Electronic cardiac pacemakers are the primary therapy for sinus node dysfunction and high-degree AV block. Although uncommon, infections, lead failures, and vascular complications do result in mortality and morbidity for some patients. Battery life limitations require device changes, complications do result in mortality and morbidity for some patients. 

Several approaches have been studied, including direct gene transfer in native cardiomyocytes to express the β2-adrenoreceptor, a dominant-negative form of the Kv2.1 potassium channel, or the classical pacemaker channels of the HCN family. Despite some promising experimental results, there are still technical limitations, and biological pacemakers are not yet suited to replace implantable pacemakers in the clinic (see Rosen et al5 and Boink and Rosen6 for an extensive review and discussion). In addition to difficulty in achieving pacing at high heart rates, major challenges relate to the fact that gene transfer must be safe; reliable; and ideally, life lasting. An alternative to gene transfer in native cardiomyocytes is the transplantation of cells with pacemaking activity. Such cells are not necessarily excitable themselves because they only need to generate a pacemaking current that is transferred to neighboring cardiomyocytes through gap junctions to evoke an action potential. This concept is called a tandem cell unit (TCU) strategy and has been analyzed previously by in vitro studies and transplantation of mesenchymal stem cells expressing the pacemaker channel HCN2.

In this issue of Circulation: Arrhythmia & Electrophysiology, Jia and colleagues combined the concept of a TCU pacing strategy with an optogenetic pacing approach in an in vitro system. The authors have generated a cell line of nonexcitable human embryonic kidney (HEK) 293 cells to express the optogenetic protein channelrhodopsin 2 (ChR2). ChR2 is a light-activated cation channel from the green algae Chlamydomonas reinhardtii, first described 2003, and is widely used in the field of neuroscience (for review, see Yizhar et al10). ChR2 opens on illumination with blue (~470 nm) light, resulting in an inward Na+ current and depolarization. In a TCU approach, this current flows through gap junctions from the illuminated nonexcitable cell to a neighboring cardiomyocyte, which is subsequently depolarized and generates an action potential. Such action potentials are then conducted throughout the functional syncytium of coupled cardiomyocytes and paces the whole heart.

Jia and colleagues8 have proven that this concept works in the culture dish in vitro. They have cocultured ChR2-expressing HEK cells with neonatal rat or adult canine cardiomyocytes and elegantly showed by dual whole-cell patch clamp experiments that coupling is indeed established and that optical stimulation of ChR2-expressing HEK cells is pacing the flanking myocytes. Using gap junction blockers, they have further proven that functional gap junctions are required for optical pacing in the TCU approach. The authors convincingly tested this approach in a 2D syncytium of myocytes using calcium mapping experiments and performed a mathematical simulation that predicted sufficient functionality of the ChR2-TCU approach in vivo.

Previous studies using transgenic mice or zebrafish demonstrated that ChR2-mediated optical pacing of the heart can be performed in vivo. However, there are a number of important challenges to overcome before ChR2-mediated optical pacing can be considered for clinical application. Apart from the technical problem of establishing a reliable method for long-term light application to the cells in the heart, the most complex problem will be to identify a safe strategy for efficient and long-lasting cell transplantation or gene transfer that allows in vivo application of ChR2 in native nontransgenic hearts. The following sections briefly discuss the major challenges and the potential advantages of optical pacing in the heart.

**Which Cells to Use?**

Because of the high proliferation potential and the associated risk of tumor formation, the HEK cells that Jia and colleagues used will not be useful for transplantation. An ideal cell type should stably engraft without immunologic rejection and effectively couple to native cardiomyocytes for life without generation of arrhythmias or tumors. Jia and colleagues show that cardiomyocytes, cardiac fibroblasts, and canine and human mesenchymal stem cells can be transfected with ChR2. In fact, mesenchymal stem cells might be a suitable cell type because they can be obtained from the patients and transplanted without immunologic rejection. In a previous study, HCN2-expressing transgenic mesenchymal...
mal stem cells were successfully used for cardiac pacing in vivo; however, long-term engraftment and contribution to pacemaking has not been investigated so far.

In addition to nonexcitable cells, cardiomyocytes could be a suitable cell type because their electric integration after transplantation was previously proven by in vivo imaging of the mouse heart. Furthermore, it has been shown that cardiomyocytes generated and purified from ChR2-expressing embryonic stem cells can be effectively paced by light in vitro. Whether such cells can be used for in vivo pacing is yet to be determined. To transfer this idea to human hearts, one should consider the use of induced pluripotent stem cells as a source of cardiomyocytes. Easily obtained from patient skin biopsy specimens, induced pluripotent stem cells could be stably transfected with ChR2 and differentiated to fully immune-compatible human cardiomyocytes. Consequently, purification and transplantation of such ChR2-expressing human cardiomyocytes represent a promising future approach of cell-based optical pacing of the heart.

**Direct Gene Transfer**

An alternative to transplanting ChR2-expressing cells would be the direct gene transfer of resident cardiomyocytes. It is known that ChR2 expression has no side effects on cardiomyocyte alteration of action potential duration or induction of cardiac hypertrophy. A major advantage of direct gene transfer is the fact that functional coupling of ChR2-expressing resident cardiomyocytes is lifelong. Experiments on the hearts of ChR2-expressing transgenic mice have shown that illumination of areas as small as 0.05 mm² (corresponding to ~50 myocytes) is sufficient for pacing, indicating that only small areas of the myocardium need to be subjected to gene transfer. Most studies using gene transfer of resident cardiomyocytes used adenoviruses or adenovirus-associated viruses, which resulted only in transient gene expression. Stable and long-lasting gene transfer requires integrating viruses that raise other limitations, such as transgene silencing, oncogene activation, or immunorejection. A safe and long-lasting gene transfer to the human myocardium has not yet been established, and further development of this field is needed before clinical applications can be proposed.

**Light Application**

The excitation light to activate ChR2 (470 nm) as well as the red-shifted variants from other species such as VChR1 from *Volvox carteri* or MChR1 from *Mesostigma viride* (~530 nm) does not penetrate deep into tissue; therefore, light stimulation through the chest wall will not be feasible. Epicardial stimulation was used to effectively stimulate ChR2-expressing mouse hearts, but it remains unclear whether this would be sufficient to activate transplanted cells deep within the myocardium. Epicardial and endocardial illumination could be performed by flexible fiberoptic light guides or direct illumination with single or flexible strips of multiple light-emitting diodes. In the future, electroluminescent foils and wires, which are highly flexible but currently do not provide enough light intensity, could potentially be applied for epicardial or intramyocardial illumination. Moreover, new ChR2 variants with higher light sensitivity, such as ChR2-T159C, might eventually make sufficient light simulation less challenging.

**Potential Advantage of Optical Pacing**

Despite these challenges, the use of ChR2 for cardiac pacing has several advantages compared to electric pacemakers and opens new possibilities. Jia and colleagues calculate that optical pacing requires less energy than electric pacing, resulting in longer battery life, and they suggest that optical fibers may be more biocompatible than electrodes.

Most patients receive ventricular electric pacemakers that stimulate only 1 point of the right ventricle. This is disadvantageous for patients with left ventricular systolic dysfunction who require cardiac resynchronization therapy with biventricular pacemakers. Optical pacing also could potentially be used for cardiac resynchronization therapy by simultaneously pacing multiple sites across the whole heart. To achieve this, cell transplantation or gene delivery must be applied to many sites of the heart, and light stimulation must be global.

**Optical Defibrillation**

In addition to cardiac pacing, ChR2 could potentially be applied for defibrillation of atrial or ventricular arrhythmias. It was previously shown that constant illumination of ChR2-expressing cardiomyocytes in vitro prolongs depolarization and refractoriness and electrically silences illuminated areas. Furthermore, halorhodopsin, an optogenetic protein with hyperpolarizing current, was successfully used to inhibit electric activity in zebrafish hearts. Such prolonged depolarizations or hyperpolarizations produced by illumination might be suitable to interrupt reentrant electric activity in the atrium or ventricle and thereby stop arrhythmias. Because the TCU approach by Jia and colleagues is unlikely to generate enough current to inhibit the electric activity of cardiomyocytes, direct gene transfer would be required. Whether epicardial expression and illumination is sufficient to interrupt a reentrant ventricular tachycardia or whether transmyocardial ChR2 or halorhodopsin activity would be required remains to be determined. Because the atrial wall is thin, transmyocardial activation is less challenging and might someday offer a means for pain-free atrial defibrillation.

**Outlook**

In summary, ChR2 can be used effectively for cardiac pacing either indirectly by a TCU approach or directly in ChR2-expressing cardiomyocytes. Future experiments in vivo are required to identify the most efficient strategy for pacing the heart by light. It is essential not only to achieve short-term pacing, but also to prove feasibility of long-term stable pacemaker function without cell rejection or tumor formation. One must admit, however, that the clinical use of optical pacing seems a distant prospect given the excellent efficacy of the existing implantable cardiac pacemakers and the technical challenges for using optogenetics in the heart in vivo.
Disclosures

None.

References


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