Bioelectricity is a universal multifaced signaling cue in living organisms

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ABSTRACT **The cellular electrical signals of living organisms were discovered more than a century ago and have been extensively investigated in the neuromuscular system. Neuronal depolarization and hyperpolarization are essential for our neuromuscular physiological and pathological functions. Bioelectricity is being recognized as an ancient, intrinsic, fundamental property of all living cells, and it is not limited to the neuromuscular system. Instead, emerging evidence supports a view of bioelectricity as an instructional signaling cue for fundamental cellular physiology, embryonic development, regeneration, and human diseases, including cancers. Here, we highlight the current understanding of bioelectricity and share our views on the challenges and perspectives.**

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INTRODUCTION

Bioelectricity refers to the cellular electrical properties intrinsically caused by the uneven distributions of ions and charged molecules across cell membranes (Burr and Northrop, 1935). Usually, there is a high concentration of potassium ions and a low concentration of sodium ions in cytosol compared with the external cellular environment—the cellular resting membrane potential forms when these chemical concentrations and their electric charge gradients reach a balance. The ion channels, pumps, gap junctions, and solute carriers are the major contributors to this uneven distribution of ions and charged molecules, such as amino acids and proteins, essential for maintaining cell physiology. The types of these contributors can be variable, given one type of tissues or cells. Thus, different types of cells may possess a characteristic resting membrane potential, which can be due to a unique profile of ion regulators or to its physiological history, as many of these ion regulators can open and close posttranslationally due to other events in the cell. From this perspective, the neuron is a specialized cell type with drastic electric changes. In this brief review, we focus on nonexcitable cells.

While bioelectricity is a phenomenon that is intrinsically rooted in individual cells, its influence is not limited to the cellular level (McCaig *et al.*, 2009). Instead, it can manifest at various levels, including the organ, tissue, and even whole body (Figure. 1). This multilevel manifestation of bioelectricity plays a crucial role in various physiological processes, such as cell migration, proliferation, differentiation, and many diseases, including cancer metastasis. One important function of bioelectric signaling is to enable cellular collectives (tissue networks) to store and process information in ways that individual cells cannot. Much as neural bioelectricity in the brain underlies the emergence of a coherent organism that adaptively navigates three-dimensional space, non-neural bioelectricity allows groups of cells to traverse anatomical morphospace during embryogenesis and large-scale regeneration. Thus, bioelectricity could have diverse and far-reaching implications in biology.

BIOELECTRICITY AND FUNDAMENTAL CELLULAR PHYSIOLOGY

The differential distributions of ions and charged molecules not only establish the cell membrane potential but also maintain cell volume. In animals, stable cell volume is maintained by pumping

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Abbreviations used: ASAP1, Accelerated Sensor of Action Potentials 1; EV, Extracellular Vesicles; GEVI, Genetically Encoded Voltage Indicator; NADH/NAD+, Nicotinamide-adenine dinucleotide; TME, Tumor Microenvironment; TNT, Tunneling nanotubes.

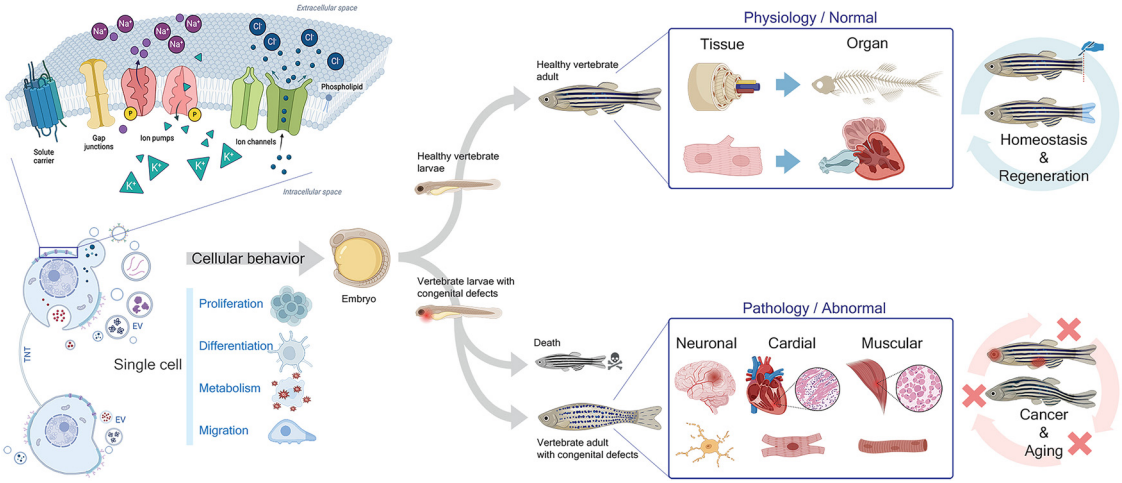

FIGURE 1: Bioelectricity is a multiple-faced signal essential from a single cell to higher levels of biological processes. Cellular bioelectric status is an overall signal with contributions from multiple ion channels, pumps, gap junctions, and solute carriers. The bioelectric sign plays essential roles in fundamental cell physiology, such as proliferation, differentiation, metabolism, and migration. Cellular bioelectricity can also be modified by EV (extracellular vesicles) and TNT (tunneling nanotube). Bioelectricity can be utilized during embryonic development as a signal for patterning and differentiation of tissues and organs. Once animals develop into adults, bioelectricity remains a critical signal for homeostasis, regeneration, and diseases such as cancers. Abnormal bioelectricity may lead to diseases such as birth defects, aging, and cancers. Some cartoons were adopted or modified from BioRender.

Na^+ out and K^+ in through Na^+/K^+ ATPase to counter the Donnan effect, unstable osmotic fluctuations caused by intracellular impermeant molecules from cell metabolism (Kay, 2017). Cellular shapes and sizes are critical for cell function and cell migration. Migrating cells are usually polarized and form leading-edge protrusions (e.g., lamellipodia, filopodia, and blebs). The local membrane potential and differential distribution of ion channels were found to contribute to the lamellipodial protrusion (Schwab, 2001). Indeed, the cell membrane potential also serves as a fundamental regulator of cell migration through calcium influx (voltage-gated ion channels), modulating cytoskeleton signaling such as ERK-Rho-kinase, and modulating cortical actin polymerization (Schwab *et al.*, 2012). Various ion channels (e.g., K^+, Na^+, Ca^{2+}, Cl^-, TRP (Transient receptor potential) superfamily, and piezo channels) have been reported to be involved in cell migration (Schwab *et al.*, 2012; Canales Coutino and Mayor, 2021).

Cellular bioelectric state and its related differential ion concentrations are also critical for cell metabolism. Intracellular ion concentration may directly affect key metabolic enzymes and essential regulator proteins. In addition, some solute carriers pair metabolites (e.g., glutamate) transport and specific ions (Zhang *et al.*, 2019; Alam *et al.*, 2023). Thus, bioelectric change inevitably influences metabolism. Furthermore, cellular membrane potential can interact with metabolic redox and energy carrier pairs (NADH/NAD^+ and ADP/ATP) through membrane ATPase and membrane-bound dehydrogenases (Schofield *et al.*, 2020).

Cell proliferation, another critical autonomous behavior, was also found to be tightly linked with cell membrane potential and ion channels (Blackiston *et al.*, 2009). Each cell type may have a distinct bioelectric status. Generally, the cell membrane potentials of less proliferative cells are more electrically hyperpolarized, and proliferating cells are more depolarized (Blackiston *et al.*, 2009; Abdul Kadir *et al.*, 2018). In addition, cell membrane potentials and potassium ion concentration undergo a rhythmic change during cell cycles (Urrego *et al.*, 2014). For example, MCF-7 mammary tumor cells were found to be depolarized toward G_0/G_1, immediately hyperpolarized during the G_1/S transition, and then further hyperpolarized during the G_2/M stage (Wonderlin *et al.*, 1995). Not only cell membrane potential but also mitochondrial membrane potential was reported to regulate cell cycle in yeasts (Gorospe *et al.*, 2023). In addition, cell membrane potential was also found essential for bacterial cell division (Strahl and Hamoen, 2010), and bacterial biofilm formation and interbacteria communications can be mediated by bioelectric signals (Koshland, 1983; Prindle *et al.*, 2015; Yang *et al.*, 2020). Recently, we have observed that zebrafish embryonic cells show hyperpolarization before cytokinesis during cleavage stages (Silic *et al.*, 2022). Alter cell membrane potential leads to cell proliferation changes, suggesting that bioelectricity may control the cell cycle, likely through the Ca^{2+} and K^+ ion flux and cyclin/cyclin-dependent kinases (Whitaker, 1995; Abdul Kadir *et al.*, 2018; Humeau *et al.*, 2018).

Cell differentiation is generally coupled with cell proliferation and cell metabolism (Agathocleous and Harris, 2013). It is not surprising that bioelectricity also regulates cell differentiation (Sundelacruz *et al.*, 2009). Mounting evidence revealed that the cell membrane potential is not only associated with cell differentiation but also plays an instructional role in multiple cell types, such as keratinocytes, neural cells, immune cells, and myocytes (Sundelacruz *et al.*, 2008). For example, hyperpolarization may trigger tyrosine dephosphorylation of the Kir2.1 channel and cause an increase of myocyte transcription factors through Ca^{2+} influx (Konig *et al.*, 2004). Another fascinating example is that stem cells can be induced to differentiate into osteogenic or neurogenic tissues using electrical stimulation matching the endogenous membrane potentials (Zhang *et al.*, 2023).

BIOELECTRICITY IN EMBRYONIC DEVELOPMENT

Embryonic development is a robust system in which a fertilized egg proliferates, differentiates, and eventually forms an entire organism. Cell-autonomous behaviors such as proliferation and differentiation are essential. The intercell interactions (short and long ranges) are also critical for multicellular organisms to pattern and

orient body axes, as well as pattern tissues such as bird feather systems (Jiang *et al.*, 2021; Tseng *et al.*, 2023) and create organs of the correct shapes and sizes (Takahashi *et al.*, 2001; Lander, 2011). Morphogen proteins and transcription factors play crucial roles in embryogenesis (Wolpert, 1969; Pearson *et al.*, 2005; Briscoe and Small, 2015; Negrete and Oates, 2021), and some of these are downstream of bioelectric cues (Vandenberg *et al.*, 2011; Pai *et al.*, 2015; Dahal *et al.*, 2017; Belus *et al.*, 2018). The role of bioelectricity is becoming evident in the developmental biology of multiple organisms such as Xenopus, zebrafish, mice, and fruit flies and has been extensively reviewed (Levin *et al.*, 2017; Harris, 2021; Levin, 2021a; George and Bates, 2022; Silic and Zhang, 2023). The ectopic expression of ion channels, as well as dominant negative or hypermorphic mutations, leads to drastic morphological structure changes in animal models and human patients bearing channelopathies (Masotti *et al.*, 2015; Adams *et al.*, 2016; Sun *et al.*, 2019). This finding suggests that bioelectricity may serve as organ-level or whole-body-level instructional signals. Bioelectric signals are critical for embryonic patterning that sets organ shapes and sizes, as reported in zebrafish fin-size – altered mutants caused by multiple K^+ channels, connexins, and solute carriers (Iovine and Johnson, 2000; Iovine *et al.*, 2005; Perathoner *et al.*, 2014; Lanni *et al.*, 2019; Silic *et al.*, 2020; Daane *et al.*, 2021; Stewart *et al.*, 2021). In addition, changes in the bioelectric state of certain embryonic regions in a developmental stage can change the identity of that region to different organs. For example, ectopic eyes were induced via expression of a constitutively conductive EXP1 cation channel, glycine-gated chloride channel, or dominant negative *Kir6.1* (*Kcnj8*) in Xenopus head or trunk regions (Pai *et al.*, 2012), including gut tissues normally thought to be incompetent to acquire eye fate in vertebrates. A similar phenotype caused by multiple channels strongly supports the idea that bioelectricity, not individual ion channels, is the key to these embryonic phenotypes—a conclusion also supported by the ability to swap vertebrate ion pumps for yeast pumps with no sequence or structural homology while keeping their functional effects in regeneration (Adams *et al.*, 2007). Indeed, consistent with early studies using microprobes on Xenopus embryos, a recent investigation using the GEVI (genetically encoded voltage indicator), ASAP1, revealed characteristic voltage changes during zebrafish embryogenesis (Silic *et al.* 2022). Moreover, it was discovered that external electrical currents could regulate tissue size and shape in vitro (Shim *et al.*, 2024). It is clear that bioelectricity plays a crucial instructional role in embryonic development. A few hypotheses, such as bioelectric code and integration with a morphogenic field, have been proposed as frameworks within which to understand how patterns of bioelectric state map onto anatomical outcomes (Tseng and Levin, 2013b; Levin and Martyniuk, 2018; Harris, 2021).

BIOELECTRICITY IN TISSUE HOMEOSTASIS AND REGENERATION

Upon completion of embryonic development, tissue, and organ-level bioelectric signaling remains functional and is critical for tissue homeostasis, which is usually achieved through regeneration and tissue turnover. Turnover is a universal process of decay and regeneration of new components across biological systems (Reddien, 2024). Electrically coupled cells and collective electrical fields may serve as regenerative cues for other chemical and mechanical signals responsive to tissue integrity changes or injury (Levin, 2009; Tyler, 2017; McLaughlin and Levin, 2018). Consistent with this notion, electric currents have been recorded in the newt limb and Xenopus tadpole tail regeneration (Borgens *et al.*, 1977b; Reid *et al.*, 2009). Moreover, the regeneration can be modulated by external electric stimulation, V-ATPase, and voltage-gated Na^+ channel, Nav1.2, in tadpole tail regeneration (Borgens *et al.*, 1977a; Adams *et al.*, 2007; Tseng *et al.*, 2010). In addition to newts and frogs, bioelectricity in regeneration was also reported in murids and zebrafish (Becker, 1972; Hechavarria *et al.*, 2010; Monteiro *et al.*, 2014; Leppik *et al.*, 2015). Similarly, electric response and wound healing have been demonstrated in epithelial cells, and the regenerative bioelectricity has been found to be mediated by PI(3)K and redox signaling (Zhao *et al.*, 2006; Reid and Zhao, 2014; Ferreira *et al.*, 2016).

In addition to vertebrates, bioelectricity is evident in planarian regeneration, where it is responsible for the patterning of the anterior-posterior axis. Targeting the endogenous bioelectric circuit in regenerating planarian fragments can produce no-head and two-head animals (Beane *et al.*, 2011; Beane *et al.*, 2013; Durant *et al.*, 2019). Strikingly, a temporary (as brief as 3 h) alteration of the bioelectric state gave rise to planaria, which have a permanently altered target morphology—their fragments continue to generate two heads in perpetuity with no further manipulation (Oviedo *et al.*, 2010; Durant *et al.*, 2017), motivating a model in which the bioelectric circuit holds anterior-posterior axis polarity as a kind of rewritable memory separate from the genetics (Pezzulo *et al.*, 2021).

Altering the bioelectric state of the blastema by sodium ionophores was able to induce complete regeneration of tails and limbs in amphibia in normally nonregenerative conditions, acting as a brief trigger that kickstarts a complex program of changes in gene expression in anatomy (Tseng *et al.*, 2010; Tseng and Levin, 2013a). The bioelectric role in regeneration is also being validated in other organisms. Fruit fly gut epithelial regeneration has also been reported to be bioelectric dependent on gap junction – mediated Ca^{2+} currents triggered by neurons (Petsakou *et al.*, 2023). Another exciting discovery is that Nav1.7 was identified as a critical chondrocyte regulator and therapeutic target for osteoarthritis, a joint degeneration disease. The Nav1.7 channel regulates the chondrocyte secretome through Ca^{2+} signaling, and blocking this channel can facilitate the progression of osteoarthritis (Fu *et al.*, 2024). As aging is naturally linked with tissue/organ homeostasis and regeneration, it is not a surprise that bioelectricity also plays a crucial role in aging (Silver and Nelson, 2018; Pio-Lopez and Levin, 2024).

BIOELECTRICAL MALFUNCTIONS AND CANCER: A FAILURE OF MULTICELLULARITY

Cancer, though defined on the pathology level, is often thought of as a disease of genome decay, accompanied by genomic instability and a myriad of mutations from chromosome changes to single nucleotide mutations. So far, only a small number of genes have been defined as cancer driver genes (oncogenes or tumor suppressor genes), which are critical for cancer formation (Stratton *et al.*, 2009). With the completion of many human cancer genomes, essentially all human genes are found mutated in some human cancer cell genomes (Ding *et al.*, 2018; Martinez-Jimenez *et al.*, 2020; Ostroverkhova *et al.*, 2023). So, it is unsurprising that many ion channels were reported in cancers, especially given that more than 400 channels are in the human genome and are widely expressed in many cell types (Hutchings *et al.*, 2019). Other models of cancer, favoring nongenetic mechanisms (Soto and Sonnenschein, 2011; Sonnenschein and Soto, 2016), likewise implicate impairments of

bioelectrical signaling as defects in the mechanisms that normally bind individual cells to a common purpose in normal tissue and organ morphostasis (Levin, 2021b).

A role for bioelectric signaling in cancer, via the role of sodium and potassium channels, is beginning to be dissected, and even some channel blockers were already in clinical trials (Ding *et al.*, 2018; Prevarskaya *et al.*, 2018; Martinez-Jimenez *et al.*, 2020; Bell *et al.*, 2023; Ostroverkhova *et al.*, 2023). The detailed correlation evidence of ion channels in cancer has already been reviewed (Montgomery *et al.*, 2020). As bioelectricity is an intrinsic signal and has a wide distribution of ion channels, it is undoubted that it has an essential cellular function in tumor initiation, progression, and metastasis from various human cancers (Yang and Brackenbury, 2013; Robinson *et al.*, 2021; Sheth and Esfandiari, 2022). Glioma, a notorious brain tumor, is another of the most studied tumors for the role of bioelectricity. Glioma cells can interact with neurons through glioma synapses and gap junctions to change neuron bioelectric activities and cause disease symptoms. Recently, the *in vivo* glioma – neuron interactions were demonstrated in awake mice using a genetically encoded Ca^{2+}reporter, GCaMP6f (Montgomery *et al.*, 2020).

In animal models, bioelectric signals have been shown to induce melanoma in the absence of genetic defects or oncogene drivers (Morokuma *et al.*, 2008; Lobikin *et al.*, 2015), as well as to normalize tumors induced by human oncogenes such as *TP53* and *KRAS* mutations (Chernet and Levin, 2013; Chernet *et al.*, 2016). Recently, a neuron-like GABA-mediated bioelectrical activity between melanoma cells and keratinocytes was revealed in the zebrafish genetic melanoma model (Tagore *et al.*, 2023). During cancer progression, cancer cells may depolarize cellular membrane potential to achieve independent cell growth and uncontrolled proliferation, and this process is tightly linked to ion channel alterations and Ca^{2+} signals (Yang and Brackenbury, 2013). Depolarized glioma cell membrane potential was able to promote tumor proliferation, and hyperpolarization reduced the glioma progression (Venkatesh *et al.*, 2019). Similarly, knockdown gap junction GJB2 could disrupt cancer cell nanotube projection and inhibit tumor invasiveness (Bahcheli *et al.*, 2024). The cancer cell bioelectric changes may also influence cell differentiations and make some cancer stem-like cells (Biagiotti *et al.*, 2006; Biella *et al.*, 2007). The tumor microenvironment (TME) usually consists of an extracellular matrix and multiple cell types, including fibroblasts, immune cells, and endothelial cells. During cancer progression, TME profoundly impacts cancer cell bioelectricity and vice versa, along with interactions between bioelectric, biochemical, and mechanical signals (see details in next section) (Sheth and Esfandiari, 2022). Consistent with this notion, neuronal regulation in many types of cancer has recently been recognized since the bioelectric contributions from neurons can be relatively measured (Gillespie and Monje, 2020; Winkler *et al.*, 2023).

Bioelectricity is also crucial for metastasis due to cancer cell bioelectric status and cancer microenvironment. (Payne *et al.*, 2019). It is well known that ion channel distribution and related cell membrane potential are key regulators for cell migration (Schwab *et al.*, 2012). Cancer cells may co-opt similar mechanisms for metastasis (Zhang *et al.*, 2024). Many studies suggested that depolarized cancer cells may tend to be more metastatic (Ribeiro *et al.*, 2020). On the tissue or organ level, bioelectricity may coordinate the metastasis from invasion to colonization through the TME and blood or lymphatic vessel environments. Collective migration and electric field were also frequently reported in cancers (Li *et al.*, 2013; Garg *et al.*, 2019; Zhu *et al.*, 2020). Thus, ion channel blockers/agonists and external electric fields may interrupt cancer development. Indeed, ion channels' function and potential as therapeutic targets are currently being investigated (Takayasu *et al.*, 2020; Abed *et al.*, 2023). In addition, electrotherapy and external electric devices have already been clinically applied to glioma based on the principle of bioelectricity (Rominiyi *et al.*, 2021).

CHALLENGES, NEW PROGRESS, AND PERSPECTIVES

One of the challenges is that the bioelectricity concept is not well accepted or even well known in many research areas. A prominent contributing factor is the misconception that bioelectricity is exclusively relevant in neuroscience, not nonexcitable tissues (Levin, 2021a). But this is gradually changing as more scientific discoveries are revealed and published. The bioelectricity subgroup in the Cell Bio 2023 is a great advance, at least to the cell biology research community (Pai *et al.*, 2024). The special topic journal, like *Bioelectricity*, is an excellent move and a landmark for the bioelectricity field. The second barrier is the largely unrevealed connection with the current biomedical research regime centered on biochemistry and genetics. Genetics alone does not determine phenotypes entirely because the bioelectrical layer of control provides a kind of physiological "software" that provides extra plasticity to the genetically encoded hardware. For example, bioelectric status changes can induce persistent alternative anatomical structures after regeneration and even ones associated with different species than the genomic default (Sullivan *et al.*, 2016; Levin, 2021a). Likewise, genetic state and phenotype diverge in animals bearing oncogenic mutation but no tumor due to bioelectric normalization.

Biochemical molecules are deemed as the mainstream signaling carriers (Lander, 2011). Bioelectricity is different from traditional biochemical signals in many ways. The bioelectric signals generally serve as overall body patterning instruction and coordinate and program overall body anatomy (Levin, 2021a). Essentially, the bioelectric control could be an epigenetic mechanism that guides morphogenesis usually through a robust and dynamic status (i.e., cellular voltage memory) (Levin *et al.*, 2017; Levin and Martyniuk, 2018; Levin, 2021a). Furthermore, there is a lack of a 1:1 relationship between bioelectric outcome and gene products. This is because bioelectricity is an overall readout of multiple contributors (channels, gap junctions, etc.) and multiple downstream output routes to biochemical machinery on the cellular level (Levin, 2014, 2021a). Bioelectric patterns were found to impact important morphogen proteins such as BMP, SHH, and FGFs, which are critical for embryonic development, adult tissue, and organ regeneration. For example, BMP signaling was found to be regulated by KCNJ2-mediated bioelectricity in mice and fruit flies (George and Bates, 2022). Cell membrane potential stabilizes SMO and regulates hedgehog (HH) signaling in fruit fly wing discs (Emmons-Bell and Hariharan, 2021; Spannl *et al.*, 2021). The Na^+ and K^+ gradients are essential for SHH secretion and SMO activation (Petrov *et al.*, 2020). Likely due to the nature of secondary messenger, calcium signaling is often reported as a common downstream pathway. The calcium signal is similar to cellular voltage change during zebrafish embryos (Chen *et al.*, 2017; Silic *et al.*, 2022). In addition to calcineurin and PI(3)K pathways, Ca^{2+} flux can modulate bioelectricity and tune the conductivity of ion channels that directly contribute to cell bioelectricity and excitability (Clapham, 2007). It is hard to distinguish the bioelectricity from the Ca^{2+} signaling, but additional transduction mechanisms, such as serotonin movement (Fukumoto *et al.*, 2005) and K-ras clustering (Zhou *et al.*, 2015),

exist for converting bioelectric information into downstream transcriptional readouts.

In addition to biochemical interactions, bioelectricity is also tightly linked with mechanical transduction, which is involved in various physiological (e.g., touching, hearing, angiogenesis, development, and regeneration) and pathological processes (e.g., cardiac hypertrophy, muscular dystrophy, and polycystic kidney) (Martinac, 2014). Some ion channels, such as TRP, PIEZO, K2P, and ENaC (epithelial sodium channel), can directly sense cellular mechanical forces and convert mechanical changes into an electric signal or Ca^{2+} flux (Martinac, 2014; Jin *et al.*, 2020). Inversely, PIEZO channels were also found to be gated by voltage (Moroni *et al.*, 2018). The extracellular matrix proteins can also directly influence ion channel conductivities on the cell membrane. For example, chondroitin sulfates were reported to modify voltage-dependent ion channels (Vigetti *et al.*, 2008). Similarly, extracellular matrix proteins can also impact connexin functions (Imbeault *et al.*, 2009). Thus, these mechanical, bioelectric, and biochemical signals can interplay and coordinately regulate complex biological processes such as stem cell differentiation and cancers (Engler *et al.*, 2006; Discher *et al.*, 2009; Karska *et al.*, 2023).

Another challenge of bioelectricity research is the still limited available experimental approaches. The overall cellular bioelectric status is an integration from multiple inputs of ion channels, pumps, gap junctions, and solute carriers. Thus, the bioelectric status is robust and dynamic and is usually a component of physiological feedback, requiring the development of bioelectric simulator platforms (Pietak and Levin, 2016), and more broadly, new conceptual frameworks borrowed from multiscale neuroscience (Pezzulo and Levin, 2015). Genetic mutation of one of these contributors is generally insufficient to cause a phenotype unless the mutation is dominant negative or hypermorphic (Silic and Zhang, 2023). This phenomenon was noticed as bioelectricity does not reduce to molecular genetics (Levin, 2014). Thus, identifying a critical endogenous contributor is hard since forward genetic screening is less effective. As bioelectricity is highly dynamic, measuring *in vivo* cellular bioelectric status is also challenging using old-fashioned chemical dyes or patch clamp techniques. Fortunately, newly developed tools such as optical probes, GEVIs, optogenetics, and chemogenetics can readily be adapted to bioelectricity research using well-established model organisms such as zebrafish (Foust *et al.*, 2023; Silic and Zhang, 2023). However, nonoptical reporter technologies will be needed to detect bioelectric states in deep tissues. Another exciting area of progress involves electroceuticals (Balasubramanian *et al.*, 2024), including pharmacological and optogenetic modalities that target tissue native bioelectric networks instead of a specific pathway (Adams *et al.*, 2013; Chernet *et al.*, 2016; Pio-Lopez and Levin, 2023).

In addition to challenges, some new frontiers of cell biology related to bioelectricity have emerged. Extracellular vesicle (EV) is one of them. EVs are cell-derived membrane-enclosed particles found in essentially all cell types. Currently, EVs are known as a universal way of intracellular communication as they can deliver soluble mediators, such as proteins (cytokines, morphogens, and microRNAs, to the direct contact of neighboring cells. EVs can be classified into exosomes or ectosomes based on their biogenesis, and they have variable sizes (Zappulli *et al.*, 2016; Salomon *et al.*, 2022; van de Wakker *et al.*, 2023). The EVs may directly distribute ion channels such as sodium channels, glutamate receptor subunits, solute transporters, connexin, and aquaporins (Faure *et al.*, 2006; Soares *et al.*, 2015; Pathare *et al.*, 2017; Barros Lamus *et al.*, 2021; Nouri *et al.*, 2021). Thus, they may modulate cellular bioelectricity. On the other hand, bioelectricity could also regulate EV formation and release. P2X7R, an ATP-gated ion channel, was found in controlling EV release (Golia *et al.*, 2023). Like EV, the cell membrane tunneling nanotubes (TNTs) are another type of intercellular communication for diverse contents from ions and proteins to organelles. However, the TNT working range (physical distance between cells) is longer than EVs. TNTs have an actin structure and portions of the plasma membrane between two cells and facilitate cytoplasm and organelle exchange (Cervantes and Zurzolo, 2021; Zurzolo, 2021; Wang *et al.*, 2024). TNTs are also related to cellular bioelectricity since connexin and Ca^{2+} flux were found to be generated and propagated within TNTs (Smith *et al.*, 2011; Vargas *et al.*, 2019). Moreover, cells connected by TNT can be electrically coupled through connexins (Wang *et al.*, 2010). This TNT-mediated electrical coupling could modulate intracellular downstream signaling molecules such as PI3K (Abounit and Zurzolo, 2012). Thus, both EVs and TNTs expand the horizon of bioelectricity.

In conclusion, bioelectricity is a universal, multifaceted signaling modality for regulating autonomous collective cell behavior, embryonic development, regeneration, and diseases such as cancers. Although there are some challenges, significant progress has been made in recent years. With newly developed tools, we expect bioelectricity to find its way into cell, developmental, and evolutionary biology, as well as many other biomedical research areas.

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