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Optogenetic Cardiac Pacing and Its Applications

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ABSTRACT

Optogenetics combines the biological techniques of optics and genetics and uses light to control the activities of living tissues such as neurons and heart. Optogenetic actuators including channelrhodopsin (ChR), halorhodopsin (NpHR), and archaerhodopsin specifically provide for neuronal or cardiac controls. The clinical translation of cardiac optogenetics will include human and larger mammalian animal model applications and ultimately optogenetics may have the power to restore normal heart rhythm.

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Optogenetic Cardiac Pacing

Optogenetics uses light to control the activities of living tissues such as neurons and heart as a new biological technique that combines optics and genetics. Optical coherence tomography (OCT) can provide novel three-dimensional (3D) imaging and when combined with OCT, optical coherence microscopy (OCM) provides high resolution imaging that are 50x - 100x greater than conventional ultrasound, MRI, or CT [1-10]. OCT systems have already been successfully used in interventional cardiology, ophthalmology and optometry and dermatology [11-13].

Optogenetic actuators including channelrhodopsin (ChR), halorhodopsin (NpHR), and archaerhodopsin can provide neuronal or cardiac control (bacterio-opsin) [14,15]. ChRs are sensory photoreceptors (rhodopsins) while NpHR is a chloride ion-specific light-gated ion and archaerhodopsin is the bacterio-opsin family of receptor proteins [14-22].

Optogenetics was named the Method of the Year 2010 and the "Breakthroughs of the Decade" by the research journals *Nature Methods and* Science, respectively (https://www.medinc.co.uk/optogenetics-breakthrough-of-the-decade-by-dr-zulfiquar/).

In 2015 the first *Drosophila* heart study using OCT was reported on the Discovery channel and in the Boston Globe (Light - powered hearts?" https://www.bostonglobe.com/lifestyle/2015/10/25/light-powered-hearts/ETWV7DZU6pwMNm1P59TLGL/story.html).

Drosophila Heart in Optogenetic Pacing

Marked morphological and functional changes in the *Drosophila* heart during development were observed in a longitudinal study. In the pupal stage the heartbeat is reduced dramatically and stops beating during pupae. A circadian clock gene *d*Cry was shown to affect heart development and functioning [23].

An optogenetic pacing system has clearly showed mCherry fluorescence signal in the heart of a ChR2-mCherry transgenic fly compared to a wild-type control fly [24]. Successful optogenetic pacing of ChR2-expressing *Drosophila* at different developmental stages was shown [25]. Red light increases the excitability of the heart tissue and flies expressing ReaChR. Optogenetic fly models were able to be tachypaced under red light stimulation [26,27].

Cardiac Arrhythmia by Optogenetic Pacing

Optogenetics can treat cardiac arrhythmias [28]. Several experimental models showed photosensitive ion channels and pumps (opsins) by optogenetic pacing of cardiac preparations and the opsins can precisely stimulate or silence electrophysiological activity in cardiac cells [29,30]. Cardiomycytes that express ChR2 can sensitively activate Ca2+ signaling properties and transgenic mice expressing ChR2 can control heart muscles *in vivo* [28,30]. Stimulation of Gs-signaling by optogenetics showed a light sensitive G_s-protein coupled receptor in mice cardiac tissue [31]. Cardiac excitable media demonstrated in heart precisely to influence cardiac function and overall dynamics [32]. Near-infrared (NIR) light has the ability to penetrate tissue and therefore has the potential to manipulate cardiovascular diseases non-invasively [33]. Greater tissue depths are achieved with the red-shifted opsins than conventional blue-sensitive channel-rhodopsins [30]. Taken together these studies have increased our understanding of cardiac physiology.

Electronic device therapy (i.e., implantable pacemakers and cardioverter–defibrillators [ICDs]) largely provide the basis for current management of cardiac arrhythmias [34]. Arrhythmias such

as atrial fibrillation (AF) can be treated by rapid antitachycardia pacing [35]. Mice cardiomyocytes showed electric shock functions in electrical defibrillation and elderly humans and *Drosophila* may similarly significantly reduce heart rate via electrical pacing [36,39]. Rhythm disturbances were associated with an increase in age and light intensity was associated with NpHR stopping the heart rate in *Drosophila* [40,41].

Glutamatergic neurons provide extensive innervation to the adult heart in *Drosophila* metamorphosis. Pacemaker action potentials was demonstrated in muscles of the first abdominal cardiac chamber [42]. *KCNQ1* mutant *Drosophila* showed abnormal contractions and fibrillations and *KCNQ1* in humans is related to myocardial repolarization [43].

Drosophila genetic screens identify genes related to their functions. EGFR signaling regulates adult cardiac function while mutants in a fly orthologue of epidermal growth factor (EGF) rhomboid 3 have enlarged cardiac chambers and the Notch ortholog weary (*wry*) is associated with dilated cardiomyopathy [44,45]. Insulin-IGF receptor signaling showed regulation of age-dependent changes in cardiac function [46].

Chronic stability and excellent biocompatibility were achieved in small animals through multimodal and multisite pacing studies [47]. Direct viral delivery and functionality of opsins in cardiomyocytes has been demonstrated *in vitro* [34]. A cell line can stably express the excitatory opsin, ChR2 *in vitro* between the ChR2-expressing donor cells and host cardiomyocytes [48]. Expression of the light activated ChR2 can stimulate heart muscle *in vitro* and in mice demonstrated precise localized stimulation, constant prolonged depolarization of cardiomyocytes and cardiac tissue, Ca2+ homeostasis, electrical coupling and arrhythmogenic spontaneous extra beats [49]. The delivery of ChR2 transgene to several ventricular sites by diffuse illumination of hearts resulted in electrical synchronization and significant shortening of ventricular activation times [50]. Cardiac nonmyocytes in mouse hearts showed myocyte AP-like signals in cryoinjured scar border tissue indicating direct evidence of effects of heterocellular electrotonic coupling in the whole heart on cardiac electrical connectivity [51]. A high vulnerability to tachycardia of optically tachypaced human induced pluripotent stem -cardiomyocytes in 3D engineered heart tissue can be effectively terminated by ryanodine receptor stabilization, sodium or potassium channel inhibition [52]. Cultured mouse embryos showed optogenetic pacing with 4D (3D+time) OCT structural and Doppler imaging, which demonstrated that embryonic hearts can provide function efficiently and produce strong blood flows [48]. Expressing the Channelrhodopsin-2 (ChR2) transgene at one or more ventricular sites in rats allowed optogenetic pacing of the hearts at different beating frequencies with blue-light illumination [50].

OCT and Its Clinical Applications in Heart

OCT requires optical stimulation to be delivered safely and with long-term efficiency. OCT can assess coronary vasculature in cardiovascular medicine and several clinical systems have become commercially available. Patients who have stable coronary artery disease can be assisted by OCT for a more detailed lumen segmentation. On the other hand, in patients with acute coronary syndrome, OCT can offer 100% detection of intraluminal thrombus with in comparison to coronary angioscopy which detected plaques in 79% and stenosis in 24% of patients [53-57].

Optogenetics in the Future

The optogenetics field has made significant progress in heart research from its inception almost a decade ago. The clinical translation of cardiac optogenetics for human application is moving towards use as a tool in larger mammalian animal models [58]. Optogenetics may restore normal heart rhythm to increase the overall quality of life and action potential duration of ChR2- or NpHR can be modulated in opsin-expressing rat cardiomyocytes [34,59-61]. Optogenetics may potentially have a therapeutic role in treating heart diseases.

Drosophila genetic screens using OCT can identify additional cardiovascular-related genes and assess pre-clinical drug development cardio toxicity, which account for approximately 20% of withdrawal of drugs from development [62,63]. Cardio toxicity measures by electrophysiology are often low through put and efficient high throughput screening tools that significantly reduce cost are needed [34,62-64]. Overall, optogenetics is high throughput and automated tool for use in evaluating cardio toxicity.

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