OnLine Journal of Biological Sciences

Literature Review

A Review of Cellular Mechanism of Pulsed Electromagnetic Field (PEMF) in Inhibiting Adipogenesis of Mesenchymal Stem Cells

[1]Puji Sari, [2]Nuzli Fahdia Mazfufah, [3]Umiatin, [1]Dwi Anita Suryandari, [1]Silvia Werdhy Lestari and [1]Luluk Yunaini

[1]Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Department of Stem Cells and Tissue Engineering Research Center, IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[3]Departement of Physics, Universitas Negeri Jakarta, Jakarta, Indonesia

Article history
Received: 04-08-2022
Revised: 05-10-2022
Accepted: 07-12-2022

Corresponding Author:
Nuzli Fahdia Mazfufah
Department of Stem Cells and Tissue Engineering Research Center, IMERI, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
Email: nuzli.fahdia@ui.ac.id

Abstract: PEMF is a method that utilizes electric current using a coil that is powered by dynamic electric current, it will cause a dynamic magnetic field in the coil. In the last few decades, it is known that PEMF has the potential to be used as a modality in the treatment of obesity. For this reason, PEMFs have been studied *in vitro* as their effect in inhibiting the adipogenesis of Mesenchymal Stem Cells (MSC). But the use of PEMF therapy in inhibiting adipogenesis still has many challenges. Understanding of the mechanisms that occur in cells that are exposed to PEMF is also still very slight. This article reviews the studies that investigated *in vitro* effect of PEMF in inhibiting adipogenesis studies and the underlying mechanism. In the future, it is hoped that this review can be used as a basic reference for *in vivo* research and clinical trials related to inhibiting adipogenesis using PEMF.

Keywords: Obesity, PEMF, Adipogenesis, MSC

Introduction

Studies have confirmed that obesity has become a serious health problem in the world (Djalalinia *et al*., 2015; Ng *et al*., 2014; Kelishadi, 2007). The prevalence of obesity from year to year continues to increase dramatically in most countries (Hruby and Hu, 2015). Data recorded by the World Health Organization (WHO) in 2016, shows that 1.9 billion adults in the world are overweight and at least 650 million people experiencing obesity (WHO, 2016).

Obesity occurs when there is excessive deposition of fat into adipose tissue (Ofei, 2005). Abnormal accumulation can cause various negative effects on the body, including cancers, type 2 diabetes, hypertension, stroke, coronary artery disease, congestive heart failure, asthma, chronic back pain, osteoarthritis, pulmonary embolism, gallbladder disease, and also an increased risk of disability (Djalalinia *et al*., 2015). Studies also found a consistent association between the high risk of premature morbidity or mortality particularly and cardio metabolic morbidity with overweight and obesity (Reilly and Kelly, 2011).

Until now, treatment for obese patients is carried out through strict dietary control, physical exercise, changing lifestyle habits, and consuming appetite suppressant drugs (Wyatt, 2013). Managing weight in a conventional manner requires at least one year and sometimes requires more time length (Benton and Young, 2017). One of the potential modalities that can be used for obesity therapy is the use of used Electro Magnetic Field (PEMF).

PEMF is a form of biophysical stimulus that utilizes an electric current indirectly. The PEMF method is a method of utilizing electric current using a coil that is powered by dynamic electric current, it will cause a dynamic magnetic field in the coil (Yadollahpour and Rashidi, 2015). The effect of PEMF has been studied further related to its ability to inhibit adipogenesis. Adipogenesis produces adipocyte cells that function for energy storage, an imbalance in the process of storing and expending energy can cause obesity (Longo *et al*., 2019).

Adipogenesis can be done *in vivo* and *in vitro*. One type of cell that can be used to observe adipogenesis *in vitro* is the Mesenchymal Stem Cell (MSC), which has the ability to differentiate into adipocyte cells (Kozlowska *et al*., 2019). At the present, the research related to PEMF and adipogenesis is still limited. It is because of the limited information related to the mechanism that underlies the effects of PEMF exposure. The lack of standardization of the PEMF variables used in the experiment also becomes

a problem. This review discusses *in vitro* studies related to PEMF exposure in inhibiting adipogenesis and its underlying cellular mechanisms.

Adipogenesis of Mesenchymal Stem Cells

Stem cells are known as cells that do not have a specialization, can renew themselves continuously, and can differentiate into several types of cells in certain physiological or experimental conditions (Wei *et al*., 2013; Chagastelles and Nardi, 2011). In general, stem cells can be categorized into several types, namely Embryonic Stem Cells (ESC), induced Pluripotent Stem Cells (iPSC), and adult stem cells (Zakrzewski *et al*., 2019). One type of adult stem cell that is widely used in the medical world is mesenchymal stem cells or human Mesenchymal Stem Cells (hMSC), this type of stem cell is multipotent and non-hematopoietic (Parekkadan and Milwid, 2010). Like other adult stem cells, mesenchymal stem cells can proliferate over a long period of self-renewal. Mesenchymal Stem Cells (MSC) have the capacity to differentiate into mesodermal, ectodermal, and endodermal lineages (Wakao *et al*., 2012).

MSC is present in almost every tissue. 25 MSC, which were first investigated, were obtained from bone marrow sources (Ullah *et al*., 2015; Orbay *et al*., 2012). Until now, MSC has been isolated from several other parts of the body, including adipose tissue (Bunnell *et al*., 2008), umbilical cord (Hassan *et al*., 2017), amnion (Araújo *et al*., 2018), chorion (Araújo *et al*., 2018), placenta decidua (Shaer *et al*., 2014), dental tissue (Huang *et al*., 2009), peripheral blood (Trivanović *et al*., 2013), synovial fluid (Harvanová *et al*., 2011), skin (Orciani and Di Primio, 2013) and Wharton's jelly (Varaa *et al*., 2019). As a result of MSC being isolated from various sources and by various means of isolation and propagation, the determination of the characteristics of MSC is inconsistent and differs from one laboratory to another (Dominici *et al*., 2006). Therefore, the International Society for Cellular Therapy (ISCT) determines standard criteria for MSC characteristics. The first criterion, MSC must be able to stick to plastic (plastic adherent) when in a standard culture using culture containers. Second, 95% of the MSC population must positively express CD105, CD73, and CD90, which are measured using flow cytometry. MSC must also have 2-5% negative expressions of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR. Third, MSC must be able to differentiate into osteoblasts, adipocytes, and chondroblasts under standard *in vitro* differentiation conditions (Dominici *et al*., 2006).

Adipogenesis refers to the process of adipose tissue formation, this process consists of many stages that begin with the expansion of MSC clones, MSC differentiation into preadipocytes, and the formation of mature adipocytes (Fink and Zachar, 2011; Chen *et al*., 2016; Kwan *et al*., 2015). MSC cultured *in vitro* with adipogenic induction media are able to complete their adipogenesis in various time frames, ranging from 7 to 21 days (Lee and Fried, 2014). Adipogenic induced cells will undergo morphological changes and have vacuoles or lipid "droplets" as a sign of differentiated cells (Lee and Fried, 2014) These cells can be retained in culture for more than 40 days, the longer the time culture and induction of adipogenic media, the size of lipid droplets will grow even greater (Kawai and Rosen, 2012). Understanding the process of differentiation can help design potential anti-obesity strategies (Lee and Fried, 2014).

Adipogenesis is a complex process, this process is integrated with other processes, which involve many transcription factors that have a specific role (Shaer *et al*., 2014; Takada *et al*., 2009). Molecular and cellular mechanisms of adipocyte differentiation have been studied in depth through *in vitro* and *in vivo* studies (Takada *et al*., 2009). After the expansion of preadipocytes, MSC will differentiate into mature adipocytes under strict control of various transcription factors, including C/EBPβ/δ and PPARγ (Takada *et al*., 2009; Moseti *et al*., 2016). PPARγ is a nuclear receptor as well as a transcription factor that plays a key role in adipogenesis (Takada *et al*., 2009). Activation of PPARγ is the initiation of adipogenesis because PPARγ is a transcription factor that activates adipogenic genes that play a role in adipogenesis (Tontonoz *et al*., 1994). Excessive PPARγ expression in fibroblast cell lines is known to initiate adipogenesis (Barak *et al*., 1999). In contrast, PPARγ defects in ESC and mouse embryonic fibroblasts are known to inhibit the occurrence of adipogenesis in fibroblast cell lines known to initiate adipogenesis (Lefterova *et al*., 2014).

PPARγ is a nuclear receptor and a ligand dependent transcription factor that play a role in the differentiation and regulation of adipose cells (Ma *et al*., 2018; Cariou *et al*., 2012). PPARγ also plays a role in the process of storing lipids, thermogenesis, active modulators of lipid metabolism, and insulin sensitivity (Tontonoz *et al*., 1994). Some lipid metabolites have been known to be involved as adipocytes. PPARγ activators, including unsaturated fatty acids or poly-unsaturated fatty acids, eicosanoids, prostaglandins, 15-deoxy-Δ12,14 prostaglandin J2 (Ma *et al*., 2018). However, these molecules are known to have a low affinity for PPARγ and their numbers are also small in adipocyte cells (Ma *et al*., 2018). This makes the physiological relevance of these molecules as endogenous PPARγ ligands (Ma *et al*., 2018). At this time, physiologically relevant PPARγ ligands remain unknown (Ma *et al*., 2018). The known exogenous PPARγ ligands are Thiazolidinediones (TZD), which is an anti-diabetes drug (Dave *et al*., 2012).

Correlation Between Adipogenesis and Obesity

Adipose cells resulting from adipogenesis have the main function of energy storage and lipid homeostasis (Choe *et al*., 2016). In mammals, adipose tissue consists of at least two types of adipose tissue, namely white adipose tissue or White Adipose Tissue (WAT) and brown adipose tissue or Brown Adipose Tissue (BAT) (Gómez-Hernández *et al*., 2016; Marcelin and Chua Jr, 2010). WAT is the dominant type of adipose tissue, WAT is located in the subcutaneous and visceral parts that surround internal organs, such as the heart, intestine, kidney, and gonad (Betz and Enerbäck, 2015). The primary function of WAT is as a storage of energy in the form of triglycerides (Betz and Enerbäck, 2015). In humans, the development of WAT occurs mostly after birth and continues throughout life.45 the BAT has a primary role as a temperature regulator.64 BAT has the ability to change chemical energy and instantly becomes hot when activated by the nervous system sympathetic (Ljungberg, 2006). BAT allows small mammals to maintain a constant body temperature at cold (Ljungberg, 2006).

Adipose tissue function in general is closely related to the process of lipid metabolism. The process of lipid metabolism begins with the process of digestion of fat consumed, this fat will go through the process of digestion and break down into fatty acids and glycerol. Glycerol is a component that dissolves in water so that glycerol will easily enter the circulatory system to the liver. Conversely, fatty acids have water insoluble properties, so fatty acids will be emulsified before going to the small intestine to be absorbed. In small intestinal epithelial cells, fatty acids are converted to triglycerides. Triglycerides are the main energy source in the body, because triglycerides have hydrophobic properties, triglycerides are transported through the circulation in a complex form called lipoprotein (Desai *et al*., 2013). In the body, lipoprotein is metabolized through two pathways, namely the exogenous and endogenous pathways (Desai *et al*., 2013).

The exogenous pathway is the pathway for transporting lipids from the small intestine to the tissues, in which the triglycerides are circulated in the form of chylomicrons (Desai *et al*., 2013). A large number of triglycerides carried in the chylomicron form are then hydrolyzed by Lipoprotein Lipase (LPL). The bond between chylomicrons and LPL causes triglycerides to be hydrolyzed into Free Fatty Acids (FFA). Most of this FFA will be used as energy in the muscles and heart or directed for re-esterification and storage in adipose tissue. Meanwhile, the remnants of the hydrolyzed chylomicron will be cleansed from the circulation by absorption by the liver through LDL Receptor (LDLR) or LDL-Receptor Related Protein (LRP) (Desai *et al*., 2013).

Endogenous lipoprotein pathways are pathways for the distribution of lipids from the liver to peripheral tissue (Desai *et al*., 2013). These pathways begin with the secretion of Very Low-Density Lipoprotein (VLDL) particles that are rich in triglycerides from liver hepatocyte cells. These particles are then hydrolyzed by LPL and become FFA which will be stored in muscle and adipose tissue. Another result of this hydrolysis is Intermediate Density Lipoprotein (IDL) particles, some of the IDL produced will go to the liver and be further metabolized by Hepatic Lipase (HL). Some LDL will carry out its role in circulation, which is to transport cholesterol to peripheral tissues. In contrast, High Density Lipoprotein (HDL) plays a role in returning excess cholesterol in the peripheral tissues to the liver (Desai *et al*., 2013).

An imbalance between energy storage and expense in adipose tissue will cause obesity (Choe *et al*., 2016). In addition, obesity can also be caused by an increase in the process of adipogenesis (Camp *et al*., 2002). An increase in the process of adipogenesis also impacts an increase in the mass of the adipose tissue followed by an increase in the number of cells fat (hyperplasia) and increased fat cell size (hypertrophy) (Wade, 2013). In this context, a decrease in adipose tissue mass can be directly related to the healing process of obesity. A decrease in adipose tissue mass is known to be able to cause loss of lipids through lipolysis and loss of mature fat cells through apoptosis (Choe *et al*., 2016).

Pulsed Electromagnetic Field

Electromagnetic modalities include all types of modalities that use electricity to produce electric and magnetic fields (Malmivuo and Plonsey, 1995). Generally, this stimulation is carried out using electronic devices outside biological networks to cause electrical effects in the network (Lu *et al*., 2015). The large electric fields that will appear on the network depend on tissue type and exposure characteristics. A magnetic field of 0.1-1 mT can produce an inductive electric field of 1-100 mV/cm in the target network. One of the electromagnetic based stimulation methods that are widely used in the health field is PEMF.

PEMF is known as a form of biophysical stimulus that utilizes an electric current indirectly. PEMF allows a dynamic electric current to flow through the Helmholtz coil so as to produce a dynamic magnetic field effect with pulsed intervals that can stimulate the microenvironment to influence physiological activity in organisms during pulsed (Haghnegahdar *et al*., 2014) These events are in accordance with Maxwell's equations expressed by the formula $\nabla \times E = dB/dt$.

Electromagnetic waves are electric currents and paired magnetic fields produced by the movement of electrons (Malmivuo and Plonsey, 1995). This radiation produces energy that moves and spreads over time (Touitou and Selmaoui, 2022). Electromagnetic radiation has a spectrum that contains a series of electromagnetic waves in different frequencies, which cannot be seen by humans with the eye naked (Touitou and Selmaoui, 2022). These electromagnetic waves can be generated, transmitted, and distributed according to three frequency ranges, namely:

The Extremely Low Frequency range (ELF) covers frequencies around the number 50-60 Hz, the intermediate frequency i.e., the frequencies produced by electrical lines and electronic electrical equipment range from 300 Hz to <10 MHz and radio frequencies (10 MHz to 300 GHz) including radar, radio broadcast, television and telecommunications (Yadollahpour and Rashidi, 2015).

Electromagnetic waves have ionization properties. Based on its characteristic ionization properties, the spectrum of electromagnetic radiation can be divided into two main categories, ionizing and non-ionizing radiation (Malmivuo and Plonsey, 1995). Waves with ionizing frequencies have enough energy to remove electrons from atoms or molecules so that they are harmful to cells (Malmivuo and Plonsey, 1995). Examples of ionizing radiation are ultraviolet, X-rays, and gamma radiation.

The use of electromagnetic waves in the health field has been known for thousands of years (Gómez-Hernández *et al*., 2016; Fukada and Yasuda, 1957). The first written document related to the application of electric waves for medical purposes was known to have existed in (Ullah *et al*., 2015; Fink and Zachar, 2011) CE when Scribonius Largus recommended the use of torpedo fish to cure headaches and gouty arthritis (Huegel *et al*., 2018).

Research into electromagnetic waves and their biological effects continues to grow. In the early 1800s, researchers in the fields of physics and biology for the first time discovered the relationship between physical forces including mechanical force, electricity, magnetism, and ultrasonic waves to the biological state of bones (Fukada and Yasuda, 1957). Departing from these findings, the use of electromagnetic waves in the field of bone healing began known. This is supported by research conducted by Fukada and Yasuda (1957) Who found an endogenous electrical effect on bone biology processes, particularly in fracture healing (Huegel *et al*., 2018).

The utilization of electromagnetic waves in the health field does not stop at the process of healing bones. Electromagnetic waves are also known to have the potential to help heal wounds in the skin and underlying tissues (Francisco *et al*., 2013), reduce postoperative pain and swelling (Janicak and Dokucu, 2015) and other forms of electromagnetic exposure can also be used to cure depression (Janicak and Dokucum 2015).

Pulsed Electromagnetic Field as a Potential Bioelectromagnetic Therapy to Inhibit Adipogenesis of Mesenchymal Stem Cells

The use of PEMF in medical therapy becomes larger. Several studies related to this problem, in more detail, are described in Table 1.

Unlike the PEMF studies on bone healing and another therapy, studies related to PEMF and their effects on cell differentiation, especially adipogenesis, have not been done much, so the response mechanism that occurs in cells due to PEMF exposure is not well known. In recent times, there have been several studies focusing on the effects of electromagnetic waves on cell differentiation, specifically in the inhibition of adipogenesis for the treatment of obesity. *In vitro* research conducted by Du *et al*. (2014) Concluded that electromagnetic wave exposure can induce osteogenesis and inhibit the differentiation of mesenchymal stem cells to become adipocyte cells (Du *et al*., 2014). Other studies conducted by Lu *et al*. (2015) Also support the same thing, namely exposure to electromagnetic waves can suppress the expression of transcription factors that support adipogenesis (Lu *et al*., 2015).

The inhibition of adipogenesis due to PEMF exposure is supported by research conducted by Du *et al*. (2014) in this study, the results were obtained that PEMF exposure with a frequency of 7.5 Hz, magnetic field magnitude 0.4 T and exposure time of 2 h/day for 15 days of adipogenesis can inhibit the expression of adipogenic genes. However, in this study, checking of gene expression was only carried out on the last day of exposure, i.e., on the 15th day so it could not be seen how the pattern of gene expression occurred during the 15 days of the exposure time. The results also showed that on the 4th and 7th day after exposure, no statistically significant differences were found in PPARγ gene expression. Although seen in the PEMF group, PPARγ expression had a lower tendency than the two control groups. There were no significant differences found on days 4 and 7 can be caused by several factors, including the micro conditions that are applied to cells. It is known that the microenvironment contributes to cell differentiation. Several studies related to PEMF and adipogenesis of MSC in more detail are described in Table 2.

The Mechanism of Adipogenesis Inhibition in Mesenchymal Stem Cells by Pulsed Electromagnetic Field

Changes in the magnetic field produced by electromagnetic exposure stimulus will produce an electric field induced in the network. Cellular and biophysical mechanisms of electromagnetic waves toward cells are still the focus of research. The high complexity of the molecular mechanisms underlying cell responses to exposure and the many types of exposure are one of the obstacles to the difficulty of elaborating on the focus of the research (Francisco *et al*., 2013).

The main theory that can explain the molecular mechanism of cell response to electromagnetic waves is the theory of changes in plasma membrane potential. Electric fields exposed to cells can act as extracellular signals to cells and will affect ion transport at the cellular level (Ross *et al*., 2015). It is known that the magnetic gradient of electromagnetic waves can penetrate deeper tissues and work directly on cells by changing the potential of the plasma membrane. Ross *et al*. (2015) this is thought to have an impact on the movements of

molecules that will pass through the plasma membrane (Ross *et al*., 2015), so as to be able to influence cell activity. In plasma membranes, there are voltage gated ion channels that can open and close in response to changes in membrane potential. When the channel is open, the ions will easily pass through the membrane, but when closed, then the flow of ions will be prevented from passing through the membrane (Fig. 1).

Table 1: The use of PEMF as a potential therapeutic tool

Frequency	Intensity	Time of exposure	Type of therapy	Results	Reference
20 to 50 Hz	2 to 5 mT	90 min per day for 3 days	*In vitro* using MCF7 breast cancer cells	The PEMF-based anticancer strategies may represent a new therapeutic approach to treat breast cancer without affecting normal tissues in a manner that is non-invasive and can be potentially combined with existing anti-cancer treatments	Crocetti *et al*. (2013)
15 Hz	1 to 10 mT	2 h per day for 7 days	*In vivo* (bone wound healing)	The PEMF at 5-10 mT can significantly accelerate wound healing and enhance the repairing ability of bone tissue	Liu *et al.* (2020)
25 Hz	2 and 10 mT	1 h per day for 21 days	*In vivo* (diabetic wounds healing)	The PEMF delivered at 10 mT can improve energy absorption capacity of diabetic wounds in the early healing phase	Huegel *et al.* (2018)
15 Hz	0 to 1.2 mT in 200 ms and returning to 0 in 24 ms	8 h per day for 28 days	*In vivo* (angiogenesis)	Therapy with Pulsed Electromagnetic Fields (PEMF) can promote angiogenesis in ischemic lesions	Pan *et al.* (2012)
20 Hz	10 mT	2 h per day for 10 days	*In vivo* (dementia)	The exposure can improve the ability of learning and memory in dementia rats	Li *et al.* (2019)
60 Hz	10 mT	6 h per day for up to 3 and 14 days	*In vivo* (ischemic stroke)	The PEMF exposure has a neuroprotective effect after ischemic stroke in mice during the recovery process	Urnukhsaikhan *et al.* (2021)
27.12 MHz Carrier Modulated by a 3-ms burst repeating at 5 Hz	6±1 V/m	30 min	*In vivo* (intracranial pressure and microvascular hunting)	The PEMF significantly dilated arterioles, increased capillary blood flow velocity and reduced MVS/ capillary ratio compared to sham treated animals These effects led to a significant decrease in tissue hypoxia, BBB degradation and neuronal necrosis	Elahian *et al.* (2018)

Table 2: Several studies about inhibition of PEMF to adipogenesis of MSC

Frequency	Intensity	Time of exposure	Source of MSC	Results	Reference
7,5 Hz	0,4 T	2 h/day until 15 days of differentiation	Umbilical cord (UC-MSC)	The PEMFs inhibited the expression of adipogenic transcription factors and adipogenic genes such PPARγ, FABP4 dan LPL	Du *et al.* (2014)
20 Hz	2 mT	6 h/day until 12 days of differentiation	Bone marrow (BM-MSC)	The PEMFs inhibited the expression of adipogenic transcription factors such as adipokines and adipocyte binding protein-2	Lu *et al.* (2015)
75 Hz	2 mT	10 min/day until 14 days of differentiation	Adipose (AD-MSC)	The PEMFs inhibited the expression of adipogenic genes such as PPARγ and ADIPOQ	Sari *et al.* (2020)

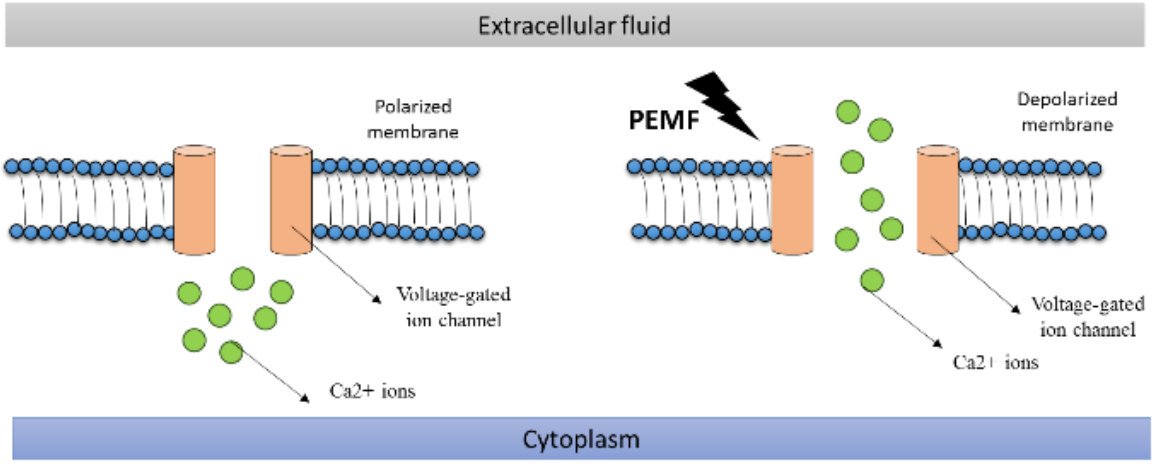

Fig. 1: Movements of molecules through the plasma

Research on cellular mechanisms and cell response to electromagnetic field stimulation has been investigated using mesenchymal stem cells (Zhang *et al*., 2016). It is known that electromagnetic field stimulation can increase cytosolic Ca_2+ concentrations via the calmodulin pathway, which then triggers cell proliferation (Zhang *et al*., 2016; Brighton *et al*., 2001). High levels of Ca_2+ in cells are believed to be the effect of activating voltage-gated Ca_2+ channels on the plasma membrane due to external electromagnetic exposure (Zhang *et al*., 2016). Mesenchymal stem cells also have other voltage-gated ion channels, such as voltage gated Na + channels and voltage gated K + channels, but to the extent that There is currently no research examining whether other voltage gated ion channels are also activated due to electromagnetic exposure. However, if you see the effect of electromagnetic exposure on the voltage-gated Ca_2+ channel, then it is possible that other voltage gated ion channels can also be activated by electromagnetic stimuli Zhang *et al*., 2016. Electromagnetic field stimulation is able to change the plasma membrane potential thereby activating voltage gated Ca_2+ channels and increasing cytosolic Ca_2+ concentrations (Araújo *et al*., 2018; Desai *et al*., 2013; Camp *et al*., 2002). Increased Ca_2+ concentrations are known to have an important role in regulating intracellular signaling activation, cell proliferation, and cell differentiation, especially in the induction process of osteogenesis and adipogenesis inhibition (Zhang *et al*., 2016; Bae *et al*., 2018).

Inhibition of adipogenesis caused by an increase in intracellular Ca_2+ can occur through the calcineurin activation pathway (Meldolesi, 2008). Through this pathway, it is known that intracellular Ca_2+ increase modulates adipocyte lipid metabolism and inhibits the early stages of adipogenesis in mice and mice (Shi *et al*., 2000). The further evaluation also concluded that an increase in intracellular Ca_2+ could inhibit the metabolism of adipocyte lipids and inhibit the initial stages of adipogenesis in mice and mice. Shi *et al*. (2000) direct adipogenesis in humans, which is characterized by decreased PPAR expression (Shi *et al*., 2000).

Calreticulin protein (CRT) is a protein that keeps the Ca_2+ concentration in the cytosol high. In this situation, Ca_2+ will bind to the Calmodulin subunit (CaM) so that it activates Calcineurin (CaN). This activation will give a specific signal to the nucleus compartment to induce the transcription Factor (F1) and block the activation of the RXR-PPARγ complex. As a result, gene transcription involved in adipogenesis is blocked and adipogenesis cannot take place (Meldolesi, 2008). Conversely, if Ca_2+ concentrations are low in the cytoplasm, the calcium/calmodulin dependent protein kinase II (CaMKII) enzyme will be activated via the c-Srk pathway. A possible consequence of CAMKII phosphorylation is the activation of another transcription Factor (F2) which promotes activation of the RXR-PPAR kompleks complex thereby inducing adipogenesis.

Inhibition of adipogenesis is antagonistic to the process of osteogenesis. If adipogenesis is inhibited, the osteogenesis pathway is induced, and vice versa. Previous studies related to the effect of PEMF exposure on osteogenesis showed that PEMF exposure was able to increase Wnt5a expression (Umiatin *et al*., 2019). Increased Wnt5a expression was known to inhibit adipogenesis by suppressing PPAR expression (Takada *et al*., 2009). The induction of osteogenesis can be triggered by the activation of the Wnt5a/β-catenin signaling pathway, this activation is followed by the inhibition of adipogenesis which is characterized by a decrease in adipogenesis markers namely PPARγ and leptin (Bae *et al*., 2018). The relationship between activating osteogenesis through the Wnt5a signaling pathway that causes adipogenesis inhibition (Takada *et al*., 2009). Activation of the non-canonical Wnt signaling pathway can directly suppress the PPARγ function in the mesenchymal stem cell differentiation process. Umiatin *et al*. (2019) this will bind to RXR-PPARγ and inhibit the process of adipogenesis (Ayala *et al*., 2018).

Research conducted by Ayala *et al*. (2018) analyzed the effects of dynamic magnetic field exposure of 17-70 mT on the potential changes in the skeletal muscle cell plasma membrane. In this study, a mathematical equation was obtained that produced a change in membrane potential of 8 mV for 1 sec. To reach the threshold for changes in membrane potential, a value of 10-15 mV is required (Ayala *et al*., 2018). Increased cytosolic Ca_2 + leads to the activation of calmodulin thereby inducing the potential for MSC differentiation in the chondrogenic pathway (Uzieliene *et al*., 2018). Increased cytosolic Ca_2 + is chained by the activation of Voltage Operated Calcium Channels (VOCC) and Transient Receptor Potential (TRP) due to electromagnetic stimulus (Uzieliene *et al*., 2018).

Conclusion

In conclusion, after reviewing the recent scientific literature, PEMF therapy can be one of the potential methods to inhibit adipogenesis. It is proven by *in vivo* experiments with positive results. But there is no standard for adipogenesis inhibition therapy using PEMF. So, it is necessary to carry out further research by paying attention to variations in physical parameters including frequency, magnetic intensity, and duration of exposure to find the optimal parameters for adipogenesis inhibition using a specific PEMF device design.

Unfortunately, to date, there have been no *in vivo* experiments related to the inhibition of adipogenesis. For further research, the *in vivo* study is highly recommended on this topic to find out whether PEMF exposure can have an effect at the organism level.

Acknowledgment

We would like to gratefully acknowledge kemenristekbud, Indonesia for provides Hibah PDUPT grant number NKB-128/UN2.RST/HKP.05.00/2020 to published this article.

Funding Information

Hibah PDUPT grant number NKB-128/UN2.RST/HKP.05.00/2020, which provided the funding to published this article.

Author's Contributions

Puji Sari: Developed the idea, looked up relevant literature, and composed the first version.

Nuzli Fahdia Mazfufah: Written the manuscript and research relevant literature.

Umiatin, Dwi Anita Suryandari, Silvia Werdhy Lestari and Luluk Yunaini: Revised and reviewed the article.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

References

Araújo, A. B., Furlan, J. M., Salton, G. D., Schmalfuss, T., Röhsig, L. M., Silla, L. M. R., ... & Paz, A. H. (2018). Isolation of human mesenchymal stem cells from amnion, chorion, placental decidua and umbilical cord: Comparison of four enzymatic protocols. *Biotechnology Letters*, *40*, 989-998. https://doi.org/10.1007/s10529-018-2546-z

Ayala, M. R., Syrovets, T., Hafner, S., Zablotskii, V., Dejneka, A., & Simmet, T. (2018). Spatiotemporal magnetic fields enhance cytosolic Ca_2+ levels and induce actin polymerization via activation of voltage-gated sodium channels in skeletal muscle cells. *Biomaterials*, *163*, 174-184. https://doi.org/10.1016/j.biomaterials.2018.02.031

Bae, Y. K., Kwon, J. H., Kim, M., Kim, G. H., Choi, S. J., Oh, W., ... & Jeon, H. B. (2018). Intracellular calcium determines the adipogenic differentiation potential of human umbilical cord blood-derived mesenchymal stem cells via the Wnt5a/β-Catenin signaling pathway. *Stem Cells International*, *2018*. https://doi.org/10.1155/2018/6545071

Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lozano, P., Chien, K. R., ... & Evans, R. M. (1999). PPARγ is required for placental, cardiac and adipose tissue development. *Molecular Cell*, *4*(4), 585-595. https://doi.org/10.1016/S1097-2765(00)80209-9

Benton, D., & Young, H. A. (2017). Reducing calorie intake may not help you lose body weight. *Perspectives on Psychological Science*. https://doi.org/10.1177/1745691617690878

Betz, M. J., & Enerbäck, S. (2015). Human brown adipose tissue: what we have learned so far. *Diabetes*, *64*(7), 2352-2360. https://doi.org/10.2337/db15-0146

Brighton, C. T., Wang, W., Seldes, R., Zhang, G., & Pollack, S. R. (2001). Signal transduction in electrically stimulated bone cells. *JBJS*, *83*(10), 1514-1523. https://doi.org/10.2106/00004623-200110000-00009

Bunnell, B. A., Flaat, M., Gagliardi, C., Patel, B., & Ripoll, C. (2008). Adipose-derived stem cells: Isolation, expansion and differentiation. *Methods*, *45*(2), 115-120. https://doi.org/10.1016/j.ymeth.2008.03.006

Camp, H. S., Ren, D., & Leff, T. (2002). Adipogenesis and fat-cell function in obesity and diabetes. *Trends in Molecular Medicine*, *8*(9), 442-447. https://doi.org/10.1016/s1471-4914(02)02396-1

Cariou, B., Charbonnel, B., & Staels, B. (2012). Thiazolidinediones and PPARγ agonists: Time for a reassessment. *Trends in Endocrinology & Metabolism*, *23*(5), 205-215. https://doi.org/10.1016/j.tem.2012.03.001

Chagastelles, P. C., & Nardi, N. B. (2011). Biology of stem cells: An overview. *Kidney International Supplements*, *1*(3), 63-67. https://doi.org/10.1038/kisup.2011.15

Chen, Q., Shou, P., Zheng, C., Jiang, M., Cao, G., Yang, Q., ... & Shi, Y. (2016). Fate decision of mesenchymal stem cells: Adipocytes or osteoblasts?. *Cell Death & Differentiation*, *23*(7), 1128-1139. https://doi.org/10.1038/cdd.2015.168

Choe, S. S., Huh, J. Y., Hwang, I. J., Kim, J. I., & Kim, J. B. (2016). Adipose tissue remodeling: Its role in energy metabolism and metabolic disorders. *Frontiers in Endocrinology*, *7*, 30. https://doi.org/10.3389/fendo.2016.00030

Crocetti, E., Sica, L. S., Schwartz, S. J., Serafini, T., & Meeus, W. (2013). Identity styles, dimensions, statuses, and functions: Making connections among identity conceptualizations. *European Review of Applied Psychology*, *63*(1), 1-13. https://doi.org/10.1016/j.erap.2012.09.001

Dave, S., Kaur, N. J., Nanduri, R., Dkhar, H. K., Kumar, A., & Gupta, P. (2012). Inhibition of adipogenesis and induction of apoptosis and lipolysis by stem bromelain in 3T3-L1 adipocytes. *PLoS One*, *7*(1), e30831. https://doi.org/10.1371/journal.pone.0030831

Desai, M., Beall, M., & Ross, M. G. (2013). Developmental origins of obesity: Programmed adipogenesis. *Current Diabetes Reports*, *13*, 27-33. https://doi.org/10.1007/s11892-012-0344-x

Djalalinia, S., Qorbani, M., Peykari, N., & Kelishadi, R. (2015). Health impacts of obesity. *Pakistan Journal of Medical Sciences*, *31*(1), 239. https://doi.org/10.12669/pjms.311.7033

Dominici, M. L. B. K., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F. C., Krause, D. S., ... & Horwitz, E. M. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*, *8*(4), 315-317. https://doi.org/10.1080/14653240600855905

Du, L., Fan, H., Miao, H., Zhao, G., & Hou, Y. (2014). Extremely low frequency magnetic fields inhibit adipogenesis of human mesenchymal stem cells. *Bioelectromagnetic*, *35*(7), 519-530. https://doi.org/10.1002/bem.21873

Elahian, B., Lado, N. E., Mankin, E., Vangala, S., Misra, A., Moxon, K., ... & Weiss, S. A. (2018). Low-voltage fast seizures in humans begin with increased interneuron firing. *Annals of Neurology*, *84*(4), 588-600. https://doi.org/10.1002/ana.25325

Fink, T., & Zachar, V. (2011). Adipogenic differentiation of human mesenchymal stem cells. *Mesenchymal Stem Cell Assays and Applications*, 243-251. https://doi.org/10.1007/978-1-60761-999-4_19

Francisco, A. C., del Mar, S. A. M., Irene, C., Sandra, R. A., Josefa, L., Elisa, R. M., ... & Isabel, N. M. (2013). Could radiotherapy effectiveness be enhanced by electromagnetic field treatment?. *International Journal of Molecular Sciences*, *14*(7), 14974-14995. https://doi.org/10.3390/ijms140714974

Fukada, E., & Yasuda, I. (1957). On Prezoletric Effect of Boné *Journal of Physical Society of Japan*,*12*(10). https://doi.org/10.1143/JPSJ.12.1158

Gómez-Hernández, A., Beneit, N., Díaz-Castroverde, S., & Escribano, Ó. (2016). Differential role of adipose tissues in obesity and related metabolic and vascular complications. *International Journal of Endocrinology*, *2016*. https://doi.org/10.1155/2016/1216783

Haghnegahdar, A., Khosrovpanah, H., Andisheh-Tadbir, A., Mortazavi, G., Saeedi, M. M., Mortazavi S., ... & Koohi, O. (2014). Design and fabrication of helmholtz coils to study the effects of pulsed electromagnetic fields on the healing process in periodontitis: preliminary animal results. *J Biomed Phys Eng*. *4*(3), 83-90.

Harvanová, D., Tóthová, T., Sarissky, M., Amrichová, J., & Rosocha, J. (2011). Isolation and characterization of synovial mesenchymal stem cells. *Folia Biol (Praha)*, *57*(3), 119-124. https://doi.org/10.1155/2017/9312329

Hassan, G., Kasem, I., Soukkarieh, C., & Aljamali, M. (2017). A simple method to isolate and expand human umbilical cord derived mesenchymal stem cells: Using explant method and umbilical cord blood serum. *International Journal of Stem Cells*, *10*(2), 184-192. https://doi.org/10.15283/ijsc17028

Hruby, A., & Hu, F. B. (2015). The epidemiology of obesity: A big picture. *Pharmacoeconomics*, *33*, 673-689. https://doi.org/10.1007/s40273-014-0243-x

Huang, G. J., Gronthos, S., & Shi, S. (2009). Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in regenerative medicine. *Journal of Dental Research*, *88*(9), 792-806. https://doi.org/10.1177/0022034509340867

Huegel, J., Choi, D. S., Nuss, C. A., Minnig, M. C., Tucker, J. J., Kuntz, A. F., ... & Soslowsky, L. J. (2018). Effects of pulsed electromagnetic field therapy at different frequencies and durations on rotator cuff tendon-to-bone healing in a rat model. *Journal of Shoulder and Elbow Surgery*, *27*(3), 553-560. https://doi.org/10.1371/journal.pone.0191074

Janicak, P. G., & Dokucu, M. E. (2015). Transcranial magnetic stimulation for the treatment of major depression. *Neuropsychiatric Disease and Treatment*, 1549-1560. https://doi.org/10.2147/NDT.S67477

Kawai, M., & Rosen, C. J. (2012). Bone marrow fat and bone mass. *Translational Endocrinology of Bone: Reproduction, Metabolism and the Central Nervous System*, 167.

Kelishadi, R. (2007). Childhood overweight, obesity and the metabolic syndrome in developing countries. *Epidemiologic Reviews*, *29*(1), 62-76. https://doi.org/10.1093/epirev/mxm003

Kozlowska, U., Krawczenko, A., Futoma, K., Jurek, T., Rorat, M., Patrzalek, D., & Klimczak, A. (2019). Similarities and differences between mesenchymal stem/progenitor cells derived from various human tissues. *World Journal of Stem Cells*, *11*(6), 347. https://doi.org/10.4252/wjsc.v11.i6.347

Kwan, Y., Su, T., Fu, X., Tse, A. K., Fong, W. F., & Yu, Z. L. (2015). Herbal remedies against adipogenesis. *Journal of Alternative Medical Research*, *1*(1), 1-7. https://doi.org/10.19104/jamr.2015.105

Lee, M. J., & Fried, S. K. (2014). Optimal protocol for the differentiation and metabolic analysis of human adipose stromal cells. In *Methods in Enzymology* (Vol. *538*, pp. 49-65). Academic Press. https://doi.org/10.1016/B978-0-12-800280-3.00004-9

Lefterova, M. I., Haakonsson, A. K., Lazar, M. A., & Mandrup, S. (2014). PPARγ and the global map of adipogenesis and beyond. *Trends in Endocrinology & Metabolism*, *25*(6), 293-302. https://doi.org/10.1016/j.tem.2014.04.001

Ljungberg, A. (2006). *The Role of PPAR [alpha] and Growth Hormone in Hepatic Lipid Metabolism and Atherosclerosis*. Department of Physiology, Wallenberg Laboratory for Cardiovascular Research, The Sahlgrenska Academy at Göteborg University.

Longo, M., Zatterale, F., Naderi, J., Parrillo, L., Formisano, P., Raciti, G. A., ... & Miele, C. (2019). Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. *International Journal of Molecular Sciences*, *20*(9), 2358. https://doi.org/10.3390/ijms20092358

Lu, T., Huang, Y. X., Zhang, C., Chai, M. X., & Zhang, J. (2015). Effect of pulsed electromagnetic field therapy on the osteogenic and adipogenic differentiation of bone marrow mesenchymal stem cells. *Genet Mol Res*, *14*(3), 11535-11542. https://doi.org/10.4238/2015.September.28.5

Liu, Y., Ning, Z., Chen, Y., Guo, M., Liu, Y., Gali, N. K., ... & Lan, K. (2020). Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature*, *582*(7813), 557-560. https://doi.org/10.1038/s41586-020-2271-3

Li, H. T., Yi, T. S., Gao, L. M., Ma, P. F., Zhang, T., Yang, J. B., ... & Li, D. Z. (2019). Origin of angiosperms and the puzzle of the Jurassic gap. *Nature Plants*, *5*(5), 461-470. https://doi.org/10.1038/s41477-019-0421-0

Ma, X., Wang, D., Zhao, W., & Xu, L. (2018). Deciphering the roles of PPARγ in adipocytes via dynamic change of transcription complex. *Frontiers in Endocrinology*, *9*, 473. https://doi.org/10.3389/fendo.2018.00473

Malmivuo, J., & Plonsey, R. (1995). *Bioelectromagnetism: Principles and applications of bioelectric and biomagnetic fields*. Oxford University Press, USA. https://doi.org/10.1093/acprof:oso/9780195058239.001.0001

Marcelin, G., & Chua Jr, S. (2010). Contributions of adipocyte lipid metabolism to body fat content and implications for the treatment of obesity. *Current Opinion in Pharmacology*, *10*(5), 588-593. https://doi.org/10.1016/j.coph.2010.05.008

Meldolesi, J. (2008). Inhibition of adipogenesis: A new job for the ER Ca_2+ pool. *The Journal of Cell Biology*, *182*(1), 11-13. https://doi.org/10.1083/jcb.200805110

Moseti, D., Regassa, A., & Kim, W. K. (2016). Molecular regulation of adipogenesis and potential anti-adipogenic bioactive molecules. *International Journal of Molecular Sciences*, *17*(1), 124. https://doi.org/10.3390/ijms17010124

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., ... & Gakidou, E. (2014). Global, regional and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, *384*(9945), 766-781. https://doi.org/10.1016/S0140-6736(14)60460-8

Ofei, F. (2005). Obesity-a preventable disease. *Ghana Medical Journal*, *39*(3), 98. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1790820/

Orbay, H., Tobita, M., & Mizuno, H. (2012). Mesenchymal stem cells isolated from adipose and other tissues: Basic biological properties and clinical applications. *Stem Cells International*, *2012*. https://doi.org/10.1155/2012/461718

Orciani, M., & Di Primio, R. (2013). Skin-derived mesenchymal stem cells: Isolation, culture and characterization. *Skin Stem Cells: Methods and Protocols*, 275-283. https://doi.org/10.1007/978-1-62703-330-5_21

Parekkadan, B., & Milwid, J. M. (2010). Mesenchymal stem cells as therapeutics. *Annual Review of Biomedical Engineering*, *12*, 87-117. https://doi.org/10.1146/annurev-bioeng-070909-105309

Pan, A., Sun, Q., Bernstein, A. M., Schulze, M. B., Manson, J. E., Stampfer, M. J., ... & Hu, F. B. (2012). Red meat consumption and mortality: Results from 2 prospective cohort studies. *Archives of Internal Medicine*, *172*(7), 555-563. https://doi.org/10.1001/archinternmed.2011.2287

Reilly, J. J., & Kelly, J. (2011). Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: Systematic review. *International Journal of Obesity*, *35*(7), 891-898. https://doi.org/10.1038/ijo.2010.222

Ross, C. L., Siriwardane, M., Almeida-Porada, G., Porada, C. D., Brink, P., Christ, G. J., & Harrison, B. S. (2015). The effect of low-frequency electromagnetic field on human bone marrow stem/progenitor cell differentiation. *Stem Cell Research*, *15*(1), 96-108. https://doi.org/10.1016/j.scr.2015.04.009

Sari, P., Yunaini, L., Luviah, E., & Mazfufah, N. F. (2020). Relative expression of PPARγ and ADIPOQ mRNA as markers of inhibition adipogenesis on mesenchymal stem cells after Pulsed Electromagnetic Field (PEMF) exposure. *Journal of Global Pharma Technology*, *12*(1), 307-317.

Shaer, A., Azarpira, N., Aghdaie, M. H., & Esfandiari, E. (2014). Isolation and characterization of human mesenchymal stromal cells derived from placental decidua basalis; umbilical cord Wharton's jelly and amniotic membrane. *Pakistan Journal of Medical Sciences*, *30*(5), 1022. https://doi.org/10.12669/pjms.305.4537

Shi, H., Halvorsen, Y. D., Ellis, P. N., Wilkison, W. O., & Zemel, M. B. (2000). Role of intracellular calcium in human adipocyte differentiation. *Physiological Genomics*, *3*(2), 75-82. https://doi.org/10.1152/physiolgenomics.2000.3.2.75

Takada, I., Kouzmenko, A. P., & Kato, S. (2009). Wnt and PPARγ signaling in osteoblastogenesis and adipogenesis. *Nature Reviews Rheumatology*, *5*(8), 442-447. https://doi.org/10.1038/nrrheum.2009.137

Tontonoz, P., Hu, E., & Spiegelman, B. M. (1994). Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. *Cell*, *79*(7), 1147-1156. https://doi.org/10.1016/0092-8674(94)90006-x

Touitou, Y., & Selmaoui, B. (2022). The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system. *Dialogues in Clinical Neuroscience*. https://doi.org/10.31887/DCNS.2012.14.4/ytouitou

Trivanović, D., Kocić, J., Mojsilović, S., Krstić, A., Ilić, V., Okić-Đorđević, I., ... & Bugarski, D. (2013). Mesenchymal stem cells isolated from peripheral blood and umbilical cord Wharton's jelly. *Srpski arhiv za Celokupno Lekarstvo*, *141*(3-4), 178-186. https://doi.org/10.2298/sarh1304178t

Ullah, I., Subbarao, R. B., & Rho, G. J. (2015). Human mesenchymal stem cells-current trends and future prospective. *Bioscience Reports*, *35*(2). https://doi.org/10.1042/BSR20150025

Umiatin, D. I. H., Wijaya, S. K., Sari, P., & Djaja, A. D. (2019, April). Design and development of pulse electromagnetic fields (PEMF) as adjuvant therapy for fracture healing: A preliminary study on rats. In *AIP Conference Proceedings* (Vol. *2092*, No. 1, p. 020028). AIP Publishing LLC. https://doi.org/10.1063/1.5096696

Uzieliene, I., Bernotas, P., Mobasheri, A., & Bernotiene, E. (2018). The role of physical stimuli on calcium channels in chondrogenic differentiation of mesenchymal stem cells. *International Journal of Molecular Sciences*, *19*(10), 2998. https://doi.org/10.3390/ijms191029

Urnukhsaikhan, E., Bold, B. E., Gunbileg, A., Sukhbaatar, N., & Mishig-Ochir, T. (2021). Antibacterial activity and characteristics of silver nanoparticles biosynthesized from Carduus crispus. *Scientific Reports*, *11*(1), 21047. https://doi.org/10.1038/s41598-021-00520-2

Varaa, N., Azandeh, S., Khodabandeh, Z., & Gharravi, A. M. (2019). Wharton's jelly mesenchymal stem cell: Various protocols for isolation and differentiation of hepatocyte-like cells; narrative review. *Iranian Journal of Medical Sciences*, *44*(6), 437. https://doi.org/10.30476/ijms.2019.44952

Wade, B. (2013). A review of pulsed electromagnetic field (PEMF) mechanisms at a cellular level: A rationale for clinical use. *Am J Health Res*, *1*(3), 51-55. https://doi.org/10.11648/j.ajhr.20130103.13

Wakao, S., Kuroda, Y., Ogura, F., Shigemoto, T., & Dezawa, M. (2012). Regenerative effects of mesenchymal stem cells: Contribution of muse cells, a novel pluripotent stem cell type that resides in mesenchymal cells. *Cells*, *1*(4), 1045-1060. https://doi.org/10.3390/cells1041045

Wei, X., Yang, X., Han, Z. P., Qu, F. F., Shao, L., & Shi, Y. F. (2013). Mesenchymal stem cells: A new trend for cell therapy. *Acta Pharmacologica Sinica*, *34*(6), 747-754. https://doi.org/10.1038/aps.2013.50

WHO. (2016). Obesity and Overweight Fact Sheet [Internet]. http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Wyatt, H. R. (2013). Update on treatment strategies for obesity. *The Journal of Clinical Endocrinology & Metabolism*, *98*(4), 1299-1306. https://doi.org/10.1210/jc.2012-3115

Yadollahpour, A., & Rashidi, S. A. M. A. N. E. H. (2015). Therapeutic applications of electromagnetic fields in musculoskeletal disorders: A review of current techniques and mechanisms of action. *Biomedical and Pharmacology Journal*, *7*(1), 23-32. https://dx.doi.org/10.13005/bpj/448

Zakrzewski, W., Dobrzyński, M., Szymonowicz, M., & Rybak, Z. (2019). Stem cells: Past, present and future. *Stem Cell Research & Therapy*, *10*, 1-22. https://doi.org/10.1186/s13287-019-1165-5

Zhang, J., Li, M., Kang, E. T., & Neoh, K. G. (2016). Electrical stimulation of adipose-derived mesenchymal stem cells in conductive scaffolds and the roles of voltage-gated ion channels. *Acta Biomaterialia*, *32*, 46-56. https://doi.org/10.1016/j.actbio.2015.12.024