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Review

Endogenous Bioelectrics in Development, Cancer, and Regeneration: Drugs and Bioelectronic Devices as Electroceuticals for Regenerative Medicine

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A major frontier in the post-genomic era is the investigation of the control of coordinated growth and three-dimensional form. Dynamic remodeling of complex organs in regulative embryogenesis, regeneration, and cancer reveals that cells and tissues make decisions that implement complex anatomical outcomes. It is now essential to understand not only the genetics that specifies cellular hardware but also the physiological software that implements tissue-level plasticity and robust morphogenesis. Here, we review recent discoveries about the endogenous mechanisms of bioelectrical communication among non-neural cells that enables them to cooperate *in vivo*. We discuss important advances in bioelectronics, as well as computational and pharmacological tools that are enabling the taming of biophysical controls toward applications in regenerative medicine and synthetic bioengineering.

INTRODUCTION

Rapid advances in genomics are revealing the mechanisms (and their evolutionary origin) underlying cellular hardware: the complement of proteins that a given cell deploys. However, fundamental gaps in our understanding remain, with respect to the software: the algorithms by which cells accomplish specific goals under a range of diverse environmental perturbations (Pezzulo and Levin, 2016). Single cells, including microscopic paramecia and the giant *Acetabularia* (a plant-like alga that is > 10 cm tall), achieve an extremely highly patterned invariant morphology without benefit of cell–cell communication or stem cell differentiation. Some animals, such as the salamander, are able to regenerate whole limbs, eyes, and other organs throughout its lifespan (as can single cells such as *Stentor*) (Birnbaum and Alvarado, 2008). As remarkable as their ability to create new patterned tissue upon injury is the fact that regeneration stops when the correct pattern has been completed. How does this system know what a correct pattern looks like (Pezzulo and Levin, 2015)? The ability to regenerate the same structure and then stop at the appropriate time requires single cells to cooperate, measuring and acting on structural information on a much larger scale (Pezzulo and Levin, 2015). Embryos can build a smaller, but morphologically identical animal body, even when >50% of the cells are removed (Cooke, 1981), and pieces cut from planarian flatworms grow precisely the needed organs, no more and no less, at the correct wound surfaces, while rescaling themselves to perfect proportions (Saló et al., 2009).

This sort of anatomical homeostasis (the ability to maintain a large-scale target morphology under a range of unpredictable damage/intervention) highlights the essential role of decision-making algorithms implemented in living tissue (Friston et al., 2015; Manicka and Levin, 2019). Genomic and biochemical signals are clearly important but are not the whole story. It has long been known that cancer cells can be reprogrammed by embryonic and regenerative environments into normal histogenesis (Hendrix et al., 2007; Mintz and Illmensee, 1975), whereas asexually reproducing planaria execute 100% perfect anatomical regeneration despite an incredibly messy genome (including mixoploidy) resulting from 400 million years of somatic inheritance and accumulation of mutations [reviewed in Levin et al. (2018)]. An exciting frontier is emerging around the basic questions of how genome-specified hardware implements robust, goal-directed, tissue-level behavior among cell collectives. In addition to its impact on our understanding of evolution, developmental cell biology, and multicellularity, this area has crucial implications for biomedicine. If we understood the information processing pathways that enable cells to harness chemistry, physics, and computation toward specific outcomes, transformative approaches could be implemented to repair birth defects, regenerate organs after traumatic injury or aging, reprogram tumors, and build artificial living machines to desired spec (Levin, 2011).

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https://doi.org/10.1016/j.isci.2019.11.023

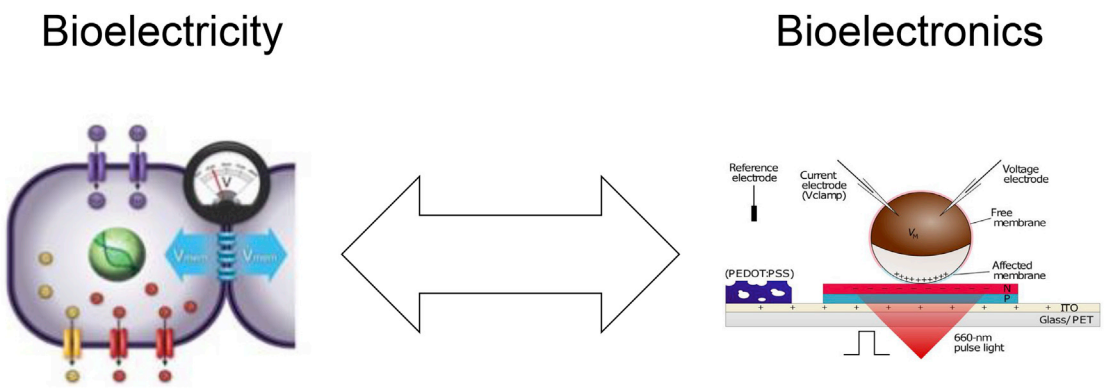

Figure 1. Bioelectric Signaling Can Communicate with Bioelectronic Devices to Affect Cell Function and Drive Growth and Patterning

It is increasingly becoming clear that important outcomes can be induced not only by rewiring the hardware (i.e., genomic editing and gene therapy) but also by providing specific *inputs* into the cellular decision-making networks. Complementing decades of focus on transcriptionally modulated chemical signals, recent work has begun to explore and exploit the role of physical forces and microenvironment in regulating cell behavior. Due to space limitations, we do not cover tensile forces and biomechanics, which have been expertly reviewed elsewhere (Beloussov, 2012; Davidson, 2012; Miller and Davidson, 2013; Navis and Bagnat, 2015; von Dassow and Davidson, 2011) but instead focus on developmental bioelectricity: ways in which non-neural cellular networks process information via electrical dynamics (Levin and Martyniuk, 2018; Mathews and Levin, 2018). Advances in novel materials, computational platforms, and bioengineering tools are greatly facilitating the discovery of fundamental new biology and novel applications in biomedicine and synthetic biology. Here, we present recent advances in target bioelectric circuits and bioelectronic devices that can stimulate them (Figure 1).

Targeting Endogenous Bioelectric Circuits: Ion Channel Targets

As in neurons, the bioelectric state of all other cells is set by the combined functionality of ion channels and pumps, which determine resting potential and electrical synapses known as gap junctions, which selectively propagate those states through the network (Figure 2). Indeed, this system of coordination is evolutionarily ancient, having an origin in coordination in bacterial biofilms (Humphries et al., 2017; Prindle et al., 2015) but extending also to algae (Bentrup and Jaffe, 1968; Jaffe, 1966; Robinson et al., 1981). Thus, ion channels and gap junctions represent critical targets for manipulation of endogenous bioelectric signaling and the downstream changes in gene expression, cytoskeletal structure, and cell behavior. Several recent applications have highlighted the potential for rational effects at the cell and tissue/organ level by manipulating endogenous bioelectric pathways (Bates, 2015; Funk, 2013; Mathews and Levin, 2017; McLaughlin and Levin, 2018).

Manipulating Ion Channels Leads to Changes in Morphogenesis

One of the functions of transmembrane potential (V_{mem}) gradients is to provide prepatterns for the creation of various complex organs (Vandenberg et al., 2011) (Figure 3). For example, the voltage distribution across the nascent face is the reason why mutations in the Kir2.1 result in channelopathies not only of the heartbeat but also of craniofacial development (Adams et al., 2016). Importantly, it is now known that a key step in this process, stem cell differentiation, is regulated by bioelectric state. Using small molecule drugs to target ion channels of mesenchymal stem cells has revealed that V_{mem} can induce (Sundelacruz et al., 2008) and in fact override bioelectric chemical inducers (Sundelacruz et al., 2013a, 2013b). Interestingly, the downstream genetic targets of bioelectric state change are highly conserved between mammalian mesenchymal stem cells (MSCs) and regenerating tissues in frog and axolotl (Pai et al., 2016) (including pathways such as bone morphogenetic proteins [BMPs], Ca^{2+} signaling, integrins, and histone deacetylase), consistent with the evolutionarily ancient origins of bioelectrical signaling.

Beyond the single-cell level, bioelectric mechanisms are an important aspect of the microenvironment that enables cells to make decisions based on the states of their neighbors. Galvanotaxis, or migration in electric fields generated by epithelia, has now been observed across taxa, from amebae (Gao et al., 2015) and

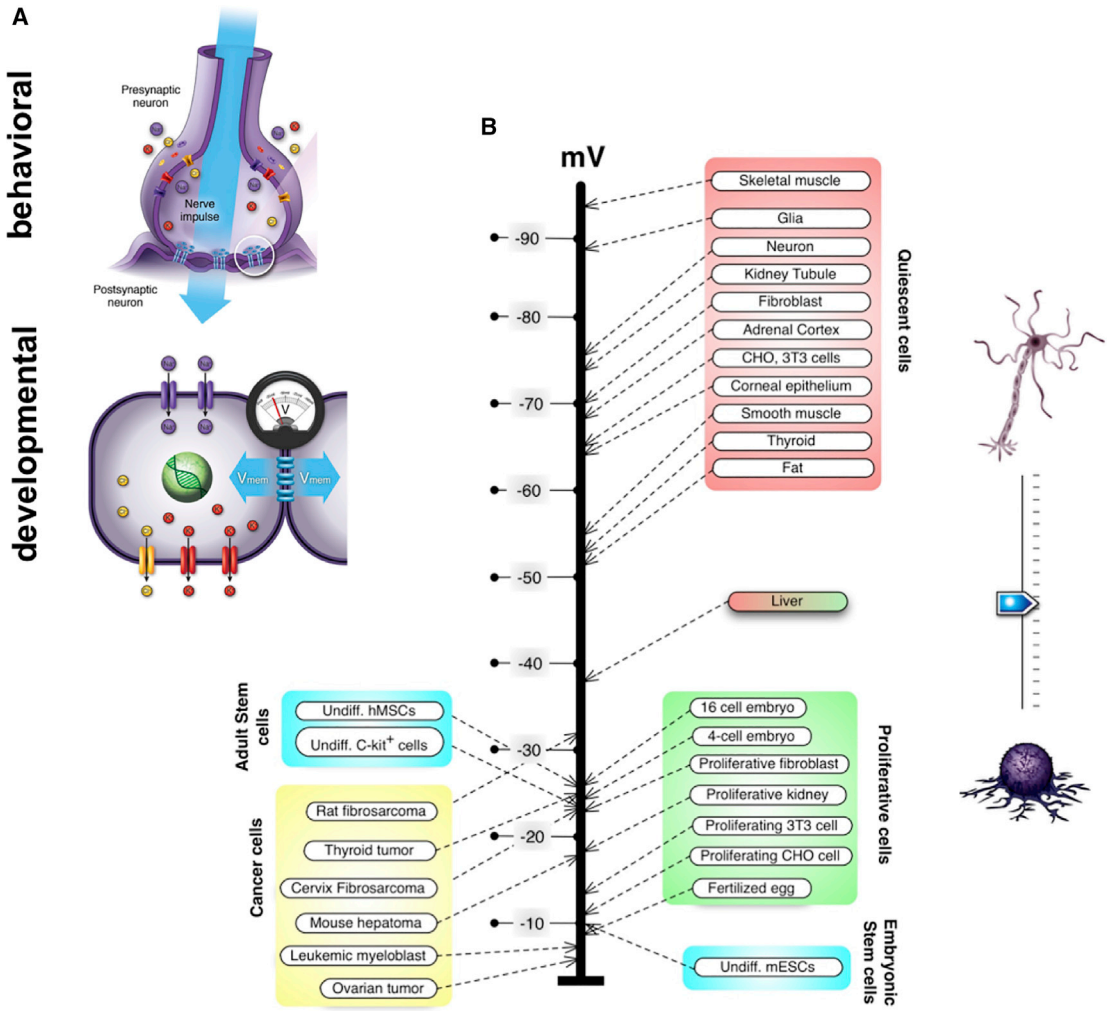

Figure 2. Bioelectric Signaling at the Cellular Level

(A) All cells, including non-neural cells, possess a resting membrane potential that is defined by the activity of its ion channels and pumps and the concentrations of various ions inside and outside of the cell. Cells communicate their individual bioelectric states via gap junctions—electrical synapses—to their neighbors, and the resulting bioelectric network guides individual and tissue-level cell behaviors. Images by Jeremy Guay of Peregrine Creative Inc. (B) Individual cell states are determined by V_{mem}; with highly plastic (embryonic, stem, and cancer) cells being relatively depolarized while mature, terminally differentiated cells are hyperpolarized. Data taken from (Binggeli and Weinstein, 1986).

fungi (Brown et al., 2019; Gow and Morris, 1995) to human cells (Feng et al., 2012; Kim et al., 2017; Ren et al., 2019), and is being exploited for wound healing applications (Reid et al., 2011; Reid and Zhao, 2014; Wang and Zhao, 2010). Classic work by Lionel Jaffe examined bioelectrics in coordinating cell activity via trans-cellular currents in models such as amebas, pollen tubes, and various kinds of eggs and embryos (Borgens et al., 1979a; Jaffe and Robinson, 1978; Jaffe and Nuccitelli, 1977; Nuccitelli and Jaffe, 1975, 1976; Nuccitelli et al., 1975, 1977; Robinson and Jaffe, 1973, 1976; Weisenseel et al., 1975). In addition to electric fields, cells also sense static charges (Beech, 1997; Strilic et al., 2010; Weihua et al., 2005) and resting potentials. For example, transplanted eye primordia normally grow one optic nerve that connects to the host. However, if the cells in the host cells are depolarized, for example by targeting chloride channels via the compound ivermectin (Figure 4), a massive hyperinnervation occurs (Blackiston et al., 2015). Characterization of the transduction mechanism revealed the involvement of serotonergic signaling directed by the differential resting potential between neighboring cells, which has also been implicated in bioelectric transformation to melanoma (Blackiston et al., 2011). This made it possible to identify small molecule drugs that improve the vision resulting from eye implants into otherwise blind animals (Blackiston et al., 2017). Remarkably, the native innervation is unchanged by such treatments, indicating that only nerve that is ectopic (recognizes

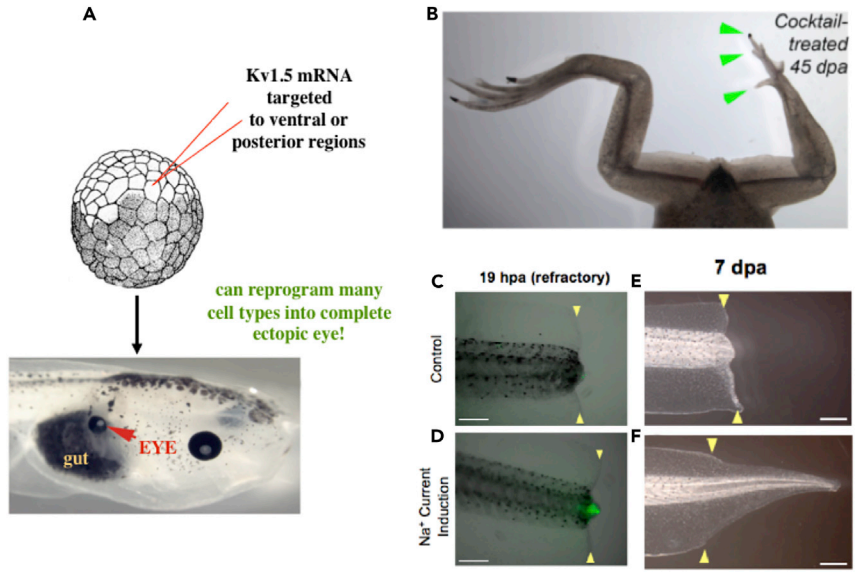

Figure 3. Bioelectric Signaling Instructs Growth and Form

(A) Ion channels that set an eye-specific bioelectric prepattern, injected as mRNA into cells of a frog embryo, can reprogram regions of the body (such as the gut) to form an eye, including its internal layers (panel taken with permission from [Pai et al., 2012]).

(B–F) (B) An ionophore applied to the leg amputation of a froglet induces the growth of a leg with toenails and toes, during stages where limbs do not normally regenerate (panel taken with permission from [Tseng and Levin, 2013]). The same ionophore can be used to trigger tail regeneration. Normal amputated tadpole tails (C) at refractory stages do not regenerate (E); however, if an influx of sodium (D) is induced, regeneration of a tail results (F). Scale bar in panels C–F is 500 μm. Panels taken with permission from Tseng et al. (2010).

that it is in the wrong location) reads and reacts to this environmental cue. Investigation into how nerves detect whether they are in their correct place, and making the decision of reading the surrounding cells' V_{mem} or not, is a major area for future investigation and will be greatly facilitated by emerging technology that implements spatial control of ionic environment with which to confront cells (Deng et al., 2016; Selberg et al., 2018).

Targeting Bioelectric States Repairs Birth Defects

Because of its central role in morphogenesis of a variety of body systems, bioelectrical signaling has also been exploited to understand, and counteract, the effects of teratogens and mutations in birth defects of the brain (Pai et al., 2015b). Nicotine and mutations of the critical neurogenesis gene *Notch* both perturb the normal bioelectric pattern observed in the nascent ectoderm. The bioelectric distribution is normally hyperpolarized in the middle of the tissue and depolarized at the edges, and this is critical—if the pattern is flattened in either direction, gene expression and brain patterning become completely abnormal. Artificially reinforcing this pattern, by activation of HCN2 channels to hyperpolarize the middle region and depolarize the lateral regions rescues normal gene expression, anatomy, and even learning rate (behavioral IQ) despite the exposure to strong teratogens and *Notch* mutation (Pai et al., 2018). In addition to the obvious biomedical implications, these data shed light on the basic biology of organogenesis: the borders of the nascent brain are set by the *differences* in V_{mem} between cell groups, showing how developmental compartments are set up by physiological gradients. Work is ongoing in our laboratory to understand how these differences (properties on a multi-scale level) are readout and transduced into changes of gene expression and cell behavior, even at a distance (Pai et al., 2015a).

Bioelectric Modulation for Regenerative Repair

Beyond embryogenesis, bioelectrics has been studied in regeneration, a process in which some species are able to re-grow complex organs following amputation (Birnbaum and Alvarado, 2008). Classic data revealed a role for trans-epithelial bioelectric gradients: shunting these reduced regenerative ability (Borgens et al., 1979b, c), whereas inducing them could cause a degree of limb regeneration in otherwise

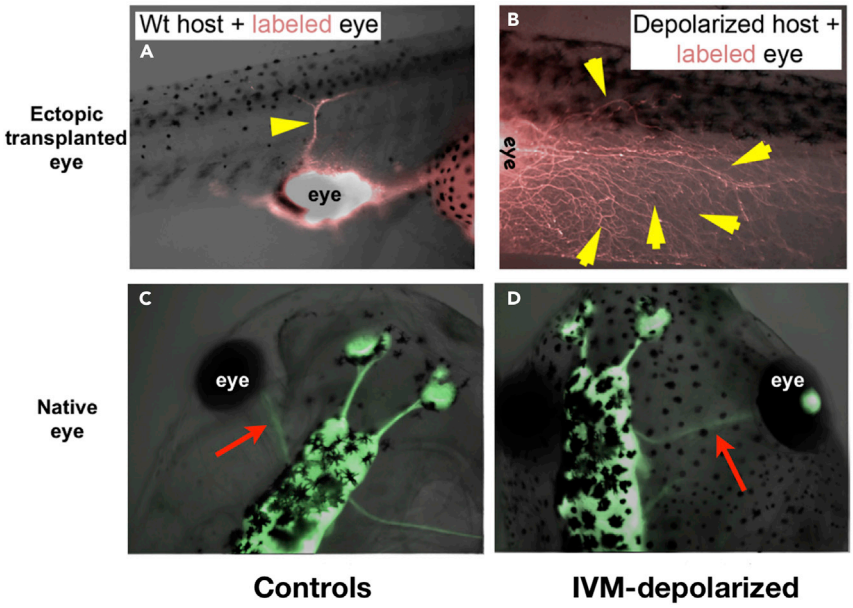

Figure 4. Bioelectric Controls of Ectopic Innervation

(A) Ectopic eye labeled with red fluorescent protein normally makes one optic nerve that reaches toward the spinal cord. (B–D) (B) When the host body is depolarized—for example, by the application of the chloride channel opener ivermectin (IVM)—massive hyper-innervation occurs. Remarkably, the normal innervation (C) is not altered in these animals (compare C to D), showing that only the ectopic nerve is paying attention to the surrounding cells' bioelectric topography and suggesting the use of local depolarization as a method to augment innervation of transplanted organs. Panels taken with permission from Blackiston and Levin (2013).

non-regenerative species (Becker and Spadaro, 1972; Bodemer, 1964; Borgens et al., 1977). The endogenous electric field appears to be critical for migration of cells toward the wound and for inducing innervation, which is known to augment regenerative capacity (Kumar and Brockes, 2012). Applied electric field approaches are now being pursued in rodents with modern markers to understand immune system and stem cell involvement in the process (Leppik et al., 2015, 2018; Mobini et al., 2017; Oliveira et al., 2019). Complementing the electric field and its directionality vector are the gradients of resting potentials in the wound region, which regulate proliferation and trigger organ-forming cascades. Managing the time course of these gradients *in vivo* has been used to induce tail (including spinal cord and muscle) regeneration via Notch and fibroblast growth factor (FGF) signaling pathways under non-regenerative conditions via ionophores that enable control of wound bioelectric state (Tseng et al., 2010). The ability to control resting potential and other aspects of the wound will be a critical component of future studies investigating the role of neurons and immune system cells as first responders to the ionic signaling that reveals the location of damage and guides downstream repair cascades (Ferreira et al., 2016, 2018; Zhu et al., 2019).

Fundamental Biology Revealed by Bioelectric Experiments

A few important lessons about basic biology are starting to emerge from the recent applications of molecular physiology methods to the control of anatomical patterning. First, it is critical to note that the driver in these cases is not a specific channel protein or gene: unlike in many biochemical pathways, the "pressure point" on the system that affords optimal control is a physiological macrostate, the resting potential of the cell. The same outcome can be induced by a range of different ion channels, as long as the resulting resting potential pattern is correct. For example, in tail and spinal cord regeneration, the endogenous V-ATPase pump can be shut down and replaced with a yeast proton pump that has no structural or sequence homology (Adams et al., 2007). This, however, rescues the regenerative ability, because the tail-building module is driven by a bioelectric state of the wound. Because ion channels can be opened and closed post-translationally, cells can be in exactly the same bioelectric state despite highly divergent gene expression; conversely, cells with identical transcriptomic or proteomic profiles can be in very different bioelectric states. This means that physiomic profiling using dyes (Adams and Levin, 2012; Deal et al., 2016; Kulkarni et al., 2018) or genetically encoded voltage reporters (Mutoh et al., 2012; St-Pierre et al., 2015) is an essential complement to biochemical profiling. It also means that in contexts such as cancer,

where bioelectrics is critical for progression and metastasis, it is incorrect to think of ion channel oncogenes as simple targets to be turned on or off—strategies must be formulated for the control of V_{mem}, which may require differential control of multiple channels in diverse cell types and microenvironments (Cervera et al., 2016a; Kale et al., 2015; Moore et al., 2017).

Indeed, many ion channels and gap junctions are themselves voltage sensitive, which can lead to the formation of multi-protein circuits with complex system-level behaviors (Cervera et al., 2016a, 2016b, 2018a, 2018b, 2018c, 2019; Palacios-Prado and Bukauskas, 2009). Some of these circuits establish robustness (resistance of cells to induced V_{mem} modulation), whereas others implement positive feedback loops that result in a kind of memory where changes to resting potential are reinforced for long-term physiological effects that last long after the stimulus is removed. Thus, it is essential to use predictive computational models to understand spatiotemporal behavior of bioelectric circuits. New open-source computational tools are coming on-line (Cervera et al., 2018c; Pietak and Levin, 2016, 2017), which facilitate bio-realistic simulation of physiological and biochemical networks. These enable the prediction of single cell- and tissue-level dynamics and allow testing of candidate strategies for inducing repair such as of brain defects (Pai et al., 2018).

An important component of tissue-level bioelectric dynamics is the fact that instructive states are not local. For example, amputation (but not puncture injury) of a leg in froglets results in rapid (~30-s long) bioelectric signal in the *contralateral* (untouched) leg, which reveals the location of the damage (proximodistal position along the limb) (Busse et al., 2018). This kind of phenomenon is general and is not merely an indicator (readout) but is functionally instructive. For example, the size of the developing brain can be determined by bioelectric state of ventral cells destined to be gut (Pai et al., 2015a), whereas the normalization of induced tumors can be achieved by hyperpolarizing channels expressed on the opposite side of the animal (Chernet et al., 2015; Chernet and Levin, 2014). As in the nervous system, bioelectrical networks enable coordination and long-range integration of information, which strongly suggests that future applications need to take into account far more than tumor or wound tissue, or even just the immediately adjacent microenvironment. Remarkably, such long-range controls via bioelectrics had been predicted by H. S. Burr as far back as the 1930s (Burr, 1941; Burr et al., 1938, 1940). However, current molecular insight into the mechanisms of long-range communication (via calcium waves, gap junction-based transport of neurotransmitters, and diffusion of second messengers such as butyrate [Chernet and Levin, 2014]) can be exploited for surrogate site diagnosis and treatment of difficult-to-reach regions.

A final emerging theme is that of modularity: simple triggers induce a self-limiting, complex cascade of signaling and morphogenesis that results in the formation of a coherent structure. For example, expression of some ion channels in the frog embryo induces a whole eye to be formed in aberrant locations, such as in the gut or on the tail (Pai et al., 2012). Similarly, recent work showed that a transient (24-h) treatment of amputated adult frog legs with a hormone (progesterone) that induces, among other pathways, bioelectrical remodeling leads to more than 12 months of limb regeneration and patterning (Herrera-Rincon et al., 2018). Similarly brief (48-h) reduction of electrical connectivity, or three-hour depolarization, in planarian flatworms permanently converts them to a double-headed phenotype that continues to regenerate entire ectopic heads in future rounds of regeneration in plain water (Durant et al., 2019; Oviedo et al., 2010). In all cases, a brief trigger induces a coherent morphogenetic event, not an out-of-control tumor, which stops growing at the correct time. Modularity has important implications for both evolution and biomedicine; identifying such "subroutine calls" *in vivo* is an important task for the field. Bioelectrical triggers for complex organs facilitate evolvability because relatively small physiological or genetic changes that result in differential V_{mem} patterns can readily induce major changes to body anatomy. Another implication is that once discovered, triggers can be used to induce organ formation long before we have the knowledge about how to make a complex structure or have the technical ability to manually assemble it from stem cell progeny.

Current and Future Techniques for Modulating Ion Channel Signaling

Most of the molecular work unraveling the roles of bioelectric signals in development and regeneration has utilized misexpression of well-characterized ion channel constructs, to gain specificity and mechanistic insight (Mathews and Levin, 2018; Pai et al., 2017, 2018; Pitcairn et al., 2017). Indeed, recent advances in optogenetics have enabled light-induced regeneration of spinal cord and tail (Adams et al., 2013, 2014, 2016; Chernet et al., 2016). However, the biomedicine is likely to be most rapidly advanced by methods not requiring genome editing or gene therapy. Therefore, the immense toolkit of ion channel drugs, many of which are already approved for human use in cardiac arrhythmias, epilepsy, and other indications,

can be re-purposed as electroceuticals in regenerative and anti-neoplastic (Cairns et al., 2018; Lobikin et al., 2015; Ozkucur et al., 2015; Tuszynski et al., 2017). An additional exciting set of directions however concerns new materials for sensing and actuation of bioelectrical signals *in vivo*.

Devices and Materials for Uncovering New Biology

Beginning with Galvani's studies on animal electricity (Galvani, 1972), bioelectronics has merged electronic devices with living systems. Advances in microfabrication and novel materials have now reduced the size of the bioelectronic interface from macroscale contacts capable of stimulating entire muscles to devices that can communicate with individual cells (Zhou et al., 2017) and membrane proteins (Misra et al., 2009; Noy, 2011; Soto-Rodriguez et al., 2016). This broad range of length scales makes these bioelectronic devices ideal means to interrogate and actuate bioelectric circuits.

Biosensors

To interrogate bioelectric circuit, bioelectronic sensors can sense electric fields, ionic concentrations, and biological markers (Fang et al., 2015; Loffler et al., 2017; Rivnay et al., 2014; Simon et al., 2016; Someya et al., 2016; Tian et al., 2018). These many types of bioelectronic sensors have been described in prior reviews (Bollella et al., 2017; Rim et al., 2017; Rivnay et al., 2014; Zhang and Lieber, 2016; Zhu et al., 2016). A comprehensive and detailed description is beyond the scope of this work. Here we will focus on cell-sensors for extracellular and intracellular recordings, or group of cells, for both extracellular detection and measurement of V_{mem} (Abbott et al., 2018; Tian and Lieber, 2019).

Extracellular Recordings

Transistor type biosensors afford signal amplification and large-scale multiplexing (Kaisti et al., 2016). A common transistor biosensor platform is the organic electrochemical transistor (OECT). A typical OECT is made by a mixture of the polymers poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) that are combined with selected enzymes or antibodies to detect molecules of interest (Strakosas et al., 2015, 2017). The main advantage of the OECT platform is its inherent sensitivity to ionic species in solution that spans from the fact that the conducting channel conducts both electrons and ions (Berggren and Richter-Dahlfors, 2007; Rivnay et al., 2016, 2018). Mixed conductivity is essential for interfacing with bioelectric circuits because electric fields and electric currents in biological systems are mostly associated with ionic concentration gradients and ionic currents rather than electrons. Water, which is ubiquitous in biological systems, is a poor electronic conductor, but ions move freely in the liquid. Therefore, the OECT platform effectively acts as a transducer between the two types of charge carriers. This transduction mechanism can be used to assess cell health and response of barrier tissue to foreign substances (Jimison et al., 2012). The layer of cells grown on top of the OECT serves as a barrier between the OECT and the electrolyte. A healthy barrier layer with intact tight junctions between the cells does not allow the passage of ions into the OECT channel when a gate potential is applied so that the modulation in the channel current is small. Upon introduction of H_2O_2, tight junctions are damaged and the OECT response is increased. Recently, OECT have been interfaced with three-dimensional tissue with tubistors (Pitsalidis et al., 2018). The tubistor is the first example of a bioelectronic device in three dimensions. The tubistor affords a more natural interface with cells of many types of tissue and the 3D tubular geometry is ideal for flow of nutrients and for mimicking tubular types of tissues such as vascular, gastrointestinal, and kidneys. With the tubistor, Owens and co-authors have monitored *in vitro* the progression of cell cultures growing into a 3D conducting scaffold (Figure 5). Given the sensitivity of OECT to ionic species and external electric fields, a similar approach can be used to monitor bioelectric circuits.

Intracellular Recording

The advent of nanomaterials and microfabrication strategies has allowed bioelectronics devices to interrogate the intracellular environment of single cells for recording of V_{mem}. These recordings are built on the well-developed technique of patch clamping that is described in detail elsewhere (Annecchino and Schultz, 2018). Most of the bioelectronic work has revolved around measuring spatiotemporal mapping of V_{mem} of electrically excitable cells, such as neurons and cardiomyocytes (Chang Liao et al., 2015), but the functionality developed with these devices can be easily expanded to other non-electrically excitable cells to monitor bioelectric circuits. Silicon nanowires with nanoscale dimensions and high aspect ratios are suitable for crossing the cell membrane and connecting the intracellular domain with minimal or no damage to the cell. These nanowires are synthesized with spatially controlled electrical properties so that a

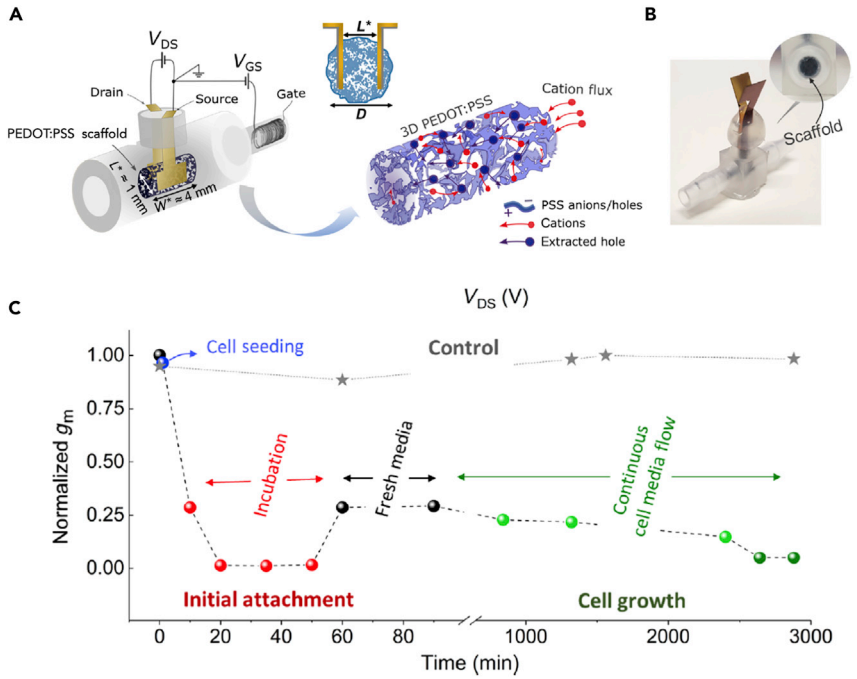

Figure 5. The Tubistor, the First Example of a Bioelectronic Device in Three Dimensions

(A) Schematic representation of the tubistor. A 3D Pedot:PSS scaffold is placed inside a microfluidic channel that comprises a source and drain contact across which V_{DS} is applied and an electrochemical gate that modulates the conductivity of the Pedot:PSS between source and drain, (B) picture of the tubistor, (C) different stages of cell growth affect the transconductance (g_m) of the tubistor that can be used to monitor cell activity (reproduced from Pitsalidis et al., 2018).

nanoscale field effect transistor is created on an individual nanowire by varying the doping levels (Tian et al., 2010). These nanowires are synthesized with a kink, so that they can be mounted on the tip of an atomic force microscope probe that is used to insert the nanowire into the individual cell. Recordings of action potential of cardiomyocytes are more precise when collected inside the cell with the silicon nanowire than when recorded in the extracellular space (Tian and Lieber, 2019).

In addition to intracellular recordings, bioelectronic devices are able to monitor the current response of membrane ion channels in artificial lipid bilayers. For example, gramicidin, bacteriorhodopsin, alamethicin, and ATPase are connected directly with carbon nanotubes (Huang et al., 2010), silicon nanowires (Misra et al., 2009), and organic field effect transistors (Angione et al., 2012) to develop biosensors with increased functionality. In these biosensors, the ion transport across the channels affects the electrostatic environment of the semiconducting material (nanotube, nanowire, conductive polymer) and is recorded as a change in current between the source and the drain electrodes. In this fashion, transport across both passive (gramicidin, alametachin) and active (rhodopsin, ATPase) is monitored as a function of extracellular environment. For example, Noy has recorded the activity of the enzyme ATPase using silicon nanowire transistors as a function of ATP concentration in solution (Misra et al., 2009). The Rolandi group has placed an artificial lipid bilayer on Pd contacts to monitor directly the transport of H^+ across the ion channels (gA) and alamethicin (ALM). In this scenario, the Pd electrode serves as a transducer between the H^+ current across the ion channels and the electronic current in the devices, thus effectively coupling the biological and electronic worlds (Hemmatian et al., 2016). With these devices, H^+ current across the gA and ALM channels is monitored and modulated with a potential difference applied across the cell membrane mimic. As expected, gA acts as a H^+ passive channel, whereas ALM H^+ transport is voltage gated and ALM only opens above the threshold voltage of approximately 70 mV. Additionally, rhodopsin integrated with these H^+–selective bioelectronic devices leads to light-driven H^+ transport across the cell membrane (Soto-Rodriguez et al., 2016).

Bioactuators

In the early experiments of Galvani and modern medical technologies, such as pace-makers and neuronal implants, bioelectric signaling to cells occurs through an electronic impulse at the electrodes that affects

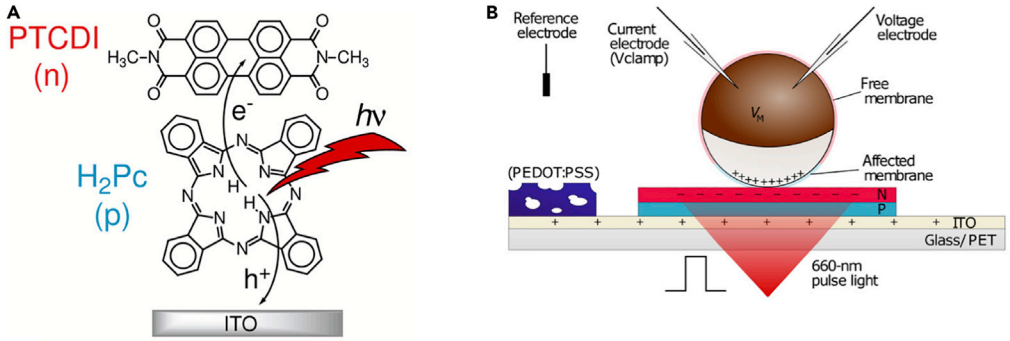

Figure 6. Schematic Representation of Organic Photocapacitor Working Principle and the Experimental Setup

(A) A photon absorbed causes electron hole separation in the polymer blend.

(B) A cell placed upon the organic photocapacitor detects a change in membrane potential when the light is turned out due to the charge separation occurring on the surface of the organic photocapacitor.

the action potential of electrically excitable cells. There have been many comprehensive reviews on neuronal (Bettinger, 2018) and peripheral nerve stimulation (Pavlov and Tracey, 2019), electroceuticals using electronic stimulations (Famm et al., 2013), and the use of electric fields for bone growth (Khalifeh et al., 2018) and wound repair (Zhao, 2009). Here, we will focus on bioelectronic devices that are able to deliver directly biophysical and chemical signals targeting specific cell functions. These signals include the electrophoretic delivery of ions and small molecules, such as neurotransmitters, to modify cell behavior (Arbring Sjöström et al., 2018). Organic electronic ion pumps composed of PEDOT:PSS are able to pump ions and small charged molecules from a reservoir well through both an ionically conductive poly-ion exchange membrane and a PEDOT:PSS electrode into bulk solution (Isaksson et al., 2007; Jonsson et al., 2016; Owens and Malliaras, 2011). This architecture pumps different charged species, such as monovalent cations, gamma-aminobutyric acid (GABA), and acetylcholine (Jonsson et al., 2015). Delivery of the neurotransmitter $GABA^+$ into the extracellular f≥lku6luid surrounding hyperactive neurons reduces epileptic activity (Williamson et al., 2015). These organic ion pumps are able to deliver multiple ions, as well as charged molecules, but their delivery efficiency becomes smaller with increasing molecule size. Simon et al. have recently demonstrated electrophoretic delivery of molecules such as acetylcholine using capillary fibers (Poxson et al., 2019). Delivery of larger molecules is enabled by a dendrimer-based ionic polymer with controlled porosity that allows for transit of larger molecules. Recently, Glowacki and co-workers (Jakešová et al., 2019) have demonstrated optoelectronic control of single cells by merging organic photocapacitors with *Xenopus laevis* oocytes. They performed electrophysiological recordings while illuminating the organic photocapacitors, showing that they are able to photoinduce transient changes between 20 and 110 mV with a time constant between 5 μs and 5 ms. These transients induce the opening of potassium channels, indicating that this device can directly control membrane protein conductivities (Figure 6).

Another strategy for controlling delivery of ions is to use PdH_x contacts that transfer H^+ across the solution contact interface for control of pH (Deng et al., 2016; Miyake et al., 2015). Using PdH_x contacts, Miyake et al. have used bioelectronics to monitor the output of the enzymatic biochemical reaction of glucose oxidation that reduces pH and have created with this reaction an enzymatic AND gate and an enzymatic flip-flop circuit (Miyake et al., 2015). In turn, Deng et al. have used the pH control spanning from the PdH_x bioelectronics devices to affect the rate and light output of the bioluminescent reaction of luciferin with luciferase (Deng et al., 2016). This reaction is faster and brighter at high pH and thus can be turned on or off using bioelectronics pH control.

A variety of nanomaterials have been developed for reading and writing bioelectric states in tissue. Particles that alter resting potential of cells by contact (Jayaram et al., 2017; Rana et al., 2016; Shin et al., 2013; Warren and Payne, 2015) offer the possibility of spatially targeted interventions without the use of transgenes. Complementing these are fluorescent nanoparticles that report the concentration of specific ions such as sodium (Dubach et al., 2009, 2011a, 2011b). An important future use of these reagents will be to understand the link between bioelectric signals regulated by K^+, Na^+, and Cl^- channels and the still poorly understood but likely important flow of electrons in living tissues (Sanjuan-Alberte et al., 2018; Sanjuan-Alberte and Rawson, 2019).

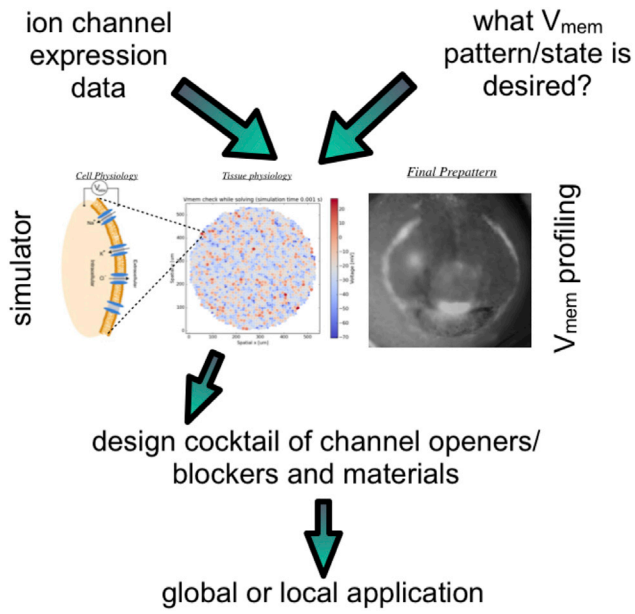

Figure 7. Bioelectric Strategies for Therapeutics

Data on ion channel expression in human tissues, together with knowledge of which bioelectric states are desired for healthy patterning and physiology, can be fed into simulators of the bioelectric circuits that enable predictions of the effects of possible ionic perturbations. The resulting suggested combinations of ion channel drugs and ion-releasing materials can be deployed *in vivo* or *in vitro* to manipulate cell and tissue-level behaviors in therapeutic or bioengineering contexts.

Opportunities and Next Steps

Proteomic and physiological profiling, together with drug screens (Sullivan and Levin, 2018), are revealing the molecular sources of relevant ion fluxes. Similarly, the transduction of changes in V_{mem} into second messenger pathways is now understood at the cellular level: voltage-regulated neurotransmitter transporters, calcium channels, KRAS clustering, and butyrate-regulated histone deacetylases convert bioelectric events into transcriptional responses [reviewed in Levin et al. (2017)]. Thus, at the scale of a single cell, bioelectric pathways are being decoded at high molecular resolution. However, the major knowledge gap concerns the tissue-level bioelectric code: the mapping between spatiotemporal distribution of ion currents, electric fields, and patterns of resting potential and the resulting morphogenetic outcomes (Levin and Martyniuk, 2018). Computational modeling of bioelectric circuits is beginning to reveal their memory, self-organizing, symmetry-breaking, and robustness properties (Cervera et al., 2015, 2016a, 2016b, 2018a, 2018b, 2018c, 2019; Garcia-Morales et al., 2017).

To understand how specific patterns of bioelectric state drive the creation and remodeling of complex organs, experiments will require the ability to read and write bioelectric states in tissues *in vivo*. Thus, newly developed ionogenic materials provide an important complement to optogenetics (Adams et al., 2013, 2016; Chernet et al., 2016), to interrogate the control of growth and form by ionic signals. This is especially relevant for model systems such as planaria, in which transient bioelectric changes can permanently change the regenerative pattern to which their stem cell progeny construct (Durant et al., 2017, 2019) but are not amenable to introduction of foreign optogenetic ion channels.

Bioelectronic devices presage the development of strategies to both monitor and guide regeneration, repair, and immune response modulation via knowledge of the bioelectronic code. To achieve the guided self-assembly needed for patterning *in vivo* or in bioengineering and synthetic morphology contexts (McNamara et al., 2016, 2018), closed-loop control would be desirable using strategies from biological control and machine learning that have been proposed to converge synthetic biology with bioelectronics (Selberg et al., 2018). Integration with optogenetic and microfluidic platforms is essential, as these are already beginning to interrogate cellular perception and decision-making mechanisms (Bugaj et al., 2017; Perkins et al., 2019). Likewise, new bioelectronic materials will be increasingly incorporated into wearable

bioreactors used for induction of complex regenerative repair (Golding et al., 2016; Hechavarria et al., 2010; Herrera-Rincon et al., 2018). Together, newly developed quantitative physiological predictive platforms (Pietak and Levin, 2016, 2017) and dynamically controlled ionogenic materials will enable the complex and nonlinear behavior of bioelectric circuits in tissues to be understood in health and disease states (Figure 7). The discovery and deployment of novel bioelectric signaling interventions are likely to have a major impact as an alternative to genomic editing and gene therapy for a wide range of applications in birth defects, regeneration, cancer, immunology, and synthetic morphology.

ACKNOWLEDGMENTS

M.L. gratefully acknowledges support from the Allen Discovery Center program through The Paul G. Allen Frontiers Group (12171), the Templeton World Charity Foundation (TWCF0089/AB55), and the Bailey Family Foundation. M.L and M.R. acknowledge funding from the Defense Advanced Research Projects Agency (DARPA), Army Research Office under Cooperative Agreement Number W911NF-18-2-0104.

AUTHOR CONTRIBUTIONS

All authors wrote and edited this review article.

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