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# Optoelectronic control of cardiac rhythm: Toward shock-free ambulatory cardioversion of atrial fibrillation

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Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia, progressive in nature, and known to have a negative impact on mortality, morbidity, and quality of life. Patients requiring acute termination of AF to restore sinus rhythm are subjected to electrical cardioversion, which requires sedation and therefore hospitalization due to pain resulting from the electrical shocks. However, considering the progressive nature of AF and its detrimental effects, there is a clear need for acute out-of-hospital (i.e., ambulatory) cardioversion of AF. In the search for shock-free cardioversion methods to realize such ambulatory therapy, a method referred to as optogenetics has been put forward. Optogenetics enables optical control over the electrical activity of cardiomyocytes by targeted expression of light-activated ion channels or pumps and may therefore serve as a means for cardioversion. First proof-of-principle for such light-induced cardioversion came from in vitro studies, proving optogenetic AF termination to be very effective. Later, these results were confirmed in various rodent models of AF using different transgenes, illumination methods, and protocols, whereas computational studies in the human heart provided additional translational insight. Based on these results and fueled by recent advances in molecular biology, gene therapy, and optoelectronic engineering, a basis is now being formed to explore clinical translations of optoelectronic control of cardiac rhythm. In this review, we discuss the current literature regarding optogenetic cardioversion of AF to restore normal rhythm in a shock-free manner. Moreover, key translational steps will be discussed, both from a biological and technological point of view, to outline a path toward realizing acute shock-free ambulatory termination of AF.

**Keywords:** atrial fibrillation, cardiology, cardioversion, engineering, optogenetics, treatments

## Introduction

Human life requires adequate blood circulation, which is maintained through rhythmic contractions of the heart. Such contractions are triggered by electrical impulses propagated across the cardiac syncytium in a highly coordinated fashion. These waves are the result of electrical currents produced by cardiomyocytes. At the plasma membrane of these excitable cells, a repertoire of voltage-gated ion channels is expressed, the opening and closing of which lead to ion fluxes that cause depolarization and subsequent repolarization of cardiomyocytes. Collectively, this process results in the generation of the cardiac action potential. This electrical impulse is rapidly propagated across cardiac tissue via intercellular connections, called gap junctions, leading to

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$Ca^{2+}$-induced $Ca^{2+}$-releases followed by coordinated contraction of the heart. Disturbances in the genesis or spreading of action potentials can induce cardiac arrhythmias. These arrhythmias are a large and growing problem worldwide, with high annual mortality and morbidity rates, and significant health care costs [1]. The most common cardiac arrhythmia is atrial fibrillation (AF), which affects 2%–3% of people worldwide, and the number of AF patients is quickly rising due to the overall aging of the population [2, 3]. AF is, despite all our efforts, still associated with an increased risk of all-cause mortality, cardiovascular mortality, ischemic stroke, ischemic heart disease, sudden cardiac death, and heart failure [4]. The substantial clinical, social, and economic burdens imposed by cardiac arrhythmias illustrate the current challenges in the management of heart rhythm disorders in general and in the treatment of AF in particular.

The present mainstays of arrhythmia treatment are antiarrhythmic drugs, cardiac ablation, and electrical cardioversion. All these treatment options have their own shortcomings and as a result are far from optimal regarding efficacy and applicability, despite their undisputed contribution to the improvement of arrhythmia management over the last few decades. Currently, electrical cardioversion—which involves application of high-voltage shocks to rapidly restore normal heart rhythm—is the best treatment option for patients in need of acute termination of AF [5]. This is especially true for the increasing number of AF patients experiencing drug-resistant and symptomatic AF recurrences, even after multiple ablation procedures. However, this procedure needs to be performed in the hospital as it requires sedation before electric cardioversion can be applied. Hence, despite being effective, this intervention adds to continuously increasing hospital workload and health care costs. Moreover, as electrical cardioversion usually only offers a temporary solution due to the high recurrence rate of AF, repetition of the procedure is often required. In this context, it is important to realize that AF duration is inversely related to the success rate of maintaining sinus rhythm by cardioversion. This finding, but also the detrimental effects of AF itself, points toward the necessity of early intervention to restore sinus rhythm [6]. This notion has received much attention lately because of new trials indicating beneficial effects of early cardioversion. However, even before this insight took hold, ambulatory cardioversion of AF was pursued by the clinical application of implantable atrial cardioverter-defibrillators, also known as atrioverters [7]. Although these devices proved to be safe and effective for the out-of-hospital electrical cardioversion of AF, their use was discontinued because of patients' intolerance to the repeated delivery of the painful electroshocks [8]. Thus, the acceptance by patients of the cardioversion procedure is a crucial parameter to consider in the conception of an acute ambulatory method for AF termination. Ideally, termination of AF is realized without the patients even noticing it, which requires the mode of termination to be at least pain-free and therefore electrical shock free.

Knowing that the traumatizing electrical shocks required for cardioversion are the culprit in realizing device therapy for AF, other methods for manipulating the electrical behavior of myocardial tissue to terminate arrhythmias have been explored [9–12]. One of these concerns the expression of light-activated ion channels or pumps in cardiomyocytes to gain optical control over their electrical behavior. This method is known as optogenetics. Its application has resulted not only in breakthrough progress in the field of neuroscience by revealing structure–function relationships in the brain but has also paved the way for new avenues of cardiovascular research, including investigations into acute shock-free termination of cardiac arrhythmias. Such optogenetic rhythm control has first been demonstrated in cell culture models of AF, revealing that brief illumination of optogenetically modified neonatal rat atrial myocytes (NRAMs) indeed resulted in acute termination of reentrant electrical activity to restore normal wave propagation [13–15]. A few years later, these results were confirmed in mouse and rat models of AF [9, 16], setting the stage for more translational studies into exploring optogenetic rhythm control as a potential basis for future ambulatory AF cardioversion. Importantly, concurrent with the development of optogenetics, the field of optoelectronics has also made huge progress. Recent advances include, for example, the development of flexible arrays containing multiple electrodes and light-emitting diodes (LEDs) for implantation and miniaturized wireless power transfer (WPT) devices for LED activation. Collectively, the progress in the fields of optogenetics and optoelectronics has created new possibilities for exploring and realizing acute shock-free termination of AF to restore sinus rhythm in an ambulatory manner.

 

In this paper, we will first discuss the current treatment options for AF and the lack of ambulatory cardioversion for immediate restoration of sinus rhythm. Next, based on current literature, optogenetics will be presented as a means to realize such out-of-hospital cardioversion by enabling optical termination of AF. The second part of this review will address the biological and technological challenges ahead of us regarding the translational steps needed to realize immediate termination of AF in the ambulatory setting.

### Atrial fibrillation: symptoms, progression, and treatment

The current medical guidelines recognize five different types of AF: first diagnosed, paroxysmal, persistent, long-standing persistent, and permanent AF [5]. A patient is first diagnosed, independently of the features of the arrhythmia, once an electrocardiogram (ECG) of AF is recorded. AF is classified as paroxysmal when it terminates spontaneously or with intervention within 7 days of onset, persistent if it lasts more than 7 days but less than 1 year, and long-standing persistent when it continues for at least 12 months. Once treatment is no longer effective in long-standing persistent AF patients, the arrhythmia is classified as permanent AF [5].

AF affects over 60 million people worldwide, and its prevalence is expected to increase, primarily due to the aging of the population [2, 17]. Moreover, AF is associated with the development of heart failure, an increased mortality risk, embolic stroke, cognitive decline, depression and an overall decreased quality of life [18–20]. Additionally, AF is associated with environmental and genetic risk factors (including common and rare genetic variants), which in some cases can lead to its early onset [2, 21, 22]. Hence, AF can be considered a multifactorial disorder involving different pathophysiological mechanisms, making its effective treatment for large patient groups a challenging endeavor [23, 24].

This notion is indeed reflected in daily clinical practice. Decision-making regarding the therapeutic approaches requires a careful evaluation of the risk of stroke, severity of symptoms, the type of AF, the characteristics of the AF trigger and substrate, existing comorbidities, and cardiovascular risk factors [5]. The different treatment options include pharmacological rhythm or rate control, cryoballoon or radiofrequency catheter ablation, and—in specific cases—invasive surgical approaches including different versions of the so-called maze procedure [5]. Despite all these treatment options, a substantial group of patients is left with symptomatic and therapy-resistant AF [25, 26]. These patients can be subjected to electrical cardioversion, which is successful in almost every patient but does not prevent recurrence of AF [27, 28]. To painlessly apply the electrical shocks, patients must first be sedated and therefore hospitalized. This results in high costs and adds strain and stress to patients, while leaving them exposed to AF longer than desired [29].

This latter notion may be important given the fact that AF is a progressive disorder, a feature which is aptly described by the expression “AF begets AF.” The worsening of AF is thought to be due to structural, metabolic, and electrical remodeling of the atria [30–32]. In terms of structural remodeling, fibrosis and fatty tissue infiltration are known to be the main pro-arrhythmic substrates worsening the course of AF [32, 33]. Both have been proven to facilitate reentry circuits driving AF and to have an impact on cardiomyocyte electrophysiology through direct heterocellular contacts and paracrine crosstalk [34, 35]. Moreover, atrial myocytes undergo AF stage-specific changes in substrate utilization for ATP production. A study by Barth et al. showed atria from patients with permanent AF to adopt a ventricular- and fetal-like gene expression profile accompanied by transcriptional downregulation of genes involved in fatty acid oxidation and concomitant upregulation of genes with a function in glucose catabolism [36]. The electrical remodeling that occurs in AF includes a shortening of the cardiomyocyte’s action potential and a decrease in absolute and effective refractory periods, which are thought to be mediated by rate-induced intracellular $Ca^{2+}$ overload [37]. The quivering of the atria during AF causes loss of the so-called atrial “kick” and a subsequent reduction in cardiac output, which may be further reduced by AF-induced irregular and/or high-rate ventricular contractions [38]. As a consequence, AF may contribute to the development or worsening of heart failure. Similarly, heart failure may promote or exacerbate AF in different ways, including by the elevation of cardiac filling pressures, dysregulation of intracellular $Ca^{2+}$ handling, and autonomic and neuroendocrine dysfunction [39]. Taken together, the progressive nature of AF emphasizes the importance of realizing ambulatory methods for AF cardioversion to prevent, halt,

and possibly even reverse the various types of remodeling favoring AF. Accumulating clinical evidence indeed indicates that early rhythm control in AF may have beneficial effects [6, 40].

As already briefly mentioned above, an international consortium attempted to treat AF using an implantable atrial defibrillator or atrioverter. The study included patients with recurrent AF who did not respond adequately to pharmacotherapy. The first stage of this study was carried out in the hospital and was aimed at evaluating the efficacy of the atrioverter in the termination of AF. The device was able to terminate 96% of 227 spontaneous AF episodes in a total number of 41 patients. Nevertheless, in 27% of these AF episodes involving 21 of the 41 patients, early recurrence of the arrhythmia was observed [41]. The efficacy of the atrioverter in ambulatory settings was first shown in four patients, which led to a restoration of sinus rhythm for 66 out of 85 AF episodes (78%), whereas the remaining 19 AF episodes were subsequently terminated inhospital using the device [42]. The safety of the atrioverter was evaluated in six patients with an increased risk of developing ventricular arrhythmias. These patients, who collectively received more than 350 electroshocks from the device, did not develop ventricular arrhythmias or any other complication of the new therapy [43]. The use of the atrioverter was shown to be beneficial in the treatment of AF, as both the occurrence of spontaneous AF events and the risk of developing episodes requiring pharmacotherapy decreased. The authors also noticed a decrease in the duration of long-lasting AF episodes [44]. Despite its clear beneficial effects, the use of the atrioverter was discontinued as the frequent arrhythmia recurrence requiring repeated painful cardioversion shocks was not tolerated by the patients [8, 42].

### Optogenetic rhythm control for shock-free acute AF termination

For acute ambulatory cardioversion of AF to be realized in patients, the method to terminate AF should be pain-free. AF termination should therefore not rely on high-voltage shocks but on a different method to manipulate cardiac electrical activity. One method that has proven to be able to realize acute termination of AF in a shock-free manner is optogenetics. This method concerns the expression of light-sensitive proteins to control a particular biological function by illumination of the targeted cells. One of the first applications of optogenetics involved the expression of light-gated ion channels to gain optical control over the membrane potential of neurons and therefore over their electrical activity with unprecedented precision and spatiotemporal resolution. In subsequent studies, other cell types, including cardiomyocytes, were optogenetically modified to control their electrical activity [45]. Currently, both light-gated ion channels and light-activated ion pumps are used to steer the activity of excitable cells. These so-called optotools are originally derived from microbial opsins [46]. The light sensitivity of optotools stems from the covalent bonding between an opsin and a retinal chromophore, a complex which is often referred to as rhodopsin. Upon light stimulation, the opsin-bound all-*trans*-retinal photoisomerizes into 13-*cis* retinal. This initiates a series of conformational changes, which leads to channel opening or pump activation allowing ion flow. The direction of the ion flow is determined by the properties of the optotool and the membrane potential of the target cell. Light exposure can thus either elicit a depolarizing or repolarizing photocurrent in optogenetically modified cardiomyocytes. Depending on the extent of the optically controlled shift in membrane potential, cardiomyocyte excitation can be partly or fully inhibited for the duration of illumination. This allows the use of optogenetics to interrupt reentrant circuits such as the ones occurring in atrial tachyarrhythmias, thereby terminating the arrhythmic activity to restore normal heart rhythm. Unlike conventional electrical methods, optogenetics offers the possibility to gain control over cellular electrical activity in a cell type-specific manner. This can be achieved by using cell type-specific promotors to drive optogene transcription. As a result, the optotool will only be expressed in the intended target cells, such as cardiomyocytes in case of the heart. In the context of cardioversion, this strategy prevents excitation of neurons and skeletal muscle cells and thereby avoids the pain caused by collateral activation of non-cardiomyocytes during electrical shock treatment. Other potential advantages of optical cardioversion include the absence of tissue damage caused by high-voltage shocks [47], but also the ability to target specific regions and to activate areas that would respond poorly to electrical stimulation due to structural heterogeneities (e.g., fibrofatty infiltrates) typically found in atria prone to fibrillation. Future research should point out whether and to what extent these advantages can indeed be realized via optogenetic rhythm

 

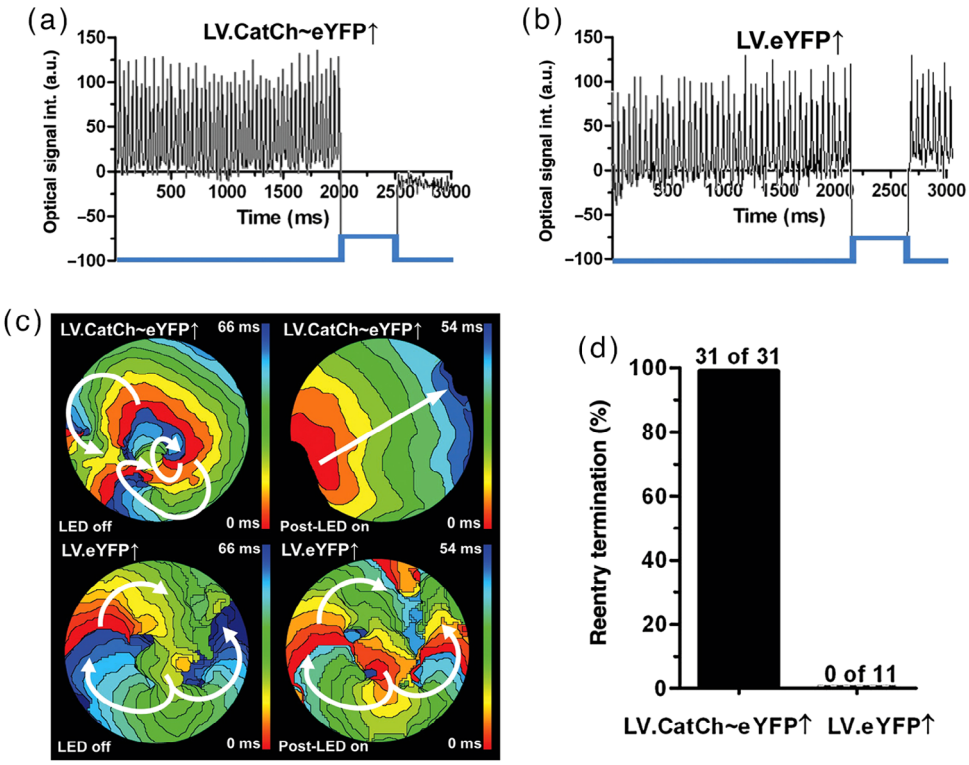


**Fig. 1** *Effective light-induced rotor termination by optogenetics in neonatal rat atrial myocyte (NRAM) monolayers. Panels A–D illustrate the termination of an electrical burst pacing-induced spiral wave in CatCh-expressing NRAM monolayers [13]. (A) Optical voltage mapping signals of an NRAM monolayer expressing enhanced yellow fluorescent protein (eYFP)-tagged CatCh or (B) eYFP (negative control sample) showing successful rotor termination only in the CatCh group after illumination using a 500 ms light pulse at an intensity of 0.038 mW/mm$^2$. (C) Typical activation maps of successful optogenetic reentry termination in a monolayer of CatCh-transduced NRAMs (top panels) but not in the control monolayer expressing eYFP (bottom panels). (D) Bar graph representing the percentage of successful reentry terminations in both experimental groups. a.u., arbitrary units; LV, lentiviral vector.*

control. Earlier research in cardiac cell cultures and rodent hearts, however, already revealed some of the favorable features of optical arrhythmia termination [9, 13, 14, 16].

### *In vitro optogenetic termination of atrial arrhythmias*

The feasibility of optogenetic termination of reentrant cardiac electrical activity was first demonstrated by Bingen et al. In this study, it was shown that stable reentrant circuits could be terminated effectively and repeatedly in monolayers of NRAMs expressing CatCh (a *Chlamydomonas reinhardtii* channelrhodopsin 2 [ChR2] variant displaying increased $Ca^{2+}$ permeability) by applying 500 ms light pulses (Fig. 1A–D) [13]. Feola et al. subsequently showed that a narrow line of illumination connecting the rotor core with an unexcitable boundary suffices to terminate reentrant waves in monolayers of CatCh-expressing NRAMs [14]. Finally, Majumder et al. provided experimental evidence on the feasibility of spatiotemporal control of spiral waves by targeting the core region of the rotor. They showed that core manipulation by dynamic patterned illumination allows attraction, anchoring, and dragging of spiral waves along predefined trajectories and could be used to annul spiral waves by directing them to non-excitable boundaries or to spiral waves of opposite chirality [15]. Alongside the proof of concept that cardiac arrhythmias could be terminated by light, the abovementioned optogenetic in vitro studies also yielded fundamental insights into cardiac arrhythmia mechanisms by exploiting the unique features of optogenetics. In favor of translational studies toward potential clinical exploration of

optogenetic termination of AF, more relevant 3D in vitro models of the diseased human atria are required in order to provide in-depth insight into the demands regarding optotool, transgene delivery, light source, and illumination pattern for safe and effective optical cardioversion.

### *In vivo optogenetic termination of atrial arrhythmia*

The feasibility of optogenetic AF termination in whole hearts was first studied by Bruegmann et al. using transgenic mice carrying an AF-promoting connexin 40 mutation and expressing ChR2 mutant H134R (ChR2-H134R) throughout the body [16]. In their study, atrial flutter or fibrillation was induced in Langendorff-perfused hearts, after which the hearts were subjected to 470 nm light pulses of 1 s and 0.4 mW/mm$^2$ covering 100 mm$^2$ of the epicardial surface of both atria. This resulted in termination rates of the atrial arrhythmias of 91% and 98% for the ChR2-H134R-positive hearts and of 13% and 25% for the ChR2-H134R-negative hearts after delivery of one and two light pulses, respectively (Fig. 2A). The authors further showed that AF could be terminated in vivo by shining 470 nm light of the same duration and intensity as in their ex vivo experiment onto the atria in open chest experiments (Fig. 2B). The authors also addressed another important translational feature—namely, optogenetic termination of atrial arrhythmias after systemic adeno-associated virus (AAV) vector-mediated ChR2-H134R gene delivery to wildtype CD1 mice. To this end, the hearts were explanted at 6–8 months post transduction and exposed to blue light pulses after AF induction. Illumination (1 s, 5 mW/mm$^2$, 100 mm$^2$) of the epicardium of both atria terminated AF with an average efficacy of 74.7% ± 9.1% in AAV-treated hearts as opposed to 24.7% ± 5.9% in untransduced hearts, whereas the spontaneous AF termination rate in illuminated ChR2-H134R-expressing hearts was 9.1% ± 3.8%.

To further explore the possible clinical application of optogenetics for AF management, Nyns et al. published a study in which optogenetic termination of AF was realized by combining several key translational aspects [9]. First, they optimized a technique called "gene painting," consisting of applying viral vector particles directly onto the atria. This local transduction method—which was pioneered by Kikuchi et al. using adenovirus vector particles [48]—significantly reduces the number of vector particles needed to genetically modify the target tissue in comparison to systemic delivery methods. It also avoids their body-wide spread, which is preferred from a safety point of view. By "painting" AAV vectors to deliver red-activatable channelrhodopsin (ReaChR) driven by a strong atrial myocyte-specific promoter (derived from human natriuretic peptide A; [NPPA] gene) onto the right atrial surface of Wistar rats, Nyns et al. reached a cardiomyocyte transduction rate of ~80% in the right atrium (Fig. 2C). Importantly, no cardiac electrophysiological abnormalities were observed in rats that had been "painted" compared to control animals. Sustained atrial tachyarrhythmia episodes (>10 s) were induced by high frequency burst electrical pacing of the atria combined with the administration of carbachol (4 $\mu$M ex vivo, 50 $\mu$g/kg in vivo). The resulting atrial flutter and fibrillation episodes could be effectively terminated by shining blue (i.e., 470 nm) light onto the right atria both ex vivo and in vivo. As previously shown by Bruegmann et al., the efficacy of atrial arrhythmia termination was influenced by the size of the area of illumination as well as by the duration and intensity of the light pulses. As expected, lower light intensities led to lower termination rates, which could be increased by applying two or three light pulses. After thoracotomy and in situ illumination of the atrial surface with an external light source, Nyns et al. achieved optogenetic termination rates of 94% for AF events and 100% for atrial flutter episodes. To further explore the clinical applicability of optogenetics in the context of AF, Nyns et al. performed optogenetic termination experiments in closed-chest conditions using an implanted light source, the activation of which relied on computer-driven automatic detection of AF. In these experiments, the implanted 470-nm LED device was combined with a reflector cup to illuminate a surface of ~28 mm$^2$ at 3.5 mW/mm$^2$ (Fig. 2D). This device was implanted in the inside of the thoracic wall facing the anterior wall of the right atrium without directly touching the heart (Fig. 2E). Once implanted, it was connected to a heart rate monitor, enabling atrial arrhythmia detection by a custom-made algorithm based on PR and RR interval irregularities in body surface ECGs (Fig. 2F). Of note, atrial flutter was not detected by this algorithm due to its inherent regularity. AF detection was followed by a preprogramed delay of 10 s before delivery of a 500 ms light pulse to ensure that AF was sustained. This resulted in a 96% success rate of optogenetic termination of AF episodes (Fig. 2G). Through these experiments, the authors demonstrated that

 

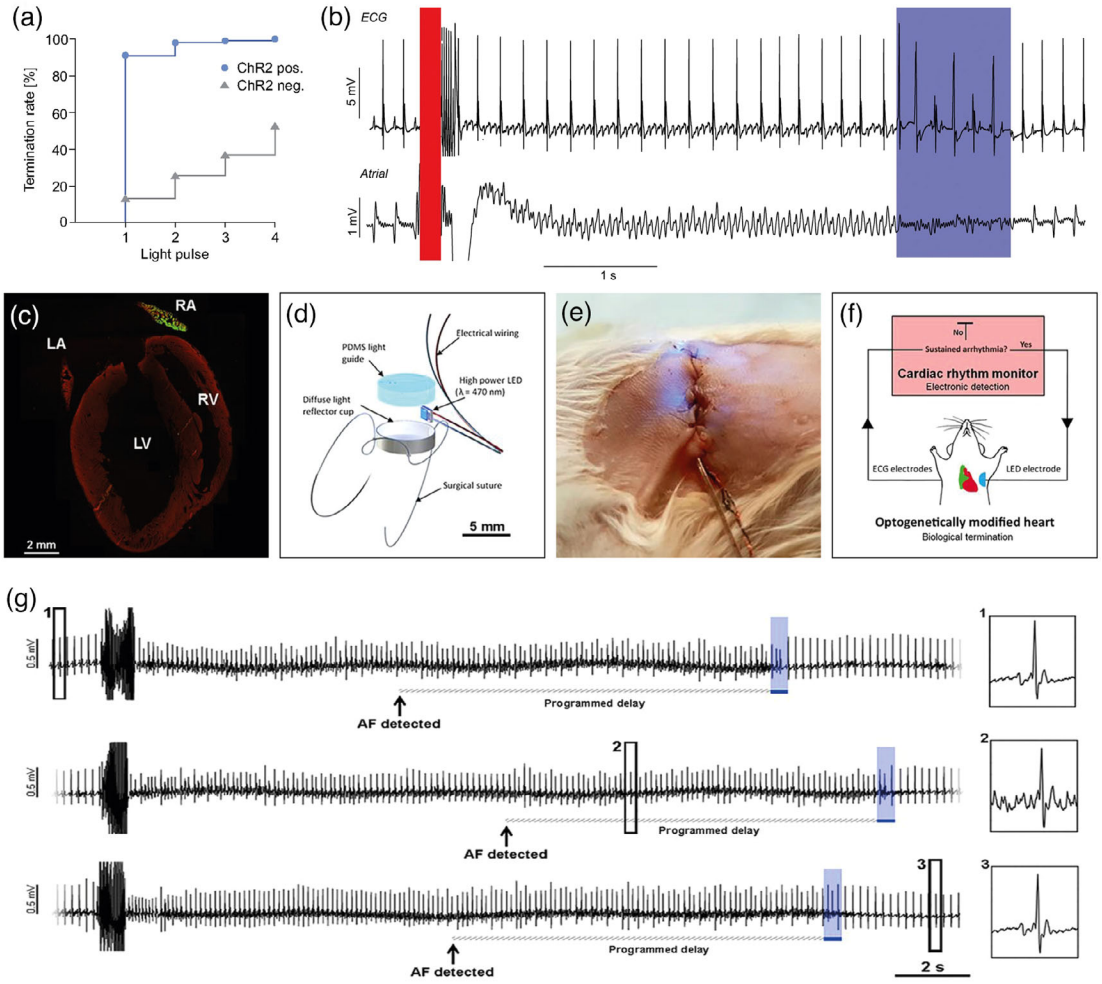


**Fig. 2** *In vivo termination of atrial arrhythmias. In vivo use of optogenetics for restoration of sinus rhythm in mice (panel A and B) and rats (panel C–G) with induced atrial arrhythmias [9, 16]. (A) Atrial fibrillation (AF) termination rates after applying 1–4 blue light pulses (1 s, 0.4 mW/mm$^2$, 100 mm$^2$) to 10 ChR2-H134R-positive hearts (blue circles; n = 101 episodes) and 6 ChR2-H134R-negative hearts (gray triangles, n = 114 episodes). (B) Electrocardiogram (ECG) (top) and endocardial atrial electrogram (bottom) showing optogenetic AF cardioversion in vivo in a ChR2-H134R$^{(+/+)}$/Cx40-A96S$^{(+/-)}$ mouse. AF was induced by high-frequency electrical stimulation (red bar) and terminated by a blue light pulse (blue bar). (C) Representative immunohistological staining of a longitudinal section of a rat heart after "gene painting" with an adeno-associated virus (AAV) vector encoding a red-activatable channelrhodopsin (ReaChR)~citrine fusion protein. Citrine (green) and cardiac troponin I (red) staining show transgene expression specifically in the right atrium. (D) Schematic view of the implantable light-emitting diode (LED) assembly. The surgical suture is shown to illustrate the fixation method. (E) Photograph of activated LED implant after surgical closure of the thoracic wall, muscle layers, and skin of an optogenetically modified rat. The electrical wires entering the wound drive the pacing electrode and LED. (F) Schematic diagram of the automated hybrid bioelectronic system consisting of the optogenetically modified right atrium (green) and a cardiac rhythm monitor. The input of the cardiac rhythm monitor consists of analog body surface ECG signals that are automatically analyzed for rhythm anomalies. Upon detection of sustained AF, an output signal is generated, switching on the implanted LED device for 500 ms. (G) Representative body surface ECG traces showing three consecutive events of successful automated in vivo closed-chest detection and termination of AF by the hybrid bioelectronic system. Upon detection of AF by the custom-made algorithm and expiration of a programed 10 s delay, the 470 nm LED implant is activated for 500 ms at 3.5 mW/mm$^2$ (blue boxes) resulting in autogenous termination of AF and subsequent restoration of sinus rhythm. Inserts highlight (1) regular sinus rhythm before optical termination of AF, (2) rapid and irregular atrial activity during AF, and (3) regular sinus rhythm after the third of three consecutive and successful attempts of optical AF termination. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.*

termination of AF and subsequent restoration of sinus rhythm could be achieved in a shock-free and automated manner by a hybrid bioelectronic system. These experiments were performed in unconscious animals, so future steps should aim at answering whether and how such a system could be applied in ambulatory settings.

### *Preparing for optogenetic termination of atrial arrhythmias in humans*

Optogenetics has been shown to be effective in terminating atrial arrhythmias in cell culture and small animal models. Its further development into a therapy for humans will depend on translational studies into the requirements for effective and safe optogenetic AF termination in patients. These studies should, for example, identify the region(s) of the (human) heart where the optogenetically induced shift in membrane potential needs to be generated for optimal optogenetic termination of AF, as this will determine both the optogene and light delivery strategy. Moreover, previous research showed that the magnitude of the shift in membrane potentials correlates with the light penetration through the myocardium and with the light sensitivity and expression level of a given optotool. Hence, considering and studying all these parameters is indispensable for a successful translation of optogenetics toward possible AF treatment in humans.

In order to study the feasibility of optogenetic AF termination in humans, Boyle et al. used patient-derived electrophysiological in silico models of fibrotic human atria reconstructed from late gadolinium-enhanced magnetic resonance imaging scans [49]. In three different models, ChR2-H134R expression was simulated in ~60% of atrial myocytes, and the efficacy of AF termination was assessed by comparing the effect of uniform light exposure of the atria with that of targeted illumination of the "critical isthmus" of the atrial tachyarrhythmia. The critical isthmus was identified by an automated, noninvasive flow-network analysis of activation patterns in patient-specific simulations. Illumination of the critical isthmus for longer than one cycle length of the arrhythmia resulted in much higher termination success rates (up to 100% with a 1000 ms light pulse) and required drastically less input power than global atrial light exposure. In a more recent in silico study from the same research group, the *Guillardia theta* anion channelrhodopsin (GtACR1) was shown to efficiently terminate AF in pathologically remodeled human atria at much lower light intensities than ChR2-H134R [50].

Nyns et al. recently studied light transmittance through tissue slices of left atrial appendages from patients [51]. In this study, the authors also showed that 567 nm light at an intensity of 0.5 $mW/mm^2$ was enough to fully activate ReaChR in optogenetically modified rat atrial tissue (Fig. 3A–C). Based on these results, applying a light intensity of 5 $mW/mm^2$ on the epicardial surface of the atria would be sufficient to fully activate the optotool in the deepest layer of the human atrial wall, which thickness is estimated at 2–2.5 mm. By successfully performing transthoracic optogenetic cardioversion of induced AF events in rats, the authors provided additional evidence for the feasibility of transmural optogenetic cardioversion of AF in humans. Collectively, these findings suggest that existing optotools and optoelectronic technologies may already be sufficient for transmural optogenetic control of atrial electrical activity in humans, thereby potentially counteracting AF.

The use of subthreshold illumination has also emerged as a potential strategy for optogenetic termination of cardiac arrhythmias. Subthreshold illumination consists in applying light pulses below the light intensity threshold needed for excitation of the optogenetically modified myocardium. Virtual and real experiments in adult mice models, together with an in silico study by Karathanos et al. using a computational model of a left human atrium with short QT syndrome, suggest that subthreshold illumination could potentially lead to atrial arrhythmia termination by prolonging the action potential duration and reducing the conduction velocity [52–54]. If these findings hold true for large AF animal models and AF patients, such a strategy could reduce the energy consumption required for optogenetic AF termination. However, care should be taken not to promote arrhythmias via such light pulses of low intensity [55].

A next step toward clinical exploration of optogenetic AF termination entails the optogenetic modification of human atrial slices. This may reveal whether and how this tissue can be optogenetically modified to gain optical control over its electrical activity and to restore normal heart rhythm after its artificial disturbance. Furthermore, additional in silico research could be performed to simulate different myocardial pathological alterations

 

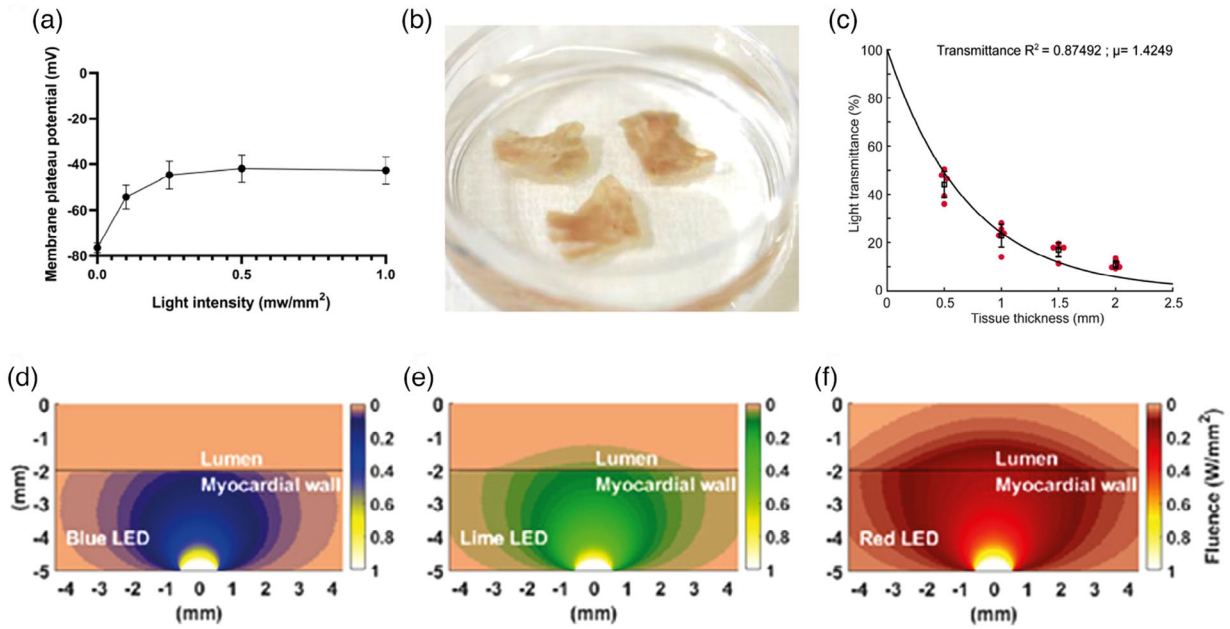


**Fig. 3** *Myocardial light penetration and optogenetic response. Evaluation of the light requirements for full optotool activation in human atria (A–C) and in silico comparison of myocardial penetration of light of different wavelengths (D–F) [51, 59]. (A) Average plateau potentials in red-activatable channelrhodopsin (ReaChR)-expressing atrial tissue of aged rats measured at the end of a 1 s lime (565 nm) light pulse of various intensities. The membrane potential depolarization saturates at 0.5 mW/mm² light intensity. (B) Tissue slices of left atrial appendage from an atrial fibrillation (AF) patient cut with a vibratome for light penetration measurements. (C) Measurements of 565 nm light penetrance in human atrial tissue samples of 0.5, 1, 1.5, and 2 mm. Experimental data were fitted to a monoexponential decay function. (D–F) Computational simulations of propagation of 470 nm (D), 567 nm (E), and 617 nm (F) light through rat myocardium.*

such as fibrosis, hypertrophy, and adipose tissue infiltration in order to study the light penetration properties associated with specific tissue compositions and organizations. The outcome of these simulations could provide insight into how light wavelength, intensity, and pattern can be tailored for a particular atrial condition in favor of optimizing optogenetic AF termination efficacy and energy consumption.

## Biological challenges to accomplish acute ambulatory AF termination by optogenetics

Optogenetics presents unique advantages for realizing shock-free termination of AF in an ambulatory manner. Nevertheless, several biological obstacles need to be overcome before optogenetic rhythm control can be considered ready for clinical exploration. First, additional research is needed regarding the desired optotool properties, including its light sensitivity, on- and off-kinetics, desensitization rate, ion conductances, intracellular trafficking, toxicity, and immunogenicity.

In addition, in terms of transgene delivery, it should become clear how to realize stable, long-term transgene expression at the target site in an effective and safe manner. These challenges will now be discussed in more detail, as well as several lines of research aimed at overcoming them.

### *Optotools*

To ensure effective optogenetic AF cardioversion, an optotool should have various characteristics including high light sensitivity, low-to-absent cellular toxicity, and electrophysiological properties compatible with human cardiac electrophysiology in general and AF termination in particular.

In order to produce the desired electrophysiological effect in cardiomyocytes, a light-activated ion channel or pump needs to be expressed at the sarcolemma. As most optotools originate from microbial organisms, they lack mammalian plasma membrane targeting signals and may encounter folding problems in mammalian cells.

Unmodified optotools are therefore often rather poorly expressed at the plasma membrane of mammalian cells and have the tendency to accumulate in compartments of the secretory pathway and/or to form intracellular aggregates. Misfolding, aggregation, and intracellular accumulation of microbial opsins can cause cellular toxicity. It is therefore important to thoroughly investigate the impact of their (over)expression on the viability and function of cardiomyocytes. Likewise, the intracellular distribution of optotools in cardiomyocytes needs to be studied and optimized if necessary. To improve transport to the plasma membrane of neurons, microbial opsins have been equipped with trafficking signals derived from the inward rectifier $K^+$ channel Kir2.1, which in many cases resulted in stronger optogenetic responses [56]. Whether these trafficking signals also improve the expression of microbial opsins at the plasma membrane of cardiomyocytes has not yet been systematically investigated. Another aspect that requires careful consideration from a functional point of view is the impact of an optotool on the intracellular ion concentrations. Most natural channelrhodopsins are nonspecific cation channels conducting a variety of different cations including protons. Due to their very high relative permeability for protons, they can acidify cells and thereby alter cellular behavior [57]. This problem can be tackled by using channelrhodopsins with low proton conductivity [58]. Channelrhodopsin activation may also lead to intracellular $Ca^{2+}$ overload. This is especially true for light-gated cation channels with high $Ca^{2+}$ conductance such as CatCh, which after prolonged overexpression caused severe cytotoxicity in NRAMs (our unpublished results). As opposed to channelrhodopsins, light-activated outward proton pumps alkalize cells, which may also negatively affect cardiomyocyte function.

As discussed above, the absorption spectrum of an optotool is also an important feature to take into account because longer wavelengths in the visible part of the electromagnetic spectrum penetrate cardiac tissue better than shorter wavelengths [59]. Accordingly, optotools that are best activated by red light can modulate electrical activity at larger tissue depths than those requiring green or blue light for maximum excitation. This has resulted in the identification and engineering of rhodopsins with redshifted activation spectra, including ReachR [60], Chrimson(R) [61], and ChRmine [62], and mutants of these proteins [63–65], to meet the requirements of particular optogenetic applications. In a recent study, the use of the ultralight-sensitive channelrhodopsin ChRmine allowed transthoracic optical pacing of mouse hearts [66].

Other desirable properties of light-gated cation channels for optogenetic termination of atrial arrhythmias include the ability to produce large plateau currents, relatively fast off-kinetics to allow a quick return to sinus rhythm after arrhythmia termination, and no or weak inactivation upon prolonged illumination or repetitive stimulation. The last issue relates to the fact that channelrhodopsins reach maximal current amplitudes when the activating light pulse is delivered after a sufficiently long dark period, which allows them to accumulate in the dark-adapted state. As a consequence, repetitive light stimulation of channelrhodopsins at frequencies relevant to the heart results in decreased current amplitudes [67]. The use-dependence of optotools is therefore a crucial parameter to consider, particularly for patients requiring frequent optogenetic interventions. In a recent study, Ördög et al. compared the performance of four different optotools in neonatal rat myocytes [68]. The findings of this elaborate study provide a framework for selecting the most suitable optotool to terminate arrhythmias. As the total photocurrent produced by an optogenetically modified cell is a function of the unitary conductance of the optotool, its expression level, and the existing ion gradients, these factors also have to be taken into account in opsin selection for terminating atrial flutters and AF.

### *Optotool expression*

Another challenge in the context of optogenetics is to force the expression of a particular optotool in the target cells. Cardiomyocytes are known to be highly resistant to physical or chemical transfection methods. Viral vector-based gene delivery, on the other hand, has been proven efficient both for in vitro and in vivo cardiac applications [69]. Lentiviral vectors (LVs) are able to transduce most proliferative as well as non-proliferative mammalian cell types, providing long-term transgene expression by stable integration of the vector DNA into the host cell genome, and possess a broad cell tropism and host range. LVs are routinely used in vitro for the transduction of primary and pluripotent stem cell-derived cardiomyocytes [70, 71]. The use of LVs is currently limited for

 

cardiac in vivo applications primarily due to their poor ability to transduce adult cardiomyocytes in vivo and their limited spread in myocardium [72]. Adenoviral vectors (AdVs) derived from human adenovirus serotype 5 can very well transduce postmitotic mammalian cells, including cardiomyocytes of rodents, pigs, nonhuman primates, and humans [73, 74]. Transgene expression from the AdV genome is inherently transient due to its episomal nature, and first- and second-generation AdVs still contain adenoviral genes, expression of which has potential cytotoxic effects in the host cell and triggers a strong inflammatory response in vivo. This problem can be (partially) overcome by the use of third-generation AdVs, which are completely devoid of adenoviral genes, and as an additional advantage of accommodating up to 37 kb of foreign DNA [75]. Despite their drawbacks, AdVs are one of the most commonly used gene delivery vectors in cardiovascular gene therapy clinical trials [73, 76]. An alternative to AdVs for in vivo transduction of (human) cardiomyocytes is the AAV-based gene therapy vectors [77]. AAV vectors have been approved as vehicles in gene therapy products for clinical use, and they are the vector of choice in an increasing number of clinical trials [78]. AAV vectors are based on a human nonpathogenic virus and do not possess viral genes. The AAV vector genome persists long-term in nondividing cells such as cardiomyocytes mainly as episomal monomeric and concatemeric circles. Therefore, unlike LVs, AAV vectors carry little risk of insertional mutagenesis due to their predominant non-integrating behavior [79]. Because AAV vectors penetrate compact tissues such as the myocardium wall rather well, they can mediate widespread transgene expression in the heart. Moreover, the engineering of cardiotropic AAV vectors with reduced liver tropism has allowed efficient myocardial transduction by systemic vector administration [80–82].

Although AdVs and AAVs are currently the best options to express a transgene in cardiac tissue, both these episomal vector systems cannot avoid transgene loss due to cell death or cell division. The latter cause of transgene loss may be of limited relevance to the human heart given the low cardiomyocyte replacement rate [83]. However, as the data on cardiomyocyte renewal in humans are based on radiocarbon dating in healthy individuals, cardiomyocyte turnover/loss may be higher in diseased hearts. To bypass the problem of transient transgene expression, optotool-encoding transposons or homologous recombination-mediated knock-in technologies could be further explored for the heart [84, 85]. The latter approach would allow targeted integration of the optogene in a specific region (a "safe haven") of the host genome, decreasing the risk of deleterious effects due to random integration of vector DNA.

The delivery method of viral vectors to the heart represents another critical factor for successful long-term and stable optogenetic modification of the myocardium. Systemic injection of AAV vector particles has been shown to be efficient in small animals and piglets for cardiac applications. Nevertheless, such an approach in patients would require a very large number of vector particles and is therefore unlikely to be pursued in the near future given the (prohibitively) high costs of vector production and the undesired spreading of vectors beyond the targeted tissue. This situation may change with the further sophistication of methods to select/engineer new, highly cell type-specific AAV capsids [86, 87]. As mentioned above, the application of viral vector particles directly onto the epicardial side of the atria through "gene painting" offers a potential alternative to systemic injection. This approach has not only been successfully applied in rats [9] but also in rabbits [88] and pigs [89], suggesting that it might also be used in humans. The downside of this strategy is that it requires, for now, a mini-thoracotomy to gain access to the atria. Moreover, the possible long-term effects of the damage inflicted to the epicardial mesothelium during the "gene painting" procedure require further investigation, as does the possible difference/gradient in transgene expression level between the epicardial and endocardial site of the atrial wall. Although not performed in the context of atrial "gene painting," a recent study demonstrated that ultrasound-guided gene delivery by ventricular intramyocardial injection allowed for optogenetically modifying cardiac ventricles in rats [90]. This administration method led to high-enough ReaChR expression in the ventricles to optogenetically terminate ventricular fibrillation events and to restore sinus rhythm in these animals. Such an approach could therefore also be explored for the local delivery of vector particles to the atria.

### *Immune response management*

In order to achieve a sustained expression of a transgene and to ensure the preservation of the

 

cardiac tissue itself, it is crucial to minimize gene therapy-related immune responses. These immune responses can be directed toward the transgene product (optotool) or toward the vector capsid and, in the case of AdVs, also toward the adenoviral proteins expressed from the vector genome. This could decrease the transduction efficacy by direct neutralization of the vector particles or by their opsonization and subsequent phagocytosis, lysis, or agglutination. In addition, a cellular immune response could be triggered toward targeted cells presenting residual viral components or (parts of) the transgene product itself.

Although AdVs very efficiently transduce a large variety of human cell types, first- and second-generation vectors in particular are known to trigger strong innate and adaptive immune responses, which limit their suitability for in vivo applications. The cell-mediated recognition of adenoviral capsid components or nucleic acid molecules occurs by Toll-like receptors and other pathogen recognition receptors [91–94]. This induces the release of pro-inflammatory cytokines and chemokines, inhibits adenovirus replication (by establishing a global antiviral state), attracts innate immune cells, and activates cells of the adaptive immune system to mount protective humoral and cellular antiviral responses [95, 96]. Moreover, due to the high prevalence of adenovirus infections among humans, patients to be treated with AdVs may possess antibodies directed against adenovirus capsid components that could limit the efficiency of AdV-mediated gene transfer [97].

AAV infections are also common among humans and trigger both innate and adaptive immune responses [98–100]. As a consequence, many humans have AAV-neutralizing antibodies, which can lead to a decreased in vivo transduction efficiency of AAV vectors [101]. Moreover, AAV vectors induce a large repertoire of innate, humoral, and cellular antiviral immune responses [101, 102]. The adaptative immune response to AAV-transduced cells can lead to their clearance, which in the case of cardiomyocytes would negatively impact cardiac function. Importantly, presentation of AAV capsid components at the surface of transduced cells by class I and II major histocompatibility complex proteins is transient due to the gradual breakdown of the capsid proteins. Therefore, immune suppression during the period that AAV vector particles are lingering in the body should help to dampen the immune response to the gene therapy.

As microbial opsins are foreign proteins to the human body, they could induce a cytotoxic T cell response. This would result in elimination of the optogenetically modified cells accompanied by a strong inflammatory reaction inflicting damage to the heart muscle. In order to prevent such detrimental effects, one could administer immunosuppressants such as the mTOR inhibitor rapamycin [9].

However, for clinical translation, the immune response-related issues most likely require more advanced solutions. The problem of preexisting (neutralizing) antibodies directed against the gene delivery vehicle can be solved by serological screening of patients and subsequent use of vector serotypes for which patients are naïve. Alternatively, viral vector-specific antibodies can be removed by plasmapheresis or immunoabsorption [98, 99, 103]. Chemical shielding, encapsulation into exosomes, or engineering of capsids displaying low immunogenicity could also help vector particles to escape antibody recognition. Moreover, due to the relation between the purity of the vector dose and the severity of the immune response, it is important to rigorously purify the vector particles (e.g., to remove producer cell contaminants and empty capsids) and to use vector preparations with a high specific activity (i.e., a low physical particle to transducing unit ratio).

A major threat for successful application of optogene therapy is the expression of the transgene in professional antigen-presenting cells (APCs). Several strategies can be employed to avoid this from happening, including the use of viral vectors that selectively bind to target cells but cannot enter APCs (i.e., transductional targeting) and the use of cell type-specific promoters to drive transgene expression (i.e., transcriptional targeting). Suitable promoters for cardiomyocyte-restricted transgene expression can be derived from, among others, the NPPA [9], TNNT2 [104], MYH6 [90], and CKM gene [13]. A third option to avoid transgene expression in APCs is to incorporate recognition sequences for microRNAs that are expressed in APCs but not in the target cells in the 3′ untranslated region of the transgene (i.e., posttranscriptional targeting) [98, 99]. Finally, as the delivery route strongly influences to which extent vector particles encounter

 

APCs, local administration (e.g., through "gene painting") is preferable over systemic infusion.

Moreover, various means of immunosuppression could be employed to overcome immune barriers impeding gene therapy. For example, targeted blockage of proinflammatory cytokines (e.g., interleukin 1, interleukin 6, and type I interferons), monoclonal antibodies disrupting the complement cascade, T-cell co-stimulation blockers, or transient mTOR inhibition may represent additional tools to subdue deleterious innate and adaptive immune responses [99, 103]. Strategies aimed at inducing immune tolerance could also be used to prevent immune rejection of optogenetically modified cells [98, 103]. In this context, it has been shown that the presentation of an antigen to the liver can provide tolerance and even abolish preexisting immune responses to this antigen [105]. For a more extensive coverage of this topic and additional ways to reduce AAV vector-related immunity, see [98, 99, 103].

In summary, further research is needed in order to optimize optotools, their myocardial delivery, and their durable expression for an optimal atrial arrhythmia termination efficacy. All kinds of pharmacological and molecular strategies are currently being pursued and may help to overcome the remaining biological hurdles for the future clinical implementations of optogene therapy. Apart from coping with the biological challenges, realization of optogenetic cardioversion of AF for long-term ambulatory application also requires a number of technological challenges to be dealt with.

## Technological challenges to accomplishing acute ambulatory AF termination by optogenetics

As previously stated, implantable devices have been proven safe and effective in monitoring and controlling cardiac rhythm [7, 8]. As a result, we believe that the generation of an implanted optical defibrillator (IOD), substituting electrical shocks with light pulses, could partially rely on the conventional technological design of devices such as ICDs and the atrioverters.

Although the optogenetic termination of AF has shown promise in small animal models, implementing this technology for ambulatory use in humans poses several technological challenges. In this section, we will address the current obstacles that need to be overcome to develop IODs for clinical application. These challenges encompass, among others, atrial arrhythmia detection, efficient light delivery, biocompatibility, and power supply. To tackle these issues and pave the way for future human applications, we will explore potential solutions based on existing technologies and discuss their feasibility.

### *Atrial arrhythmia detection*

A reliable detection method of atrial arrhythmias is critical for successful implementation of an automated ambulatory optogenetic cardioversion system of atrial arrhythmias. Conventional atrioverters measure the global electrical cardiac activity by sensing electrodes located in the same leads as the electrodes delivering the electrical shock [106]. For the optical cardioversion approach, the most efficient way to deliver light is to place LEDs in direct contact with the epicardium. This implies that there would be no direct electrical connection between the heart and the device. Therefore, alternative measurement methods for atrial arrhythmia detection should be adopted in the context of IODs. An attractive possibility would be to overlay the high-density LED array with a high-density transparent electrode array for direct epicardial positioning [107]. Such an integrated system would allow precise spatiotemporal monitoring and modulation of the cardiac bioelectricity. Recently published array systems for these purposes could be integrated into the IOD design [108–110].

Ideally, an IOD should be able to detect all types of atrial arrhythmias, including AF and flutter events. Out of the 16 AF detection algorithms reported from clinical applications, only four can detect both AF and atrial flutter [111]. This is because most of the detection algorithms are based on RR interval variability, whereas atrial flutter events often present a stable RR interval. Considering that flutter events could evolve into AF, it is necessary to design algorithms allowing efficient detection of both AF and flutter [112, 113]. Based on human digital body surface ECG recordings, new strategies for atrial arrhythmia detection involving artificial intelligence (AI) have recently been developed. These strategies allow the detection of all atrial arrhythmia subtypes (including atrial flutter and AF) at an average accuracy close to 95%, whereas an average accuracy of only 87% was reported for the existing commercial algorithms [111, 114]. Moreover, the use

of AI allowed the prediction of atrial arrhythmias before their occurrence at an accuracy of 83.3% [115]. Additionally, with the development of wearable electronics, AI-based detection algorithms could be trained using different data sources including but not limited to body surface ECGs, noise-coupled ECGs, photoplethysmograms, and mechanical recordings [116–118]. Therefore, AI-based algorithms provide an attractive option for the detection of atrial tachyarrhythmias in the context of IOD development. Such an AI method would have to be specifically trained with the recording method chosen for IODs, as mentioned above. However, as AI models need to be extensively trained before reaching high accuracy [119], this will require the acquisition of a large and annotated database for training.

### *Sufficient light delivery*

To effectively activate an optotool and terminate arrhythmias in vivo, sufficient light should be delivered in order to penetrate the atrial wall [60]. Based on previous work, the estimated light intensity to perform an optical cardioversion should be at least 5 $mW/mm^2$ to fully activate an optotool at the deepest layer of a human atrial wall using 565 nm light [51]. This intensity is five times higher than noon sunlight under a clear sky in the summer [120]. High-power micro/mini-LEDs (mLEDs) are currently the best option for delivering high-intensity light in an implantable form, due to their small size, longevity, high-power density, and high energy efficiency (therefore limiting heat production). A single blue mLED can reach an efficiency of 60%, providing a maximum of 10 $mW/mm^2$ light intensity at the focal plane [121]. By arranging the mLEDs in the form of arrays, customized light patterns could be created for different patient situations.

Along with the high light intensity required, the choice of the wavelength of the light is another key factor for successful termination. As previously mentioned, light of different colors differs in their ability to penetrate (heart) tissue (Fig. 3D–F) [59]. It is therefore crucial to consider this parameter, acknowledging that the light needs to fully penetrate the human atrial wall (with a thickness of approximately 2 mm) [122]. Apart from penetrance, the efficiency of LEDs varies for each wavelength depending on the junction materials and light generation methods, with blue LEDs having the highest efficiency [123]. Thus, the penetrance of the light and the efficiency of the light source should both be taken into consideration in the selection of the optotool and illumination strategy.

### *Biocompatibility and coating materials*

To ensure reliable optical termination of atrial arrhythmias in patients, the light should be delivered straight onto the heart. This means that the LEDs need to be placed in close contact with the myocardium. As a result, similar to atrioverters, the electrical components as well as the mLEDs have to be implanted into the patient's body. In order to avoid electrical shorting and ionic contamination, the electrical components of the IOD need to be isolated from the body fluids [124]. Additionally, the substrate and coating materials of mLEDs should be flexible to maintain electrical connections and alignment of mLEDs to the targeted areas, as the heart and body move constantly. Soft materials, such as polydimethylsiloxane (PDMS), polyimide, and parylene C (poly-chloro-*p*-xylylene), have proven to be stable in simulated body environments during extended periods of up to 20 months [125–127]. PDMS implants remained stable in a clinical study of 180 days [128]. Moreover, soft materials enable origami-folding techniques which would decrease the size of the mLED array structure during its implantation and could allow a less invasive surgical procedure [129, 130]. Alongside electrically isolating the device from the body, the physiological response secondary to the implantation of a foreign body can potentially lead to inflammation and tissue remodeling, including the development of fibrosis and encapsulation of the device components [131]. Such tissue remodeling could, when occurring in the myocardium, lead to new arrhythmogenic substrates and decrease the cardioversion efficacy of the optoelectronic system. By choosing the right biocompatible materials and using steroid-eluting coatings, the device could last for a long time and ideally as long as needed [131, 132].

### *Power consumption optimization*

A normal electrical AF cardioversion via the atrioverter requires on average 3 J of energy per shock [7]. However, if global atrial illumination is needed for optogenetic cardioversion of AF, by applying a 500 ms light pulse on the whole human atria (representing a surface area of approximately 180 $cm^2$), it would require more than 90 J of energy [133]. This estimation is based on the light intensity of 5 $mW/mm^2$ required to obtain the maximum

 

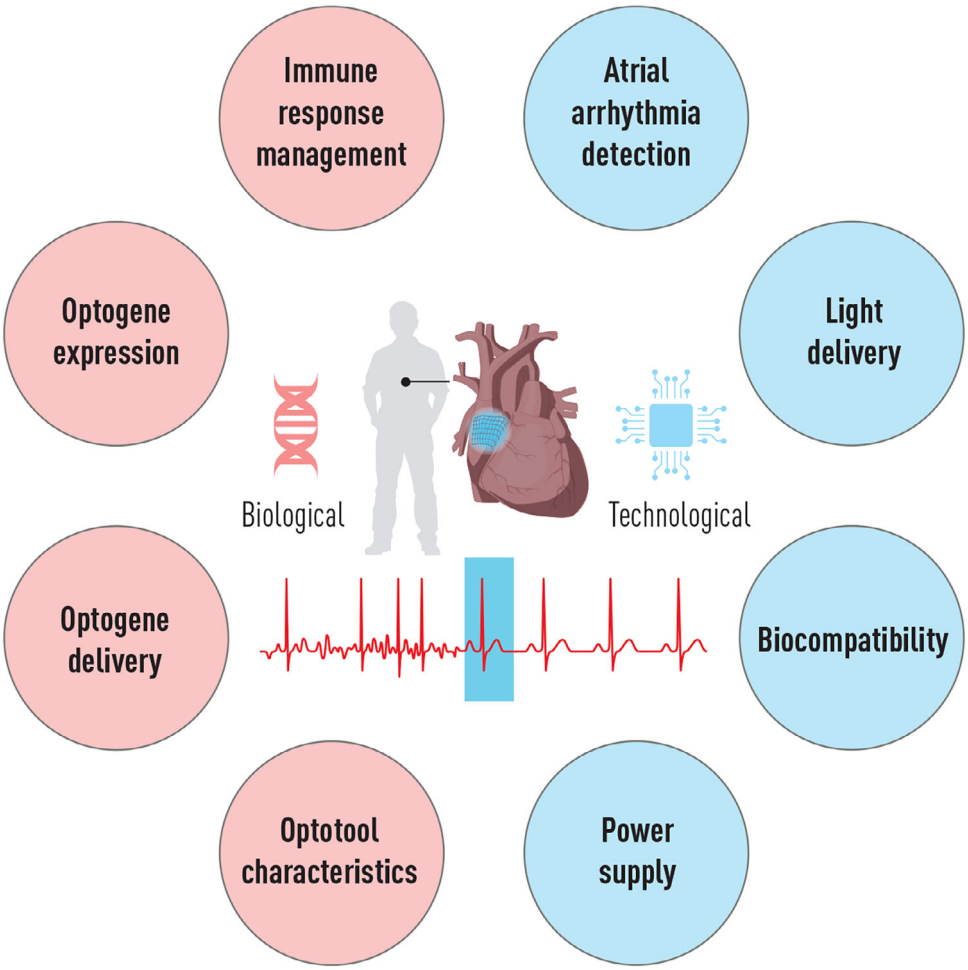


**Fig. 4** *Biological and technological challenges toward acute ambulatory atrial fibrillation (AF) termination by optogenetics. Graphical representation of the different biological and technological challenges that need to be overcome for translational application of optogenetics in AF termination.*

electrophysiological effect in atrial cardiomyocytes considering the thickness of the human atria, as previously mentioned [51]. However, as already shown in in vitro studies, specific local illumination patterns also had a high atrial arrhythmia termination efficacy [14], for example, using a striped illumination pattern resulted in a similar optogenetic termination efficacy as a global atrial illumination. The use of tailored illumination patterns would drastically reduce the power consumption of the IOD, thereby favoring longer battery life. Moreover, this would also reduce the viral vector dose, as now only the LED-illuminated area would be transduced to express an optotool, further adding to the cost-efficiency of such a targeted intervention. Nonetheless, we believe that further research is needed to identify the most suitable illumination patterns for optogenetic termination of atrial arrhythmias in vivo.

Apart from reducing energy consumption, another way to delay or avoid surgical battery replacement could be achieved by the design of a rechargeable system. The battery of IODs could be recharged using energy harvesting methods, such as WPT, nanogenerator, or ultrasound [134–136]. These power harvesting strategies could allow the patients to recharge their IODs by transcutaneous energy transfer during the night or even to harvest energy ambulatory [124]. Overall, power harvesting methods for IODs could solve the high-power demand issue associated with such systems,

would enable further miniaturization of IODs, and could avoid battery replacement procedures.

## Conclusion

Despite the progressive and detrimental nature of AF, its current repertoire of treatment options lacks the possibility of immediate restoration of sinus rhythm in an automated manner outside of the hospital. For such acute ambulatory cardioversion, the mode of AF termination should be pain-free and therefore not rely on high-voltage electrical shocks. Optogenetics enables such pain-free cardioversion through the targeted expression and activation of light-sensitive ion channels or pumps in the atria, thereby effectively replacing the electrical shock by a light pulse for AF termination. Although the experimental results so far are promising, a number of biological and technological issues still need to be addressed before the full translational potential of optogenetics can be determined and clinical exploration can be considered (summarized in Fig. 4). These mainly concern sustained non-toxic optogene expression at a sufficient level and harmless and appropriate light delivery for safe, effective, and durable optoelectronic rhythm control. Further research into these matters is warranted given the high potential to realize a pain-free method for acute ambulatory cardioversion of AF, thereby profoundly impacting the quality of life of AF patients and associated health care costs.

## Author contributions

Vincent Portero and Shanliang Deng equally contributed to this review. Antoine A. F. de Vries and Daniël A. Pijnappels equally directed this review. All authors critically reviewed this manuscript.

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## Conflict of interest statement

None of the authors have conflicts of interest to declare.

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