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REVIEW

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Optogenetics in cardiology: methodology and future applications

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Abstract

Optogenetics is an emerging biological approach with the unique capability of specific targeting due to the precise light control with high spatial and temporal resolution. It uses selected light wavelengths to control and modulate the biological functions of cells, tissues, and organ levels. Optogenetics has been instrumental in different biomedical applications, including neuroscience, diabetes, and mitochondria, based on distinctive optical biomedical effects with light modulation. Nowadays, optogenetics in cardiology is rapidly evolving for the understanding and treatment of cardiovascular diseases. Several in vitro and in vivo research for cardiac optogenetics demonstrated visible progress. The optogenetics technique can be applied to address critical cardiovascular problems such as heart failure and arrhythmia. To this end, this paper reviews cardiac electrophysiology and the technical progress about experimental and clinical cardiac optogenetics and provides the background and evolution of cardiac optogenetics. We reviewed the literature to demonstrate the servo type, transfection efficiency, signal recording, and heart disease targets in optogenetic applications. Such literature review would hopefully expedite the progress of optogenetics in cardiology and further expect to translate into the clinical terminal in the future.

Keywords: Cardiomyocyte, Cardiac electrophysiology, Optogenetic stimulation

Introduction

Over the last few decades, the development of adeno-associated virus (AAV) as a vector for gene delivery and therapy has advanced rapidly [1, 2]. Recently, the AAV-based drugs such as Luxturna and Zolgensma were approved by the FDA for use in the USA, signifying important milestones for the establishment of AAV-based therapeutics in the clinics [3]. The success of using AAV for gene therapy is due to several characteristics, including its nonpathogenic nature, good safety profile, and ease of production to clinical grade.

AAV-based optogenetics is a novel strategic approach applied in the cardiac engineering field, involving the delivery of microbial light-sensitive proteins called opsins to excitable cardiomyocytes, thus enabling either light-based depolarization or hyperpolarization [4]. This technology has already been applied to optical systems in the clinical-diagnostic field and therapeutic strategies for cardiovascular disease.

Recently, a rapid increase in publications revealed many newly identified opportunities in cardiac optogenetics. The optogenetic technology has expanded outside neuroscience and into areas like cardiovascular research that has surprisingly remained largely unexplored [5, 6]. In this review, we will describe the state of methodology and applications based on cardiac electrophysiology study as well as therapeutic insights into the impact of cardiac optogenetics on heart diseases.

Cardiac electrophysiology

Cardiac activity sensing

Electrocardiography (ECG)

Microbial-type rhodopsins have been discovered in archaea, prokaryotes, and eukaryotes. Bacteriorhodopsin,

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a light-driven proton pump, or channelrhodopsin-1 (ChR1), a recently identified light-gated proton channel, is a membrane ion transport protein [7]. Channelrhodopsin-1 (ChR1) and channelrhodopsin-2 (ChR2) were found in the algae species, Chlamydomonas reinhardtii, and shown to be involved in the generation of photocurrents of this green alga [8]. ChR2 is an optimized light-switched cation-selective ion channel of ChR1. It opens rapidly after absorption of a photon to generate a large permeability and desensitizes in continuous light to a smaller steady-state conductance. As a result, ChR2 has been a unique tool to study the electrical properties of biological cells, including neurons, muscle cells, and cardiomyocytes, simply by low-light illumination [7, 9].

Cardiac optogenetics allows non-invasive investigation of heart electrophysiology in vivo through ECG recording. In order to observe timely and spatially controlled photostimulation of ChR2 hearts in vivo, a fiber optic delivering a specific spectrum of light pulses, generated by a time-controlled light-emitting diode, was used in open-chest mice preparation during non-invasive ECG monitoring (Fig. 1a). [10] Cardiac photopacing was mapped by delivering light pulses with the fiber tip placed close to the epicardium of different heart regions (Fig. 1b). The optical fiber delivered time-controlled light pulses, allowing synchronization with a specific time point of the ECG recording.

Optical mapping

The Langendorff Perfused heart model is an experimental procedure to excise the heart and then cannulate through the aorta to connect to a constant-flow Langendorff-perfusion apparatus [11–13]. The heart can be retrogradely perfused via the coronary artery. After an initial 10–20 min of perfusion following cannulation, the dye was injected at a distance upstream from the aortic cannula for coronary perfusion. The heart was perfused with a light-sensitive dye such as di-4-ANBDQPQ [14], di-4-ANBDQBS, and di-4-ANEQ(F)PTEA dye loading solution [15, 16]. Incubation with dye was performed for 10 min at 37 °C and constantly bubbled with 95% O_2/5% CO_2. After dye loading, the perfusate was replaced with fresh Tyrode's solution containing blebbistatin to reduce contraction. For image, cessation of motion is a significant part to perform the stable image because it avoids distortion of the signals due to bubbles and motion of the solution. During image recordings, the excitation lights were switched on to excite opsin-expressed cardiomyocytes by exposure to a specific light spectrum. Dual-dye imaging plays a vital role in applying optical mapping techniques for optogenetic study. A multistream light switcher is required to automatically switch between the red (Vm signals) and blue (ChR2 activation) light-emitting-diodes (LEDs) to excite dye-loaded cells. Simultaneously, the calcium indicator (Rhod-2 AM) can also be added for the recording of calcium transient signal. Dichroic mirrors were used to combine the red and blue lights to pass through the lens and an excitation filter

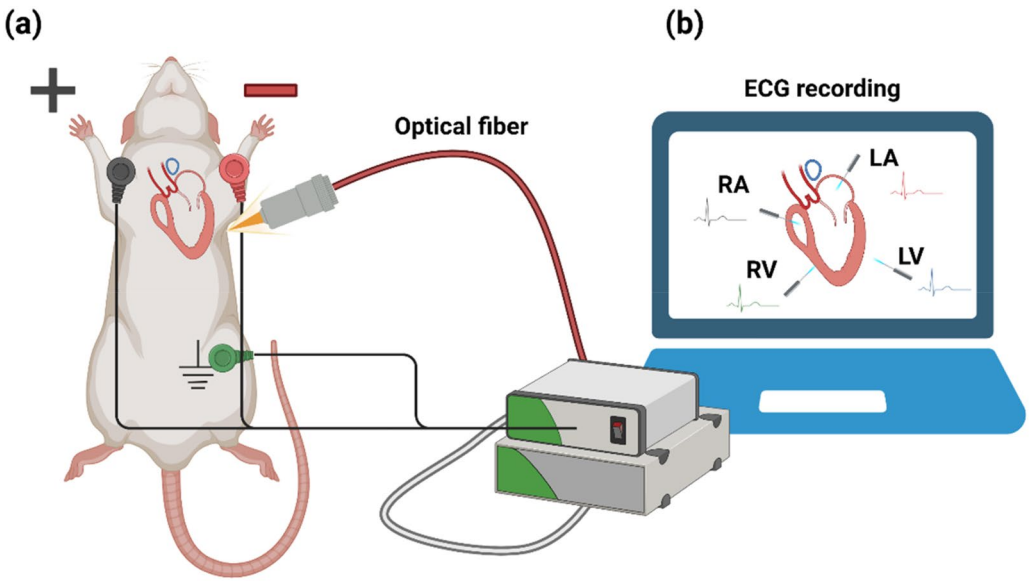

Fig. 1 Non-invasive investigation of cardiac electrophysiology with continuous ECG recording. **a** Representation of the setup system and epicardial photostimulation in mice. **b** ECG tracing of heart excitation originated by epicardial light stimulation of different regions of the myocardium

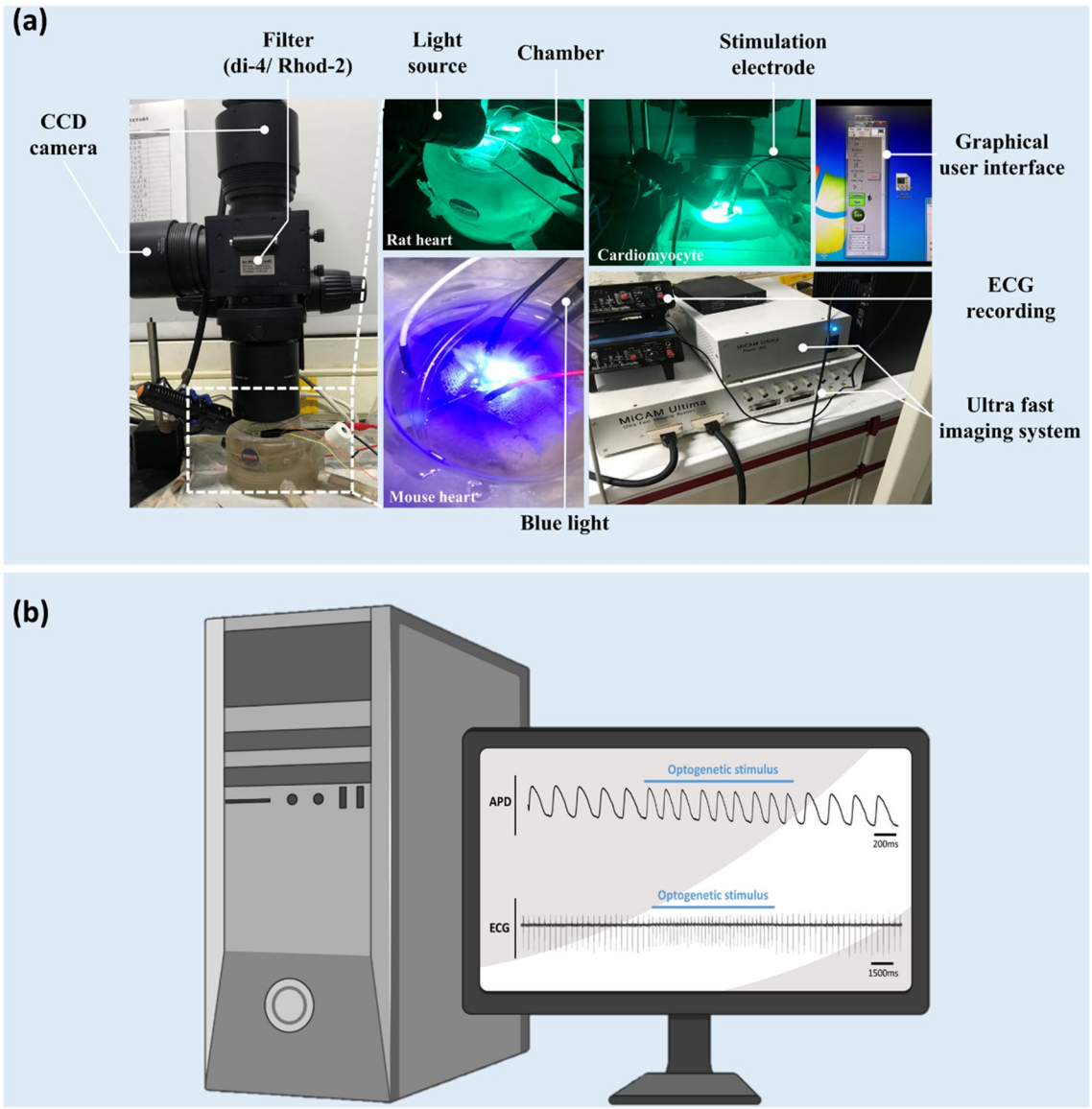

Fig. 2 Optical mapping system for cardiac optogenetic experiments. **a** Optical mapping system setup, **b** transmembrane potential (Vm) signal and ECG recording

(di-4/ Rhod-2) (Fig. 2A). Various red and blue excitation filters were used to optimize excitation-ratiometry measurements [17].

Fluorescence images were collected with a fast camera lens and taken with a high-speed electron-multiplying charge-coupled device camera. Excitation ratiometry was achieved by synchronizing the green and blue LEDs with the camera frame exposure (Fig. 2a). The green (peak wavelength = 530 nm) was placed around the imaging chamber to uniformly illuminate the preparation and applied to excite Ca^{2+} sensitive dye, excite the voltage-sensitive dye, respectively. A custom-designed system equipped with an EMCCD camera was used for multi-light source optical mapping (Fig. 2a). A multi-bandpass emission filter was placed in front of the camera lens to avoid bleed-through of the excitation light and to selectively make the fluorescence emission pass through. During image recordings, baseline transmembrane potential (Vm) was collected when channelrhodopsin-2 (ChR2)-expression cells were activated by exposure to blue light (470 nm), and ECG was recorded simultaneously (Fig. 2b) [14, 17, 18].

Cardiac treatment

Direct electrical stimulation

The cardiac influence of direct electrical stimulation on the autonomic nervous system has been experimentally demonstrated. Many topics regarding autonomic nervous system stimulation for significant cardiovascular disease therapy have been widely explored, especially in arrhythmia and heart failure [19]. However, direct electrical stimulation could cause predefined current waveforms and a non-specific ionic identity that could unexpectedly activate the threshold of membrane potential. Moreover, persistent electrical current injection is unsuitable because electrochemical reactions transfer electrons between the metal electrode surface and the solution to induce potentially harmful chemical species, including reactive oxygen species at the cathode, that diffuse into the tissue. The electrode itself is also damaged by corrosion during anode stimulation [20]. The mass of the chemical product formed at the electrode-solution interface is directly released into tissue proportional to the electrical charge delivered during the current injection. Minimizing the contribution of irreversible Faradaic reactions and damage is a severe side effect to overcome during electrical stimulation.

Magnetic stimulation

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive therapy technique, which is commonly applied to neurological disorders such as epilepsy [21], depression [22], Alzheimer's disease [23], and Parkinson's disease [24]. TMS uses the time-vary magnetic field in the coil to induce an electrical field on the brain or nerve, according to Faraday's law. The nerve will be activated to produce the action potential to modulate the biology mechanism. Wang et al. [25] developed a low-cost, miniature rTMS system and apply on the small animal model to investigate the influence of rTMS on heart rhythm. The coil was placed above the mouse's head and continuously stimulated the vagus nerve. The heart function was modulated by continuous vagus nerve rTMS, resulting in decreasing heart rate (Fig. 3). The optimized frequency of rTMS-induce pronounced RR-interval prolonging effect was 20 Hz, consistent with the dominant frequency of direct vagus nerve stimulation in clinical studies. In addition, simulation of the eddy current in the mouse brain also validates the feasibility of the current density of 25.4 $\mu A/mm^2$ that exceeds the activation threshold of the vagus nerve of 5.6 $\mu A/mm^2$. Consequently, rTMS stimulation is a non-invasive treatment for modulation of the vagus nerve to reduce the irregular heart rhythm, but the penetration depth of the magnetic field is limited, and the electric field is difficult to manipulate to specific areas. Optogenetics provides a breakthrough approach to improve the penetration depth and control the unique part through adjusting of light illumination spectrum and selected expression of opsins.

Optical stimulation

In addition to magnetic stimulation, the optogenetic technique is a fantastic biomedical technique that provides an alternative to electrical and magnetic stimulation. Optical stimulation mediates the genetically expressed light-sensitive ion channels such as Channelrhodopsin-2 (ChR2) (Fig. 4). It uses certain opsin and external light sources with specific wavelengths to modulate membrane voltage and activate AP in cardiac cells or the whole heart, and optical excitability similarly varies with cell type.

In comparison with other stimulation, optical pacing can employ much longer pulses without undesirable side effects. Light-gated ion channels can be applied in a multicellular and in vivo setting to produce persistent depolarizing current without such limitations. For example, the pacemaking frequency of ChR2-expressed Purkinje fibers could be tuned with a large dynamic range using very low irradiances at constant illumination after

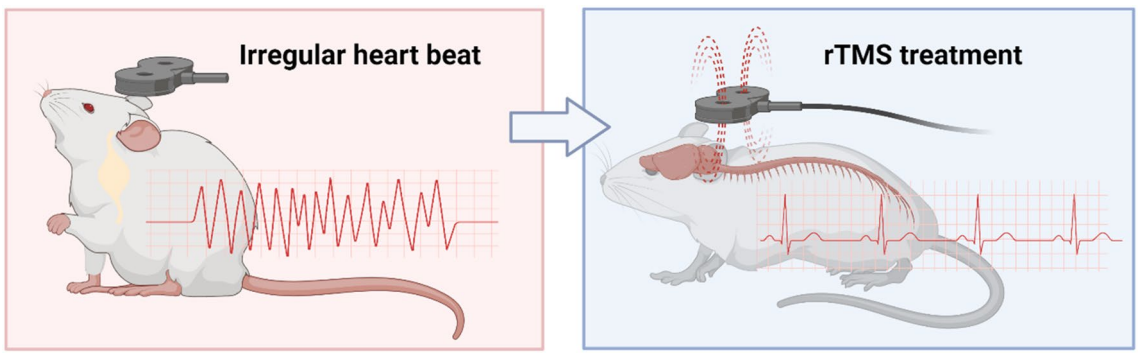

Fig. 3 rTMS treatment activates vagus nerve to inhibit arrhythmia through magnetic stimulation. Figure created with BioRender.com

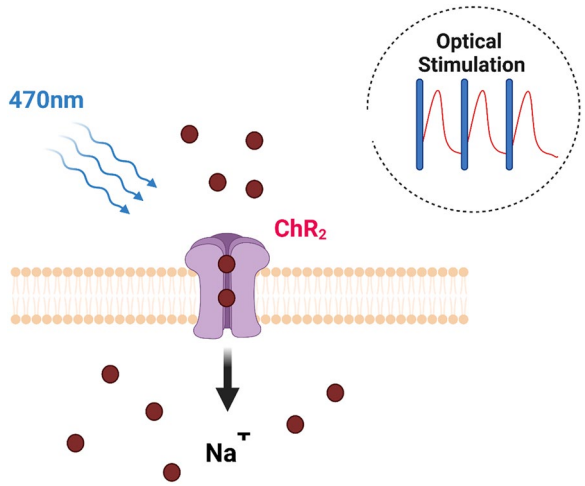

Fig. 4 Light-gated ion channel modulation by optogenetic stimulation and burst action potential. Figure created with BioRender.com

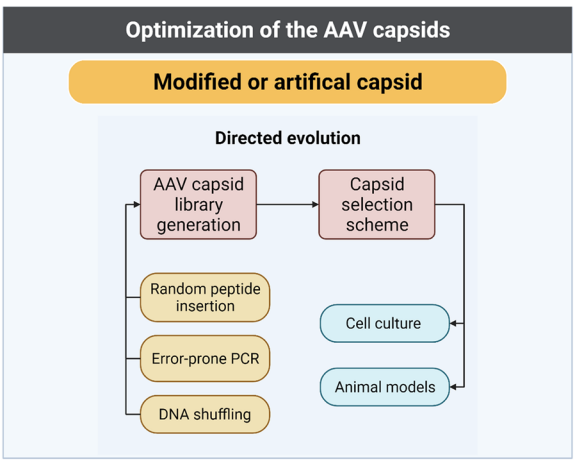

Fig. 5 The AAV capsid is the primary determinant of cell/tissue tropism

expressing ChR2. These results illustrated an advantage and attractive new application of cardiac optogenetics [26]. Moreover, coexpression of both depolarizing and hyperpolarizing opsins is the bidirectional approach to controlling cellular spiking using multiple [27] or single [28] constructs. This novel concept is suitable for endogenous optical pacemaking of the heart that includes the sinoatrial node, atrioventricular node, and the aforementioned Purkinje fibers. Therefore, due to specific and high-spatial-resolution stimulation using the light spot, optogenetic has a very high potential to apply to cardiology and cardiovascular disease therapy in the future.

Optogenetic in cardiology

Serotype

Numerous AAV serotypes have been identified from humans and other species that express a wide range of tissue and cell tropisms, determined mainly by each serotype's primary attachment receptor and co-receptor specificity [29]. The distinct serotypes have been widely screened for their ability to transduce many types of target cells and organs, but the reduction in transduction efficiency and often cross-reactive are common in the human population, limiting the widespread clinical use of many serotypes clinically due to neutralizing AAV vectors by anti-AAV antibodies [30, 31]. Therefore, it is important to optimize and choose the specific AAV capsid being used for each therapeutic application individually.

Several strategies have been developed to optimize the AAV capsid for gene delivery, including rational design and directed evolution. Methods to identify novel capsids with enhanced target specificity and low immunogenicity are divided into two broad categories, including native/fossil capsid isolation and capsid engineering [32]. Native/fossil capsid isolation is conduced through cell culture for live virus isolation, cell/tissue PCR amplification, or genome mining. This review mainly focuses on AAV capsid engineering that relies on modifying key structural and genetic elements via rational design or directed evolution for gene delivery. Rational design is a known aspect of AAV biology and structure that is dominantly modified on surface-exposed regions of the AAV capsid. Directed evolution is an approach to identify variants with distinct properties through coupling library-generated AAV capsid diversity and a selection scheme (Fig. 5). Strategies to produce AAV capsid libraries include error-prone PCR, DNA shuffling, and random peptide insertion. The libraries are then screened in cell culture-based systems, animal models, or a combination of both.

AAV capsid engineering approaches that produce novel capsids derived from natural or artificial AAV capsids with optimized target tissue specificities and minimal antigenicity are an attractive solution to improve vector tropism for cells and tissues that harbor persistent viral infections [29, 30]. The potential advantages that would be beneficial for the clinical trial are the ability to give lower effective AAV doses, avoid vector sequestration in non-target tissue, and evade pre-existing anti-vector humoral and cellular immune responses. [33–35].

AAV tropism is an essential factor affecting transduction efficiency dictated by AAV capsid proteins. The AAV serotype and the cell surface determine the transduction efficiency because AAV transduction mechanism is through the AAV capsid's interaction with cell surface

proteins and glycans. Moreover, as cell surface molecules vary across species, the efficiency of AAV varies across species and strains [36, 37]. Specific serotypes and route of delivery were dominant factors for optogenetic experiments. AAV1, AAV6, AAV8, and AAV9 have been reported as the most cardiotropic serotypes through systemic delivery [38]. Christina M. Ambrosi et al. had reported that in vitro AAV6-mediated transgene expression is superior to the use of AAV1 and AAV9 in rat and human cardiomyocytes [39]. However, in vivo AAV9-mediated transduction expression in the adult rat heart provides robust, predominantly cardiomyocyte-specific transgene delivery [39]. Also, Inagaki K et al. demonstrated that AAV9 was the most efficient serotype for cardiac gene delivery in mice. [40, 41].

Transfection efficiency

Recent studies have reported that a single intravenous dose of AAV9 makes a 200-fold increase in myocardial transduction efficiency compared with AAV1, confirming the superiority of AAV9 for cardiac gene transfer [41–43]. Intravenous vector administration can induce certain AAV serotypes to efficiently transduce cardiomyocytes, revealing that these AAV vectors affect transcytosis, depending on the serotype [42]. Vogt et al. had reported on cardiac optogenetics that systemic delivery of AAV9 established robust and long-lasting expression in mice [44]. However, the invasive transduction approach has not been extended to larger animals, which are suitable candidates for cardiac electrophysiology study because of the size and ease of endoscopic access. [45].

Christina M. Ambrosi et al. had compared the transfection efficacy of AAVs 1 and 9 in adult rats. The results indicated that AAV9-mediated infection through systemic delivery in the adult rat for four weeks resulted in ventricular opsin expression, but AAV1-mediated infection showed no cardiac transgene expression [39, 46, 47]. AAV9-mediated mCherry expression in the adult rat heart provides robust, predominantly cardiomyocyte-specific transgene delivery. However, excised hearts, brains, livers, and kidneys showed little to no signs of AAV-mediated infection in all animals.

In addition to system delivery, direct intramyocardial AAV injection also results in the transduction of cardiomyocytes, but the effect is typically more localized and restricted to the injection site itself. AAV9 transduction is restricted to the myocardium and transduced to the liver and skeletal muscle, even after their increased cardiotropism [48, 49]. To improve the cardiac specificity of variants, modified receptor-binding properties with improved transduction profiles partially evade neutralizing antibodies [50]. Also, constructing a random viral display peptide AAV library could allow subsequent in vivo selection of cardiotropic AAV variants [51]. Alternatively, through DNA shuffling of different AAV serotype capsid genes, this variant exhibited enhanced transduction to cardiac muscle and diminished tropism to the liver after systemic administration [52]. Overall, several functionally diverse AAV9 variants were identified and displayed a 10- to 25-fold lower gene transfer efficiency in the liver but transducing the myocardium as efficiently as AAV9. The emerging strategies were developed to enhance tissue tropism and produce AAVs that can evade neutralizing antibodies.

Signal recording

Optogenetic sensing is to measure the cardiomyocyte electrophysiological signal during the optogenetic stimulation. The current tools for optogenetic sensors include patch-clamp, microelectrode array (MEA), and optical mapping. Patch-clamp is the invasive intracellular technology for membrane potential measurement (Fig. 6a). The external voltage is applied across the cell membrane, and membrane current is measured to depict the ion channel and transfer. Wang et al. [53] developed multiple patch-clamp recording systems to decipher neuronal circuitry for understanding brain function. In cardiology in optogenetic applications, patch-clamp provides real-time action potential (AP) waveform depiction during optogenetic stimulation. Gruber et al. [54] utilized optogenetic and patch-clamp tools to explore the optogenetic modulation of cardiac AP properties for arrhythmias study. However, patch-clamp is very high-cost equipment, and its measurements must require a well-trained operator with biological experience. MEA is fabricated by photolithographically patterned electrodes that enable high throughput for culture-cardiomyocytes studies due to multiple electrode sensing functions (Fig. 6b). Nussinovitch et al. [55] cultured the neonatal rat cardiomyocytes monolayers on MEA culture plates to record electrical conduction and automaticity in optogenetic stimulation. The main advantage of MEA is to provide multiple wells signals recording in parallel. In contrast, the drawbacks of MEA are high-cost peripheral equipment and dish consumption [56]. Optical mapping utilizes fluorescence imaging-based technology to record fluorescence signals of di-4-ANEPPS dye (excitation wavelength of 475 nm) cardiomyocytes (Fig. 6c). Optogenetics technique combines optical mapping to provide the high-spatial–temporal resolution image for AP and electrical conduction path of the cardiomyocyte [57]. Klimas et al. [58] designed an all-optical dynamic cardiac electrophysiology framework to perform the different experimental optogenetic models, including cardiomyocytes and whole heart. Although all-optical electrophysiology provides precise optogenetic actuation and sensing in cardiology,

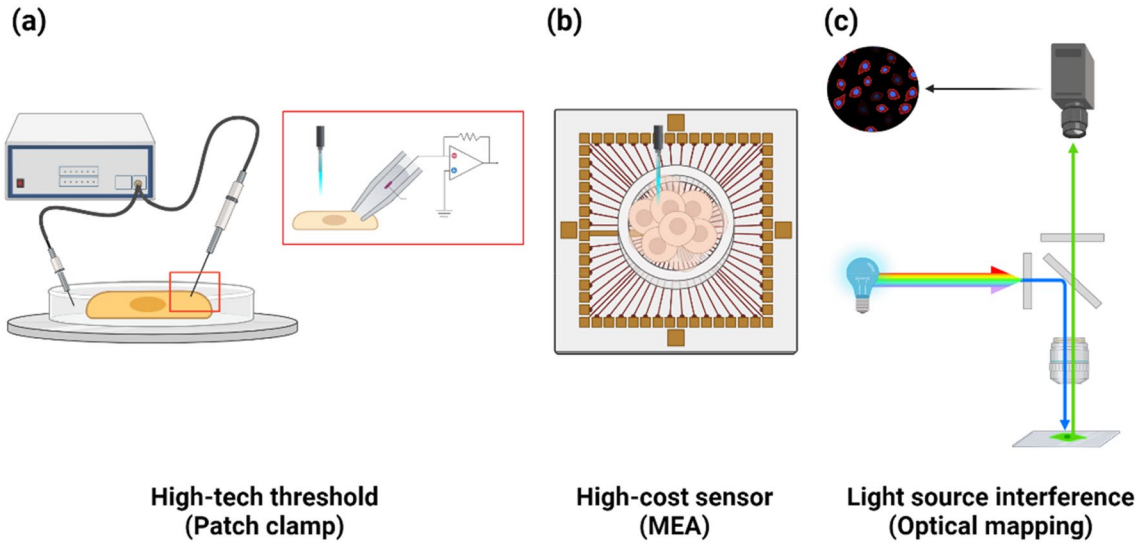

Fig. 6 Optogenetic sensing tool for signal recording. **a** Patch-clamp, **b** microelectrode arrays, **c** optical mapping. Figure created with BioRender.com

some technical challenges remain unsolved, especially in wavelength overlap between ChR2 and di-4-ANEPPS. Recently, Wang et al. 59, 60] presented a low-cost, miniature, novel impedance plethysmography technique to record the impedance of cardiomyocytes in response to the cell electrical behavior under optogenetic stimulation [61, 62].

Heart disease target in optogenetic application

Soon after introducing the optogenetic technique in neurobiology, it has rapidly drawn significant interest and evolved quickly to become a vital tool in basic cardiovascular research, especially in cardiac electrophysiology. The optogenetic application in cardiac diseases mainly focuses on using light-activated ion channels and pumps to manipulate the transmembrane potential of cardiomyocytes to control their excitability or inhibition. Light-sensitive opsin in cardiomyocytes modulates the membrane potential by illuminating the heart with a precisely controlled light beam, transforming the light source into a cardiac pacemaker [63, 64]. Optogenetic control ranges from tailored stimulation to inhibition and provides novel and unique research possibilities regarding cardiac pacing, resynchronization, and arrhythmia termination [65].

Atrial fibrillation (AF) patients present with increased mortality, decreased quality of life and increased risk of heart failure development and progression [66]. However, restoring and maintaining sinus rhythm is beneficial for early AF progression because sustained AF is associated with left atrium structural and functional remodeling, rendering future sinus rhythm restoration strategies inefficient [67, 68]. In the past, electrical cardioversion and implantable cardioverters may be options to treat AF. However, long-term electric stimulation may be related to side effects such as frequent shocks and associated pain.

Moreover, ventricular tachyarrhythmias are characterized by fast and uncoordinated electrical excitations of the myocardium, resulting in pump failure and sudden cardiac death [69]. The current state-of-the-art therapy for these life-threatening disorders is to receive an implantable automatic defibrillator to deliver high-energy electrical shocks. Clinical studies have reported the life-saving potential of this therapeutic approach. At the same time, it may induce significant adverse effects such as structural damage of cardiac tissue worsening the underlying cardiac disease, severe pain because of nonselective excitation of nerves and muscles with subsequent psychological trauma, and increased mortality [70–72]. Therefore, alternative approaches are imperative that enable pain-free and less deleterious termination of lethal arrhythmia.

More recently, in different experimental models, light-gated opsin transgene in cardiomyocytes allowed control of their beating rate in response to blue light exposure [44, 65, 73, 74]. Optogenetics may provide a novel solution to cardioversion and arrhythmia defibrillation, providing pain-free shocks to restore sinus rhythm. Optogenetic-based pacemaking activity is the ability to open the ChR2 channel in response to blue light illumination and result in a robust depolarizing transmembrane

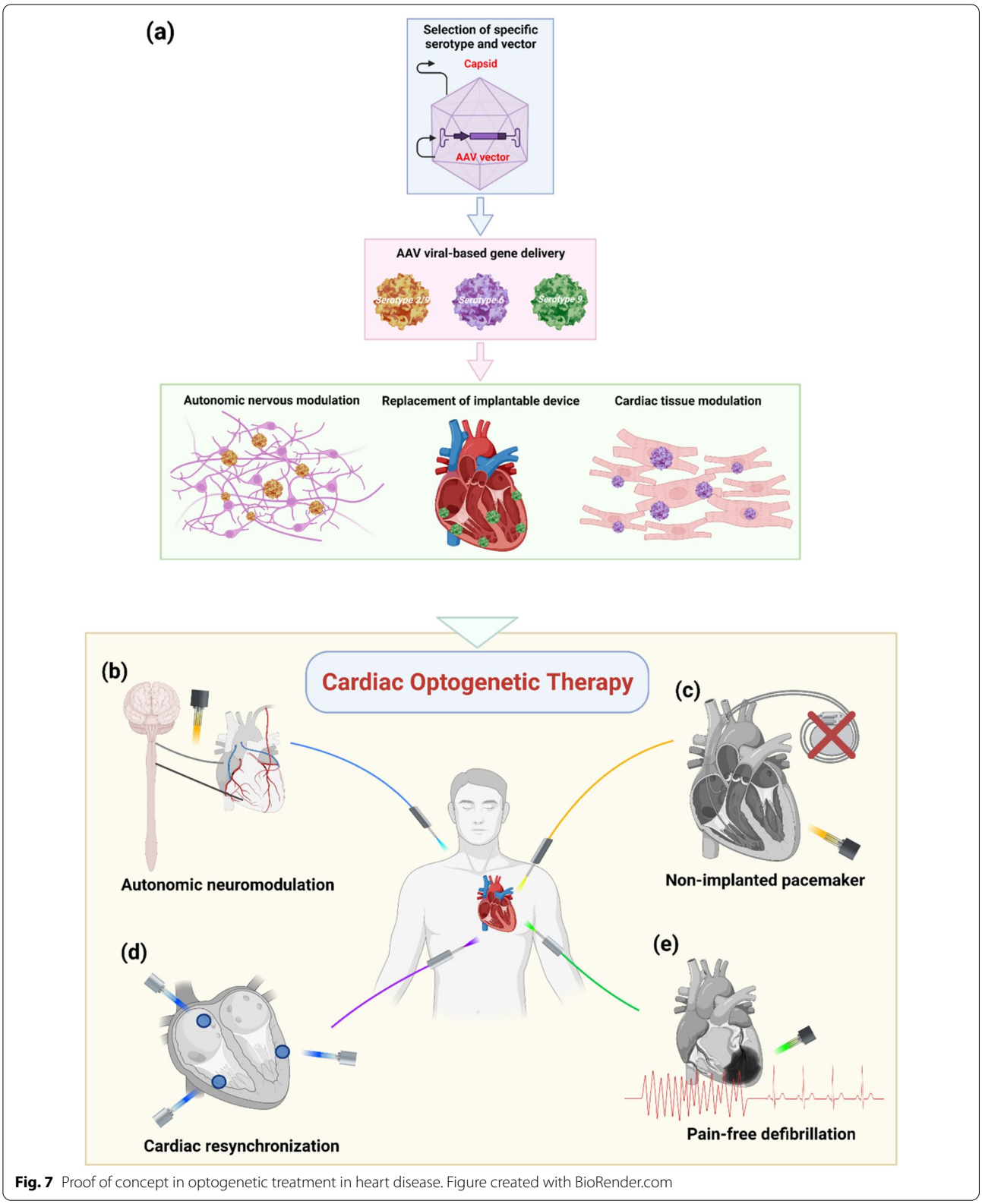

Fig. 7 Proof of concept in optogenetic treatment in heart disease. Figure created with BioRender.com

current that can lead to the development of an action potential in genetically modified cardiomyocytes. [75].

The different opsins can be delivered to the heart using a gene therapy approach such as a viral vector-based delivery (Fig. 7a). Several studies have made proof of concept for the feasibility of using optogenetic tools for cardiological applications. Optogenetic tools can be used for neuromodulation, including inhibition of the sympathetic or potentially activation of parasympathetic inputs to the heart, thereby increasing electrophysiological stability and protecting against myocardial ventricular arrhythmia (Fig. 7b) [76, 77]. Christoph C. Vogt et al. and Udi Nussinovitch et al. [44] demonstrated optogenetic pacing of mouse hearts that provide unique ability to bidirectionally control beating rate by either depolarizing or repolarizing stimuli (Fig. 7c). Optogenetics is also applied to cardiac resynchronization therapy in that diffused light and opsin expression have the potential to affect the unlimited number of distant loci (Fig. 7d) [65]. Painless defibrillation is different from the excruciating and nonselective electrical shocks currently used to terminate the arrhythmia [78]. ChR2-mediated transmural depolarization of the myocardium causes an interrupt of voltage-dependent Na + channels throughout the myocardial wall and blocks wavefront propagation into illuminated cardiac tissue. Tobias Bruegmann et al. [79] showed that optogenetic defibrillation is highly effective in the murine heart and potentially achieves nondamaging and pain-free termination of ventricular arrhythmia (Fig. 7e). Optogenetics in cardiac disease therapy is promising and valuable for delivering ChR2 to the heart by using various light-gate opsins strategies for neuromodulation, cardiac pacing, resynchronization, and even pain-free defibrillation.

Conclusion

This review has demonstrated the methodology for collecting cardiac electrophysiologic signals and the dominant role of AAV in optogenetic applications. A common approach was to monitor cardiac rhythms by ECG measurement in real time. The ECG can be acquired before, during, and after tissue illumination to measure changes in heart rate. Upon illumination, ECG could exhibit the dynamic alteration of the heart excitation, such as delays in atrioventricular conduction and sudden and dramatic reductions with the restoration of normal heart rate after cessation of illumination. The ECG recording system can record the ECG through four electrodes positioned as specified by Einthoven's triangle. It is controlled wirelessly through a host computer where the data can be recorded from freely moving animals during photostimulation.

Optical mapping is a powerful tool for investigating cardiac physiology and pathophysiology. The system requires only a single camera and single excitation light to simultaneously acquire voltage and calcium signals from whole heart preparations and other physiological models, including neurons and isolated cardiomyocytes. Until recently, the implementation of simultaneous mapping in two excitation lights with two fluorescent probes has been technically challenging for cardiac optogenetics. Experimental protocols using optical mapping can be used for performing dual calcium-voltage recording, combined with an optogenetic approach, to study membrane potential, intracellular Ca^{2+} transients, and pacemaker activity. The protocol demonstrated robust tissue dual-dye loading, light-programmed pacing, and high-resolution optical mapping. The combination of optogenetics and optical mapping techniques with high temporal and spatial resolution provides a valuable tool for studying cardiac physiology and pathophysiology in the animal model.

Cardiac treatment is for cardiovascular disease therapy, including direct electrical stimulation, magnetic field-induced eddy current, and optogenetic stimulation. Electrical stimulation has yielded encouraging early clinical results on the therapeutic modulation of cardiac autonomic tone and regulation of irregulated heart rhythm. However, several patients are excluded from electrical stimulation therapy because there is concern that electrical stimulation could induce electromagnetic interference with pacemakers and implanted cardioverter defibrillators. An assessment of benefit and harm is necessary to support the decision to use electrical stimulation in these patients. Moreover, magnetic stimulation is a vital therapeutic tool used in non-invasive stimulation. The electric field induced by the time-varying magnetic field in a stimulating coil could activate nerve fibers in the brain, resulting in depolarization or hyperpolarization of the autonomic neurons. Although magnetic stimulation is a non-invasive therapeutic strategy for cardiovascular disease, the penetration of depth of the magnetic field is limited, and it is difficult to fully stimulate specific targets. Therefore, optogenetic stimulation provides a high-spatial-time resolution for specific light spot stimulation. Such optical-based biological technique is expected to translate into the clinic for precise treatment in cardiology in the future.

In the recent market, recombinant AAV gene therapies have been approved in Europe and the USA, which are landmark achievements in the history of modern science. The emergence of a new class of therapies for monogenic disorders had been considered as an opportunity for untreatable diseases. Cardiac gene transfer is an attractive strategy for developing novel heart

disease treatments. AAV vectors are suitable candidates to mediate opsin expression in animal models and are being evaluated for human gene therapy. However, the AAV capsid protein, which forms the virions' outer shell, is the primary determinant to display the best cardiac tropism, transduction efficiency, and immunogenicity. Gene delivery of antiviral therapeutics to specific anatomical sites is vital to accumulate and retain AAV viruses for the next generation of antiviral therapies. Therefore, comprehensive strategies are to identify AAV vectors to improve transduction efficiencies, alternate tropisms, reduced sequestration in non-target organs, and reduced immunogenicity and have produced AAV capsids that are currently under evaluation in pre-clinical and clinical trials.

Optogenetic technology provides a promising therapeutic strategy with spatiotemporally precise tools for stimulation, sensing, and analysis of function in cells, tissues, and organs. It is beneficial to offer low-energy and localized approaches due to the use of light-gated opsins. The tool has been applied in many neurological diseases, but it has also evolved exceptionally well in animal cardiac research, both in vitro and in vivo now. Implantable optogenetic devices are being extensively developed to study particular electrophysiological phenomena in cardiovascular disease with precise control. Optogenetic arrhythmia cardioversion might be achieved by inducing a conduction block or filling the excitable gap. Light-sensitive opsin expression and appropriate illumination with an exposure time longer than the arrhythmia cycle length are necessary for successful arrhythmia termination. Cardiac optogenetic implanted devices are tempting due to the possibility of delivering pain-free shocks with out-of-hospital biological cardioversion. However, several issues need to be overcome, including immune reactions, loss of opsin expression, applicability and safety in humans, long-term behavior, and anticoagulation requirements. Nevertheless, there is undeniable that cardiac optogenetics is the most promising and robust technology that brings the novel opportunity of therapeutic approaches for unsolved issues in cardiovascular diseases.

Abbreviations

AAV: Adeno-associated virus; AF: Atrial fibrillation; AP: Action Potential; ChR2: Channelrhodopsin-2; ECG: Electrocardiogram; LED: Light-emitting diode; MEA: Microelectrode array; rTMS: Repetitive transcranial magnetic stimulation.

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Competing interests

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