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REVIEW

Stem Cells and Physical Energies: Can We Really Drive Stem Cell Fate?

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Summary

Adult stem cells are undifferentiated elements able to self-renew or differentiate to maintain tissue integrity. Within this context, stem cells are able to divide in a symmetric fashion, feature characterising all the somatic cells, or in an asymmetric way, which leads daughter cells to different fates. It is worth highlighting that cell polarity have a critical role in regulating stem cell asymmetric division and the proper control of cell division depends on different proteins involved in cell development, differentiation and maintenance of tissue homeostasis. Moreover, the interaction between cells and the extracellular matrix are crucial in influencing cell behavior, included in terms of mechanical properties as cytoskeleton plasticity and remodelling, and membrane tension. Finally, the activation of specific transcriptional program and epigenetic modifications contributes to cell fate determination, through modulation of cellular signalling cascades. It is well known that physical and mechanical stimuli are able to influence biological systems, and in this context, the effects of electromagnetic fields (EMFs) have already shown a considerable role, even though there is a lack of knowledge and much remains to be done around this topic. In this review, we summarize the historical background of EMFs applications and the main molecular mechanism involved in cellular remodelling, with particular attention to cytoskeleton elasticity and cell polarity, required for driving stem cell behavior.

Key words

Stem cells • Cell polarity • Epigenetic • Electromagnetic fields • Physical stimuli

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Historical background

Since the ancient times, the idea of using electric currents and magnets for healing have intrigued humanity. In China, more than 2000 years ago, “magnetic stones” were advocated to correct health imbalances (Unschuld 2003). In 46 aC, Scribonius Largus, a roman physician, mentioned the use of torpedo fish for treatment of headaches and gout in his Compositiones Medicae (Bennett *et al*. 1986). In the last two centuries, with the development of the electromagnetic theory, there has been a surge increase in the interest to the interaction of electromagnetic fields (EMFs) and various life processes. Particularly into the 20th century, plenty of bio-effects of electric, magnetic, and electromagnetic fields on human beings, animals and cells have been described either in vitro or *in vivo* conditions (Berg 1997, Berg 1999, Hönes

et al. 1998). EMFs have reportedly therapeutic potentials for a wide variety of diseases including musculoskeletal diseases, cancer treatment, neurological disorders, wounds (Yadollahpour and Rashidi 2014, Shahpari *et al.* 2012, Ottani *et al.* 1988, Kloth and Feedar 1988).

The most famous and controversial pioneers in this field are probably Georges Lakhovsky and Antoine Priore. In the 30's, Lakhovsky invented the Multiple Wave Oscillator, which he claimed would revitalize and strengthen the health of cells, using resonance effects produced by his device (Lakhovsky 1992). Priore built in the 50's a series of electromagnetic devices producing a strong magnetic field for the purpose of treating cancer and disease. He reported to have treated a number of animals with cancer with his last most important device funded by French government, but this device broke down before it could be applied to human patients (Graille 1984). But both encountered big difficulties with the traditional medical community and these devices disappeared, even if there are still revivals in different forms even nowadays.

Concerning the field of regeneration, the most famous scientist and absolute pioneer is surely Alexander Gurwitsch. In 1922, he discovered that living cells separated by quartz glass were able to communicate vital-cell information. Numerous experiments suggested that this information was transmitted by invisible light waves in a UV frequency spectrum passed by quartz and stopped by window glass. Gurwitsch coined the phrase "mitotic" wave since it was observed during enzymatic reactions and mitosis. He also determined that muscle tissue, cornea, blood and nerves are all transmitters of this special energy. His work is the first documented evidence of "biophotons," coherent light emitted by animal and plant cells intensively studied later by Fritz-Albert Popp, and became the basis for the design of later bioelectromagnetic therapy devices (Gurwitsch 1968, Popp *et al.* 2002). Other important contributions are due to H. Saxton Burr and F. Stuart Northrope, who studied the role of bioelectric signals in embryonic development and regeneration, assuming the existence in 1935 of a bioelectrical field that they called "L-field" (Burr and Northrop 1939).

Significant steps forward in this topic were made by Robert Becker. Becker was intrigued by the capacity of the salamander to regenerate a paw from bones, muscles or nerves. Therefore, he decided to study the electric potential around the wounds, finding very small currents (2-3 μA), for some days first negative, and then positive. Blocking these currents stops the regeneration of the tissue. His theory about the observations is fascinating and revolutionary: after a fracture or a wound, two electric currents are produced by the organism. One is coming from the injured tissue (bone or muscle), the other comes from the nerves. Both together meet and build a sort of battery, a "living electrode". In this way, the organism makes his own diagnosis, like X Rays, and leads the regrowth. At this point an incredible phenomenon takes place: specialized cells like erythrocytes are able to de-differentiate. In other terms, they acquire again like in the embryonal state, the capability to transform in any kind of cell. This phenomenon is unfortunately not possible for mammals, because they do not possess enough nervous cells, mostly concentrated in the brain. In 1971, with an external electric current, Becker succeeds in obtaining the beginning of regeneration on a mice paw, but for unknown reasons, the process stops along the way (Becker 1972). Becker popularized these and other advances in our understanding of the role of electric and magnetic fields in healing and regeneration in two books, The Body Electric (Becker 1985) and Cross Currents (Becker 1990).

Today, Michael Levin and colleagues at Tufts University are probably the most important active research group continuing to forward the exploration of bioelectromagnetics and physiology. Their research focuses on the genetic and biophysical mechanisms that enable decision-making during the complex development of the embryo and of individual organs and harnessing these insights towards developing new solutions for regenerative medicine and cancer suppression. By demonstrating that patterns in the electrical activity of biological cells act as key regulators of a variety of critical processes such as embryogenesis, regeneration, tumorigenesis and metastatic transformation, and that electrical patterning plays key roles in regenerative processes such as limb regeneration in salamanders, eye induction, craniofacial patterning, and head-tail polarity in planaria, Levin has shown that patterns of bioelectric signaling constitute "…*an autonomous layer of control not reducible to a biochemical or genetic account of cell state*" (Levin 2014).

Cell polarity

Adult stem cells from different tissues are known for self-renewing and for their capability to

interact with their niche, maintaining a quiescent state, or undergoing differentiation toward a specific phenotype (Voog and Jones 2010). Mesenchymal stem cells can be isolated from different tissue, as bone marrow (Gnecchi and Melo 2009), adipose tissue (Araña *et al.* 2013), umbilical cord (Balzano *et al.* 2019), dental pulp (Ranganathan and Lakshminarayanan 2012). Stem cell fate can be modulated by chemical and physical intracellular or extracellular stimuli, that act through activation or repression of specific molecular pattern controlling cell proliferation and/or differentiation (Cruciani *et al.* 2019). Cell polarity is a fundamental property of migrating cells, and is related to the membrane organization and cytoskeleton arrangement that occur in response to different kind of signals (Goehring and Grill 2013, Cheng and Zygourakis 2007). Polarization and the capability to redistribute their cellular organelles and proteins, is essential for stem cell division and migration or adhesion to extracellular matrix (ECM) (Florian and Geiger 2010). In stem cells, the balance between symmetric and asymmetric division is aimed at regulating cell viability, proliferation and tissue homeostasis, and is perfectly controlled during the entire organism life (Egger *et al.* 2011, Wignall 2015). Asymmetric division results in two different daughter cells, one of which maintains the same self-renewal features of the mother cell and the other one with a reduced regenerative potential, that undergo differentiation toward a specific cell type (Murke *et al.* 2015). Dysregulation in this system leads to loss of tissue function or to uncontrolled cell proliferation, responsible for cancer initiation and progression (Muthuswamy and Xue 2012, Gómez-López *et al.* 2014). Moreover, stem cells lose their regenerative potential, dividing mainly in a symmetrical manner during their life (Jung and Brack 2014). Aging of hematopoietic stem cells (HSCs), as well as of mesenchymal stem cells from different tissues, is associated with loss of polarity and Wnt pathway dysregulation. This pathway is involved in hematopoiesis, thus its proper activation is important for homeostasis maintenance (Schultz and Sinclair 2016). Some authors demonstrated that a single-daughter cell transplant in mouse with aged HSCs, results in restoring asymmetric divisions and in rejuvenating their daughter stem cells (Florian *et al.* 2018). Endogenous electrochemical signals, as ion fluxes or membrane potential gradients, exert a key role in the activation of specific ion transporters and in regulating cell polarity during wound healing and regeneration processes (Campetelli *et al.* 2012, Ikehara *et al.* 1998, Goudarzi *et al.* 2010). Membrane potential is dynamically regulated in order to guide import and export of anions and cations through the membrane, modulating the composition of extra-cellular liquids (Levin 2012). Recent studies on electric field responses in biological systems provided the ability of exogenous electric fields (EFs) to orient cell polarity, migration and division, also affecting the behavior during tissue regeneration (Chang and Minc 2014, Funk *et al.* 2009, Zhang *et al.* 2013, Pesce *et al.* 2013). For example, the Radio Electric Asymmetric Conveyer (REAC) technology, delivering radioelectric fields of 2.4 GHz, is able to optimize ions fluxes and drive cellular asymmetry and polarization, directly interacting with intrinsic cell electric field (Maioli *et al.* 2016b). Moreover, it has been demonstrated that embryonic stem cells stimulation by REAC, was able to exert a biphasic effect, optimizing the expression of pluripotency-related genes, or implementing stem cell differentiation potential without the use of chemical agents (Maioli *et al.* 2012). The same technology was able to act on human induced pluripotent stem cell (iPS) obtained from urine, increasing the achievement of a cardiogenic phenotype (Basoli *et al.* 2019). Within this context other studies revealed that iPS stimulation with EFs, exhibited an increase in cell migration and expression of pluripotent markers, unravelling a novel technique to facilitate stem cell therapy and migration of transplanted stem cells (Zhang *et al.* 2011).

Cytoskeleton and membranes

Cytoskeleton is a complex dynamic structure inside the cytoplasm of cells, which is responsible for cell movements, giving them the capability to resist to deformation, mediating signal transduction from ECM (Fletcher and Mullins 2010). Cytoskeleton rearrange its structure, according to environmental physical and chemical stimuli, through polymerization cycles of actin structures (Stricker *et al.* 2010). In stem cell biology, cytoskeleton exerts a central role in differentiation processes, plasticity, mechanotransduction and niche activities (Vining and Mooney 2017). In response to signals received from their microenvironment, unspecialized stem cells start to differentiate into mature specialized cell, involving cytoskeletal reorganization, fiber formation, expression of adhesion molecules and cell division (Ambriz *et al.* 2018). Several signaling and adhesion molecules connect the cytoskeleton with the

ECM, thus being involved in membrane mechanotrasduction (Schwartz and DeSimone 2008). Among these Members of the mitogen-activated protein kinase (MAPK) family, extracellular signal-regulated kinase (ERK), integrins, selectins, and laminin receptors can be identified (Liu and Lee 2014). Variation in cytoskeletal structures, microtubules and actin filaments, lead to functional changes, altering intracellular signaling cascade, as during cellular senescence, leading to a different expression of Cyclin-dependent kinase inhibitors p16 and p21 (Moujaber *et al.* 2019, Hernandez-Segura *et al.* 2019). Aged cells show loss of proliferation and migration, along with a reduced regenerative potential (Rao and Cohen 1990). Some authors demonstrated that the application of repeated electromagnetic field shock (REMFS) on aged T lymphocytes and fibroblast cell lines, exerts an anti-aging effect promoting Hsp90 release from the heat shock transcription factor (HSF1), inducing an increase in the number of proliferating cells (Perez *et al.* 2008a). The activation of cellular responses and repairing systems, mediated by REMFS-induced HSF1, could represent an important approach for managing Alzheimer and other age-related diseases (Perez *et al.* 2019, Perez *et al.* 2008b). Within this context, the exposure to electromagnetic fields induce a constant modulation of neural system, improving learning and memory impairment by stimulating neurogenesis in mice (Sakhaie *et al.* 2017, Leone *et al.* 2014), and implementing the achievement of a neurogenic phenotype in PC12 cell dopaminergic model (Maioli *et al.* 2015). When exposed to REAC technology, human adipose-derived stem cells (ADSCs) exposed to prolonged culturing senescence, showed a significant downregulation in the expression of senescence-associated genes (p16INK4, p53, p19) and β-galactosidase (Maioli *et al.* 2014a, Rinaldi *et al.* 2012). The anti-aging effect of REAC was mediated by the transcription of the catalytic telomerase subunit (TERT) and pluripotency-related genes. (Rinaldi *et al.* 2014), thus preserving cell commitment to different phenotypes.

Influence on genes and epigenetics

In vitro stem cell differentiation can be induced by chemical stimuli, such as small molecules that act modifying self-renewal, inducing cell differentiation and reprogramming (Ding and Schultz 2004, Li *et al.* 2013, Mansour *et al.* 2012, Mattout *et al.* 2011). Stem cell fate is controlled by multiple signaling pathways, involving miRNAs (Gangaraju and Lin 2009, Balzano *et al.*2018), and other epigenetic events, such as DNA methylation and chromatin remodeling, still not completely yet understood (Lacagnina 2019, Bhuvanalakshmi *et al.* 2017, He *et al.* 2019). Growth factors, hormones, cytokines and other pharmacological agents can be added to the culturing medium to influence stem cell commitment toward specific phenotype (Christ *et al.* 2013, Maioli *et al.* 2016a). In particular molecules as melatonin and vitamin D are able to influence adipogenic and osteogenic differentiation processes in ADSCs cultured in specific conditioned media, by activating a specific epigenetic program and regulating gene expression (Basoli *et al.* 2017, Santaniello *et al.* 2018). Bone Morphogenetic Protein 9 (BMP-9) is able to induce osteogenic differentiation in mesenchymal stem cell by interacting with Wnt and IGF signaling pathways (Luther *et al.* 2011). Synthetic variant of pyrrolopyrimidine TWS119 is able to induce neurogenesis in embryonic stem cells by modulating serine/threonine kinase GSK-3β functions (Ding *et al.* 2003). Physical stimuli, as low frequency electromagnetic fields (ELFEFs) have been recently applied in the area of stem cells in the attempt to optimize cell growth and differentiation (Maioli *et al.* 2013a), without the need of chemical agents (Ross *et al.* 2015). Stem cells exhibit different responses to EFs depending on their differentiation status, by modulation of transcription factors and of genes involved in cell cycle regulation (Belyaev *et al.* 2005, Sun *et al.* 2009, Liu *et al.* 2015). REAC technology was able to induce the commitment of ADSCs toward neuronal, skeletal muscle and cardiac phenotypes, as well as a direct reprogramming of human fibroblasts toward the same lineages, creating an ideal environment for cell differentiation to be used in regenerative medicine (Maioli *et al.* 2014b, Maioli *et al.* 2013b). EFs has a stimulatory effect on osteoblasts mineralization, associated with an enrichment in cellular differentiation and bone tissue-like formation (Diniz *et al.* 2002, Kang *et al.* 2013). MSCs exposed to ELFEFs exhibited an increase in the expression of neurogenic markers, in the yield of the neuronal (Cho *et al.* 2012, Meng *et al.* 2009) and astrocyte differentiation by modulating SIRT1 expression (Jeong *et al.* 2017). ELFEFs mechanism of action involves mainly an increase in cytosolic Ca^{2+}concentration and the activation of Ca^{2+}-binding proteins, leading to DNA methylation and histone post-transcriptional modifications in neurogenesis and osteogenesis processes (Leone *et al.* 2015). Moreover, the

increased calcium concentration due to magnetic field-mediated activation of voltage-gated calcium channels, promotes MSCs migration in injured sites, representing a valid way to increase the MSCs engraftment in clinical applications (Zhang *et al.* 2018).

Physical energies and biological systems

Several studies demonstrated the success of magnetic fields in driving stem cell commitment towards different phenotypes, and the chance to enroll the derived differentiated elements in clinical practice (Facchin *et al.* 2018). The interaction between EFs and biological systems generate responses at cellular and tissue levels, encompassing activation of specific molecular programs (Funk *et al.* 2006). In particular, considering its role in physiological regulation and in the organism response to the environment, nervous system represents a topic of interest for many researchers (Ramsay and Woods 2014). It has been previously demonstrated that ELFEFs improve neurogenesis and hippocampus-dependent memory in mice (Mastrodonato *et al.* 2018). In addition, they are able to induce proliferation and inhibit NSCs apoptosis, showing high potential in treatment of degenerative and psychiatric disease, as Parkinson, obsessive-compulsive disorder, epilepsy and other more (Cui *et al.* 2017, Mazzini *et al.* 2018). Nevertheless, EFs, being able to induce chondrogenic differentiation of progenitor cells and regeneration of articular cartilages can be also a valuable tool for *in vivo* management of musculoskeletal system, osteoarthritis, and chondral damages (Collodel *et al.* 2013, Sanna Passino *et al.* 2017, Hiraki *et al.* 1987). Physical stimuli find application also in cardiac-related diseases. Actually *in vivo* studies, both in mice and in humans, demonstrated that exposure of injured heart to EFs protects myocardium from the damages induced by oxidative stress, preserving metabolic activity in cardiomyocytes (Biały *et al.* 2018, Ma *et al.* 2013). Finally, although the mechanism of action is not completely understood yet, clinical studies in humans revealed that magnetic fields can be successfully applied, together with conventional therapies, for rheumatic diseases, anxiety, depression and pain care (Hattapoğlu *et al.* 2019, Zwolińska *et al.* 2016).

Conclusion

Stem cells represent an important resource for regenerative medicine and tissue repairing. Nowadays, many clinical trials involving stem cell transplantation for regeneration of functional organs are taking place, thanks to stem cell immunomodulatory activity and their capability to migrate toward injured tissues. EFs application could have an important role in regulating biological responses to the environment, by direct modulating stem cells fate in their own niche, or improving human intrinsic biological self-healing capability, without the need of cell transplantation.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

EMFs, electromagnetic fields; ECM, extracellular matrix HSCs, hematopoietic stem cells; EFs, electric fields; REAC, Radio Electric Asymmetric Conveyer; iPS, induced pluripotent stem cell; REMFS, repeated electromagnetic field shock; ADSCs, adipose-derived stem cells; ELFEFs, low frequency electromagnetic fields MSCs, mesenchymal stem cells; NSCs, neural stem cells.

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