Biological Pacemakers Obtained Through Cellular Differentiation for the Restoration of Sinoatrial Node Function. A Systematic Review

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Abstract. Heart disease is one of the leading causes of death in the world. Heart disease has a wide variety of causes. Recently, the development of biological pacemakers obtained by cell differentiation for the restoration of sinoatrial node function has emerged for those heart diseases that are related to the sinoatrial pacemaker. The research is aimed at observing the action potential (AP) and I_f current relationship based on their depolarization and hyperpolarization in preclinical models. The present work aims to identify AP and I_f current values from recent reports to determine whether cardiomyocytes derived from embryonic hESC-CMs or induced pluripotent iPSC-CMs fulfill the function of pacemaker cells in the sinoatrial node in preclinical models based on the criteria of the Preferred Reporting Items for Systematic Reviews. Results show that the PA values were reflected between −40 mV and 60 mV and the I_f current ratio between −60 mV and −80 mV increased heart rate in preclinical models. Findings from this review will be informative for researchers seeking to prioritize future advances in the development of biological pacemakers.

Keywords: Biological pacemaker · Sinoatrial node · Action potential

1 Introduction

One of the leading causes of death in the world is heart disease, which causes 17.3 million deaths per year among men and women. By 2021, cardiovascular diseases ranked second among the ten leading causes of death in Mexico. Several research groups, supported by biomedical engineering, bioengineering and nanomedicine, are interested in developing medical technologies or new therapies to advance in the treatment of heart disease.

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In recent years, several treatment alternatives have been developed for cardiac diseases, among which the biological pacemaker deserves special mention. The main biological pacemaker of the heart is the sinoatrial node or SA node, which is responsible for generating an electrical impulse that travels through conduction channels allowing the atria and ventricles to contract correctly so that blood pumping is carried out in the heart. However, there are many cellular and molecular mechanisms that allow the electrical activity of the NSA pacemaker, such as the I_f (funny current), which is expressed in highly active cardiac regions and can be defined as a mixture of sodium-potassium channels that allow the slow depolarization of pacemaker cells in the diastolic phase.

The main objective of the development of biological pacemakers in the laboratory is to obtain cells capable of generating an action potential to replace the dysfunctional pacemaker cells of the sinoatrial node, which would allow the heart to maintain its proper rhythm (Fig. 1) [1–8]. A clear example of these new methodologies is the preclinical trial performed in [9] where cardiomyocytes derived from embryonic stem cells (hESC-CMs) were transplanted into primate monkeys with myocardial problems, which had successful results, improving left ventricular ejection by 10%. However, the challenge to develop new ways of obtaining cells for the treatment of cardiac diseases persisted. In [10] tested the expression of NSA pacemaker cell activity autologously in the right ventricle in adult canine models. These were monitored for two weeks in which they presented a rate of 90 ± 17 bpm and more than 80% of the isolated cells were estimated to be viable. In addition, a sample of 18 cells was taken where it was observed that they presented an I_f dependent inward current, and the hyperpolarization point was activated at -105 mV and at $+15$ mV for deactivation.

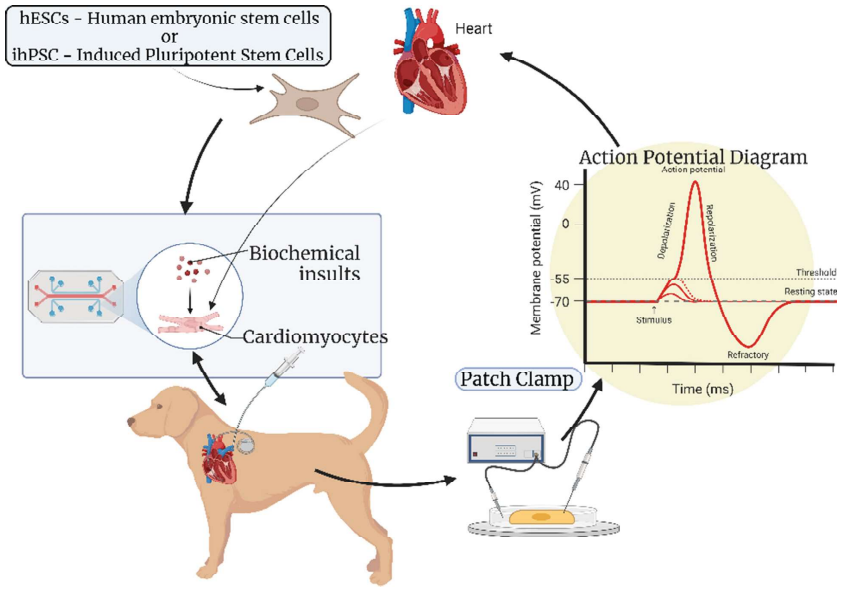

Fig. 1. Representation of the development of biological pacemakers.

Later, in 2017, a different process was carried out to obtain adult pluripotent stem cells, in [11] created induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from human hair follicle keratinocytes, which were implanted in canine models with atrioventricular block. The action potentials were recorded from the concentration of the cells where approximately 25% presented a spontaneous contraction, likewise the behavior of the I_f and it was determined that the automaticity of the cardiomyocytes depends on this current to carry out the stimulation of the heart.

As can be seen, cell therapy has emerged as an alternative to create new treatments for the reduction of cardiac diseases. Therefore, there is a need to detect the best suitable cell differentiation pathways to replace dysfunctional cells in the sinoatrial node by paying attention to the control of electrophysiological parameters, which is why in this work the following research question has been posed: Do cardiomyocytes derived from embryonic or pluripotent cells fulfill the function of pacemaker cells in the sinoatrial node in preclinical models? The findings of this review will be informative for investigators seeking to advance biological pacemakers.

2 Methods

This review was guided by the protocol published in [12]. A systematic search was carried out on PubMed and ScienceDirect during the period from 2011 to 2021. The search strategy was "(Biological Pacemaker" OR Pacemaker) AND ("Stem Cell" OR "Embryonic stem cell" OR Myocyte OR "Cell Therapy" AND ("Action potential" OR hyperpolarization). Once the search was finished, the articles were collected, and two authors (J.A.M.M. and C.C.G.) separately evaluated titles/abstracts for trial admissibility using a priori selection criteria. Inclusion criteria were, the study involves cell differentiation of induced pluripotent stem cells or embryonic stem cells is carried out; the study reports at least one result of the electrical potential or current I_f or derived from measurements of the cell studied; and the study is a preclinical assay in animal model. The exclusion criteria established were: The date of publication is prior to 2011; the paper does not have or does not claim to have been approved by an ethics committee; the paper is a publication other than a research article, such as a systematic review, meta-analysis, editorial commentary, or conference proceedings; the full text is not available. The duplicates were removed, and the authors separately evaluated the admissibility of the retrieved full-text trials. Other authors (D.L.F.G, R.E.G.L., and V.G.F.) verified the information synthesized in tables and made recommendations on the data retrieved from the selected records.

3 Results

Initially, the search yielded 2994 records. After screening, 668 publications were identified for detailed review, of which 69 were included in the present systematic review (Fig. 2). However, after thorough and detailed reading, we discarded 36 papers because the content did not focus on biological pacemakers and 29 papers did not present action

potential or I_f current results. Finally, 4 papers met the established criteria, whose information was tabulated to show the relevant results where the use of iPSC-CMs and hiPSCs allowed the development of biological pacemakers generating electrical impulses, improving the heart rate according to the species (Table 1). Likewise, these papers present the derivation and differentiation process of each cell used in each source, after obtaining cardiomyocytes. It should be noted that each report presented a different cell differentiation process, as shown in Table 2.

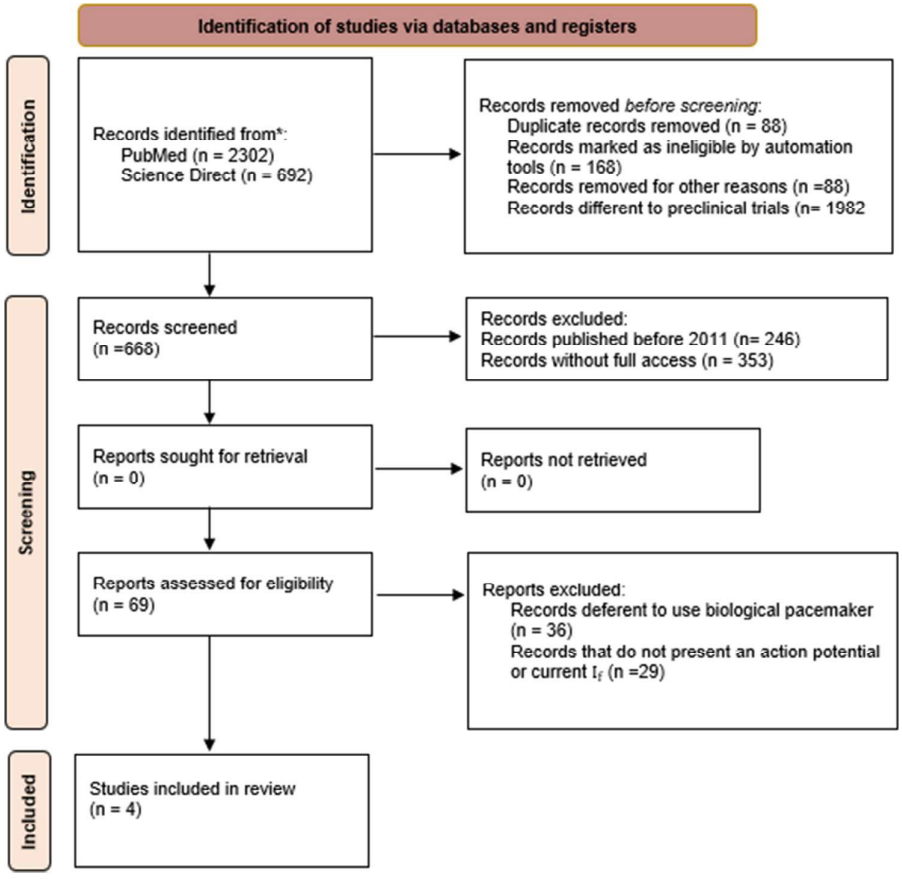

Fig. 2. PRISMA flow diagram for scoping reviews.

In the papers identified, the species were induced to total or partial heart block, causing an interruption or delay in the transmission of electrical impulses from the atria to the ventricles, affecting the pumping capacity of the heart, since the aim was to attack this cardiac anomaly after the injection of these cells, where it was possible to determine whether the injection of iPSC-CMs and hiPSCs caused an improvement in the electrical system as well as in the heart rate according to the species.

Table 1. Electrophysiological values obtained in the animal models using the indicated cells. NA indicates not available.

Ref.	Animal model (n) and cells type	Action potential (mV)	funny current, I_f	Heart rate (bpm)
[11]	Canine (13) - iPSC-CMs	−20	−60	80
[13]	Murine (21) - hiPSC	−40.4	NA	79.9 ± 2.8
[14]	Canine (NA)- hiPSCs	−60.1 ± 2.4	−60	NA
[15]	Canine (6) - sinoatrial node cells	−60	−105	90

In [11], the heart rate was monitored for 13 consecutive weeks, where a considerable improvement was observed in the canine models used, after carrying out the implantation of the cells. It was observed that the rate of the biological pacemaker increased during the first weeks, and then remained stable in weeks 4 and 5 at 40 bpm. However, the trend increased for the remaining weeks obtaining a frequency of 80 bpm in weeks 12 and 13. In [13] after carrying out the injection of cells in murines, these presented a heartbeat of 79.9 ± 2.8 bpm for 10 samples, which remained constant during the 28 days of study. In [8] the heart rate obtained was 90 ± 17 bpm after carrying out the monitoring in 3 canine models.

In [11] and [15] highlighted the importance of the I_f current in the automaticity of cardiomyocytes. Likewise, it was observed that two of four recordings presented an action potential of approximately −40 mV and −40.4 mV, in canine and murine respectively and two other results of −60 and −60.1 mV only in canine models. In order to carry out the measurement of these parameters, the Patch Clamp technique was used.

In [14], the value of I_f was −84.6 mV, where the cardiomyocytes studied presented a phase 4 depolarization and spontaneous beats, observing that the I_f prevented complete repolarization until the resting potential, because cardiac cells do not present a resting potential like other cell types, due to the presence of the diastolic phase that depolarizes the cell membrane until the activation threshold of the Ca^{2+} inward current is reached, thus proving the presence of the electrophysiological characteristics for slow response cells, as well as ease of promoting the automaticity of these cells. For the action potential obtained, this behaved in the same way as in [11], the AP of the NSA presented a less negative maximum diastolic potential (MDP) and with smaller amplitudes and it was observed that 54% of the 59 cardiomyocytes showed a ventricular-type AP, 22% showed a nodal-type AP and 24% showed an atrial-type AP. It was observed the highest current and BP values in [15]. The current I_f obtained was −105 mV and the AP was obtained at −60 mV expressing an inward current, which was activated in hyperpolarization at −105 mV and its deactivation at 15 mV. It should be noted that the authors used cardiomyocytes directly.

Limitations of the present systematic review are related to the heterogeneity between studies and limited full access to various sources of evidence that may contain information relevant to the subject under study.

Table 2. Cell derivation and differentiation reported in each source of evidence.

Ref.	Derived from	Cell differentiation
[11]	Human keratinocytes	iPSCs were spontaneously differentiated to cardiomyocytes
[13]	Human fibroblast	hiPSCs were co-cultured in a layer of END-2-cells
[14]	Human fibroblast	hiPSCs differentiated into cardiomyocytes, expression of Sox-7, Oct-4, Lin-28
[15]	Sinoatrial node cells	Cardiomyocytes

4 Conclusion

The present systematic review methodically analyzed for the first time the impact of biological pacemakers in the cardiology field. Biological pacemakers show revolutionary potentials to develop new methods of care for cardiac conditions. Derivation and differentiation of the cells indicated in each source of evidence is heterogeneous. There is no concordance among the animal models on the origin and differentiation of the cells. Therefore, it is still necessary to generate scientific evidence in animal models for cell therapy to be imposed as an alternative to create new treatments to address heart disease related to the sinoatrial pacemaker. Further research is required to determine the safest and most appropriate cell differentiation pathway to replace dysfunctional cells in the sinoatrial node and achieve control of electrophysiological parameters. This will determine that cardiomyocytes derived from embryonic or pluripotent cells fulfill the function of pacemaker cells in the sinoatrial node in preclinical models. Although research maturation remains a major concern, this type of scientific breakthrough may represent a step forward in meeting the expectations of patients and surgeons.

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