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“Beauty is a Light in the Heart”¹: The Transformative Potential of Optogenetics for Clinical Applications in Cardiovascular Medicine

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Abstract

Optogenetics is an exciting new technology in which viral gene or cell delivery is used to inscribe light sensitivity in excitable tissue to enable optical control of bioelectric behavior. Initial progress in the fledgling domain of cardiac optogenetics has included *in vitro* expression of various light-sensitive proteins in cell monolayers and transgenic animals to demonstrate an array of potentially useful applications, including light-based pacing, silencing of spontaneous activity, and spiral wave termination. In parallel to these developments, the cardiac modeling community has developed a versatile computational framework capable of realistically simulating optogenetics in biophysically detailed, patient-specific representations of the human heart, enabling the exploration of potential clinical applications in a predictive virtual platform. Towards the ultimate goal of assessing the feasibility and potential impact of optogenetics-based therapies in cardiovascular medicine, this review provides (1) a detailed synopsis of *in vivo*, *in vitro*, and *in silico* developments in the field and (2) a critical assessment of how existing clinical technology for gene/cell delivery and intra-cardiac illumination could be harnessed to achieve such lofty goals as light-based arrhythmia termination.

Optogenetics is an emerging biomedical technology in which light-sensitive proteins (opsins) functioning as ion channels, ion pumps, or signaling receptors, are expressed in excitable tissue to enable precise, localized, and low-energy optical control of cellular behavior (1-3). This approach has been widely adopted in experimental neuroscience (4) and earnest exploration of novel clinical applications in neural engineering has already begun, including potential light-based treatment methods for epilepsy, depression, and Parkinson's disease (5). In contrast, researchers working in the cardiovascular field have been relatively slow in embracing the idea that optogenetics might be a valuable tool for new clinical applications (6,7). Light-based stimulation could overcome several limitations of

¹Excerpt from a quotation attributed to the poet Khalil Gibran, 1883–1931.

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conventional electrotherapy for arrhythmia treatment; for example, since optogenetics enables selective stimulation of tissue targeted for opsin expression, optical defibrillation could circumvent the excitation of skeletal muscles surrounding the heart during high-energy shocks, thus alleviating the pain associated with it (8). Moreover, the amplitude and duration of electrical stimuli are limited by electrochemical reactions at the pacing site (9); since optogenetics-based control of membrane behavior does not involve Faradaic charge transfer, novel and potentially useful stimulation regimes are possible, such as continuous low-amplitude depolarization.

The first steps towards such novel clinical applications are being taken in the research community. Light sensitization and optical modulation of behavior has been demonstrated *in vitro* in mammalian heart cells and tissue and in the beating hearts of transgenic mice; simultaneously, a major effort has been invested in the development of a comprehensive infrastructure to enable predictive computer simulations of cardiac optogenetics in biophysically detailed multi-scale models, ranging from light-sensitized cells and monolayers to patient-specific models of the entire heart (10). This article begins with a review of the most relevant findings in the latter two areas, with particular emphasis on information of immediate relevance to the development of clinical applications for cardiac optogenetics. Then, we assess the landscape of techniques already in use or under development that could be employed in the clinic for light sensitization of heart tissue and intra-cardiac illumination; the latter approaches are today's building blocks for tomorrow's potential optogenetics-based therapies. Finally, we provide concrete examples of light-based clinical approaches that might be enabled by this technology based on speculative *in vitro* and *in silico* experiments that have already been carried out.

Light Sensitization of Cardiac Cells and Tissue *In Vitro*

Much of the early work in cardiac optogenetics has been concerned with the use of channelrhodopsin-2 (ChR2), a non-selective cation channel that produces depolarizing transmembrane current in response to illumination with blue light (3). Since ChR2-mediated photocurrent (I_{ChR2}) is sufficient to elicit action potentials (APs) in transduced (i.e., ChR2-expressing) mammalian cardiomyocytes, many studies have sought to demonstrate the usefulness of optogenetics-based stimulation as an alternative to the traditional electrical methods for cardiac tissue excitation. The first such study was conducted by Bruegmann et al. (11), who generated a stable line of light-sensitive mouse embryonic stem cells (ESCs) via electroporation-based transfection with a plasmid carrying the ChR2 gene. ESCs were then differentiated into spontaneously beating embryoid bodies consisting of light-sensitive cardiomyocytes. This preparation was used to demonstrate that blue light could be used to modify electrical behavior, either by entraining or accelerating activation rate (using pulsed or continuous illumination, respectively). In the same study (11), transgenic mice were generated from the ChR2-rich ESCs and employed in a proof-of-concept experiment for optogenetics-based pacing *in vivo*. Specifically, in an open-chest configuration, the right atrium was illuminated with pulses of blue light from a focused LED; this resulted in ectopic atrial pacing (as evidenced by altered P-wave morphology) and entrained ventricular activation at a rate higher than sinus rhythm (450 beats per minute [bpm], versus control rate 350-400bpm; see Fig.1A). Moreover, illumination at three different locations in the

ventricles resulted in three unique QRS complex morphologies (Fig.1B). In a subsequent study (12), Arrenberg et al. confirmed the feasibility of optogenetics-based cardiac pacing in transgenic animals using a zebrafish model.

The next milestone in the field was achieved by Abilez et al. (13), who used a lentiviral transfection to express ChR2 in a line of human ESCs. These cells were then differentiated into light-sensitive cardiomyocytes, which were plated on microelectrode arrays; blue light pulses applied to these preparations were shown to induce one-to-one entrainment of electrical activation and mechanical contraction.

Notably, not all approaches for the inscription of light sensitivity in heart tissue involve ChR2-expressing myocytes as in the studies above: it is also possible to develop a separate line of opsin-expressing donor cells which can then be co-cultured with normal cardiomyocytes (or, potentially, grafted into heart tissue) to form an optically excitable heterocellular syncytium. Jia et al. (14) pioneered this tandem cell unit (TCU) technique using ChR2-rich human embryonic kidney cells (HEK+ChR2) to photosensitize monolayers of neonatal rat ventricular myocytes (NRVMs). Functional electrical coupling was attributed to the formation of gap junctions between cells in donor and host tissue regions; this assertion was supported by the fact that connexin-43 was expressed abundantly in co-cultured HEK+ChR2 cells compared to control HEK cells. Importantly, the authors also demonstrated the feasibility of using both optogenetics-based stimulation and optical mapping of transmembrane voltage in the same tissue preparations. Subsequent work by Nussinovitch et al. (15) provided further support for the applicability of TCUs in cardiac optogenetics by showing that ChR2-expressing NIH-3T3 fibroblasts (Figs.2A-B) were an equally feasible vehicle for inscribing light sensitivity in cardiac tissue. This study also demonstrated the possibility of bi-directional light-based control of membrane potential (i.e., both depolarization and hyperpolarization) in cardiac cell monolayers using a combination of excitatory and inhibitory opsins. In addition to ChR2, the authors transduced donor cells with ArchT, a proton pump that produces hyperpolarizing current in response to red-light illumination (Fig.2C). This enabled the use of light stimuli with non-overlapping spectra (i.e., light used to excite ChR2 did not affect ArchT and vice-versa) to optically pace (Fig.2D; pulsed illumination) or silence spontaneous activation (Fig.2E; constant illumination) in the same hybrid monolayers of NRVMs and 3T3+ChR2+ArchT cells.

This type of optogenetics-enabled selective control of bioelectric excitation in cardiac cell monolayers has yielded innovative protocols for high-throughput *in vitro* identification of specialized tissue regions. Arrenberg et al. (12) used transgenic zebrafish expressing both ChR2 and the inhibitory opsin Halorhodopsin (NpHR, a chloride pump that generates outward membrane current when activated by orange light) to demonstrate dynamic light-based modulation of heart rate. Patterned application of illumination in a 24-point grid covering each heart then enabled accurate, automated identification of sinoatrial and atrioventricular node locations. Although it remains unknown whether this type of approach could be used to map conduction system structures in larger, more structurally complex hearts, successful expression of inhibitory opsins in mammalian cell culture (15) suggests that it may be a fruitful avenue for future research.

A recent development in the optogenetics field is the use of opsins that impart light-based control of cell signaling as opposed to transmembrane potential modulation; for example, melanopsin is a photosensitive g-protein coupled receptor (GPCR) that is natively expressed in the human eye, but not in the heart. Beiert et al. (16) produced mouse ESCs expressing this opsin, which were then differentiated into spontaneously-beating cardiac cell monolayers. In these preparations, pulses of extraordinarily low-energy blue light illumination (1,000 times less bright than the stimuli used to excite ChR2) perturbed G_q signaling, leading to distinct intracellular Ca^{2+} “puffs”; this in turn led to irregular pacemaker activity and arrhythmia. Such techniques could facilitate selective, repeatable, and in-depth probing of the regulatory mechanisms that control critical subcellular networks such as the calcineurin/NFAT-dependent pathway, which mediates atrial fibrillation (AF)-associated downregulation of L-type calcium channel expression, leading to arrhythmogenic abbreviation of atrial AP.

Finally, it is noteworthy that molecular optimization of opsins to achieve more favorable characteristics for neuroscience applications has led to an ever-growing “toolbox” of components that could have a major impact on cardiac optogenetics. A recently developed ChR2 variant that could prove highly useful for cardiac optogenetics is ReaChR, which has 2-5x larger photocurrent and opens in response to red-light illumination, which has a higher penetration depth ($\approx 2\times$) than the blue light required to optically stimulate ChR2 (17). Both of these attributes are relevant to cardiac optogenetics applications, since they promise the ability to elicit robust excitatory current in cells deeper inside opaque heart tissue, and with lower input energy (7); however, it should be noted that ReaChR has very slow channel kinetics compared to ChR2 (17), which could lead to significant AP prolongation, making this opsin a poor choice for cardiac pacing applications. Other new types of ChR2 that remain to be tested for cardiac applications, including a red light-sensitive variant with faster kinetics (Chrimson) (18), may be able to overcome this problem.

Biophysically Detailed Cardiac Optogenetics Modeling

Like many other aspects of modern cardiac research and therapy (19), the field of optogenetics stands to benefit greatly from the development and application of multiscale computational models. The first step towards such models is to characterize the biophysical behavior of opsins in response to illumination in the physiological milieu; paralleling *in vitro* applications, efforts towards this type of description have thus far focused on the characteristics of ChR2. In their seminal work, Nagel et al. (1) identified ChR2 as a light-sensitive ion channel permeable to Na^+ , Ca^{2+} , and K^+ and suggested that its molecular properties could be explained by a photocycle with at least one conductive (open) state and two closed states with different degrees of refractoriness. Bamann et al. (20) then studied the spectral absorption characteristics of ChR2 and showed that illumination with blue light ($\lambda=470-490\text{nm}$) resulted in maximal activation of the light-based gating mechanism. Analysis of voltage-clamped cells expressing ChR2 revealed that I_{ChR2} magnitude was potentiated by the irradiance (energy content) of the applied light stimulus (21); the same study concluded that a four-state Markov model with two open states of differing ionic conductance was required to accurately represent I_{ChR2} morphology. Most recently, Williams et al. (22) quantitatively characterized I_{ChR2} in HEK cells and studied light-

induced APs in ChR2-rich guinea pig ventricular myocytes to derive the most rigorously validated ChR2 model to date, including detailed representations of both light- and voltage-sensitive properties. As anticipated by previous studies (1,21), measurements suggested a four-state ChR2 photocycle (Fig.3A). Close examination of I_{ChR2} dynamics indicated that illumination applied to dark-adapted channels (state C1) initiated a rapid (millisecond-scale) transition to a high-conductance state (O1); with sustained illumination, ChR2 transitioned to a lower-conductance (light-adapted) state (O2), giving rise to a current profile with distinct peak and steady-state phases (Fig.3B&C). Importantly, the authors of this study also observed and incorporated in their model strong current rectification at membrane potentials above 0mV (the reversal potential of ChR2), which was shown to be particularly important to correctly represent I_{ChR2} contributions during the upstroke, notch, and plateau phases of light-triggered APs in cardiac cells (Fig.3D). The model was thoroughly validated using a novel optical AP clamp methodology, which was described in detail in a subsequent report (23).

Incorporating light-sensitive current models in organ-scale simulations of cardiac behavior to simulate optogenetics in the intact heart is a non-trivial task that must be carefully approached to properly account for all key experimental observations. Boyle et al. developed a comprehensive yet flexible framework to accurately represent opsin delivery and illumination in biophysically-detailed models of any spatial scale, ranging from single cells to the beating heart (10). This process necessitated novel developments at all levels of the existing multi-scale hierarchy for modeling cardiac electrophysiology (Fig.4). At the cell level, a detailed model of I_{ChR2} was added to the set of equations representing the ionic currents governing trans-membrane behavior in light-sensitive cells. By adjusting the specific formulations used for non-optogenetic currents, different delivery modes were modeled; for viral gene delivery (GD), ChR2 was directly expressed in myocytes (as in transgenic animals) while for cell delivery (CD), ChR2 was expressed in donor cells (as in the TCU technique). At the tissue level, a stochastic algorithm was used to generate spatial distributions of light-sensitive cells that reflected the wide variety of heterogeneous patterns that have been observed *in vitro* for gene and cell therapy (24-26). Finally, at the organ level, an experimentally-validated exponential decay approximation of light attenuation due to energy absorption and photon scattering in blood and heart tissue (27) was used to ensure that the three-dimensional distribution of optical energy delivered by simulated light sources was realistic. Notably, this approximation did not incorporate the effect of backscattering, which is known to give rise to a sub-surface peak in the light energy delivered ≈ 0.2 - 0.8 mm below the illuminated surface (28,29). In theory, a Monte Carlo approach could be incorporated in the simulation framework to represent this effect; however, it is unclear whether the greatly increased computational burden would be worthwhile since the primary light-related constraint for optogenetics-based stimulation is the fact that visible light penetrates very poorly in cardiac tissue, a detail modeled adequately by the simple exponential approximation and others, such as the steady-state photon diffusion equation (27).

The individual components (photosensitive current characteristics, distribution of light-sensitive cells resulting from GD or CD, light attenuation) of the cardiac optogenetics

simulation framework discussed above were firmly grounded in experimental observations and, when possible, directly validated by *in vitro* findings (10). Nonetheless, it must be emphasized that many physiological factors remain unknown (e.g., whether light-sensitivity can be successfully inscribed in mammalian hearts *in vivo* and, if so, whether the resulting expression rate will be adequate to enable light-based control of membrane behavior). As such, a critical advantage of the modeling framework is that it was developed as a set of modular enhancements to the existing platform for cardiac simulations; this approach ensured that individual components of the framework, such as the particular opsin representation or the light attenuation model, were interchangeable with new versions, e.g., different light-sensitive protein behavior and/or illumination properties (10). Consequently, as new developments emerge in the field of optogenetics—including, but not limited to, characterization of opsin delivery to the heart *in vivo*—they can be integrated in simulations on an as-needed basis.

In addition to insights regarding potential clinical applications of cardiac optogenetics, which will be discussed at the end of this review, simulations based on the above-described framework (10) have already yielded important findings regarding the basic determinants of optical stimulation efficiency (i.e., the threshold level of light energy required to elicit a propagating electrical response) in ChR2-expressing heart tissue (10). Modeling showed that both delivery mode and the spatial distribution of light-sensitive cells affected the value of this threshold. Specifically, the optical efficiency of tissue with ChR2 expressed by GD was predominantly determined by the overall density of transduced cells; in contrast, for CD, there was an efficiency penalty associated with low-density diffuse spatial patterns of light-sensitized tissue due to the reduced interface between ChR2-expressing donor cells (which acted as current sources during illumination) and surrounding host myocytes (which acted as sinks). As such, simulations suggest that the CD approach would be more suitable for applications where light-sensitive cells were highly clustered (e.g., as the result of a myocardial injection); preliminary *in vitro* work in cardiac cell monolayers with ChR2 expressed by GD and CD supported this model prediction (30).

Feasibility of *In Vivo* Light Sensitization Via Gene Or Cell Therapy

Although significant progress has been made in the development of reliable *in vitro* methods to inscribe light sensitivity in cardiac tissue, it remains unknown whether such procedures can be employed to achieve robust opsin expression in patient hearts. Ambrosi et al. (30) performed a direct injection of adeno-associated virus (AAV) carrying the gene for green fluorescent protein (GFP) in the heart of an adult rat; upon examination 4 weeks post-procedure, GFP expression was observed in the vicinity of the injection site. This offers preliminary evidence that gene therapy might be a viable avenue for *in vivo* opsin delivery in other mammalian hearts, including those of humans. AAV-based techniques have been used to induce safe, long-term cardiac expression of proteins unrelated to optogenetics (e.g., SERCA2a – the ionic pump responsible for replenishing sarcoplasmic reticulum Ca^{2+} stores) (31). Several different *in vivo* GD methods have been used effectively, including direct injection into the myocardium (24,30), systemic delivery via intravenous infusion (25), and application of viral vectors to the epicardial surface by “painting” (32). However, as discussed in the previous section, there are major concerns regarding the spatial

distribution of opsin-expressing cells, which could significantly constrain the potential therapeutic value of optogenetics-based actuation of cardiac tissue behavior (10). Finally, any GD method used for light-sensitization would need to overcome potential complications and side effects of cardiac gene transfer (e.g., presence of neutralizing antibodies for AAV, oncogenic transformation and immunogenic response for adenoviral and lentiviral delivery), which are well documented in the literature (31).

Light-sensitized donor cell lines, including human ESCs, have been generated *in vitro* (13-15), but it remains unclear whether delivery of such cells to the beating heart could be a feasible approach for enabling optogenetics-based therapy in patients. Many clinical trials have shown that stem cells can be infused via intracoronary (33) or direct myocardial (34) injection in diseased hearts without significant adverse outcomes, such as tumor formation or immunological response. However, therapeutic benefits in these studies were attributed to indirect mechanisms (i.e., activation of downstream mechanisms) and not long-term survival of injected cells (33,34); this high attrition rate is a major concern in the context of CD for optogenetics applications, since light-sensitive donor cells would need to remain in the heart. This issue may be resolved by ongoing efforts to improve long-term engraftment rates (35); for example, long-term survival of genetically modified donor myocytes was recently demonstrated in primate hearts, although these grafts were arrhythmogenic (36). Finally, it remains unclear whether donor cell-to-myocyte electrical coupling will occur *in vivo*, which would be a pre-requisite for light-based stimulation. Overall, these hurdles to the effective use of CD for optogenetics must be carefully considered when selecting an appropriate delivery mode for *in vivo* therapy, especially since simulation findings (see above) suggest that the usefulness of CD would be limited to localized infusion of light-sensitive cells at discrete optical pacing sites (10).

Finally, an emerging concern is the possible toxicity of opsins themselves. Miyashita et al. (37) compared different approaches for expressing ChR2 in rat pyramidal neurons and found that *in utero* DNA electroporation resulted in abundant, long-term transgene expression but also abnormal axonal swelling that was not seen in cells transduced with GFP only. Viral GD of ChR2 to the same cells resulted in lower expression rates but did not have any deleterious effects. This suggests that there is a threshold level for safe cellular ChR2 expression, which is highly relevant in the context of cardiac applications, where robust photocurrent is needed to ensure light-based depolarization. More research is needed in this area to better understand how intrinsic opsin toxicity might limit therapeutic usage.

Feasibility of *In Vivo* Intra-Cardiac Illumination for Optical Stimulation

As discussed in previous articles (7,10), direct delivery of focal light beams to the heart surface is not an intrinsically difficult problem, since it is expected that devices commonly used for clinical procedures such as angiography could be retrofitted to emit light instead of absorbing it. The level of energy required to activate opsins expressed at the illuminated surface is too low to damage tissue by heating and can be achieved with microscopic inorganic light emitting diodes (μ -ILEDs) (38). However, an important constraint for optical stimulation is that the penetration depth of visible light in cardiac tissue is quite low, particularly for the blue illumination used to excite ChR2, which has an approximate spatial

decay constant of $\sigma \approx 0.57$ mm (27). In situations where transmural excitation of opsins proves necessary, this attenuation effect will be a major barrier to the efficacy of optogenetics-based treatments; fortunately, the problem can be overcome via multiple avenues. First, the use of red-light sensitive opsins, such as ReaChR (17) and Chrimson (18), described above, could effectively double the penetration depth of optical stimuli. Second, Kim et al. (39) demonstrated the manufacturing of ultra-thin, waterproof arrays of μ -ILEDs; it has been speculated that such ultra-thin optoelectronic devices could potentially be implanted in the myocardium to deliver light stimulation in the transmural depth.

Other clinically relevant optogenetics-based applications, such as wide area optical excitation (analogous to defibrillation shocks) to terminate arrhythmias, will necessitate approaches capable of illuminating multiple points throughout the heart simultaneously to elicit a distributed response. Exciting new developments in the field of biocompatible integumentary membranes could provide a novel technique for illuminating multiple points across the cardiac surface; as shown in Fig.5, Xu et al. used a μ -ILED embedded in such a membrane to achieve illumination in a Lagendorff-perfused rabbit heart (40). The development of multi- μ -ILED versions of this technology for *in vivo* applications, such as optoelectronic membranes that conform to the human epicardial and/or endocardial surfaces, would unquestionably enable novel applications in cardiac optogenetics.

Another important question that has never been explicitly addressed is whether the relative energy requirements for optogenetics-based stimulation of heart tissue compare favorably to conventional electrical methods. Working with small μ -ILED arrays, Kim et al. reported that an input power of 5mW was required to illuminate a 0.024mm^2 area with sufficient output photon flux to robustly excite ChR2 ($E_c = 10\text{mW/mm}^2$) (38). Based on this analysis, the estimated energy required for reliable optogenetics-based stimulation of a 1mm^2 cardiac tissue region for 1 second is 208.3mJ. Although the illumination properties for specific clinical applications such as arrhythmia termination are not yet known, this provides a meaningful starting point for comparison with electrotherapy. Based on this estimate, illumination for ChR2 excitation with an array of 960 square-shaped μ -ILEDs measuring $500\mu\text{m}$ on each side with a relatively long pulse duration of 300ms would consume the same amount of energy ($\sim 15\text{J}$) as a typical shock from an internal cardioverter defibrillator device (41); although no published studies have explored the feasibility of light-based cardioversion, Boyle et al. (7) suggest, in a recent editorial, that this type of configuration might be sufficient to terminate AF. This conceptual argument in favor of therapeutic feasibility is noteworthy because selective light-based stimulation of opsin-expressing cardiac tissue (and not other surrounding muscle) would make the defibrillation process completely painless – a major improvement compared to conventional electrical approaches.

Possible Clinical Applications of Cardiac Optogenetics

Tissue-Selective, Low-Energy Pacing

A major potential advantage of optogenetics is that it can enable selective electrical control of specific cell or tissue types that can be targeted via customized gene therapy vehicles; Tsubota et al. (42) demonstrated light-based control of AP spiking in a subset of cerebellar neurons when ChR2 and NpHR were delivered with a promoter for that cell type.

Development of analogous promoters to facilitate opsin expression in the Purkinje system (PS) exclusive to other cardiac tissue would provide a novel pacing technique with very low energy requirements and exquisite precision. This type of approach could have major implications for techniques like His bundle pacing which is superior to conventional methods for resynchronizing ventricular contraction in many heart failure patients, but is seldom used due to high pacing thresholds and the fact that electric stimulation often excites both the His bundle and the ventricular septum, leading to abnormal activation patterns (43). Boyle et al. (10) used a detailed model of the rabbit ventricles and PS to show that both of these problems could be addressed by a PS-selective optogenetics-based alternative, since optical stimuli have very low energy requirements and could be designed to excite only the opsin-expressing His bundle.

Dynamic Modulation of Action Potential Duration

Many heart rhythm disorders are caused by pathologically abbreviated or elongated APs but pharmacological therapies to address such conditions are imprecise and lack specificity. A potentially beneficial use of optogenetics is to apply long, low-amplitude light stimuli to dynamically modulate AP shape in opsin-expressing cells. Karathanos et al. (44) recently used simulations to show that light-based therapy produced better results than drug treatment for restoring healthy AP duration in a model of atrial cells under the conditions of short QT syndrome; these findings are in agreement with recent *in vitro* findings that demonstrated the use of optogenetic tools to shape APs in light-sensitized NVRM monolayers (45). At the organ scale, however, simulations showed that the efficacy of this therapy was greatly hindered by light attenuation effects; of particular concern was the fact that energy loss due to attenuation and photon scattering in the atrial wall resulted in more prominent AP elongation near the illuminated surface (the endocardium) than in the tissue depth. This gave rise to a prominent gradient in AP duration that could be a substrate for reentry induction, although no instances of arrhythmia were observed (44). Overall, the study concluded that further work is needed to overcome intrinsic limitations of the optogenetics-based approach; computer models provide an excellent platform for executing this type of optimization, since the range of potential configurations that can be tested is extensive.

Pain-Free, Low-Energy Arrhythmia Termination

As discussed above, light-based defibrillation is the most tantalizing potential application for cardiac optogenetics because it could offer a completely pain-free alternative to the powerful electric shocks used by existing devices while also consuming significantly less energy. Excitingly, preliminary results suggest that this is a very worthwhile avenue to pursue. Bingen et al. (46) showed that fibrillatory activation patterns induced in rat atrial myocyte monolayers expressing calcium-translocating ChR2 (CatCh) could be reliably terminated using long (500ms) blue light pulses with extremely low intensity ($38\mu\text{W}/\text{mm}^2$); the arrhythmia termination mechanism was light-induced reduction of excitability, which slowed the activation rate and reduced wavefront curvature, causing rotors to meander towards monolayer boundaries where spontaneous termination occurred. Additionally, unpublished results discussed by Boyle et al. (7) claimed that simulated AF in an MRI-based patient-specific left atrial model with simulated ChR2 expression could be terminated by

stimulation with a long-duration (300ms) light pulse (1mW/mm^2) delivered uniformly to the endocardial surface. Although promising, this approach must be explored in further detail with more realistic intra-cardiac light sources, such as the multi- μ -ILED arrays discussed earlier in this review, both to evaluate whether the approach could be used to terminate AF in patients and to determine whether the technology might be appropriate to treat lethal ventricular arrhythmias.

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Fig.1.

Illumination records (blue) and ECG lead II signals (black) from transgenic mice derived from ChR2-expressing stem cells. (A) Pacing via pulsed light stimulation of the atria entrained cardiac activation at 450bpm, which was faster than the resting heart rate (approx. 350bpm); insets 1 and 2 also show that the morphology of the P wave was distinct during optically-paced beats. (B) Focal illumination of three different ventricular locations also achieved tachypacing (450bpm) with distinct QRS complexes due to different activation patterns compared to sinus excitation. Reproduced with permission from Bruegmann et al. (11).

Fig.2.

Acceleration or silencing of spontaneous activation in cardiac cell monolayers with light sensitivity inscribed by delivery of ChR2 and ArchT-rich fibroblasts (3T3 cells). (A-C) Imaged cardiomyocyte/3T3 hybrid monolayers plated electrode microarrays (inter-electrode spacing: 200μm); ChR2 (green) and ArchT (orange) expression patterns are highlighted in B and C, respectively. (D) Entrainment of monolayer activation at 100Hz with a sequence of 20 flashes of blue light (470nm). (E) Suppression of spontaneous activation with a 30 s long red-light illumination (624 nm). Modified with permission from Nussinovitch et al. (15).

Fig.3.

(A) 4-state Markov model representing the response of ChR2 to blue-light illumination. Green and red circles are conductive and non-conductive states; blue arrows are light-sensitive transitions. See text for further details. Modified with permission from Boyle et al. (10). (B-C) Measured and simulated I_{ChR2} traces evoked by light stimuli (blue bars) at different holding potentials. (D) Dynamics of I_{ChR2} during a light-evoked action potential; start and end of illumination indicated by blue dots. Modified with permission from Williams et al. (22).

Fig.4.

New techniques to simulate optogenetics at each level of the existing hierarchy for realistically modeling cardiac electrophysiology. At the cell level, non-light-sensitive ionic currents (yellow) are generic and interchangeable depending on simulation requirements. At the tissue level, stochastically distributed light - sensitive elements (blue) are shown in human ventricular tissue with photosensitization via localized injection at the apical endocardium. At the organ level, light attenuation effect is illustrated by the 3D distribution of light energy delivered to the heart (irradiance, E_e) in an MRI-based human ventricular model. E_e indicates the irradiance applied at the endocardial surface. Modified with permission from Boyle et al. (10).

Fig.5.

Top: Key steps in the design and fabrication of 3D multifunctional integumentary membranes (3D-MIMs). Bottom: Proof-of-concept for a 3D-MIM containing multiple sensors and actuators, including one μ -ILED; a similar device with multiple light sources could be used to achieve spatially-distributed illumination of the entire opsin-rich heart surface. Scale bars, 500 μ m. Reproduced with permission from Xu et al. (40).