



# Role and mechanism of micro-energy treatment in regenerative medicine

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**Abstract:** With the continuous integration and intersection of life sciences, engineering and physics, the application for micro-energy in the basic and clinical research of regenerative medicine (RM) has made great progress. As a key target in the field of RM, stem cells have been widely used in the studies of regeneration. Recent studies have shown that micro-energy can regulate the biological behavior of stem cells to repair and regenerate injured organs and tissues by mechanical stimulation with appropriate intensity. Integrin-mediated related signaling pathways may play important roles in transducing mechanical force about micro-energy. However, the complete mechanism of mechanical force transduction needs further research. The purpose of this article is to review the biological effect and mechanism of micro-energy treatment on stem cells, to provide reference for further research.

**Keywords:** Micro-energy medicine (MEM); stem cells; mechanical force; integrin; regeneration

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## Introduction

Micro-energy medicine (MEM) is an emerging integrated medicine that uses external devices to generate mechanical waves, electromagnetic waves and other forms of energy to prevent and treat diseases or sub-health states (1). In 2011, American Association for the Advancement of Science (AAAs) regarded integrated medicine as the "third revolution in life science" after cell and molecular biology and genomics, of which MEM is an important representative (2). Under concept of micro-energy, the core

of life is no longer just the body, but energy. The intensity of body energy is directly reflected in cell viability. MEM can improve the vitality of cells in the body, through a variety of technical means, to achieve the goal of disease treatment and prevention (1). Recently, the application fields of MEM have been gradually extended to cardiology, urology, endocrinology, burn plastic surgery, oncology, even cosmetology. In addition to enhancing local blood circulation, stimulating peripheral nerve growth, anti-inflammatory and analgesic effects, micro-energy treatment can also activate, transform and recruit stem cells to

participate in the repair and regeneration of target organs and tissues (1). Compared with traditional treatments, micro-energy therapy, as a safer means, doesn't produce drug-related side effects and surgery-related complications.

Regenerative medicine (RM) is an emerging interdisciplinary field of life science and engineering, concentrated on repairing, replacing or regenerating injured tissues and organs to restore the damaged function caused by any reasons (3). The RM related technologies and methods include, but are not limited to, stem cell therapy, organ reconstruction *in vitro*, etc. (4). The applications of stem cell therapy in RM contain exogenous cell transplantation and endogenous cell activation (5). Stem cell transplantation has achieved remarkable results and been widely carried out, but there are still some problems after transplantation, such as the decline of cell survival rate and regeneration ability, immune rejection, ethical and tumorigenic problem. Therefore, it is difficult to generally and safely use stem cell banks for regenerative therapy in clinical (6). The body's innate healing response may also be leveraged to promote regeneration, although adult humans possess limited regenerative capacity. Many mammalian organs contain a subpopulation of undifferentiated stem cells (SC) involved in tissue replenishment and repair, e.g., the vascular endothelium can be repaired by the mature vessel wall-resident endothelial basal cells (stem cells) that migrate to the injured area. In a way, tissue resident stem cells are so-called endogenous stem cells. Owing to without immune rejection and ethical problem, Endogenous stem cells have attracted much attention in recent years (7). However, the problems, such as low numbers *in vivo*, slow cell proliferation and differentiation, are still existed.

Micro-energy therapy can promote the migration, proliferation and differentiation of stem cells from different sources, stimulate cells to secrete cell factors and regulate immune progress, to solve above problems. So, MEM has been widely used in the field of stem cell therapy and research. At present, the researches of the effect about MEM on the biological behavior of stem cells mostly stay at the phase of tissue and cell characterization after applying treatment. In order to further understand the regulation effect and mechanism of MEM on stem cells, this paper reviews the related research in recent years.

### Application classification of MEM

The common treatment methods in MEM include low-intensity pulsed ultrasound (LIPUS), low-intensity

extracorporeal shock waves therapy (Li-ESWT), and low-frequency pulsed electromagnetic fields (PEMF).

### Li-ESWT

Shock wave is a kind of bi-directional sound wave with frequency at 16Hz-20MHz, consisted by a short-time extremely high positive amplitude and a long-term low amplitude negative amplitude, transmitting energy to a remote anatomical target with minimal effect on the tissue located between the generator and the target (8). It has the characteristics of high peak pressure (up to 100 MPa or higher), rapid pressure rise (<10 ns) and short duration time (<10  $\mu$ s) (8), which can be divided into focused and divergent types. Shock wave disperses considerably less energy at 0.03–0.1 mJ/mm<sup>2</sup>, is unfocused, and fewer shocks are administered. Li-ESWT, with usually less than that of 0.1 mJ/mm<sup>2</sup> (0.09 mJ/mm<sup>2</sup> most common) energy flux density (EFD) (9), can produce mechanical force, micro-cavitation and thermodynamic effects to generate biological roles. Its energy density can be calculated as EFD  $\times$  number of shock. Li-ESWT with 0.09mJ/mm<sup>2</sup> shocked 50–400 times in each session can generate 4.5–36 mJ/mm<sup>2</sup> cumulative energy. Recently, Li-ESWT has been used in fracture healing, pain relief, arthritis treatment, soft tissue healing and revascularization, without causing damage at the site of action (10-13). Meanwhile, Li-ESWT also participates in the treatment of muscle diseases, heart disease, nonhealing or delayed healing wounds, and erectile dysfunction (ED) (14-17).

### Low frequency PEMF

PEMF is a dynamic form of applied energy, which includes both magnetic and electric fields. Cells usually have a complex charge system, which is a potential target of electromagnetic action. PEMF can transfer energy and regulate the biological behavior of cells, which is achieved through the bioelectric fields produced by inductive coupling (18). PEMF, with fundamental (burst) frequency from 0 to 300 Hz, the pulse frequency about 4 kHz, and magnetic field amplitude from some mT to several tens of mT, has the characteristics of intermittent emission, square or trapezoidal waveform, and high safety (19,20). As a micro-energy therapy method, PEMF can be used in bone healing, osteoporosis treatment and prevent (21,22), and also act to improve angiogenesis, promote blood circulation and treat soft tissue injury, Parkinson's disease and other diseases (23-25).

## LIPUS

Ultrasound is a form of energy transfer for mechanical vibration, with a frequency from  $2 \times 10^4$  to  $2 \times 10^9$  Hz in transverse or longitudinal (26). When spreads in the organism, a certain dosage of ultrasound can cause changes of the organism function and structure, that is the biological effect of ultrasound. By adjusting the relevant parameters, scholars have found that ultrasound at the intensities of 0.05–0.50 W/cm<sup>2</sup> can be used in imaging medicine, and 0.03–1,000 W/cm<sup>2</sup> intensity ultrasound can be used for surgery and therapy (27).

As a widely used method in MEM, LIPUS emits low-intensity ultrasound ( $<3$  W/cm<sup>2</sup>) in the mode of pulse wave (100–1,000 Hz). The pulse period has ON and OFF signal periods, which are referred to as the duty cycle of the signal (26). LIPUS has good tissue penetration and low thermal effect, and realizes its therapeutic roles by mechanical effect. Most researchers used LIPUS with ultrasound intensity at 30 mW/cm<sup>2</sup>, pulse ratio 1:4 at 1,000 Hz, and frequency at 1.5 MHz in their studies, and the cumulative energy (in J/cm<sup>2</sup>) of LIPUS equals ultrasound intensity (in W/cm<sup>2</sup>) times time (in seconds). Since the application time of LIPUS is about 2–20 min, and the intensity range is from 0.03 to 1.00 W/cm<sup>2</sup>, thus the commonly used energy density of LIPUS is about 2–150 J/cm<sup>2</sup> (27). In 1994 and 2000 respectively, the U.S. Food and Drug Administration (FDA) approved LIPUS as a safe and non-invasive method for the treatment of fresh fracture healing and nonunion (28). Moreover, LIPUS is also effective in promoting fibroblast differentiation, cartilage tissue, ligament, tendon repair and other treatments (29–33). Sports medicine research found that LIPUS is conducive to the penetration of topical drugs through the skin to injured site, reducing athlete recovery time (34).

## Biological effects of MEM on stem cells

MEM could be capable of improving stem cell proliferation, self-renewal and other biological behaviors achieved by regulating cell cycle and promoting the expression of related genes (35,36). LIPUS treatment significantly increased the expression of differentiation-related genes (such as osteocalcin) of mesenchymal stem cells and boosted their osteogenic differentiation. Meanwhile, endothelial differentiation inducer combined with LIPUS could more obviously advance the von Willebrand factor (vWF) expression and revascularization than didn't (37).

The results not only proved that LIPUS could improve stem cell differentiation, but also suggested that LIPUS combined with other stem cell differentiation inducing factors could increase the multi-directional differentiation ability of stem cells. MEM could also regulate the migration of mesenchymal stem cells to the treated site through rearranging cytoskeleton and expressing cell migration-related genes (such as ITGA8), *in vivo* and *in vitro*. Simultaneously, the recruited stem cells secreted a large number of cellular active factors, such as nerve growth factor (NGF) and vascular endothelial growth factor (EGF), to promote the damaged nerves and vessels repair (38,39). Literature review found that in a certain range, the biological efficacy of MEM on stem cells increased with the increase of its dosage, but excessive energy beyond a threshold would lead to some negative results, for example, cell apoptosis (40,41).

## Effect of MEM on exogenous stem cells

Exogenous stem cells used for stem cell transplantation in RM usually have the cross-system, cross-derm differentiation potential and high self-renewal ability, which can be divided into two types: embryonic stem cells and adult stem cells. Embryonic stem cells have great medical potential as omnipotent stem cells, but they cannot be used for clinical treatment owing to the ethical problems. Therefore, researchers gradually focus on adult stem cells, including bone marrow mesenchymal stem cells (BMSCs), adipose-derived stem cells (ADSCs) and others.

## Effect of MEM on BMSCs

BMSC, a kind of adult stem cell deriving from bone marrow, is more suitable for stem cell transplantation than other cells because of its advantages such as convenient material acquisition, low immune rejection and easy foreign gene transfection and expression. Therefore, BMSCs have been expected to be an ideal tool in the field of cell therapy in RM. However, some defects in BMSCs practical application are also existing, for instance low efficiency of proliferation and differentiation, low survival rate, and small number of cells homing to the target organs (42). Many works regarding BMSCs have demonstrated MEM could effectively improve above problems.

MEM-mediated BMSCs transplantation is most widely used in fracture researches. In a study, researchers found that *in vitro* LIPUS (unfocused plane waves, frequency 1.5 MHz, duty cycle 1:4, spatial average-temporal average

intensity  $30\text{mW}/\text{cm}^2$ , pulse repetition frequency 1 kHz for pulse duration of 200  $\mu\text{s}$ , 20 minutes/day, 7 days) increased the stromal cell derived factor-1 (SDF-1) and C-X-C chemokine receptor type 4 (CXCR4) mRNA levels about 1.6 times and 4.3 times than control group without LIPUS treatment respectively and promoted migration of BMSCs in cell migration assay ( $P=0.003$ ). Compared with injection of BMSCs alone in vivo, LIPUS combined with BMSCs transplantation significantly improved the earlier fracture healing and callus formation (43).

Moreover, Sansone *et al.* (44) proposed a novel approach for the treatment of atrophic bone non-unions via parallel applications of Li-ESWT and BMSCs transplantation. Li-ESWT could be employed to induce up-regulation and expression of several angiogenic and osteogenic growth factors, which promoted BMSCs replication and differentiation (44). Another research about PEMF and BMSCs demonstrated that PEMF (20 Hz/2 mT) significantly advanced the activity of the alkaline phosphatase (ALP) ( $P<0.01$ ) and the expression of osteocalcin (osteocalcin and osteopontin) in BMSC, while inhibited adipogenic differentiation of BMSCs by reducing adipogenic factor expression (45). In addition, icariin as a means of regulating osteogenic differentiation of BMSCs has been reported (46), so whether icariin combined with MEM can enhance the regulatory effect of BMSCs is worthy of further study.

MEM combined with BMSCs also plays a role in treating spinal cord, kidney, heart injury and diabetic ED. Ning *et al.* (47) found that BMSCs stimulated by LIPUS (10, 30, 50, 70  $\text{mW}/\text{cm}^2$ , 3 min/d, 3 days) had higher cell viability in cell proliferation, especially cells in 50  $\text{mW}/\text{cm}^2$  group. It can be seen that LIPUS has the optimum intensity for stem cell action. Meanwhile, LIPUS treatment enhanced BMSCs migration ability ( $P<0.0001$ ) and cell neurotrophic factors expression ( $P<0.0001$ ) than control in vitro. BMSCs stimulated by LIPUS could increase the content of brain-derived neurotrophic factor (BDNF) and NGF ( $P<0.0001$ ), reducing reactive gliosis and recovering rat locomotor function. Furthermore, the cavity formation of injured spinal cord rates in LIPUS-treated BMSCs group reduced 20% than BMSCs transplantation group ( $P<0.01$ ) (47). The recent research suggested that LIPUS [frequency 1MHz, duty cycle 10%, peak negative pressure 0.35 MPa ( $0.6\text{ W}/\text{cm}^2$ ), micro-bubble dosage  $10^6/\text{mL}$ , and total ultrasound sonication time 30 s] combined with microbubbles could promote CXCR-4 modified BMSCs homing to acute renal injury tissue of rat ( $P<0.01$ ),

participating in tissue repair, and recovering renal structure and function (48). Chang and other scholars (49) conducted a control study about the effect of the dog model of myocardial infarction treated by BMSCs transplantation, received LIPUS along with microbubbles treatment or not. Results demonstrated that the pretreated BMSCs more gathered in the coronary artery, especially in the infarcted area, and the group of BMSCs transplantation received ultrasound-mediated microbubble destruction showed the most improvement in heart function and percentage of perfusion defect area to total left ventricular perfusion area ( $P<0.05$ ). In 2017, Shan *et al.* (50) found that combination of Li-ESWT and BMSC improved erectile function of diabetic erectile function rats more effectively than BMSC transplantation alone. The reason might be related to that Li-ESWT favored the survival of transplanted BMSCs, increased stromal cell-derived factor-1 expression and enhanced revascularization in the diabetic cavernous tissue (50).

#### Effect of MEM on ADSCs

Compared with BMSCs, the ADSCs' proliferation and differentiation potential have no advantage. However, ADSCs sources are richer and easier to obtain, so that the researches on ADSCs are increasing in recent years. There are also still difficulties in ADSCs transplantation, and the roles of MEM on ADSCs transplantation have been widely reported. Jiang *et al.* (51) found that ADSCs treated by LIPUS (frequency 1.0 MHz, pulse repetition frequency 1 kHz for pulse duration of 200  $\mu\text{s}$ , at  $100\text{ mW}/\text{cm}^2$ ) displayed higher mRNA expression levels of runt-related transcription factor 2 (Runx2), osteocalcin (OCN), ALP and bone sialoprotein (BSP) genes than ADSCs injection alone ( $P<0.05$ ), which suggested LIPUS can induce the osteogenic differentiation of ADSCs (51). Another study proved ADSCs pretreated by Li-EWST had potent therapeutic effect in diabetic bladder dysfunction by secreting VEGF and NGF to improve bladder innervation and revascularization. Compared with untreated ADSCs injection, injecting treated ADSCs also enhanced the migrations of endogenous stem cells to injured sites ( $P<0.05$ ) (52). The roles of PEMF in regulating the osteogenic ability of human adipose-derived mesenchymal stromal cells (hADSC) have been also reported (53). Nasb *et al.* (54) conducted the first clinical study about LIPUS on ADSCs therapy in OA patients. Patients were randomly divided into 3 groups in a ratio of 1:1:1, respectively treated by injecting ASDC, injecting saline with LIPUS treatment (pulsed frequency



1 MHz and  $0.5 \text{ w/cm}^2$  intensity, 20 min/day, 5 days/week for 8 weeks), and injecting ASDC with the same LIPUS treatment (same above). But the results have not been obtained. Many ways can enhance the efficacy of ADSCs transplantation in the treatment of ED. As one of the above methods, icariin can greatly improve the survival rate of transplanted ADSCs, and increase the expression of vWF and  $\alpha$ -SMA (54). In addition, another study also found that icariin II could also repair penile nerve by inducing ADSCs to differentiate into Schwann cells (SCs), and thus enhance erectile function (55). However, the research of MEM along with ADSCs for ED therapy is rare.

#### Effect of MEM on other stem cells

There are some studies on the relation between other stem cells transplantation therapy and MEM. Ling and other scholars (56) isolated human amnion-derived mesenchymal stem cell (hAD-MSC), treated them with LIPUS (frequency 0.25 MHz, burst width sine wave 200  $\mu\text{s}$ , duty cycle 20%, pulse repetition frequency, 1 kHz), and then injected them into the caudal vein of rats with ovarian insufficiency. Results revealed that LIPUS promoted hAD-MSCs to express and secrete growth factors *in vitro*. And LIPUS-pretreated hAD-MSC transplantation increased the weight of reproductive organs, improved ovarian function, and reduced ovarian inflammation than untreated hAD-MSC transplantation (all  $P < 0.01$ ) (56). As a class of stem cells with multi-directional differentiation potential obtained from somatic cell reprogramming, induced pluripotent stem cells (iPSCs) have less immunogenicity and do not involve ethical issues. Xia *et al.* (57) conducted a randomized, sham-controlled study using LIPUS and iPSCs for nerve injured rat. LIPUS combined with iPSCs injection enhanced the repair of transected sciatic nerve in rats than iPSCs transplantation and LIPUS treatment alone ( $P < 0.05$ ). Meanwhile, this study demonstrated that LIPUS obviously promoted the neural differentiation of iPSCs *in vitro* (57). But iPSCs may have potential problems because of its modified genes. Therefore, longer-term observation and research for the safety of iPSCs therapy are also needed.

#### Effect of MEM on endogenous stem cells

MEM combined with exogenous stem cell transplantation has achieved many good results in basic and animal experiments for a variety of diseases, repaired the injured tissues and significantly improved their functions. However, due to the existence of immunogenicity, tumorigenic risk

and potential ethical problems, the clinical transformation of exogenous stem cell transplantation is difficult. Compared with exogenous stem cell transplantation treatment, individual endogenous stem cell therapy can effectively avoid above risks. Therefore, scholars turn their attention into the treatment of endogenous stem cells, but there are also still some problems, such as small number of endogenous stem cells, lag of cell activation and slow differentiation process. These are the reasons why the studies on the effect of MEM on endogenous stem cells are increasing as time goes on.

#### Effect of MEM on myogenic stem cells

The relatively unified structure and the simple cells composition of muscle tissue make it easier to track the biological behavior of myogenic stem cells. At the same time, endogenous muscle-derived stem cells are safe on muscle tissue repair, and also their behaviors could be regulated by micro-energy treatment.

According to recent study, Yang *et al.* (58) used LIPUS [intensity 0, 100, 200, and 300  $\text{mW/cm}^2$ , pulse on and off time ratio of 1:4 (200  $\mu\text{s}$ :800  $\mu\text{s}$ ) at 1,000 Hz and frequency at 1.7 MHz, 3 minutes each time, every 2 days for 7 times] to treat rats of stress urinary incontinence (SUI). The results demonstrated that in 200  $\text{mW/cm}^2$  and 300  $\text{mW/cm}^2$  groups, the bladder capacity (BC) and leak point pressure (LPP) were restored to a normal level, and 200  $\text{mW/cm}^2$  LIPUS promote the regeneration of striated muscle ( $P < 0.05$ ), that plays important role in urinary continence function. Furthermore, 200  $\text{mW/cm}^2$  LIPUS could also enhance activation of satellite cell myogenic differentiation ( $P < 0.05$ ) (58). In 2018, Wu *et al.* (59) found that Li-ESWT (0.06  $\text{mJ/mm}^2$ , and 300 shocks at 3 Hz) improved urethral muscle regeneration of SUI rats model ( $P < 0.05$ ), and significantly increased EdU+ endogenous progenitor cells in the muscle layer, particularly the striated muscle. Moreover, Li-ESWT could also promote rat myoblast to format myotube *in vitro* (59). Kang *et al.* (60) also proposed that compared with untreated Zucker Fatty rats (a rat model of obese-related SUI), micro-energy acoustic pulses (at 0.033  $\text{mJ/mm}^2$ , 3 Hz for 500 pulses, two times a week for 2 weeks) significantly increased H3P+ cells in pelvic floor muscle and urethra of ZF ( $P < 0.01$ ). Furthermore, micro-energy acoustic pulses promote stem cells differentiation into satellite cells in rat pelvic layer muscle and urethra (60). These results indicate that MEM, act to endogenous stem cells, represents a potential novel non-invasive therapy for SUI.

In the aspect of ED therapy, in 2016, Behr-Roussel *et al.* (61) had made the assumption that Li-ESWT could trigger the activation of various intracellular signaling pathways causing up-regulation of numerous angiogenic factors (i.e., VEGF) to recruit stem cells and promote their differentiation to form neovascularization, and then improve erectile function. Moreover, in 2019, scholars identified and localized the penile stem/progenitor cells (PSPCs) with multiple differentiation characterize. Meanwhile, they found that Li-ESWT (0.033 mJ/mm<sup>2</sup>, 1 Hz, 50 pulses) enhanced expression of  $\alpha$ -SMA from 0.14±0.01 to 0.31±0.02 (P<0.001) of PSPCs, and provoked cells to differentiate into smooth muscle cells rather than skeleton muscle cells *in vitro* (62,63). Both animal and cell experiments have verified the positive effect of Li-ESWT treatment for ED by acting endogenous stem cells. In addition, almost ED patients experienced improvements in their erectile condition following Li-ESWT therapy (64), which supported the above conclusion.

#### Effect of MEM on neural stem cells

The repair of spinal cord and nerve injury can be achieved by activating neural stem cells. The biological effects of MEM on neural stem cells have also been reported. Compared with the other mechanical waves, the PEMF is more penetrating and safer in the neural tissue.

Hei *et al.* (65) performed a sham-control trial using PEMF (1 mT, different frequency) for SCs. the result demonstrated that in condition of 50 Hz, 1 h/d SCs was increased from 4th–7th day, and mRNA expression of BDNF and S100 was significantly increased at the same condition from first week to third week (P<0.05) than other conditions of PEMF treatment. In addition, researchers also used PEMF (1 mT, different frequency) to treat never injured rats, and then counted axon and neurons labeled with Dil in damaged never. They found that PEMF with different frequency (50 Hz 1 h/day, 50 Hz 12 h/day, 150 Hz 1 h/day and 150 Hz 12 h/day) could also significantly regenerate axon and neurons, and in 50Hz 1Hr/day condition, the regeneration was most obvious. The results of animal and cell studies are consistent in the best suitable frequency (65). Another study, Vincenzi *et al.* (66) found that the contents of hypoxia-inducible factor-1 (HIF-1), reactive oxygen species (ROS) and inflammatory cytokines in rat injured nerves were decreased by PEMF, which was greatly reducing neuron-Like and microglial cells death and apoptosis.

The protective effect on hypoxic injury of neural stem cells and the role of promoting nerve regeneration lead

PEMF to a potential treatment for cerebral ischemia and other nerve injury diseases.

#### Mechanism of MEM action on stem cells

Ultrasound and shock waves can generate thermal energy through mechanical wave vibration, while electromagnetic waves use non-ionizing radiation to cause local temperature rise. Although suitable thermal energy can regulate the expression of cell-related factors and biological behaviors, such as cell metabolism, these effects are too weak. Thus, thermal effect is not discussed as the main mechanism.

Many studies have considered mechanical effects as the most important biological effects of ultrasound and shock waves on stem cells. Stem cells recognize mechanical signals through certain cell membrane proteins or cell structures, and respond to activate related protein pathways within the cell, causing changes in function of key proteins and gene expression. The process of receiving mechanical signals, activating cellular signaling pathways, and conduction between different cells can be divided into three phases, including: mechanical coupling phase, biochemical coupling phase, and signal transmission phase (67).

How cells transduce the mechanical force into biochemical stimulation is not completely understood. At present, it is commonly believed that the cell structures participating in mechanical force transduction include integrins, ion channels, specific cytoskeleton proteins, cell junction molecules, and certain mechanical sensitivity protein complexes. In these structures, integrins, mechanosensitive ion channels are studied more widely.

In a study of mechanical signal activating mechanosensitive Ca<sup>2+</sup> channels, it was found that the stimulation of mechanical force with appropriate intensity caused cytoskeleton proteins to pull ion channels, leading to channels open and Ca<sup>2+</sup> influx (68). Li-ESWT can also stimulate connexin 43 hemichannels to release ATPs, which activate P2Y1 purinergic receptors and then trigger the PLC-IP3-IP3R cascade to release Ca<sup>2+</sup> into the cytoplasm (69). Moreover, Gao *et al.* (70) found that dental pulp stem cells (DPSC) presented membrane ion channels Piezo1, participating in DPSC proliferation mediated by LIPUS. In 2018, Zhao *et al.* (71) revealed that piezo1 channel is a propeller-shaped three-bladed trimeric architecture, consisted by central ion-conducting pore and the peripheral blade-like structures. When piezo1 was stimulated by mechanical force, its peripheral blade-like structures and the distal parts of beam changed

dramatically, while the central pore area and the central part of beam showed only slight displacement, and these piezo1 structural changes conformed to the principle of leverage. Combined with previous researches, they proposed the piezo1 mechanosensitive mechanism hypothesis that transmembrane helical unit (THU), the structural units of peripheral blade, and beam were regarded as the mechanical force sensor and mechanical transmission device respectively, to regulate channels open or close, which caused changes about intracellular cation concentration, especially  $\text{Ca}^{2+}$ , to regulate cell behaviors (71). Increase of  $\text{Ca}^{2+}$  within cells can affect the synthesis of cell growth factors and gene expression by regulating  $\text{Ca}^{2+}$ -dependent enzyme activity and activating downstream signaling pathways (69). Other mechanosensitive ion channels include MscL, YAP, etc. (72,73). Pilla (74) found that electromagnetic fields can also activate cation channels, to further activate the second messenger and affect gene transcription, eventually realizing the role of PEMF in regulating cell activity.

Recently, studies demonstrated that ultrasound effect on stem cells to cause cell proliferation and differentiation is closely related to the integrin in the cell membrane (75), and Li-ESWT can also induce stem cell differentiation mediated by integrin (76). In the inactivated state, the integrin conformation is usually in a folded condition and exhibits low affinity for ligand binding. When cells encounter certain activation signals, related regulatory proteins in the cell, such as talin and kindlin proteins, are induced to bind to cytoskeletal protein and the intracellular region of integrin, inducing integrin conformation to change from a folded state to an extended state, and hence increasing ligand-binding affinity (77,78). The ligand (such as fibrin, laminin, glassin) binding to integrin can also further induce conformational changes and clustering of integrin on the cell membrane surface (79), thereby activating multiple protein kinases and phosphatases binding to integrin intracellular regions. These macromolecular protein complexes contain focal adhesion kinase (FAK), Src kinase, integrin linked kinase (ILK), etc. Most studies focused on FAK and FAK-mediated downstream pathways, including FAK-Ras-MAPK, FAK-PI3K-AKT/PKB, FAK-STAT1, FAK-GTPase, etc. (80). FAK conformation changes lead to its autophosphorylation, which make downstream MAP3Ks, Ras phosphorylation, and then activate MAPK and PI3K-AKT pathways. As one intracellular signaling pathway participating in differentiation of myogenic cell, activated P38 MAPK promotes the activity of transcription factors

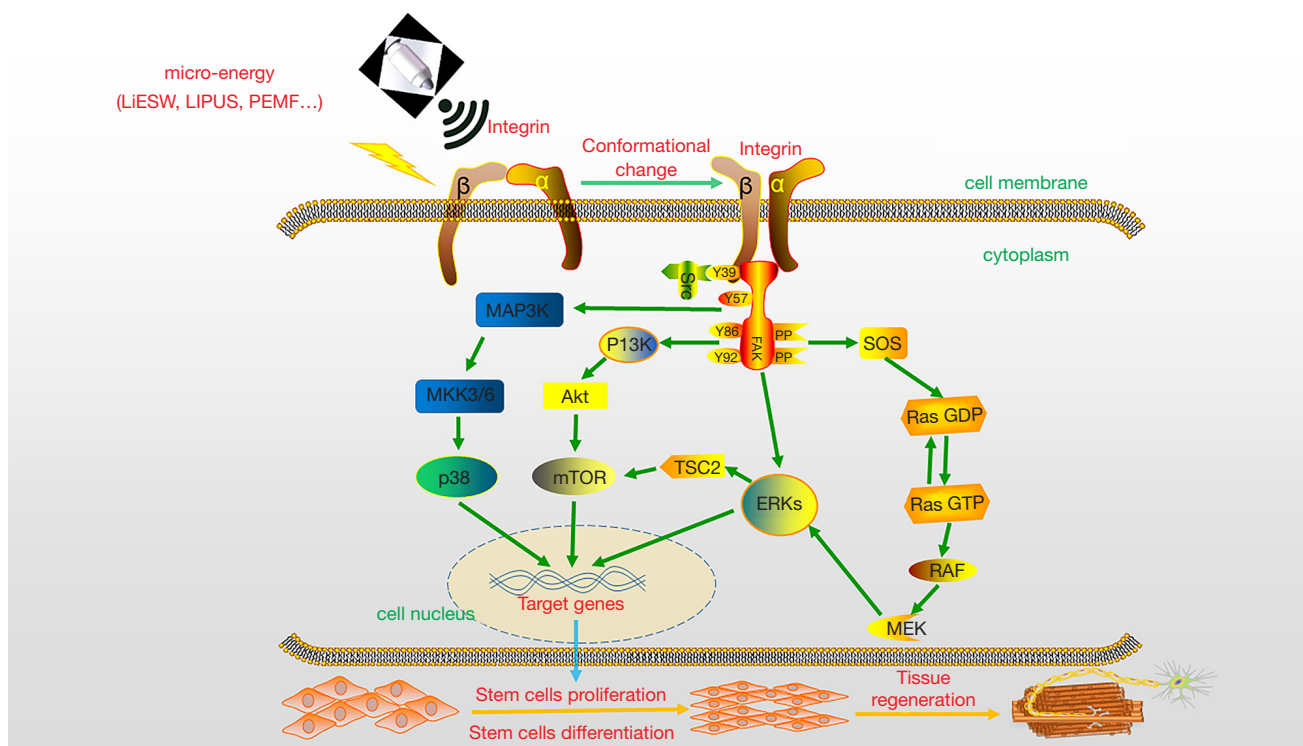
of the MyoD families and takes part in the remodeling of muscle-regulatory related chromatin (81), and also phosphorylates E47, promoting its heterodimerization with MyoD. The MyoD-E47 heterodimerization activates the muscle-specific transcription (82). Furthermore, PI3K/AKT signalling pathway plays a role in the control of stem cells pluripotency and differentiation. Active PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3), and then recruits and phosphorylates AKT, as well as activates mTORC2 (83). Activated AKT can improve the activation of mTORC1, which regulates cell cycle-regulated protein expression and promotes cell cycle conversion from G0 to G(Alert). The transition of quiescent stem cells into G(Alert) functions positions stem cells to respond rapidly under conditions of injury and stress, priming them for cell cycle entry (84). Meanwhile, mTORC1 can also increase stem cell activity and promote cell proliferation (85). In addition, mTORC2 maintains cell survival and metabolism (86). Other identified pathways activated by micro-energy treatment include: JNK, Wnt, PERK/ATF, ATP/P2X7, Notch (87). And many important pathways have yet to be discovered. The processes of Micro-energy mechanical force transduction mediated by integrin are as shown in *Figure 1*, including three maintain signaling pathways.

Ultrasound (or shock wave) microbubbles, exceed a certain threshold, can cause a sonoporation effect, that is, the above methods make the cell membrane surface temporarily disruption, which promote extracellular molecules to enter the cell, causing changes in cell biological behaviors (88). This effect is widely used in experimental studies such as cell transfection and drug infusion, and also provides a novel assumption than traditional opinion of mechanical force transduction.

In order to improve the therapeutic theory of MEM, it is of great significance to clarify the mechanism of action of MEM on cells and completely understand the specific ways and regulatory process of the effect.

### Limitation

Although increasing scholars affirm the effects of MEM, there are more and more worries. MEM can regulate cell functions through different mechanisms such as mechanical effects and electromagnetic effects, but there is no uniform standard for the indexes. Currently, there are a variety of MEM instruments used for basic research or clinical



**Figure 1** Important role and mechanism of micro-energy treatment in regenerative medicine. LIPUS, low-intensity pulsed ultrasound; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B (PKB), also known as Akt; Src, Src is short for sarcoma, a proto-oncogene tyrosine-protein kinase Src; TSC2, tuberous sclerosis complex 2; RAS, molecules of MAPK/ERK pathway; RAF, molecules of MAPK/ERK pathway; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin complex 1; FAK, focal adhesion kinase; SOS, Son of Sevenless; MAP3K, MAP kinase kinase kinase; MKK3/6, MAP kinase kinase 3/6; p38, p38 MAPK.

therapy, such as the MTS Dermagold (Atlanta, GA, USA), WBL-ED (Beijing, china), TY-PEMF-CS (Shanghai, china) and others, and also their parameters criterion and ranges are not coincident. The FDA stipulated the safety range of diagnostic ultrasound, including mechanical effect index, thermodynamic effect index and exposure time (89), but the non-imaging medicine safety standard for MEM has not been proposed. The cells with different types have different response and tolerance for micro-energy treatment, and the difference is more significantly in animal experiments. Human tissue structure is more complex, so parameters setting is more difficult. Improper stimulation would damage tissues and cells, even lead to more fearful potential consequences, so it is notably important to understand the mechanism of MEM on cells.

**Summary**

The related studies on the biological effects of MEM on

stem cells have proved that MEM is a safe and effective treatment in RM. Meanwhile, due to the numerous insurmountable risks of exogenous stem cell transplantation, endogenous stem cell micro-energy might be important target of micro-energy in regeneration. Micro-energy regulates stem cells mainly through its mechanical effects. Stem cells recognize and transduce cell surface mechanical force signals to intracellular biochemical signals through specialized cellular structures, and further activating a large number of downstream signaling pathways to achieve the regulation of cell biological behaviors, such as proliferation and differentiation. Integrins as important cell membrane mechanosensitive proteins play key roles in the above micro-energy mechanical force transduction process. At the same time, other possible mechanical force transduction ways and intracellular related downstream signaling pathways have been deeper and more extensive in recent years Thus, existing researches make the mechanisms by which micro-energy treatment regulates the involvement



of stem cells in tissue and organ regeneration clearer, and also propose possible directions and challenges for future research. In conclusion, the integration of life sciences, physics and engineering will significantly promote the advancement of MEM in RM and bring good news for human health.

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## Footnote

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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