium channels and calcineurin.^[126–128] Exogenously expressed HCN2 ion channels can rescue nicotine-induced brain patterning defects in *Xenopus* embryos via local and long-range bioelectric signaling.^[3,129]

Bioelectric signaling can be distinguished from traditional ion channel biology in that it is not necessarily the manipulation of individual ion channels that drives downstream responses but rather the resultant change in spatial voltage gradients; the same phenotypes can be obtained via modulation of a wide range of channels, as long as the appropriate voltage distribution is induced in the tissue.^[125,130]

In addition to manipulating intrinsic electric signals, exogenous ESTim has also been shown to improve regeneration (especially nerves),^[131] as it can mimic the biophysical processes of regeneration observed in spontaneously regenerative vertebrate species.^[118] Daniel Cohen's lab has developed SCHEEPDOG (Spatiotemporal Cellular HERding with Electrochemical Potentials to Dynamically Orient Galvanotaxis); a next-gen electrobioreactor to dynamically program electrical cues and induce directed and large-scale collective cell migration underlying key multicellular processes such as morphogenesis and healing.^[132,133]

3.4.1. Challenges

Cracking the bioelectric code at the systems level and mapping electrical properties to downstream changes in gene expression and biomechanical properties has huge potential in developmental and regenerative biology. The main challenge with this field is that this is still a nascent area of science that is being explored only by a handful of academic researchers. We do not fully

Table 2. Avenues to explore for driving future innovation and development of electroceutical interventions.

Category	Time framework and focus	Academia	Biotech/Pharma	Government, Healthcare system
Bioelectronic medicine (BEM)	Near to midterm: Translation into clinic	<ul style="list-style-type: none"> Develop detailed anatomical and functional maps that illustrate how peripheral nerves control organ function, develop ways of reading and writing neural signals and decode mechanism of action of BEMs This gap is being partially addressed by a \$21M NIH-funded clinical program called Research Evaluating Vagal Excitation and Anatomical Links (REVEAL) [134], powered by AI to study the anatomical connections and functional outcomes of Vagus nerve stimulation Progress technology innovations to improve and expand applications of BEMs E.g., Development of continuous monitoring sensors to enable sense and respond mechanisms in BEMs 	<ul style="list-style-type: none"> Generate proof of efficacy of precise BEMs Clinical readouts from SetPoint's RESET-RA and Galvanic's splenic stimulation study (expected in 1–3 years) and other devices in development Develop strategies (e.g., biomarker) for patient stratification and indication selection to determine patient groups that are likely to benefit from BEMs Position BEMs for commercial success to serve as a precedent for next generation of BEMs 	<ul style="list-style-type: none"> Public and private funding bodies to sponsor research to deconvolute the mechanisms by which BEMs work and invest in clinical trials Stakeholders to work together to share challenges and advances both from a technological perspective but also issues related to pricing, regulation, compliance, scalability, etc. Evaluate the value of BEMs in the healthcare system

(Continued)

Table 2. (Continued)

Category	Time framework and focus	Academia	Biotech/Pharma	Government, Healthcare system
Bioelectroceuticals (nondevice-based therapeutics)	Near to midterm: Elucidating the bioelectric code, acquiring electronic datasets	<ul style="list-style-type: none"> More academics to join the field and help decode the rules by which bioelectricity operates at various levels, develop better tools for screening electroceuticals and better integration with genetics, with the goal of moving these concepts from the lab to clinic Explore use of bioelectricity for diagnostic applications <p>E.g., tumor detection</p> <ul style="list-style-type: none"> Organize conferences, workshops, and special issues in journals to call for proposals in these categories. Currently <i>Bioelectricity</i> is the only peer-reviewed journal dedicated to bioelectroceuticals 	<ul style="list-style-type: none"> Share ion-channel drugs and compounds targeting specific ion-channel/ion-channel families with academics to test new hypotheses and decode MoA AstraZeneca, Boehringer Ingelheim, Merck offer tool compounds to academics and calls for proposals via their Open Innovation programs Academic-industry collaborations to accelerate research in this space Translate research from academic labs to industry setting (e.g., via startups) Morphochemicals, a startup in this space, is focused on reprogramming native bioelectric signaling to improve amputation stump health, limb regeneration, and organogenesis[135] They have recently demonstrated that acute (24-h) exposure to a multidrug treatment delivered by a wearable bioreactor (BioDome) can significantly restore function of an amputated adult <i>Xenopus laevis</i> hind limb by activating the endogenous morphogenetic cascade[136]. Scale R&D by utilizing the drug discovery machinery that exists within Pharma (such as high throughput screening technologies) or by developing novel “electronic” technologies Start-ups are developing novel semiconductor-based technology to perform live cell, electrical and electro-chemical imaging of cell cultures[137] Utilize advances in technology (such as synthetic biology) to introduce synthetic receptors or to re-direct native ion channels to regulate bio-electrical communication 	<ul style="list-style-type: none"> Public and private funding bodies to recognize bioelectroceuticals and fund research in this space
Long term: Translation into clinic				<ul style="list-style-type: none"> Provide guidance on regulatory pathways for bioelectroceuticals E.g., guidance for use of bioelectricity to prevent metastasis Generate proof of efficacy in the clinic

understand the encoding of complex system level states by endogenous bioelectric patterns, there is a dearth of electromic datasets on bioelectric patterns in diverse tissues in health and disease. As more researchers explore this field, developing better imaging technologies and computational tools for processing electromic data,^[61] additional insights will be generated to help progress this field. Electromics is the systemic, large-scale analysis of bioelectric signaling at various physiological levels in healthy and diseased states.

4. Outlook

Of the four levels at which we propose that electroceuticals can be considered, the most focus has been given to organ level bioelectronic medicines. Regardless of this disparity, progress in all fields will require collaboration by all players in the life science ecosystem, including academia, innovators, biotech, pharma, regulators, and healthcare systems. **Table 2** outlines suggestions to catalyze further innovation and translation for electroceutical products.

We envision bright prospects for electroceuticals to efficiently detect, treat a wide range of diseases, regenerate tissues, and organs, and even alleviate the effects of aging^[138] in the future. For ultimate commercial viability, selecting diseases with high unmet need where electroceuticals can demonstrate improved and sustained efficacy and safety over current therapies while maintaining patient convenience, patient life, scalability, and addressing pricing and reimbursement challenges all will be important. For this vision to be realized, we need to better understand the rules by which bioelectricity operates and encodes actionable information at various physiological levels as described in the ontology above and the relationship between them. Therefore, we call for collaboration to deconvolute the molecular mechanisms by which electroceuticals function as an urgent, next step to maximize the potential of this exciting field for the benefit of patients.

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Conflict of Interest

S.B. and D.W. are employees of AstraZeneca. M.L. is co-founder of a company (Morphoceuticals) which seeks to induce regenerative repair via bioelectric approaches; his lab has a sponsored research agreement with Morphoceuticals.

Keywords

bioelectricity, bioelectronic medicine, electroceutical, ion channels, membrane potential, morphoceuticals, neuromodulation

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