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Review

The use of electrical stimulation to induce cardiac differentiation of stem cells for the treatment of myocardial infarction

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Ischemic heart disease resulting from a myocardial infarction (MI), is a major health issue. Stem cell therapies may play an important role in this field. However, cardiomyocytes induced from stem cells are characterized by low rates of differentiation and immaturity. After transplantation into the damaged heart, they may even increase the risk of arrhythmias. Studies have demonstrated that electrical stimulation (ES) can promote the cardiac differentiation of stem cells. This review summarizes the latest research on the effects of applying different electrical stimulation (ES) parameters to different types of stem cells and the related mechanisms that may be involved.

Keywords

Electrical stimulation; Stem cells; Myocardial infarction; Cardiac differentiation

1. Introduction

Cardiovascular disease is a major world-wide health concern [1]. When an acute myocardial infarction (MI) occurs, the number of viable cardiomyocytes will decrease. The formation of fibrous scar tissue and the remodeling of ventricular tissue after a myocardial infarction, results in myocardial dilatation, aneurysm formation and will eventually lead to heart failure. Currently, there is no treatment that can reverse all the damage in the infarcted myocardium to improve heart function. Therefore, there is a need for new treatment methods to improve cardiomyocyte viability to preserve heart function. Transplanting cardiomyocytes derived from stem cells into an area of myocardial infarction to repair the damaged myocardium and restore heart function is a promising treatment for myocardial infarction [2, 3].

Cardiomyocytes (CM) derived from stem cells are characterized by low rates of differentiation and immaturity. Following transplantation into a damaged heart, they may further increase the risk of arrhythmias [4, 5]. During myocardial contraction, pacemaker cells send out rhythmic electrical signals from the sinoatrial node, which are transmitted to the cardiomyocytes through the cell gap junctions, and then transmitted to other areas of the heart through the highly conductive Purkinje fibers. During this process, electrical signals and gap connections are vital to the heart's synchronized contractions [6]. In recent years, electrical stimulation (ES) has received widespread attention due safety, convenience, and the avoidance of drugs. Therefore, it has become a promising method to induce stem cell cardiac differentiation [7, 8], which will further promote the clinical application of cardiac stem cells in the treatment of myocardial infarction.

2. Factors affecting the differentiation of stem cells by exogenous electrical stimulation

Cardiomyocytes form gap junctions through the intercalated disk, which is conducive to the conduction of electrical stimuli between the cells and the rhythmic contraction in accordance with electrical signals [9]. In order to promote the cardiac differentiation of stem cells, it is important to optimize the parameters of the applied electric stimulation when applying ES to simulate cardiac electrophysiological signals [10].

2.1 Pulse direction

Pulse direction can be divided into monophase pulse and biphase pulse. Although monophase pulse can effectively stimulate cardiomyocytes to produce action potentials, it can produce reactive oxygen species which can result in tissue damage. Biphasic pulses can effectively prevent tissue damage, but the hyperpolarization of the biphasic pulses generated may suppress action potential initiation [11].

Sven *et al.* [12] applied monophasic pulses (amplitude: 1 V, pulse duration: 5 ms) at a stimulation frequency of 10 Hz to fetal rat cardiomyocytes for 6 consecutive days. ES promoted cell morphological elongation and parallel arrangement as well as the expression of connexin 43 (Cx43). Pietronave *et al.* [13] applied monophasic (2 ms, 1 Hz, 5 V amplitude) or biphasic (2 ms, 1 Hz, $\pm$ 2.5 V) square-wave pulses to human cardiac progenitor cells (hCPCs). They found that the

biphasic ES observably induced the upregulation of early cardiac transcription factors, including Myocyte Enhancer Factor 2D (MEF2D), GATA-binding protein 4 (GATA-4), and Nirenberg, Kim gene 2 homeobox 5 (Nkx2.5), in addition, it also promoted the de novo expression of the late cardiac sarcomeric proteins, troponin T, cardiac alpha actinin, and sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA 2a). Both treatments increased the expression of Cx43 and its relocation to the cell membrane, but biphasic ES produced faster and more effective results. In summary, biphasic pulse ES may be more beneficial to the differentiation process of stem cells in the myocardium.

2.2 Electric field intensity and duration

For the same electric field strength, the cell apoptosis rate is higher when the pulse duration is longer [14]. Therefore, the optimal combination of intensity and duration is crucial.

In order to explore the effect of the duration of ES on the myocardial differentiation of human cardiosphere-derived cells (hCDCs), Nazari *et al.* [15] applied a charge balance biphasic pulse (I = 2 mA, V = 150 mV, f = 1 Hz) to hCDCs for 1, 3, and 5 days respectively, ES for 5 min and 10 min every day. The study found that the expression of cardiac troponin T (cTnT) and GATA4 was the highest in the 5 d and 10 min group, but the expression of α-Myosin Heavy Chain (α-MHC) decreased. Ma *et al.* [16] applied 1 V/cm, 5 Hz parameters to human induced pluripotent stem cells (hiPSCs) for 30 days after applying ES, and found that the expression of cardiogenic genes was upregulated, the functional maturation of hiPSC-derived CMs (hiPSC-CMs) was facilitated, and the cardiac functions of ischemic heart was improved when the hiPSC-CMs were injected into the infarcted myocardium.

2.3 Electrical stimulation initiation

The impact of ES depends on the degree of differentiation of the cells during the first ES. If applied too early, ES will inhibit the accumulation of myocardial protein and produce undesirable contraction behavior. If it is applied too late, ES no longer contributes to the functional development of cardiomyocytes.

Radisic *et al.* [17] applied ES to neonatal rat ventricular myocytes of 1, 3, and 5 days. After the 1 d-old ventricular myocytes are electrically stimulated, the contents of Cx43 and α-MHC decrease, resulting in low-differentiated monolayer cardiomyocytes, which cannot produce synchronous contraction. In contrast, the 5 d-old ventricular myocytes only establish gap links after being electrically stimulated, and have no effective contractile function. Finally, it was found that the ventricular myocytes of 3 d-old neonatal rats had better contractility and gap linkage after ES. Ronaldson-Bouchard *et al.* [18] applied ES to human cardiac tissues grown from early-stage human iPSC-derived cardiomyocytes (day 12, immediately following the first spontaneous contractions) or late-stage hiPSC-CMs (day 28, matured in culture), and found that the early-stage hiPSC-CMs show obvious plasticity, which indicates that the responsiveness of hiPSC-CMs to ES declines as differentiation progresses. Chen *et al.* [19] applied ES to embryoid bodies of 4 d, 7 d, and 12 d, which corresponded to the three stages of stem cell differentiation in the early, middle, and end stages. The ES parameters were the same except that the current intensity is 10, 30, 60 μA. Low amplitude ES has little effect on stem cells in the early stage of differentiation, but under the highest amplitude of 60 μA ES, their β-Myosin Heavy Chain (β-MHC) levels increase observably. For stem cells in the intermediate differentiation stage, the expression of β-MHC and troponin-T increased when stimulated by 30 μA, but high amplitude stimulation at 60 μA was not conductive to the cardiac marker expression. In the terminal stage, the troponin-T levels increased as the stimulus amplitude increased. This demonstrates that embryonic stem cells respond differently to ES at different differentiation stages, and that the effects of ES depended strongly on the time of its initiation.

2.4 Electrical stimulation frequency

The frequency of ES can affect the contraction and contractile behavior of cardiomyocytes. ES with a frequency of 1–2 Hz can induce a transient surge of intracellular Ca^{2+} levels, and at the same time promote sarcomere development and maturation [7]. There is evidence [8] that the heart rate of hiPSC-CMs changes with the frequency of ES. Even after ES is stopped, the heart rate of hiPSC-CMs can be maintained and continue to conduct to the surrounding cells. Therefore, in the process of differentiation or maturation, because hiPSC-CMs have electrophysiological plasticity, it is easy to generate cardiomyocyte-like cells with a heart rate different from that of the cardiomyocytes surrounding the transplanted myocardium, which may lead to arrhythmias. Therefore, the frequency of ES should be optimized.

Tandon *et al.* [20] applied monophasic pulses of 3 V/cm, pulse width 2 ms, and frequencies of 1, 3, and 5 Hz to the cardiac tissue of neonatal rat ventricular myocytes. Studies have shown that cardiac tissues stimulated at 3 Hz frequency had the highest tissue density, the highest concentrations of cardiac troponin-I and Cx43, and the best contractility. Zhang *et al.* [21] applied ES to hiPSC-CMs after 2 days of cell preculture. Cardiomyocytes were first stimulated at 1 Hz for 5 continuous days, and followed by 1 Hz daily step-up stimulation until the frequency reached 6 Hz. And after maintaining stimulation at 6 Hz for 2 days, the researchers continued to apply 1 Hz stimulation for 14 days. The results indicated that, after ES, hiPSC-CMs highly expressed the Cx43 and achieved a more mature phenotype, as confirmed by the more organized sarcomeres. Marc *et al.* [22] tested two pacing protocols: biphasic pulses with a frequency at 0.5 Hz for the entire period and pacing at 2 Hz during the first week and 1.5 Hz thereafter, a field strength of 2 V/cm and a pulse width of 4 ms, act on hiPSC-CMs respectively. The high frequency paced human engineered heart tissues (hEHTs) had markedly higher forces. Ronaldson-Bouchard *et al.* [18] also applied two schemes of ES. Under the condition that the other ES parameters are the same, 3 weeks at 2 Hz and 2 weeks at a

frequency increasing from 2 Hz to 6 Hz by 0.33 Hz per day, followed by one week at 2 Hz. The intensity-trained tissues had higher maturation of contractile function. Nunes *et al.* [23] utilized two different protocols of ES (5 V/cm, 1 ms, monophasic square waveform) to hiPSC-CMs: the stimulation frequency was progressively and daily increased from 1 to 3 Hz (Low frequency ramp-up regimen) and from 1 to 6 Hz (High frequency ramp-up regimen). They found that compared with the low frequency ramp-up regimen, the high frequency ramp-up regimen within one week could further enhance the structure and electrophysiological function of the engineered myocardial tissue.

3. Effect of endogenous electrical stimulation on the differentiation of stem cells

The biomimetic conductive microenvironment may affect the development of the heart by promoting electrical conduction between cardiomyocytes *in vitro*.

You *et al.* [24] found that when seeding neonatal rat cardiomyocytes on Au nanoparticles homogeneously synthesized throughout a polymer templated gel without ES, the expression level of Cx43 was significantly increased. Similarly, Wang *et al.* [25] found that flexible and conductive graphene sheets can promote the intrinsic electrical propagation by mimicking natural biomimetic conductive microenvironment without applying external ES, not only regenerating CMs *in vitro*, but also accelerating the maturation of functional CMs. These results highlight the possibility of conductive substrates affecting the development of cardiomyocytes.

4. Related mechanisms of electrical stimulation in inducing cardiac differentiation of stem cells

Although studies have shown that ES can play a key role in the process of heart development and maturation, the mechanisms involved are not yet fully understood.

4.1 Previous research

(1) Wu Chang-xue *et al.* [26] found that as the time of ES progressed, the expression of myocyte enhancer factor 2C gene was up-regulated, which in turn promoted the expression of troponin I and the formation of cardiomyocytes.

(2) Genovese *et al.* [27] found that the expression of follistatin (FST) was up-regulated in hMSCs after ES, and demonstrated that short-term ES promotes the differentiation of cardiomyocytes of hMSCs. In recent years, FST has been proven to be the key to muscle development, differentiation, and regeneration. It can promote cell proliferation and limit fibrogenesis by participating in the repair of mesodermal- and endodermal- tissues [28].

4.2 New progress

(1) Liang Li *et al.* [29] found that during ES, one of the mechanisms for the cardiac differentiation of bone marrow mesenchymal stem cells (BMSCs) is the upregulation of transforming growth factor-β1 (TGF-β1) expression, which promotes the expression of Cx43 and actinin alpha 2 (ACTN2), and is regulated by the TGF-β1 inhibitor pirfenidone.

(2) He *et al.* [15] applied ES to human cardiosphere-derived cells (hCDCs) and found that the differentiation of hCDCs is highly dependent on the synchronous transmission of electric current through the optimally aligned and elongated cardiomyocytes, which is called a "Syncytium". When a pulsatile electric current with constant amplitude is applied, the aligned hCDCs is perpendicular to the direction of the current. The aligned cardiomyocytes start to act like a capacitor that is charged and discharged in response to the applied electric stimulation. This pulsatile property of cardiomyocytes facilitates the formation of synchronous myofiber contractions.

(3) Crestani *et al.* [30] found that the expression of cardiac intercalated disk proteins (Nebulin-related-anchoring protein (Nrap) and β-catenin) increased after hiPSC-CMs were applied ES. Wnt/b-catenin signaling pathway has been proven to promote the cardiac differentiation of hiPSCs and the gap junction formation [31]. Nrap expression is involved in the process of myogenic muscle fiber generation and cell-cell connection and refinement [32]. This suggests the possible activation of Wnt/β-catenin pathway under the action of ES.

(4) Ma *et al.* [16] found that the possible mechanism of ES to promote cardiac differentiation of hiPSCs is to activate the Ca^{2+}/PKC/ERK pathways. Western blotting showed that ES upregulated Ca^{2+} binding proteins (Calmodulin (CAML) 1 and 3), enhanced PKC expression and ERK phosphorylation, and downregulated histone deacetylase 1 (HDAC1). Both of the above effects can be eliminated or even reversed by calcium signal inhibitors. Calcium signal inhibitors include verapamil (Ca^{2+} ion channel blocker), W-7 (CAML antagonist) and TMB-8 (intracellular Ca^{2+} antagonist). The specific inhibition of HDAC1 gene expression in BMSCs by RNAi causes an increase in the mRNA expression levels of genes related to myocardial development and structure [33]. Therefore, ES may enhance the cardiac gene expression of hiPSCs by activating intracellular Ca^{2+} signals and downstream genes.

(5) Kanwal Haneef *et al.* [34] found that applying ES to stem cells on 3D collagen scaffolds can promote the expression of cardiac markers, and found that 3D collagen scaffolds have natural extra cellular matrix (ECM) capabilities, providing structural strength and resistance to deformation. ECM is mainly composed of collagen that gives structural strength to the left ventricle. The latest research [28] suggests, this may be due to the following reasons: Glycosaminoglycans (GAGs) are widely distributed in ECM. GAGs are rich in ionizable carboxyl and sulfate groups, which have the characteristics of charge transfer. GAGs act as an electrical conduit from the ECM to the cell during electro-stimulatory applications. At the same time, ES will increase the synthesis of proteoglycans, each of which contains a specific GAG side chain. In

contrast, collagen tissue has piezoelectric properties; so that when the tissue is compressed, it generates an electric charge. It is caused by highly aligned fibrillar type I and II collagen packing and the cross-linked structures, mainly exists in bone and cartilaginous tissues [35], and the myocardium [36].

5. Future directions

Under different conditions, different ES parameters may correspond to different differentiation of myocardial stem cells, such as atria, ventricles, conduction cells, etc. Chan *et al.* [37] found that biphasic pulse stimulation with electric field strength of 6.6 V/cm, pulse width of 2 ms, and frequency of 1 Hz is the best ES parameter for hiPSC-CMs. Hernández *et al.* [38] found that cardiac differentiation induced by ES is affected by the cell line used. Crestani *et al.* [30] found that hiPSCs were more likely to differentiate into cardiac conduction cell phenotype under ES, with the increase of connexin 40 (Cx40) expression and the reduction of Cx43 expression. The *Irx3* gene plays a key role in the precise regulation of intercellular gap junction coupling and impulse propagation in the heart. It can directly suppress Cx43 transcription and indirectly activate Cx40 transcription [39].

Areas of future research include determining the ES conditions corresponding to the model standards of different stem cells, in order to further improve our understanding of the mechanism of ES induced differentiation, and to employ tissue engineering for cardiac differentiation of stem cells by ES. This has the potential for the clinical application of ES to minimize damage after an MI, and reduce the incidence of chronic heart failure.

6. Conclusions

In conclusion, ES is one of the effective methods to solve the low differentiation rate and immaturity of cardiomyocytes derived from stem cells. In clinical practice, electrical stimulation programs should be individually tailored according to different stem cells to produce the best promotion effect. In addition, if other stimuli are applied at the same time as electrical stimulation, it may have a synergistic or antagonistic effect, such as mechanical stimulation, matrix, etc.

Author contributions

YD drafted the manuscript and participated in its design and so on. JM and FZ conceived of the study, and participated in its coordination and helped to draft the manuscript and so on. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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