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Review Article

Promising application of magnetic nanoparticles and electromagnetic fields in articular cartilage-related diseases: an overview

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Abstract:

Articular cartilage-related diseases are a growing problem worldwide. Although there are several therapeutic options available for these diseases, the repair of damaged articular cartilage remains an intractable issue. Therefore, a strong need exists for new approaches such as offered by tissue engineering. However, this approach is also associated with some drawbacks including poor regeneration of cultured cells. Recently, magnetic nanoparticles and electromagnetic fields (EMFs) have been suggested to influence on different cellular processes such as proliferation and differentiation and thus are able to overcome these drawbacks of tissue engineering. In this article, we review the current knowledge on the definition and characteristics of magnetic nanoparticles and EMFs. We also discuss the beneficial effects of magnetic nanoparticles and EMFs in various *in vitro* and *in vivo* studies.

Keywords: Articular cartilage; Cellular processes; Electromagnetic fields; Magnetic nanoparticles; Tissue engineering**.**

1. Introduction

Cartilage is a resistant and elastic tissue that covers the ends of long bones at joints and is classified into three types including hyaline cartilage, elastic cartilage and fibrocartilage [1]. Articular cartilage is the hyaline cartilage composed of a dense extracellular matrix with a sparse distribution of highly specialized cells named chondrocytes. One important feature of the articular cartilage is that unlike most tissues, it lacks blood vessels, nerves or lymphatic [2]. Structurally, four zones are organized within the articular cartilage from top to bottom: superficial zone, transitional zone, deep zone and calcified zone. Because of differences in component density and cell morphology, each zone has different biological and mechanical properties [3].

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The superficial zone occupies about 10-20 % of the articular cartilage and has large content of collagen and low content of proteoglycans as well as narrow and round chondrocytes. Functionally, this zone is responsible for most of the tensile properties of cartilage which enable it to resist sheer and tensile forces imposed by articulation [4, 5]. The thickness of the next zone, the transitional zone, is about 40-60 % of the articular cartilage and is characterized by collagen fibers with larger diameter and round chondrocytes with 15-25 μm diameter. This zone is the first line of resistance to compressive forces [6]. The deep zone occupies 20-50 % of the articular cartilage and unlike other zones, it has the highest concentration of proteoglycans and the lowest concentration of water. This zone contains round chondrocytes as well as large and tubular collagen fibers oriented vertically. The deep zone provides the greatest resistance to compressive forces due to the high content of proteoglycans [7]. The deepest zone of articular cartilage, the calcified zone, separates the articular cartilage from the subchondral bone and has mineral crystals of calcium phosphate [4, 5]. This zone contributes to secure the cartilage to the bone by anchoring collagen fibers of the deep zone to the subchondral bone [8].

Generally, the principal function of articular cartilage is to transfer loads to the subchondral bone, resulting in a smooth, low-friction and sliding motion of that joint [9, 10]. To better evaluating the biomechanical behavior of articular cartilage, it is worth mentioning that the tissue is considered as a biphasic medium. In other words, the articular cartilage is composed of two phases: a fluid phase and a solid phase. The fluid phase is the greatest phase and consists of water and inorganic ions (e.g., sodium, calcium, chloride and potassium). The solid phase contains collagen, proteoglycans, lipids, phospholipids and non-collagenous proteins [3]. Therefore, based on its major function, damage to the articular cartilage often severely affects the daily life of patients [11]. However, the articular cartilage is not able to regenerate or repair itself because of limited blood supply to the cartilage and thus, it requires interventions [12]. Although various therapeutic options for articular cartilage damage are presented in the clinic, there are some serious drawbacks associated with their applications, plus none of them guarantees complete recovery [13].

Recently, tissue engineering has emerged as an interdisciplinary field since it combines aspects of medicine, cell and molecular biology, materials science and bioengineering to regenerate, repair or replace damaged tissues or organs due to trauma and diseases such as osteoarthritis afflicting the musculoskeletal tissues (e.g., articular cartilage) [14, 15]. The three central ingredients of tissue engineering include 1) stimulating factors for morphogenesis (e.g., transforming growth factors), 2) cells (e.g., mesenchymal stem cells (MSCs)) which respond to stimulating factors and 3) the scaffold of extracellular matrix which delivers cells to the damaged site and then, provides optimum diffusion of nutrients and encourages cellular communication [16-18]. Despite the great hope that tissue engineering has made, rapid and complete regeneration is a very challenging problem, because transplanted cells are easily lost in host tissues and have low survival rates [19, 20]. Thus, to overcome these challenges, the manipulation of cellular processes including proliferation, migration and differentiation seems necessary and vital [21, 22]. In this regard, several studies have suggested that biophysical cues can influence cellular processes and also have some other benefits such as cost-

effectiveness, long life, easy to characterize and high reproducibility [23-27]. As biophysical cues, mechanical and electrical stimulation has been shown to trigger cells themselves to deliver signals by intrinsic pathways and consequently direct cell processes. Therefore, the application of mechanical and electrical stimulation can be effective strategy to alleviate some of the problems which currently prevail in tissue engineering [28-30].

In this review, we discuss the definition and characteristics of magnetic nanoparticles and electromagnetic fields as mechanical and electrical stimulation, respectively. We also review various experimental studies which have investigated the beneficial effects of magnetic nanoparticles and electromagnetic fields on different cell processes. Therefore, the current review provides insights into the magnetic nanoparticles and electromagnetic fields and may help scientists when aiming the treatment of articular cartilage-related diseases.

2. Magnetic nanoparticles

2.1. The definition and characteristics

Magnetic nanoparticles are usually composed of magnetic iron oxide cores coated with shells made of some biocompatible organic materials (e.g., dextran) [31, 32]. The size of the cores is often between a few nanometers and several tens of a nanometer while the size of the coated cores may increase up to a few hundred nanometers (Figure 1). However, even the magnetic nanoparticles with coated cores are much smaller than cells and thereby are suitable for use in biomedical fields such as tissue engineering [33]. The magnetic nanoparticles can be moved to the cell membrane or intracellular components of a particular tissue using an external magnetic field gradient and thus they are able to non-invasively regulate numerous cellular processes via mechanical forces. Although there are a variety of ways to apply mechanical forces to cells, the magnetic nanoparticles provide a controllable level of force to be varied in different regions of a tissue-engineered construct, for example, by changing the magnetic field geometry and/or the number of particles attached to each cell in each region [34-36]. In addition to this, the magnetic nanoparticles can be applied to guide cell adherence into a scaffold or a predetermined shape. In this line, a layer composed of coated magnetic particles with proteins is held in place using a magnetic field. Cells are seeded on top of the layer via binding to the proteins present in the layer and then cultured. After that, the magnetic field is removed, leading to the detachment of the cultured cell layer [37]. This cell layer can be used in the production of tubes, where a rotating cylindrical magnet attracts the 2-D cell layer and then rolls it into the desired configuration [38]. The magnetic nanoparticles are also used in cell seeding into porous 3-D scaffolds [39]. The cells attached to the magnetic nanoparticles are inserted into the porous scaffolds. Then, using a permanent magnet placed underneath the scaffolds, the penetration depth of the magnetically tagged cell suspension is improved.

Figure 1: Schematic illustration of core-shell structure.

2.2. The experimental studies related to the effects of magnetic nanoparticles

Kobayashi et al. in 2008 indicated that under the direction of an external magnetic force, magnetically labeled MSCs were successfully accumulated in the desired area of osteochondral defects of the patella. These magnetically labeled MSCs also had the capacity to differentiate into chondrocytes in a chondrogenic differentiation medium containing transforming growth factor-beta (TGF-β) 1 and bone morphogenetic protein (BMP) 2 [40]. Another study of same authors in the next year demonstrated that the use of magnetically labeled MSCs and an external magnetic device caused the formation of a cell layer containing an extracellular matrix on degenerated human cartilage [41].

One study done by Zhang et al. in 2015 showed that the incorporation of magnetic nanoparticles into hybrid hydrogels made of collagen type II, hyaluronic acid and polyethylene glycol led to a response to an external magnet while maintaining structural integrity, suggesting that these magnetic nanocomposite hydrogels could move to tissue defect sites under remote magnetic guidance. Another interest finding of this study was that the presence of magnetic nanoparticles did not affect cell morphology and adhesion [42]. In the next year, Luciani et al. observed that MSCs magnetically condensed into a porous scaffold showed all the hallmarks of chondrogenesis with significant increases in synthesis of proteoglycans and expression levels of collagen type II, collagen type XI and aggrecan, indicating a great step forward in replacement tissue for cartilage defects [43]. The study of Huang et al. in 2018 found that the use of magnetic nanoparticles in bone marrow-derived MSCs increased the cell growth and regulated the cell processes within scaffolds. Moreover, the application of magnetic nanoparticles in these cells considerably enhanced the expression levels of sex determining region Y-box 9 (SOX9), aggrecan and collagen type II, demonstrating the differentiation of bone marrow-derived MSCs into hyaline chondrogenic cells [44] (Table 1).

3. Electromagnetic fields (EMFs)

3.1. The definition and characteristics

Based on the Maxwell's equation of Ampere's law, one way to generate a magnetic field is by a wire current [45]. In addition to this, there is another way which is by an electric field. Indeed, a stationary charge generates an electric field in the surrounding space and when it is moving, a magnetic field is also produced [46]. Conversely, an English physicist, Michael Faraday, reported that changing magnetic field also produces an electric field. It is worth mentioning that electric and magnetic fields travel together through space as waves of electromagnetic radiation with changing fields mutually sustaining each other. This mutual interaction of electric and magnetic fields creates EMFs which are the fundamental forces of nature and exist in space apart from the charges or currents that may be associated with them [47]. EMFs are classified into two groups: natural and non-natural. The sources of natural EMFs include stars, earth, atmospheric discharges and biological processes (e.g., signal transitions in the nervous system). The sources of non-natural EMFs themselves are categorized into two types: ionizing and non-ionizing.

Table 1. Experimental studies evaluating the effects of magnetic nanoparticles.

Authors	Manuscript title	Year	Effects
Kobayashi et al.	"A novel cell delivery system using magnetically labeled mesenchymal stem cells and an external magnetic device for clinical cartilage repair."	2008	The accumulation of mesenchymal stem cells in the desired area of osteochondral defects of the patella
Kobayashi et al.	"Augmentation of degenerated human cartilage *in vitro* using magnetically labeled mesenchymal stem cells and an external magnetic device."	2009	The formation of a mesenchymal stem cell layer containing an extracellular matrix on degenerated human cartilage
Zhang et al.	"Magnetic nanocomposite hydrogel for potential cartilage tissue engineering: synthesis, characterization, and cytocompatibility with bone marrow derived mesenchymal stem cells."	2015	The movement of hybrid hydrogels to tissue defect sites
Luciani et al.	"Successful chondrogenesis within scaffolds, using magnetic stem cell confinement and bioreactor maturation."	2016	The increases in synthesis of proteoglycans and expression levels of collagen type II, collagen type XI and aggrecan in mesenchymal stem cells The promotion of chondrogenesis potential of mesenchymal stem cells
Huang et al.	"Development of magnetic nanocomposite hydrogel with potential cartilage tissue engineering."	2018	The increases in cell growth and expression levels of sex determining region Y-box 9, aggrecan and collagen type II in bone marrow-derived mesenchymal stem cells The promotion of chondrocyte differentiation of bone marrow-derived mesenchymal stem cells

The ionizing radiation has enough energy to remove electrons, negatively-charged particles, from the orbit of an atom, resulting in the atom to become charged or ionized whereas the non-ionizing radiation dose not have enough energy to cause ionization [48].

The three key characteristics of EMFs are frequency, intensity and waveform. The unit of frequency in the SI system is Hertz (HZ). Based on the frequency, the non-ionizing radiation can be divided into four categories: static fields (0 Hz), extremely low-frequency fields (0-300 Hz), intermediate frequency fields (300-100 KHz) and radiofrequency fields (100 KHz–300 GHz) [49]. The intensity unit of EMFs in the SI system is Tesla (T). For example, the strength of earth's natural geomagnetic field is less than 0.05 mT; whereas the strength of the field during a magnetic resonance imaging (MRI) test is about 1.5-3 T. When applying EMFs for therapeutic purposes, a range of 1 μT to 10-20 mT is usually used. However, it has been demonstrated that in conditions such as transcranial stimulation, more intensity (up to 8T) is needed [50]. In the case of the waveform, there are two common forms: sinusoidal and pulse. A sinusoidal wave is continuous and has smooth periodic oscillations. In contrast, a pulse wave is non-sinusoidal and produces very short signals with fast ascending and descending slopes to control the time at which something happens. Mostly, two groups of EMFs are used in biomedical fields. First, sinusoidal, low-frequency EMFs (50 Hz and 60 Hz); second, pulsed EMFs (PEMFs) with different frequencies (usually 1-100 Hz) [51].

3.2. The experimental studies related to the effects of EMFs

3.2.1. *In vitro* studies

Sakai et al. in 1991 showed that PEMFs promoted the proliferation and glycosaminoglycan synthesis in both rabbit costal growth cartilage cells and human articular cartilage cells [52]. The study of De Mattei et al. in 2001 found that the application of PEMFs (75 Hz, 2.3 mT) could induce cell proliferation in human articular chondrocytes [53]. In 2003, De Mattei et al. investigated the effects of EMFs on chondrocytes of bovine articular cartilage in the presence of interlekin-1 beta (IL-1β), a major pro-inflammatory cytokine involved in osteoarthritis. The results of this study demonstrated that EMFs increased anabolic activities and proteoglycan synthesis in bovine articular cartilage explants. Moreover, these beneficial effects of EMFs were maintained even in the presence of IL-1β, suggesting that EMFs were able to counteract the catabolic activities of the cytokine [54]. Therefore, this study indicated that EMFs had a chondroprotective effect on the articular cartilage. In the next year, De Mattei et al. in another study reported that exposure of bovine articular cartilage explants to EMFs (75 Hz; 1.5 mT) for 24 h in the presence of insulin growth factor-I (IGF-I) stimulated proteoglycan synthesis [55].

The study of Nicolin et al. in 2007 showed that the proliferation rate of human chondrocytes under PEMFs (75 Hz; 2 mT) exposure was markedly enhanced [56]. In 2009, Sun et al. observed that the proliferation and multi-lineage differentiation potential of human bone marrow-derived MSCs were increased when the cells were exposed to PEMFs (8 h per day for 3 days) [57]. Mayer-Wagner et al. examined the effects of EMFs on chondrogenic differentiation of cultured human MSCs in the presence of human fibroblast growth factor 2 (FGF-2) and human TGF-β3. The findings of this study in 2011 revealed that human MSCs treated with EMFs exhibited considerable increases in the expression

levels of collagen type II and content of glycosaminoglycans/deoxyribonucleic acid (DNA), confirming improved chondrogenic differentiation of human MSCs [58]. Alessia Ongaro et al. investigated the effects of EMFs during chondrogenic differentiation of MSCs derived from bovine synovial fluid in the presence of IL-1β and TGFβ-3. The results of this study in 2012 found that EMFs significantly enhanced the mRNA expression levels of aggrecan and collagen type II, leading to increased proteoglycan synthesis [59]. In the next year, Esposito et al. indicated that in umbilical cord Wharton's jelly-derived MSCs, the application of PMEFs increased cell division and density and induced early differentiation into cartilaginous tissue [60].

Chen et al. examined the effects of PEMFs on chondrogenesis of human adipose-derived stem cells cultured in a chondrogenic microenvironment. The findings of this study in 2013 showed that the application of PEMFs markedly increased the expression levels of SOX-9, collagen type II and aggrecan in the cells. Moreover, PEMFs enhanced the deposition of sulfated glycosaminoglycans in human adipose-derived stem cells as well as chondrogenic differentiation of the cells [61]. In 2014, Hilz et al. found that mechanical stimulation in combination with EMFs more effectively increased the expression levels of proteoglycans and collagen type II/collagen type I ratio and content of glycosaminoglycans/DNA in bovine chondrocytes compared to mechanical stimulation alone [62]. In the study of Yi et al. in 2016, the application of EMFs with 45 Hz frequency in human chondrocytes led to increases in the mRNA expression levels of SOX9. EMFs also considerably enhanced the expression levels of aggrecan and collagen type II alpha 1 chain (COL2A1) and thereby promoted proteoglycan synthesis in the extracellular matrix of cells [63]. Furthermore, in 2016, T. Saito et al. demonstrated that the application of EMFs with an average intensity of 1.5 mT and gradient of 0.03 mT/mm for 7 days (12 h per day) increased the synthesis of extracellular matrix in chondrocytes [64]. In the same year, the study of Kavand et al. found that the application of PEMFs (8 h/day for 21 days) in combination with TGFβ-1 increased the deposition of extracellular matrix molecules in MSCs [65].

In 2017, Parate et al. demonstrated a significant increase in the mRNA expression levels of SOX9 in MSCs exposed to PEMFs. In addition, PEMFs promoted the expression levels of aggrecan and collagen type II, leading to increased deposition of proteoglycans in the extracellular matrix of cells [66]. In the same year, one study done by Redeker et al. showed that stimulation of osteoarthritic chondrocytes with low-frequency EMFs every 8 h for 45 min during 7 days remarkably increased the expression levels of aggrecan and COL2A1 [67]. The findings of Tu et al. study in 2018 indicated that pretreatment of bone marrow-derived MSCs with EMFs enhanced the capacity of proliferation and differentiation into the chondrogenic lineage in these cells [68]. Moreover, in 2018, Mayer-Wagner et al. reported a significant improvement of the chondrogenic potential of human MSCs when exposed to the combination of low frequency-EMFs (3 times per day for 21 days) and simulated microgravity [69]. In the next year, the study of Huang et al. found that PEMFs promoted chondrogenic differentiation of MSCs as shown with increased expression levels of collagen type II, aggrecan and SOX9 [70]. In 2020, Escobar et al. demonstrated that stimulation of rat chondrocytes with EMFs markedly increased the proliferation of cells and promoted glycosaminoglycan synthesis in the extracellular matrix [71] (Table 2).

Table 2. *In vitro* experimental studies evaluating the effects of electromagnetic fields.

Authors	Manuscript title	Year	Effects
Sakai et al.	"Effects of pulsing electromagnetic fields on cultured cartilage cells."	1991	The promotion of proliferation and glycosaminoglycan synthesis in rabbit costal growth cartilage cells and human articular cartilage cells
De Mattei et al.	"Effects of pulsed electromagnetic fields on human articular chondrocyte proliferation."	2001	The promotion of proliferation in human articular chondrocytes
De Mattei et al.	"Effects of electromagnetic fields on proteoglycan metabolism of bovine articular cartilage explants."	2003	The increases in anabolic activities and proteoglycan synthesis in bovine articular cartilage chondrocytes
De Mattei et al.	"Effects of physical stimulation with electromagnetic field and insulin growth factor-I treatment on proteoglycan synthesis of bovine articular cartilage."	2004	The stimulation of proteoglycan synthesis in bovine articular cartilage explants
Nicolin et al.	"*In vitro* exposure of human chondrocytes to pulsed electromagnetic fields."	2007	The increase of the proliferation rate of human chondrocytes
Sun et al.	"Effect of pulsed electromagnetic field on the proliferation and differentiation potential of human bone marrow mesenchymal stem cells."	2009	The increases in the proliferation and differentiation potential of human bone marrow-derived mesenchymal stem cells
Mayer-Wagner et al.	"Effects of low frequency electromagnetic fields on the chondrogenic differentiation of human mesenchymal stem cells."	2011	The increases in the expression levels of collagen type II and content of glycosaminoglycans/deoxyribonucleic acid in human mesenchymal stem cells The promotion of chondrogenic differentiation of human mesenchymal stem cells

Alessia Ongaro et al.	"Electromagnetic fields counteract IL-1b activity during chondrogenesis of bovine mesenchymal stem cells."	2012	The increases in the expression levels of aggrecan and collagen type II and proteoglycan synthesis
Esposito et al	"Differentiation of human umbilical cord-derived mesenchymal stem cells, WJ-MSCs, into chondrogenic cells in the presence of pulsed electromagnetic fields."	2013	The increases in division and density in umbilical cord Wharton's jelly-derived mesenchymal stem cells The induction of differentiation into cartilaginous tissue in umbilical cord Wharton's jelly-derived mesenchymal stem cells
Chen et al.	"Electromagnetic fields enhance chondrogenesis of human adipose-derived stem cells in a chondrogenic microenvironment *in vitro*."	2013	The increases in the expression levels of sex determining region Y-box 9, collagen type II and aggrecan and deposition of sulfated glycosaminoglycans in human adipose-derived stem cells The promotion of chondrogenic differentiation of human adipose-derived stem cells
Hilz et al.	"Influence of extremely low frequency, low energy electromagnetic fields and combined mechanical stimulation on chondrocytes in 3-D constructs for cartilage tissue engineering."	2014	The increases in the expression levels of proteoglycans and collagen type II/collagen type I ratio and content of glycosaminoglycans/deoxyribonucleic acid in bovine chondrocytes
Yi et al.	"Effects of electromagnetic field frequencies on chondrocytes in 3D cell-printed composite constructs."	2016	The increase of expression levels of sex determining region Y-box 9, aggrecan and collagen type II alpha 1 chain in human chondrocytes The promotion of proteoglycan synthesis in the extracellular matrix of human chondrocytes
T. Saito et al.	"Effect of the gradient magnetic field stimulation on extracellular matrix synthesis of chondrocyte."	2016	The increase of synthesis of extracellular matrix in chondrocytes
Kavand et al.	"Extremely low frequency electromagnetic field in mesenchymal stem cells	2016	The increase of deposition of extracellular matrix molecules in mesenchymal stem cells

	gene regulation: chondrogenic markers evaluation."		
Parate et al.	"Enhancement of mesenchymal stem cell chondrogenesis with short-term low intensity pulsed electromagnetic fields."	2017	The increase of expression levels of sex determining region Y-box 9, aggrecan and collagen type II in mesenchymal stem cells The increase of deposition of proteoglycans in the extracellular matrix of mesenchymal stem cells
Redeker et al.	"Effect of electromagnetic fields on human osteoarthritic and non-osteoarthritic chondrocytes."	2017	The increase of expression levels of aggrecan and collagen type II alpha 1 chain in osteoarthritic chondrocytes
Tu et al.	"The legacy effects of electromagnetic fields on bone marrow mesenchymal stem cell self-renewal and multiple differentiation potential."	2018	The increases in capacity of proliferation and differentiation into the chondrogenic lineage in bone marrow-derived mesenchymal stem cells
Mayer-Wagner et al.	"Effects of single and combined low frequency electromagnetic fields and simulated microgravity on gene expression of human mesenchymal stem cells during chondrogenesis."	2018	The improvement of the chondrogenic potential of human mesenchymal stem cells
Huang et al.	"Magnetic enhancement of chondrogenic differentiation of mesenchymal Stem Cells."	2019	The increase of expression levels of collagen type II, aggrecan and sex determining region Y-box 9 in mesenchymal stem cells The promotion of chondrogenic differentiation of mesenchymal stem cells
Escobar et al.	"*In vitro* evaluation of the effect of stimulation with magnetic fields on chondrocytes."	2020	The increase of proliferation in rat chondrocytes The promotion of the synthesis of glycosaminoglycans in the extracellular matrix of rat chondrocytes

3.2.2. *In vivo* studies

Ciombor et al. examined the effects of PEMFs on osteoarthritis in Hartley guinea pigs. The findings of this study in 2003 revealed that the application of PEMFs (1 h/day for 6 months) maintained the morphology of articular cartilage, decreased the cartilage neoepitopes and suppressed the matrix-degrading enzymes in animals with osteoarthritis. Moreover, the treatment with PEMFs considerably decreased and increased, respectively, the number of cells immunopositive to interleukin-1 (IL-1) and TGF-β [72]. The study of Fini et al. in 2008 showed that the use of PEMFs (6 h/day for 6 months) in aged Dunkin Hartley guinea pigs markedly slowed the progression of osteoarthritis lesions in the knee cartilage of animals [73]. In 2009, Boopalan et al. indicated that the application of PEMFs twice a day for 6 weeks in the osteotomized femur of Wistar rats enhanced the expression levels of TGFβ-1 and TGFβ-2, leading to fracture healing [74]. In the same year, Fernando et al. reported that the use of PEMFs (30 min/day for 20 days) caused an increase in the thickness of knee joint cartilage in Wistar rats [75].

One study done by Boopalan et al. in 2011 demonstrated that the application of PEMFs (1 h/day for 6 weeks) significantly enhanced the expression levels of collagen type II and synthesis of proteoglycans in the knee joint of rabbits subjected to osteochondral defects [76]. Sotelo-Barroso et al. investigated the effects of PEMFs on cellularity and gene expression of the distal femoral metaphyseal articular cartilage in Wistar rats. The results of this study in 2015 showed that the application of PEMFs (30 min/day for 20 days) caused a remarkable increase in the cartilage cellularity. PEMFs also enhanced the expression levels of collagen type XI, SOX6 and aggrecan, leading to the support of joints, promotion of differentiation and increase of proteoglycan synthesis, respectively [77]. The study of Parate et al. in 2020 found that the application of EMFs increased the production of paracrine factors and thereby promoted cartilage regeneration [78]. In 2020, Stefani et al. indicated that exposure of osteoarthritis defects to PEMFs stimulated cartilage repair [79] (Table 3).

4. Conclusion

According to the beneficial effects of magnetic nanoparticles and EMFs in the mentioned studies of the present review, they might be regarded as valuable adjuncts to tissue engineering for the treatment of articular cartilage-related diseases. However, to establish an appropriate treatment regimen, a better understanding of the underlying mechanisms in the sense of cellular pathways/events triggered is needed. Thus, more systematic studies are absolutely necessary and vital to evaluate the health benefits of magnetic nanoparticles and EMFs in the clinic.

Disclosure of interest

The authors report no conflict of interest.

Table 3. *In vivo* experimental studies evaluating the effects of electromagnetic fields.

Authors	Manuscript title	Year	Effects
Ciombor et al.	"Modification of osteoarthritis by pulsed electromagnetic field—a morphological study."	2003	The maintenance of the morphology of articular cartilage, decreases in the cartilage neoepitopes and number of cells immunopositive to interleukin-1, suppression of the matrix- degrading enzymes and increase of the number of cells immunopositive to transforming growth factor-beta in Hartley guinea pigs with osteoarthritis
Fini et al.	"Effect of pulsed electromagnetic field stimulation on knee cartilage, subchondral and epyphiseal trabecular bone of aged Dunkin Hartley guinea pigs."	2008	Slowing the progression of osteoarthritis lesions in the knee cartilage of aged Dunkin Hartley guinea
Boopalan et al.	"Pulsed electromagnetic field (PEMF) treatment for fracture healing."	2009	The increase of expression levels of transforming growth factor-beta 1 and 2 and fracture healing in the osteotomized femur of Wistar rats
Fernando et al.	"Effects of pulsed electromagnetic fields on the cartilage joint thickness of distal femoral metaphysis in the rat."	2009	The increase of the thickness of knee joint cartilage in Wistar rats
Boopalan et al.	"Pulsed electromagnetic field therapy results in healing of full thickness articular cartilage defect."	2011	The increases in the expression levels of collagen type II and synthesis of proteoglycans in the knee joint of rabbits subjected to osteochondral defects
Sotelo-Barroso et al.	"PEMF effects on chondrocyte cellularity and gene expression of the rat distal femoral metaphyseal articular cartilage."	2015	The increases in the cartilage cellularity and expression levels of collagen type XI, sex determining region Y-box 9 and aggrecan, support of joints, promotion of differentiation and increase of proteoglycan synthesis in the distal femoral metaphyseal articular cartilage in Wistar rats
Parate et al.	"Pulsed electromagnetic fields potentiate the paracrine function of mesenchymal stem cells for cartilage regeneration."	2020	The increase of the production of paracrine factors and promotion of cartilage regeneration
Stefani et al.	"Pulsed electromagnetic fields promote repair of focal articular cartilage defects with engineered osteochondral constructs."	2020	The stimulation of cartilage repair in osteoarthritis defects

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