Bone Bioelectricity and Bone-Cell Response to Electrical Stimulation: A Review

Taylor deVet,[a] Akiv Jhirad,[a] Laura Pravato,[a] & Gregory R. Wohl[a,b,*]

[a]McMaster School of Biomedical Engineering, McMaster University, Hamilton, Ontario, Canada; [b]Department of Mechanical Engineering, McMaster University, Hamilton, Ontario, Canada

*Address all correspondence to: Gregory R. Wohl, PhD, P.Eng., Department of Mechanical Engineering, McMaster School of Biomedical Engineering, McMaster University, Hamilton, Ontario, Canada; Tel.: +905-525-9140, E-mail: wohlg@mcmaster.ca

ABSTRACT: It is hypothesized that bone cells can sense mechanical force in the extracellular network via an electrical signal. This has led to the use of electrical stimulation (ES) to improve fracture repair and mitigate bone loss. Although overlap exists in bone maintenance and fracture healing mechanics, the processes involved in both are very different, resulting in dissimilar behaviors from the cells. Osteocytes are the most abundant cell type in bone tissue, and their basic structure and lineage are fairly well understood, but much debate is present regarding their behavior, with even less known about their behavior in electrical environments. A wide range of research exists on cell behavior under different types of ES, but it is difficult to draw conclusions due to the large variance in stimulation parameters, cell types, and origins (locations and species). By exploring behavior of multiple bone-cell types under different forms of ES, as well as mechanical stimulation through fluid flow, we can determine more about cell reactions to stimuli. In turn, a better understanding of cell response has the potential to improve and broaden therapeutic applications of ES for bone healing and bone loss mitigation, and enhance outcomes for osseointegration into implantable medical devices. These require greater understanding of the bone cellular environment from an electrical perspective as well as cellular responses to ES.

KEY WORDS: electrical stimulation, bone, fracture repair, pulsed electromagnetic field, capacitive coupling, *in vitro*, *in vivo*

I. INTRODUCTION

The effect of electrical stimulation (ES) on bone tissue and bone healing has been of great interest since Fukada and Yasuda reported on load-induced electrical potentials in the late 1950s.[1] Bone tissue deformation is directly correlated to electrical signal that is created on either side of the bend. Bone surfaces under compression produce negative potentials that cause tissue formation, and areas under tension produce positive potentials that cause resorption.[1–4] Loading rate and load directly correlate to the magnitude of the generated charges.[1–3] The electric field that is generated due to stress is reduced to almost zero when weight bearing is absent, and in such cases, the bones will start to deteriorate.[2] This was directly related to earlier studies by Bassett and Becker showing that misaligned fractures in children had new bone deposited on the concave side, and the older bone was removed from the convex side, allowing the misalignment to straighten with time.[3] Many studies since have demonstrated that ES has a significant effect on outcomes in fracture healing,[5] spinal fusion,[6,7] and healing of osteotomies[8] as well as aiding in delayed unions postfracture.[9]

It has since been hypothesized that bone cells can sense mechanical force in the extracellular network through an induced electrical signal, causing cell proliferation and cytodifferentiation.[10,11] It has also been proposed that bone cells are electrically sensitive with a positive charge, inducing chemotaxis in osteoblasts, resulting in bone building.[12] A few theories describe what cells sense to initiate this movement. First, most cells have negative membrane potentials that allow direct current (DC) ES to propel them in one direction, referred to as galvanotaxis.[13] The side of the membrane facing the anode becomes hyperpolarized and attracts free calcium ions that cause the membrane to contract and propel the cell toward the cathode.[14] Secondly, the cells may be able to sense ion movement in their environment, allowing them to migrate and reorient

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themselves accordingly.[14] Ion movement due to fluid flow creates a stress-generated potential (SGP) in the extracellular matrix and a change in charges around the cells. The change in charge distribution is a factor in calcium response theory, indicating that the cell's reaction is most likely a combination of these two phenomena.

Due to the potential ability for electrical signals to influence bone-cell behavior, ES became a popular research topic for applications in bone repair[5,6,8,9,15–18] and for mitigating bone loss.[19–26] However, this research has not progressed as far as expected because osteocyte response remains largely misunderstood. Our purpose here is to determine the next steps for investigation by reviewing current research on the electrical environment that bone cells experience.

II. BONE TISSUE COMPOSITION

Bone tissue is comprised of a hydroxyapatite mineral phase and an organic phase of collagen, water, proteins, and cells.[27] Although studies have confirmed that the electrical effect in bone is not entirely biological,[1,3] the exact cause of the bioelectric effect is still under debate. Both collagen and hydroxyapatite exhibit piezoelectric effects in specific settings and have a unique bioelectric effect when they interact. Dry bone properties are almost identical to those of dry collagen,[28,29] because they comprise the bulk of the organic portion of the tissue. Typically, bone is only considered piezoelectric when it is dry; that is because collagen behaves piezoelectrically when dry, and the piezoelectricity drops drastically when it becomes wet.[30] When collagen becomes saturated with water, it aligns more symmetrically and electric potentials becomes short circuited.[28–31] *In situ*, hydroxyapatite limits the amount of water that collagen can absorb and allows it to maintain some of its piezoelectricity when saturated, because it alters the fiber orientation.[28] Collagen has an abundance of electrons, whereas hydroxyapatite has very few, and it is proposed that junction bending between the two generates an electric potential (it behaves similarly to a positive–negative [p–n] junction in a semiconductor).[2]

When hydroxyapatite is removed from the bone matrix, the amount of electricity that is generated by deformation significantly decreases but does not disappear, indicating that the hydroxyapatite carries the bulk of the load, but collagen still experiences an increase in strain under compressive loads.[1,2,32] Collagen is an important component for the cells because it allows them to detect the *direction* of the stress within the matrix as opposed to just the magnitude from hydroxyapatite.[1] This indicates that the electricity generated from mechanical deformation in bone results from a combination of stress on the collagen fibers and hydroxyapatite crystals, in addition to creation of these p–n junctions.[2]

III. SGPS

A secondary hypothesis on bone bioelectricity is the creation of SGPs, first reported in the 1960s, shortly after Yasuda and Fukada's initial breakthrough.[32] Interfaces that separate two different phases of material automatically create an electric potential, because one phase is usually more electronegative than the other.[31] When bone is compressed, a negative charge spreads throughout the matrix, causing cations in the interstitial fluid to be attracted to the negatively charged surfaces and leaving a net surplus of anions in the extracellular fluid.[33] These streaming potentials can be caused by differences in voltage, pressure, and concentration gradient within the channels of the bone[2,13,28,34,35] but are only referred to as SGPs when they are mechanically generated. To diffuse the built-up charge within the matrix, ions redistribute until the charges are balanced. This causes the current to dissipate and no net movement occurs,[4,34] as no free ions are left to create a streaming potential.[29]

Evidence points to SGPs as a combination of the piezoelectricity of matrix and streaming potentials.[32,36] SGP relaxation times are too long for classical piezoelectricity to be the dominant factor.[32] Conversely, if streaming potentials were dominant, the conductivity should affect SGP relaxation time, which is also not the case.[32] This demonstrates that both bone anatomy and composition play a part in its bioelectric behavior.

IV. FRACTURE REPAIR

A unique feature of bone tissue is its ability to repair from fracture. Any realignment, relocation, or even introduction of an implant initiates regeneration and osteoinduction[10,37] that creates a combination of electrical, chemical, and mechanical stimuli on the tissue. Under normal conditions, the bone metaphyseal region is electronegative and the midshaft is isopolar.[12] When a fracture occurs, the entire bone becomes more electronegative, with the metaphysis remaining the most electronegative.[12] The fracture site becomes very negatively charged as it collects electrons[11,38] and anions, causing an ionic current flow to the injured area.[39] Free ions move along the concentration gradient, causing both a chemical and electrical shift and creating local electrical fields of 1–2 V/cm as the ions move into surrounding cells.[10] The ion movement takes the form of current loops that enter through the injury site and exit through intact bone upstream.[39]

Pulsed electromagnetic field (PEMF) and direct electric field stimulation have recently been used to accelerate the fracture healing rate. PEMFs have been shown to create more of a stable initial callus, resulting in a faster healing[40] by recruiting more immature mesenchymal stem cells (MSCs) that will differentiate into preosteoblasts.[37] Direct ES has been shown to do the same, with callus that grows substantially thicker and forms weeks earlier when the cathode is on the injured bone.[41] Because the cathode is the negative electrode, it causes the fracture site to become significantly more negative than normal, presumably amplifying the flow of ions and cells to the site. With more cells and building materials present, the bone heals more quickly.

V. ES

A. Whole Bone (*In Vivo*)

1. Electrode Polarity

A multitude of studies has shown that whole-bone ES produces osteogenesis at the cathode electrode,[2,41–47] specifically in small areas that closely surround the electrode.[40] These findings support those of basic bone behavior studies that found bone areas with the most negative electric potentials have the greatest amounts of bone formation,[33] also agreeing with studies that show application of a negative charge to a fracture improves healing.[41] This phenomenon takes place as increased cell proliferation occurs at the cathode, with an increase in osteoid and new bone formation under DC application.[48,49] Electrical stimuli have been shown to cause bone formation that is fairly disorganized,[42,45] and similar to periosteal bone,[41,48] intramembranous bone,[43] or a cartilaginous or fibrous-type tissue that resembles metaplastic- or osteoblastic-type bone.[44] This indicates that the osteoblasts are more active with stimulation[43,48] but also that other cell types are also more active. Throughout the stimulation process, the bone becomes more organized as a controlled remodeling process occurs.[50] When stimulation ceases, the bone is resorbed through osteolysis.[42]

Activity at the anode has shown mixed results, including bone destruction[44,48] or no tissue change at the insertion site.[2,18] Osteoclasts have been shown to migrate toward a positive charge, which could explain increased bone resorption,[13] but they have higher membrane resistance when compared to osteoblasts, indicating that they are less electrically sensitive.[51] Osteoclast migration and increased necrosis around the anode may be linked, because more remodeling occurs at the anode to eliminate necrotic bone.[48]

The lack of response at the anode has been hypothesized to be due to electrical signal parameters. If stimulating signal is not similar enough to natural signal, the bone may not react similarly to the predicted *in vivo* behavior.[50] Charge distribution during growth or the healing environment in bone is focused on negative charge relocation at the injury site. Positive charge movement is a means to counterbalance the negative charge. A large positive charge focus in one location is inconsistent with the naturally occurring charge phenomenon, and so this could explain why the accumulation of positive charge does not illicit a natural response in the same way as does negative charge.

2. Signal Parameters

Although overall consensus seems that current of 1–20 μA is optimal to stimulate bone formation,[10] a review of several *in vivo* whole-bone stimulation studies suggests that a very wide range of current magnitude can be used to achieve the same results. Studies using current magnitudes from as little as 20 pA up to as high as 100 mA have all shown the ability to stimulate bone formation, although sometimes the bone is poorly organized.[41,42,47,50] However, others have demonstrated osteolysis due to excessive current above only 20 μA.[18,41,52] This speaks to the need to control and carefully specify parameters used in studies. A summary of studies can be found in Table 1.

The amount of bone that is formed around a negative electrode is related to current density and charge,[46] rather than just the current itself. Alternating current (AC) signals are commonly considered to mimic endogenous signals, but no evidence has shown that an AC signal performs better than a DC signal because it delivers less charge overall.[52] Using equivalent current values but altering signal shape has had greater effect on the amount of bone formation, with results consistently showing that a DC signal is optimal.[10,46–48] A DC signal allows for the highest charge buildup at the cathodic site as well as the most consistent flow of ions, resulting in the greatest formation of bone.

3. Bone Loss Mitigation

Bone loss and the processes associated with bone remodeling are biologically different from fracture repair. Moreover, bone loss paradigms could respond differently based on underlying mechanisms (e.g., disuse osteopenia vs. postmenopausal osteoporosis, which is hormonally driven). Several different mechanisms including PEMF, direct ES, and capacitive coupling have been tested to mitigate bone loss in different animal models.[20,23–26] A PEMF uses two coils in a Helmholtz configuration that induces an electromagnetic (EM) field between the coils when an electric current is applied. Simple sinusoidal and complex PEMF waveforms were compared using an isolated (disuse) Turkey ulna model.[25] The simplest, lowest-frequency sinusoidal waveform (15 Hz frequency; 0.08 mV amplitude) resulted in the greatest increase in cortical area relative to both an ES control group and the contralateral, intact ulnae within group. A similar study was conducted to examine the effect of pulse power on the cortical bone area and demonstrated a maximum osteogenic effect between 0.01 and 0.04 T^2/s.[26] Additionally, within–group comparison of the study's contralateral ulnae showed a –13% difference in cortical area in the control group and a 12.3% difference in the group that underwent the 0.01-T^2/s stimulation protocol. In a sciatic denervation rat model of disuse osteopenia, ES capacitive coupling resulted in significant reduction in percent of cortical porosity, increased cortical area, and increased cortical thickness of the denervated bone when compared to the unstimulated, denervated control.[24]

A common and well-established model of human postmenopausal osteoporosis is the ovariectomized (OVX) female rat.[53] Although remodeling processes that led to bone loss were similar to the disuse model, underlying systemic hormonal mechanisms that drove bone loss likely influenced bone responsiveness to EM stimulation. Nevertheless, studies using OVX rats have shown positive response to EM stimulation. Using a 1-mT magnetic field signal at 50 Hz frequency, Sert et al.[20] reported a significant increase in tibial bone cortical thickness in OVX rats. Similarly, Chang and Chang used a 2-mV/cm electrical field at 7.5-Hz frequency and reported significant increases in trabecular bone volume fraction, trabecular thickness, and trabecular bone formation rate in the proximal tibial metaphysis of OVX rats.[23] The use of capacitive coupling has also been shown to produce global effects on OVX rats that have had whole-body stimulation within a 1.5-MHz, 30-mW/cm^2 electric field.[19] These experiments found increased global bone mineral density (BMD), spinal BMD, and lower limb BMD in the treatment group compared to the control group. The same research group showed that low-intensity ES mitigated osteocyte apoptosis due to OVX in rats, with ES OVX rats having similar levels of osteocytes to those in the group with intact ovaries.[54]

TABLE 1: Reviewed studies of whole-bone *in vivo* ES

Model	Stimulation parameters (μA)	Stimulation duration	Experiment duration (d)	Results	Ref.
Chick tibia	10	24 h	10	Cathode: thicker periosteum and proliferation of osteoblasts; anode: more osteoclasts and osteoblasts gathered	48
Dog	10	24 h	21	Bone formation around cathode poorly organized but organization increased at 21 d; more osteoblasts collected around cathode	50
Dog	20	24 h	7, 15	Increased matrix formation around titanium implant	119
Human	10, 20	24 h	14	Increased ALP generation and enhanced fracture repair	122
Human	10, 20	24 h	84	20 μA helped heal nonunion fractures	123
Mouse	10	5 min	28	Increased new blood vessels on d 14; overall increase in fibroblasts	124
Rabbit	10, 30	24 h	35	Improved fracture fusion	125
Rabbit	100	24 h	3–28	Increased BMP-2, -6, and -7 and TGF-β	126
Rabbit	5–40	24 h	21	0–20 μA increased osteogenesis; 50–100 μA led to severe damage	127
Rabbit	0–100	24 h	14	4–6 μA: No change in tissue; 15–20 μA: periosteal osteogenesis near cathode and increased callus formation; >100 μA: bone tissue destruction and charred surrounding tissue	41
Rabbit	20	24 h	28	Osteoblastic bone formation	42
Rat	10	5 min	10–90	Increased collagen production, angiogenesis, and matrix calcification	128
Rat	5, 10, 20	24 h	8	10 μA: 50% thicker bone formation; 20 μA: 80% thicker bone formation	47

ALP, alkaline phosphatase; BMP, bone morphogenetic protein; TGF, transforming growth factor.

B. Cell Stimulation (*In Vitro*)

The stimulus of an external electric field results in a large voltage drop across a cell membrane but a small voltage drop in the cytoplasm as the plasma membrane becomes polarized, creating a large local electric field at the membrane only.[55] This capacitive property of charge holding allows the cell to regulate its internal environment, shielding it from potentially damaging changes. The cellular membrane's negative voltage renders almost all cells sensitive to ES and should cause migration toward the anode,[50] but almost all bone cells exhibit migration toward the cathode,[12] indicating that other factors control

migration. A relatively important finding from an *in vitro* study showed that bone adaptation seems to be controlled by recruiting more cells, not by altering the response of an individual cell—an all or nothing process[56]—and that bone cells seem to have a refractory period for stimulation, with specific frequencies ideal for maximal stimulation response.[57]

1. Osteoblasts

Most commonly, osteoblasts have been stimulated with PEMFs. Some studies found a reduction in cell proliferation but an increase in alkaline phosphatase (ALP) activity, collagen synthesis, osteocalcin levels,[58–61] and growth factors such as vascular endothelial growth factor (VEGF)[62] that use both DC[63] and capacitive coupling[64] stimulation. Exposure time to the PEMF has also been shown to positively correlate with expression levels of bone morphogenetic protein (BMP)-2 and -4 from osteoblasts.[65] BMP-2 and -4 are crucial factors in skeletal repair and regeneration,[66] and their roles are complemented by VEGF, without which impaired bone formation and suppressed blood vessel development in bone would occur.[67] Collectively, the increased expression of these biomolecules through ES administration could enhance bone formation and healing[68] and mitigate the loss of bone mass *in vivo*.[69]

Conversely, other studies have found that PEMFs increase osteoblast proliferation but do not affect cellular differentiation.[48,70–78] The extreme variance in these results shows how sensitive the cells are to the EM stimulus, because waveform shape and duration greatly affect their behavior. PEMFs can cause both positive, negative, and no change in cell activity, depending on how they are applied. A summary can be found in Table 2.

Direct ES can also be applied through capacitive coupling of the culture environment. This has no adverse effect on osteoblasts, with numbers remaining stable through experiments.[51] Interestingly, the number and productivity of enzymes in the osteoblasts are higher on the side of the cell closest to the negative electrode, indicating asymmetrical activity.[79] Osteoblasts have been shown to move toward a circuit cathode's negative charge,[14,43,50,51] and this is carried out through the growth of lamellipodia on the cathodic side of the cell.[13,51]

All methods of ES—capacitive, inductive, and magnetic coupling—cause increased DNA synthesis in preosteoblasts, but capacitive coupling can maintain this increased activity throughout the duration of the stimulus.[80] This may be due to the different types of responses that originate from each of these stimulation types. Capacitive coupling causes Ca^{2+} ion movement through voltage-gated channels, whereas inductive coupling and EMFs cause Ca^{2+} to be released through intracellular stores. The intracellular release increases cytosolic calcium concentrations and may involve the calcium/calmodulin pathway.[80]

2. Osteocytes

Osteocytes are the most abundant type of cell within bone tissue. Although their basic structure and lineage are fairly well understood, much debate continues regarding their behavior.[81] Osteocytes, surrounded by tightly packed collagen fibers,[82] can control bone structure up to 1 μm around the lacuna that they inhabit.[2,52] The matrix directly around the osteocyte does not become fully mineralized, forming the lacuna in which the cell resides to create an interconnected set of canaliculi channels.[83] Osteocytes are presumed to detect and communicate strain when subjected to shear stress by the movement of fluid past their cellular processes.[81] An osteocyte's sensory ability is further confirmed based on the fact that its cellular processes are mainly on the mineralized side, as opposed to the vascular side of the cell,[82] indicating that communication occurs through the matrix and not through changes in blood flow. Cellular processes of neighboring cells are connected through gap junctions that fill with fluid. The fluid is saturated with proteoglycans and ions that allow for communication.[84] The gap junctions can be directly regulated by electric fields.[58] Although PEMFs do not affect the number of cells present,[85] they do influence the amount of communication factors through gap junctions. Osteocytes can have a twofold increase in prostaglandin E_2 and an overall increase of transforming growth factor (TGF)-β1 and NO^{2-} with exposure to a PEMF.[58,85]

TABLE 2: Reviewed studies on *in vitro* cell stimulation

Cell type	Current type	Stimulation parameters	Stimulation duration	Study duration (d)	Results	Ref.
AD MSCs	DC	200 μA	4 h	21	Cells produced 100% more calcium	129
BM MSCs	DC	100 mV/mm	1 h	3, 7, 14	Increased osteogenic differentiation, collagen, calcium deposits, osteopontin expression, Osterix, and calmodulin genes; with > 7 d stimulation, results lasted post-testing	130
BM MSCs	DC	200 mV/mm	1 h	21	Increased ERK1/2 phosphorylation, osteopontin expression, and cell proliferation	107
BM MSCs	DC	Stimulated media, 200 mV/mm	1 h	21	Increased osteopontin expression and cell proliferation	107
BM MSCs	DC	10–600 mV/mm	2–10 h	1	Stimulation through salt bridges caused anodal migration	131
BM-MSC chondrocytes	PEMF	0.7 V, 35 V/m, 1-KHz sine	45 min × three 225- min breaks	7	No change in metabolism; increased collagen II production	77
Human osteoclasts	PEMF	15 Hz	4 h	9	Decreased cells and nuclei per cell	71
MC3T3-E1	CC	60 kHz, 20 mV/cm, 50% duty cycle	24 h	1	Increased BMP-2 and ALP expression; increased mRNA expression for BMP-2, -4, -5, -6, and -7	132
MC3T3-E1	DC	200 mV/mm	1 h	21	Increased SPP1 and BMP-2 mRNA; decreased cell density around electrodes	107
MC3T3-E1	CC	60-Hz sine, 44.81-V PTP, 2.0 V/m, 300 A/cm	30 min–24 h	1	Increased DNA expression; Ca^{2+} ion movement through voltage-gated channels	80
MC3T3-E1	DC	100 mV, 0.1 V/cm, 50–800-Hz square wave	1 h	1, 3, 7	200–400 Hz produced most cells; increased ALP and collagen I; ES + IGF: highest calcium deposits, RUNX2, collagen I, and OPN	133
MC3T3-E1	Direct	1 kHz, 500 mV	24 h	14	Increased proliferation and cell alignment with field	73
MC3T3-E1	PEMF	15-Hz pulses, 4.3 kHz, 22.5 G, 0.16 V/m	30 min–24 h	1	Increased DNA; Ca^{2+} released through intracellular stores	80

TABLE 2: (*continued*)

Cell type	Current type	Stimulation parameters	Stimulation duration	Study duration (d)	Results	Ref.
MC3T3-E1	PEMF	2 mT, 15 Hz	2 h	3	Increased cellular attachment to implant; cytoskeleton better developed	72
MC3T3-E1	PEMF	4 mT, 15 Hz	30 min	2	Increased cellular proliferation	74
MC3T3-E1	PEMF	100 V, 1.5-μA square wave	1 h	12	Increased cellular proliferation and adhesion	75
MC3T3-E1	Direct, charged film	50–250 μA	4 h	7	Increased metabolism after 7 d	134
MC3T3-E1	PEMF	15 Hz, 5 mT	2 h	1	Increased intracellular calcium transients	135
MG63	CC	100 mV/mm	2 h	1	Increased proliferation on cathode	136
MG63	PEMF	5-ms pulses at 15 Hz, 0–18 G	8 h	4	Increased PGE2, osteocalcin, and ALP production; decreased proliferation but more differentiated phenotype	59
MLO-Y4	PEMF	5-ms pulses at 15 Hz, 0–18 G	8 h	4	No change in cell number and osteocalcin levels; increased PGE2 and TGF-β1; initially increased then decreased cellular activity and Cx43 production	85
MLO-Y4	PEMF	15 Hz; 0, 5, and 30 G	2 h	3	5 G inhibited cellular apoptosis, increased dendritic length and OPG mRNA, and decreased RANKL mRNA levels; 30 G promoted MLO-Y4 apoptosis; media from 5 G inhibited osteoclasts and caused apoptosis	86
Mouse bone marrow	PEMF	7.5 Hz, 4.8–12.2 μV/cm	2 h	9	Osteoclasts appeared after 5-d stimulation; 4.8 μV/cm reduced osteoclast recruitment and decreased resorption area percentage; 12 μV/cm increased osteoclast recruitment and bone resorption area percentage	90
Mouse bone marrow	PEMF	7.5 Hz, 3 μV/cm	8–16 h	1	Accelerated osteoclast apoptosis	91
Mouse bone marrow	PEMF	1.6 mT; 4.8 and 9.6 μV/cm	24 h	8	Increased osteoclast-like cell formation; extremely low fields suppressed osteoclast recruitment	92

TABLE 2: (*continued*)

Mouse osteoclasts	PEMF	8 Hz, 3.8 mT	40 min	5	Prevented RANKL-induced osteoclast cell formation	118
NIH3T3, MG-63	CC	2 μA	5–30 min	1	Increased migration and proliferation	137
Osteoblasts (hFOB 1.19)	Direct	4.2 A/m^2	1 h	21	Enhanced proliferation	138
Primary osteoblasts from human femoral head	PEMF	0.4 mT, 14.9 Hz	24 h	3–10	Increased ALP activity and proliferation	76
Primary osteoblasts from human femoral head	PEMF	320 Hz, 3 mT	45 min × three times	1	Increased collagen I; decreased viability	77
Primary osteoblasts from human knee	PEMF	0.3 V	3 h	3	Decreased ALP activity	120
Rabbit osteoclasts	DC	1-V/mm salt bridge	17.2 h	1	Lamellipodia orientation caused migration toward cathode	51
Rat calvaria	CC	60-kHz sine, 1–20 mV/cm	6 h	1	Increased proliferation	57
Rat calvaria	PEMF	3-Hz varying amplitude	5 min	1	Increased DNA synthesis and cAMP production; caused large voltage drop across cell membrane	55
Rat calvaria	PEMF	15 Hz, 1 G, 0.1 mT, 2 mV/cm	14 h	1	Increased osteoblast proliferation; decreased ALP production	70
Rat calvaria osteoblasts	DC	100-μA/cm^2 salt bridge	1 h	10	Increased proliferation and calcium entering cell and released from endoplasmic reticulum; formed osteocyte network; some osteoblast-like cells transitioned to osteocytes-like states	98
Rat calvaria osteoblasts	PEMF	15 Hz, 9.6 G	1.5 h	1	Higher protein adsorption and increased proliferation	78
RCJ 1.20; RCB 2.2 A	DC	1-V/mm salt bridge	17.2 h	1	Lamellipodia orientation caused migration toward cathode	51

TABLE 2: (*continued*)

Cell type	Current type	Stimulation parameters	Stimulation duration	Study duration (d)	Results	Ref.
ROS 17/23	PEMF	5-ms pulses at 15 Hz, 0–18 G	24 h	3	No change in cell number or osteocalcin levels; increased PGE2 and TGF-β1; increased then decreased cellular activity and Cx43 expression	85
Saos-2	Direct contact	100–400 mV/mm	2–8 h	1	High voltage in conductive scaffold caused negative cellular morphology changes; decreased proliferation and ALP production	61
Saos-2	Direct contact	10 mV/mm	4 h	1	Enhanced proliferation initially but less at end; increased matrix mineralization	63
Saos-2	PEMF	15 Hz, 2 mT	10 min, 3 h	1	No RANKL change; increased OPG	117

AD, adipose derived; ALP, alkaline phosphatase; BM, bone marrow; BMP, bone morphogenetic protein; cAMP, cyclic adenosine monophosphate; CC, constant current; Cx43, connexin 43; DC, direct current; ERK, mitogen-activated protein kinase; ES, electrical stimulation; hFOB, human fetal osteoblastic; IGF, insulin-like growth factor; mRNA, messenger RNA; MSC, mesenchymal stem cell; OPG, osteoprotegerin; OPN, osteopontin; PEMF, pulsed electromagnetic field; PGE, prostaglandin E_2; PTP, peak-to-peak; RANKL, receptor activator of nuclear factor-κB; ROS, reactive oxygen species; RUNX, runt-related transcription factor; SPP1, secreted phosphoprotein 1; TGF, transforming growth factor.

Interestingly, the protein Connexin 43 creates gap junctions and is produced in lower volume with PEMF application but increases with shear stress.[85] This indicates that PEMFs do not increase connectivity among cells but may augment communication among already connected cells.

PEMFs on the murine osteocytic cell line murine long bone osteocyte y4 (MLO-Y4) at 5 G inhibit cellular apoptosis and increase the length of cellular dendrites. They reduce receptor activator of nuclear factor-κB ligand levels and increase messenger RNA for osteoprotegerin (OPG), both of which control cellular apoptosis. A 5-G field strength also reduced the number of osteoclasts, in addition to decreasing their ability to resorb bone when using conditioned media from osteocyte-like cells.[86] This study proposed that cell cilia are responsible for sensing the electrical environment around them, which corresponds to other theories that cilia are used to respond to changes in fluid flow around them. A summary of these results is found in Table 2.

3. Osteoclasts

Osteoclasts are critical to bone tissue maintenance but are not closely related to osteocytes and osteoblasts, which are from the same lineage. Osteoclasts are more closely related to macrophages and have different behavioral traits. Although osteocytes and osteoblasts work together, osteocytes and osteoclasts usually counteract each other. Osteoclasts can become inhibited by osteocytes,[87,88] and in portions of bone with increased resorption, osteoclasts degrade osteocytes.[89]

In terms of ES, a big difference is the migration direction of osteoclasts. They tend to migrate or collect at the anode, which could explain increased anodal bone resorption and remodeling,[13,48,51] but they have higher membrane resistance when compared to osteoblasts, indicating that they are less electrically sensitive.[51] PEMFs can cause cells collected from bone marrow to differentiate into osteoclasts,[90–92] but correct parameters must be used for collection because extremely low PEMFs suppress osteoclast recruitment[92] and can also induce apoptosis.[86,91] For example, Chang et al. demonstrated that a 4.8-μV/cm PEMF decreased OPG production and osteoclast recruitment but increased resorption area percentage and OPG production.[90] Increasing signal strength to 12 μV/cm had the opposite effect.[90]

VI. ES SIDE EFFECTS

Although cells seem to be directly sensitive to ES *in vitro*, speculation exists that the faradaic by-products that are created in the culture environment can influence cell responses. This involves introducing pH changes, hydrogen peroxide, reactive oxygen species, and chlorine into the stimulation environment.[93] Adding a balanced electrical stimulus into an *in vivo* or *in vitro* environment can cause severe pH shifts.[94,95] Specifically, although there may be no net pH change there is a pH decrease that occurs at the anode and a pH increase at the cathode.[94,96–98] The pH changes are directly caused by reactions that occur at the electrodes.[95] Electrodes cause both faradaic and nonfaradaic reactions by using different methods to rebalance charge. Nonfaradaic reactions have no electron transfer; rather, they redistribute charged molecules in the electrolyte.[99] Faradaic reactions cause electron transfer between electrode and electrolyte, resulting in reduction or oxidation.[99]

Hydroxide is created at the stimulation cathode,[98,100] causing a reduction in water in the surrounding environment to create hydrogen peroxide.[97,100,101] In extreme cases, the cathode causes hydrogen gas formation, with amount formed directly correlated to electrode voltage.[94,95,97] In some media types, a large amount of free chlorine is created through ES, which creates hypochlorite, a very strong oxidizing agent.[94,96]

Electrode selection is also very important because faradaic reactions can cause them to dissolve and release metal ions in the medium.[95] Platinum is used most often because of its stability, but side effects of high current stimulation with platinum electrodes on tissue are similar to tissues on exposure to platinum salts, indicating that they may be dissolving.[95] Pt^{-} ions are also powerful oxidizing agents that can be reduced by organic species in the surrounding environment, causing cellular necrosis.[94]

The formation of hydrogen peroxide is of interest, because it can stimulate VEGF production[102]

and increase osteoblast activity and proliferation.[98,100] Osteoblasts can experience up-regulation of factors such as runt-related transcription factor 2, secreted phosphoprotein 1, and BMP-2.[101] This can initiate a transition toward an osteocyte-like state with formation of an osteocyte-like matrix when introduced to increased concentrations of hydrogen peroxide.[98]

The effects of too much stimulation can directly affect cell viability, specifically through pH changes.[101] ES has been found to inhibit and kill bacteria around electrodes, potentially due to the pH changes or electrochemical reactions.[93,103] Specifically, microampere DC stimulation is more effective than other ES in preventing bacteria around the cathode.[104] The amount of chlorine and by-products created from ES has the same potential to kill bacterial cells as the current itself, indicating that chlorine may cause cell death, not the ES.[96]

Osteoblasts can withstand more basic environments, and at a pH of ~ 7.6, they increase their production of collagen, ALP, and thymidine.[105,106] An increase in DNA production also occurs at a pH of 7.0–7.2 and 7.6–7.8.[105] Conversely, an increase in pH decreases creation of osteoclastic β-glucuronidase.[106] This basic environment decreases calcium flow from bone by decreasing osteoclastic activity and up-regulating osteoblastic activity, which could explain increased osteoblast activity in more basic *in vitro* settings.[106]

Generation of any of these side effects is important to consider when using ES, and stimulation signal parameters must be monitored to protect against faradaic side effects. Creation of hydrogen peroxide specifically is directly proportional to ES pulse width, frequency, and voltage in the environment.[107] Changing the stimulus to a biphasic signal reduces the by-products but does not fully eliminate them.[95,99] Faradaic reactions that occur at the cathode are not direct reversal reactions to electrode corrosion at the anode.[99] Phase length also affects how much of the reversible reactions can be reversed before the phase switches back.[94] Rather than voltage, constant current better controls the charge balance, to minimize reactions.[94]

VII. DISCUSSION

A. Signal Type

Some overarching issues arise from ES studies of bone cells. An important issue is the need for consistency in stimulation parameters, whether mechanical, fluid induced, or electrical. There is a general lack of consistency in parameters or missing information on the parameters used. It is difficult to compare results between studies with such variation in procedures and inconsistency in defined signal parameters. This is most obvious when investigating the abundance of PEMF studies on cells, because each use differing frequency, field strengths, and stimulation methods.

This is not unique to PEMF studies. Across all ES types, very little consistency exists on the use or reporting of signal parameters, and this may partly be because researchers are unsure of which parameters are important for cell stimulation.[55] A consensus seems to exist that DC stimulation is preferred over high-frequency AC signals,[10,48] but only controversial support is evident regarding signals, such as cyclical DC or low-frequency AC signals.[57] Alternating electrical signals could act as a pump to move ions and waste toward and away from cells in the absence of vasculature,[2] which may be beneficial, but some studies use a media pump to mitigate this issue.[108,109] AC signals are commonly considered for mimicking endogenous signals but no evidence shows that this is better, because charge seems to be the more important factor, not signal shape.[52] Oscillating fields have been shown to inhibit cyclic adenosine monophosphate responses, rendering cells less productive,[10] but others directly contradict this with increased cell proliferation.[71–78]

Most of the current studies on ES of bone cells are focused on PEMFs, with little to none on direct stimulation. Interestingly, many studies on PEMFs actually use electrodes that are in direct contact with culture media or in contact through salt bridges. The field strength is being controlled, but aspects associated with direct ES must be considered in this case due to both faradaic and nonfaradaic reactions that will occur in increased amounts as a result of the direct contact. This speaks to the need to use common terminology in the field as well as a better, broader

understanding of how each stimulation technique should be used.

B. Bulk Piezoelectricity

It is true that bone tissue exhibits bioelectric behaviours but it is not clear how these signals are experienced or interpreted at the cell level. Different components that combine interact to create an overall piezoelectric response that generates local potential differences of up to 6 mV.[33] However, the signal generators are very small, in many cases too small to measure,[2] making it very difficult to determine exactly how much of the tissue response each cell will see. Free ions move along fracture concentration gradients, creating local electrical fields of 1–2 V/cm as the ions move into surrounding cells.[10] When bone is loaded, the shear experienced at the cellular surface is 0.8–3 Pa, indicating that only a small fraction of the forces that are exerted on the tissue make it to the cells.[32] If this principle is extended to electrical signals, the amount of charge that gets through to the cells is a small fraction of the local electric field. Due to the minuscule nature of this value, there has been limited research into the actual electrical environment that the cell experiences. The cells are presumably very sensitive to their environment and could be very sensitive to the type of signals to which they respond or sense. The lack of research in this area may explain why osteocyte behavior is still not well understood. Additional work is needed to characterize the electrical signal environment of the cells and determine the nature of electrical signals that they receive *in situ*.

C. Cell Types

Discovery and development of immortalized bone-cell lines such as the Saos-2 osteoblast-like line and MLO-Y4 osteocyte-like line occurred after a peak in ES research activity.[110–113] This contributed to much variance among studies and has resulted in research on nonspecific cell types. The varied cell types range from MSCs to cocultures of osteocyte–osteoblast–osteoclasts that were harvested from a wide variety of bones and species; some are listed in Tables 1 and 2. The range of harvest locations yields a wide variety of results, because different bones have dissimilar environmental conditions, resulting in cells with differing sensitivity. Nevertheless, many results are consistent across the multitude of cell types, giving generalizable information on ES cell responses in the mesenchymal lineage.

The majority of studies performed with specific bone cells and ES are done with osteoblast and osteoblast-like cells. Furthering understanding of any cell type is important, but a large gap in studies on osteocytes exists, with most researchers referring to their behaviors as "poorly understood" and avoiding their use in investigation. This could be due to availability of cells themselves, seeing as it is easier to harvest osteoblasts from bone directly, or recent osteocyte-like line development during the past decade.[114,115]

D. Fracture Versus Maintenance

Overlap is present in the mechanics of bone maintenance and fracture healing, but the processes involved in fracture healing and bone remodeling are quite different. The presence of a strong electrically negative site to attract cells and ions in a fracture is unavailable in remodeling to the same magnitude. This may be a reason why for fracture healing ES has been so successful compared to direct stimulation for bone mass maintenance. The smaller electrical stimuli present in habitual bone remodeling processes are more difficult to enhance, because the stimuli are likely to be locally activating osteocytes that are not at a strength necessary to cause cellular migration. Additionally, the fractures studied at highest frequency are osteotomies or critical fracture—the most severe cases. The extreme nature of the injuries indicates that a stronger electrical response will be present, which is easier to enhance with exogenous ES that produces fewer adverse effects.

E. Reproducibility

Cells are environmentally sensitive, which makes it difficult to reproduce the same results even when using consistent ES on bone cells.[12] The medium in which the cells are grown is not created with

electrical properties in mind and adding electrical current causes medium fluctuations to which the cells quickly react. Although most studies do not report on precipitates in cell media, evidence shows that various calcium deposits occur.[13,47] This leads to a change in the free ions in the medium. Coupled with ion movement from convection currents, this can alter the local pH and greatly affect the cells, independently of electrical current effects.[47] Additionally, bone adaptation seems to be controlled by recruitment of additional cells, not by altering the response of an individual cell, making that an all-or-nothing process.[56] If the number of cells in a study is not closely controlled, results can vastly differ. This may be why early studies that harvested cells from bone, rather than experimenting on immortalized cell lines, produce so much variability. The amount and type of cells that are harvested from the tissue can vary significantly from study to study and even within the same experiment.[116]

F. Stimulation Delivery

Variance in cell ES studies may result from the way in which the stimulation is delivered. Many studies use homemade stimulation apparatuses that are developed with various types of function generators and electrode types. Some use industrial products such as a Physio-Stim,[117] Orthofix spinal stimulator,[71] and union-2000A stimulator,[118] which are used clinically for nonunion fractures. These are then adapted in the lab for use on cell cultures, using titanium implants[119,120] and their components such as the ASNIS S-series stimulation screw system.[77] Current studies on ES in cell culture seem to focus on the use of piezoelectric materials to build scaffolds and films to grow three-dimensional cultures that can include ES.[73,75,121]

VIII. CONCLUSIONS

On the basis of the above research on the electrical nature of bone tissue, the ability of bone cells to sense external stimuli, and the response of whole bone to ES, an abundance of evidence supports the hypothesis that osteocytes respond to electrical stimuli. It makes sense that an electrical signal such as the endogenous electrical charge in the tissue created *in vivo* could activate the cells in the same way if it was applied *in vitro*. The ability to do so would allow us to increase our understanding of the osteocyte and how it can maintain bone homeostasis through the control of surrounding cells. Because cells showed no adverse effects after application of electrical charge,[51] it is interesting that there is not more research into this area for osteocytes.

A wide range of research uses direct ES of cells but none specifically on osteocytes. Investigations that study osteocyte electrical behavior primarily use PEMF as the stimulus; none use direct DC stimulation. This should be further explored to understand osteocyte responses to electrical charge and characteristics of the electrical signal (magnitude, frequency) to which the osteocyte responds *in vivo*. Potential exists to improve and broaden therapeutic applications of ES for bone healing and bone loss mitigation. ES methods have potential to improve fracture healing and outcomes for osseointegration into implantable medical devices. This requires a better understanding of the bone cellular environment from an electrical perspective as well as the cellular responses to ES.

REFERENCES

1. Fukada E, Yasuda I. On the piezoelectric effect of bone. J Phys Soc Jpn. 1957 Oct 15;12(10):1158–62.
2. Bassett CAL. Electrical effects in bone. Sci Am. 1965;213(4):18–25.
3. Bassett CAL, Becker RO. Generation of electric potentials by bone in response to mechanical stress. Science. 1962 Sep 28;137(3535):1063–4.
4. Marino AA, Becker RO. Piezoelectric effect and growth control in bone. Nature. 1970 Oct;228(5270):473–4.
5. Brighton CT, Hozack WJ, Brager MD, Windsor RE, Pollack SR, Vreslovic EJ, Kotwick JE. Fracture healing in the rabbit fibula when subjected to various capacitively coupled electrical fields. J Orthop Res. 1985;3(3):331–40.
6. Kane WJ. Direct current electrical bone growth stimulation for spinal fusion. Spine (Phila, PA 1976). 1988;13(3):363–5.
7. Akhter S, Qureshi AR, Aleem I, El-Khechen HA, Khan S, Sikder O, Khan M, Bhandari M, Aleem I. Efficacy of electrical stimulation for spinal fusion: A systematic review and meta-analysis of randomized controlled trials. Sci Rep. 2020;10(1):1–12.
8. Fredericks DC, Nepola JV, Baker JT, Abbott J, Simon

B. Effects of pulsed electromagnetic fields on bone healing in a rabbit tibial osteotomy model. J Orthop Trauma. 2000;14(2):93–100.
9. Saltzman C, Lightfoot A, Amendola A. PEMF as treatment for delayed healing of foot and ankle arthrodesis. Foot Ankle Int. 2004;25(11):771–3.
10. Chao PHG, Roy R, Mauck RL, Liu W, Valhmu WB, Hung CT. Chondrocyte translocation response to direct current electric fields. J Biomech Eng. 2000 Jun 1;122(3):261–7.
11. Huang CP, Chen XM, Chen ZQ. Osteocyte: The impresario in the electrical stimulation for bone fracture healing. Med Hypoth. 2008 Jan;70(2):287–90.
12. Isaacson BM, Bloebaum RD. Bone bioelectricity: What have we learned in the past 160 years? J Biomed Mater Res A. 2010;95:1270–9.
13. Duncan RL, Turner CH. Mechanotransduction and the functional response of bone to mechanical strain. Calcif Tissue Int. 1995 Nov;57(5):344–58.
14. Mycielska ME, Djamgoz MBA. Cellular mechanisms of direct-current electric field effects: Galvanotaxis and metastatic disease. J Cell Sci. 2004;117:1631–9.
15. Nabil Y, Abdalfattah S, Lotfy M. A clinical study on the effect of electric stimulation on segment transfer distraction osteogenesis for mandibular reconstruction. Egypt J Oral Maxillofac Surg. 2014;5(1):10–5.
16. Otter MW, McLeod KJ, Rubin CT. Effects of electromagnetic fields in experimental fracture repair. Clin Orthop Relat Res. 1998;355:S90–104.
17. Inoue N, Ohnishi I, Chen D, Deitz LW, Schwardt JD, Chao EYS. Effect of pulsed electromagnetic fields (PEMF) on late-phase osteotomy gap healing in a canine tibial model. J Orthop Res. 2002;20(5):1106–14.
18. Hassler CR, Rybicki EF, Diegle RB, Clark LC. Studies of enhanced bone healing via electrical stimuli. Comparative effectiveness of various parameters. Clin Orthop Relat Res. 1977;124:9–19.
19. Lirani-Galvão APR, Bergamaschi CT, Silva OL, Lazaretti-Castro M. Electrical field stimulation improves bone mineral density in ovariectomized rats. Brazilian J Med Biol Res. 2006 Nov;39(11):1501–5.
20. Sert C, Deniz M, Düz MZ, Akşen F, Kaya A. The preventive effect on bone loss of 50-Hz, 1-mT electromagnetic field in ovariectomized rats. J Bone Miner Metab. 2002;20(6):345–9.
21. Eser P, De Bruin ED, Telley I, Lechner HE, Knecht H, Stüssi E. Effect of electrical stimulation-induced cycling on bone mineral density in spinal cord-injured patients. Eur J Clin Invest. 2003;33(5):412–9.
22. Eyres KS, Saleh M, Kanis JA. Effect of pulsed electromagnetic fields on bone formation and bone loss during limb lengthening. Bone. 1996;18(6):505–9.
23. Chang K, Chang WHS. Pulsed electromagnetic fields prevent osteoporosis in an ovariectomized female rat model: A prostoglandin E2-associated process. Bioelectromagnetics. 2003;24(3):189–98.
24. Brighton CT, Katz MJ, Goll SR, Nichols CE, Pollack SR. Prevention and treatment of sciatic denervation disuse osteoporosis in the rat tibia with capacitively coupled electrical stimulation. Bone. 1985;6(2):87–97.
25. McLeod KJ, Rubin CT. The effect of low-frequency electrical fields on osteogenesis. J Bone Joint Surg Ser A. 1992;74(6):920–9.
26. Rubin CT, McLeod KJ, Lanyon LE. Prevention of osteoporosis by pulsed electromagnetic fields. J Bone Joint Surg Ser A. 1989;71(3):411–7.
27. Boskey AL. Bone composition: Relationship to bone fragility and antiosteoporotic drug effects. Bonekey Rep. 2013;2:447.
28. Anderson JC, Eriksson C. Piezoelectric properties of dry and wet bone. Nature. 1970 Aug;227(5257):491–2.
29. Anderson JC, Eriksson C. Electrical properties of wet collagen. Nature. 1968;218:166–8.
30. Reinish GB, Nowick AS. Piezoelectric properties of bone as functions of moisture content. Nature. 1975 Feb;253(5493):626–7.
31. Konikoff JJ. Origin of the osseous bioelectric potentials: A review. Ann Clin Lab Sci. 1975;5:330–8.
32. Ahn AC, Grodzinsky AJ. Relevance of collagen piezoelectricity to "Wolff's Law": A critical review. Med Eng Phys. 2009;31(7):733–41.
33. Rubinacci A, Tessari L. A correlation analysis between bone formation rate and bioelectric potentials in rabbit tibia. Calcif Tissue Int. 1983 Dec;35(1):728–31.
34. Hung CT, Allen FD, Pollack SR, Brighton CT. What is the role of the convective current density in the real-time calcium response of cultured bone cells to fluid flow? J Biomech. 1996 Nov 1;29(11):1403–9.
35. Salzstein RA, Pollack SR, Mak AFT, Petrov N. Electromechanical potentials in cortical bone-I. A continuum approach. J Biomech. 1987 Jan;20(3):261–70.
36. Singh S, Saha S. Electrical properties of bone. A review. Clin Orthop Relat Res. 1984;166:249–71.
37. Albrektsson T. Osteoinduction, osteoconduction and osseointegration. Eur Spine J. 2001;10(2):96.
38. Becker RO. The bioelectric factors in amphibian-limb regeneration. J Bone Joint Surg Am. 1961;43A(5): 643–56.
39. Rubinacci A, Villa I, Dondi Benelli F, Borgo E, Ferretti M, Palumbo C, Marotti G. Osteocyte-bone lining cell system at the origin of steady ionic current in damaged amphibian bone. Calcif Tissue Int. 1998 Oct 1;63(4):331–9.
40. Bassett CAL, Pawluk RJ, Pilla AA. Augmentation of bone repair by inductively coupled electromagnetic fields. Science. 1974;184(4136):575–7.
41. Anisimov AI. Action of direct current on bone tissue. Bull Exp Biol Med. 1974 Sep;78(3):1069–71.
42. Brighton CT, Hunt RM. Ultrastructure of electrically induced osteogenesis in the rabbit medullary canal. J Orthop Res. 1986;4(1):27–36.
43. Shandler HS, Weinstein S, Nathan LE. Facilitated healing

of osseous lesions in the canine mandible after electrical stimulation. J Oral Surg. 1979 Nov;37(11):787–92.
44. Friedenberg ZB, Andrews ET, Smolenski BI, Pearl BW, Brighton CT. Bone reaction to varying amounts of direct current. Surg Gynecol Obstet. 1970 Nov;131(5):894–9.
45. Norton LA, Moore RR. Bone growth in organ culture modified by an electric field. J Dent Res. 1972 Sep 9;51(5):1492–9.
46. Brighton CT, Friedenberg ZB, Black J, Esterhai JL, Mitchell JEJ, Montique F. Electrically induced osteogenesis. Relationship between charge, current density, and the amount of bone formed: Introduction of a new cathode concept. Clin Orthop Relat Res. 1981;161:122–32.
47. Treharne RW, Brighton CT, Korostoff E, Pollack SR. An in vitro study of electrical osteogenesis using direct and pulsating currents. Clin Orthop Relat Res. 1979;145:300–6.
48. Noda M, Sato A. Appearance of osteoclasts and osteoblasts in electrically stimulated bones cultured on chorioallantoic membranes. Clin Orthop Relat Res. 1985 Mar 1;193:288–98.
49. Cochran GVB. Experimental methods for stimulation of bone healing by means of electrical energy. Bull NY Acad Med J Urban Heal. 1972 Aug;48(7):899–911.
50. Bassett CAL, Pawluk RJ, Becker RO. Effects of electric currents on bone in vivo. Nature. 1964 Nov 1;204(4959):652–4.
51. Ferrier J, Ross SM, Kanehisa J, Aubin JE. Osteoclasts and osteoblasts migrate in opposite directions in response to a constant electrical field. J Cell Physiol. 1986;129(3):283–8.
52. Marino AA. Direct current and bone growth. In: Marino AA, editor. Modern bioelectricity. 1st ed. New York, NY: Marcel Dekker, Inc.; 1988. p. 657–709.
53. Jee WS, Yao W. Overview: Animal models of osteopenia and osteoporosis. J Musculoskel Neuron Interact. 2001;1(3):193–207.
54. Lirani-Galvão APR, Chavassieux P, Portero-Muzy N, Bergamaschi CT, Silva OL, Carvalho AB, Lazaretti-Castro M, Delmas PD. Low-intensity electrical stimulation counteracts the effects of ovariectomy on bone tissue of rats: Effects on bone microarchitecture, viability of osteocytes, and nitric oxide expression. Calcif Tissue Int. 2009 Jun 21;84(6):502–9.
55. Korenstein R, Somjen D, Fischler H, Binderman I. Capacitative pulsed electric stimulation of bone cells. Induction of cyclic-AMP changes and DNA synthesis. Mol Cell Res. 1984 Apr;803(4):302–7.
56. Lewis KJ, Frikha-Benayed D, Louie J, Stephen S, Spray DC, Thi MM, Seref-Ferlengez Z, Majeska RJ, Weinbaum S, Schaffler MB. Osteocyte calcium signals encode strain magnitude and loading frequency in vivo. Proc Natl Acad Sci. 2017 Oct 31;114(44):11775–80.
57. Hung CT, Allen FD, Pollack SR, Brighton CT. What is the role of the convective current density in the real-time calcium response of cultured bone cells to fluid flow? J Biomech. 1996;29(11):1403–9.
58. Aaron RK, Boyan BD, Ciombor DM, Schwartz Z, Simon BJ. Stimulation of growth factor synthesis by electric and electromagnetic fields. Clin Orthop Relat Res. 2004;419:30–7.
59. Lohmann CH, Schwartz Z, Liu Y, Guerkov H, Dean DD, Simon B, Boyan BD. Pulsed electromagnetic field stimulation of MG63 osteoblast-like cells affects differentiation and local factor production. J Orthop Res. 2000 Jul;18(4):637–46.
60. Stein GS, Lian JB. Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. Endocr Rev. 1993;14(4):424–42.
61. Meng S, Rouabhia M, Zhang Z. Electrical stimulation modulates osteoblast proliferation and bone protein production through heparin-bioactivated conductive scaffolds. Bioelectromagnetics. 2013 Apr 1;34(3):189–99.
62. Hopper RA, Verhalen JP, Tepper OT, Mehrara BJ, Detch R, Chang EI, Baharestani S, Simon BJ, Gurtner GC. Osteoblasts stimulated with pulsed electromagnetic fields increase HUVEC proliferation via a VEGF-A independent mechanism. Bioelectromagnetics. 2009 Apr;30(3):189–97.
63. Griffin M, Sebastian A, Colthurst J, Bayat A. Enhancement of differentiation and mineralisation of osteoblast-like cells by degenerate electrical waveform in an in vitro electrical stimulation model compared to capacitive coupling. PLoS One. 2013 Sep 11;8(9):e72978.
64. Hartig M, Joos U, Wiesmann HP. Capacitively coupled electric fields accelerate proliferation of osteoblast-like primary cells and increase bone extracellular matrix formation in vitro. Eur Biophys J. 2000;29(7):499–506.
65. Bodamyali T, Bhatt B, Hughes FJ, Winrow VR, Kanczler JM, Simon B, Abbott J, Blake DR, Stevens CR. Pulsed electromagnetic fields simultaneously induce osteogenesis and upregulate transcription of bone morphogenetic proteins 2 and 4 in rat osteoblasts in vitro. Biochem Biophys Res Commun. 1998;250(2):458–61.
66. Bragdon B, Moseychuk O, Saldanha S, King D, Julian J, Nohe A. Bone morphogenetic proteins: A critical review. Cell Signal. 2011;23(4):609–20.
67. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med. 1999;5(6):623–8.
68. Peng H, Wright V, Usas A, Gearhart B, Shen H-C, Cummins J, Huard J. Synergistic enhancement of bone formation and healing by stem cell–expressed VEGF and bone morphogenetic protein-4. J Clin Invest. 2002;110(6):751–9.
69. Yakar S, Rosen CJ, Beamer WG, Ackert-Bicknell CL, Wu Y, Liu J-L, Ooi GT, Setser J, Frystyk J, Boisclair YR, LeRoith D. Circulating levels of IGF-1 directly regulate bone growth and density. J Clin Invest. 2002;110(6):771–81.
70. Chang WHS, Chen LT, Sun JS, Lin FH. Effect of

pulse-burst electromagnetic field stimulation on osteoblast cell activities. Bioelectromagnetics. 2004;25(6):457–65.
71. He Z, Selvamurugan N, Warshaw J, Partridge NC. Pulsed electromagnetic fields inhibit human osteoclast formation and gene expression via osteoblasts. Bone. 2018 Jan 1;106:194–203.
72. Jing D, Zhai M, Tong S, Xu F, Cai J, Shen G, Wu Y, Li X, Xie K, Liu J, Xu Q, Luo E. Pulsed electromagnetic fields promote osteogenesis and osseointegration of porous titanium implants in bone defect repair through a Wnt/β-catenin signaling-associated mechanism. Sci Rep. 2016 Aug 24;6(1):1–13.
73. Min Y, Liu Y, Poojari Y, Wu JC, Hildreth BE, Rosol TJ, Epstein AJ. Self-doped polyaniline-based interdigitated electrodes for electrical stimulation of osteoblast cell lines. Synth Met. 2014 Dec 1;198:308–13.
74. Esmail MY, Sun L, Yu L, Xu H, Shi L, Zhang J. Effects of PEMF and glucocorticoids on proliferation and differentiation of osteoblasts. Electromagn Biol Med. 2012 Dec 1;31(4):375–81.
75. Tian J, Shi R, Liu Z, Ouyang H, Yu M, Zhao C, Zou Y, Jiang D, Zhang J, Li Z. Self-powered implantable electrical stimulator for osteoblasts' proliferation and differentiation. Nano Energy. 2019 May 1;59:705–14.
76. Barnaba S, Papalia R, Ruzzini L, Sgambato A, Maffulli N, Denaro V. Effect of pulsed electromagnetic fields on human osteoblast cultures. Physiother Res Int. 2013 Jun 1;18(2):109–14.
77. Hiemer B, Krogull M, Bender T, Ziebart J, Krueger S, Bader R, Jonitz-Heincke A. Effect of electric stimulation on human chondrocytes and mesenchymal stem cells under normoxia and hypoxia. Mol Med Rep. 2018 Aug 1;18(2):2133–41.
78. Wang J, An Y, Li F, Li D, Jing D, Guo T, Luo E, Ma C. The effects of pulsed electromagnetic field on the functions of osteoblasts on implant surfaces with different topographies. Acta Biomater. 2014 Feb 1;10(2):975–85.
79. Matsunaga S. Histological and histochemical investigations of constant direct current stimulated intramedullary callus. J Japan Orthop Assoc. 1986 Dec;60(12):1293–303.
80. Brighton CT, Wang W, Seldes R, Zhang G, Pollack SR. Signal transduction in electrically stimulated bone cells. J Bone Joint Surg Ser A. 2001;83(10):1514–23.
81. Aarden EM, Nijweide PJ, Van Der Plas A, Alblas MJ, Mackie EJ, Horton MA, Helfrich MH. Adhesive properties of isolated chick osteocytes in vitro. Bone. 1996 Apr;18(4):305–13.
82. Palumbo C. A three-dimensional ultrastructural study of osteoid-osteocytes in the tibia of chick embryos. Cell Tissue Res. 1986 Oct;246(1):125–31.
83. Burger EH, Klein-Nulend J. Mechanotransduction in bone—Role of the lacunocanalicular network. FASEB J. 1999 May 1;13(9001):S101–12.
84. Knothe Tate ML, Adamson JR, Tami AE, Bauer TW. The osteocyte. Int J Biochem Cell Biol. 2004 Jan;36(1):1–8.
85. Lohmann CH, Schwartz Z, Liu Y, Li Z, Simon B, Sylvia VL, Dean DD, Bonewald LF, Donahue HJ, Boyan BD. Pulsed electromagnetic fields affect phenotype and connexin 43 protein expression in MLO-Y4 osteocyte-like cells and ROS 17/2.8 osteoblast-like cells. J Orthop Res. 2003 Mar;21(2):326–34.
86. Wang P, Tang C, Wu J, Yang Y, Yan Z, Liu X, Shao X, Zhai M, Gao J, Liang S, Luo E, Jing D. Pulsed electromagnetic fields regulate osteocyte apoptosis, RANKL/OPG expression, and its control of osteoclastogenesis depending on the presence of primary cilia. J Cell Physiol. 2019;234(7):10588–601.
87. Tanaka K, Matsuo T, Ohta M, Sato T, Tezuka K, Nijweide PJ, Katoh Y, Hakeda Y, Kumegawa M. Time-lapse microcinematography of osteocytes. Miner Electrolyte Metab. 1995;21(1-3):189–92.
88. Kramer I, Halleux C, Keller H, Pegurri M, Gooi JH, Weber PB, Feng JQ, Bonewald LF, Kneissel M. Osteocyte Wnt/β-catenin signaling is required for normal bone homeostasis. Mol Cell Biol. 2010;30(12):3071–85.
89. Bronckers ALJJ, Goei W, Luo G, Karsenty G, D'Souza RN, Lyaruu DM, Burger EH. DNA fragmentation during bone formation in neonatal rodents assessed by transferase-mediated end labeling. J Bone Miner Res. 1996 Dec 3;11(9):1281–91.
90. Chang K, Chang WHS, Huang S, Huang S, Shih C. Pulsed electromagnetic fields stimulation affects osteoclast formation by modulation of osteoprotegerin, RANK ligand and macrophage colony-stimulating factor. J Orthop Res. 2005 Nov;23(6):1308–14.
91. Chang K, Chang WHS, Tsai MT, Shih C. Pulsed electromagnetic fields accelerate apoptotic rate in osteoclasts. Conn Tissue Res. 2006 Jul;47(4):222–8.
92. Rubin J, McLeod KJ, Titus L, Nanes MS, Catherwood BD, Rubin CT. Formation of osteoclast-like cells is suppressed by low frequency, low intensity electric fields. J Orthop Res. 1996 Jan 1;14(1):7–15.
93. Dauben TJ, Ziebart J, Bender T, Zaatreh S, Kreikemeyer B, Bader R. A novel in vitro system for comparative analyses of bone cells and bacteria under electrical stimulation. Biomed Res Int. 2016;2016:5178640.
94. Brummer SB, Turner MJ. Electrical stimulation of the nervous system: The principle of safe charge injection with noble metal electrodes. Bioelectrochem Bioenerg. 1975;2(1):13–25.
95. Brummer SB, Robblee LS, Hambrecht FT. Criteria for selecting electrodes for electrical stimulation: Theortical and practical considerations. Ann NY Acad Sci. 1983 Jun 1;405(1):159–71.
96. Sandvik EL, McLeod BR, Parker AE, Stewart PS. Direct electric current treatment under physiologic saline conditions kills Staphylococcus epidermidis biofilms via electrolytic generation of hypochlorous acid. PLoS One. 2013 Feb 4;8(2):e55118.
97. Brighton CT, Adler S, Black J, Itada N, Friedenberg ZB.

Cathodic oxygen consumption and electrically induced osteogenesis. Clin Orthop Relat Res. 1975;107:277–82.
98. Wang Q, Zhong S, Ouyang J, Jiang L, Zhang Z, Xie Y, Luo S. Osteogenesis of electrically stimulated bone cells mediated in part by calcium ions. Clin Orthop Relat Res. 1998;(348):259–68.
99. Merrill DR, Bikson M, Jefferys JGR. Electrical stimulation of excitable tissue: Design of efficacious and safe protocols. J Neurosci Meth. 2005;41:171–98.
100. Bodamyali T, Kanczler JM, Simon B, Blake DR, Stevens CR. Effect of faradic products on direct current-stimulated calvarial organ culture calcium levels. Biochem Biophys Res Commun. 1999 Nov 2;264(3):657–61.
101. Srirussamee K, Mobini S, Cassidy NJ, Cartmell SH. Direct electrical stimulation enhances osteogenesis by inducing BMP2 and SPP1 expressions from macrophages and preosteoblasts. Biotechnol Bioeng. 2019;116(12):3421–32.
102. Cho M, Hunt TK, Hussain MZ. Hydrogen peroxide stimulates macrophage vascular endothelial growth factor release. Am J Physiol Heart Circ Physiol. 2001 May;280(5):H2357–63.
103. Ehrensberger MT, Tobias ME, Nodzo SR, Hansen LA, Luke-Marshall NR, Cole RF, Wild LM, Campagnari AA. Cathodic voltage-controlled electrical stimulation of titanium implants as treatment for methicillin-resistant Staphylococcus aureus periprosthetic infections. Biomaterials. 2014 Feb 1;41:97–105.
104. Asadi MR, Torkaman G. Bacterial inhibition by electrical stimulation. Adv Wound Care. 2014 Feb;3(2):91–7.
105. Kaysinger KK, Ramp WK. Extracellular pH modulates the activity of cultured human osteoblasts. J Cell Biochem. 1998;68(1):83–9.
106. Bushinsky DA. Metabolic alkalosis decreases bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts. Am J Physiol. 1996 Jul;271(1 Pt 2):F216–22.
107. Srirussamee K. Direct electrical stimulation approaches for bone repair and regeneration [dissertation]. Manchester, UK: University of Manchester; 2019.
108. Kumar A, Nune KC, Misra RDK. Electric field-mediated growth of osteoblasts: The significant impact of dynamic flow of medium. Biomater Sci. 2016 Jan 1;4(1):136–44.
109. Kumar A, Nune KC, Misra RDK. Understanding the response of pulsed electric field on osteoblast functions in three-dimensional mesh structures. J Biomater Appl. 2016 Oct 1;31(4):594–605.
110. Kodama HA, Amagai Y, Sude H, Kasai S, Yamamoto S. Establishment of a clonal osteogenic cell line from newborn mouse calvaria. Jpn J Oral Biol. 1981;23:899–901.
111. Kato Y, Windle JJ, Koop BA, Mundy GR, Bonewald LF. Establishment of an osteocyte-like cell line, MLO-Y4. J Bone Miner Res. 1997;12:2014–23.
112. Kato Y, Boskey AL, Spevak L, Dallas M, Hori M, Bonewald LF. Establishment of an osteoid preosteocyte-like cell MLO-A5 that spontaneously mineralizes in culture. J Bone Miner Res. 2001;16(9):1622–33.
113. Rodan SB, Imai Y, Thiede MA, Wesolowski G, Thompson D, Bar-Shavit Z, Shull S, Mann K, Rodan GA. Characterization of a human osteosarcoma cell line (Saos-2) with osteoblastic properties. Cancer Res. 1987;47:4961–6.
114. Kalajzic I. A new osteocytic cell line, raising new questions and opportunities. J Bone Miner Res. 2019;34:977–8.
115. Divieti Pajevic P. New and old osteocytic cell lines and 3D models. Curr Osteop Rep. 2020;18:551–8.
116. Qadan MA, Piuzzi NS, Boehm C, Bova W, Moos M, Midura RJ, Hascall VC, Malcuit C, Muschler GF. Variation in primary and culture-expanded cells derived from connective tissue progenitors in human bone marrow space, bone trabecular surface and adipose tissue. Cytotherapy. 2018 Mar 1;20(3):343–60.
117. Borsje MA, Ren Y, De Haan-Visser HW, Kuijerd R. Comparison of low-intensity pulsed ultrasound and pulsed electromagnetic field treatments on OPG and RANKL expression in human osteoblast-like cells. Angle Orthod. 2010 May 1;80(3):498–503.
118. He J, Zhang Y, Chen J, Zheng S, Huang H, Dong X. Effects of pulsed electromagnetic fields on the expression of NFATc1 and CAII in mouse osteoclast-like cells. Aging Clin Exp Res. 2015 Feb 1;27(1):13–9.
119. Bins-Ely LM, Cordero EB, Souza JCM, Teughels W, Benfatti CAM, Magini RS. In vivo electrical application on titanium implants stimulating bone formation. J Periodont Res. 2017 Jun 1;52(3):479–84.
120. Portan DV, Deligianni DD, Papanicolaou GC, Kostopoulos V, Psarras GC, Tyllianakis M. Combined optimized effect of a highly self-organized nanosubstrate and an electric field on osteoblast bone cells activity. Biomed Res Int. 2019;2019:7574635.
121. Ribeiro C, Moreira S, Correia V, Sencadas V, Rocha JG, Gama FM, Gómez Ribelles JL, Lanceros-Méndez S. Enhanced proliferation of pre-osteoblastic cells by dynamic piezoelectric stimulation. RSC Adv. 2012 Nov 28;2(30):11504–9.
122. Masureik C, Eriksson C. Preliminary clinical evaluation of the effect of small electrical currents on the healing of jaw fractures. Clin Orthop Relat Res. 1977 May 1;4:84–91.
123. Brighton CT, Black J, Friedenberg ZB, Esterhai JL, Day LJ, Connolly JF. A multicenter study of the treatment of non-union with constant direct current. J Bone Joint Surg Ser A. 1981;63(1):2–13.
124. Mendonça JS, Neves LMG, Esquisatto MAM, Mendonça FAS, Santos GMT. Comparative study of the application of microcurrent and AsGa 904 nm laser radiation in the process of repair after calvaria bone excision in rats. Laser Phys. 2013;23(3):035605.
125. France JC, Norman TL, Santrock RD, McGrath B, Simon BJ. The efficacy of direct current stimulation for lumbar intertransverse process fusions in an animal model. Spine (Phila, PA 1976). 2001;26(9):1002–7.
126. Fredericks DC, Smucker J, Petersen EB, Bobst JA, Gan JC, Simon BJ, Glazer P. Effects of direct current electrical

stimulation on gene expression of osteopromotive factors in a posterolateral spinal fusion model. Spine (Phila, PA 1976). 2007 Jan;32(2):174–81.
127. Friedenberg ZB, Zemsky LM, Pollis RP, Brighton CT. The response of non-traumatized bone to direct current. J Bone Joint Surg Ser A. 1974;56(5):1023–30.
128. Fonseca JH, Bagne L, Meneghetti DH, dos Santos GMT, Esquisatto MAM, de Andrade TAM, do Amaral MEC, Felonato M, Caetano GF, Santamaria M, Mendonça FAS. Electrical stimulation: Complementary therapy to improve the performance of grafts in bone defects? J Biomed Mater Res B Appl Biomater. 2019 May 28;107(4):924–32.
129. Zhang J, Li M, Kang ET, Neoh KG. Electrical stimulation of adipose-derived mesenchymal stem cells in conductive scaffolds and the roles of voltage-gated ion channels. Acta Biomater. 2016 Mar 1;32:46–56.
130. Eischen-Loges M, Oliveira KMC, Bhavsar MB, Barker JH, Leppik L. Pretreating mesenchymal stem cells with electrical stimulation causes sustained long-lasting pro-osteogenic effects. Peer J. 2018 Jun 11;6:e4959.
131. Zhao Z, Watt C, Karystinou A, Roelofs AJ, McCaig CD, Gibson IR, De Bari C. Directed migration of human bone marrow mesenchymal stem cells in a physiological direct current electric field. Eur Cells Mater. 2011;22(0):344–58.
132. Wang Z, Clark CC, Brighton CT. Up-regulation of bone morphogenetic proteins in cultured murine bone cells with use of specific electric fields. J Bone Joint Surg Ser A. 2006;88(5):1053–65.
133. Qi Z, Xia P, Pan S, Zheng S, Fu C, Chang Y, Ma Y, Wang J, Yang X. Combined treatment with electrical stimulation and insulin-like growth factor-1 promotes bone regeneration in vitro. PLoS One. 2018 May 10;13(5):e0197006.
134. Zhang J, Neoh KG, Hu X, Kang ET, Wang W. Combined effects of direct current stimulation and immobilized BMP-2 for enhancement of osteogenesis. Biotechnol Bioeng. 2013 May 1;110(5):1466–75.
135. Tong J, Sun L, Zhu B, Fan Y, Ma X, Yu L, Zhang J. Pulsed electromagnetic fields promote the proliferation and differentiation of osteoblasts by reinforcing intracellular calcium transients. Bioelectromagnetics. 2017 Oct 1;38(7):541–9.
136. Gittens RA, Olivares-Navarrete R, Rettew R, Butera RJ, Alamgir FM, Boyan BD, Schwartz Z. Electrical polarization of titanium surfaces for the enhancement of osteoblast differentiation. Bioelectromagnetics. 2013 Dec 1;34(8):599–612.
137. Konstantinou E, Zagoriti Z, Pyriochou A, Poulas K. Microcurrent stimulation triggers MAPK signaling and TGF-β1 release in fibroblast and osteoblast-like cell lines. Cells. 2020 Aug 19;9(9):1924.
138. Ercan B, Webster TJ. The effect of biphasic electrical stimulation on osteoblast function at anodized nanotubular titanium surfaces. Biomaterials. 2010 May 1;31(13):3684–93.