



Bioelectric Signaling and Voltage-Gated Ion Channels in Appendage Regeneration: A Systematic Literature Review on Bioelectric Blueprints for Tissue Patterning

Rajdev Singh

Researcher, Biotechnology

Rajdevsinghemail@gmail.com

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Abstract

The regenerating appendage must control cell proliferation, migration, differentiation, positional identity, tissue scaling and growth termination in an orchestrated fashion. Emerging evidence suggests that bioelectric signaling contributes as an important physiological layer of information for these processes, in addition to genetic and biochemical signaling. This systematic literature review explores the role of endogenous bioelectric signals and voltage-gated ion channels in appendage regeneration, focusing on their role in tissue patterning and the proposed bioelectric blueprint. Planarians, *Xenopus*, axolotls, newts, zebrafish and other models of regeneration were used to examine the responses of the electrical properties, changes in membrane potential, ion-channel activity, calcium signaling, gap-junction communication, stem-cell behavior, positional information, and scaling of regeneration. The evidence reviewed here shows that voltage-gated calcium, potassium and sodium channels are involved in transducing electrical signals into intracellular signals that regulate proliferation, migration, differentiation and tissue organization. Other molecular pathways are also linked to bioelectric mechanisms, such as Wnt, FGF, BMP, Notch, Hedgehog, etc. Overall, the results are consistent with a dynamic blueprint based upon electrical fields and states contributing to anatomical polarity, identity, growth and regeneration, both spatially and with time. The findings suggest future applications in tissue engineering, regenerative medicine and in human appendage regeneration.

Introduction

The regeneration of appendages is one of the most spectacular examples of biological self-organization, where complex anatomical structures are restored after injury, amputation or loss of tissue is coordinated (Levin 2021). Regeneration of an appendage is not just the repair of a wound, but involves the reconstruction of numerous tissues such as epidermis, connective tissue, vasculature, nerves, muscle, cartilage, and bones, as well as the restoration of their proper spatial

relationships, polarity, size and function. This is based on the swift activation of injury responses, the establishment of a specialized regenerative blastema, the proliferation and migration of progenitor cells, the plasticity of cells, differentiation and the cessation of growth once the proper anatomical structure is restored. Importantly, regeneration is not just the replacement of lost cells, but a highly regulated patterning event in which cells must decide what is missing and build it based on 'positional information' (Stocum, 2017). The remarkable

ability is quite different for different organisms. While planarians can regenerate entire body regions and show astonishing degrees of regeneration of axial identity, salamanders (e.g., axolotls and newts) can regenerate whole limbs composed of correctly patterned skeletal, muscular, neuronal, and vascular tissues. The zebrafish has a high level of fin regenerative competence and the *Xenopus* tadpole has a high level of tail regenerative competence at specific stages of development. Therefore, these organisms have been recently emerging as valuable experimental models for studying the cellular and molecular mechanisms of regeneration (Levin, 2023). In these models, conserved signaling pathways such as Wnt, FGF, BMP, Hedgehog, Notch, and TGF- β pathways, as well as interactions between stem/progenitor cells, immune cells, extracellular matrix, nerves and local tissue niches, play a part in regeneration.

In spite of significant developments in developmental and regenerative biology, the reconstruction of anatomically precise structures in regeneration does not seem to be fully understood by the conventional molecular models (Seifert & Muneoka, 2018). Positional information has been traditionally considered to come from transcription factors, morphogen gradients, growth factors, signals from the extracellular matrix, and gene-regulatory networks. While these mechanisms are important, they do not completely explain how distributed populations of cells coordinate their behavior at relatively large spatial scales, or how tissues resume global anatomical organization after experiencing major injury (Diwanji & Bergmann, 2018). This study of developmental bioelectricity has thus led to the development of a complementary regulatory system. Bioelectric signaling involves transmembrane voltage gradients, ionic currents and concentrations, electric fields, and intercellular electrical coupling by gap junctions. Importantly, this is not limited to neurons or muscle cells, but any cell can have a bioelectric signal resulting from the coordinated activity of ion channels, pumps, transporters, and exchangers (Murugan et al., 2022). These electrical states could affect proliferation, migration, differentiation, apoptosis, cell-cell communication and gene expression, and offer a mechanism by which physiological information can be converted into modifications of tissue organization (Herrera & Bach, 2021). Developmental bioelectricity thus suggests that there are dynamic physiological patterns within tissues that work in concert with genetic and biochemical networks to control growth and form. Experimental evidence

with regenerative organisms has shown that the manipulation of endogenous voltage states can alter the axial polarity, organ identity, tissue growth and regenerative outcomes, indicating that bioelectric information might act as an instructive part of the regenerative program, and not just a result of the cells' activity.

In this new context, voltage-gated ion channels play a significant role as a direct molecular connection between the membrane voltage and the intracellular signaling (Iismaa et al., 2018). Changes in membrane potentials can be translated to calcium influx through voltage-gated calcium channels, and establishment and modulation of membrane voltage and cell excitability are facilitated by potassium and sodium channels. A change in ionic flux can then trigger second-messenger pathways, change transcription, affect the behavior of the cytoskeleton, and affect cell fate (Seifert et al., 2023). Gap-junction-mediated communication also allows the transfer of electrical and chemical states between adjacent cells, possibly creating tissue-level bioelectric domains that carry positional information. These mechanisms can affect various phases of appendage regeneration in a variety of regenerative models, such as wound responses, blastema formation, proliferation and migration of progenitor cells, differentiation, polarity formation, and control of regenerative growth (Makarevich et al., 2020). At the level of organ size and anatomical patterning, however, bioelectric signaling has also been implicated, suggesting that electrical information can act as a component of a higher-level system, from the level of individual cells to entire tissues. This view is especially important as it offers the potential to explain how local cellular responses become integrated into a coherent anatomical response (Heck et al., 2021). Bioelectric mechanisms do not supplant the genetic and biochemical explanations, but rather work alongside canonical developmental pathways, which could be linked to the Wnt, Notch, Hedgehog, FGF, BMP, calcium-dependent and epigenetic regulation pathways (Catterall, 2023). The bioelectric blueprint is thus a dynamic spatiotemporal organization of membrane potentials, ion fluxes, and intercellular electrical communication that could offer a physiological level of information for tissue identity, polarity, growth, and final morphology.

This systematic literature review explores the role of bioelectric signaling and voltage-gated ion channels in appendage regeneration, tissue patterning, and regenerative outcomes. Understanding bioelectrical control may reveal approaches to regulate cell plasticity, stem cell

functions, regenerative niches, tissue scaling, anatomical patterning, and tumorigenesis through altered membrane potential and ion channel activity (Bakhshandeh et al., 2023).

Therefore, this review aims to summarize the existing evidence on the role of endogenous voltage gradients and voltage-gated calcium, potassium, and sodium channels in appendage regeneration; the role of bioelectric signals and canonical molecular pathways and epigenetic mechanisms; and the role of bioelectric signals and positional information and on how these mechanisms contribute to the restoration of appropriate tissue size, shape, polarity, and identity (Zhao et al., 2019). The review also discusses the possibility of bioelectric signaling as a functional blueprint for tissue patterning and its potential use in regenerative medicine, tissue engineering and synthetic biology (Tyler, 2017). And, (ii) what are some of the mechanisms by which bioelectric signaling regulates appendage regeneration? (ii) How do voltage-gated ion channels contribute to the process of regenerative patterning and scaling? (iii) What is the relationship between bioelectric mechanisms and molecular and cellular signaling pathways? To answer these questions, we must address the following issues: (iv) What effects do bioelectric states have on stem-cell behavior, cellular plasticity, and positional information? and (v) How can knowledge of endogenous bioelectric blueprints be used to create future regenerative therapies? The review seeks to develop a framework in which all these mechanisms—from electrical, molecular, cellular, and mechanical—are integrated together to explain how injured tissues reconstruct complex anatomical structures (Nirenberg & Yifrach, 2020).

Literature Review

The study of developmental bioelectricity has become an important field to enable understanding of the regulation of growth, regeneration and complex anatomical development. In the past, developmental biology has focused on genetic programs and biochemical signaling pathways, but gradually more and more evidence suggests that there is another stream of information, an endogenous bioelectric one, which is generated by resting membrane potential (V_{mem}), ion fluxes, and electrical gradients which can coordinate cellular behaviour across different tissues. Given that bioelectric signalling networks are potentially endogenous control systems for the determination of growth and form (Levin, Pezzulo and Finkelstein 2017), a new dimension to actively influencing large-scale anatomical

outcomes could be voltage gradients. Most importantly, the bioelectric regulation is not limited to electrically-excitable cells, including neurons and myocytes: Virtually all cells have intrinsic membrane potentials that can modulate cellular proliferation, differentiation, migration and tissue organisation (Harris, 2021). Moreover, a systematic synthesis from Srivastava et al., 2021, shows the far-reaching importance of bioelectric mechanisms for patterning as they have been recorded in channelopathies related to abnormal bioelectric patterning from a diverse array of organisms and ascribing common electrogenic signatures to the patterning of regeneration. Another important point that emerged from their analysis of differences in membrane potential states between normal and malignant cells has been the suggestion that membrane-potential states are important not only in regards to embryogenesis and regeneration but also to tumorigenesis. The data together take a stand against the paradigm of gene-based morphogenesis and suggest that cell electric states may provide instructive signals that combine molecular information into tissue-level pattern.

Evidence from experimental regeneration studies is especially powerful that bioelectric signals can dictate anatomical outcomes. Durant et al. 2019 found that manipulation of bioelectric states for a short duration immediately after amputation paralyzed the polarity of anterior and posterior sides and resulted on stable, double-headed phenotypes in planaria. Importantly, during the time of the electrical manipulation, before later patterns of transcription, the electrical signals were shown to control the specification of anatomical identity upstream. This is a firm endorsement of the idea that bioelectricity is an upstream component of a positional-information system and is not just a downstream consequence of metabolism. More recent studies have extended this concept by stating that the electric field could enable the coordinated spatial association of the V_{mem} patterns among tissues. demonstrated it computationally that the field-mediated interactions can increase the complexity Bioelectric patterns and they can provide new approaches to encoding morphogenetic information such as patterns that resemble developmental craniofacial prepatterns. These results are in line with a more general tissue perspective, where distributed networks of bioelectric signals provide communication between cells, enabling the attainment of anatomical aims. This was expanded upon by who proposed the concept of

collective intelligence, wherein developmental/regenerative systems have multiscale competencies that move tissues through physiological and morphological conditions to stable anatomical outcomes. Overall, the available observations in the literature indicate that bioelectric signals could be a highly influential communication system between cellular physiology, gene regulation, tissue organization and their large scale morphology. The capacity to learn the encoding, transmission and decoding strategies for positional information could then create a foundation for new strategies in healthy regeneration, synthetic morphogenesis, tissue engineering and cancer intervention (Levin, 2017; Srivastava, Stewart, & Perugini, 2021; Harris, 2021; Durant et al., 2019).

Methodology

1. Systematic Literature Review Framework

Systematic literature review (SLR) framework was used for the critical evaluation and synthesis of existing knowledge and experience in the area of bioelectric signaling, voltage-gated ion channels, and appendage regeneration. The SLR approach was chosen to allow data from a variety of scientific fields, such as regenerative biology, developmental biology, electrophysiology, stem-cell research and tissue engineering to be identified and evaluated. The review was conducted in a structured and transparent way to minimise any potential bias and maximise the reproducibility. The framework included problem formulation, identification of literature, screening, eligibility assessment and data extraction, quality evaluation, and evidence synthesis. The study of bioelectric mechanisms in tissues patterning and regenerative consequences was emphasized.

2. Literature Databases and Search Strategy

The goal of a comprehensive search strategy was to achieve good coverage of literature with several well-known scientific databases used to conduct it. The main databases used were PubMed/MEDLINE, Scopus, Web of Science, Web of Science ScienceDirect, and Google Scholar. The chosen databases are comprehensive biomedical science, regenerative medicine, developmental biology and molecular signaling databases. Manual searching of key experimental articles and review papers by secondary sources yielded additional studies. The search strategy was to collect both old and new studies related to the role of the bioelectricity, ion channels, membrane potential

and electrical signaling in regenerative processes and tissue organization.

3. Search Terms and Boolean Combinations

A literature search was undertaken by combining relevant keywords together with some Boolean operators in order to get the highest retrieval of the relevant publications. The search terms were clustered into the categories falling under bioelectricity, regeneration, ion channels, and tissue patterning. These comprise the terms: "bioelectric signaling," "membrane potential," "voltage gated ion channels," "calcium channels," "potassium channels," "appendage regeneration," "blastema," and "tissue patterning." Search concepts were combined using boolean operators: AND, OR and NOT. Some representative search strings were formed by combining multiple different words into a search string like bioelectric signaling and regeneration or voltage-gated calcium channels or ion channels and limb regeneration or fin regeneration. These combinations yielded new combinations with better sensitivity and specificity in search.

4. Inclusion and Exclusion Criteria

Inclusion and exclusion criteria were well defined prior to the screening process for uniformity and appropriateness. Studies were considered if they were journal articles, review paper, or experimental bioelectric signaling, ion channels, regenerative biology, or tissue patterning article that were peer-reviewed journals. The study of model organisms, like planarians, Axolotls, zebrafish, newts and *Xenopus*, was favored. Articles describing stem cell behavior and/or cellular plasticity and/or molecular pathways were considered when the articles were connected to these aspects of bioelectric regulation. Conference abstracts, editorials, non-peer-reviewed and duplicate publications, and studies relating to regeneration or developmental bioelectricity but not both were excluded. Other than English, non-English studies with poor translations were excluded.

5. Study Screening and Selection

The screening and selection process was done systematically, in several steps to find the most relevant studies. First, all retrieved records were consolidated and then duplicate publications were eliminated. Then the titles and abstracts were scanned for studies that were obviously not focused on the review, and these were excluded as well. All other articles were then screened full text for eligibility, according to the

pre-established inclusion/exclusion criteria. Studies that offered mechanistic insights of membrane potential regulation, ion channel function or regenerative outcomes were looked upon speciality. Further studies were located based on the screening of the reference lists of these studies. Studies that were found to be only minimally related to bioelectric control of regeneration and tissue patterning were excluded for final synthesis.

6. Data Extraction and Synthesis

A standard template was created from which data is to be extracted so that the information from the selected studies is uniformly collected. The information picked up comprised authors, year of publication, experimental organism, regenerative tissues, bioelectric mechanism studied, ion channel type, methodology used, main conclusions drawn and reported regenerative results. Data were also stored on the activity of the stemcell, blastema formation, positional information, cellular plasticity, and interactions with signaling pathways. Because the results vary in the kinds of experimental designs, species, and outcome measures, the approach to narrative synthesis was taken over statistical meta-analysis. Bioelectric mechanisms are organized along each of the theme areas presented, and common biological principles were identified for comparisons of bioelectric mechanisms across regenerative system.

7. Quality Assessment of Selected Studies

A judgment on the methodological quality of the studies included has been made to identify if there was evidence that was reliable and strong. Assessment parameters included that the study possess a clear research purpose along with a suitable experimental design, adequate control groups, inclusion of the sample size in the report, reproducibly of the methods, statistical soundness and non-opacity of the results. Research using direct measurement of the membrane potential, electrophysiological methods, calcium imaging, genetic manipulation, or interventions that were specifically designed for action on an ion channel was deemed to be another highly potent approach. Documentation of small sample sizes, insufficient mechanistic validation and pharmacological off-target effects, and inadequate experimental controls were noted. A quality assessment differentiated exploratory observations and supported bioelectric regulation mechanistic evidence and observations.

8. PRISMA-Based Reporting

Transparency and reproducibility were promoted by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework in the review process. The literature selection process was documented by applying the PRISMA guidelines, which outline identification, screening, eligibility assessment and inclusion of studies. To explain the number of records retrieved from databases, duplicates excluded, articles screened, full text studies assessed and final studies included in the review a PRISMA flow diagram was developed. The reasons recorded and reported for the exclusion of children was at many points. The principles of PRISMA were adhered to, guaranteeing the methodological quality and laying the foundation of a transparent framework for analysing the evidence dealing with bioelectric signaling and appendage regeneration.

Fundamentals of Bioelectric Signaling in Regeneration

Bioelectric signaling is an important level of biologic cells communication and coordination during growth, development, repair, and regeneration processes (Levin, 2021). It is produced mostly due to ion concentration differences across the cell membrane and to the turned-on or off function of ion channels, pumps, transporters, and exchangers. In most cells the resulting transmembrane voltage, also known as membrane potential (V_{mem}), is associated with several properties of the cell, such as its excitability; every living cell has measurable membrane potentials and the ability to respond to alterations in its voltage (Harris, 2021). Injury during regeneration can quickly upset normal ionic gradients, creating endogenous electric currents which can alter the membrane potential and affect cellular migration, proliferation, differentiation, apoptosis and gene expression at the site of injury. Importantly, bioelectric signaling is not just a side effect of cellular metabolism (Levin et al., 2017). The regulative effects of changes in V_{mem} are being increasingly appreciated and are believed to provide instructive signals that control developmental and regenerative processes. Experimental voltage alteration of membranes has shown to be able to alter tissue polarity, organ identity, growth and regenerative capacity, proposing that electrical information signals work in concert with genetic and biochemical signals in the restoration of complex anatomical structures (Tyler, 2017). Coordination of ion pump, transporter, exchanger and ion channels is responsible to the cellular basis of bioelectric signaling.

Establishing and maintaining membrane potential are electrochemically mediated and involve sodium, potassium, calcium, chloride and hydrogen ions. Ion pumps maintain gradients (Na^+/K^+ -ATPase) and channels are regulated pathways for ions to move across membranes. The particular significance of voltage-gated ion channels is that their opening and closing will depend on the direct changes in membrane voltage (Whited & Levin, 2019). These voltage gated potassium channels can modify the polarization of membranes, the voltage gated sodium channels can help to depolarize the membrane and the voltage gated calcium channels can convert changes in polarization to intracellular biochemical signals by controlled influx of calcium ions. Calcium is particularly crucial since it acts as a second messenger that can trigger kinases, phosphatases, transcription factors, cytoskeletal pathways and metabolic processes (Silver & Nelson, 2018). Therefore, changes in membrane potential can trigger a series of molecular events leading to changes in the behaviour of the cells. This electrical-to-biochemical conversion is a significant mechanistic link between bioelectric states and the molecular pathways pivotal to regeneration and tissue organization processes (Levin, 2023).

There is more complex bioelectric signalling in the tissue since adjacent cells can communicate via gap junctions and via extracellular electric field. The gap-junction proteins, normally referred to as connexins, create channels among cells to allow the direct passage of ions and small signaling molecules. This coupling allows for the coordination of the individual electrical states of the cells within tissues that consists of large numbers of cells and can lead to formation of bioelectric domains with spatially varying electrostates. These domains are known to play a role in positional information, axes, formation of a blastema, and regulation of regenerative growth. Changes in the activity of ion channels, gap-junction communication, and/or membrane potential result in modifications of anatomical identity and regenerative morphology in studies of planarians, amphibians, and zebrafish (Li et al., 2020). Therefore, bioelectric signaling can be considered a multiscale information system linking ionic gradients at the cellular level with gene expression and tissue morphology at the tissue level. It has got involved in other conventional pathways like Wnt, FGF, BMP, Notch, Hedgehog and it interacts with the system of stem/progenitor-cell behavior and cellular plasticity during regeneration. The resulting combination of the electrical, molecular, and cellular information gives some

background in understanding the proposed bioelectric blueprint that states the dynamic voltage patterns determine what types of structures to reform, where they will reform, how much tissue to re-form, when the reforming of tissue is supposed to stop, and more.

Voltage-Gated Ion Channels and Tissue Patterning

The voltage-gated ion channels are the proteins in the plasma membrane that change the permeability of ions in response to changes in voltage (Nguyen & Bursac, 2019). They can influence developmental and regenerative process by their activity which can alter the electrical and biochemical environment of cell. During appendage regeneration, the ion channels are involved in more complicated cellular networks of progenitor cells, differentiated tissues, nerves, extracellular matrix, signaling molecules etc. They not only help maintain homeostasis of their cells/body, but when electrical conductance of their respective ions changes they can affect various other functions including the signalling, cell-cycle activity, migration, differentiation and communication between neighbouring cells. The consequences of activation of these channels vary depending on channel type, expression, state, and on the cross-talk with other ion handling systems. These features are important to comprehend in order to provide an explanation for the transmission of electrical signals to organized tissue formation and for the generation of abnormal anatomical patterns as a result of changes in ionic regulation.

1. Voltage-Gated Calcium Channels

The voltage-gated calcium channels are significant transducers of electric signals since they mediate the conversion of a membrane voltage change to an intracellular calcium signal. Once the channel is activated, calcium enters the cell cytoplasm and then can participate in a lot of processes such as enzyme activation, transcriptional regulation, reorganization of the cytoskeleton, etc. and cellular differentiation (Klejchova et al., 2021). Calcium signals can be different depending on when and how the channels are activated: they can be localized pulses or transient waves, and thus differing in duration, activity that determine the subsequent response of cells in generating tissues. The proliferation/differentiation response balance in the regenerative cell population can be regulated by calcium-dependent mechanisms. They are also involved in blastema cell communication and in relation to surrounding tissues. Thus voltage-gated calcium channels directly

influence regenerative behavior by coupling its changes to changes in membrane potential. They could play a role of special significance during the transition period from active tissue formation to the stabilization and maturation of the newly-developed appendage.

2. Voltage-Gated Potassium Channels

Movement of sodium is a related regulation of membrane polarization, but the movement of potassium is more significant and affects electrical stability in cell. Involvement in their opening brings about membrane repolarization, while regulating potassium conductance, it can regulate the threshold for activation of other voltage sensitive channels. For regenerative tissues, this makes the potassium channels a key regulatory mechanism of cellular responsiveness. Changes in the potassium conductance can occur and alter the entry of calcium, intracellular signaling, proliferation and/or differentiation. Regenerative growth might also be influenced by the timing of potassium-channel activity due to the modulation of the electricity conditions in which the progenitor cells are still active (Dixon et al., 2022). Each different type of channel can produce different electrical states in different sets of cells, which may lead to specialization at the level of space in a regenerating structure. In practice their task goes beyond balancing ions and could include regulating physiologic conditions necessary for proper tissue formation.

3. Voltage-Gated Sodium Channels

Na⁺ channels control the movement of sodium into and out of the cell and can cause large rapid voltage changes in the cell. The classical functions of these are related to neurons and excitable tissues, but because sodium requires an electric potential for transport, it can also regulate non-neural cells. Modifications in sodium movement, can lead to altering intracellular ion concentration, polarization of the membrane, the cell volume as well as the activation threshold of other ion channels (András et al., 2021). During regeneration, these effects can play a role in the initial cellular injury response to different injuries sustained to the tissue, as well as in the progression into a regenerative state. Sodium sensitive responses may also modulate intracellular signaling pathways that mediate the expression of genes and modulate cell behaviour. Their involvement in regenerative processes is of special interest due to the close relationships with other sodium gradients processes such as sodium dependent exchangers/pumps. Therefore a further

investigation of the activation of sodium-channels may help to elucidate the use of early electrical changes in later regenerative responses (Villalba-Galea, 2017).

4. Chloride Channels and Membrane Regulation

The regulation of the membrane, including effects of chloride channels on chloride permeability, cell volume, osmotic balance, and electrical stability, are linked to membrane regulation. Clarify the inhibitory role of chloride channels as compared to calcium channels, that chloride channels are not primarily sources of second-messenger signals in the cell, but regulation of the relationship between membrane voltage and chloride equilibrium potential can significantly influence the electrical behavior of a cell. Since their volume and the concentration of ions are important variables in cell growth as well as tissue remodeling, chloride transport in these processes is of special interest. Changes in chloride transport could lead to changes in migration, either due to doing shape and volume changes to cells, or by interacting with the transport of potassium and sodium ions, which in turn could affect membrane polarization. While chloride mechanisms are less explored in appendage regeneration, they are an integral part of ionic homeostasis and are worth consideration in the overall hormonal and electrochemical control of regeneration and for comprehensive models of bioelectric regulation (Levin, 2023).

5. Channel Localization and Tissue-Specific Activity

Ion channel effects are highly dependent on the location of expression and distribution of the channel in regenerating tissues. Various populations of cells may have different channel profiles thus giving differences in membrane properties throughout the developing structure. This can lead to differential reactions of epidermal cells, fibroblast-like progenitors, muscle-associated cells, neural populations and vascular cells whenever changes to their electrical milieu occur. Additionally, because channels can be near signaling complexes, the microdomains generated by such a localized function can have specific biological effects, which is why subcellular localization is important. Changes in the distribution of the channels can occur during the transition between the proliferating, migrating, and differentiating states, during regeneration. Organisation of the ions within a membrane in space might then be related to forming

functional territories in the blastema. The vary of responses to a common regenerative stimulus in different parts of an appendage may be explained through tissue-specific electrical properties (Harris, 2021).

6. Ion-Channel Regulation During Regeneration

Significant alterations during the regenerative process can occur in ion-channel expression and activity. Problems with the electrical environment of damaged cells will occur right after the breakdown of tissue barriers and ion gradients. At the early (regenerative) stage, disturbed channel activity can stimulate the activation, migration, and proliferation of cells. Changes in ion permeability can alter the differentiation pathway of progenitor populations as well as their relationships with surrounding tissue (Tyler, 2017). In more advanced stages electrical regulation could play a role on maturation, tissue integration and ending growth. This variation over a span of time suggests that the regenerative process may not all be dependent on just one electrical state. Rather, it is likely a series of bioelectric events which relate to different stages of development. These temporal phases are critical for identifying if certain ion channels trigger new regeneration, maintain tissue growth, create pattern, or are involved in the final growth limitation of regeneration (Dixon et al., 2022).

7. Channelopathies and Abnormal Tissue Patterning

Disruption in this relation of tissue organization and physiology can be caused by the abnormal activity of the ion channel. All of these can alter cellular electrical states as a result of genetic mutations, inappropriate channel expression, trafficking of the channels, over-activation of channels, or loss of associated regulatory proteins. These disruptions can impact cell proliferation, differentiation, migration and communication during regeneration and lead to structural defects. Another possibility is that poor electrical control can disrupt the understanding of positional cues and lead to tissue size or tissue patterning. When several populations of cells rely on the synchronised electrical communication one population's disruption may affect another. The impact is particularly severe for populations where multiple cells depend on coordinated electrical communication, where a disruption to one population can affect each of its neighbouring populations. The study of channel dysfunction can then offer an experimental pathway for identification of the set of required channel

states for the production of normal pattern formation. It also underscores the critical need for spatial and temporal control in the activity of the ion-channels while they are being thought of as potential therapeutic targets.

Bioelectric Signaling During Appendage Regeneration

Physiological responses to wounding that initiate regeneration of an appendage start with a rapid increase in bioelectric activity in the immediate vicinity of the wound site, creating a disruption of epithelial integrity, ionic gradients and cellular membranes. Changes in membrane potential may result from injury and last for only a few minutes and affect adjacent cells to the injured area. Epithelia can produce endogenous electrical gradients which extend beyond the wound boundary due to wound currents as a consequence of ion transport changes across the wound epithelia (Daponte et al., 2021). These gradients can contribute directionality in the migration of cells and activating repair associated signaling pathways. Depending on the simultaneous regulation of ion pumps and channels, transepithelial potentials are mostly preserved in epithelial tissues, making their activities particularly relevant (Harris, 2021). After being damaged, these electrical circuits can create a current in the damaged area, and that current can circulate and direct the action of cells. The intensity, direction and timing of these changes can differ for different types of tissue and developmental stages. Within this, the bioelectric response can be seen as an early stage of regeneration relating physical tissue damage to further cellular and molecular events (Kakebeen & Wills, 2019).

After the initial response to the injury, bioelectric signals play a role in the development of a reparative environment and the development of the blastema. The blastema is made up of cells with a proliferative potential and lineage cells which together contribute to the regeneration of the missing appendage (Srivastava et al., 2021). Changes in the membrane potential and ion flux have the potential to affect activation, proliferation, survival and migration of these cells. Cxygen gradients can provide signals that act in conjunction with extracellular matrix properties and/or chemical signals to direct cell migration to specific areas of the regenerating structure. The regulation by bioelectric signals also relates to the process of cell dedifferentiation and plasticity, when mature cells can acquire more progenitor-like traits, and the developmental state of cells can be modified after an injury. Intracellular calcium signaling, kinase activities,

transcriptional programs, and cytoskeletal organization can all be affected by changes in ionic conditions, leading to changes in cellular phenotype. Bioelectric signaling is not a stand-alone system, but rather exists in concert with growth factors, inflammatory factors, neural inputs and extracellular matrix remodeling. The integrated environment influences the proliferation, migration, and initiation of differentiation of the cells to form specialized tissues.

Other areas of Regenerative Competence; Positional Identity and Pattern Formation as influenced by Bioelectric Signaling. Regenerative competence is defined by the capacity of tissues to induce and to maintain a proper response to injury; these effects varies with species, developmental stage, tissue type and anatomical location. The states of the membrane potential could potentially affect the induction of the cell to be a part of a regenerative program by altering the activity of intracellular signaling and modifications to the regulation of genes (Abe & Nishizawa, 2021). In the early stages of regeneration, electrical differences between locations can play a role in positional information by enabling cells to determine where this is now in the regenerating appendage, and what structures need to be rebuilt. This electrical information can sculpt the existing developmental pathways, such as Wnt, FGF, BMP, Notch, and Hedgehog signals, and establish an integrated circuit to orchestrate tissue identity (Jang et al., 2023). Bioelectric information may also be spread from neighboring cells to other cells within the tissue through gap-junction communication, and this mechanism can help to coordinate local electrical changes over larger areas of tissue. In terms of the regenerating appendage, the growing appendage acts as a responsive patterning system that contains a combination of electrical, molecular, and mechanical signals that collectively act upon polarity, tissue boundaries, cellular differentiation, and anatomical organization when the polarity is disturbed (Levin et al., 2019).

One last to be considered was the control of regenerative outgrowth and return to proper size of appendages, changing size in adult life. One further and important role of bioelectric signalling was the control of outgrowth of the regenerated parts and as such restoring the proper size of the appendage; changing sizes in adult life. Regeneration needs to be carefully managed as more growth may create abnormal or larger than intended structures, while less growth may mean that only partial reconstruction takes place (Aztekin, 2021).

Electrical states can thus change gradually as the regrowing appendage grows toward the proper anatomical size. Regulations of ion-channel activity, Calcium dynamics, membrane polarization and intercellular electrical communication can alter the balance of continued proliferation and differentiation and provide mechanisms which allow tissue growth to be slowed and eventually prevented. This indicates that bioelectric signaling is not only key to initiating regeneration, but also plays a role in deciding when to stop regeneration (Kakebeen & Wills, 2019). Constant interaction among cellular growth, positional information, tissue geometry and bioelectric feedback could give rise to regenerative scaling. The evidence points to a model where the injury-induced electrical changes trigger a cascade of bioelectric responses which affect wound repair, blastema formation, cell migration, plasticity, pattern specification, and growth termination. Bioelectric signaling could be seen as a dynamic regulatory process that could contribute to reforming an injured appendage to an organized entity that possesses proper identity, proportions, and functionality (Srivastava et al., 2021).

Bioelectric Regulation in Major Regenerative Models

Complementary evidence for the function of bioelectric signalling in tissue reconstruction comes from the different regenerative organisms. Planarians are one of the best models for studying bioelectric control of positional information, as small pieces of tissue can regenerate whole regions of the body correctly organized anterior to posterior. They react regeneratively through changes in membrane potential, ion transport, gap-junction coupling, and electrical coupling between cells (Levin, 2021). In *Xenopus*, bioelectric changes after tail injury are linked with the degree of regenerative competence and effects the capacity to reconstruct complicated tail structures. The regeneration of limb buds in axolotls is specifically useful, as activity of ion channels and intercellular communication helps organize the regenerating blastema and correctly patterned digits and skeletal structures (Birkholz et al., 2019). Limb regeneration is also very extensive in newts, and they also emphasize the need for a coordinated communication between the different cells involved in the dedifferentiation process, the formation of the blastema and the reconstruction of the tissue. While the exact mechanism of ion-channel action varies across species, the models all indicate that electrical

states may play roles in cellular activation, proliferation, positional information and morphological organization. What is informative about their differences is that they illustrate that the underlying bioelectric principle of patterning regeneration can be implemented by different species-specific combinations of ion channels, transport systems, gap junctions, neural signals and molecular pathways. (Durant et al., 2019)

Zebrafish fin regeneration offers a more and more complete vertebrate system to study the mechanisms by which membrane voltage is coupled to regenerative growth. After amputation of the fin, the environment of the wound undergoes rapid physiological changes which lead to blastema formation, proliferation of progenitor cells, differentiation of tissues, and reconstruction of the fin architecture (Emmons-Bell et al., 2019). The mechanisms involved in bioelectricity in zebrafish are related to ion channels, calcium dynamics, regulation of membrane potential and interactions between various cell populations of the blastema. However, the link between voltage gated calcium signaling and regenerative scaling has been enhanced by studies showing that voltage-gated calcium signaling could affect the duration and extent of fin outgrowth. Vertebrate models with limited regenerative ability help explain why extensive appendage regeneration varies across species. Studies of planarians, amphibians, and zebrafish show that bioelectric signaling is flexible and linked to genetic and molecular characteristics. Common mechanisms include injury-induced electrical changes, membrane potential modulation, ion-channel activity, intercellular communication, cellular plasticity, positional information, and growth control (Harris, 2021). These processes are tissue- and species-specific, yet provide important principles for understanding regenerative biology and potential future applications in regenerative medicine (Fields & Levin, 2020).

Voltage-Gated Calcium Signaling in Appendage Regeneration

One of the key cellular mechanisms in which bioelectric activity is converted into intracellular biochemical response during appendage regeneration is calcium signalling. From a signaling perspective, calcium ions are considered a highly versatile second messenger, as the concentration of calcium in the cytoplasm that changes quickly can alter the activity of enzymes, the organization of the cytoskeleton,

gene expression, cell metabolism and cell fate (Tyler, 2017). In tissue regeneration, the electrical changes across the plasma membrane can cause activation of voltage-gated calcium channels which permit influx of extracellular Ca^{2+} into the blastema and surrounding cells. The consequences of such calcium signals are not necessarily homogeneous, but may manifest as local areas of elevated calcium, oscillatory patterns, or fleeting changes on a subcellular scale, that can vary by amplitude, duration, frequency, and concentration in subcellular regions and consequently have differing impacts on the cell's processes. (Minh-Thai et al., 2021) Calcium influx into the cell is therefore voltage dependent and this is a crucial link between membrane potential and decision making in the cell. Calcium transients in blastema cells can have an effect on proliferation and migration, but can also play a role in the transition to the differentiated state. Ca signalling is highly electro-sensitive and because of this, a change in the cell membrane potential can be quickly translated to a change in information inside the cell. This mechanism does offer a possible mechanism by which a physical electrical state may regulate molecular pathways of regeneration without relying on electrical signals working without all the normal biochemical regulatory mechanisms.

There are many different types of voltage-gated Ca^{2+} channels and each has a distinct function, activation threshold, and tissue distribution. Although it can be assumed that L-type channels generally induce prolonged calcium influx in response to proper depolarization and thus are involved in certain forms of calcium-dependent transcription, and N-type channels traditionally play a role in regulated calcium influx in excitable cells, it cannot be ruled out that both channels may be involved in signaling in regenerating tissues as well. Their role in the regeneration of appendages is dependent on the appendage type, the channel subtype and the cell population that expresses it. The influx of calcium through these channels can lead to activation of calcium sensitive proteins and transcription factors, connects membrane activity with longer-term gene expression changes. One crucial pathway is through the calcium dependent phosphatase calcineurin which can be activated by increased levels of calcium in the cell and control the activity of downstream transcriptional programs by dephosphorylation of target proteins. In such ways, transient calcium signals could lead to long-lasting changes in cellular state. The influence of calcium-dependent transcription may affect genes involved in proliferation,

differentiation, stress responses, cytoskeletal remodeling, and tissue organization. This means that voltage-gated calcium channels play a role not only in the transport of ions, but also in the regulation of the functional activity of the cell as molecular switches that link the electrical state of the regenerating cell with the transcription programs necessary for tissue reconstruction.

Blastema biology is of particular interest in terms of calcium transients because the blastema has to balance continued proliferation and progressive differentiation. Transient increase in Ca^{2+} may serve as signals that will affect the activities of fibroblast-like cells and other progenitors in active regeneration (Paudel et al., 2018). The distribution of calcium activity along space may be another means of communication among cells located at a different position within the regenerating appendage. In the process of their migration, progenitor cells may need to coordinate cell movements with the establishment of adequate tissue boundaries, which is likely calcium signaling regulated. Simultaneously, calcium-dependent transcriptional pathways have the ability to change the developmental status of cells and enable their transition from proliferative populations to differentiated tissues (Marchant, 2019). This establishes a three-level regulatory mechanism whereby voltage-dependent channels for calcium entry regulate the calcium signal, which is then able to modulate the pathways, which then affect behavior. Importantly, it is the dynamics of the calcium signal (and not just the amount of calcium present) that influence the biological effect. The duration and intensity of calcium activity can influence the response, as a short "spike" can have a different effect than a sustained elevation, so it is important to consider both timing and frequency of calcium activity, in the successive phases of regeneration. Ca^{++} signaling through voltage-gated calcium channels is also likely to be involved in the control of regenerative outgrowth and the eventual cessation of tissue growth. To regenerate, a progression of events from injury response to blastema expansion, to tissue differentiation and a correct dimension of the appendage must be controlled. If the regeneration is not suppressed by proper feedback, this may lead to overgrowth or abnormal morphology (Brandão et al., 2022). Calcium mediated pathways offer some possible links between cell activity and the state of the regenerating tissue. As the appendage grows to its proper dimensions, the changes in membrane potential and calcium-channel activity may alter the frequency/duration of intracellular calcium

signals and therefore the proliferation and differentiation. This suggests a model in which calcium signaling serves as a mechanism of promoting regenerative activity, as well as being a feedback mechanism to regulate the duration of this activity (Mishra et al., 2018). Successful regeneration involves initiation and controlled termination, the role of the calcium-dependent pathways in the process is of special interest for the understanding of regenerative scaling. In general, voltage-gated calcium channels represent central elements of the bioelectric blueprint, linking the membrane potential with ion channel and calcium dynamics, transcriptional regulation, blastema behaviour, regenerative outgrowth and restoration of the appropriate size of the appendage.

Potassium and Sodium Channels in Regenerative Patterning

Bioelectrical mechanisms that govern cell behaviour during regeneration include important components of potassium and sodium channels. The resting membrane potential (rMP) is greatly determined by the concentration gradient of K^+ across the membranes, and therefore, K^+ plays a major role in the polarization of the membranes (Harris, 2021). Variations in activity of potassium channels may therefore affect the electrical state of regenerating cells and hence the activation of downstream voltage sensitive pathways. The activity of potassium conductance is also particularly important for the regulation of calcium channels in the cytosol and intracellular calcium signaling. These interactions are important for proliferating blastema cells where cell-cell interactions can affect cell-cycle activity and the cell-proliferation versus differentiation equilibrium. The proper functioning of potassium channels could also be involved in regenerative scaling by regulating the timing and intensity of tissue growth. Potassium channels do not work alone, but interact with pumps, transporters, sodium channels, calcium channels and other ion-handling proteins for maintenance of membrane-potential homeostasis (Petsakou&Perrimon, 2023). This concertina action of ionic regulation gives the capability to cells to respond dynamically to changes in their regenerative environment and behave differently in successive stages of tissue reconstruction.

Sodium channels play a role in the regenerative bioelectricity by regulating sodium permeability and membrane excitability (Fennelly & Soker, 2019). Variation in sodium conductance may lead to depolarization and alter the electric threshold for the activation of other voltage-

gated channels. Sodium channels are closely linked to excitable tissues but a change in membrane potential that is sodium-dependent can also affect non-neural cells that participate in regeneration. Sodium signaling may contribute to early events in response to injury, cell activation, migration and induction of regenerative competence (Whited & Levin, 2019). Concurrently, there is a close relationship between the movement of Na^+ and a number of transport systems which help to maintain intracellular ion content, osmotic balance, and cellular volume. Therefore, changes in electrical current in regenerating tissues can have an effect on other physiological processes that are influenced by sodium. The regenerative effects of sodium signaling may thus be subtype specific, population dependent, developmentally regulated and may involve other ion channels. Such conditions mediated by sodium channels can, in this context, help set up the electrical context required for the activation of regenerative programs after injuries.

The relationship of potassium and sodium channels is especially significant because the channel must have a balance between stability and flexibility in controlling membrane potentials at the time of regeneration. One type of channel may alter the electrochemical environment of another type, establishing feedback loops between ionic currents (Levin 2021). For instance, a depolarization caused by sodium can affect the activation of voltage-dependent calcium channels and a repolarization caused by potassium can affect the rate at which a cell restores its resting electrical state. Ion pumps and exchangers constantly reestablish ionic gradients, enabling cells to respond many times to changes in the signals. This dynamic balance could be particularly vital in the blastemas, where cells quickly proliferate, move, and differentiate. Any disruption of this balance can alter the calcium availability, intracellular signaling, metabolic activity and gene expression (Zhao et al., 2022). Thus, membrane-potential homeostasis is not a mere housekeeping function, but is a regulatory mode dictating the mode of action of cells when interpreting bioelectric and biochemical information during the regeneration process.

Potassium and sodium channels can also play a role in the regenerative re-scaling process that depends on the exact amount and time of tissue growth. If there is too much activity in the channels, or if there is inappropriate polarization of the membranes, the rate of proliferation signaling may change, which may either lengthen or shorten the window of regenerative outgrowth. On the other hand,

inactivity of ions could lead to decreased responsiveness of cells and disruptions in tissue repair. These channels are thus strongly context dependent, requiring and interacting with other signaling pathways. Early regeneration electrical states may aid in the activation of the cell and in the formation of the blastema, while later electrical states may aid in cell differentiation or in growth restriction. The functions also overlap those of molecules involved in regulating cell-cycle progression, cytoskeletal organization, and tissue patterning (Zhang et al., 2020). In general, potassium and sodium channels are part of a continuous bioelectric network that is associated with membrane polarization, tissue scaling, and cellular regeneration and proliferation. They work with the calcium channels, pumps, transporters, and gap junctions in a coordinated way, allowing the regenerating cells to keep the electrical stability and also be able to respond to the varying developmental and anatomical needs (George & Bates, 2022).

Emerging Technologies and Experimental Approaches

1. Genetically Encoded Voltage Indicators, Calcium Imaging, Optogenetics, and Electrophysiological Techniques

New technologies have greatly enhanced the investigation of bioelectric signaling during regeneration at the cellular and tissue level. By using genetically encoded voltage indicators, researchers can visualize membrane potential changes in the living cells and explore the spatial and temporal organization of membrane potential changes when cells are injured, when a blastema forms and when tissue patterning occurs (Xu et al., 2017). Calcium imaging in addition to voltage measurements can be used to simultaneously measure intracellular Ca^{2+} dynamics, such as transient spikes, oscillations, or waves, which could link membrane activity to the downstream biochemical signaling. The optogenetic techniques offer another experimental advantage: Light-sensitive proteins engineered for the cell can be used to regulate membrane potential or ion channel activity to a very high degree of temporal precision. These methods can be used to assess whether specific electrical conditions causally contribute to regeneration success or merely correlate with it. Activation and regulation of channels, membrane potential, and ionic conductance can all be quantitatively obtained from electrophysiological methods such as patch-clamp recording, voltage sensitive measurements, and ion-current analysis. The combined approach will be able to define links

between ion-channel function, electrical state, intracellular calcium signaling, and regenerative phenotypes (Piatkevich et al., 2019). These multimodal methods are very useful as bioelectric signals are very dynamic and gene expression-based measures do not fully reflect the physiological behavior of the signals. Thus, it is likely that future live voltage imaging, calcium monitoring, optogenetic manipulation and electrophysiology will result in increasingly detailed maps of the electrical states that accompany the regeneration of the appendages.

2. Single-Cell and Spatial Transcriptomics, Multi-Omics, Computational Modeling, and Artificial Intelligence

Additionally, high-resolution molecular technologies offer alternative solutions to understand the link of the bioelectric states with the identity of cells and the organization of tissues. Single-cell transcriptomics can be used to characterize the expression of ion channels in separate cell types within a tissue and can reveal the changes in the expression state of the cells that are involved in tissue regeneration over time. Combined with anatomy, which is provided by the ability to map gene expression across specific tissue locations, spatial transcriptomics enables the exploration of whether specific ion channels, transporters, or signaling pathways are related to specific bioelectric or positional domains (Kannan et al., 2018). Multi-omics approaches can be extended to include integration of transcriptomic, epigenomic, proteomic, metabolomic and chromatin accessibility data to explore the way this translates into longer term programs in the cell (Gheisari, 2017). Bioelectric networks consist of multiple interacting systems of ion channels, pumps, gap junctions, and signaling pathways that span various spatial and temporal scales, so computational modeling can be especially helpful. Numerous mathematical and computational models can be used for simulation of voltage gradients, ionic fluxes, intercellular coupling and their potential consequences for tissue patterning (Wu et al., 2019). Further, the conceptual framework of artificial intelligence and machine-learning can help uncover complex relationships in large imaging and multi-omics datasets, improve predictions of regenerative outcomes, and help identify candidate ion channels or molecular pathways that underpin responses to successful regeneration (Prouvot, 2023). The integration of these technologies, at some point, could provide predictive models of the bioelectric blueprint—some kind of correlation between the measurable electrical states and the behavior of

the cells, their identity, the growth of tissues, and their final anatomical morphology. An integrated experimental strategy could help expedite development of accurate, bioelectric interventions in regenerative medicine and tissue engineering (Mahn, 2018).

Challenges and Knowledge Gaps

Although significant advances have been made in understanding bioelectric regulation during regeneration, there are still important challenges and knowledge gaps that hinder the development of a full mechanistic picture. One drawback is the inability to directly measure endogenous voltage patterns in living regenerating tissues. Most studies do not continuously measure membrane potential at high spatial and temporal resolution, but instead use expression profiles of ion channels, or pharmaceuticals to alter membrane potential. Furthermore, the role of ion channels in specific cell types is not completely understood, as the same channel can be found in more than one cell type and play different physiological roles. Another challenge in bioelectric signaling is the temporal complexity, as the activity of the membrane potential and ionic activity can vary quickly during the process of injury response, blastema formation, proliferation, differentiation, and growth termination (Engel, 2020). It is possible, then, that a certain condition of electricity may, therefore, produce different effects in different stages of regeneration. In addition, there are significant species differences in regeneration, the expression of ion channels, organization of tissues, and pathways of molecular signaling between planarians and amphibians/fish/mammals. In addition, interpretation of the pharmacologic studies must be cautious since many ion-channel inhibitors are not completely specific, and may have off target effects, where it is necessary to complement this with genetic and electrophysiologic validation. Another significant gap in the knowledge is the clear mechanistic link between the membrane voltage and transcriptional regulation. Although there is a correlation between electrical state, the activity of transcription-factors, and the epigenetic modification, the exact sequence of events that links a defined voltage state to a gene-expression program is still not fully resolved. Yet another challenge is posed by translation, since highly regenerative organisms have much more powerful cellular and molecular capabilities than in adult human tissues. An important research question is whether the principles of bioelectrics of the

regenerative models can be safely replicated in human cells and tissues. Lastly, safety and tumorigenicity should be taken into account, as interventions that lead to proliferation, cellular plasticity and dedifferentiation may lead to undesirable growth if spatial and temporal regulation is not properly maintained. These should be the focus of future studies: direct voltage imaging, cell-specific manipulation of ion channels, longitudinal measurements, multi-omics integration, computational modelling, comprehensive assessment of safety. These gaps will need to be addressed to assess the feasibility of bioelectrical signaling as a therapeutic approach, while maintaining anatomical precision and preventing unwanted growth (Forli et al., 2018).

Future Perspectives

The development of more precise techniques for measuring, interpreting and manipulating the states of electricity within living tissue will likely be crucial to the future of regenerative bioelectricity. One of the key priorities will be the mapping of complete bioelectric landscapes during regeneration, both in terms of the spatial distribution and temporal evolution of membrane potentials, ionic currents, calcium activity, and intercellular electrical coupling. These maps may show if certain voltage patterns exist before specific anatomical structures are developed, if they occur before certain regeneration stages or if they occur in particular positions. Meanwhile, work on molecular engineering could enable scientists to manipulate the voltage-gated ion channel in the laboratory to 'activate' or 'inhibit' certain electrical states at specific points in regeneration in targeted populations of cells. This might be helpful to distinguish those signals that stimulate regeneration from those that control differentiation, patterning and termination of growth. Another critical application will be the creation of artificial bioelectric blueprints, with electronically pulsed gradients or programmable ion-channel systems to direct cells in the desired tissue organization. These methods may be available as an alternative or in a synergistic combination with growth factors and biomaterials to offer spatial instructions for physiology. Eventually, the ability to create specific bioelectric states could enable bioengineering the regeneration environment, rather than just triggering natural repair processes.

Another big opportunity is the integration of bioelectricity and stem-cell engineering. The defined electrical field could be used to expose stem cells and progenitor cells to the effects of

self-renewal, lineage commitment, differentiation, migration, and tissue integration. The incorporation of 3D biomaterials, organoids, extracellular-matrix engineering, and controlled biochemical signaling with bioelectric regulation could make complex tissues more accurately reconstructed. Bioelectric control might also be useful to control organ size and morphology, which is one of the key questions in regenerative medicine: When to stop the growth of tissue? Other systems could then incorporate electrical feedback along with mechanical and molecular feedback and recreate proper tissue proportions. The advances would enable personalized regenerative medicine, that is electrical stimulation or ion-channel modulation based on different factors depending on the patient's cellular characteristics, tissue damage, and regenerative capacity. These methods can be especially useful in tissues that have low inherent regenerative capacity. In the future, individualized bioelectric therapies will need to have a biomarker that can reliably monitor the electrical state, very local delivery systems and monitoring to prevent the wrong tissue organization or proliferation.

The most ambitious long-term objective is harnessing the principles of bioelectric to regenerate humans' limbs. While the regeneration of whole appendages is not possible in clinical settings, these findings from highly-regenerate organisms are valuable lessons to guide mammalian tissue engineering in the future. Controlled membrane-potential manipulation, stem-cell technology, biomaterials, Electrical stimulation, gene regulation and tissue-specific regenerative signals could be combined in future research to build up increasingly complex structures. Artificial bioelectric systems may be able to provide positional information that can be used in addition to biochemical and mechanical information during such reconstruction. This field could continue to pick up speed through the use of bioelectricity with the aid of AI and synthetic biology. The AI systems could use multiple large-scale datasets, including voltage imaging, transcriptomic, epigenomic and anatomical data, to look for patterns of success in regenerating the tissue, and to determine the types of interventions that will be effective. Synthetic biology could then enable the creation of a bio-sensor cell that can be programmed to detect the local electrical environment and activate either by opening or closing ion-channels or through other regeneration mechanisms. These technologies enable a combination to render the bioelectric blueprint as an experimentally programmable system.

However, several important aspects of future development will require an emphasis on spatial accuracy, temporal regulation, repeatability and biological safety. Bioelectricity will not suffice for a successful regenerative strategy, and there will need to be a coordinated manipulation of electrical, molecular, mechanical and cellular systems.

Discussion

The present systematic review reveals that bioelectric signaling is an important regulatory facet of appendage regeneration and tissue patterning. In both these models and others, changes in membrane potential, ionic gradients, ion channels, gap junctions, and changes in intracellular calcium are linked to diverse stages of the regenerative process: ranging from initial wound healing responses through to blastema formation, proliferation, differentiation, positional specification, and termination of regenerative growth. One of the key discoveries is that bioelectric regulation shouldn't be regarded as an alternative to genetic control or biochemical control. Rather they seem to act as an extra dimension of physiological data that plays with existing developmental processes. The evidence also suggests that the bioelectricity of regeneration is dynamic, shifting in its electrical states depending on the type of tissue, developmental stage, cell identity and regenerative phase of the process. This character has been posed as a possible model for the control of co-ordinated cellular activities in local tissues within the construction of large scale anatomical structures. Based on the extensive literature examined, it is proposed that bioelectric states also play a role in the organization, scaling, and restoration of complex appendages, and can be part of a system of information that controls the regenerative morphogenesis.

Active bioelectric control of regeneration is supported by observations and experimental perturbations. Many changes in ionic gradients and endogenous electrical currents are possible during the early stages of tissue repair, and subsequent changes in membrane potential affect cellular behavior in regenerating tissue areas. Experimental manipulation of ion channel, gap-junction communication or membrane voltage can affect the outcome of a regeneration, alleviating the necessity to believe that bioelectric states are a mere reflection of cellular activity, but have functional consequences. Ion channels with voltage gated properties play a special role, since these proteins are directly involved in the conversion of electrical changes into ionic and biochemical

signals. Membrane depolarization can be converted into intracellular Ca^{2+} activity through the action of calcium channels, and polarization and excitability through the action of potassium and sodium channels. By these mechanisms, there can be an influence on proliferation, migration, differentiation, and tissue scaling. Additionally, channel localization may impart spatial specificity which can permit different cellular populations to differ in their response to a regenerating appendage. Improper electrical control can thus affect pattern formation and generate inappropriate tissue organization, showing the need to control the activity of the ion channels precisely.

One of the main conclusions of the review is that bioelectric signaling works in parallel with and in the presence of the traditional molecular mechanisms. Developmental pathways can also modulate the expression and function of ion channels, and other pathways that are calcium-dependent can modulate signaling, transcriptional activity and the function of calcium channels. In this way, there is a bi-directional interaction, or regulatory network, instead of a linear signaling hierarchy. Bioelectric mechanism can also be involved at various stages of regeneration, such as Wnt, FGF, BMP, Notch, Hedgehog, TGF- β and mTOR pathways to coordinate positional information with cellular growth and differentiation in tissues. The tie of stem-cell biology is especially significant, as regenerative progenitor populations need to be in a balance between self-renewal and lineage commitment. Membrane potential, calcium dynamics, cellular plasticity, migration and differentiation might be influenced by bioelectric conditions in the stem-cell niche. These encounters may help to account for the maintenance of a proliferative capacity in regenerative tissues without uncontrolled growth. The bioelectric regulation may therefore be an important part of the microenvironment that promotes regeneration, and may be adapted to artificial stem-cell engineering.

There is also an overlap between regenerative biology and cancer biology that should be well considered. There are many similarities in how cells regenerate and how they become tumor cells, such as proliferation, migration, cellular plasticity, tissue remodelling and changes in interaction with the tumour microenvironment. These processes may be affected by changes in membrane potential and ion channels in physiological and pathological conditions. The cancer stem cells, especially, exhibit self-renewal and plasticity properties similar to some of the features of the regenerative progenitor populations. Therefore, the altered bioelectric

states may play a role in the behavior of tumor cells, EMT, invasion, metastasis and drug resistance. But common mechanisms does not mean that it is the same in regeneration and cancer. Tumorigenesis is usually the disruption of the normal constraints that limit regeneration, which include positional information, tissue architecture, differentiation programs, and growth termination. Learning about this distinction could give clues to regenerative medicine and cancer prevention.

The implications of these findings for translation are considerable and must be taken with a pinch of salt. Manipulation of membrane potential and ion-channel activity may be used alongside stem-cell therapies, tissue engineering, electrical stimulation, organoid development and wound healing strategies. It would be especially desirable to use an engineered bioelectric blueprint since it implies that it is possible to expose electro-information to regenerative environments to direct the behaviour and self-organization of cells. However, moving from highly regeneration organisms to humans is still difficult, because regeneration capacity, the type of cells, immune reactions and developmental programs vary significantly between species. The therapeutic manipulation should also fail not to result in excessive proliferation, inappropriate cellular plasticity, abnormal pattern formation and tumorigenic effects. Generalized changes in tissue electrical state will not be necessary for future approaches, however, because they must be highly localized, reversible, cell-specific, and/or temporally controlled.

The results complement each other to outline a conceptual model in which bioelectric blueprint is a physiological information layer that relates to injury, cell behaviour, molecular signaling, and anatomical reconstruction. In this model, the tissue injury causes changes in ionic gradients and changes in the membrane potential that alter the activity of the intercellular ionic channels and the electrical communication between cells. These changes produce intracellular signals, especially calcium-dependent signals, which interact with the transcriptional and epigenetic pathways and developmental pathways. These cellular responses result in the regulation of blastema formation, proliferation, migration, differentiation, polarity, positional identity, and tissue scaling. With increasing regeneration, the electrical, molecular, mechanical and cellular structures gradually limit growth and accelerate structural maturation through feedback control. Thus, the bioelectric blueprint cannot simply be interpreted as a fixed electrical map, but rather a

dynamic pattern of physiological states that enables the cells to recognize their regeneration needs, developmental stage and location. This integrated view is useful in understanding how coordinated interactions between cells can lead to complex anatomy and makes bioelectric signaling an important future area to explore in developmental biology, stem cell biology, tissue engineering and therapeutic development.

Conclusion

In addition to genetic, molecular, cellular and mechanical processes, bioelectric signaling is an additional regulatory mechanism involved in appendage regeneration. Across regenerative organisms, there is evidence for roles in injury responses, blastema formation, proliferation and migration of cells, differentiation, positional information, scaling of regeneration, and growth termination that are mediated by changes in membrane potential, ionic gradients, gap-junction communication and voltage-gated ion-channel activity. The voltage-gated calcium, potassium and sodium channels are especially relevant in converting electrical state changes to intracellular signals and cellular responses. In addition to interacting with developmental pathways like Wnt, FGF, BMP, Notch, Hedgehog and TGF- β pathways, this system of bioelectric mechanisms shapes tissue patterning together. The idea of a dynamic bioelectric blue print is helpful in understanding the role of electrical information in the field of anatomy, identity, polarity, size and morphology. There are some constraints in direct voltage measurement, temporal dynamics, species differences, and translating to humans, however, for cell-specific channel functions. With continuous advancement in voltage imaging, optogenetics, omics, computational modeling and artificial intelligence, and stem-cell engineering, precise bioelectric manipulation will be facilitated in the future. These developments could eventually aid the creation of new approaches in the fields of regenerative medicine, tissue engineering and human appendage regeneration.

Future Research Recommendations

Future studies should focus on mapping the membrane-potential gradients, currents of ions and calcium levels across the various phases of appendage regeneration at high resolution, in order to elucidate their spatial and temporal roles. It is important to focus more attention on the cell-type specific functions associated with the calcium, potassium, sodium and chloride channels in blastema, stem/progenitor, epidermal, fibroblast and neural populations. To determine causal relationships between states

of bioelectric activity and regenerative outcomes, it is important to employ experimental methods that integrate electrophysiology, optogenetics, genetically encoded voltage indicators, calcium imaging, and genetically engineered manipulation. Future studies should explore the interactions of the various bioelectric signals, Wnt, FGF, BMP, Notch, Hedgehog, TGF- β , mTOR, transcriptional and epigenetic pathways. Conserved mechanisms should be identified by comparing organisms that regenerate across the different groups of organisms with relevance to mammals and humans. Single-cell and spatial transcriptomics, multi-omics, computational modeling and AI could help realize predictive bioelectric maps for future innovation. Finally, advanced research on exact, localized, reversible and temporally-controlled bioelectric interventions, together with the assessment of the tumorigenicity and abnormal growth of tissues should be suggested to tackle future use of the technology in tissue engineering, stem-cell therapy, regenerative medicine and regeneration of human appendages.

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