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VIEWPOINT

Biophysical signalling from and to the (stem) cells: a novel path to regenerative medicine

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We are a part of and we play a part in the vibratory nature of the Universe, involving both electromagnetic vibrations and sounds. There is now compelling evidence that our cells produce and sense energies like magnetic fields, and they do generate and perceive mechanical oscillation.[1–3] These inherent mechanical motions may fall within the audible or subsonic range, prompting further dissection of their multifaceted mode of action. Despite the increasing need to gain new mechanistic insights, what is relevant is the awareness that our cells, and their subcellular structures, are continuously oscillating.

Any essential feature in our cells is modulated in a rhythmic fashion. To take cytosolic calcium homeostasis, a major signalling trait, as an example: it is fashioned upon waves, which means that cells exhibit rhythmic calcium oscillations, and that their rhythm can be likened to an input that provides information by itself.[4]

Biomolecular recognition is also insolvably linked to the oscillatory nature of subcellular components. The conventional view is that signalling molecules have to interact like a key in a lock to trigger an event. This is certainly the case, but there is also evidence indicating that cellular reactions exhibit a timely, wide-ranging connectedness which is too fast to be explained solely by the simple molecular diffusion in water. Most water molecules are bound to subcellular structures, which are constantly moving and oscillating, such as the cytoskeleton and the nucleoskeleton, forming a sort of textile network, encompassing the nucleus, mitochondria, and the endoplasmic reticulum, that would create serious problems to a merely diffusive trafficking of signalling molecules.

If we think of proteins in physical terms, we may see their α-helices as being like springs and the turns between them as connectors, making the system in a single protein capable of vibrating in a sort of phase resonance. This oscillator (the protein) is like a metronome, which, owing to molecular motors such as kinesins or dyneins, is able to move along the cyto-nucleo-skeleton, where the microtubules act as an elastic network dissipating the major rhythmic differences among the various oscillators that make up the ensemble of signalling molecules.[5,6] This context facilitates and promotes the achievement of synchronization phases, with each element of the network remaining individual and, at the same time, becoming aware of what is occurring in the system, because of its inherent connectedness.

This entire approach takes a glimpse on both nanomechanics and quantum field theory because the major challenge is not looking at the single event *per se*, but rather is investigating how multiple patterning can share information.

Consonant with this view, the cellular microtubuli, due to their intrinsic vibration modes and electrical polarity, are now regarded as a system capable of generating high-frequency electric fields with radiation characteristics.[7] This oscillating field appears to be important for the intracellular organization and intercellular interaction. Electrodynamic activity of a variety of cells in the frequency region from kHz to GHz has been experimentally detected, expecting the microtubules to be the source of this activity.[7] Intriguingly, multilevel switching properties have been detected at the single microtubule level.[8] In particular, memory states have been shown to form in this sort of nanowire whose protein arrangement symmetry is related to the conducting state written in the micrutubule itself.[8] This element can be viewed as a device that has been experimentally shown to be able to store and process information, being an analogue of the flash memory switch in a computer chip.

The cellular ability to generate and handle electromagnetic patterning is also the underpinning for considering 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-MFs) resulted in the transcriptional modulation of an endorphinergic system,[9] which was previously shown to be essential in the regulation of myocardial growth, cytosolic calcium oscillatory patterns, and myofilament responsiveness to calcium. ELF-MFs were also found to induce a high throughput of cardiogenesis and a

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remarkable increase in spontaneously beating cardiac myocytes from mouse embryonic stem (ES) cells.[10]

More recently, we discovered that radioelectric fields of 2.4 GHz could be delivered to cultured cells by the aid of a Radio Electric Asymmetric Conveyer (REAC). The REAC technology generates radioelectric asymmetrically conveyed microcurrents in tissues, without depth limits. The sum of these radioelectric-induced microcurrents produced by the device, and the electromagnetic field generated by the cell culture or patient's body, are concentrated by the asymmetric conveyer-probe (ACP) of the device, in order to optimize their bioelectrical activity. This innovative strategy was found to induce remarkable biological effects at many interconnected levels, from gene modulations up to functional remodelling. REAC treatment enhanced pluripotentiality expression and led to high-yield commitment towards myocardial, neuronal, and skeletal muscle differentiation in both mouse ES cells[11] and human adipose-derived mesenchymal stem cells (hADSCs).[12] We found that commitment to the same lineages could be efficiently afforded by REAC-delivered radioelectric fields in human skin fibroblasts.[13] For the first time, human non-stem somatic adult cells were reprogrammed to lineages in which they would never otherwise appear, avoiding potentially risky gene delivery by viral vectors, and without the requirement for cumbersome and expensive chemistry, as is needed in reprogramming induced by non-integrating technologies. Moreover, somatic cell reprogramming by the REAC technology involved a transient overexpression of stemness genes, followed by their down-regulation,[13] without freezing the exposed cells into an embryonic-like intermediate, which may entail the persistence of stray cells with potential tumorigenic drift.

Intriguingly, exposure of hADSCs to REAC-conveyed radioelectric fields was able to reverse their senescence *in vitro*.[14] REAC treatment significantly decreased the number of hADSCs positively stained for senescence-associated β-galactosidase along multiple culturing passages.[14] After 30 passages in culture, the REAC treatment was able to enhance *TERT* gene expression, encoding the catalytic core of telomerase, increasing the telomere length, with full recovery of the multilineage potential of hADSCs.[14] The antisenescence effect of REAC also involved the activation of a telomerase-independent pathway, leading to an up-regulation in *Bmi-1* gene expression and stemness-related genes and proteins.[14] The ability of REAC to act on both telomerase-independent and telomerase-dependent patterning to optimize stem cell ability to cope with progression of senescence may have important biomedical implications. In fact, stem cells, like any other cell in the body, undergo senescence, which hampers their own self-renewal and differentiation potential, and may account for the age-associated decline in the self-healing potential of tissues and organs. Moreover, senescence from prolonged stem cell expansion *in vitro*, as is carried out to enhance the cell number prior to transplantation, significantly impairs expression of pluripotency/multipotency, leading to a consistent decline in the multilineage repertoire and in the yield of differentiated cells. The ability of REAC to act as a 'time machine' on the chronobiology of stem cells may act as the basis for future approaches in tissue rejuvenation and may optimize the therapeutic outcome of expanded cells.

Compounding the potential to exploit the REAC technology in different contexts of regenerative medicine is its ability to afford neurological and morphofunctional differentiation in PC 12 cells,[15] a rat adrenal pheochromocytoma cell line displaying metabolic features of Parkinson's disease. The REAC action was mediated by the transcriptional activation of neurogenic genes, such as neurogenin-1, β3-tubulin, and nerve growth factor, and was associated with a consistent increase in the number of cells expressing both β3-tubulin and tyrosine hydroxylase.[15]

These findings open up new perspectives for the use of physical energies in the treatment of neurodegenerative diseases.

On the whole, biophysical signalling 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 in a self-healing programme for damaged tissues.

Within this context, we have shown and patented for the first time the ability of cells to express 'vibrational' (nanomechanical) signatures of their health, and their multilineage repertoire.[16] A large variety of biological processes rely upon the nanomechanical properties of subcellular structures, such as the microtubular network and, more in general, the cytoskeleton and nucleoskeleton whose intrinsic rhythmic behaviour imparts features characteristic of connectedness and synchronization modes that can be transmitted up to and recorded from the cell surface. Atomic force microscopy (AFM) can be used to acquire information on cellular nanomechanical properties,[16,17] providing the opportunity to identify vibrational signatures that can be used to afford lineage-specific commitments in undifferentiated cells.

In conclusion, new therapeutic approaches may develop in the 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 any tissue of our body (tissue-resident stem cells). Due to the diffusive nature of this energy, (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 need for stem cell transplantation. Heart failure, a multi-aetiological syndrome with different pathophysiological paths converging in common biological alterations, both cardiac and systemic, holds promise for becoming one of the most interesting experimental areas.

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References

1. Albrecht-Buehler G. Rudimentary form of cellular 'vision'. *Proc Natl Acad Sci USA* 1992;**89**:8288–8292.
2. Albrecht-Buehler G. A long-range attraction between aggregating 3T3 cells mediated by near-infrared light scattering. *Proc Natl Acad Sci USA* 2005;**102**:5050–5055.
3. Uzer G, Thompson WR, Sen B, Xie Z, Yen SS, Miller S, Bas G, Styner M, Rubin CT, Judex S, Burridge K, Rubin J. Cell mechanosensitivity to extremely low-magnitude signals is enabled by a LINCed nucleus. *Stem Cells* 2015;**33**:2063–2076.
4. Nivala M, Ko CY, Nivala M, Weiss JN, Qu Z. The emergence of subcellular pacemaker sites for calcium waves and oscillations. *J Physiol* 2013;**591**:5305–5320.

5. Martens EA, Thutupalli S, Fourrière A, Hallatschek O. Chimera states in mechanical oscillator networks. *Proc Natl Acad Sci USA* 2013;**110**:10563–10567.
6. Schaap IA, Carrasco C, de Pablo PJ, Schmidt CF. Kinesin walks the line: single motors observed by atomic force microscopy. *Biophys J* 2011;**100**: 2450–2456.
7. Havelka D, Cifra M, Kučera O, Pokorný J, Vrba J. High-frequency electric field and radiation characteristics of cellular microtubule network. *J Theor Biol* 2011;**286**:31–40.
8. Sahu S, Ghosh S, Hirata K, Fujita D, Bandyopadhyay A. Multi-level memory-switching properties of a single brain microtubule. *Appl Phys Lett* 2013;**102**:123701.
9. Ventura C, Maioli M, Pintus G, Gottardi G, Bersani F. Elf-pulsed magnetic fields modulate opioid peptide gene expression in myocardial cells. *Cardiovasc Res* 2000;**45**:1054–1064.
10. Ventura C, Maioli M, Asara Y, Santoni D, Mesirca P, Remondini D, Bersani F. Turning on stem cell cardiogenesis with extremely low frequency magnetic fields. *FASEB J* 2005;**19**:155–157.
11. Maioli M, Rinaldi S, Santaniello S, Castagna A, Pigliaru G, Gualini S, Fontani V, Ventura C. Radio frequency energy loop primes cardiac, neuronal, and skeletal muscle differentiation in mouse embryonic stem cells: a new tool for improving tissue regeneration. *Cell Transplant* 2012;**21**:1225–1233.
12. Maioli M, Rinaldi S, Santaniello S, Castagna A, Pigliaru G, Delitala A, Bianchi F, Tremolada C, Fontani V, Ventura C. Radio electric asymmetric conveyed fields and human adipose-derived stem cells obtained with a non-enzymatic method and device: a novel approach to multipotency. *Cell Transplant* 2014;**23**:1489–1500.
13. Maioli M, Rinaldi S, Santaniello S, Castagna A, Pigliaru G, Gualini S, Cavallini C, Fontani V, Ventura C. Radio electric conveyed fields directly reprogram human dermal-skin fibroblasts toward cardiac-, neuronal-, and skeletal muscle-like lineages. *Cell Transplant* 2013;**22**:1227–1235.
14. Rinaldi S, Maioli M, Pigliaru G, Castagna A, Santaniello S, Basoli V, Fontani V, Ventura C. Stem cell senescence. Effects of REAC technology on telomerase-independent and telomerase-dependent pathways. *Sci Rep* 2014;**4**:6373.
15. Maioli M, Rinaldi S, Migheli R, Pigliaru G, Rocchitta G, Santaniello S, Basoli V, Castagna A, Fontani V, Ventura C, Serra PA. Neurological morphofunctional differentiation induced by REAC technology in PC12. A neuro protective model for Parkinson's disease. *Sci Rep* 2015;**5**:10439.
16. Gimzewski JK, Pelling A, Ventura C. INTERNATIONAL PATENT: International Publication Number WO 2008/105919 A2, International Publication Date 4 September 2008. Title: Nanomechanical Characterization of Cellular Activity.
17. Pelling AE, Sehati S, Gralla EB, Valentine JS, Gimzewski JK. Local nanomechanical motion of the cell wall of *Saccharomyces cerevisiae*. *Science* 2004;**305**:1147–1150.