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Bologna, April 24, 2019

Prof. Ying Dou,
Science Editor, Editorial Office
World Journal of Stem Cells

Subject: *Review* Article
Manuscript NO: 46336

Dear Professor Ying Dou,

We are submitting the revised version of our review manuscript entitled *"Physical Energies to the Rescue of Damaged Tissues"* by Federica Facchin, Silvia Canaider, Riccardo Tassinari, Chiara Zannini, Eva Bianconi, Valentina Taglioli, Elena Olivi, Claudia Cavallini, Marco Tausel, Carlo Ventura, for consideration by the Editor and Reviewers of *World Journal of Stem Cells.*

We would like to thank the Editor and the Reviewers for their precious time and invaluable comments. We have carefully addressed all the comments. The corresponding changes and refinements made in the revised paper are highlighted in the manuscript and summarized in our response below. English language has been revised and spell corrections have been made.

REPLY TO EDITOR

We would like to thank the Editor for his precious time and invaluable comments.
We understand his suggestion and the motivations of his requests. Below, we have carefully addressed all comments showed in 46336-edicted file.

Editor's comments in 46336-edicted file:
Please provide the manuscript documents in word version so that we can edit.

AUTHORS' RESPONSE:

We have provided the manuscript documents in word version.

Editor's comments in 46336-edicted file:
Please revise the manuscript according to the review report and my comments.
And answer all of the reviewers' comments carefully (point-to-point).

AUTHORS' RESPONSE:
We have revised the manuscript according to the review report and Editor's comments. We have replied point-by point to the comments.

Editor's comments in 46336-edicted file:
Also, please check and revise the manuscript according to the CrossCheck report, especially part 7, 9,13,14.

AUTHORS' RESPONSE:
We have checked and revised the manuscript according to the CrossCheck report. In order to remove similarity with the manuscript entitled "Seeing Cell Biology with the Eyes of Physics", Nano World Journal, 2017 of Carlo Ventura, we have changed the manuscript where it was requested (parts entitled 1). Below we reply point-by point to the comments.

In the **Introduction**

the sentences:
"Accordingly, by the aid of innovative approaches, such as the Resonant Recognition Model (RRM), DNA has been found to exhibit wide-ranging electromagnetic resonance frequencies from THz to KHz spectra[36]. The RRM is based upon the initial observation that periodicities in energy distribution of delocalized electrons within a given protein are essential for its function, and activity, like protein-protein, or protein-DNA interaction, as it occurs following transcription factor binding to, and bending of DNA, two major steps in transcriptional modulation[37]. 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[37,38]. These theoretically calculated spectra have been confirmed experimentally[39,5]. Moreover, RRM allows designing of new peptides with desired spectral characteristics, and biological activities[40]. The RRM enables these spectral characteristics, which fall within the infrared and visible light spectra, to be calculated[37]."

have been replaced with the sentences:
"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 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[39,5]. Another advantage in the use of RRM is the chance of excogitating novel peptides with unprecedented spectral features and bioactivities[40]."

The sentences:
"The overall scenario is emerging of an intracellular environment where complex

nanoarchitectonics are designed within a network of micro-tubules and -filaments which are far away from simply being the cytoskeleton. These elements may rather be acting 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."

have been replaced with the sentences:
"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."

The sentences:
"Here, we will discuss these issues with particular regard to the stem cell biology, within the biomedical perspective of using physical energies to govern (stem) cell fate. We will focus on mechanobiology, and the chance of using mechanical waves to elicit self-repairing mechanisms and tissue rescue in a number of pathological conditions. We will dissect the ability of specially conveyed electromagnetic fields to optimize stem cell potency and differentiation, reversing stem cell aging and promoting a multilineage repertoire in both human adult stem cells, and non-stem somatic cells."

have been replaced with the sentences:
"Here, we will discuss these issues with particular regard to the 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."

In the **"CELLULAR MICROTUBULES: A NETWORK OF OSCILLATORS THAT SYNC AND SWARM"** paragraph

the sentences:
"Scanning tunneling Microscopy (STM), coupled with an ad-hoc designed artificial cell-like environment arranged to pump electromagnetic frequencies to microtubules growing onto a nanoelectrode array, has provided evidence for the occurrence of specific resonance modes between the applied frequency and tubulins, tubulin dimers, as well as the whole microtubules[5]. STM also revealed that specific "tunneling current images" are produced by microtubules as resonance patterns in response to electromagnetic frequencies in the MHz domain[5]. A peculiar conformational patterning can be therefore elicited in microtubules by remotely applying an electromagnetic field: electro-mechanical coupling occurs at microtubular level as a function of defined resonance patterns between the frequencies of the incoming electromagnetic fields and those developed by the microtubules themselves[5]. These findings add further support for consideration of microtubules as an intracellular bioelectronic circuit. Akin to such perspective, is the theoretically calculated proposal for the emission of high-frequency electric fields with radiation characteristics from microtubules[14] and even the experimental detection of multi-level memory-switching properties at the level of a single brain microtubule[59]."

have been replaced with the sentences:
"Scanning Tunneling Microscopy (STM), coupled with and 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[5]. STM analysis also provided evidence that the resonant tunneling currents elicited by microtubules occurred in response to electromagnetic fields applied within a MHz range[5]. 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[5]. These observations further support the idea that microtubules may act as an intracellular bioelectronic circuit. Consonant with such perspective, are (i) theoretical calculations considering the microtubules as elements generating electric fields of high frequency and radiation features[14], and (ii) 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, equatable to a memory switch device with a near-to-zero hysteresis loss[59] (Figure 1)."

In the **"BIOMOLECULAR RECOGNITION PATTERNING"** paragraph

the sentences:

"The actual speed of molecular interplay within complex cellular decisions (i.e. the commitment towards defined stem cell lineages, and terminal differentiation), as conceived on the assumption of diffusive mechanisms, would be unpredictably influenced by the fact that, owing to the presence of different glycosaminoglycans (i.e. hyaluronic acid), the intracellular environment is not comparable to an aqueous salt solution. Such environment should be rather considered as a non-homogenous, aqueous gel, whose composition and diffusive features are continuously changing in response to the cellular metabolic patterning. Is there a way to reconcile the current understanding of microtubular dynamics with a novel view of intra- and inter-cellular connectedness that may result more consonant with the astonishing speed at which cells forge their fate? Vast majority of signaling proteins exhibit helix-turn-helix modules, which can be reckoned as oscillators, with helices acquitting the role of oscillating springs, and the turns behaving as connectors between oscillators. A single protein can be viewed as a phase-resonant vibrating entity[7]. Near terahertz field microscopy (TFM) has made possible to detect protein vibrations, midget motions essential for Life[7]."

have been replaced with the sentences:
"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 microtubular role in tuning intra- and inter-cellular 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]. Terahertz near-field microscopy (TFM) has been exploited to detect protein vibrations, midget motions essential for Life[7]."

The sentences:
"Cell proteins not only diffuse through water, but they can "walk" onto microtubular tracks availing of "molecular motors", such as kinesins and dyneins[35]. Signaling molecules can be equated to oscillators traveling across the cytoskeletal web, with the microtubules (and other intracellular filaments) dissipating vibrational differences among oscillators."

have been replaced with the sentences:

“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 sentences:
“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 pure mechanical changes can be remotely tuned in a precise structural fashion by the remote application of electromagnetic fields[5].”

have been replaced with the sentences:
“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].”

The sentences:
“Protein cavities would appear as domains where an electric vector from an electromagnetic field and its magnetic component may interact with suitable structures within the cavity itself. Albeit protein cavities may entail nanotopographies sensitive to electric resonance, the issue as to whether these cavities may act as sensors generating resonances in the presence of an electromagnetic fields remains an open and difficult to answer question.”

have been replaced with the sentences:
“Protein cavities would appear as domains arranged for the interaction of the electric and magnetic components of an incoming electromagnetic field. However, 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.”

In the **“Cellular nanomotions: vibrational signatures to direct stem cell fate”** paragraph

the sentences:
“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-skelethon, which will be reflected in remarkable changes in nanomechanical patterning recordable at the cell surface. Within this context, we have first shown and patented the cell ability to convey at their surface nanomechanical signatures of their adaptive states (i.e. their healthy status, or the growth within a hostile environment) and differentiating potential[100].”

have been replaced with the sentences:
“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 Microsocpy (AFM) to afford a nanomechanical characterization of cellular activity, detecting defined signatures corresponding to the cellular healthy or non-healthy status, or to specific differentiating pathways[100].”

In the **“Electromagnetic fields”** paragraph

the sentences:
“In this regard, we first provided evidence that extremely low-frequency pulsed magnetic fields acted on adult ventricular cardiomyocytes to induce the transcription of an endorphinergic system[101], which was previously found to be essential in cytosolic calcium[102] and pH[103] homeostasis, in the regulation of myocardial growth[104-106] and the orchestration of stem cell cardiogenesis[107-109].”

have been replaced with the sentences:
“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 an 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].”

The sentences:
“We found that a radio-electric field of 2.4 GHz, a frequency used worldwide for the Internet, could be conveyed to cultured (stem) cells via ad hoc designed Radio Electric Asymmetric Conveyer (REAC)[111].”

have been replaced with the sentences:
“We found that a radioelectric field of 2.4 GHz, the same frequency used in Wi-Fi (wireless fidelity) technologies, can be conveyed in vitro to stem and somatic cells via an ad hoc designed Radio Electric Asymmetric Conveyer (REAC)[111].”

The sentences:
“Another breakthrough coming from analysis of biological effects produced by REAC conveyed radioelectric fields was the observation that this treatment was able to reverse the senescence of human adult stem cells in vitro[118].”

have been replaced with the sentences:
“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].”

The sentences:
“(iiiii) akin to its pleiotropic functions, HA has been used in the form of mixed ester of butyric and retinoic acids to promote cardiogenesis in both mouse ES cells[125] and human adult stem cells in vitro and in vivo[126-128], even affording efficient myocardial repair in vivo without stem cell transplantation in infarcted rat hearts[129].
Compelling evidence relates impairment in stem cell polarity with stem cell aging, as well cancer development[124]. In Drosophila, aged germ line stem cells exhibited reduced self- renewal capability as a consequence of centrosome misorientation and altered cell polarity within their stem cell niche[124,130]”.

have been replaced with the sentences:
“(iiiii) 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 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]."

Editor's comments in 46336-edicted file:
Please check and confirm that there are no repeated references

AUTHORS' RESPONSE:
We confirm that there are no repeated references in the manuscript.

Editor's comments in 46336-edicted file:
Please provide the decomposable figure of Figures, whose parts are movable and editable. So you can put the original pictures in ppt and submit it in the system.

AUTHORS' RESPONSE:
We submit in the system Figure 1 (new), 2 and 3 in ppt, in order to provide movable and editable images.
We also submit three Table (1, 2 and 3) that resume shock waves, electromagnetic field and photobiomodulation studies, respectively, at the end of the relative paragraphs of the manuscript.
In Table 3 and in the paragraph entitled "Photobiomodulation", we decided to add the citation of the paper of Blatt et al. (2016).
Therefore, in the manuscript now appear the following sentences:
"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[179]. The LLLT action was mediated by an increase in the number of circulating c-kit+ stem cells during the first 48 hours post-infarction[179]. These beneficial effects resulted to be a long-lasting outcome of the laser treatment, since they were documented 90 days after the infarct induction[179]. 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".

REPLY TO REVIEWERS

Response to Reviewer 1 Comments

REVIEWER'S GENERAL COMMENT:
The manuscript by Facchin and colleagues is a detailed review of various physical energies and how they regulate biological processes. The authors extrapolate there findings to focus on how different physical forces may be of use in regenerative medicine. The evidence supporting the use of these technologies in regenerative medicine is the weakest part of the manuscript. Overall, this is a fascinating review of an area that needs far more attention than is currently afforded in the biological sciences. The large number of studies that the authors have pulled together that support the role of mechanical forces, electric fields, electromagnetic fields, electromagnetic radiation and photobiomodulation in regulating biological processes has reached the point where traditional biologists can no longer ignore the importance of these signals in translating their research findings. It is hoped that this review will prompt further research into these intriguing areas.

AUTHORS' RESPONSE:
We would like to thank the Reviewer for her/his favorable opinion about our paper and for the enclosed comments. We are pleased that our paper was found fascinating.

Changes and refinements have been made in the revised paper and they are highlighted in the manuscript.
Moreover, the manuscript was checked for grammatical errors and English improvement were made.

Response to Reviewer 2 Comments

REVIEWER'S GENERAL COMMENT:
Dear Authors, the reviewer would like to thank the authors for the interesting topic. The manuscript is relatively well prepared. However, it may be easily reader for the readers. Addition of several images regarding the data with tables may improve the quality of the manuscript. Thank you very much.

AUTHORS' RESPONSE:
We would like to thank the Reviewer for her/his precious time and invaluable comments. We are pleased that our paper was found interesting.
We agree with the Reviewer that additional figure and table could improve the quality of the manuscript. We now added a Figure (the new Figure 1) that summarizes the role of microtubules as cellular oscillators and the Table 1, 2 and 3 that resume shock waves, electromagnetic field and photobiomodulation studies, respectively.
Changes and refinements made in the revised paper are highlighted in the manuscript.
Moreover, in order to correct English language and style, the manuscript has been revised.

We hope to have properly understood the minor and major revisions requested and we remain at your disposal for any further information you may require.

Hoping that you will consider the revised version suitable for publication in *World Journal of Stem Cells*

Thank you in advance for your consideration.

Yours sincerely,

Carlo Ventura, M.D., Ph.D.
University of Bologna, Italy