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Review

Effects of electricity on the regenerative potential of mesenchymal stromal cells and their secretomes

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SUMMARY

Mesenchymal stromal cells (MSCs) are fibroblast-like cells that can be isolated from various adult tissues, including bone marrow and adipose tissue. Known for their regenerative potential, MSCs exert therapeutic effects largely through their secretome, a complex mixture of biomolecules and extracellular vesicles that mediates processes such as tissue repair, immunomodulation, and inflammatory regulation. This review critically examines how electrical stimulation (ES) can enhance the regenerative effects of MSCs. ES is a technique that applies an electrical field to cells to alter their behavior through the activation of various signaling pathways and by affecting the composition of the intracellular microenvironment and MSC secretome. Additionally, the integration of biomaterials such as 3D hydrogels and conductive scaffolds and how they may further amplify these effects is highlighted. By evaluating current ES-based protocols and their effects on diverse paracrine functions, this review provides a comprehensive overview of how ES can maximize the regenerative potential of MSCs.

BACKGROUND

The scientific community has intensively explored the potential of mesenchymal stromal cells (MSCs), particularly in regenerative medicine and as a source of cell-derived biologics.[1] In this context, soluble biomolecules and extracellular vesicles (EVs) secreted by MSCs, collectively known as the secretome, can be utilized to mediate paracrine effects through various biological mechanisms.[1] Over the past decade, physical stimulation techniques have been developed to modulate cell fate and enhance the regenerative potential of stem cell secretomes. Electrical stimulation (ES), in particular, has been extensively studied in recent years as a means of improving the regenerative functions of MSCs *in vitro*.

This review focuses specifically on the various parameters used in ES and their effects on MSCs and their secretomes. We provide a critical review of published data and summarize the most favorable conditions for enhanced regenerative outcomes. Furthermore, this review consolidates the existing literature concerning the effects of ES on the secreted factors themselves and discusses why the secretomes or EVs may be more desirable for clinical applications than stimulated MSCs alone.

MESENCHYMAL STROMAL CELLS

MSCs are stromal cells that self-renew and possess multi-lineage differentiation potential. They can be isolated from various tissues such as bone marrow, umbilical cord, amniotic fluid, and adipose tissue. They can also be extracted from alternative sources such as the endometrium and menstrual blood.[2,3] The use of MSCs as therapeutics is becoming increasingly prevalent compared to other stem cell types due to their wide range of tissue sources and the relative ease of harvesting.[2] However, some reports suggest that the tissue source influences the regenerative potential of MSCs, and in many cases, it may determine the effectiveness in treating diseases. In this context, bone-marrow-derived MSCs (BM-MSCs) are the preferred MSC type for use in bone regeneration, as their osteogenic differentiation potential appears to be higher than that of other MSC sources.[4] In contrast, umbilical-cord-derived MSCs have demonstrated superior therapeutic effects to BM-MSCs in the treatment of type 1 diabetes, while adipose-derived MSCs (AD-MSCs) may be more suitable for therapies requiring larger cell quantities, since the source material is more abundant and their proliferation rates may be higher.[5,6] Although all MSCs express common markers, their overall gene expression profiles differ.[4,6] Both AD-MSCs and BM-MSCs are positive for CD73 and negative

for the hematopoietic marker CD45.[4] However, other markers such as CD106, an adhesion molecule found on vascular cells, are expressed by BM-MSCs, but not by AD-MSCs.[4] Emerging evidence indicates that age-related osteoporosis is associated with a significant decline in the quantity, differentiation potential, and anti-inflammatory capabilities of BM-MSCs.[7] This highlights that experimental design and clinical treatment strategies should carefully consider the MSC source, both in terms of harvesting site and donor type, to ensure the cellular characteristics are best suited to the intended therapeutic outcome.[6]

MSCs have been intensively investigated for their regenerative properties, with the first MSC-based clinical trial conducted in 1995.[8] Until recently, the principal MSC sources employed in clinical trials were BM-MSCs, umbilical-cord-derived MSCs, and AD-MSCs. However, additional factors such as patient age, sex, the feasibility of MSC extraction, and the presence of somatic mutations significantly influence therapeutic outcomes.[8] The diversity of isolation techniques and variability in source characteristics often complicate direct comparisons between studies. To address these inconsistencies, Dominici et al. proposed in 2006 a set of minimal criteria to define MSCs for research purposes.[9] These criteria comprise adherence to plastic under standard culture conditions; expression of specific surface antigens; and *in vitro* differentiation into osteoblasts, adipocytes, and chondroblasts.[9]

Numerous studies have proposed that MSCs might possess the ability to cross the germ layer boundary, potentially differentiating into ectodermal and endodermal cells. In this context, several independent research groups have specifically claimed neuronal differentiation.[10–12] However, the majority of these studies relied heavily on morphological changes and marker expression and lacked comprehensive functional data directly comparing MSC-derived cells to functional neurons. Notably, MSCs can exhibit neuron-like morphology when exposed to cellular stress and may express neuronal markers even after differentiating into osteogenic and adipogenic lineages.[13] The absence of true neuronal differentiation is further supported by studies demonstrating a lack of functional integration and differentiation of MSCs within brain tissue.[14] Similarly, it has been suggested that MSCs could undergo endodermal differentiation, specifically into pancreatic islet-like cells.[10,15] Despite numerous attempts to demonstrate such cross-lineage differentiation, no convincing functional evidence supports the claim that MSCs can differentiate into cell types beyond the mesenchymal lineage without direct reprogramming via forced expression of ectodermal or endodermal factors or fusion with other cell types.[16,17]

Nevertheless, multiple clinical trials have demonstrated that MSCs can alleviate symptoms of diseases affecting tissues derived from all three germ layers.[18] This effect is often attributed to "bystander effects," in which MSCs contribute to endogenous regeneration through the modulation of paracrine factors.[1] These effects are generally understood to be mediated by the MSC secretome.[1]

MSC SECRETOMES

The MSC secretome contains a wide diversity of biomolecules, such as cytokines, chemokines, growth factors, nucleic acids and peptides, as well as EVs.[19] Importantly, the profiles of MSC secretomes differ depending on their source and the age of the donor.[20,21] The wide variety of secreted molecules can mediate different cellular and systemic effects, and some are secreted only in specific microenvironments or in response to certain signals.[22] The immune response can be modulated through secreted factors within the soluble fraction of the MSC secretome, such as transforming growth factor β (TGF-β) and metalloproteinase-processed C-C motif chemokine ligand-2.[22] TGF-β contributes to the activation of regulatory T cells, while metalloproteinase-processed C-C motif chemokine ligand-2 suppresses T cell chemotaxis and activation.[22,23] In addition, key growth factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor-1 are found in the soluble fraction of the secretome and play crucial roles in regeneration.[22,24,25]

The regenerative and immunomodulatory effects of the secretome are also observed in response to EVs isolated from secretome preparations.[22,26,27] EVs are defined as small membrane-bound particles surrounded by a lipid bilayer that are released from cells.[28] EVs are heterogeneous in terms of size, cargo, and function and can be divided into multiple subtypes.[28] These include particles that are classified as either small EVs, usually described as being < 200 nm in diameter, or large EVs, which are > 200 nm in diameter.[28] EVs can transfer proteins, nucleic acids, and lipids between cells, which in turn can influence various physiological and pathological functions in both secreting and target cells.[27] There is significant interest in the nucleic acid content of EVs, particularly microRNAs (miRNAs) and mRNAs, as these molecules, protected from degradation in the circulation by the EV membrane, have the potential to modulate gene expression in recipient cells upon delivery.[22] This is important, as certain miRNAs play roles in regeneration, cell cycle regulation, and immunomodulation.[22] miR-196a, for example, is one of several miRNAs present in BM-MSC-derived EVs and is involved in activating osteogenic gene expression.[22,29]

EVs have been shown to transfer their luminal cargo, including proteins and nucleic acids, through endocytosis, direct fusion with the membrane, or micropinocytosis.[30,31] Through these processes, EVs facilitate the direct transfer of biomolecules such as functional RNAs.[30] This has been demonstrated in a study by Ridder et al., in which glioma cells engineered to express Cre recombinase were shown to release EVs containing functional Cre mRNA that can be actively utilized by recipient cells.[30]

However, there is emerging evidence that EVs can also influence target cells via external modulation, rather than solely by transmitting their luminal cargo.[32]

One mechanism by which can EVs exert their influence involves interactions between membrane-bound ligands and cognate receptors on target cells. By displaying distinct proteins and lipids on their surfaces, EVs can engage these receptors and trigger downstream signaling cascades, such as the canonical Notch pathway.[33] Sheldon et al. were the first to show that endothelial cell-derived EVs carry the Notch ligand Delta-like 4, which can bind to the corresponding receptor on recipient cells.[34] In addition, a recent review by Tan et al. proposed "extracellular modulation by EV attributes" as a mode of action.[32] Their

compelling hypothesis suggests that MSC-derived EVs primarily exert therapeutic effects by modulating the extracellular environment through surface-bound enzymes and ligands, rather than by being internalized into target cells. This extracellular reprogramming would enable broad, efficient tissue repair and immunoregulatory responses at low EV doses.

Given the importance of EVs as a therapeutic tool in regenerative medicine, there is increasing interest in physical modulation of MSC-derived EVs to alter their functional characteristics.[35] ES represents a particularly promising approach for enhancing the therapeutic potential of EVs. Wu et al. identified the primary effects of ES on EVs as including increased molecular production, enhanced cardioprotective effects in cardiac MSCs, and elevated concentrations of bone morphogenic protein (BMP)-2, which would be valuable for osteogenesis.[35] Although these alterations to EVs via external stressors, such as ES, remain largely unexplored, they demonstrate considerable therapeutic promise.

The therapeutic potential of MSC secretomes

The variety of molecules and vesicles within the secretome mediates a wide array of paracrine effects in biological processes such as tissue repair, proliferation, control of inflammation, immunomodulation, migration, neovascularization, and anti-fibrotic effects.[36–39]

Bone regeneration

In the absence of acute fracture, concurrent pathologies, and prolonged treatment with glucocorticoids, bone tissue is able to "regenerate" without much assistance when fractured or broken.[40] When there is an injury to the bone, inflammatory cells produce cytokines and growth factors, which then influence MSCs to migrate toward the injury site.[41,42] The MSCs first form pre-osteoblasts and then osteoblasts, culminating in terminal osteogenic differentiation.[43] The MSC secretome can enhance osteogenic differentiation and bone regeneration through mechanisms such as growth factor secretion, enhanced angiogenesis, and recruitment of endogenous cells.[44–47] Shanbhag et al. investigated how the MSC secretome enhanced guided bone regeneration compared to an established growth factor therapy involving leukocytes and platelet-rich fibrin-conditioned media.[44] In the MSC secretome, growth factors such as insulin-like growth factor 1, TGF-β, and connective tissue growth factor were all found to be present; these are essential for stimulating bone formation.[44] The secretome also contained BMPs, which induce osteogenic differentiation and enhance the formation of new bone tissue.[44]

Angiogenic factors in the secretome, such as basic fibroblast growth factor and angiogenin, promote angiogenesis, which is crucial for bone regeneration.[45,46] Improved blood vessel formation is important in regeneration, as it promotes the delivery of nutrients and cells needed in this process.[44] As such, utilizing the secretome to enhance vascularization is of particular interest in regenerative medicine.[44]

Cellular senescence

Cellular senescence is a process in which cells cease dividing and undergo phenotypic alterations such as tumor-suppressor activation and changes to the secretome.[48] In MSCs, this process tends to occur during the growth phases of the cell cycle.[48–51] In response to DNA damage, senescence can occur during the G1 phase, preventing DNA replication in damaged cells, or in the G2 phase, where mitosis is blocked.[51] The secretome of healthy, non-senescent MSCs exhibits regenerative and anti-senescent effects on other cells due to its immunomodulatory, regenerative, and anti-apoptotic abilities.[52] However, when MSCs become senescent, the secretome and EVs promote senescence in neighboring cells, adversely impacting tissue repair and regeneration.[50,52] Senescent AD-MSCs have been demonstrated to produce EVs in significantly greater quantities and of larger size than those isolated from healthy cells..[53] Interestingly, EVs from senescent MSCs contained 2.3-fold less total RNA than those from healthy cells and have been demonstrated to induce to secondary senescence in recipient cells.[53,54]

Various nuclear stressors, such as oxidative stress, DNA damage, proliferative exhaustion, or other intra- and extracellular stressors, can trigger the initiation of cellular senescence, or the secretion of the senescence-associated secretory phenotype (SASP).[49,52] The SASP is a complex, heterogeneous phenotype characterized by the secretion of various bioactive factors, including inflammatory cytokines, chemokines, growth factors, proteases, and EVs from senescent cells.[52] These secreted factors can significantly influence neighboring cells and contribute to age-related diseases.[52] The SASP can subsequently induce senescence either through an autocrine mechanism that reinforces senescence within the MSCs themselves or via a paracrine effect that induces secondary senescence in adjacent healthy cells.[52] For example, interleukin-1β (IL-1β) and TGF-β can induce paracrine senescence by increasing reactive oxygen species production and altering the DNA damage response signaling pathway in neighboring cells, which would typically preserve genomic and proteomic homeostasis.[49,55] Elevated secretion of SASP factors can also impair the immune response, consequently reducing the clearance of senescent cells from the affected areas and leading to outcomes such as organ damage or hyperinflammation.[51]

Immunomodulation

MSC secretome has been shown to affect both the innate and adaptive immune responses. It suppresses T cell proliferation and reduces the secretion of specific pro-inflammatory cytokines such as interleukin-2 (IL-2).[56,57] During a normal adaptive immune response, released IL-2 binds to cell surface receptors on activated T cells, increasing T cell proliferation through the mammalian target of rapamycin (mTOR) signaling pathway.[56] A reduction in IL-2 secretion would inhibit T cell proliferation and consequently lead to a weakened inflammatory response.[58] This, in turn, can promote tissue repair by suppressing hyperinflammation.[58]

MSCs can also modulate B cell function, both directly and indirectly, through similar mechanisms. One such mechanism involves B cell activation in the presence of AD-MSCs, leading to reduced differentiation of B cells into plasmablasts and increased induction of regulatory B cells.[59] These regulatory B cells secrete anti-inflammatory cytokines such as IL-10 and TGF-β, which can directly inhibit the proliferation and cytokine

production of various immune cells, such as $CD4^+$ and $CD8^+$ T cells, thereby limiting chronic inflammatory conditions and autoimmune diseases.[60]

Within the innate immune response, MSCs can interfere with components of the immune system, such as dendritic cells, monocytes, macrophages, and natural killer (NK) cells.[61,62] The MSC secretome can reduce the maturation and function of dendritic cells by downregulating specific maturation markers, such as CD38, CD80, and CD83.[57] Although reduced dendritic cell maturation can impair antigen presentation and cytokine production, it may also help prevent autoimmunity by limiting T cell activation against the body's own cells.[57,63]

MSCs and their secretome influence monocytes and macrophages by polarizing them toward an anti-inflammatory M2 phenotype and decreasing tumor necrosis factor-α (TNF-α) levels.[64] NK cells remove infected and diseased cells during the immune response and activate macrophages.[65] The secretome can modulate this function by increasing the secretion of interferon-γ (IFN-γ), which in turn enhances macrophage activity and improves the clearance of infected cells.[57,65]

Therefore, the effects of the MSC secretome on both the adaptive and innate immune response can be beneficial or detrimental, depending on the specific context and conditions.

Anti-inflammatory effects

The MSC secretome has been shown to reduce inflammation in cartilage, muscles, and lung tissue.[1,21,66] When MSCs are stimulated with the pro-inflammatory cytokine TNF-α, they secrete factors that contribute to the reduction of inflammation.[67] This reduction in inflammation can occur through the secretion of TNF-α stimulated gene/protein-6 (TSG-6), which suppresses the early inflammatory response via the p38 and mitogen-activated protein kinase (MEK) pathways.[67,68] Choi and colleagues cultured human BM-MSCs and then incubated the cells with TNF-α.[62] After 18 h of incubation, they observed an increased expression of TSG-6 and found that secreted TSG-6 attenuated mouse peritonitis through decreased toll-like receptor 2/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling in macrophages.[62]

Dedier and colleagues studied the anti-inflammatory effects of the secretome of BM-MSCs primed with IL-1β.[69] The priming resulted in a notable alteration of the secretome composition, with a significant upregulation of IL-6 compared to the secretome released by untreated cells. Subsequent *in vitro* experiments demonstrated that this elevated IL-6 exhibited anti-inflammatory properties. IL-6 exhibits a dual nature in inflammation, acting as both a pro-inflammatory cytokine and an anti-inflammatory myokine depending on its concentration and the presence of other inflammatory factors.[70] While IL-6 can promote chronic inflammation and autoimmune responses, it can also exert immunosuppressive effects and contribute to the resolution of acute inflammatory processes.[70] In this context, Dedier and their group discovered that the primed BM-MSC secretome promoted the reduction of inflammation partly through the IL-6 pathway, suggesting that IL-6 acts as an anti-inflammatory myokine when its secretion is triggered by MSC secretomes.[69]

Electrical stimulation

Multiple studies have investigated the impact of culture conditions on the viability of the MSCs and the regenerative potential of MSC secretome, particularly the effect of 2D versus 3D cell culture and ES.[1,71–73]

ES is a physical stimulation approach involving the application of an electrical or electromagnetic field to cells in order to alter their behavior or function, such as their proliferation or differentiation characteristics.[41]

Fukada and Yasuda laid the groundwork for the investigation of ES on bone tissue when they tested the piezoelectric effect in bones.[74] Bone piezoelectricity occurs when stress is applied to the bone, leading to collagen generating an electrical signal, which positively impacts the bone's self-regeneration ability.[74,75] When bones are damaged, for example, after a fracture or a minor break, the tissue can regenerate without much assistance.[40] However, when large volumes of bone tissue are broken or missing, this regeneration process is not easily replicated.[40]

Within clinical practice, exogenous electric fields have been used to increase the piezoelectric effect via polarization and promote healing and recovery after surgery.[76] A fracture healing patch device utilizes a pulsed electromagnetic field (PEMF) current, and in acute distal radius fractures, this device has been shown to promote osteoblast differentiation and maturation.[76,77] The *in vivo* ES protocols were adopted before any in-depth studies on the effects *in vitro* were completed. As a result, the research community began investigating the molecular mechanisms involved.[41]

When an electric or electromagnetic field is applied, the charged molecules on the cell membrane are redistributed, triggering specific voltage-gated ion channels to open.[78–80] Ions such as Ca^{2+}, Na^+, K^+, and Cl^- move in or out of the cell, thus altering the membrane potential of the cell.[78,79] This change in potential triggers the cytoskeleton to alter its shape, which then influences multiple cellular processes such as migration.[78] Changes in membrane potential and ion concentrations also have the ability to activate multiple intracellular signaling pathways (Figure 1).[78–80]

An influx of Ca^{2+} can trigger Ca^{2+}-dependent signaling pathways such as the phosphoinositide 3-kinase/Ak strain transforming (PI3K/Akt), mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK), and the Ca^{2+}/calmodulin pathways (Figure 1).[78,79] These pathways regulate gene expression and cellular processes such as growth, proliferation, differentiation, and migration.[78]

The influx of Ca^{2+} ions caused by ES is important during osteogenic regeneration, as, when there is an injury to the bone, inflammatory cells secrete various chemokines, cytokines, and growth factors, which in turn cause MSCs to migrate to the injury site.[41,42] As MSCs are the primary precursors for bone-forming cells, once they reach the injury site and adhere to the bone, they differentiate into osteoblasts.[41,42] The PI3K/Akt pathway is a key regulator of osteoblast differentiation.[43] The influx of Ca^{2+} ions can activate the pathway in a multitude of ways; for instance, increases in intracellular Ca^{2+} can directly activate PI3K, while activation of cell receptors can lead to phosphorylation events that release Ca^{2+} from intracellular stores in a feedback loop.[81] Additionally, when Ca^{2+} ions bind to calmodulin,

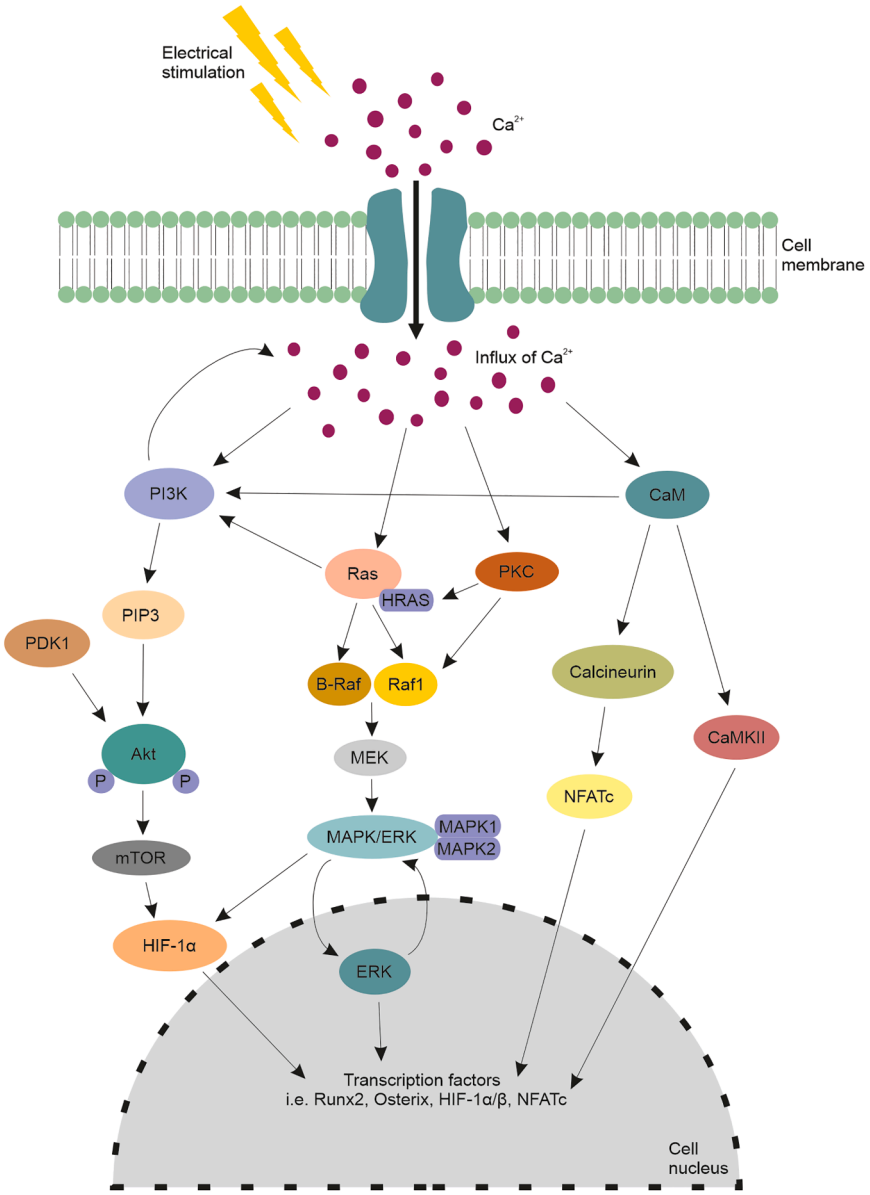

Figure 1. How electrical stimulation and subsequent influx of calcium ions can activate various signaling pathways (PI3K/Akt, MAPK/ERK, and calmodulin)

Once activated, the PI3K/Akt pathway phosphorylates PI3K, recruiting and activating Akt through PDK1 and mTOR, which in turn enhances the expression of osteogenic markers. The MAPK/ERK pathway is activated by an influx of Ca^{2+} ions, leading to the activation of small GTP-binding proteins such as Ras, which begins the signaling cascade and eventually the transcription of proteins involved in osteogenesis, such as Runx2 and Osterix. The calmodulin pathway begins with the calcium-binding protein calmodulin, activating CaMKII and the calcineurin pathway. Ca^{2+}, calcium ions; PI3K, phosphoinositide 3-kinase; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PDK1, 3-phosphoinositide-dependent protein kinase 1; Akt, Ak strain transforming; mTOR, mammalian target of rapamycin; HIF-1, hypoxia-inducible factor 1; Ras, rat sarcoma virus; PKC, protein kinase C; Raf, rapidly accelerated fibrosarcoma; MEK, MAPK/ERK kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; CaM, calmodulin; CaMKII, calcium/calmodulin-dependent protein kinase II; NFATc, nuclear factor of activated T cells; Runx2, runt-related transcription factor 2.

this protein can activate PI3K.[81] Once PI3K is activated, it phosphorylates phosphatidylinositol (4,5)-biphosphate to phosphatidylinositol (3,4,5)-triphosphate (PIP3), which then triggers the phosphorylation of Akt through 3-phosphoinositide-dependent protein kinase 1 (PDK1) (Figure 1).[82] In turn, PDK1 activates the mTOR pathway which upregulates hypoxia-inducible factor-1α (HIF-1α) (Figure 1).[82,83] As a vital component of osteogenesis, HIF-1α promotes angiogenesis by stimulating the secretion of VEGF as well as stimulating osteoblast activity.[83]

When ES alters cell membrane potential, pathways such as MAPK/ERK, which can affect differentiation, are also affected (Figure 1).[84,85] The MAPK/ERK pathway can be activated by a multitude of stimuli, such as growth factors, G protein-coupled receptors, and an influx of Ca^{2+}.[86] One activation mechanism is the influx of ions that leads to the activation of small guanosine triphosphate (GTP)-binding proteins like Ras, which then triggers the rest of the pathway.[87] This pathway increases the upregulation of runt-related transcription factor-2 (Runx2) and Osterix (Figure 1).[84,85] Both Runx2 and Osterix are regarded as "master" osteogenic factors. Runx2 is a vital transcription factor in the process of osteogenesis, as it is responsible for the activation of various differentiation marker genes such as osteocalcin (OCN), while Osterix is necessary for preosteoblasts to differentiate into mature osteoblasts along with increasing transcriptional activity of key osteogenic promoters such as alkaline phosphatase (ALP) and bone sialoprotein.[85,88] Furthermore, the activation of ERK signaling is known to lead to a variety of other cellular responses, including proliferation.[86]

The Ca^{2+} influx induced by ES also modulates the calmodulin signaling pathway (Figure 1). Upon Ca^{2+} binding, calmodulin activates multiple key enzymes that drive osteogenesis.[89] This protein activates calmodulin-dependent kinase II (CaMKII), which subsequently enhances the transcriptional activity of Osterix. Additionally, calmodulin activates calcineurin, promoting the dephosphorylation and nuclear translocation of nuclear factor of activated T cells (NFAT), which cooperates with Osterix to trigger the expression of osteogenic genes, including OCN and osteopontin (OPN).[89]

When using ES to influence MSC differentiation, the electric field is applied through the culture medium, either in a tissue

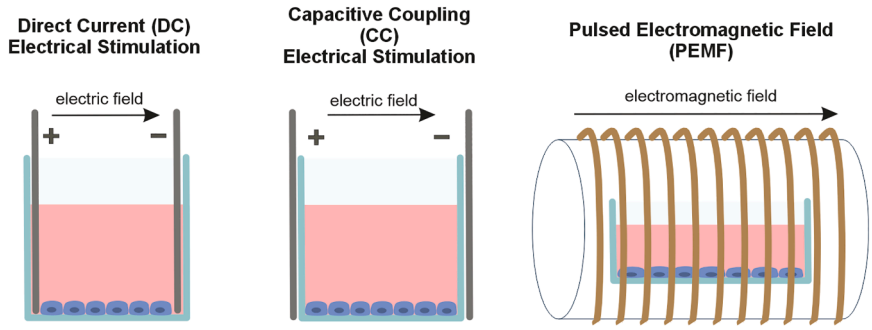

Figure 2. Overview of three electrical stimulation approaches: direct current, capacitive coupling, and pulsed electromagnetic field

The figure presents the advantages/disadvantages of each method and a schematic representation of the devices. In direct current ES, electrodes are placed directly in the medium to create an electric field for cell stimulation. A disadvantage of this method is that chemical reactions can occur within the medium at the electrodes, and negative byproducts are produced. Capacitive coupling involves electrodes being placed on the outside of the wells to create an electric field, and unlike DC-ES, there is no interaction between media and electrodes. PEMFs involve a current flowing through a solenoid to create the electromagnetic field. Similar to CC-ES, there is no direct contact between electrodes and media.

culture plate (2D) or a non-conductive scaffold (3D).[41] While ES has been more thoroughly researched in 2D conditions, combining ES with 3D cell culture conditions such as hydrogels and conductive scaffolds is becoming a more common experimental practice.[90]

There are three main methods involved in applying this electric field, broadly termed direct coupling (DC), capacitive coupling (CC), and inductive coupling (IC).[41] One of the primary methods of IC is PEMF (Figure 2).[41,91]

DC-ES setups are commonly made from cell culture dishes or wells that have conductive electrodes placed directly into the cell culture medium, and these electrodes operate at different parameters that can vary between experiments (Figure 2).[41,92] As the electrodes are directly immersed in the culture medium, this technique has the highest risk of generating so-called Faradaic products, such as reactive oxygen species at the electrodes that can lead to pH changes.[92] The parameters that can be varied in experiments include the pulse duration (the length of time that each group of electric pulses lasts), the frequency (the number of pulses per second), the voltage (the strength of the stimulation delivered), and the length of stimulation (the total time the cells are exposed to the electrical pulses).[41,92]

CC-ES is a less invasive stimulation method than DC-ES, whereby an electric field is generated between two parallel capacitor plates that sit on the edges of the cell culture chamber or well, without making contact with the cell culture medium (Figure 2).[41] The electric field generated is then transmitted through the cell culture medium, effectively stimulating the cells throughout the culture.[41,93] As these devices have no direct contact between the media and the electrodes, they have the advantage of avoiding the formation of reactive species that can occur in DC-ES.[93]

In IC-ES, the electrodes also do not make contact with the cell culture medium and instead use conductive coils or solenoids around the cell culture system to induce the electric field, avoiding the production of undesirable by-products that can be generated when the current is applied.[41,94] Within this stimulation technique, PEMF stimulation sends an electric current through a solenoid, which creates a magnetic field at its center, perpendicular to the applied electric field (Figure 2). This stimulation method has been used effectively in both *in vitro* and *in vivo* treatments.[94] In this context, PEMFs have been utilized in cartilage explants *in vitro* and in cartilage models *in vivo* to enhance the synthesis of the extracellular matrix and cytokines such as TGF-β.[94] The advantage of PEMF stimulation is that it is non-invasive, whether for cell culture or use in animal/human tissue.

While studies involving PEMFs often have differing stimulation protocols, either through duration of stimulation or intensity of pulses, the results observed *in vitro* tend to follow the same precedent of increased ALP activity, cytokine production, and an increase in type I collagen secretion.[94] These observations therefore indicate a positive effect of PEMF on the osteogenic potential of stem and/or bone cells.[94]

The cell signaling mechanisms discussed previously and detailed in Figure 1, together with studies examining various stimulation methods, support the concept that ES of MSCs represents a promising approach to enhance the therapeutic efficacy of MSC-based treatments, particularly in tissue regeneration processes such as osteogenic differentiation, proliferation, and migration. ES can enhance differentiation into specific lineages and improve tissue integration compared to non-stimulated MSCs.[41]

However, several challenges and considerations must be addressed when applying ES to MSCs. Currently, no standardized ES protocol exists, and while pre-clinical data appear promising, further research is required to translate stimulated MSCs into widespread clinical application.[41,95] Protocol variations encompass not only the specific parameters of ES devices, such as frequency, pulse characteristics, and stimulation duration, but also distinct cellular priming methods, including 3D culture, TNF-α treatment, IFN-γ exposure, hypoxia licensing, and various pharmacological interventions.[96]

As the regenerative effects of ES have become increasingly studied in recent years, establishing standardized protocols for stimulating MSCs tailored to specific therapeutic outcomes represents the next critical step in developing ES-augmented MSC therapies.

Table 1 summarizes the ES methods and parameters, along with their effect on the relevant regenerative properties of MSCs, from the literature reviewed in this paper.

Impact of ES on the regenerative potential of MSCs

Proliferation and viability

ES is well known to alter cellular proliferation.[97] To identify optimal parameters for direct current ES (DC-ES) that maximize both proliferation and viability of human AD-MSCs, Kämmerer

Table 1. Summary of general electrical stimulation protocols applied to MSCs

Reference	ES type	Parameters	Duration of treatment	Cell type	Other alterations, e.g., growth media or scaffold	Summarized effect on regenerative potential
Kämmerer et al.[97]	DC	1.7 V AC, 20 Hz	continuous for 24 h, 72 h, and 7 days	human AD-MSCs	unaltered growth medium	increased proliferation at day 3
Srirussamee et al.[98]	DC	2.2 V	1 h/day for 10 days	human BM-MSCs	unaltered growth medium	increase in proliferation
Griffin et al.[99]	CC	15 Hz, 62.5 ms pulse width, 10 mV/mm electric field	3 h/day for 5 days	human BM-MSCs	unaltered growth medium	enhanced cellular proliferation
Bicer et al.[71]	DC	10 Hz, 0.04 ms pulse duration, 0.1 V/cm	30 min/day for 21 days	human AD-MSCs	both 2D and 3D anionic nanofibrillar cellulose hydrogel conditions stimulated	osteogenesis: increased ALP activity at day 7 and increase in calcium deposit accumulation at day 21; adipogenesis: average size of lipid droplets decreased
Leppik et al.[40]	DC	100 mV/mm	1 h/day for 21 days	rat AD-MSCs	β-tricalcium phosphate (β-TCP) scaffolds in an osteogenic medium	increased expression of TGF-β1, BMP-2, OPN, and calmodulin
Bagheri et al.[100]	PEMF	1.3 ms pulse duration, 75 Hz frequency	continuous exposure for 28 days	BM-MSCs	unaltered growth medium	enhanced ALP activity, increased OCN production, and upregulated expression of Runx2, Dlx5, and Osterix
Martini et al.[101]	PEMF	1.3 ms pulse duration, 75 Hz frequency	continuous exposure for 28 days	human BM-MSCs	cell culture medium supplemented with BMP-2	enhanced ALP activity; increase in expression of BMP-2, BMP-6, and OCN
Jansen et al.[102]	PEMF	15 Hz, 1 Gauss EM field, 5 ms pulse bursts	continuous up to 14 days	Human BM-MSCs	osteogenic medium	increased mineralization at days 9 and 14; increased mRNA levels of BMP-2, TGF-β1, OCN, and bone sialoprotein
Liu et al.[103]	DC	4.4 V, 200 mV/mm electric field	1 h/day for 3 days	human AD-MSCs	unaltered growth medium	increased proliferation and increased adipogenesis
Kwon et al.[104]	DC	0, 1, 5, or 25 V/cm, 8 ms duration, 5 Hz frequency	3 days	unspecified mouse MSCs	unaltered growth medium	increase in gene expression of type II collagen, aggrecan, and Sox9
Liu et al.[105]	DC	1, 5, and 10 V/cm, 8 ms duration, 5 Hz frequency	1 h/day for 3 days	human AD-MSCs	cells seeded on poly(3,4-ethylene dioxythiophene)-poly(styrene sulfonate) (PEDOT:PSS)	increased expression of type II collagen and Sox9
Zhang et al.[106]	EMF	7.5, 15, 30, 50, and 70 Hz/1 mT EMF	continuous exposure for 24 h	human BM-MSCs	treated with verapamil and PF-573228	increased MSC migration in an intracellular calcium-dependent manner
Lee et al.[107]	PEMF	1,200 μA current	Continuous exposure for 3, 6, and 9 h or unspecified pulsed ES	human AD-MSCs	unaltered growth medium	increase in cell viability when exposed to pulsed ES and an increase in directional cell migration after 3 h of simulation

(*Continued on next page*)

Table 1. *Continued*

Reference	ES type	Parameters	Duration of treatment	Cell type	Other alterations, e.g., growth media or scaffold	Summarized effect on regenerative potential
Vincenzi et al.[108]	PEMF	1.3 ms pulse duration, 75 Hz frequency	24 h	human osteoblasts	1 ng/mL IL-1β for 24 h post-stimulation	activation of adenosine receptors inhibited the IL-1β-stimulated release of pro-inflammatory cytokines IL-6 and IL-8
Ross et al.[109]	PEMF	5.1 Hz, 0.04 mT magnetic field intensity, and 0.07 mV/cm induced electric field intensity	5 min	unspecified MSCs	unaltered growth medium	increased secretion of IL-3, IL-4, and IL-10 and decreased secretion of IL-1β, IL-6, and IL-17A
Li et al.[110]	DC	sinusoidal alternating current of 5 μA, 50 μA, 100 μA, 500 μA, 5 mA, and 10 mA; 50 Hz frequency	6 × 2 min exposure for 1 h/day	rat AD-MSCs	unaltered growth medium	increased cell number and higher levels of intercellular adhesion molecule-1 and VEGF

The table includes ES-induced various regenerative effects, including the following: proliferation and viability, osteogenic differentiation, adipogenic differentiation, chondrogenic differentiation, migration, and control of inflammation. Abbreviations: DC, direct current; CC, capacitive coupling; PEMF, pulsed electromagnetic field; AC, alternating current; AD-MSCs, adipose-derived mesenchymal stromal cells; BM-MSCs, bone marrow-derived mesenchymal stromal cells; ALP, alkaline phosphatase; TGF, transforming growth factor; BMP, bone morphogenic protein; OPN, osteopontin; OCN, osteocalcin; IL, interleukin; VEGF, vascular endothelial growth factor.

et al. subjected these cells to continuous stimulation at 1.7 V alternating current (AC) and 20 Hz for 24 h, 3 days, and 7 days.[97] Under these conditions, stimulated AD-MSCs exhibited comparable growth to non-stimulated cells at 24 h; by day 3, however, cell numbers had increased 4.5-fold and surface coverage had risen 2.7-fold. Prolonged stimulation to day 7 led to a pronounced decline in both metrics, indicating potential adverse effects of continuous ES.

Further evidence of toxicity associated with prolonged DC-ES was provided by Kwon et al., who applied DC fields of 1–25 V/cm with 8 ms pulses at 5 Hz.[104] After 3 days, fewer than 5% of cells exhibited damage; by day 7, however, cell death approached 50%.

Such loss of viability may result from electrode corrosion, which alters the culture medium and induces morphological changes in cells, or from electrochemical byproducts such as hydrogen peroxide (H_2O_2) and other free radicals generated during DC-ES.[41,97]

The role of H_2O_2 in ES-mediated effects was further explored by Srirussamee and their group, who applied 2.2 V DC for 1 h daily over 10 days to BM-MSCs.[98] The authors observed enhanced proliferation and metabolic activity in stimulated cells, yet differentiation remained unaffected.

In contrast, another study demonstrated that capacitive coupling ES (CC-ES) enhances proliferation and migratory capacity of BM-MSCs in an *in vitro* wound-healing assay simulating fracture repair.[99] These findings suggest that CC-ES may boost cell growth without the deleterious effects observed with DC-ES.[41]

To harness the benefits of ES for prolonged MSC culture, future studies should refine stimulation modalities that maintain cell viability over extended periods. Proposed strategies include using isolated chambers linked by agar salt bridges to external electrodes or combining continuous and pulsatile stimulation to prevent late-stage declines in proliferation.[41,97]

Osteogenic differentiation

There is great interest in investigating the effects of ES on osteogenic differentiation. A study by Bicer and colleagues demonstrated that exposure to electrical fields through DC-ES stimulation positively affects the osteogenic potential of AD-MSCs in both 2D and 3D anionic nanofibrillar cellulose hydrogel conditions.[71] The AD-MSCs were cultured in 6-well tissue culture plates that were attached to an IonOptix C-pace EP system, which was run at a frequency of 10 Hz, pulse duration of 0.04 ms, and a voltage of 0.1 V/cm for 30 min per day over a 21-day period.[71] Osteogenic differentiation was evaluated using multiple assays: ALP activity was quantified on day 7, while calcium mineralization was assessed via alizarin red S staining at day 21. Additionally, immunocytochemistry was employed to detect the expression of osteogenic markers OCN and OPN at the conclusion of the 21-day period. Under 2D conditions, there was a significant increase in ALP activity at 7 days, as well as increased calcium deposit accumulation and OCN fluorescence ratio at 21 days. In contrast, no significant difference in the OPN fluorescence ratio was observed between the control and stimulated cells at any time point during the experiment. In 3D hydrogels, a similar increase in ALP activity was seen when stimulated with ES, along with a significantly higher level of

calcium mineralization. At day 14, AD-MSCs cultured in 3D hydrogel and subjected to ES exhibited elevated OPN levels; however, this difference in OPN expression was no longer significant by day 21.

Leppik et al. investigated the effect of DC-ES on rat AD-MSCs that were seeded onto a β-tricalcium phosphate (β-TCP) scaffolds in osteogenic medium.[111] The authors demonstrated that DC-ES at 100 mV/mm for 1 h daily increased the expression of TGF-β1, BMP-2, OPN, and calmodulin by day 21 (the end of the experiment). This enhanced gene expression induced by ES positively influenced AD-MSC osteogenesis, as demonstrated through histological analysis, mechanical testing, and gene expression studies of rat femur healing *in vivo*. Furthermore, the scaffold demonstrated promising osteoinductive properties in both preclinical animal models and clinical applications.[112] The osteoinductive properties of β-TCP scaffolds, their ability to stimulate bone formation in non-osseous sites, make them particularly promising tools for bone tissue healing, especially when combined with electrical stimulation.[112,113]

IC-ES is a common stimulation method, although there is no general agreement on the optimal conditions for stimulating MSCs. Studies have employed varying pulse durations, frequencies, stimulation periods, and magnetic field intensities, with several investigations utilizing pulsed PEMFs for continuous cellular stimulation. Bagheri and colleagues used PEMF of 1.3 ms and a frequency of 75 Hz for 28 days to study the effects of IC-ES on human BM-MSCs.[100] Continuous exposure to PEMFs over a 21-day period resulted in enhanced ALP activity, increased OCN production, and upregulated expression of osteogenic transcription factors, including Runx2, Dlx5, and Osterix. This study also indicated that the Notch signaling pathway, which regulates skeletal development and cell fate, plays a role in PEMF-stimulated osteogenic differentiation in MSCs.

In another study, human BM-MSCs were continuously exposed to PEMF for 28 days.[101] The pulsed signal applied to the cells mirrored that of the previous study mentioned, employing a 1.3 ms pulse duration and 75 Hz frequency. However, this study diverged by supplementing the cell culture medium with BMP-2. They found that PEMF effects were associated with an increase in ALP activity and increased expression of BMP-2, BMP-6, and OCN. These studies, along with others, consistently demonstrated that PEMF exposure alone is insufficient to induce differentiation.[102] Instead, additional osteogenic stimuli, such as BMP-2 supplementation in the culture medium, are often required to promote this process.[41,100–102]

Adipogenic differentiation

Adipogenic differentiation is a complex cellular process involving the differentiation of MSCs into lipoblasts and preadipocytes before finally differentiating into mature adipocytes.[114]

Liu and their group followed the DC-ES protocol used by another group, stimulating human AD-MSCs for 3 days and assessing proliferation, adipogenic differentiation, and stemness.[103,115] The authors demonstrated that, after exposure of AD-MSCs to ES, cells maintained their normal morphology and expression of the stemness markers and retained their ability to differentiate into adipocytes.[103]

The study by Bicer and colleagues also investigated the effects of DC-ES on the adipogenic differentiation of AD-MSCs after 21 days.[71] Adipogenic differentiation was assessed via labeling the lipid droplets with oil red O and subsequent microscopy-based analysis of lipid droplet size. Electrically stimulated AD-MSCs exhibited a slight, albeit statistically non-significant, reduction in oil red O staining compared to their non-stimulated counterparts. Moreover, the average size of lipid droplets decreased under ES conditions. These findings suggest that ES can reduce adipogenic differentiation of AD-MSCs, which, while disadvantageous in adipogenesis, could be advantageous when osteogenic or chondrogenic differentiation is the desired cell fate.

Chondrogenic differentiation

Although the positive effects of ES on osteogenesis and cartilage repair are well known, only a few studies have investigated the effects of ES on chondrogenesis.

Kwon and colleagues observed the expression levels of various chondrogenic markers (type I collagen, type II collagen, aggrecan, and Sox9) in MSCs when exposed to ES.[104] This study applied chronic stimulation under electric fields of 0, 1, 5, or 25 V/cm, a duration of 8 ms, and a frequency of 5 Hz. After 3 days, quantitative real-time RT-PCR analysis showed a significant increase in the gene expression of type II collagen, aggrecan, and Sox9, suggesting that ES can promote chondrogenic differentiation of MSCs.

Liu et al. examined the effects of ES on chondrogenic differentiation of AD-MSCs seeded on poly(3,4-ethylenedioxythiophene)-poly(styrene sulfonate) (PEDOT:PSS).[105] Interestingly, unlike in the study mentioned previously, upregulation in type II collagen and Sox9, but not aggrecan, was observed.[104,105] Therefore, the results of this study support the suggestion that ES can promote chondrogenic differentiation of human AD-MSCs. However, the degree of differentiation seems to differ depending on the stimulation parameters and culture conditions.

Migration

Cellular migration is a complex process that is coordinated by various scaffold, adaptor, and adhesion proteins such as actin, myosin, integrin, and tensin, as well as signaling molecules like protein kinase C, mitogen-activated protein kinases, focal adhesion kinase, Rho GTPase, and Rho kinase.[106] Increasing MSC migration is of therapeutic interest as it could improve the efficiency of MSC engraftment in bone and cartilage repair or enable spatially precise delivery of their secretomes.[106] However, promoting MSC migration through ES is still a relatively new avenue.

Zhang and colleagues exposed human BM-MSCs to low-frequency electromagnetic fields for 24 h and subsequently treated them with the L-type calcium channel blocker verapamil.[106] Following this, cell migration and the expression levels of cell-adhesion proteins, as well as the Rho GTPase protein activity, were assessed via G-LISA (GTPase-linked immunosorbent assay) small GTPase activation assays. The researchers concluded that ES promoted MSC migration in an intracellular calcium-dependent manner that was mediated by focal adhesion kinase activation.

Lee and colleagues investigated how ES influences the directional migration of AD-MSCs, a process also known as electrotaxis.[107] In this study, electrotaxis of AD-MSCs was assessed in customized agar-salt electrotaxis chambers. The cells were exposed to an electric current of 1200 μA for 3, 6 and 9 h

continuously or to an unspecified pulsed stimulation. Compared to the unstimulated control, cell viability dropped to approximately 80% after 6 h of continuous ES and declined further to 40% after 9 h. In contrast, cells that received pulsed stimulation showed a smaller reduction in viability with no significant cell death, suggesting that prolonged ES exposure decreases viability and triggers apoptosis. The authors then sought to identify the optimal ES duration for promoting directional cell movement. They found that applying ES for 3 h was sufficient to direct cell migration toward the anode, both during stimulation and for 6 h afterward.

Control of inflammation

Vincenzi et al. studied the effect of PEMFs on inflammation in osteoblasts.[108] They found that PEMFs led to the activation of adenosine receptors, which are known to play a vital role in regulating inflammation via the inhibition of pro-inflammatory cytokine release. This has been shown to significantly reduce inflammation in osteoblasts.

One study used PEMFs to modulate immunomodulatory properties in MSCs to improve tissue regeneration.[109] The authors exposed MSCs to PEMFs for 5 min and observed increased secretion of anti-inflammatory cytokines IL-3, IL-4, and IL-10. Additionally, PEMF stimulation decreased the secretion of pro-inflammatory cytokines IL-1β, IL-6, and IL-17A from MSCs. They concluded that PEMFs may help regulate the immune response by controlling the production and stability of inflammatory cytokines. This balance is crucial, as its disruption can transform a normal immune response into a chronic one, potentially leading to autoimmune diseases.

Li and colleagues investigated the effects of DC-ES on paracrine functions of rat AD-MSCs by exposing them to 50 Hz stimulation in six 2-min sessions at various alternating currents for 1 h per day.[110] This stimulation caused a pronounced increase in AD-MSC cell number, as well as significantly higher levels of intercellular adhesion molecule-1 (ICAM-1) and VEGF. ICAM-1 is essential in the immune response, as it regulates leukocyte recruitment from circulation to inflammatory sites, while VEGF is essential for angiogenesis.[116] These results indicate that ES can modulate the levels of factors involved in regeneration in MSC secretomes.

Knowledge regarding the impact of ES on the immunomodulatory potential of MSC secretomes and EVs remains limited. However, Zhang and colleagues reported that DC-ES with a frequency of 0.5 Hz, pulse width of 5 ms, and voltage of 1.5 V/1.8 cm increased the secretion of cardioprotective EVs from cardiac MSCs.[117] These results suggest a potential positive effect on immunomodulation through ES, as increased EV production could inhibit the proliferation of B cells and NK cells.[118] To confirm this, further research is needed to assess the effects of ES on paracrine factors released by MSCs.

Conclusions

In summary, ES has been shown to modulate the paracrine activity of MSCs and their secretomes, particularly with respect to angiogenesis, proliferation, and regenerative capacity. However, the precise impact on MSC biology and secretome composition depends heavily on the chosen stimulation modality and specific ES parameters, such as stimulation duration and voltage. For enhancing osteogenic differentiation, direct-current ES (DC-ES) appears most effective. Nevertheless, DC-ES carries drawbacks, including the generation of Faradaic byproducts and the reduction of adipogenic differentiation, an outcome beneficial for osteogenesis but undesirable in therapeutic contexts that require adipogenesis, such as obesity and related metabolic disorders. PEMFs present a similar dilemma. Although they exert anti-inflammatory effects, prolonged exposure may compromise cell viability.[119] Furthermore, the effects of ES on cellular senescence and on the regenerative potency of the secretome remain underexplored. Consequently, to harness physical stimulation for augmenting the therapeutic potential of the MSC secretome, further research is required to optimize ES protocols. Critically, these protocols should be designed to balance multiple paracrine functions simultaneously to ensure that any adverse outcomes do not outweigh the desired clinical benefits.

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DECLARATION OF INTERESTS

The authors declare that they have no competing interests that have influenced the work reported in this review.

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