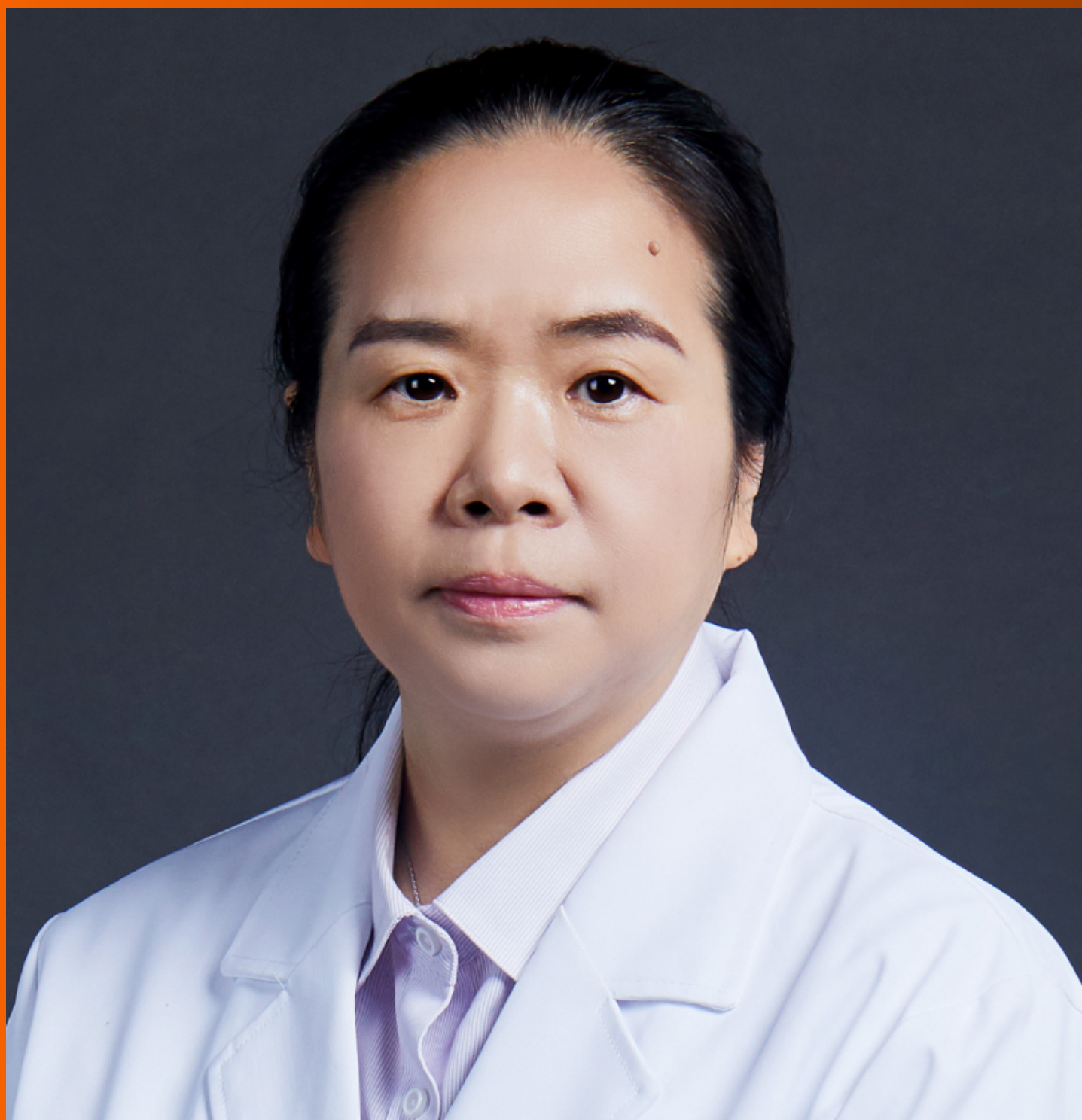


# World Journal of *Stem Cells*

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## Unveiling the morphogenetic code: A new path at the intersection of physical energies and chemical signaling

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### Abstract

In this editorial, we discuss the remarkable role of physical energies in the control of cell signaling networks and in the specification of the architectural plan of both somatic and stem cells. In particular, we focus on the biological relevance of bioelectricity in the pattern control that orchestrates both developmental and regenerative pathways. To this end, the narrative starts from the dawn of the first studies on animal electricity, reconsidering the pioneer work of Harold Saxton Burr in the light of the current achievements. We finally discuss the most recent evidence showing that bioelectric signaling is an essential component of the informational processes that control pattern specification during embryogenesis, regeneration, or even malignant transformation. We conclude that there is now mounting evidence for the existence of a Morphogenetic Code, and that deciphering this code may lead to unprecedented opportunities for the development of novel paradigms of cure in regenerative and precision medicine.

**Key Words:** Physical energies; Stem cells; Bioelectricity; Electromagnetic radiation; Mechanical forces; Morphogenetic code

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**Core Tip:** The capability of biological systems to create dynamically evolving shapes, up to large-scale anatomy, raises a number of fundamental questions that are only partially addressed in terms of molecular signaling. Physical energies, including mechanical and electromagnetic waves, afford substantial control of somatic and stem cell fate under normal and pathological conditions. This editorial focuses on the remarkable role of bioelectricity in shape generation, and maintenance, up to growth

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regulatory patterning that lead to the specification of tissues/organs and of the whole individual. Implications of bioelectrical signaling in tissue regeneration and in the control of malignant transformation are also discussed.

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## INTRODUCTION

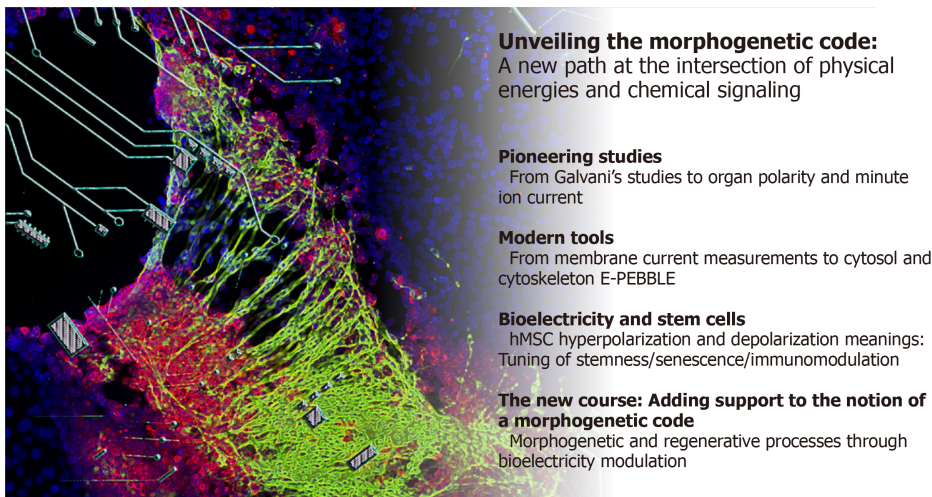
There is increasing, compelling evidence that cellular dynamics and fate are fashioned by the capability to generate physical signaling, other than biochemical reactions[1]. Both somatic and stem cells produce mechanical and electric waves[2-5] to elaborate intra- and inter-cellular communications and orchestrate complex developmental pathways[6-9]. Compounding the complexity of this emerging picture, cells are also exploiting the ability of a selected number of molecules or molecular complexes to behave as chromophores, that is generating electromagnetic radiation in the form of light to orchestrate targeted signaling processes[1,10-13]. To this end, the list of molecules than can be actually deemed as chromophores includes a number of components in major ion channels involved in cytosolic calcium handling[14,15], essential players in redox signaling[16], such as multiple NAD(P)H oxidoreductases [17], as well as molecules involved in biochemical pathways leading to the formation of gasotransmitters, like nitric oxide (NO)[18,19].

Concerning the cellular interior, there is now evidence that microtubuli act as a major source for the generation of both mechanical waves and electromagnetic signaling[20-22]. The mechanical buckling of microtubuli, coupled with their inherent electric polarity, is a major determinant in the spreading of mechano-electrical signaling across the cellular boundaries[22]. Microtubuli are themselves displaying chromophore characteristics[23], a trait that may further contribute long-range intercellular connectedness through electromagnetic radiation (light). Overall, microtubuli can be viewed as a sort of bioelectronic circuit, whose oscillatory patterns exhibit the features of both synchronization and swarming[24,25]. These mechanisms may play a remarkable role in a form of biomolecular recognition that transcends the lock-and-the-key scheme of interaction, being rather based upon the participation of molecules in the construction of signaling processes through a mechanism of resonance. Novel hypotheses are now being formulated, considering the resonant vibrational profiles associated with the helix-loop-helix structure shared by signaling peptides and transcription factors[26-29], and the possibility that microtubuli act as a viscoelastic matrix assembling these molecules into synchronous resonating clusters [25,30].

Taking into account these considerations, physical energies could be deployed to control cell behavior including the biology of stem cells. In this regard, we have provided evidence that the fate of stem cells, and their rescuing potential can be remarkably affected by electromagnetic fields[31-34], even reversing senescence in vitro[35,36], and mechanical forces[37]. Comprehensive review analyses focusing on the rescue of damaged tissues by the aid of physical energies, also including the use of shock waves and photobiomodulation, can be found elsewhere[1,38,39].

On the whole, a common feature arising from the inherent mechanical vibration of electrically polarized elements (*i.e.*, the cytoskeleton and virtually all the electrically charged subcellular components), and from the concerted activity of cellular ion channels, is the generation of endogenous bioelectric fields with radiation characteristics[1,40].

In this editorial, we focus on the role of bioelectricity as a constitutive element directing the development and assembly of shapes, from the subcellular/cellular level, up to large-scale anatomical patterning (Figure 1).



**Figure 1 Graphical abstract: Symbolic representation of cellular bioelectronic circuitries in morphogenetic patterning.** hMSC: human mesenchymal stem cell.

## THE PIONEERING STUDIES: EVIDENCE FOR AN ELECTRO-DYNAMIC FIELD IN LIVING ORGANISMS

Bioelectricity is a term coined to identify the ability of electric fields endogenously generated in living cells to afford modulation of biological patterning from the cellular up to the tissue and organ level[40]. In all the cells and tissues, a part of electrically driven signaling originates from ion channels and related ion fluxes[40,41]. The differential distribution of resting potential across tissues represents an ancestral and conserved modality, highly integrated and connatural with chemical structures, in the establishment of cell signaling networks[42]. Bioelectricity plays a major role in the scaling up dynamics responsible for embryogenesis, and tissue regeneration, while altered non-coherent bioelectrical patterning appears to be involved in the onset of degenerative or malignant states[43].

The first studies on bioelectricity sink their roots in the beginnings of the 18<sup>th</sup> century. Seminal discoveries in this field emerged with the studies by Luigi Galvani, who showed the feasibility to achieve muscle twitching by touching the muscle with a deviating cut sciatic nerve in the absence of metal electricity, definitely providing evidence for animal electricity[44-46]. Through his experiments Galvani also unknowingly discovered the injury current and the injury potential[47].

Later in 1840's Emil du Bois-Reymond[48] provided crucial advancement in the bioelectricity, when he was able to show the existence of macroscopic levels of electrical activities in frog, fish and human bodies, while recording defined electric currents in living tissues and organisms by the aid of galvanometers made of insulated copper wires. He discovered the action potentials[48,49], and at the same time, he was able to demonstrate the existence of less fluctuating electricity at wound level, conclusively showing the injury current and potential[50].

Bioelectricity studies received a fundamental boost in the early 20<sup>th</sup> century, when the pioneering work of Harold Saxton Burr provided clear-cut evidence for the crucial role bioelectric fields play in the control of biological shapes, and embryonic development.

In his studies, Burr and Mauro[51] used a voltmeter, accurately dissecting voltage gradients in developing embryos[52], as well as at the level of malignant tissues[53]. He also extended his studies to plants, providing remarkable advancement in the understanding of the role exerted by bioelectricity in plant physiology and diversity [54,55].

Dr. Burr has shown that all living forms, of any species, generate and are embedded within electrodynamic fields, which can be quantitatively assessed and mapped under physiological, as well as diseased states[56-60]. He conceived that such Fields of Life or L-Fields constitute the fundamental blueprints of all kind of Life forms. Dr. Burr was also firmly convinced that the systematic assessment of L-Fields would have yielded unprecedented insights into the biophysical dynamics, even those associated with mental conditions, before symptoms of illness develop, paving the way for new strategies of Predictive and Precision Medicine.

In 1940's Dr. Langman, from the New York University and Bellevue Hospital Gynecological Service, performed a voltmeter-based assessment of the L-Field to screen more than 1000 women suffering from a variety of symptoms in the generative-urinary tract. The subjects who exhibited significant voltage gradient difference between the cervix and ventral abdominal wall were subjected to deeper analyses and laparotomy, as it was reported in 1949 in a paper published by Langman and Burr [53]. They found a hundred and two cases where there was a significant shift in the voltage gradient, suggesting malignancy. Surgical and biopsy confirmation was found in ninety-five of the one hundred and two cases [53,60].

These findings led Dr. Burr to perform additional studies, and found that changes in the L-Field not only allowed monitoring of tissue/nerve injury, but that precise L-Field signatures were associated with, or they may have even determined, the wound healing process [59].

Dr. Burr's studies discovered relevant implications and applications in the assessment of female menstrual cycle, also showing that the movement of ovulation can thoroughly be monitored electrically [58]. Somehow these studies, by providing remarkable information about the chronobiology of ovulation anticipated essential issues within, and also provided essential knowledge to a number of fields that would have been experiencing terrific development over the years, including modern gynecology, family planning, birth control and *in vitro* fertilization.

Revisiting Burr's work shows that he was a fantastic visionary pioneer of studies that only today are creating progressive evidence for the existence of a morphogenetic field and for the need to believe in this potential as an unprecedented chance to access a real comprehensive view on how biological systems acquire coherence [61]. Burr focused on their capability to create dynamically evolving shapes that, while sharing enormous similarities with the simplest microorganisms and our eukaryotic cells, nevertheless entail the evolutionary unfolding not only to complexity, like in multicellular organisms, but to the deeper meaning of biological forms and shapes. This includes the inherent susceptibility of biological forms and shapes to create further contexts and being then guided by those contexts to orchestrate the coherent morphologies and functions of the entire individual [61].

A fundamental merit of Burr was not only his pioneering work, but his ability to bring science at a subtle line where science itself should find the courage to accompany the scientist to the unrestrainable need of merging with other disciplines, like Arts, Philosophy and Religion, in the effort of accepting other view points to explore the mystery of Life and Universe.

Following Burr, applied electric fields were shown to interfere with the regeneration pattern in the planaria by Marsh and Beams [62], leading to head or tail formation at cut locations, and resulting in body polarity reversion.

Other remarkable contributions to the field of bioelectricity were brought about by Lionel Jaffe and Richard Nuccitelli, who afforded quantitative and non-invasive measurement of extracellular minute ion currents by their vibrating probe [63], further elucidating the role of bioelectric signaling in the control of developmental pattern [64-68]. Then, Borgens *et al* [69,70], Cone and Tongier [71], Cone and Cone [72], Stillwell *et al* [73], and McCaig *et al* [74] confirmed and extended Burr's findings in wound healing, limb regeneration, embryogenesis, and organ polarity.

These pioneering studies on bioelectricity led to seminal discoveries and set the basis for unprecedented ways of approaching fundamental issues in the unfolding of living organisms. Nevertheless, the narrative and implications set forward by bioelectricity studies have until very recently been left in the shadow, despite the inability of chemical and molecular approaches to answer fundamental questions of biology itself. As a result, the field of bioelectricity has been severely penalized in terms of biology education, popularity among scientists and funding opportunity.

Only recently, the need for more transdisciplinary and non-traditional approaches has emerged as an unavoidable path in Science, also favored by the novel developments and biological applications of Computer Science and AI. Additionally, these new synergies could benefit from the development of novel probes, allowing detection and 3D imaging of electric microcurrents in the form of fluorescent signals even at the single cell level.

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## MODERN TOOLS FOR STUDYING THE BIOELECTRIC SIGNALING IN LIVING CELLS AND TISSUES

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Until quite recently, the assessment of intracellular electric fields was relying upon the



use of patch clamp, voltage clamp, or voltage dyes, allowing the measurement of electric pattern at the cellular membrane level. This site of investigation, although of remarkable relevance, only represents about 0.1% of the overall cellular volume. Moreover, with most of these membrane-associated tools cumbersome calibration approaches are needed. The recent development of nano-sized photonic voltmeter, initially referred to as E-PEBBLE (photonic explorer for biomedical use with biologically localized embedding), is now making affordable a 3D, and timely investigation of the electric patterning within the whole cellular volume[75-78]. These nanodevices can be calibrated extracellularly and can be subsequently targeted to the inside of any living cell or subcellular compartment, without further calibration. E-PEBBLEs embed Di-4-ANEPPS, a ratiometric, fast responding voltage-sensitive dye, whose fluorescence spectrum shifts in response to electric field changes[75,76]. The dye is encapsulated within a silane-capped mixed micelle, and it is therefore easily taken up by living cells. The possibility to address such shift ratiometrically, remarkably minimizes signal-to-noise ratio problems. These nanodevices exhibited an enhanced targeting efficiency when they are linked to multiple surface-conjugated targeting moieties. Interestingly, the use of these nanodevices provided evidence for the existence of intracellular electric fields that were not merely confined to the cellular membranes, but they were also ensuing within, and spreading throughout the cytosol [75-78]. These findings are consistent with the observations discussed above in the present article, showing that microtubules and microfilaments are electrically charged/polarized, and highly vibrating entities, behaving as electric power transmission lines for intra- and inter-cellular communication.

The chance for monitoring also non-membrane electric patterning inside living cells, has been challenging consolidated dogmas, holding promise for further dissecting cellular dynamics through the eyes of Physics. Worthy to note, the chance for combining E-PEBBLEs with confocal microscopy analysis is now opening the perspective to integrate subcellular chemical with physical signaling.

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## BIOELECTRICITY IN STEM CELL DYNAMICS: TUNING STEMNESS, SENESCENCE, PARACRINE AND DIFFERENTIATING PATHWAYS

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The use of specific ratiometric fluorescent dyes, including DiBAC<sub>4</sub> and CC2-DMPE, has allowed a thorough analysis of ion channel-orchestrated bioelectric patterning in stem cell dynamics. In particular, membrane potential has been found to be essential in conducting the commitment of human mesenchymal stem cells (hMSCs) towards the osteogenic and adipogenic fates[79,80]. Data yielded with the voltage-sensitive dye DiBAC<sub>4</sub>, revealed a characteristic trait of hyperpolarization in differentiating hMSCs, as compared with undifferentiated cells[79]. A causal role of hyperpolarization could be inferred by the observation that the differentiating process could be abrogated by disrupting the hyperpolarization progression by depolarizing hMSCs with two different strategies, including cell culturing in the presence of high [K<sup>+</sup>], or ouabain, a specific inhibitor of Na<sup>+</sup>/K<sup>+</sup> ATPase pump in the plasma membrane. Conversely, an upregulation in the expression of osteogenic markers was obtained when hMSCs were exposed to the KATP channel openers pinacidil and diazoxide, two compounds known for inducing hyperpolarization in different cell types. These findings strongly indicate that bioelectric fields play a major role in stem cell differentiation[79]. Bioelectric signaling not only is essential as a functional regulator of stem cell differentiation, but it also plays a relevant role in the maintenance of the differentiated state [81]. Depolarization of hMSC-derived osteoblasts and adipocytes resulted in the downregulation of bone and fat tissue markers, and therefore in phenotypic loss, even in the presence of specific differentiating factors for each commitment[81]. This observation suggests that bioelectric signaling might have overridden the molecular signaling in the maintenance of a differentiated state. The observed phenotypic suppression was not associated with an upregulation of stemness genes. Rather, the depolarized osteoblasts could be committed to competent adipocytes[81]. Thus, depolarization could be exploited to improve the transdifferentiation potential in hMSC-derived cells, without restoring stem-like signatures.

From a more general perspective, these results indicate that tuning of the bioelectric properties of stem cells may emerge as a novel approach to finely direct their therapeutic potential. Accordingly, the development of protocols to obtain electrically enriched hMSCs has provided the chance to isolate stem cells in which distinct electric states and ionic properties were associated with defined stemness and regenerative potential[82]. In these studies, hMSCs were sorted on the basis of their fluorescence

intensity for the trans-membrane potential indicator tetramethylrhodamine ethyl ester (TMRE). Subpopulations of electrically enriched hMSCs were found to differentially express genes involved in stemness, senescence, immunomodulation, and autophagy [82]. In particular, hMSCs with low levels of TMRE, accounting for a depolarized membrane potential, exhibited a reduced expression of senescence-associated markers, while overexpressing genes encoding autophagy and immunomodulatory players [82]. These findings indicate that hMSC sorting based upon cellular bioelectric properties would both allow stem cell enrichment for distinct features, and provide unprecedented strategies for selected cell therapy outcomes.

The relevance of bioelectricity in stem cell biology is further highlighted by the functional role of transmembrane potential ( $V_{mem}$ ) in the regulation of stem cell proliferation and migration. In neurosphere-derived neural precursor cells (NPCs), IKIR and IKDR channels are involved in establishing a hyperpolarized resting  $V_{mem}$  of about -80 mV[83]. Depolarization through modulated IKIR enhanced NPC mitosis and neurosphere size[83]. Similarly, in both human and mouse embryonic stem (ES) cells, IKDR are expressed and exert a permissive role in proliferation, that can be antagonized by  $K^+$  channel blockers[84].

The use of dyes of the DiBAC family also allowed to establish that bioelectricity is deeply involved in cell migration and communication. In fact, a combination of optical membrane-potential measurements with mechanical stimulation showed that the physical bridging provided by tunneling nanotubes (TNTs), embedding microtubules and to a lesser extent actin filaments, mainly acted as a form of electric connectedness [85,86]. Thus, neuronal migration and differentiation, as well as long-distance neuron-astrocyte communication, are supported by a form of bioelectronic circuitry through TNT-mediated depolarization. Consonant with these studies, modulation of potassium channels has been shown to control stem cell migration and invasiveness[87].

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## THE NEW COURSE: ADDING SUPPORT TO THE NOTION OF A MORPHO-GENETIC CODE

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While these findings point at the role of bioelectricity at cellular level, it is now becoming evident that the seminal intuitions and discoveries of Harold Saxton Burr and the studies of Scientists who continued to work in the groove traced by him, have laid the foundation to understand how bioelectric fields are a part of a morphogenetic code.

Cellular electric fields, electromagnetic and light radiation, as well as nanomechanical oscillations, are now emerging as vibrational signatures, imparting informational messages that contribute to the onset, unfolding and continuous remodeling of forms and of their inherent functions.

It is now clear that understanding the genetic, or protein level of cell signaling would only minimally help taking a glimpse on the most complex informational hierarchies underlying biological morphologies. Rather, considering biological systems as part of the vibrational nature of the Universe is now fostering more transdisciplinary efforts. The work of Scientists from multiple disciplines is gathering data showing that physical energies may act as a sort of software program driving the transition from nanoarchitectonics and supramolecular interactions to cellular shapes, and positioning, up to growth regulatory patterning that lead to the specification of tissues/organs and of the whole individual.

The observation that a wide-ranging spectrum of channelopathies, and consequent derangement in ion transport, are unequivocally linked to deep subversion of normal morphogenesis, leading to multi-organ crumbling and failure[88,89], should have been supported over time the notion that signaling through physical energies must had been regarded as a major determinant in the establishment of forms and functions. Nevertheless, discussing about forms, the “forma mentis” of Scientists has been slowly changing according to these evidence, and only recently a large body of compelling data is boosting molecular biologists to think in terms of biophysical signaling. The outstanding work performed by Michael Levin and his Coworkers is now providing clear-cut evidence for the intimate connection between molecular signaling, gene transcription and bioelectric signaling[90]. Levin’s work is yielding fundamental data in showing that a continuous tuning of cellular patterning with the whole organism requirements underlies the establishment of shapes throughout embryogenesis[91-95] and the preservation of forms during the development of a hostile environment (*i.e.*, injury or tumorigenesis)[96-100]. Understanding the informational mechanisms that govern the establishment of complex anatomy has required a paradigm shift from the

observation of local cell communication to the attempt of approaching biological intelligence as a computational process that entails a network of maps encoded by physical energies. By this approach, Levin's group have shown the feasibility of modeling somatic computation *via* non-neural bioelectric networks, and that the spreading of multifaceted ion fluxes over space and time merges with molecular signaling in non-excitable cells[101,102]. This strategy has been further refined by the use of voltage sensitive dyes in combination with defined extracellular ionic solutions, an approach that has allowed investigating the consequences of resting membrane potential manipulation on cellular dynamics[78-80]. The development of targeted 4D imaging and related data analysis has also been part of studies that highlighted membrane potential as a tunable tool in the modulation of calcium-primed signaling in *Xenopus* embryogenesis[101,103-105]. Further contribution to the understanding of these dynamics, and to the deployment of control strategies of bioelectric patterning, came from the development of the BioElectric Tissue Simulation Engine (BETSE) by Pietak and Levin[106]. BETSE proved effective as a multiphysics simulator, and for a predictive spatio-temporal profiling of bioelectric patterns from the modeling of ion channel and gap junction activity. These approaches have been at the basis of a number of interrelated findings showing a crucial role of defined bioelectric patterns in: (1) The establishment of morphogenetic patterning during embryo development [107-110]; (2) The deployment of optogenetics in developmental biology, through the use of light to handle ion flux-dependent voltage and signals in embryo development [101]; (3) The physiological control of the large-scale mechanisms operating in tissue regeneration[111,112]; (4) Regenerative processes from the level of wound healing, up to the rescue of brain defects induced following animal model exposure to teratogenic or mutagenic agents[113]; and (5) The establishment of micro-environmental signals suitable for revealing, inducing or even counteracting cancer onset and progression[96, 97,114].

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## CONCLUSION

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The journey for understanding how Physics may orchestrate molecular and cellular patterning up to contribute shapes and anatomical homeodynamics has started many years ago. Nevertheless, only recently we are facing the re-discovery of the potential for using physical energies to afford efficient modulation of cell signaling, tissue patterning and rescue.

Considering the diffusive properties of such physical stimuli, we may also envision a novel strategy of regenerative medicine relying upon the reprogramming of stem cells *in situ*, where they are resident in all tissues of the human body. This approach may hold promise for affording tissue regeneration without cell or tissue transplantation, but rather boosting our own self-healing potential. So far, the effects elicited at the stem cell level by endogenous electric patterning, or by mechanical vibration and electromagnetic radiation (including light), have been investigated mainly in mouse ES cells, and hMSCs. Nevertheless, besides hematopoietic stem cells, other tissue-resident multipotent elements, such as pericytes[115,116], and cells exhibiting pluripotency features in the adulthood, as the "multilineage-differentiating, stress-enduring" cells[117-119], may conceivably be targeted by mechanical and/or electromagnetic stimulation. Addressing this issue may disclose novel perspectives in regenerative and precision medicine, and should be the subject for future investigations.

We hope that our efforts will lead to a progressive awareness among the scientific community of the chance of using bioelectricity, electromagnetic radiation and nanomechanics to afford efficient re-setting in the epigenetics up to modulate tissue morphogenesis and regeneration, even offering chances to control oncogenesis and metastatic dissemination.

Progression within this context may be supported by the development of a transdisciplinary endeavor led by a novel generation of committed Scientists, and by the availability of targeted funding platforms.

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