

Physical energies to the rescue of damaged tissues

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Abstract

Rhythmic oscillatory patterns sustain cellular dynamics, driving the concerted action of regulatory molecules, microtubules, and molecular motors. We describe cellular microtubules as oscillators capable of synchronization and swarming, generating mechanical and electric patterns that impact biomolecular recognition. We consider the biological relevance of seeing the inside of cells populated by a network of molecules that behave as bioelectronic circuits and chromophores. We discuss the novel perspectives disclosed by mechanobiology, bioelectromagnetism, and photobiomodulation, both in term of fundamental basic science and in light of the biomedical implication of using physical energies to govern (stem) cell fate. We focus on the feasibility of exploiting atomic force microscopy and hyperspectral imaging to detect signatures of nanomotions and electromagnetic radiation (light), respectively, generated by the stem cells across the specification of their multilineage repertoire. The chance is reported of using these signatures and the diffusive features of physical waves to direct specifically the differentiation program of stem cells *in situ*, where they already are resident in all the tissues of the human body. We discuss how this strategy may pave the way to a regenerative and precision medicine without the needs for (stem) cell or tissue transplantation. We describe a novel paradigm based upon boosting our inherent ability for self-healing.

Key words: Stem cells; Physical energies; Mechanical forces; Electric fields; Electromagnetic fields; Electromagnetic radiation; Photobiomodulation; Damaged tissues

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Core tip: Rhythmic oscillatory patterns permeate the entire universe and sustain cellular dynamics. Our cells encompass a seemingly infinity of rhythms, unfolding at the nanomechanical and electric level in the microtubular network. Essential signaling molecules are shown to behave as chromophores, supporting the absorbance and emission of light. Photobiomodulation is a rapidly growing area of inquiry for both deciphering novel signaling mechanisms and affording unprecedented clinical applications. The deployment of the diffusive features of physical energies is leading to a regenerative/precision medicine, based upon the reprogramming *in situ* of tissue-resident stem cells, without the needs for cell or tissue transplantation.

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INTRODUCTION

We are immersed in and we are a part of the oscillatory nature of the universe. In today's physical age, on the threshold of the 4th Industrial revolution, most basic issues will be about electronics, machines, and the future of what we call artificial intelligence (AI). Science is increasingly looking at cell biology with the eyes of physics and electronics, providing compelling evidence that life is embedded within oscillatory patterns that create coherent rhythms, now recordable at cellular, subcellular, and even molecular levels. In addition to expressing rhythmically their molecular dynamics, cells are able to organize their decisions and fate by detecting and deploying the physical energies that permeate nature, including extremely weak mechanical vibrations (nanomotions), magnetic fields, and electromagnetic radiations (light).

As in the universe, in biological organisms, rhythmic oscillations and synchronization of oscillatory patterns are an essential requisite for recognition and connectedness. Sophisticated approaches, including atomic force microscopy (AFM)^[1-4], scanning tunneling microscopy (STM)^[5,6], terahertz field microscopy (TFM)^[7], and hyperspectral imaging (HSI)^[8-10] are now providing a dynamic picture of the cellular environment at a nanoscale level, showing that mobile elements of the cyto- and nucleo-skeleton are dancing with patterns that display features of coherence, short- and long-range signal propagation, networking, and memory. Tubulin dimers, and microtubules are now emerging as the constituents of a highly dynamic web, acting both as a source for the generation and the context for the interplay of physical energies^[5,6]. These energies include mechanical forces^[11-13] as well as the production of electric and very likely electromagnetic fields, with radiation characteristics^[5,6,14], and even the occurrence of electromagnetic radiation (light), as a result of biophysical dynamics of a number of molecules increasingly regarded as chromophores^[15-17]. To this end, the list of intracellular chromophores is now progressively increasing, including flavins, flavoproteins, and cytochromes^[18-22], which are thought to be involved in the generation of reactive oxygen species (ROS) and nitric oxide^[19,23-25], behaving as major pleiotropic conductors in cell biology.

Although it is not clear to what extent chromophores are expressed in mammalian cells compared to insects, there is now evidence for the presence of different members of the opsin (a group of cis-retinal dependent G-protein coupled receptors) family in mammalian cells, controlling crucial downstream signaling pathways involving members of the family of transient receptor potential cation channels (TRPs)^[26-28]. TRPs are a superfamily of multiple members, which have been shown to be selectively activated by defined wavelengths of light, playing a major role in cellular dynamics^[29-33], as photoentrainment and modulation of cellular circadian rhythms^[34].

These new achievements in science pose the more general issue of how and to what extent signaling molecules may be viewed as both generators and sensors of physical energies. They also highlight the particular relevance of the identification of frequency region selectivities for inducing defined morphological and functional paths, by precisely tuning the delivery at the cellular or tissue level of specific patterns/

signatures of frequencies, wave forms, and pause intervals for each energy alone (mechanical, electric-electromagnetic or light) or in combinatorial modes.

Within such a dynamic landscape, signaling molecules, like small peptides, based upon their intrinsic helix-turn-helix repeated modules, may be viewed as oscillatory entities^[7], walking onto microtubular and microfilament routes in close association with molecular motors^[35]. The cellular environment acquires notation of an intracellular niche whose characteristics are forcing Scientists to revisit their knowledge and interpretation of crucial issues that include the biomolecular recognition patterning, the inherent meaning and implication of cell polarity, the modalities through which cellular information is built and unfolded, and the determination of complex cellular decisions and fates. Accordingly, the use of innovative approaches, such as the Resonant Recognition Model (RRM), has led to the conclusion that DNA can also be viewed as an oscillatory entity resonating with electromagnetic frequencies spanning from THz to KHz^[36]. RRM relies upon the finding that the function of proteins may be controlled by periodic distribution in the energy of their delocalized electrons, affecting protein dynamics, or protein-DNA interplay, a fundamental step in DNA remodeling and epigenetic control operated by a wide variety of transcription factors^[37]. To this end, RRM also postulated that protein conductivity could be associated with defined spectral signatures, resulting from electromagnetic radiation/absorption patterns generated by the flow of electric charges through the protein backbone^[37,38]. Interestingly, spectral signatures postulated on the basis of RRM have been verified and supported by experimental evidence^[5,39]. Another advantage in the use of RRM is the chance of excoigitating novel peptides with unprecedented spectral features and bioactivities^[40].

The overall scenario is emerging of an intracellular environment where complex nanoarchitectonics are fashioned within a dynamic assembly of microtubules and microfilaments. These elements can now be regarded as a bioelectronic circuit embedding a multitude of signaling molecules that, besides interacting only with lock-and-key modalities, may also behave as actuators capable of generating phase coherent oscillatory patterns where the building blocks of information arise from the facilitation or dumping of the transfer of physical forces.

Here, we will discuss these issues with particular regard to stem cell biology and the use of physical energies to control stem cell decisions and afford somatic cell reprogramming. We will highlight the relevance of mechanobiology, and the possibility to use mechanical waves to elicit self-repairing mechanisms and tissue rescue in a number of pathological conditions. We will describe the effectiveness of radioelectric fields in enhancing the differentiating potential of stem cells, even reversing their senescence patterning. We will address the various facets of using electromagnetic radiation (light) of defined wavelengths to orchestrate selectively stem cell commitment and tissue repair. We will describe the innovative use of AFM and HSI to decipher the cellular emission of vibrational patterns, in terms of mechanical vibration (AFM) or electromagnetic radiation (HSI), corresponding to specific signatures of growth regulatory and differentiation processes. We will highlight the potential for exploiting the diffusive features of these energies and convey vibrational signatures in the form of nanomechanical motions and/or light patterns to the stem cells *in situ* to afford their reprogramming where they already are, resident in all tissues of the human body. We will finally discuss how this strategy will involve the development of novel interfaces between the human body and machines, as well as AI, paving the way to a precision regenerative medicine without the needs for (stem) cell or tissue transplantation, a novel paradigm based upon boosting our inherent ability for self-healing.

CELLULAR MICROTUBULES: A NETWORK OF OSCILLATORS THAT SYNC AND SWARM

There is increasing evidence that cells and subcellular domains are mechanosensitive. Mechanobiology is a growing area of interest that deals with the mechanical processes in biological systems. It ranges from cellular mechanics to molecular motors and single molecule binding forces. In addition to tuning the stiffness and shape of cell scaffolding and substrates, mechanical cues and mechanosensitivity are attracting much attention as they represent the context for sensing a wide variety of different stimuli, including osmotic changes, gravity, electromagnetic fields, (nano) motions falling both in an audible range (sound), or even fashioned at subsonic or ultrasonic levels.

The frequency-dependent transport of mechanical stimuli by single microtubules and small networks has been recently studied in a bottom-up approach, using

optically trapped beads as anchor points^[41]. When microtubules were interconnected to linear and triangular geometries to perform microrheology by defined oscillations of the beads relative to each other, a substantial stiffening of single filaments was detected above a characteristic transition frequency of 1-30 Hz, depending upon the molecular composition of the filament itself^[41]. Below such frequency range, filament elasticity was only controlled by its contour and length persistence. This elastic pattern showed networking features, with the longitudinal momentum being facilitated through linear microtubular constructs *in vitro*, while the lateral momentum was dumped so that the linear construct behaved as a transistor-like, angle dependent momentum filter^[41]. These *in vitro* experiments also showed that the overall geometry of the microtubular network was a remarkable cue, since closing the construct circuitry by imposing a triangular shape resulted in stabilization of the microtubular elements in term of the overall molecular architecture and direction of oscillation. These findings suggest that within intact cells microtubular dynamics may afford generation and fine tuning of mechanical signals with a stronger degree of force generation and/or filtering and more flexibly than expected^[41]. The complexity in the deformation pattern of microtubules is now prompting further studies to unravel their mechanics through sophisticated atomistic approaches^[42].

A major feature of microtubular networks is their ability to exhibit synchronization patterns and even manifest a collective behavior. Synchronization may be viewed as a form of self-organization that occurs in multiple natural and technological systems, from spontaneously excitable cells, like pacemaker cells and neural cells, to coupled lasers, metallic rods, or even robots. On a molecular scale, the observation that simple mixtures of microtubules, kinesin clusters, and a bundling agent assemble into structures that produce spontaneous oscillations, suggests that self-organized beating may be a generic feature of internally driven bundles^[43]. These synthetic cilia-like structures exhibit self-assembling at high density, leading to synchronization and metachronal traveling waves, reminiscent of the waves seen in biological ciliary fields^[43]. From governing motility in simple protists to establishing the handedness of complex vertebrates, highly conserved eukaryotic cilia and flagella are essential for the reproduction and survival of many biological organisms. Likewise, the emergence of synchronization patterns in eukaryotic microtubules may be essential in the generation and spreading of nanomechanical and electric signaling orchestrated by these nanowires. Despite the fact that synchronization of oscillatory patterns appears to result from intrinsic properties of microtubules under critical, timely/spatial bundling conditions, the intimate mechanism by which individual components coordinate their activity to produce synchronized oscillatory patterns remains unknown.

Another form of self-organization is swarming insects, flocking birds, or schooling fish, where individuals also move through space exhibiting a collective behavior without remarkably changing their internal state(s)^[44]. In their pioneer work, Sumino *et al.*^[45] have shown that an artificial system of microtubules propelled by dynein motor proteins self-organizes into a pattern of whirling rings. They found that colliding microtubules align with each other with high probability. As a function of increasing microtubular density, the alignment ensued in self-organization of microtubules into vortices of defined diameters, inside which microtubules were observed to move in both clockwise and anticlockwise fashion^[45]. Besides exhibiting these spatial traits, the phenomenon also evolved on timely bases, since over time the vortices coalesced into a lattice structure. The emergence of these structures appeared to be the result of smooth, reptation-like motion of single microtubules in combination with local interactions (collision dependent nematic alignment)^[45]. These discoveries have put forward the issue of previously unsuspected universality classes of collective motion phenomena that are mirrored even at the subcellular level, where microtubules have shown the capability, at least *in vitro*, to behave as swarming oscillatory elements, whose phase dynamics and spatial/temporal dynamics are coupled.

The possibility that microtubules may not only generate and propagate mechanical signals but that they may also be implicated in electric signaling acting as biological nanowires is suggested by the fact that tubulin has a large dipole moment. As a result, microtubules will exhibit a large cumulative dipole moment, imparting features of electrostatic polarity and functional directionality^[46]. Within the microtubules, tubulin dimers have highly electronegative C-termini, attracting electrically positive counterions, a mechanism that may account for the observed amplification of ionic signaling^[47-49]. These hypotheses have received significant support from the observation that microtubules were able to modify remarkably the electric conductance in solutions with varying concentrations of microtubules made by different concentrations of tubulin and tubulin dimers at a frequency range of alternating electric fields between 1 kHz and 10 MHz^[46]. A consistent increase in

solution conductance was observed at an alternating current frequency of 100 kHz, this effect being directly proportional to the concentration of microtubules in solution. Like mechano-transduction, the alternating current frequency dependent response of microtubules-containing electrolytes was also finely regulated, exhibiting a concentration independent peak in the conductance spectrum at 111 kHz, an observation that suggests the presence of intrinsic electric signaling properties of microtubules in aqueous environments^[46]. Intriguingly, these properties did not result from conductance patterns elicited by their building blocks, since tubulin dimers exhibited a completely different behavior by decreasing solution conductance at 100 kHz under similar conditions^[46].

The microtubular wall is interspersed by nanopores formed by the lateral arrangement of tubulin dimers. The application of patch clamp technique to *in vitro* generated two-dimensional microtubular sheets revealed that voltage-clamped sheets generated cation-selective oscillatory electrical currents whose magnitude depended on the holding potential, ionic strength, and composition^[50]. The oscillations progressed through various modes including single and double periodic regimes and more complex behaviors with prominent fundamental frequencies. In physiological potassium concentrations, oscillations represented remarkable changes in conductance that were also affected by the prevalent anion^[50]. Current injection elicited voltage oscillations, showing excitability similar to action potentials^[50], suggesting a functional role of wall nanopores in the handling of the electrodynamic capabilities of microtubules.

Microtubules have also been shown to form bundles, particularly in neurons. Intriguingly, bundles of brain microtubules have been recently reported to behave as bio-electrochemical transistors that form nonlinear electrical transmission lines^[51]. These bundles were shown to generate electrical oscillations and bursts of electrical activity similar to action potentials^[51], indicating that electrical oscillatory patterns represent an inherent microtubular feature. These findings may have remarkable biomedical implications in the unfolding of both neuronal and non-neuronal functions. These may include the fine tuning of cytoskeleton-regulated ion channels and may even play a role in higher brain functions, including memory and consciousness.

The biomedical implications of considering the microtubular network as a bioelectronic circuit are further inferred by other observations showing that exposure to alternating electric fields between 100–300 kHz of strength approximately 1.0–2.5 V/cm is able to arrest cell mitosis^[52]. This finding has led to a Food and Drug Administration approved treatment for glioblastoma multiforme^[53], with the electric field effects on microtubules being considered as the main underlying mechanism of action^[52,54,55]. This clinical outcome prompts strong motivation to pursue additional studies aimed at further elucidating the electric signaling features of mammalian microtubules. For this purpose, contributions due to the dipole moments, charges, van der Waals, and solvation energy have been taken into account to dissect and explain microtubular energy balance^[56], and optomechanical approaches have been proposed for monitoring microtubule vibration patterns^[57]. Moreover, alterations of collective terahertz oscillations have been found to be induced in tubulin by anesthetics, correlating with their clinical potency^[58]. This observation may have implications for anesthetic action and post-operative cognitive dysfunction.

There is now evidence that resonance modes not only occur in microtubules at the (nano) mechanical level but can even be detected at the level of their electric conductivity. Even more intriguingly, mechanical and electromagnetic resonance modes can coexist and affect each other within the microtubular network. STM, coupled with an *ad-hoc* designed cell replica developed to deliver electromagnetic fields of defined frequencies to microtubules growing on platinum nanoelectrodes, has shown that tubulins, tubulin dimers, and microtubules exhibited electric conductivity profiles resonating only with specific electromagnetic frequencies applied to the *in vitro* system^[9]. STM analysis also provided evidence that the resonant tunneling currents elicited by microtubules occurred in response to electromagnetic fields applied within a MHz range^[9].

These findings indicate that microtubules can generate specific electromechanical oscillations as a consequence of a resonant response to defined electromagnetic frequencies produced or delivered within their environment^[9]. These observations further support the idea that microtubules may act as an intracellular bioelectronic circuit. Consonant with such perspective are (A) theoretical calculations considering the microtubules as elements generating electric fields of high frequency and radiation features^[14]; and (B) experimental assays demonstrating that even a single brain microtubule behaves as a nanowire harboring “memory states” depending on its protein arrangement symmetry, coupled with conductivity state embedded in the microtubule itself, equitable to a memory switch device with a near-to-zero hysteresis

loss^[59] (Figure 1).

BIOMOLECULAR RECOGNITION PATTERNING

The microtubular network and its sync and swarming behavior may help develop a novel hypothesis on biomolecular recognition within the intracellular environment. The “key-and-lock” dynamics, while fitting the description for the interaction of few molecules in aqueous solutions, fails to adequately describe and predict the collective behavior of a high number of different signaling players that cohabit the intracellular environment and share overlaying space and time domains of interaction to afford integrated cellular decisions. In addition, the time needed for cellular proteins to create productive interaction through intracellular diffusion mechanisms would be highly unpredictable on large-scale colliding bases. The high speed and fine coordination of molecular interplay within complex cellular decisions, including stem cell differentiation, cannot be solely explained on the basis of molecular diffusion and collision within the intracellular environment. At this level, a diffusive mechanism would become hampered and highly unpredictable, due to the synthesis and accumulation of a wide variety of glycosaminoglycans, such as hyaluronan, imparting the features of an aqueous gel dynamically modifying its composition and diffusive properties in response to cell metabolism.

The growing discernment of a microtubular role in tuning intracellular and intercellular communication may offer a clue to formulate novel hypotheses on the mechanisms underlying the astounding speed at which cellular fate is devised. The vast majority of signaling proteins exhibit helix-turn-helix modules, where the helices can be reckoned as oscillating springs, and the turns can be viewed as inter-oscillator linkers. A single peptide becomes a vibrational element capable of phase-resonant oscillatory patterns^[7]. TFM has been exploited to detect protein vibrations, midge motions essential for Life^[7]. These observations suggest that, like violin strings or pipes of an organ, proteins can vibrate in different patterns within our cells^[7]. Cell proteins not only diffuse through water, but they can “walk” onto microtubular tracks availing of kinesins and dyneins motors as their molecular machines^[35]. Signaling peptides can be therefore regarded as a multitude of oscillatory devices using molecular machines to move along the microtubular net, with the microtubules acting themselves as multi-level connections affording efficient phase synchronization between multiple oscillators.

The resonant behavior described in microtubules^[5] holds promise for remarkable impact in further elucidation of biomolecular recognition patterning. The chance of using a selective frequency region to induce defined morphological patterns in microtubules has shown that mechanical patterns can be precisely orchestrated through the remote application of electromagnetic fields^[5]. Therefore, the finding that local density states in tubulin dimers, microtubules, and possibly other proteins can be modified by changing the frequency of their electromagnetic exposure entails that unfurling of protein structure into rhythmic resonance patterns may result as a relevant inherent mechanism sustaining both intracellular, and intercellular communication. Dissecting the resonance patterns intervening within clusters of signaling molecules, and between such molecules and the microtubular networks, and providing suitable methods to investigate the establishment of collective behavior among oscillators that undergo both sync and swarming will likely represent a novel paradigm for investigating the onset and spread of informational processes in biological systems.

Despite continuous progress in investigating this complex matter, the intimate origin of the observed resonant behavior remains largely elusive, particularly if the correspondence between electromagnetic and mechanical oscillation assessed *in vitro* is translated into an *in vivo* setting.

During electromagnetic exposure of protein and protein complexes, in case of electromagnetic resonance, photons would be expected to find domains in the protein structure amenable for both electric and magnetic absorption. Protein cavities would appear as domains arranged for the interaction of the electric and magnetic components of an incoming electromagnetic field. Although nanotopography within these cavities may be suitable for electric resonance, the issue as to whether these cavities may act as sensors generating resonances in the presence of an electromagnetic field remains an open and difficult to answer question.

A remarkable challenge is represented by the fact that, different from simplified *in vitro* systems, the intact cellular level electromagnetic and nanomechanical oscillations are supposed to be highly interconnected with none of their resonance-elicited responses separately emerging as electrical, magnetic, or mechanical. Within this

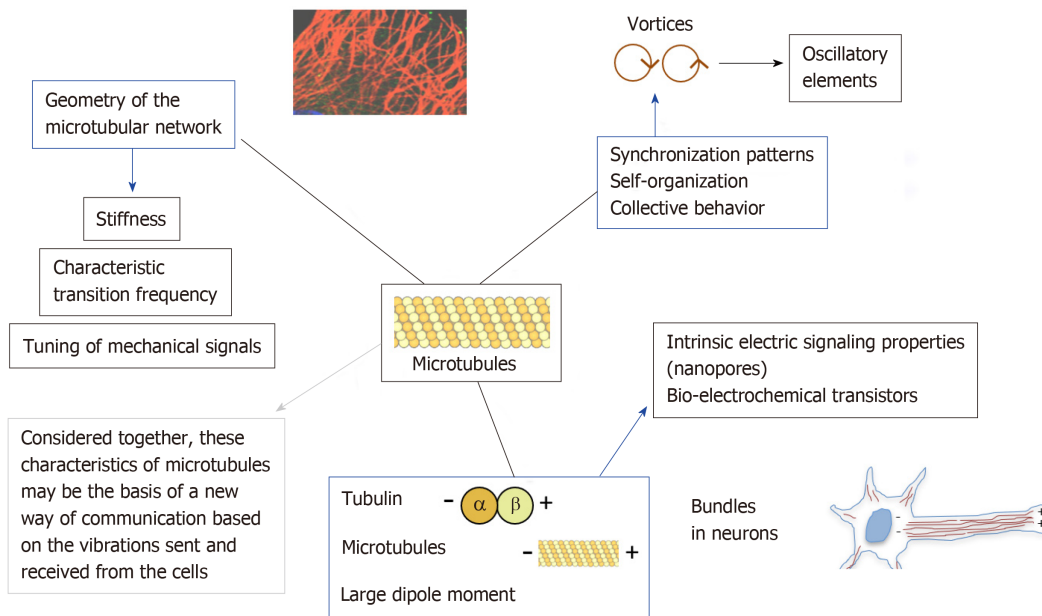


Figure 1 Cellular microtubules: A network of oscillators that sync and swarm. Microtubules are emerging as major players in crucial cellular activities, on the basis of a number of interrelated characteristics. These include: (A) The transfer of mechanical waves, changing their stiffness, and the transmission of longitudinal and lateral momentum on the basis of the frequency of their oscillation and the geometry afforded by their timely 3D assembly and disassembly within the cells; (B) The onset and propagation of electric fields and signaling, depending upon the large dipole moment of tubulin, developing both electrostatic polarity and functional directionality, and upon the lateral arrangement of tubulin dimers to create nanopores, interspersing the microtubular wall, and generating cation-selective oscillatory electrical currents; (C) The generation of bundles, as shown in brain microtubules, behaving as bio-electrochemical transistors forming nonlinear electrical transmission lines; (D) The ability to resonate mechanically in the presence of electromagnetic fields of defined frequencies, retaining memory states coupled with conductivity states, like a memory switch device; and (E) The property of synchronizing their oscillatory pattern and swarming into vortices, affecting the vibrational features of signaling peptides moving across the microtubular network by the aid of molecular motor machines, thus modulating biomolecular recognition patterning.

context, a significant step forward may be provided by the recent invention of an atomic resolution scanning dielectric microscopy capable of seeing a single protein complex operating live at resonance in a single neuron without touching or adulterating the cell^[6].

Overall, while our view of intracellular and intercellular connectedness is dramatically evolving over time, a novel paradigm is emerging, which considers the cellular and subcellular structures as senders and receivers of electromagnetic and nanomechanical fields. Unfolding this new paradigm may lead to the use of physical energies to orchestrate complex cellular decisions. Translating this perspective at the level of stem cells would result in unprecedented implication in precision regenerative medicine, as discussed below.

PHYSICAL ENERGIES TO THE RESCUE OF DAMAGED TISSUES: CAN TISSUE RESIDENT STEM CELLS BE A TARGET?

Targeting stem cells with mechanical vibrations

Mechanical signals minutely travel inside and across our cells, with our molecular players and the cyto-nucleo-skeleton behaving as sender and receiver of patterns manifesting with different frequencies, wave form and intensity, as well as pause intervals that are also essential in shaping the ensuing cellular responses. Complexity of biological systems is now emerging through a new landscape made of symmetry breaking, switching among various oscillatory patterns, synchronization of oscillators into clusters, and the swarming of oscillatory clusters that exhibit a collective behavior without necessarily altering their internal states. These dynamics are deeply inbred in a wide variety of cellular nanomotions, including those in the mitotic spindle, the bidirectional organelle transport, and the trafficking of molecular motors and signaling molecules. Within this environment, shaping of mechanical signaling has the advantage of bridging nanomotions with the generation and transfer of information processes across long distances in short time frameworks.

Can this growing body of knowledge be translated into a biomedical application? A positive answer came from the pioneer studies of Rubin *et al*^[60], showing that low-

level mechanical stimuli could be delivered to afford strengthening and normalization of anabolic activity in long bones^[60-62]. These authors showed that brief daily treatments with mechanical vibrations of high-frequency and very low magnitude could inhibit adipogenesis, while promoting osteogenesis *in vivo*^[63] as well as in isolated human stem cells *in vitro*^[64,65], up to the point of maintaining the osteogenic potential in bone marrow stem cells, and advancing trabecular bone formation during reambulation^[66]. Mechanical vibrations can also be deployed to modulate nuclear signaling and afford significant transcriptional responses. In this regard, an important role is played by the so-called “Linker between Nucleoskeleton and Cytoskeleton”, a mechanosensitive network of molecules implicated in chromatin remodeling and epigenetic modification upon nuclear transfer of extremely low-magnitude mechanical signals^[67-69]. Based upon consideration that an electromagnetic field may originate from the electrical polarity of microtubules that form the mitotic spindle, a model enabling calculation of such electromagnetic field coupled to acoustic vibrations of the mitotic spindle has been recently developed to describe mitotic spindle kinetics^[70], still a poorly understood phenomenon of critical relevance in both physiological and pathological (*i.e.*, cancer) states.

These findings clearly indicate that modulation of cell mechanosensitivity has relevant biomedical implication and may pave the route for developing novel approaches to stem cell biology and regenerative medicine.

Shock waves

Shock waves are mechanical pressure waves with defined wave profile used in clinical practice since more than 30 years^[71]. Low-energy shock wave therapy (SWT) has been successfully used *in vivo* to treat problematic wound-healing disturbances^[72-74], tendinopathies, and non-healing bone fractures^[75-77]. The ability of SWT to promote tissue regeneration has been initially ascribed to the activation of angiogenic pathways sustained in a paracrine fashion by local release of trophic mediators^[78-81]. These findings have fostered extensive investigations to extend further the potential clinical contexts susceptible for SWT application and to elucidate the underlying mechanistic bases. The application of extracorporeal or epicardial shock waves to a porcine model of myocardial infarction has led to a significant improvement of vascularization with increased number of capillaries and arterioles at the infarct border zone, ameliorating ischemia-induced dysfunction and left ventricular function^[82,83]. Subsequent studies in infarcted mice indicated that the ability of SWT to prevent left ventricular remodeling and failure through the induction of angiogenesis involved a complex circuitry, encompassing the mechanical stress-induced release of the antimicrobial peptide LL37, its ability to form complexes with nucleic acids, and the release of RNA/protein complexes converging to the activation of Toll-like 3 receptors^[84].

The possibility that the angiogenic action of SWT may occur through stem mobilization from the bone marrow has been suggested in studies providing evidence that the beneficial effect of SWT in a hindlimb ischemia model was associated with the mobilization of endogenous endothelial progenitor cells into the systemic circulation^[85,86]. Recent studies in an animal model of chronic myocardial ischemia, using wild-type mice receiving bone marrow transplantation from green fluorescent protein donor mice, demonstrated that besides local angiogenesis, cardiac SWT was also inducing the recruitment of bone marrow resident endothelial cells to the damaged myocardium^[87]. This response was associated with enhanced expression of the chemoattractant stromal cell-derived factor 1 in the ischemic myocardium and serum. *In vitro* analyses revealed that the ability of SWT to induce endothelial cell proliferation, their enhanced survival, and capillary sprouting was dependent on both vascular endothelial growth factor (VEGF) 2 and heparan sulfate proteoglycan^[87]. Besides affecting the release of stored VEGF reservoir bound to heparan sulfate proteoglycan, facilitating VEGF binding to its receptors, SWT has been shown to induce angiogenesis by acting at the transcriptional level, triggering the gene and protein expression of VEGF and endothelial nitric oxide synthase^[88]. The tight dependence of these responses upon a mechano-sensing/transduction mechanism could be inferred by the finding that (A) SWT enhanced the phosphorylation of caveolin-1; (B) it increased the expression of *HUTS-4*, which represents β -1 integrin activity; and (C) knockdown of either caveolin-1 and β -1 integrin suppressed SWT induced enhancement of human umbilical vein endothelial cell migration *in vitro*^[88].

These molecular findings can also be viewed as a reverse story - from the bed to the bench side - as they provide a mechanistic underpinning on a number of studies that were earlier conducted in patients with severe coronary artery disease, showing that SWT was able to ameliorate myocardial ischemia in patients with severe coronary artery disease^[89]. Accordingly, a double-blind and placebo-controlled study demonstrated that SWT improved chest pain and myocardial function without any

complication or side effects in patients with severe angina, leading to the conclusion that SWT was an effective, safe, and non-invasive option for these patients^[90]. Following these initial clinical studies, the molecular dissection of mechano-transduction and signaling patterning primed by SWT served as a driving force for further expanding the clinical application of SWT. In a human study, low-energy cardiac SWT was found to suppress left ventricular remodeling and enhance myocardial function in patients with acute myocardial infarction, suggesting that extracorporeal SWT may represent a safe, effective adjunctive therapy to primary percutaneous coronary intervention^[91].

A remarkable perspective for using SWT in regenerative medicine approaches is now emerging from an increasing variety of studies conducted *in vitro* in isolated stem cells as well as in animal models of tissue damage. Evidence includes the ability of SWT to promote the osteogenic differentiation in adipose- and bone marrow-derived mesenchymal stem cells^[92-94] and the proliferation and migration of rat adipose derived stem cells^[95]. Shock wave treatment induced ATP release, increased Erk1/2 and p38 MAPK activation, and enhanced proliferation *in vitro* in different stem cell types, including C3H10T1/2 murine mesenchymal progenitor cells and primary human adipose tissue-derived stem cells (hADSCs)^[81]. Purinergic signaling-induced Erk1/2 activation was found to be essential for the proliferative effect of shock waves, which was further confirmed by *in vivo* studies in a rat wound healing model, where shock wave treatment induced proliferation and increased wound healing in an Erk1/2-dependent fashion^[81]. Interestingly, converging genetic and molecular evidence has been provided that the miR-138-FAK-ERK1/2-RUNX2 machinery can be generally activated by extracorporeal SWT in tendon-derived and adipose derived stem cells, promoting efficient osteogenic differentiation irrespective of the stem cell origin^[93]. *In vivo*, SWT promoted the induction of endogenous neural stem cells and functional improvement after spinal cord injury in rats^[96]. Recruitment and induction of endogenous stem cells were also at the basis of the capability of SWT to ameliorate voiding function and improve the innervation and vascularization in a rat model of diabetic bladder dysfunction^[97] (Table 1).

Exogenous stem cell transplantation is often hampered by the invasiveness of delivery in certain districts, such as the central or peripheral nervous system, and by other limitations that need to be addressed, including the ideal stem cell source, the timing and modality of delivery, and uncertain fate, especially after transplantation in complex tissues.

In this regard, SWT is emerging as a feasible, efficacious, non-invasive, safe, and cost-effective tool to elicit mechanical signaling and multiple interconnected downstream patterning that converge in optimization of the intrinsic potential of tissue rescue, through the activation of rapid release of trophic mediators, as well as the recruitment and induction of endogenous tissue resident and/or mobilized stem cells.

Cellular nanomotions: Vibrational signatures to direct stem cell fate

It is now clear that cytoskeletal and organelle biology is tightly coupled and that complex connections are dynamically featured at the level of cellular membranes, from the endoplasmic reticulum and the Golgi up to the plasma membranes. The rhythmic patterning and the unfolding of synchronization and swarming modes within the microtubular network are fast remodeling dynamics of the intracellular environment and behave as a vibrational system capable of force generation leading to rhythmic membrane movement.

The nanomechanical oscillatory features of subcellular structures, including the cellular membranes, govern essential biological processes in living cells, including, for example, organelle translocation, nanovesicular assembly and motion, as well as the trafficking and release/cellular exchange of signaling molecules^[98]. AFM is based upon the use of a scanning probe that detects local mechanical features, nano-architectonics, and even thermal or electrical characteristics with a particular probe (tip) positioned very close to a target. The extremely close vicinity between the tip and the target sample allows conducting fine analyses even within a nanoscopic environment. The possibility to use AFM in an aqueous milieu makes this system suitable for characterizing living cells, or their subcellular components, with subnanometer resolution grades. These features have made possible to apply AFM to the analysis of cellular/subcellular nanomotions under physiologic conditions, sensing or conveying weak forces with extreme sensitivity^[99].

In yeasts or bacteria, cell growth, morphogenesis, and metabolic activity are coupled with characteristic nanomotions that coalesce at the cell surface with defined vibrational blueprint^[99]. To this end, a novel area of inquiry has been developed and referred to as “sonocytology”, which is based upon the initial observation that nanomechanical motions detected from these small cells could be transformed into

Table 1 Low-energy shock wave therapy studies

Low-energy shock wave therapy	Conditions	Biological effects	References number
<i>In vivo</i> studies	Wound-healing disturbances, tendinopathies, and non-healing bone fractures	Activation of angiogenic pathways with local release of trophic mediators	[72-77]
	Myocardial infarction in animal models	Improvement of vascularization at the infarction border zone; Mobilization of endogenous progenitor cells from bone marrow into the systemic circulation and to the damaged myocardium; Increase in VEGF gene and protein expression with endothelial cell proliferation	[82-88]
	Human severe coronary artery disease or severe angina	Improvement of myocardial ischemia and chest pain	[89-90]
	Human acute myocardial infarction	Suppression of left ventricular remodeling and enhancement of myocardial function	[91]
	Spinal cord injury in rats	Induction of endogenous neural stem cells and functional improvement	[96]
	Diabetic bladder dysfunction in rat model	Improvement of voiding function; Enhancement of innervation and vascularization	[97]
			[92-94]
<i>In vitro</i> studies	Adipose- and bone marrow-derived mesenchymal stem cells	Induction of osteogenic differentiation	[81,95]
	Murine adipose derived stem cells	Stem cell proliferation and migration in an Erk1/2-dependent fashion	

audible sounds, following accurate amplification of AFM cantilever vibration, providing a thorough mechanistic analysis of cellular activity^[99]. This approach can also be extended to the analysis of complex adaptive behavior in eukaryotic cells. For instance, *in vitro* cardiogenesis, the process of differentiation of stem cells into spontaneously beating cardiomyocytes, entails a major remodeling of the microtubular network, and overall of the cyto- nucleo-skeleton, which will be reflected in remarkable changes in nanomechanical patterning recordable at the level of cellular plasma membrane. In this regard, we have shown and patented for the first time the possibility of using atomic force microscopy (AFM) to afford a nano-mechanical characterization of cellular activity, detecting defined signatures corresponding to the cellular healthy or non-healthy status or to specific differentiating pathways^[100]. In particular, we found that stem cells express nanomechanical patterns that can be harvested by AFM and processed into vibrational signatures of their commitment along defined lineages^[100]. Our ongoing work is based upon the development of high-fidelity multifrequency mechanical transducers capable of conveying back such signatures to undifferentiated stem cells to direct their commitment towards specific fates. Differently from SWT, this strategy would allow orchestration of the differentiating potential of stem cells on the basis of specific nanomechanical codes, instead of relying upon non-specific, empirically designed, and high-intensity mechanical waveforms.

Electromagnetic fields

A significant biomedical deployment of the “nanoworld” described above is the chance of using physical energies to modulate cellular dynamics and fate. In this regard, we first provided evidence that extremely low-frequency pulsed magnetic fields acted on adult ventricular cardiomyocytes to induce the expression of endorphin genes and peptides^[101], playing a major role in intracellular calcium^[102] and pH^[103] handling, in the regulation of myocardial growth^[104-106] and the orchestration of stem cell cardiogenesis^[107-109]. In mouse embryonic stem (ES) cells, extremely low-frequency pulsed magnetic fields induced the transcription of cardiogenic and cardiac specific genes and proteins, ensuing into a high-throughput of spontaneously beating cardiomyocytes^[110].

We found that a radioelectric field of 2.4 GHz, the same frequency used in wireless fidelity technologies, can be conveyed *in vitro* to stem and somatic cells *via* an *ad hoc* designed radio electric asymmetric conveyer (REAC)^[111]. Thanks to its probe, tissue or cell exposure to REAC induce local microcurrents that are attracted and conveyed back to the treated targets without depth limit^[111]. The sum of these microcurrents

elicited in the patient's tissue target *in vivo*, or in isolated cells *in vitro*, are concentrated by the asymmetric conveyer-probe of the device, optimizing tissue or cellular bioelectrical activity^[111]. This innovative approach proved effective in the modulation of stem cell biology at multiple intertwined layers, including the transcription of stemness genes, the expression of tissue-restricted genes and proteins, and the commitment or terminal differentiation along different lineages. In mouse ES cells^[111], as well as hADSCs, REAC exposure optimized the expression of pluripotency and multipotency, respectively, and primed a consistent increase in the yield of stem cells committed along myocardial, skeletal muscle, and neuronal fates^[111,112]. Interestingly, following REAC exposure, even human skin fibroblasts could be committed to the same lineages^[113]. This observation shows the feasibility of directing human somatic cells to fates in which these cells would never spontaneously appear. This approach did not require methods that so far cannot be easily translated into a clinical practice, such as the use of lentiviral vectors for target gene delivery or the somatic cell reprogramming by cumbersome non-integrating technologies. In addition, REAC-mediated reprogramming of somatic cells involved a biphasic effect on the transcription of stemness genes - a rapid overexpression followed by a down regulation^[113] - mimicking the embryogenetic patterning, where the onset of multi-lineage commitment follows, and requires, the transcriptional shutdown of these genes^[114-117]. This observation not only may account for the relatively high yield of commitment observed in each lineage (about 10%-15% of the REAC exposed cell population was oriented towards cardiogenesis, skeletal myogenesis, or neurogenesis)^[113], but it implies that the transcriptional inhibition of the stemness genes would avoid freezing of the REAC treated cells into an embryonic-like state, which may potentially evolve into malignant cells.

Another breakthrough coming from analysis of biological effects produced by REAC conveyed radioelectric fields was the observation that this treatment proved effective in reversing human stem cell senescence^[118]. In fact, a significant decrease in the number of hADSCs expressing senescence-associated β -galactosidase, a marker of cellular senescence, could be observed following REAC exposure throughout long-term cell culture, extended up to the 30th passage^[118]. At the 30th passage in culture, REAC-treated hADSCs showed a remarkable overexpression of the *TERT* gene, encoding the catalytic core of telomerase. This effect was paralleled by an increase in telomere length and telomerase activity, with complete restoration of the ability to differentiate along multiple lineages^[118]. The antisenescence effect of REAC also involved the activation of a telomerase-independent route, as shown by the increase in the transcription of *Bmi-1*, a pleiotropic inducer of stemness genes and proteins, which, accordingly, were found to be upregulated even at the latest 30th passage in the exposed cells^[118].

These observations may also be relevant at the biomedical level. It is now generally accepted that the progressive senescence of tissue resident stem cells across our life span may be responsible for the impairment in tissue self-healing potential. Moreover, from a cell therapy perspective, the strategy of prolonging stem cell culture to yield a high number of transplantable elements, involves the paradox of promoting cellular senescence, thus mocking the initial aim of the cellular expansion of increasing the change of post-transplant tissue recovery.

The "time machine" effect elicited by an electromagnetic energy on stem cell chronobiology may not only prompt innovative approaches for tissue rejuvenation, but it may provide the opportunity of affording (stem) cell expansion procedures without undesired senescence of the cultured cells.

In separate studies, we found that the antisenescence action of REAC was counteracted by 4-methylumbelliferone, a powerful inhibitor of type-2 hyaluronan synthase (HAS2)^[119]. The main implication of this finding lies on the fundamental role of HA in maintaining cell polarity and on the possibility of using electromagnetic energy as a tool to optimize cell polarity at the stem cell level. The intracellular role of HA is highlighted by many interrelated observations: (A) The cardiovascular differentiation of ES cells is abrogated by suppression of HAS2^[120]; (B) Embryogenesis itself is suppressed by HAS2 knockout due to lethal cardiovascular abnormalities^[121]; (C) Intracellular HA acts as docking anchor for hyaluronan binding proteins (hyaladherins), mainly including protein kinases and tissue-restricted transcription factors, favoring targeted phosphorylation steps that are essential for transcriptional efficiency^[121-123]; (D) Most of HA mediated interactions encompass molecular motors and are executed at the level of microtubules, providing a dynamic environment that sustains and directs cell polarity^[124]; (E) Akin to its pleiotropic functions, HA has been used in the form of mixed ester of butyric and retinoic acids to induce a cardiogenic program of differentiation in mouse ES cells^[125]; and (F) In human mesenchymal stem cells, *in vitro*, as well as *in vivo* models of myocardial infarction^[126-128], even affording efficient myocardial repair *in vivo* without stem cell transplantation in infarcted rat

hearts^[129].

Compelling evidence relates impairment in cellular polarity to stem cell senescence, or the development of an oncogenic risk^[124]. Senescent stem cells in *Drosophila* exhibited reduced self-renewal capability as a consequence of centrosome misorientation and altered cell polarity within their stem cell niche^[124,130]. The relevance of preserving cell polarity in biological systems is further highlighted by the results of targeted mutation of tumor suppressor p53 in mammary stem cells, where symmetric division and oncogenesis develop in tight association with cell polarity loss^[131].

Collectively, these observations point at maintenance of cell polarity as to an underlying attribute for an optimal health. The fact that the antisenesescence effect of REAC treatment depended upon intracellular HA availability indicates that proper delivery of electromagnetic fields may represent a tool for optimizing cell polarity in cells and tissue. Such a possibility entails the perspective of conveying radioelectric fields to afford a “one component (cell polarity) - multiple target (stem cell pluripotency, reprogramming, and rejuvenation)” strategy of boosting our self-healing potential.

Deepening the interest for the use of electromagnetic energy in cell biology, the REAC approach also proved effective in inducing the neurological and morphofunctional differentiation of PC12 cells, a rat cell line of pheochromocytoma, retaining metabolic characteristics of Parkinson’s disease^[132]. The REAC effect included the transcriptional up-regulation of *neurogenin-1*, *β3-tubulin*, and *nerve growth factor*, a set of neurogenic genes, and increased the number of both *β3-tubulin* and tyrosine hydroxylase expressing cells. The induction of a neurogenic phenotype was associated with the appearance of neuron-like cells^[132]. Worthy to note, the differentiating effect of REAC was paralleled by a decrease in the number of PC12 tumor cells, while our previous studies showed no decrease in viability of normal human skin fibroblasts and hADSCs. On the whole, these observations suggest that the REAC treatment may be beneficial in the handling of Parkinson’s disease, and further studies are currently ongoing by our Group on this direction (Table 2).

Photobiomodulation

The origin of the term Photobiomodulation (PBM), considered as the possibility of using light to afford modulation of biological processes and tissue healing in various pathological conditions, probably dates back to 1967, when the Hungarian scientist Endre Mester tried to replicate an experiment performed at that time by McGuff in Boston^[133,134]. The latter had been using a beam from ruby laser to eliminate a tumor previously implanted in a rat. Mester was not aware that the system built for him was delivering a ruby laser of only a minimal fraction of power of the laser used by McGuff. As a result, Mester failed to affect the implanted tumor but, he came up with hair regrowth and wound healing at the site of the tumor implantation^[135-138]. Initially referred to as “low level laser therapy (LLLT)”, this kind of approach was successfully applied over time to afford wound healing and counteract inflammation and pain in orthopedic diseases. Following the recent consensus that light-emitting diodes could successfully replace the use of coherent lasers, the term LLLT has been employed to identify generally a “low level light therapy”. Since 2015, the acronym LLLT has been replaced by the term PBM, based upon the difficulty of expressing “low level” as a defined quantity range.

The biological responses elicited by PBM raise the major question as to whether our cells may also use and process electromagnetic signals by themselves, instead of only sensing an exogenously applied electromagnetic radiation (light). A positive answer to this question came from the seminal discovery of Guenter Albrecht-Buehler in 1992, when he analyzed the cellular behavior of Baby hamster kidney cells inoculated sparsely on one face (s-face) and grown as a confluent layer on the opposite face (c-face) of the same thin glass film, using specially prepared substrates to make the confluent cells preferentially oriented and lined up along parallel stripes^[139]. Albrecht-Buehler found that after 7 h and in the absence of visible light most of the cells on the s-face traversed with their long axes and with defined angles the direction of the whorls of the confluent cells on the opposed c-face^[139]. This cell behavior was abolished by a thin metal coating, absorbing visible and infrared light across the range of wavelengths. On the contrary, the orienting pattern was maintained after coating of the s-face by a thin silicone coating of the glass, which strongly absorbed in the blue end of the visible spectrum but was transparent for red and infrared light^[139]. These findings indicated that cells are capable of detecting the orientation of each other by the generation and processing of signals carried by electromagnetic radiation penetrating a thin glass but not a thin metallic film. Moreover, the results from these studies provided evidence that the wavelength of such radiation was likely in the red to infrared range. The author concluded that “the ability of cells to detect the direction of others by electromagnetic signals points to a rudimentary form of cellular

Table 2 Electromagnetic field studies

Electromagnetic fields	Conditions	Biological effects	References number
Extremely low-frequency pulsed magnetic fields	Adult ventricular cardiomyocytes	Induction of the expression of endorphin genes and peptides; Control of intracellular calcium and pH homeostasis; Regulation of myocardial growth; Orchestration of stem cell cardiogenesis	[101-109]
	Mouse embryonic stem (ES) cells	Induction of cardiogenesis, cardiac gene and protein expression, ensuing into a high-throughput of spontaneously beating cardiomyocytes	[110]
Radioelectric field of 2.4 GHz (REAC)	Mouse ES cells, hADSCs and human skin fibroblasts	Optimization in the expression of pluripotency/multipotency; Increase in commitment along myocardial, skeletal muscle, and neuronal fates, with a biphasic effect on the transcription of stemness genes	[111-117]
	hADSCs	Reduction of senescence-associated β -galactosidase expression; Overexpression of the <i>TERT</i> gene associated with an increase in telomerase activity; Overexpression of the <i>BMI1</i> gene; REAC effects counteracted by chemical inhibition of type-2 hyaluronan synthase	[118-120]
	PC12 cells, a rat cell line of pheochromocytoma	Induction of the neurological and morphofunctional differentiation; Up-regulation of neurogenic genes; Decrease in PC12 cells	[132]

ES cells: Embryonic stem cells; hADSCs: Human adipose tissue-derived stem cells; REAC: Radio electric asymmetric conveyor.

“vision”^[139]. Subsequent studies performed by Albrecht-Buehler were published in 2005, showing that near infrared light scattering in dynamic cells was exploited to afford long-range attraction in culture, even when cells were initially seeded in single non-aggregated elements and located randomly onto the tissue culture dish^[140]. Nevertheless, cells were able to detect each other within a certain range and move together to form aggregates. In this study the author described a valid assay to calculate the value of range, which resulted to be much larger than one cell diameter and remarkably dependent upon the measured intensity of near-infrared light scattering produced by the cultured cells^[140]. For the first time, this milestone study showed that “near-infrared light scattering by the cells mediate a long-range attraction between them, which does not require physical contact and enables them to detect each other’s presence”.

More than 20 years later, light has been conveyed in the form of PBM to afford wavelength-dependent selective regulation of differentiation and proliferation in hADSCs. In these stem cells, PBM with blue (420 nm) or green (540 nm) light was found to promote osteoblastic differentiation^[141]. The effect was mediated at the transcriptional level, recruiting a gene program of osteogenesis, including RUNX2, osterix, and the osteoblast protein, osteocalcin. The 420 nm and 540 nm wavelengths were more effective in stimulating osteoblast differentiation compared to red or near infrared PBM^[141]. Intracellular calcium was higher after exposure to light of 420 nm and 540 nm, an effect that could be inhibited by capsazepine (CPZ) and SKF96365 (SKF), which also inhibited osteogenic differentiation^[141]. These effects were likely mediated by the activation of light-gated calcium ion channels, as it was inferred by the ability of both CPZ and SKF to suppress the hADSC response to blue or green light^[141]. Members of the superfamily of TRPs channels appear to be the class of light-gated ion channels responsible for the differentiating action of blue and green PBM. Consistent with this view, TRPs are present in almost all known living forms^[142]. In particular, the vanilloid TRP sub-class (TRPV) was identified as including the receptor TRPV1, specific for capsaicin^[143]. TRPV1, exogenously expressed in *Xenopus*, has been shown to be activated by red and even to a greater extent by green light^[144]. It is now evident that multiple TRPVs play a relevant role in many biological responses, including pain, inflammation, and regulation of pressure and heat^[145]. Opsins are currently believed to represent the major group of intracellular light-sensitive proteins

(chromophores) involved in selective photon absorption (especially blue and green photons) and subsequent activation of TRPs^[146,147]. In particular, melanopsin, which relies upon 11-cis retinal isomerization (peaking at 479 nm), is potentially expressed in hADSCs and has been shown to form a pigment maximally sensitive to blue light (with a peak at 479 nm), supporting activation of G(q/11) and G(i/o) signaling cascades, ultimately promoting TRPs-mediate raise in intracellular calcium^[22]. Melanopsin has also been targeted by therapeutic devices utilizing blue light to treat jet-lag, affective disorders, depression, and insomnia^[148-150]. The fact that SKF, a non-selective TRP inhibitor, was more effective than the selective TRPV1 inhibitor CPZ in abrogating the effects of green and blue light on hADSC osteogenesis^[141] suggests that TRPV1 may not be the only light-gated ion channel involved in the osteogenic patterning triggered by blue or green PBM in hADSCs.

Further compounding the complexity and selectivity of PBM in stem cell biology is the finding that red (660 nm) or near-infrared (810 nm) light was able to stimulate, while PBM with blue (415 nm) or green (540 nm) light was found to inhibit the proliferation of hADSCs^[151]. In these experiments, PBM with blue/green light produced a CPZ-inhibitable increase in intracellular calcium, and in the amount of ROS, while red/near-infrared light produced a comparable lower increase in intracellular calcium and ROS levels^[151]. Moreover, the slight raise in intracellular calcium elicited by red/near-infrared PBM could not be blocked by CPZ. At the same dose of irradiation (3 J/cm²), blue/green light decreased cellular ATP, lowering both mitochondrial membrane potential and intracellular pH, which may account for a significant increase in ROS, while red/near-infrared PBM had the opposite effect^[151]. In the same study, the possibility that the blue/green light may have decreased hADSC proliferation by activating TRPV1 ion channel and increasing calcium and ROS was inferred by the observation that TRPV1 was expressed in hADSCs, and CPZ itself, as well as the antioxidant N-acetylcysteine, abolished the inhibition of proliferation induced by blue/green PBM. These findings also highlighted the subtle differences between the effects produced by blue and green PBM, being their action superimposable in reducing hADSC proliferation and intracellular ATP, while the blue light triggered a more pronounced increase in ROS and drop in mitochondrial membrane potential, as compared with green PBM^[151]. It was hypothesized that different sensitivities of mitochondrial chromophores may at least account for these differential responses, with red/near-infrared light conversely increasing intracellular ATP and only inducing low levels of ROS.

Taken together, these findings point at the possibility of deploying the diffusive features of PBM to afford a fine tuning of stem cell dynamics and suggest that the ability of PBM to promote tissue repair previously observed *in vivo* may have involved *in situ* reprogramming of tissue-resident stem cells.

Various forms of PBM have been proved effective in ameliorating the outcome of acute stroke in both animal models^[152-157] and humans. In a number of, although not all, controlled clinical trials enrolling patients with ischemic stroke associated with measurable neurological defects, 810 nm laser light conveyed to shaved head induced a significant and long-lasting neurological improvement, especially for patients with moderate and moderate-to-severe stroke^[158-160].

Application of near-infrared light in animal models of traumatic brain injury (TBI) has been consistently shown to rescue neurological performance and reduce the size of brain lesions in different research laboratories^[161,162]. In some studies, the favorable effect of PBM was selectively induced at defined wavelengths, with 665 nm and 810 nm proving the most effective, and with lack of improvement at 730 nm and 980 nm^[162]. Based upon this window of selectivity, the chromophore cytochrome c oxidase has been proposed as the putative target responsible for the observed tissue repair^[163]. These results were further confirmed in TBI animal models subjected to PBM with either pulsed or continuous wave lasers, with the group treated with 10 Hz pulsed waves exhibiting the most pronounced improvement^[164]. The link between these findings and the observed *in vitro* action of PBM on stem cell dynamics is supported by the finding that PBM was found to increase neuroprogenitor cells in mouse dentate gyrus and subventricular zone^[165], along with an increase in brain derived neurotrophic factor after 7 d of treatment, followed by a later increase in synapsin-1, underlying a significant enhancement in synaptogenesis and neuroplasticity^[166]. Also, learning memory was increased in both mouse and rat models of TBI^[167]. These beneficial outcomes were further improved by the combinatorial delivery of PBM and metabolic substrates, including pyruvate and lactate, which were supposed to ameliorate the mitochondrial function^[168]. Clinical trials, although still performed in a limited number of patients with TBI, appear to confirm in human subjects the results initially yielded in experimental animal models, as it has been indicated by post-treatment improvement of both language^[169] and cognitive performance^[170] as well as brain tissue recovery, as assessed by anatomical magnetic resonance imaging and

perfusion single-photon emission computed chromatography^[171].

Another major field of potential clinical application of PBM deals with the treatment of neurodegenerative diseases. In animal model of Alzheimer's disease (AD), established through the generation of amyloid beta precursor protein transgenic mice, the number of amyloid beta plaques was remarkably diminished by the application of 810 nm PBM^[172]. The behavioral pattern of affected mice was also improved, together with a consistent decrease in the expression of pro-inflammatory cytokines^[172]. The rescuing effect of PBM was associated with an increase in mitochondrial function and ATP levels^[172]. Despite these encouraging results in experimental animal models, surprisingly no major clinical trials have been conducted using PBM in AD patients. In a small-sample, preliminary (so far only appeared in abstract form) but randomized controlled trial, in patients with moderate-to-severe AD (Mini-Mental State Examination scores ranging 5-24), the combinatorial delivery of PBM through the trans-cranial and intranasal routes afforded a significant improvement (5-point Mini-Mental State Examination score) after 12 wk, with a concomitant improvement in Alzheimer's Disease Assessment Scale - Cognitive assessment^[173]. In a recently published study, where patients with AD were subjected to intravascular PBM (final positioning of the optic fiber emitter lined up to the distal site of anterior and middle cerebral arteries), authors reported a significant, long-lasting (up to 7 years) enhancement of cerebral microcirculation and cognitive improvement^[174].

Another set of studies performed in both acute and chronic mouse models of pharmacologically-induced Parkinson's disease revealed a remarkable increase in the number of dopaminergic neurons^[175]. These studies were confirmed in a tau transgenic mouse model of Parkinson's disease^[176]. The only available clinical trial presented so far in the abstract form, enrolling subjects whose age ranged between 18 and 80 years, reported a significant improvement of the investigated indicators of balance, including gait, cognitive function, and speech^[177].

Remarkably, LLLT (808 nm) has been recently applied *in vivo* to the tibia and iliac bones of pigs subjected to experimental acute myocardial infarction, leading to a significant reduction in cardiac scarring, with increased density of small blood vessels in the infarcted area, and consistent improvement of heart function^[178]. The LLLT action was mediated by an increase in the number of circulating c-kit⁺ stem cells during the first 48 h post-infarction^[178]. These beneficial effects resulted to be a long-lasting outcome of the laser treatment, since they were documented 90 d after the infarct induction^[178]. These findings provided the first evidence on the use of light radiation to afford a non-invasive cardioprotection of the heart in the acute phase post myocardial infarction, mediated by endogenous stem cell proliferation and recruitment to the ischemic heart, without resorting to stem cell transplantation strategies (Table 3).

On the whole, experimental and clinical evidence strongly support the feasibility and the remarkable degree of efficacy of PBM in a number of major pathological conditions that so far cannot be solved even by the most advanced pharmacological and/or surgical procedures.

CONCLUSION

Enhancing our self-healing potential

Cells are increasingly regarded as sensors and transducers of physical energies. Novel and probably unprecedented therapeutic strategies are emerging and will be further deployed in the near future, unfolding (stem) cell biology through the eyes of physics, electronics, and likely bioelectronics (Figure 2). Future approaches will increasingly aim at providing valuable, non-invasive, and cost-effective opportunities for boosting our inherent ability of self-healing. Owing to the diffusive features of mechanical, (electro)magnetic, and light waves, (stem) cell reprogramming may occur in place, without necessarily using transplantation (cells/tissue) procedures to rescue damaged tissues.

We envision the application of high-tech devices, including AFM, TFM, STM, and fast HSI cameras, to acquire specific signatures from mechanical and electromagnetic radiation (light) patterns of stem cells oriented towards defined fates (Figure 3). We also foresee the development of smart actuators, capable of delivering the acquired individual (*i.e.*, mechanical, or electromagnetic) or combinatorial signatures to diseased tissues, selectively driving the fate and rescuing potential of resident stem cells, to afford a precision endogenous regenerative medicine (Figure 3).

Physical tissue stimulation and the merging of human body with machines. May

Table 3 Photobiomodulation studies

Photobiomodulation	Conditions	Biological effects	References number
LLLT	Tumor transplantation in rats	Failure to affect the implanted tumor; Stimulation of hair regrowth and wound healing	[135-138]
Cell-generated electromagnetic (light) signals	Baby hamster kidney cells on thin glass film	Cell migration and orientation afforded by endogenous generation and processing of signals carried out by electromagnetic radiation (light)	[139]
Near-infrared light scattering	Cell culture	Near-infrared light scattering by cells mediates long-range attraction between them and aggregation within the culture system	[140]
PBM with blue (420 nm) or green (540 nm) light	hADSCs	Promotion of osteoblastic differentiation; Overexpression of a gene program of osteogenesis; Increase of intracellular calcium mediated by the activation of light-gated calcium ion channels	[141,142]
PBM with red (660 nm) or near-infrared (810 nm) light	hADSCs	Induction of cell proliferation; Maintenance of low ROS level	[151]
PBM with blue (415 nm) or green (540 nm) light	hADSCs	Inhibition of cell proliferation; Increase of low ROS level; Lowering of mitochondrial membrane potential and intracellular pH	[151]
Various forms of PBM	Acute stroke in animal models	Improvement of the outcome of acute stroke	[152-157]
PBM with 810 nm laser light	Human moderate-to-severe stroke associated with neurological defects	Long-lasting neurological improvement	[158-160]
Near-infrared light scattering (665 nm and 810 nm)	Traumatic brain injury in animal models	Rescue of neurological performance and reduction of the size of brain lesions; Increase of neuroprogenitor cells in mouse dentate gyrus and subventricular zone; Increase of learning memory; Improvement of mitochondrial function	[161-168]
Near-infrared light scattering (665 nm and 810 nm)	Human traumatic brain injury	Improvement of both language and cognitive performance, as well as brain tissue recovery	[169-171]
PBM with near-infrared (810 nm) light	Alzheimer's disease in animal models	Reduction of amyloid beta plaques; Decrease in the expression of pro-inflammatory cytokines; Increase in mitochondrial function, and ATP levels	[172]
PBM with near-infrared (810 nm) light	Human Alzheimer's disease	Improvement in Alzheimer's Disease Assessment Scale - Cognitive assessment; Enhancement of cerebral microcirculation	[173-174]
PBM with near-infrared (810 nm) light	Parkinson's disease in animal models	Increase in the number of dopaminergic neurons	[175-176]
PBM with near-infrared (810 nm) light	Human Parkinson's disease	Improvement of the investigated indicators of balance, including gait, cognitive function, and speech	[177]
LLLT	Acute Myocardial infarction in the pig	Reduction of scarring; Improvement of heart function; Stem cell mobilization and recruitment to the ischemic heart	[178]

LLLT: Low level light therapy; hADSCs: Human adipose tissue-derived stem cells; PBM: Photobiomodulation.

future approaches change the way we are connected to ourselves?

In the next few years we will probably experience a fundamental transformation in the way we live, work, and relate to each-other. The so-called 4th Industrial revolution will trace the future of electronics, machines, and AI and may even change the way we are connected to and perceive ourselves. Future electronics will be focused more and more on health technologies and will be directed inwards, with respect to human beings, trying to promote self-healing mechanisms. Most of currently available technology has been pointing outwards, being focused of making available

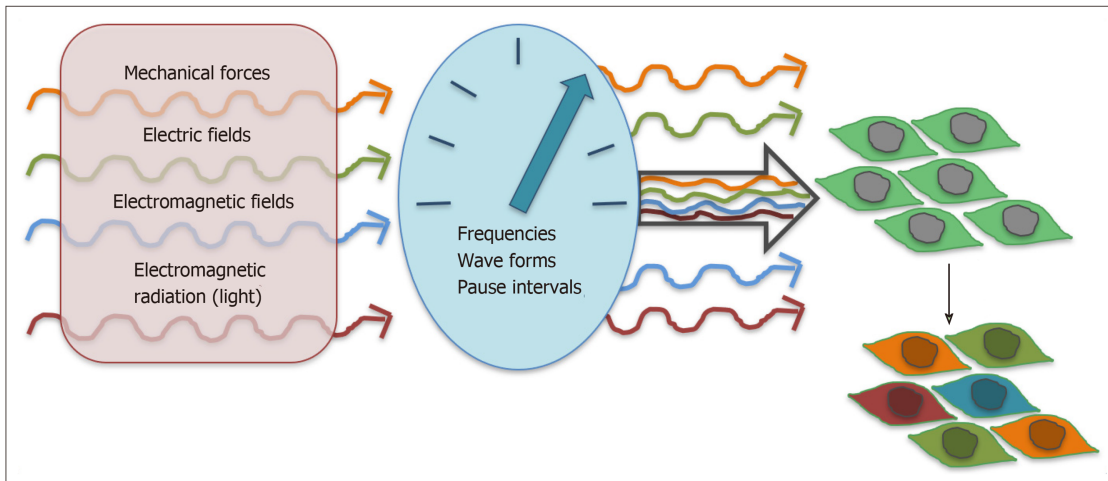


Figure 2 (Stem) cells as sensors and transducers of physical energies. A growing number of signaling molecules inside and outside the cell has been shown to behave as mechanosensors/transducers and chromophores (see details in the text). These players modulate complex dynamics controlling multifaceted responses, including proliferation, migration, and differentiation.

“something that accomplishes something”.

Emerging future technologies will promote novel interfaces of human beings with machines and AI. What if we may take signatures/codes of our (stem) cell ability to cope with a hostile environment (*i.e.*, oxidative stress, hypoxia) or differentiate along the most complex fates, like the heart, the brain and so on? What if that piece of a stem cell code may be integrated with evolving forms of machines and AI to promote endogenous tissue regeneration? Within this futuristic scenario we may start exploring the synchronistic interchange of who we are and what we are able to become.

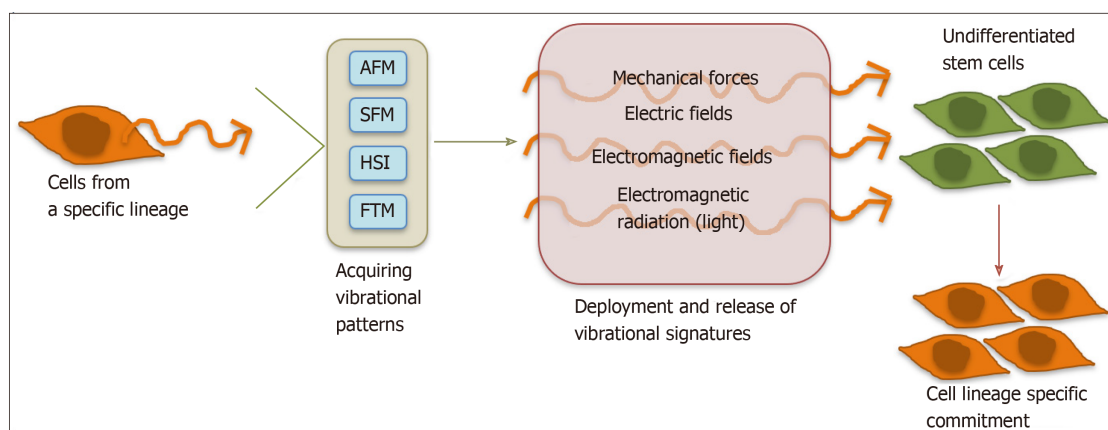


Figure 3 Harvesting and releasing vibrational signatures to afford commitment of undifferentiated stem cells towards targeted fates. Atomic force microscopy, terahertz field microscopy, scanning tunneling microscopy, and hyperspectral imaging can be used to harvest detailed oscillatory patterns, including mechanical, electric, and light waves. Computer analysis is then performed to acquire vibrational signatures from the investigated patterns. *Ad hoc* designed transducers are finally developed to provide high-fidelity and timely release of the acquired signatures onto undifferentiated (stem) cells to prime their lineage specific commitment. AFM: Atomic force microscopy; STM: Scanning tunneling microscopy; HIS: Hyperspectral imaging; TFM: Terahertz field microscopy.

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