Some Electrical Stimulation Methods for Articular Cartilage Regeneration

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Abstract. Bioelectrical signals can regulate a wide range of cellular activities in living organisms, including division, differentiation, etc. The physiological properties of stem cell cells are regulated by artificial input of electrical stimuli, including electric and electromagnetic fields, to promote cartilage repair by inducing stem cell cellular differentiation toward cartilage. Electrical stimulation (ES) stimulates cartilage regeneration at the cellular level through three mechanisms: intricate interactions of the physical environment, growth factors (GFs), and signal transduction cascades. The relevant ways in which bioelectrical stimulation regulates cellular function are the subject of this review. The non-invasive nature of this and the fact that it is not dependent on exogenous growth factors offer great promise for the clinical application of ES. However, the precise mechanisms underlying how ES interacts with cells are not well understood and need a lot more investigation. Similarly, there is a certain variability in the means and parameters of ES in in vivo and in vitro experiments, which poses a great challenge for clinical applications. Here some feasible means of ES and specific ES parameters to provide ideas for subsequent clinical applications are reviewed.

Keywords: Electrical Stimulation, Articular Cartilage Regeneration, Chondrogenic Differentiation.

1. Introduction

Joints are specific professional organs that enable fluid movement as well as recurring discomfort. Under normal settings, the articular cartilage and its extracellular matrix (ECM) can endure high circulation stresses. Articular cartilage consists of a layer of the joint and is principally responsible for its unique biomechanical properties. Articular cartilage is a kind of hyaline cartilage composed of chondrocytes and an ECM that includes type II collagen, proteoglycans, and other matrix proteins. Depending on the form and arrangement of the chondrocytes and the direction of the collagen fibers, the articular hyaline cartilage is divided into four zones: the calcified zone, the intermediate zone, the superficial zone, and the transitional zone [1].

Articular cartilage has problems to regenerate autonomously in vivo for cartilage lesions cannot reach subchondral bone and are consequently inaccessible to blood supply and bone marrow mesenchymal stem cells (MSCs). Under the continuous DC current and electric potential, which encourages cell migration to the site of damage, the bioelectric field produced by the body plays a critical role in wound healing. Moreover, a voltage gradient of -10 to -90 mV, known as the "action potential," may drive altered proliferation and differentiation of distinct cell types through signaling across the cell membrane. Researchers have been interested in the possibility of employing electrical fields (EF) in cells to accelerate the development and differentiation of biological systems. It is well-known that tissue regeneration starts with cell growth and proliferation [2].

Electrotherapeutics seems to be a safe and successful therapy for collagen-containing tissues in the majority of instances among the therapeutic techniques utilized for cartilage restoration [3].

In spite of this, electrotherapeutic applications are difficult since the specific mechanism of action is unclear, resulting in an almost infinite number of stimulation parameter combinations (e.g., waveform type, voltage, current, phase, frequency, etc.) [3]. Electrical current stimuli have considerable physical changes on cartilage defects, according to Snyder et al. and Aaron et al. discovered that electro - magnetic stimuli encourages the expression of TGF-β family genes [3,4]. In addition, an increase in chondrocyte proliferative ability and extracellular matrix formation has been described [5]. It has been demonstrated that chondrocytes are very sensitive to ES. Specifically,

electric currents may hasten the repair of substantial cartilage defects in adult animals [5]. Moreover, cell culture experiments have established a clear relationship between electromagnetic fields and the enhanced creation of extracellular matrix molecules [6]. In addition, microcurrent on tissue healing stimulates ATP production as well as nutrient transport intracellularly [4].

Electrical currents produce stimuli, increase the amount of TGF-β receptors as well as collagen fibers, which includes establishing the bioelectrical equilibrium of cartilage at the damage sites [3]. In addition, the placement of the electrodes during stimulus has the effect of altering the machinery that the cell uses to produce proteins as well as the migration of various cell types to the area where they were injured [7].

understanding how biophysical stimuli affect the biology of hyaline cartilage not only provides helpful ideas on the influences of these stimuli on cartilage physiology in vivo, it also provides novel resources for tissue engineering and regenerative medicine, both of which are centered on the creation of novel therapies for the injury treatment in articular cartilage and growth plate.

In this paper, the methodologies of ES and the active signaling pathways are described in order to highlight the possibility of more extensive future uses of ES for cartilage regeneration.

2. Typical ES methods

Direct current (DC), capacitive coupling (CC), electromagnetic field (EMF), inductive coupling (IC), and pulsed electromagnetic fields (PEMF) are some of the ES techniques that are used in in in vitro and in vivo studies to stimulate cartilage repair.

3. In vivo studies

Osteoarthritis (OA) is a widespread, age-related, diverse group of illnesses which can be characterized pathologically according to discrete regions of articular cartilage loss in synovial joints. This loss of cartilage is followed by different degrees of osteophyte development, subchondral bone alteration, and synovitis. Joint cartilage in good health distributes both static and dynamic stresses and decreases friction. Sparsely dispersed cartilage cells are responsible for the upkeep of a cartilage matrix that is abundant in collagen and proteoglycans. It is very necessary for such cartilage to have a matrix of excellent quality in order for it to maintain its functional qualities [8]. Therefore, there is an urgent need for a novel clinical instrument capable of treating cartilage damage.

Bioelectricity plays a vital function in the healing process. A direct current (DC) EF is engaged once a wound is sustained. This endogenous EF is responsible for directing cell migration to the edge of the wound. In contrast, when EF is inhibited, wound healing is impeded [9]. In prior experiments, three weeks of continuous femur electrification around the cathode in rabbits resulted in new bone growth [10]. Using bimetallic devices in rats to generate a potential difference of roughly 70 mV and an estimated amperage of 6 nA in vitro and in vivo, 71% of the experimental specimens had indications of hyaline cartilage sprouting from the residual articular cartilage rim, with as much as 0.5-1mm of advancement at 3 weeks [11]. These findings cleared the path for the use of ES in osteoarthritis patients [12]. DC ES, rather than encouraging the formation of scar tissue, prompted a regenerative response, which resulted in the growth of new bone, cartilage, and blood vessels [13]. Low-voltage DC stimulation of amputated rat forelimb stumps significantly increased vascularization. In a second study, ES generated changes in extracellular matrix deposition, with collagen fibrils that were less thick, and changed macrophage reactivity by shifting the ratio of M1 to M2 macrophages. An increment of proliferating cells was observed in ES-treated stumps 7 days after amputation. Transcriptome analysis significantly validated the histology results, which played a crucial role in embryogenesis and regeneration [14].

Aaron et al. employed EMF to perform tests with a similar focus [15]. The magnetic field applied was a pulse-burst of 4.5ms duration repeated at 15 bursts/s with a maximum magnetic field of 16 G. Compared to control osicles, EMF exposure increased the amount of TGFP protein by 32%. The

mRNA levels of EMF-exposed oocytes were 2.5 times higher than those of the control group. Compared to control ossicles, EMF-exposed ossicles had a 23 percent increase in active protein deposition in the extracellular matrix and a 3.5-fold increase in immunopositive cells. It shown that EMF at a dosage that stimulates chondrogenesis and endochondral bone formation may create persistent low levels of TGFP.

Microcurrent stimulation, according to Zuzzi et al., can accelerate healing processes of non-articular hyaline cartilage [16]. In the research, 3-mm punches were used to produce full-thickness, cylindrical cartilage lesions in animals under anesthesia. After 24 hours, the treatment group received daily 5-minute administrations of a continuous electric current for 1 Hz/20 A. Compared to the control group, the overall number of cells from connective tissue in the defect area significantly grew in 21–35 days in the treatment group. At all times, the treatment group had larger fraction of volume covered by birefringent collagen fibers.

4. In vitro studies

Armstrong et al. showed in early studies that time-varying EF delivered to growth plate chondrocyte pellets may improve cell uptake and proteoglycan production (evaluated by [35S] sulfate absorption) [17]. Different capacitively linked EFs were used to cultivate pellets made of isolated bovine growth plate chondrocytes. The selected signals of peak-to-peak (P-P) voltages were 0, 10, 100, 250, 500, 750, 1000, and 1500 V at 60 kHz. The effects of these varied voltages on cell proliferation and matrix formation were evaluated with the use of [3H] thymidine and [3SS] sulfate uptake, respectively. The levels of cyclic AMP were measured before and after the increases in thymidine or sulfate absorption to see whether or not there was a correlation between the two. There was a discernible increase in the rate of cell growth when the P-P voltage was increased to 500V, 750V, and 1000 V. The EFs fell somewhere in the region of 1.5 to 3.0 x 10-2V/cm. There was a significant statistically significant increase of 66% in absorption when compared to the control results when the field was determined to be 4.5 x10-2 V/cm. The peak-to-peak voltage was also 1,500 V. In addition, Brighton et al. reported experiments of growth plate chondrocytes from newborn calf exposed to an AC signal of 500 V P-P at 60 kHz for two days [18]. Compared to non-stimulated controls, stimulated chondrocytes exhibited a 130% increase in thymidine uptake. Other newborn calf growth plate chondrocytes were treated with 500 V P-P at 60 kHz for 2.5, 5.0, 10.0, and 20.0 minutes, and CAMP levels were measured. Chondrocytes activated for 2.5 and 5.0 minutes exhibited a 142.8% and 394.5% increment, respectively, above the control group. They concluded that the little proliferation in cAMP in growth plate chondrocytes as a result of the capacitively coupled signal may indicate that the electrical signal was sub-optimal and that a more effective signal would result in a more significant increase in CAMP, or that CAMP was either an extreme weak secondary messenger created in response to the applied signal, or that it even was not one.

The aforementioned preliminary investigations give the fundamental research concepts for future in vitro ES of cartilage regeneration.

Krueger et al. have created a device that, once programmed, can function as an automated ES system [19]. It was primarily composed of software, a control and signaling module as well as a sample carrier (cell chamber) with electrodes. The stimulation device offers twelve independently programmable pulses inside twelve wells. The stimulation system may be implemented in many ways thanks to the modular architecture. It is possible to generate peak-to-peak voltages of up to 42 V and frequencies of up to 2 MHz. The average of the calculated values for all signal generators yielded a relative output voltage variation of 3% from the setpoint. Based on the capacitive coupling of the alternating EF, field strengths drop from the electrodes to the center of the well and from the electrode interface to the surface of the medium. In addition, this electrode design may simulate the distribution of physiological EFs in cartilage tissue, since the distribution of cartilage matrix components is likewise heterogeneous. Initial findings by Krueger et al. indicate that the applied EFs result in a frequency-dependent decrease in collagen type I protein production in human dedifferentiated

chondrocytes [19]. To better comprehend the signaling pathways involved in cartilage regeneration in response to ES, more research was done.

According to the findings of Hiemer and colleagues, the presence of ES led to an increment of mRNA encoding collagen type II and aggrecan in human chondrocytes grown in hypoxic settings [20]. Using titanium electrodes and an alternating EF, the cells were subjected to a kind of ES that lasted for almost seven days (700 mV, 1 kHz). Compared with the understimulated control ($P = 0.0188$) and the ES control ($P = 0.04$), the exposure of human chondrocytes to an EF under hypoxia resulted in a 1.5-fold proliferation in the release of collagen type II.

Kwon et al. found that an EF strength of 5 V/cm caused progressive aggregation of MSCs to convert to a compact structure after three days [21]. This result was found to be similar to chondrogenic media effect containing GFs like TGF-s and insulin. Following treatment for three days, ES dramatically increased the expression of genes of chondrogenic markers, as shown by a 66-, 43- and 35-fold rise in COL2A1, AGC and SOX9 expression, respectively. These results suggest that ES can induce MSCs to differentiate into hyaline chondrogenic cells and that ES treated MSCs are able to regenerate hyaline cartilage even without additional chemical agents. Hyaline cartilage is a type of cartilage that is found in the hyaline layer of the articular cartilage. ES causes Ca2+/ATP oscillations, which, for the first time, results in MSC chondrogenesis without supplementation of exogenous cytokines or GFs. Additionally, successful electrical stimulation regimens for producing MSC chondrogenesis are presented as a consequence of this discovery. P2X4 signaling has now been shown to promote ATP oscillation and chondrogenesis driven by electrical stimuli. In addition, TGF- and BMP signaling have both been shown to increase ES-driven chondrogenesis, but each of these signals has a unique effect on ES-driven condensation. These findings were recently published in the journal Cell Reports.

Chang et al. describe a remarkable method for producing chondrocytes for cartilage regeneration utilizing canine adipose-derived mesenchymal stem cells (ADSCs) [22]. ADSCs from canines were induced to generate very compact prechondrogenic condensation in the absence of smaller-sized aggregates by stimulation of 10 V/cm for varying durations at 2 Hz in their academics. ES induces intracellular Ca2+ oscillation in ADSCs even without an external stimulus at the micromass aggregation stage, which ultimately results in prechondrogenic condensation. ES produced condensation of canine ADSCs resulted in a phenotype equivalent to the embryonic phase of chondroblasts, hence impacting cartilage regeneration. According to the research presented in the next section, ES may stimulate direct prechondrogenic cells and have a role in the healing of hyaline cartilage. This would be accomplished by activating cartilage matrix and plasticity of chondroprogenitor cells. On human cells, comparable experiments have been undertaken. The employment of 1 kHz, 20 mV/cm capacitively linked EF for 20 minutes induced chondrogenesis in ADSCs, whereas low frequency electric field stimulated chondrogenesis and at the same time also partially reduced type I and type X collagen production [23].

Lee et al. found that human dermal fibroblasts (HDF) may be changed directly to hyaline chondrogenic cells under ES [24]. ES induces condensation of HDFs and rises levels of chondrogenic marker expression, such as type II collagen, aggrecan, and Sox9, while simultaneously reducing expression levels of type I collagen when applied at 5 V/cm and 10 Hz of varied length. This occurs without exogenous GFs or gene transduction. It was shown that embryonic stem cells can reprogramme HDFs straight into hyaline chondrogenic cells by dramatically raising chondrogenic marker levels while significantly reducing fibroblastic marker expression in HDFs. These findings are based on what is described in the following paragraphs.

Vaca-González et al. used computational analysis to obtain cell culture medium dielectric constants and experimental geometric configurations using a finite element approach to the experimental setup [25]. An EF of 4 mV/cm administered for 30 minutes enhanced the number of cells. Chondrocyte glycosaminoglycan production remained steady after 5 h of exposure to an 8 mV/cm EF, with no notable changes in cell population or molecular synthesis after 1 h. This method may be used to improve the proliferative potential of in vitro-cultured chondrocytes or to drive

molecular synthesis. Additionally, it provides a novel way to assess EFS and control proliferation, death of cells, and glycosaminoglycan creation.

5. Conclusion

Various types of ES, including but not limited to DC, CC, EMF, and IC or PEMF, have been tested in past research. These methods provide a variety of therapy strategies for cartilage damage. In contemporary experimental circumstances, PEMF and other low voltage conditions are the primary ES techniques (ranging from 700mV to 10V). This is mostly due to the fact that higher voltages cause cultivated cells to become less active or die. However, it should be noted that the design of the ES parameters in each experiment was insufficiently rigorous, and that some experiments were unable to cover all parameters of energization time, current frequency, and current intensity, while the comparison of data under multiple ES conditions was not examined in each experiment. However, there is clear doubt that ES in animals may greatly enhance exponential expression of cartilage gene markers and activate associated signaling pathways. This allows the cultivation of chondrocytes without the use of external growth agents. However, the particular regulatory processes have yet to be investigated.

References

[1] T. Aigner and J. Stöve, "Collagens - Major component of the physiological cartilage matrix, major target of cartilage degeneration, major tool in cartilage repair," *Adv Drug Deliv Rev*, vol. 55, no. 12, pp. 1569 – 1593, Nov. 2003.

[2] T. Jiang, E. J. Carbone, K. W. H. Lo, and C. T. Laurencin, "Electrospinning of polymer nanofibers for tissue regeneration," *Progress in Polymer Science*, vol. 46. Elsevier Ltd, pp. 1 – 24, Jul. 01, 2015.

[3] M. J. Snyder, J. A. Wilensky, and J. D. Fortin, "Current applications of electrotherapeutics in collagen healing.," *Pain Physician*, vol. 5, no. 2, pp. 172 – 181, 2002.

[4] R. K. Aaron, B. D. Boyan, D. M. Ciombor, *et al.*, "Stimulation of Growth Factor Synthesis by Electric and Electromagnetic Fields," *Clin Orthop*, vol. 419, pp. 30 – 37, 2004.

[5] W. Wang, Z. Wang, G. Zhang, *et al.*, "Up-regulation of chondrocyte matrix genes and products by electric fields," in *Clinical Orthopaedics and Related Research*, 2004, vol. 427, no. SUPPL.

[6] M. de Mattei *et al.*, "Effects of Pulsed Electromagnetic Fields on Human Articular Chondrocyte Proliferation."

[7] B. Car, T. Brighton, W. Wang, and C. C. Clark, "The Effect of Electrical Fields on Gene and Protein Expression in Human Osteoarthritic Cartilage Expiants," 2008.

[8] Paul A Dieppe and L Stefan Lohmander, "Pathogenesis and management of pain in osteoarthritis," 2005. [Online]. Available: www.thelancet.com.

[9] B. Song, M. Zhao, J. v Forrester, and C. D. Mccaig, "Electrical cues regulate the orientation and frequency of cell division and the rate of wound healing in vivo." [Online]. Available: www.pnas.orgcgidoi10.1073pnas.202235299.

[10] J. I. Ryaby, "Clinical Orthopaedics and Related Research Number 355S, pp S205-S215 Clinical Effects of Electromagnetic and Electric Fields on Fracture Healing."

[11] B. Baker, J. Spadaro, A. Marino, and R. O. Becker, "Electrical Stimulation of Articular Cartilage Regeneration," *Ann N Y Acad Sci*, vol. 238, no. 1, pp. 491 – 499, 1974.

[12] R. E. Fary, G. J. Carroll, T. G. Briffa, *et al.*, "The effectiveness of pulsed electrical stimulation (E-PES) in the management of osteoarthritis of the knee: A protocol for a randomised controlled trial," *BMC Musculoskelet Disord*, vol. 9, 2008.

[13] L. P. Leppik *et al.*, "Effects of electrical stimulation on rat limb regeneration, a new look at an old model," *Sci Rep*, vol. 5, Dec. 2015.

[14] K. M. C. Oliveira *et al.*, "Electrical stimulation shifts healing/scarring towards regeneration in a rat limb amputation model," *Sci Rep*, vol. 9, no. 1, Dec. 2019.

[15] R. K. Aaron, S. Wang, and D. M. K. Ciombor, "Upregulation of basal TGFβ1 levels by EMF coincident with chondrogenesis - Implications for skeletal repair and tissue engineering," *Journal of Orthopaedic Research*, vol. 20, no. 2, pp. 233 – 240, 2002.

[16] D. C. Zuzzi, C. de C. Ciccone, L. M. G. Neves, *et al.*, "Evaluation of the effects of electrical stimulation on cartilage repair in adult male rats," *Tissue Cell*, vol. 45, no. 4, pp. 275 – 281, 2013.

[17] P. F. Armstrong, C. T. Brighton, and A. M. Star, "Capacitively Coupled Electrical Stimulation of Bovine Growth Plate Chondrocytes Grown in Pellet Form," Raven Press.

[18] C. T. Brighton and P. F. Townsend, "Increased cAMP Production After Short-Term Capacitively Coupled Stimulation in Bovine Growth Plate Chondrocytes," Orthopaedic Research Society, 1988.

[19] S. Krueger *et al.*, "Establishment of a New Device for Electrical Stimulation of Non-Degenerative Cartilage Cells In Vitro Establishment of a New Device for Electrical Stimulation of Non-Degenerative Cartilage Cells In," *International Journal of Molecular Sciences Article Vitro. Int. J. Mol. Sci*, vol. 22, p. 394, 2021.

[20] B. Hiemer *et al.*, "Effect of electric stimulation on human chondrocytes and mesenchymal stem cells under normoxia and hypoxia," *Mol Med Rep*, vol. 18, no. 2, pp. 2133 – 2141, Aug. 2018.

[21] H. J. Kwon, G. S. Lee, and H. Chun, "Electrical stimulation drives chondrogenesis of mesenchymal stem cells in the absence of exogenous growth factors," *Sci Rep*, vol. 6, Dec. 2016.

[22] C. Y. Chang *et al.*, "Novel method to repair articular cartilage by direct reprograming of prechondrogenic mesenchymal stem cells," *Eur J Pharmacol*, vol. 911, Nov. 2021.

[23] M. Mardani, S. Roshankhah, B. Hashemibeni, *et al.*, "Induction of chondrogenic differentiation of human adipose-derived stem cells by low frequency electric field," *Adv Biomed Res*, vol. 5, no. 1, p. 97, 2016.

[24] G. S. Lee, M. G. Kim, and H. J. Kwon, "Electrical stimulation induces direct reprogramming of human dermal fibroblasts into hyaline chondrogenic cells," *Biochem Biophys Res Commun*, vol. 513, no. 4, pp. 990 – 996.

[25] J. J. Vaca-González, J. M. Guevara, J. F. Vega, *et al.*, "An In Vitro Chondrocyte Electrical Stimulation Framework: A Methodology to Calculate Electric Fields and Modulate Proliferation, Cell Death and Glycosaminoglycan Synthesis," *Cell Mol Bioeng*, vol. 9, no. 1, pp. 116 – 126.