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Seeing Cell Biology with the Eyes of Physics

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Abstract

Rhythmic oscillatory patterns permeate the entire universe and sustain cellular dynamics at biological level. The intracellular environment is now regarded as a complex nanotopography embedding "intracrine" signals that encompass the intracellular action of regulatory molecules coupled with the newly discovered functions of the microtubuar network. Microtubuli, are far away from simply being a part of the cytoskeleton, since they produce both nanomechanical motions and display electromagnetic resonance modes that may turn local events into non-local, long-ranging paths. The possibility that microtubuli form a bioelectronic circuit prompts rethinking of biomolecular recognition as a result of synchronization of the oscillatory patterns of proteins sustained and orchestrated by resonance modes brought about by the microtubular network itself. Here, we discuss these issues within the biomedical perspective of using physical energies to govern (stem) cell fate. We focus on the ability of specially conveyed electromagnetic fields to afford optimization of stem cell polarity and pluripotency, reversing stem cell aging and promoting a multilineage repertoire in human adult stem cells, as well as in human non-stem somatic cells. We discuss the use atomic force microscopy and hyperspectral imaging for deciphering the nanomotions generated by (stem) cells during their growth and differentiation. We highlight the potential for unraveling vibrational signatures that can be exploited to direct the differentiation and self-healing potential of tissue-resident stem cells *in vivo*. In conclusion, seeing stem cell biology with the eyes of Physics may help developing a Regenerative/Precision medicine afforded through the stimulation of the natural ability of tissues for self-healing, without the needs of stem cell transplantation.

Keywords

Stem cells, Regenerative medicine, Precision medicine, Physical energies, Electromagnetic fields, Nanomechanical motions, Mictrotubuli, Microfilaments, Exososmes, Hyaluronan, Intracrine patterning, Endorphins, Aging, Cell polarity, Self-healing

Background

Regenerative Medicine, and the efforts to deploy this new perspective into a tailored system for individual patient's needs, which may fulfill the requirement for a Precision Medicine, are paving the way to unprecedented chance to cure diseases we are all afraid of, including cardiovascular diseases, neurodegenerative disorders, diabetes and its complications, difficult-to-heal wounds, and cancer.

In our body, we renew a consistent amount of cells in just a few months, underlying inherent self-healing capabilities. To this end, all tissues embed population(s) of resident stem cells that live and grow as pericytes [1] in a perivascular location within a complex environment named "the niche", entailing both chemical (trophic mediators) and physical cues [2]. A consistent amount of signaling peptides in the extracellular space are not naked molecules, but rather reside together with miRNA, long-chain RNA, and other molecules inside exosomes [3]. These are nanovescicles interconnected by a network of filaments whose effective nature still remains elusive, and can be viewed as timely delivery machines of pockets of information [3, 4]. Our view of cell-to-cell connectedness is currently evolving accordingly. We are moving from the original idea of endocrine, paracrine and autocrine signaling to the new dimension of an *intracrine* world of cell regulation. To this end, we first demonstrated the presence of nuclear endorphin receptors specifically activated by dynorphin peptides [5], triggering the induction of nuclear protein kinase C isoforms to afford major developmental decisions in stem cell cardiogenesis [6, 7]. This intracrine route of signaling imparts features characteristic of wide-ranging transcriptional regulation and cell memory [5-10] and may not only represent the molecular plight receiving the exosome–mediated trafficking. Rather, the intracrine world may even contribute to the deployment of an "intracellular niche", a complex nanotopography where signaling molecules interplay with a network of filaments and microtubuli which are far away from simply being the cytoskeleton. Scanning tunneling Microscopy (STM), coupled with a special artificial cell-like environment designed to pump electromagnetic frequencies to microtubuli growing onto a nanoelectrode array, has shown the existence of defined resonance modes between the tubulin protein, as well as the microtubular structures, and the applied frequencies [11]. Moreover, STM showed that specific "tunneling current images" are produced by microtubuli as resonance patterns in response to electromagnetic frequencies in the MHz domain [11]. A peculiar conformational patterning can be therefore elicited in microtubuli by remotely applying an electromagnetic field: electro-mechanical coupling occurs at microtubular level as a function of defined resonance modes between the frequencies of the incoming electromagnetic fields and those developed by the microtubuli themselves [11]. These findings suggest that microtubuli can be conceived as an intracellular bioelectronic circuit. Consonant with this view is the evidence for the emission of high-frequency electric fields with radiation characteristics from microbuli [12] and even the detection of multi-level memory-switching properties at the level of a single brain microtubule [13]. Moreover, the hollow fibers of microtubuli have been shown to be filled by uniquely arranged water molecules [14-17]. This atomic water channel has been shown to resonantly integrate all the microtubular proteins around it, up to the point that the microtubule irrespective of its size functions like a single tubulin molecule [14]. These data suggest that a water channel residing inside the microtubuli may act governing their tantalizing electronic properties [14]. DNA itself may be considered as an electrically charged vibrational structure. In the nucleus, this macromolecule continuously assembles into multifaceted domains through the action of transcription factors and molecular motors imparting nanomechanical oscillatory traits that are essential for the storage and processing (expression) of genetic information. Accordingly, using an innovative approach that has been developed over the last two decades, named Resonant Recognition Model (RRM), DNA has been found to exhibit wide-ranging electromagnetic resonance frequencies from THz to KHz spectra [18]. The RRM relies upon the initial observation that periodicities in energy distribution of delocalized electrons within a given protein are essential for its function, and activity, including protein-protein interaction, or protein-DNA interaction, as it occurs in the case of transcription factor binding to, and bending of DNA, two major initiating steps in the modulation of gene expression [19]. The introduction of the issue of protein conductivity in RRM development, led to the consideration that a charge moving through the protein backbone would generate an electromagnetic irradiation or absorption with spectral signatures corresponding to energy distribution along the protein [19, 20]. These theoretically calculated spectra were confirmed experimentally [11, 21]. Moreover, RRM allows designing of new peptides with desired spectral characteristics, and biological activities [22]. The RRM enables these spectral characteristics, which were found to be in the range of infrared and visible light, to be calculated [19].

These recent findings, have been intriguingly anticipated long time ago, by the seminal discovery that cells are able to communicate non solely by chemical means, but even through near-infrared light emission [23] and that cell migration and aggregation *in vitro* remarkably depends upon the cellular ability to handle light scattering within this spectrum of electromagnetic radiation [24].

How can biomolecular recognition be framed within this context? The old "key-and-lock mechanism", while suitable to describe the molecular interaction on a very restricted scale of couples of, or few interactive proteins, is not adequate to represent the collective behavior of variegated populations of signaling players co-habiting the intracellular niche and sharing overlaying spaces and times of interaction to generate coherent cellular decisions. Moreover, the timing required for cellular proteins to interact as a result of their diffusion modes through the aqueous intracellular environment would be substantially unpredictable on large-scale interactive bases. The speed of cellular reactions and ultimate decisions (i.e. stem cell fate and terminal differentiation) based upon the assumption of diffusive model(s) would be variably and unpredictably affected by the fact that, due to the presence of crucial intracellular glycosaminoglycan (i.e. hyaluronic acid), the intracellular environment is not equitable to an aqueous salt solution, but rather to a non-homogenous, aqueous gel whose composition and intrinsic diffusive properties are continuously adapting to the fluctuation in cellular metabolic patterning. Is there a way to reconcile the current understanding of the intracellular niche and intracrine physiology with a novel vision of intra- and inter-cellular connectedness that may reasonably be more adherent to the astonishing speed at which cells shape their decisions and morphogenetic behavior? Most signaling proteins exhibit helix-turn-helix repeats that can be

viewed as oscillatory entities, the helices behaving like springs, with the turns acting as connectors between oscillators. A single protein can be considered as a phase-resonant vibrating system [25]. Near terahertz field microscopy has captured protein vibrations, tiny motions essential for Life [25]. This finding suggests that, like violin strings or the pipes in an organ, the proteins in the human tissues vibrate in different patterns [25]. Cellular proteins not only diffuse through water, but they predominantly "walk" on the microtubules by the aid of "molecular motors", such as kinesins and dyneins [26]. Signaling molecules can be regarded as oscillators moving across the cytoskeletal web, with the microtubules (and likely actin filaments) dissipating vibrational differences between oscillators.

The Origin and Relevance of Resonant Behavior

The microtubule resonant behavior described by Bandyopadhyay and coworkers [11] will no doubt have a crucial impact in our future understanding of biomolecular recognition patterning. Of particular relevance, the existence, and the identification of a *frequency region selectivity* for inducing defined morphological paths reveals that pure mechanical changes can be remotely tuned in a precise structural (anatomical) mode by employing electromagnetic fields remotely [11]. Hence, the evidence that changing the electromagnetic frequency exposure can affect the local density state in the tubulin, microtubuli and potentially in many other proteins, implies that the deployment of protein structure(s) into rhythmically manifesting resonance modes may represent a major underlying mechanism for both intracellular, and intercellular communication. The unfolding of resonant modes between the signaling molecules "dancing" on microtubular networks and between such molecules and the microtubular circuitry can be foreseen as the new field of enquiry to understand the origin, propagation, and dynamic interconnection of informational processes at biological level.

Live demonstration of one to one correspondence between electromagnetic and mechanical oscillations and the complexity of multiform resonant modes expected in an *in vivo* cellular, tissue, and whole-body environment send us back to a very fundamental question: what is the origin of the observed resonant behavior? This is still an open and controversial issue. Electric and magnetic fields can impact on proteins and protein complexes. Within the context of an electromagnetic exposure, if electromagnetic resonance occurs, photons may be expected to find domains susceptible for both electrical and magnetic absorption. To this end, protein cavities may be regarded as regions where an electric vector from an electromagnetic wave and its magnetic component may interact with *suitable-for-absorption* structures within the cavity.

Although protein cavities may be highly sensitive nanotopographies for electrical resonance modes and chains, whether such cavities may also be sensitive to generate resonances under an electromagnetic field remains an open and difficult to prove issue. A major challenge has been creating the opportunity to investigate different types of resonant behaviors at the same time, since electromagnetic and nanomechanical waves are interconnected and interdependent in our cells with none of their resonant-induced behaviors being separately electrical, mechanical, magnetic or ionic. The recent invention of an *atomic resolution scanning dielectric microscopy* is now providing the unprecedented chance to observe a single protein complex operating live at resonance modes within a single-cell context without cell derangement [27]. By the aid of this novel technology, electromagnetically triggered electrical, mechanical, thermal and ionic resonant vibrations have been imaged in a protein inside an axon core of a neuron. As a result, it was experimentally observed that a protein molecule expresses unique configurations for each resonance frequency [27].

Overall, a new picture of cellular dynamics is emerging, made of mutant vibrations, along with the vivid hypothesis that we are now facing a new way of thinking connectedness at the intra- and inter-cellular level, reconciling old hypothesis with new visions. Such visions may be summarized into the chance and perspective of seeing cell biology with the eyes of Physics. Considering the cells as senders and receivers of electromagnetic and nanomechanical fields discloses the potential of using these physical energies to afford major tuning of cell behavior. When unfolded at the stem cell level, such a perspective could result into unprecedented implications for Regenerative and Precision Medicine.

Stem Cells as a Target for Electromagnetic Fields

A major implication of unfolding the "nanoworld of stem cells" described above is the possibility to direct the cellular fate with physical energies. Within this context, we first demonstrated that exposure of adult ventricular cardiac myocytes to extremely low-frequency magnetic fields (ELF-MF) resulted in the transcriptional activation of an endorphinergic system [28], which was previously shown to be essential for cytosolic calcium [29] and pH homeostasis [30], as well as for myocardial growth [31-33] and stem cell cardiogenesis [6, 7, 34]. ELF-MF were also found to induce a high-throughput of cardiogenesis and a remarkable increase in spontaneously beating cardiac myocytes from mouse embryonic stem (ES) cells [35].

More recently, we discovered that radio-electric fields of 2.4 GHz, the same frequency used worldwide to connect through the Internet, could be delivered to cultured stem cells by a Radio Electric Asymmetric Conveyer (REAC) [36]. The REAC technology generates radio electric asymmetrically conveyed microcurrents in tissues, without depth limits [36]. The sum of these radio electric-induced microcurrents elicited by the REAC apparatus in the cell culture or patient's body are concentrated by the asymmetric conveyer-probe of the device, in order to optimize their bioelectrical activity [36]. This innovative strategy has been shown to modulate stem cell dynamics at multiple interconnected levels, from the expression of stemness-related and tissue-restricted genes, up to functional remodeling of stem cells. REAC exposure

optimized the expression of pluripotency and promoted high-yield commitment towards myocardial, neuronal and skeletal muscle fates in both mouse embryonic stem cells [36] and human adipose derived mesenchymal stem cells (hADSCs) [37]. Commitment towards the same lineages has been achieved by REAC treatment of human skin fibroblasts [38]. For the first time, human non-stem somatic adult cells were directed to fates into which they would never otherwise be committed to, avoiding practices that cannot so far be readily envisioned in a clinical setting, such as the gene delivery by lentiviral vectors, or the cellular reprogramming by cumbersome and expensive chemistry, including the use of non-integrating technologies. Moreover, somatic cell reprogramming by the REAC technology was mediated by a transient overexpression of pluripotency genes, followed by their down-regulation [38], without halting the treated cells into an embryonic-like state which may drift into cancer or unwanted cell types.

Intriguingly, the exposure to properly conveyed radio electric fields was able to reverse the senescence of human adult stem cells *in vitro* [39]. REAC treatment significantly decreased the number of human adipose-derived mesenchymal stem cells (hADSCs) positively stained for senescence-associated β-galactosidase along multiple culturing passages [39]. After 30 passages in culture, the REAC treatment was able to re-express the TERT gene, encoding the catalytic core of telomerase, increasing the telomere length, with full recovery of the multilineage potential of hADSCs [39]. The antiaging action of REAC also encompassed the induction of a telomerase-independent pathway, upregulating the transcription of BMI-1, an orchestrator of the expression of stemness related genes and proteins, which also resulted to be induced by the REAC treatment of senescent stem cells [39].

These findings may have important implications. Stem cells progressively age while we age, significantly accounting for age-associated decline in self-healing potential of tissues and organs. Moreover, while subjecting stem cells to prolonged *in vitro* expansion to increase their number prior to transplantation, we also promote their senescence, which paradoxically hampers their rescuing potential after transplantation. The chance of using electromagnetic energy as a "time machine" to affect stem cell chronobiology may lead to future approaches of molecular rejuvenation *in vivo*, also offering the chance for (stem) cell expansion procedures devoid of the risk for concomitant cell aging.

Compounding this picture, electromagnetic fields have been shown to afford neurological and morphofunctional differentiation in PC 12 cells [40], a rat adrenal pheochromocytoma cell line displaying metabolic features of Parkinson's disease. The action of electromagnetic fields was mediated by the transcriptional activation of neurogenic genes, as neurogenin-1, β3-tubulin and nerve growth factor (NGF), and was associated with a consistent increase in the number of cells expressing both β3-tubulin and tyrosine hydroxylase [40]. More recently, we have shown that the antiaging effect of REAC conveyed electromagnetic fields could be significantly opposed by stem cell exposure to an inhibitor of type 2 hyaluronan synthase (HAS2) [41]. This finding points at the relevant pleiotropic role of hyaluronic acid (HA) and glycosaminoglycans in the intracrine regulation of cell polarity and at the chance of using physical energies to preserve and direct this fundamental attribute of Life. HA has been used as a component to promote cardiogenesis in mouse embryonic [42] and human adult stem cells of different origin *in vitro* and *in vivo* [43-45] and to induce cardiac repair *in vivo* without stem cell transplantation in a rat model of myocardial infarction [46]. Accordingly, HAS2 suppression abolished the capability of human ES cells to differentiate *in vitro* along the cardiogenic and vasculogenic lineages [47]. HAS2 knockout suppressed mouse embryo survival and growth owing to lethal cardiovascular abnormalities [48]. In the intracellular niche, HA is a docking place for hyaluronan binding proteins initially referred to as hyaladherins, then shown to include tissue-restricted transcription factors and their related protein kinases [48-54]. Most of these interactions require molecular motors and are deployed at the level of cellular microtubuli and microfilaments which form a major dynamic environment to establish and preserve cell polarity [55]. There is now increasing evidence bridging altered stem cell polarity and stem cell aging, as well cancer [55]. In Drosophila, aged germ line stem cells showed misoriented centrosomes leading to altered polarity with respect to their stem cell niche, and reduced self-renewal activity [55, 56]. A close association between disruption of stem cell polarity and oncogenesis emerged by experiments of targeted mutation in tumor suppressor p53, increasing the symmetric division in mammary stem cells with substantial loss in cell polarity [57].

Overall, cell polarity is emerging as a universal attribute for healthy life. The dependence of the favorable effects of electromagnetic fields from the intracellular availability of HA suggests that optimization of stem cell polarity may be an underlying pleiotropic mechanism for their action. This hypothesis deserves further investigation, since it harbors the chance of using electromagnetic fields as a tool to achieve a "one component (cell polarity) – multiple target (stem cell pluripotency, reprogramming, and rejuvenation)" strategy to enhance our self-healing potential.

Deciphering the Nanomotions of Stem Cells: A Step towards Directing Differentiation with a Vibrational Signature

Life avows in a world of vibrations: rhythm is critical in all forms of life. The diurnal, seasonal, lunar and solar cycles, and the resonant oscillations of electromagnetic fields of our own planet shape a symphony of rhythms in which life manifests itself and evolves. These natural rhythms have been integrated into a wide variety of biological responses in humans and animals. Our life entails a seeming infinity of rhythms, with vibrations at the atomic and molecular levels and within biochemical reaction rates.

Intra/inter cellular motility appears to be coordinated through mechanical signals passing between and regulating the activity of motors, microtubuli and filaments. These signals are carried by forces and sensed through the acceleration of protein-protein dissociation rates. Mechanical signaling can lead to spontaneous symmetry breaking, switching, and

oscillations, and it can account for a wide range of cell motions such as mitotic spindle movements, and bidirectional organelle transport and the establishment of collective behaviors, as those afforded by cell signaling networks. Because forces can propagate quickly, mechanical signaling is ideal for coordinating motion and information over large distances.

Starting from the pioneering work of Clinton Rubin and coworkers, showing that low mechanical signals were able to strengthen long bones *in vivo* [58], and that the anabolic activity of bone tissue, suppressed by disuse, could be normalized by brief exposure to extremely low-magnitude mechanical stimuli [59,60], it has become progressively evident that mechanical vibration was able to orchestrate major cell signaling networks. Adipogenesis was found to be inhibited by brief daily exposure to high-frequency, extremely low-magnitude signals [61] while these signals were found to act in a non-invasive fashion to influence stem cell fate, promoting bone and suppressing the fat phenotype [62]. Bone structure and B-cell populations, crippled by obesity, have been found to be partially rescued by brief daily exposure to low-magnitude mechanical signals [63], and low-level vibrations can retain bone marrow osteogenic potential and promote recovery of trabecular bone during reambulation [64].

Cells and tissues are extremely sensitive to mechanical vibrations [65, 66], as shown by the peculiar dynamics of specialized structures, such as the LINC (Linker between Nucleoskeleton and Cytoskeleton) governing cell mechanosensitivity to extremely low-magnitude signals, and their conveyment into the nucleus to elicit chromatin remodeling and epigenetic modifications essential for the regulation of the transcriptional machinery [67]. Intriguingly, an electroacustic behavior has been recently proposed to explain the mitotic spindle dynamics [68].

Overall, we are progressively becoming aware of the fact that (stem) cells may store mechanical vibrational signatures of: (i) their health status, (ii) the commitment along multiple lineages, (iii) the acquirement of terminally differentiated outcomes, and (iv) the release/exchange of building blocks of information as afforded by intercellular trafficking of signaling proteins/molecules through exosomal routes.

Biophysical signaling from and to the (stem) cells offers a clue to reinterpret our future approaches to regenerative medicine, indicating that physical energies can be delivered to stem and somatic cells to engage them into a self-healing program for damaged tissues. The intrinsic rhythmic behavior of the cytoskeleton and the nucleoskeleton conveys characteristics of connectedness and synchronization modes that can be transmitted up to and recorded from the cell surface.

Within this context, we have demonstrated and patented for the first time the ability of cells to express "vibrational" (nanomechanical) signatures of their health and differentiating potential [69]. Many biological processes taking place inside the living cell rely on the nanomechanical properties of cellular substructures and the cell membrane or wall itself. By the aid atomic force microscopy (AFM) it is now possible to gain information on the integrity and local nanomechanical properties of mammalian and microbial cellular membranes under normal and stressed metabolic conditions. The AFM is a scanning probe microscope that measures a local property, such as topography, mechanical properties, thermal and electrical properties, optical absorption or magnetism, with a probe or "tip" placed very close to the sample. The small probe-sample separation makes it possible to take measurements over a small area. Because the AFM can image biological samples at sub-nanometer resolution in their natural aqueous environment, it has potential for characterization of living cells. Using the AFM, it has been possible to observe living cells under physiologic conditions, detecting and applying small forces with high sensitivity [70]. In yeast and bacterial cells, cellular activity, metabolism, growth and morphogenetic changes were associated with defined nanomechanical activity, merging to the cell surface up to the generation of defined patterns of vibrations [69]. "Sonocytology" is the term that has been introduced to identify a novel area of inquiry based on the fact that in these small cells, after an accurate process of amplification, given the frequency range of nanomechanical motions recorded by AFM, the vibrations could be transformed into audible sounds, providing a thorough assessment of mechanistic cellular dynamics [69, 70]. More complex eukaryotic cells can also be investigated by this approach. For example, stem cells directed to cardiac myocyte differentiation begin to beat at a point in differentiation. This beating motion requires a major reorganization of the cell cytoskeleton and in turn a significant change in cellular nanomechanical properties. Concerning the cytoskeleton, it is now evident that transferring of mechanical vibration to the subcellular environment triggers the mobilization of ionic species and the generation of ionic fluxes and induced microcurrents, ultimately ensuing in the appearance of oscillating electromagnetic fields [11]. Therefore, application of mechanical vibration is expected to generate endogenous electromagnetic fields. This perspective is highly consonant with the complex dynamics in microtubular networks described above.

Hyperspectral imaging (HSI) is also currently emerging as a major tool to afford automated unbiased, non-invasive monitoring of cellular vibration patterning [71, 72]. HSI provides measurement of the electromagnetic radiation reflected from an object or scene (i.e., materials in the image) at many narrow wavelength bands. By the aid of a peculiar photocamera adapted to the stage of an inverted microscope it is now possible to use a dedicated software for "floating point" analyses of pixel reflection at all given wavelengths, affording spatial resolution of fluctuations in pixel luminance and chrominance, corresponding to a pixel-related spectral signature [73-75]. In this regard, HSI may offer several advantages over AFM in recording the vibrational pattern of cells, as HSI is not affected by the bias introduced by the contact modes of the AFM cantilever with the cell surface, which may itself suppress weaker nanomotions, erasing relevant vibrational information.

Future Perspectives

In conclusion, new therapeutic approaches may develop in a near future based upon the use of physical energies

(electromagnetic fields, sound vibration, light) to target directly the stem cells where they are *in vivo*, in all tissues of our body (tissue-resident stem cells). Due to the diffusive features of these energies, (stem) cell reprogramming may occur *in situ* paving the way to a Regenerative Medicine afforded through the stimulation of the natural ability of tissues for self-healing, without the needs of stem cell transplantation.

We forecast a near future where a new generation of electromagnetic and vibrational devices may unfold the use of physical energies to create a Regenerative/Precision Medicine characterized for being: (i) non-invasive, (ii) custom designed and personalized, but at the same time (iii) deliverable on a large-scale bases and (iv) extremely cost-effective.

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