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Optogenetic Medicine: Synthetic Therapeutic Solutions Precision-Guided by Light

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Gene- and cell-based therapies are well recognized as central pillars of next-generation medicine, but controllability remains a critical issue for clinical applications. In this context, optogenetics is opening up exciting new opportunities for precision-guided medicine by using illumination with light of appropriate intensity and wavelength as a trigger signal to achieve pinpoint spatiotemporal control of cellular activities, such as transgene expression. In this review, we highlight recent advances in optogenetics, focusing on devices for biomedical applications. We introduce the construction and applications of optogenetic-based biomedical tools to treat neurological diseases, diabetes, heart diseases, and cancer, as well as bioelectronic implants that combine light-interfaced electronic devices and optogenetic systems into portable personalized precision bioelectronic medical tools. Optogenetics-based technology promises the capability to achieve traceless, remotely controlled precision dosing of an enormous range of therapeutic outputs. Finally, we discuss the prospects for optogenetic medicine, as well as some emerging challenges.

Imagine a world where we can treat diseases simply with a beam of light, instead of with conventional pharmacotherapy by taking pills or injections. This might not be as far from reality as you think. Technological development and applications of light-based bioelectronics, in the form of electronic devices that interface with optogenetics and biological systems, are already experiencing rapid growth (Kozai and Vazquez 2015; Shao et al. 2017; Wang et al. 2017). Indeed, the parallel growth of optogenetics and synthetic biology is generating great enthusiasm for innovative technology platforms with the potential to achieve precise spatiotemporal control and manipulation of cellular behaviors through light stimulation, both in vitro and in vivo (Pathak et al. 2013). Optogenetics can be defined as the application of optical and genetic principles to control various cellular functions, including transgene activation (Ye et al. 2011), genome activation/editing (Nihongaki et al. 2015a,b, 2017), perturbation of intracellular signaling pathways (Zhang and Cui 2015), stimulation or inhibition of metabolic pathways (Kemmer et al. 2010; Rossger et al. 2013; Xie et al. 2016; Ye et al. 2017), and cell migration (Weitzman

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and Hahn 2014), in response to light irradiation. This field has recently attracted great attention because of the unmatched properties of light as a traceless, remotely controllable trigger signal that enables quantitative, spatiotemporally, well-defined modulation of cellular activities (Niopek et al. 2014; Baumschlager et al. 2017).

Bioelectronic medicine is a multidisciplinary research field that brings together molecular medicine, bioengineering, and electronics to develop intelligent control and sensing technologies for disease diagnosis and therapy. In the past, bioelectronic medicine has focused mainly on neuroscience, seeking to develop nerve-stimulating and sensing technologies to regulate biological processes and treat disease by using electrical stimulation (Obeso et al. 2001; Lozano et al. 2002; Frank et al. 2007). However, in 2005, scientists from Stanford University reported a ground-breaking application of fast optogenetic stimulation to control action potentials in neurons with millisecond precision by blue-light stimulation (Boyden et al. 2005). One year later, scientists from Wayne State University used an optogenetic tool to successfully restore visual responses in animals (Bi et al. 2006). By 2010, optogenetics was already rated as the method of the year by *Nature Methods* (Editorial 2010b), whereas *Science* rated it as one of the major breakthroughs of the preceding decade (News Staff 2010a).

With the flourishing development of optogenetics, multichromatic optogenetic devices have been developed that are activated by different wavelengths of light, including blue (Boyden et al. 2005; Bi et al. 2006; Wu et al. 2009; Ye et al. 2011; Wang et al. 2012), ultraviolet (Favory et al. 2009), green (Kainrath et al. 2017), red (Levskaya et al. 2009; Heyes et al. 2012; Kaberniuk et al. 2016), and far-red light (Shao et al. 2017, 2018). These optogenetic devices have been used to treat not only neurological diseases, but also non-neurological diseases; applications include treatment of diabetes (Ye et al. 2011; Wang et al. 2012; Shao et al. 2017), oncotherapy (Xu et al. 2014; He et al. 2015), heart disease (Cohen et al. 2017; Jiang et al. 2017), genome activation (Nihongaki et al. 2015b, 2017; Polstein and Gersbach 2015; Shao et al. 2018), and genome editing (Hemphill et al. 2015; Nihongaki et al. 2015a; Jain et al. 2016; Taslimi et al. 2016). The rapid development of optogenetics has enormously extended the scope of traditional bioelectronic medicine (Khalil and Collins 2010). Here, we highlight the latest state-of-the-art optogenetic devices for biomedical applications.

A BRIEF SUMMARY OF THE AVAILABLE PHOTORECEPTORS

A light-responsive channel protein based on channelrhodpsin-2 (ChR2) was the first to be used in optogenetics (Malone 2012). Since then, at least a dozen light-responsive proteins, also called photoreceptors, have been introduced (Sancar 1994; Guo et al. 1998; Freedman et al. 1999), including ChR2 (Boyden et al. 2005; Hight et al. 2015), FKF1-GI (Yazawa et al. 2009), PHYB-PIF (Levskaya et al. 2009), AsLOV2 (Wu et al. 2009), melanopsin (Ye et al. 2011), VVD (Wang et al. 2012), DRONPA (Zhou et al. 2012), CRY2-CIB1 (Kennedy et al. 2010), UVR8-COP1 (Favory et al. 2009), Chronos (Klapoetke et al. 2014), Chrimson (Klapoetke et al. 2014), BphP1-PpsR2 (Kaberniuk et al. 2016), CPH1 (Heyes et al. 2012), CBD (Kainrath et al. 2017), and BphS (Shao et al. 2017). These photosensitive proteins can be subdivided into several categories, including light-responsive ion channels, light-responsive G-protein-coupled receptors (GPCRs), light-responsive protein–protein interaction domains, and light-responsive caging groups. Here we describe some representative optical systems that are frequently used for different purposes. The light sensor protein ChR2 from *Chlamydomonas reinhardtii* is a representative light-responsive ion channel that was first applied to neuronal optogenetics (Nagel et al. 2003; Boyden et al. 2005; Bi et al. 2006). Later on, Chronos with kinetics faster than previous channelrhodopsins as well as high light sensitivity was discovered (Klapoetke et al. 2014). This new version opsin enables to achieve high-frequency neural activation and may be used to manipulate auditory pathways in future optogenetic-based neuroprostheses (Hight et al. 2015). The light-responsive GPCRs are mainly animal

opsins (Terakita et al. 2015), such as melanopsin (Ye et al. 2011). In 2009, the light-responsive interacting proteins FKF1-GI (Yazawa et al. 2009) and PHYB-PIF (Levskaya et al. 2009) were discovered in *Arabidopsis thaliana*. They are activated by blue light (<500 nm) and far-red light (740 nm), respectively. However, the PHYB-PIF system requires tetrapyrrole chromophores that are absent in mammalian cells. Therefore, we need to add an extra pigment to the system to activate the light-sensor proteins. Since then, the Vivid (VVD) system (Wang et al. 2012) based on blue-light-responsive protein–protein interaction has been developed and used for controlling transgene expression. This system was further transformed and developed into an advanced version named the pMag-nMag system (Kawano et al. 2015), which was engineered to control gene editing, including blue-light-controlled clustered regularly interspaced palindromic repeats (CRISPR)-Cas9/dCas9 devices (Nihongaki et al. 2015a, 2017). The light sensor protein CRY2-CIB1 is also a blue-light-responsive heterodimerization system derived from cryptochromes of the plant *A. thaliana*. CIB1 is a basic helix–loop–helix protein, and CRY2 binds to CIB1 in its photoactivated state (Liu et al. 2008). This system has been widely used and exploited in several truncated and mutated versions (for example, CRY2PHR [photolyase homology region of CRY2] and CIBN [amino terminus of CIB1] [Kennedy et al. 2010] and CRY2 L348F and W349R [Taslimi et al. 2016]). In addition, a near-infrared (NIR) light-responsive system has recently been engineered based on biliverdin (BV) phytochrome BphP1 and its natural partner PpsR2 from *Rhodopseudomonas palustris* bacteria (Kaberniuk et al. 2016). A light-responsive caging system based on the light, oxygen, and voltage domain (LOV domain) (Christie et al. 1999) is also a kind of optogenetic system: light stimulation induces a conformational change that releases the protein to interact with its target protein. In 2017, a far-red light-activated bacterial phytochrome protein (BphS) was engineered to develop a fully orthogonal and biocompatible far-red light-controlled transgene expression device (Shao et al. 2017). The properties of these systems are summarized in Table 1.

OPTOGENETIC TOOLS FOR APPLICATIONS IN NEUROSCIENCE

In 1979, Francis Crick stated that it would be important to develop a method to manipulate individual components of the brain (Crick 1979). Finally, in 2005, Karl Deisseroth first showed that microbial opsin genes could be used to optogenetically control neuronal activity (Boyden et al. 2005). With the rapid development and convergence of microbial opsin engineering and modular genetic methods, optogenetics has come to be widely used for the optical control of defined cells in the field of neuroscience (Deisseroth et al. 2006; Adamantidis et al. 2014). Three major classes of optogenetic constructs have been developed for the control of neural activity. ChR2 from *Chlamydomonas reinhardtii* has fast kinetics, so that specific action potentials can be triggered with pulses of blue light at a range of frequencies related to neural signaling (Fig. 1A). As with ChR2, the inhibitory halorhodopsin from *Natronomonas pharaonic*, a light-sensitive, chloride-pumping halorhodopsin, can be activated by yellow-light illumination (580–590 nm), allowing hyperpolarization of neurons to inhibit individual action potentials (Fig. 1B) (Han and Boyden 2007; Zhang et al. 2007b; Gradinaru et al. 2010). In addition, a set of chimeric opsins (optoXRs) were engineered by replacing the intracellular loops of the rhodopsin with the intracellular loops of GPCRs. These synthetic optoXRs could change their conformation to activate downstream biochemical cascades, including either Gs or Gq pathways, upon green light (500 nm) illumination (Fig. 1C) (Airan et al. 2009).

These optogenetic systems hold great promise as versatile tools for probing defined neurons and for optical control of neural circuitry, both in normal operation and in disease states. Although electrical stimulation has been used to control the functions of discrete brain regions and to treat multiple neurological diseases, it has unwanted side effects such as off-target and toxic to neuronal cells (Burn and Tröster 2004; Zhang

Table 1. Summary of different photoreceptors

Optogenetic system	Chromophore	Mechanism	Size (AA)	λon (nm)	λoff (nm)	Ta(l/2)	Td(l/2)	References
ChR2	Retinal	Channel opening	737	470–480	-	~0.2 msec	~15 msec	Boyden et al. 2005
FKF1-GI	FMN	Dimerization	FKF1:619, Gl: 1173	450–490	-	~5–10 min	~ h	Yazawa et al. 2009
PHYB-PIF	Phycocyanobilin	Dimerization	PHYB: 908, PIF: 100	650	750	1.3 sec	4 sec	Levskaya et al. 2009
AsLOV2	FAD	Caging	143	450–490	-	~ sec	30–50 sec	Wu et al. 2009
Melanopsin	Retinal	Gq-protein activation	534	450–490	-	~ msec	~ sec	Ye et al. 2011
Magnets	FMN	Dimerization	pMag: 150, nMag: 152	450–490	-	1.5 sec	6.8 sec	Kawano et al. 2015
DRONPA	Internal Cys62, Tyr63, and Glu64	Tetramerization/caging	257	500	400	~ sec	~ sec	Zhou et al. 2012
CRY2-CIB1	FAD	Dimerization	CRY2: 535, CIB1: 170	450–490	-	~10 sec	~12 min	Kennedy et al. 2010
UVR8-COP1	Internal Trp285 and Trp233	Dimerization	UVR8: 440, COP1: 675	280–315	-	~ h	Irreversible	Favory et al. 2009
Light–oxygen–voltage (LOV)	FAD	Dimerization	222	450–490	-	<10 sec	<50 sec	Christie et al. 1999
BphPl-PpsR2	Biliverdin	Dimerization	BphPl: 732, PpsR2: 465	730–780	640–660	3–30 sec	15 min (dark), 1.6× faster using λoff	Kaberniuk et al. 2016
CPH1(CPH1S)	Phycocyanobilin	Dimerization	513	660	720	No reported	No reported	Heyes et al. 2012
Chronos	Retinal	Channel opening	326	470	-	No reported	No reported	Klapoetke et al. 2014
Chrimson	Retinal	Channel opening	351	660	-	No reported	No reported	Klapoetke et al. 2014
CBD	AdoCbl	Tetramer dissociation	200	545	-	No reported	No reported	Kainrath et al. 2017
BphS	Biliverdin	Second messenger c-di-GMP pathway activation	681	680–810	-	~15 sec	90 sec	Shao et al. 2017

λ, wavelength; Ta1/2, half-life of association; Td1/2, half-life of dissociation; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; AA, amino acid residue.

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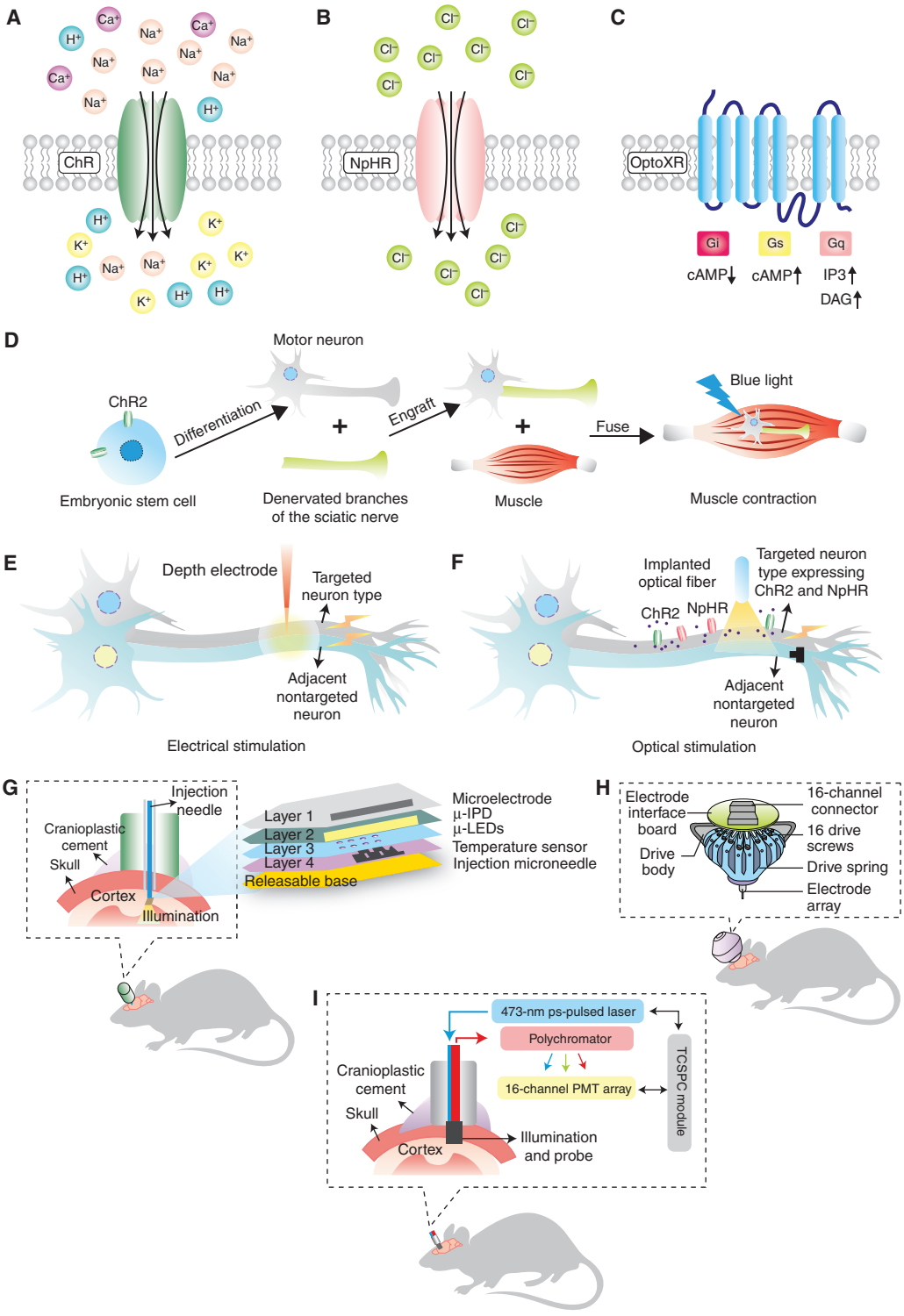

Figure 1. (*See legend on following page.*)

et al. 2007a), and is not capable of targeting genetically specified cell types. In contrast, recent optogenetic techniques integrate optics and genetics to control neural activity within targeted cell types in a temporally controlled and rapidly reversible manner. Bryson et al. (2014) implanted motor neurons derived from murine embryonic stem cells containing the light-sensitive ion channel ChR2 into partially denervated branches of the sciatic nerve of adult mice; upon 470-nm blue-light illumination, ChR2 motor neurons could be recruited to not only reinnervate denervated muscle fibers, but also produce graded muscle contractions in an optically controllable manner with high temporal resolution. Thus, the integration of regenerative medicine and optogenetics opens up the prospect of restoring muscle function after traumatic injury or disease (Fig. 1D). In addition, deep-brain stimulation (DBS) is a therapeutic option for Parkinson's disease (PD) and other neurodegenerative disorders, although its mode of action is still a matter of controversy (Fig. 1E). Gradinaru et al. (2009) used optogenetic technology based on the ChR2-NpHR system to systematically dissect the distinct circuit elements of 6-hydroxydopamine (6-OHDA)-induced PD model rats, overcoming the disadvantages of traditional strategies for stimulation (Fig. 1F) (Zhang et al. 2007a).

With the development of optogenetics in the field of neuroscience, novel devices are required to integrate optical control of neural activity and electrical readout, and optical readout of population and single-cell neural activity in freely behaving animals (Deisseroth et al. 2006; Fenno et al. 2011; Warden et al. 2014; Ledochowitsch et al. 2015). Current optical control devices have been developed to stimulate and record neural activity with wireless control of intracranial light delivery based on light-emitting diode (LED) light sources. However, these wireless systems have undesirable electrical artifacts and cause heating during stimulation, because the LED is

Figure 1. Optogenetic tools for control of neural activity. Many different types of opsins are used for control of neural activity. (*A*) The microbial, light-sensitive protein *Chlamydomonas reinhardtii* channelrhodopsin-2 (ChR2) allows sodium ions to enter the cell under blue-light illumination (activation maximum ~470 nm). (*B*) An archaeal light-driven chloride pump (NpHR) from *Natronomonas pharaonic* allows chloride ions to enter the cytoplasm when activated by yellow light (activation maximum ~580 nm). (*C*) Opsin-receptor chimaera (OptoXR) enables G-protein-mediated intracellular signaling cascades to be modulated, eliciting patterns of cellular activity under green-light illumination (activation maximum ~500 nm). (*D*) Optogenetic stimulation of muscle contraction. Murine embryonic stem-cell-derived motor neurons expressing the light-sensitive ion channel ChR2 are engrafted into partially denervated branches of the sciatic nerve of adult mice, enabling restoration of muscle function after optical stimulation using finely controlled pulses of 470-nm blue light. (*E*) In vivo electrical stimulation. Electrode-based deep brain stimulation (DBS) methods stimulate all neurons within a given volume, resulting in unwanted side effects. (*F*) In vivo optical neural stimulation. Genetic targeting of ChR2 or halorhodopsin (NpHR) into disease-specific cell types enables cell-specific neuromodulation and avoids stimulation of nontargeted cells. (*G*) Injectable optoelectronic device for light delivery to the brain in free-moving animals. The wireless multifunctional optoelectronic system contains layers for sensing electrophysiology (1: microelectrode), detecting light sources (2: microscale inorganic light-emitting diode, μ-IPD), collecting optical stimulation (3: microscale inorganic photodetector, μ-ILED), and sensing temperature (4: serpentine Pt resistor), all bonded to a releasable substrate for injection (microneedle). Multiple μ-ILED light sources can be independently controlled, enabling precise delivery of light to the neural tissue in freely behaving animals. (*H*) Stereotrode and tetrode microdrives for electrophysiological recordings in behaving mice. The tetrode microdrive assembling 16 individually movable electrodes can integrate either a fixed or freely movable optical fiber, enabling precise optical control and stable recordings of neural activity in mouse cortex or the hippocampus with only minimal impact on natural behavior. (*I*) Optical-neural devices for monitoring neural activity in freely behaving mammals. The time-correlated, single-photon-counting (TCSPC)-based fiber-optic system includes a 473-nm picosecond-pulsed laser, a polychromator, a 16-channel photomultiplier (PMT) array and a TCSPC module. A multimode optical fiber is implanted into the target area in the mouse brain to monitor neural activities from neurons expressing genetically encoded calcium ion or voltage indicators. This is particularly suitable for optogenetic control of the bulk tissue in freely behaving animals.

located very close to the site of stimulation. To address these issues, a wireless system based on ultrathin microscale inorganic LEDs (μ-ILEDs) has been developed, allowing independently addressable light sources to be directly injected into the brain. These wireless devices incorporate multisite optical stimulation, electrophysiology, and temperature sensors and actuators, which can be mounted onto an injection needle and injected deep into brain tissue with perfect coregistration. A particular advantage of these multifunctional optoelectronic systems is their small size, which may facilitate optical control of smaller neural circuits, reduce tissue damage, and minimize gliosis and immunoreactivity (Fig. 1G).

Wireless control of intracranial light delivery is expected to open up new vistas for future technological innovation, particularly for animals that are very sensitive to handling and are not adapted to wired devices (Kim et al. 2013). In a recently developed system for optogenetic stimulation in freely behaving animals, fiber-optic light delivery was integrated with stereotrodes or tetrodes. A tetrode microdrive was composed of up to 16 individually movable electrodes, which incorporated either fixed or adjustable optical fibers, enabling fine control over the position of target neurons and providing high-quality recordings of each neural circuit. This lightweight (2 g) device with 16 tetrodes was shown to be suitable for probing the concerted functions of neurons at multiple target sites in behaving mice, rather than individual neurons (Fig. 1H) (Voigts et al. 2013).

As genetically encoded fluorescence-based indicators of neural activity and devices designed to detect these fluorescence signals are developed, new devices have been designed for monitoring bulk population neural activity in freely behaving mammals using single fibers or fiber bundles. In vivo imaging methods allow long-term recording of identified single neurons to probe the dynamics of neural activity. Costa's group used fiber optics and time-correlated single-photon counting (TCSPC) to study striatal pathways in action initiation. Single-mode and multimode fibers were inserted into the target area of the brain and fluorescence signals were recorded from neurons expressing genetically encoded GCaMP3, a calcium indicator (GECI), in behaving mice. This system is well suited for optogenetic control of bulk population neural activity in freely behaving mammals, because the flexibility and minimal weight of the fiber do not greatly interfere with natural behaviors (Fig. 1I) (Cui et al. 2013). As molecular tools and hardwares continue to advance, optogenetic control systems should accelerate progress in both basic neuroscience and translational technologies.

OPTOGENETIC DEVICES FOR ONCOTHERAPY

In recent years, immunotherapy has become an effective treatment for various forms of cancer. Immunotherapy based on adoptive cell transfer (ACT) can mediate tumor regression in patients with metastatic cancer. However, these therapies have limitations because of poor trafficking of the transferred cells to the target tissue sites (Restifo et al. 2012; Vivier et al. 2012; Kalos and June 2013). To overcome this limitation, optogenetic systems have been developed to enable immune cells to recognize tumor-specific antigens and eradicate tumor cells.

One example is an optical oncotherapy system based on coupling rhodopsin α subunit and chemokine receptor-4 (CXCR4) α subunit, which can induce intracellular chemokine signals and T-cell migration toward a target tumor under 505-nm light illumination. Using this system, PA-CXCR4 can recruit the adoptively transferred $CD8^+$ T cells to target tumors in vivo, leading to a reduction of tumor growth after light stimulation. This kind of photoactivatable chemokine receptor system may open new doors for cancer immunotherapy through optical control of trafficking of T cells to target specific tissues, using light of a specific wavelength (Fig. 2A) (Xu et al. 2014).

Zhou et al. (2012) also used NIR light as a trigger to control Ca^{2+}-responsive gene expression in dendritic cells (DCs) or other immune cells for immunotherapy. The NIR-responsive optogenetic system was composed of a Ca^{2+} release-activated Ca^{2+} (CRAC) channel and lanthanide-doped up-conversion nanoparticles

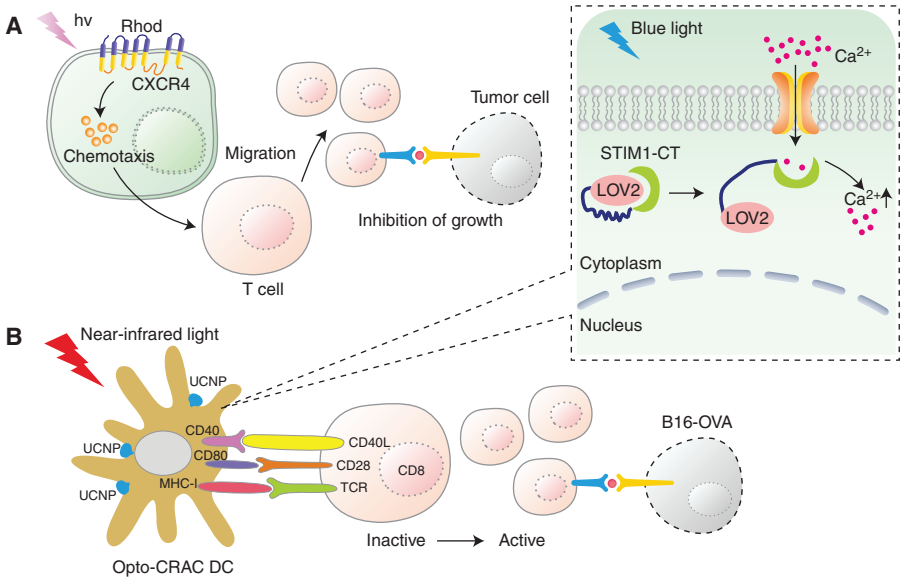

Figure 2. Synthetic optogenetic devices for oncotherapy. (*A*) Optical control of a chemokine receptor for cancer immunotherapy in murine melanoma. Under 505-nm light illumination, the chimeric photoactivatable chemokine receptor composed of the rhodopsin α subunit and chemokine receptor-4 (CXCR4) is activated to recruit transferred cells to the targeted tumor, promoting local effector functions and reducing tumor growth. (*B*) A near-infrared (NIR)-stimulatable optogenetic system (termed Opto-CRAC) designed for cellular immunotherapy. Opto-CRAC constructs can be created by fusing the endoplasmic reticulum (ER) Ca^{2+} sensor stromal interaction molecule 1 (STIM1), which can gate Ca^{2+} release-activated Ca^{2+} (CRAC) channels through its cytosolic domain (STIM1-CT), with the photoswitch light–oxygen–voltage (LOV)2 domain. In the dark, STIM1-CT fragments are kept inactive by docking to the LOV2 domain. Upon illumination with blue light, STIM1-CT fragments are exposed by undocking and unwinding of the LOV2 carboxy-terminal Ja helix, consequently activating Ca^{2+} channels to trigger Ca^{2+} influx across the plasma membrane. When coupled to lanthanide-doped, up-conversion nanoparticles, which have the capability to convert NIR to visible light, the device can wirelessly activate Ca^{2+}-dependent signaling and optogenetically modulate immunoinflammatory responses. The photoactivatable calcium ion influx in Opto-CRAC dendritic cells (DCs) facilitates immature DC maturation and improves antigen presentation, thereby sensitizing T lymphocytes to tumor antigens and promoting antigen-specific immune responses to specifically kill tumor cells in the B16-OVA melanoma model.

(UCNPs) to convert NIR light into blue light. The Opto-CRAC channel contains an ORAI calcium release-activated calcium modulator 1 (ORAI1) and a synthetic stromal interaction molecule 1 (STIM1) fused with a blue-light-responsive LOV2 domain. Under blue-light irradiation, the Jα helix of the carboxy terminal of the LOV2 domain was undocked to expose the STIM1-CT fragment, which could stimulate the ORAI1 Ca^{2+} channel and thus initiate calcium-dependent transgene expression to induce immune responses. In addition, when the Opto-CRAC system was injected into a B16-OVA murine model of melanoma, CD8 T cells were substantially up-regulated to selectively inhibit tumor growth after light stimulation. This wireless Opto-CRAC system seems to be a promising tool for cancer immunotherapy (Fig. 2B) (He et al. 2015).

OPTOGENETIC DEVICES FOR THE TREATMENT OF CARDIOVASCULAR DISEASES

Cardiovascular disease is one of the most common causes of death and disability throughout the world (Flores-Mateo et al. 2009). Although pharmacological advances and surgical strategies can reduce mortality, many patients face a substantial risk of further cardiovascular events,

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including death and myocardial ischemia (Velazquez et al. 2011). Thus, a new therapeutic approach for the treatment of myocardial injury remains a critical need. Joseph Woo's group at Stanford University presented an innovative biological system that rescued the myocardium from acute ischemia by utilizing photosynthesis of the cyanobacterium *Synechococcus elongatus*. The cyanobacterium *S. elongatus* was directly injected into the ischemic territory of an acute myocardial infarction rodent model. After light illumination, *S. elongatus* produced oxygen during photosynthesis, and this increased tissue oxygenation, metabolic activity, and cardiac performance. Furthermore, this photosynthetic system is not toxic and does not induce any significant pathological immune response. This approach using photosynthetic bacteria to provide much-needed oxygen to ischemic cardiomyocytes is a promising option that may have great potential for the treatment of ischemic disease and microvascular disease (Fig. 3A) (Cohen et al. 2017).

In addition, many cardiac diseases are associated with loss of tissue excitability, resulting in heart rhythm disorders (Ambrosi et al. 2015). Implantable electronic cardiovascular devices (IECDs), including pacemakers, cardioverters,

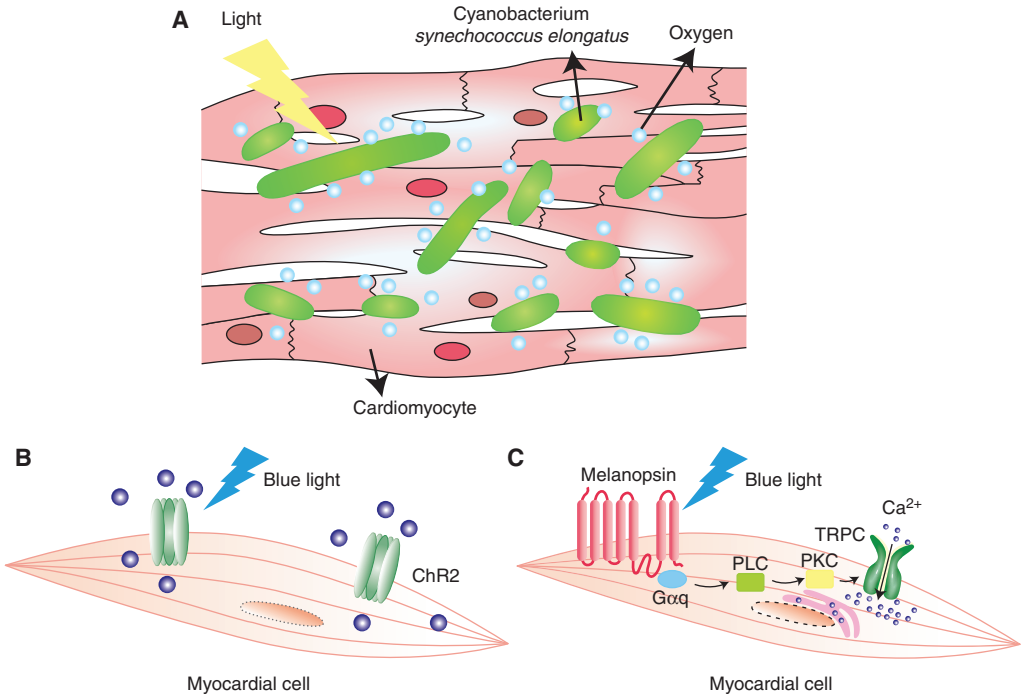

Figure 3. Schematic diagrams of optogenetic devices for treating cardiovascular diseases. (*A*) Cyanobacterium photosynthetic system for correcting myocardial ischemia. The cyanobacterium *Synechococcus elongatus* is directly injected in the hearts of acute myocardial infarction model rats. Under light illumination, cyanobacterium *S. elongatus* can generate oxygen during photosynthesis to increase metabolic activity and improve ventricular function, thus mitigating acute tissue ischemia. (*B*) Optogenetic pacemaker for potential cardiac pacing and resynchronization therapies. The optogenetic pacemaker uses light-sensitive proteins such as the nonselective cationic channel ChR2 to control cardiac excitability. The ChR2 transgene is delivered by adeno-associated virus (AAV) 9 at one or more ventricular sites in rats and the optical fiber is implanted into the target areas, which optogentically pace the treated heart under blue-light illumination. (*C*) Optogenetic pacemaker for modulating cardiomyocyte activity. Melanopsin is a light-activated, Gq-protein-coupled receptor that is stimulated by blue light (470 nm) to increase the beating rate via the G-protein-signaling pathway. PLC, Phospholipase C; PKC, phosphokinase C.

or defibrillators are widely used to address these cardiac diseases (Kolossov et al. 2006; Jung et al. 2008). In addition, biological approaches have recently been developed to restore pacemaking ability by delivering excitatory ion channels. An optogenetic-based strategy provides a tool to selectively interrogate the transduced cells to produce an excitatory ion. Bruegmann et al. (2010) first used ChR2, a light-activated nonselective cation channel, to stimulate heart muscle both in vitro and in vivo. This method enabled precise stimulation of the illuminated area and long-term depolarization of cardiomyocytes, leading to alterations of pacemaking and arrhythmogenic spontaneous extrabeats (Sazani et al. 2002; Bruegmann et al. 2010). Nussinovitch and Gepstein (2015) reported that an optogenetic approach could be used to control cardiac excitability in vivo as well. In this study, a ChR2 transgene was integrated into an adeno-associated virus (AAV) 9 vector, which was injected into one or more myocardial sites. An optical fiber–coupled monochromatic LED (450 nm) was used to deliver flashes of blue light to the site of the ChR2 transgene for cardiac pacing. Dual-site and multisite optogenetic pacing with blue-light-enabled synchronous ventricular activation and shortened ventricular activation times. These findings highlight the unique potential of optogenetics for cardiac pacing and resynchronization therapies (Fig. 3B) (Nussinovitch and Gepstein 2015).

Another example of optogenetic control of the cardiac system involves the use of a hybrid experimental/computational method. Stable transgenic human embryonic stem cells (hESCs) expressing ChR2 could be differentiated into cardiomyocytes, as assessed by patch-clamping, multielectrode array recordings, and video microscopy. Upon optical stimulation, the channel opens and allows sodium ions to enter the cell, producing an action potential. A computer simulation model of the light-paced heart was created to simulate this effect. This computational model allows us to predict the activation sequences in different pacing sites of the human heart, offering the possibility to treat various cardiac disorders associated with depression, schizophrenia, pain syndromes, and arrhythmia (Abilez et al. 2011). Nussinovitch et al. (2014) also designed an optogenetic system based on the light-activated ion channel ChR2 alone or together with the light-sensitive hyperpolarizing proton pump archaerhodopsin-T (Arch-T). This approach enabled optogenetic pacing of cardiac tissue, synchronization of cardiac-tissue electrical activity, decrease of electrical activation time, and inhibition of the cultures' electrical activity under continuous monochromatic red-light illumination (Nussinovitch et al. 2014). This proof-of-concept study implies the suitability of optogenetics for pacemaking and resynchronization therapy applications and for the development of novel antiarrhythmic strategies.

In addition, melanopsin, another light-activated, Gq-coupled receptor, was used for cardiac pacing and arrhythmia therapies. Beiert et al. (2014) developed a new optogenetic tool based on melanopsin to investigate the effects of the Gq-signaling cascade on pacemaking in cardiomyocytes with high spatial and temporal resolution, both in vitro and in vivo. Under 470-nm, blue-light illumination, photostimulation of melanopsin-induced phospholipase C (PLC) activity and IP3 generation, leading to Ca^{2+} release and increased spontaneous pacemaking activity (Fig. 3C). These findings further showed the potential value of optogenetics for cardiac pacing and arrhythmia therapies.

OPTOGENETIC DEVICES FOR DIABETES THERAPY

Diabetes mellitus is a complex and progressive disease characterized by chronically high blood glucose concentrations, and affects at least 415 million people worldwide (IDF Diabetes Atlas Group 2015). In diabetic patients, control of blood glucose homeostasis is traditionally achieved with a strict regimen of food control along with lifelong injections of insulin (for insulin-deficient type 1 diabetic [T1D] patients) or glucagon-like peptide-1 (GLP-1) analogs (for insulin-resistant type 2 diabetic [T2D] patients) at daily to weekly intervals (Holz et al. 1993; Grundy 2006; Barrera et al. 2011; Heng et al. 2015; Guy 2016; Beck et al. 2017). These strategies combine high cost with low patient convenience.

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Two promising, optogenetics-based therapeutic strategies for treating diabetes have been developed. The first is an ingenious approach by Ye et al. (2011) who engineered a blue-light-controlled optogenetic device to regulate blood glucose homeostasis. A blue-light-responsive G-protein-coupled receptor, melanopsin, was ectopically expressed in HEK-293 cells; when triggered by blue light, it activates a transcription factor, the nuclear factor of activated T cells (NFATs), by phosphorylation via an intracellular signaling cascade. This signal cascade was rewired to a synthetic NFAT-controlled promoter to drive shGLP-1 expression. These optogenetically engineered cells were then microencapsulated and implanted into a mouse model of human type 2 diabetes. The mice illuminated with blue light showed improved blood glucose homeostasis (Fig. 4A).

Another study later confirmed that diabetes therapy by light-controlled insulin expression was possible (Wang et al. 2012). Wang et al. constructed another version of the optogenetic device (LightOn) based on the LOV domain, VVD, which forms a rapidly exchanging dimer upon blue-light activation. The DNA-binding domain Gal4 (1–65) without the dimerization domain was fused to the VVD domain and a DNA activation domain VP16 (transcription activator viral protein 16) to form a hybrid transactivator (GAVV). Upon blue-light illumination, the transactivator GAVV forms a dimer, which binds to the promoter Gal (UASG) to initiate transgene expression. They tested the LightOn system in diabetic mice and showed that mice illuminated with blue light can trigger insulin expression to enhance blood glucose homeostasis (Fig. 4B). Both optogenetic systems provide new methods for the treatment of diabetes compared with traditional strategies. One day, diabetic patients may be able to regulate their blood sugar balance just with a beam of light.

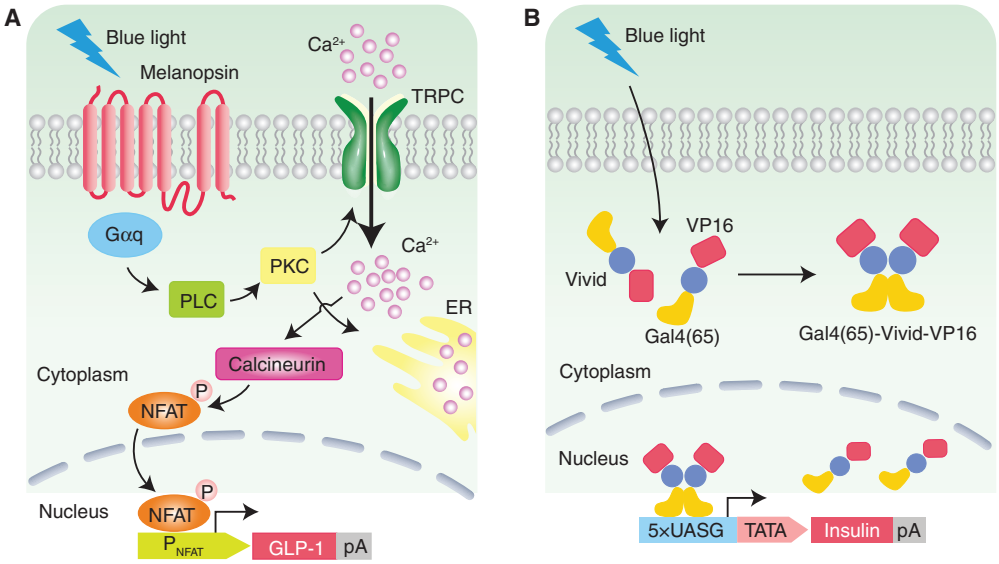

Figure 4. Synthetic optogenetic devices for diabetes therapy. (*A*) Blue-light-controlled optogenetic designer cells for diabetes therapy. Upon blue-light illumination, melanopsin (a Gαq-type G-protein-coupled receptor) is activated to initiate the downstream signaling cascade via phospholipase C (PLC) and phosphokinase C (PKC), which in turn triggers calcium influx through transient receptor potential channels (TRPCs). The elevation of intracellular Ca^{2+} activates the transcription factor NFAT (nuclear factor of activated T cells) to induce transgene expression via an NFAT-responsive promoter (P_{NFAT}). (*B*) A blue-light-induced transcription system based on the light–oxygen–voltage (LOV) domain for diabetes therapy. Upon blue-light activation, Vivid (VVD) changes its structure to induce dimerization of the Gal4 (65)-VVD-VP16 fusion protein, binding to the UASG sequence and initiating transcription of the target gene. ER, Endoplasmic reticulum.

OPTOGENETIC DEVICES FOR GENE EDITING AND EPIGENOME REGULATION

Blue-Light-Controlled Devices for Gene Editing

Site-specific DNA recombination systems are powerful tools that are frequently used in genome engineering to manipulate transgenes in chromosomes of specific cells, tissues, or organs (Akopian and Stark 2005). Cre recombinase is the most frequently used site-specific recombinase; it catalyzes a directional DNA recombination between two *loxP* sites (Sternberg 1981) and enables site-specific manipulations of DNA such as deletion, insertion, inversion, or exchange of a target gene by different *LoxP* sites, leading to a variety of conditional genome-editing possibilities. To achieve conditional and temporal control of genome engineering in living systems, chemically inducible Cre-*LoxP* recombination systems have been developed (Kellendonk et al. 1996; Casanova et al. 2002; Jullien et al. 2003; Sando et al. 2013). However, these approaches cannot meet the criteria for high spatiotemporal resolution. In addition, the inducers can have side effects on living organisms such as cytotoxicity and perturbation of intracellular signaling as a result of off-target effects (Chen et al. 2002; Roshangar et al. 2010; Laplante and Sabatini 2012).

Recently, two blue-light-inducible Cre–*loxP* systems have been developed (Kennedy et al. 2010; Schindler et al. 2015; Kawano et al. 2016). Kennedy et al. (2010) reported genetically encoded blue-light-inducible, protein-interaction modules based on *A. thaliana* cryptochrome 2 and CIB1 that dimerize on blue-light exposure with subsecond time resolution and subcellular spatial resolution. By taking advantage of split Cre recombinase and blue-light-dependent dimerization of plant photoreceptor cryptochrome 2 (CRY2) and its binding domain CIB1, they have developed Cre recombinase-mediated DNA recombination by blue light (Fig. 5A) (Kennedy et al. 2010). However, the performance of the CRY2–CIB1 split Cre device is unsatisfactory, as the recombination efficiency is low both in vitro and in vivo. Therefore, Kawano et al. (2016) developed another blue-light-inducible Cre–*loxP* recombination system (PA-Cre) for light-inducible DNA recombination in living systems. PA-Cre is based on reassembly of split Cre domains fused to the Magnet system (Kawano et al. 2015), a blue-light-dependent dimerization system containing two photosensitive domains, pMag and nMag, which were engineered from a flavin-binding fungal photoreceptor (Fig. 5B) (Zoltowski et al. 2007). This PA-Cre device efficiently induced DNA recombination with high spatiotemporal precision upon illumination. Despite this development of light-controlled Cre recombinase for genome engineering, the phototoxicity of blue light to mammalian cells and poor penetrative ability through human tissues severely restrict the utility of these devices for further translational research and clinical applications. More advanced optogenetically controlled Cre recombination systems are needed.

The CRISPR-Cas (CRISPR-associated) system in bacteria has provided powerful tools for programmable genome engineering (Cong et al. 2013b; Mali et al. 2013; Wang et al. 2013). In particular, CRISPR-Cas9 from *Streptococcus pyogenes* has emerged as a powerful technology for targeted genome modifications (Cong et al. 2013a; Jinek et al. 2013; Mali et al. 2013). The Cas9 nuclease can bind to and cleave a target DNA sequence to induce DNA double-strand breaks under the guidance of sgRNAs (Hsu et al. 2013, 2014). However, persistent high expression of Cas9 greatly increases off-target cleavage, which is a serious issue (Fu et al. 2013; Pattanayak et al. 2013). Therefore, chemical control of the nuclease activity of Cas9 has been developed, such as doxycycline-regulated Cas9 expression (Gonzalez et al. 2014; Dow et al. 2015) and rapamycin-inducible split-Cas9 (Zetsche et al. 2015). But, these chemicals have adverse side effects, and as they diffuse freely, they cannot be rapidly removed. For example, rapamycin can perturb the endogenous rapamycin mTOR signaling pathway (Laplante and Sabatini 2012). To address these issues, scientists have developed several photoactivable CRISPR-Cas9 systems to achieve spatiotemporally controlled genome editing. Jain et al. created a syn-

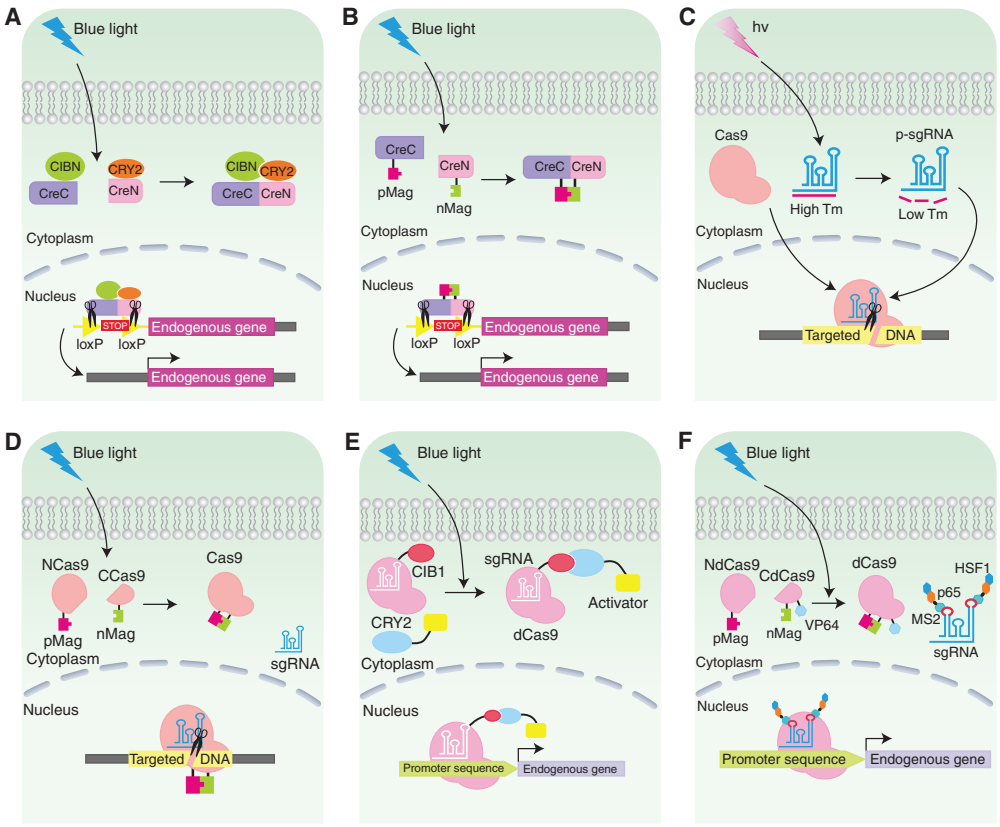

Figure 5. Schematic diagrams of optogenetic devices for genome engineering. (*A*) Blue-light-activated Cre recombinase as a tool for spatial and temporal control of gene editing. The blue-light-sensitive Cre recombinase system containing a CIBN-CreC domain and a CreN-cryptochrome 2 (CRY2) domain enables spatiotemporal control of gene editing based on CRY2–CIB dimerization under blue-light illumination. (*B*) In the dark, Cre is split into two fragments that have no catalytic activity for *loxP* sites. Under blue-light illumination, Cre quickly recovers its catalytic activity as a result of complementation of the CreN and CreC domains induced by the light-dependent dimerization of pMag and nMag, leading to recombination of DNA sequences flanked by two *loxP* sites. (*C*) UV-light-activated clustered regularly interspaced palindromic repeats (CRISPR) with light-mediated unveiling of sgRNAs (CRISPR-plus) system in human cells. The photocleavable oligonucleotides hybridize to the target region of the sgRNAs, leading to high melting temperature (Tm) and preventing the sgRNAs from binding to the target DNA. Under UV illumination, the photocleavable oligonucleotides are cleaved and the sgRNAs are released to target the specific DNA sequence owing to lower Tm, leading to light-activated target DNA cleavage. (*D*) Blue-light-inducible CRISPR-Cas9 system for optogenetic genome editing. The photoactivatable Cas9 (paCas9) is engineered by fusing split Cas9 fragments with photoinducible dimerization domains (pMag and nMag). Upon blue-light illumination, pMag and nMag heterodimerize to reassociate the split Cas9 fragments, thereby recovering RNA-guided nuclease activity, enabling light-inducible targeted genome editing. (*E*) Blue-light-mediated CRISPR-dCas9 transcription system based on CRY2/CIB1. The CRISPR-Cas9-based photoactivatable transcription system contains two fusion proteins and sgRNAs. One of them is composed of dCas9 and CIB1 that bind to the target sequence via sgRNAs, whereas the other consists of the photolyase homology region of CRY2 and the transcriptional activator domain. In the dark, the dCas9–CIB1 complex binds to the targeted gene promoter via sgRNAs, whereas the activator probe is free in the nucleus and cannot activate gene expression. Under blue-light illumination, the downstream gene transcription is initiated by heterodimerization of CRY2PHR and CIB1. (*F*) Blue-light-inducible split-dCas9-based transcription system for photoactivation of endogenous genes. The photoactivatable dCas9 (padCas9) system contains three chimeric proteins, the amino-terminal fragment of dCas9 fused with positive Magnet (pMag), the carboxy-terminal fragment of dCas9 fused with VP64 and negative Magnet (nMag), and MS2-coat protein fused with p65 and HSF1 activator domains (MS2-p65-HSF1). Upon blue-light illumination, pMag and nMag are heterodimerized, which enables reassociation of the split Cas9 fragments and activation of endogenous gene expression by sgRNAs-mediated recruitment of VP64, p65, and HSF1.

thetic UV-light (365 nm)-responsive "CRISPR-plus" (CRISPR-precise, light-mediated unveiling of sgRNAs) system to photocage the activity of the sgRNAs (Jain et al. 2016). This system takes advantage of a key oligonucleotide element, which is complementary to the seed sequence of sgRNAs, to remain inactive, and this can be cleaved by UV light to release the sgRNAs (Fig. 5C). Recently, Nihongaki et al. (2015a) engineered a blue-light-controlled Cas9 (paCas9) that was generated by fusing two split Cas9 fragments (amino-teminus residues 2–713 and carboxyl-teminus residues 714–1368 of Cas9) with the two photoinducible domains pMag and nMag. In the dark, the pMag-N-Cas9 and the nMag-C-Cas9 are separated and do not interact. Under blue-light illumination, pMag and nMag heterodimerize, making the split Cas9 fragments active to edit target genes (Fig. 5D) (Nihongaki et al. 2015a). Nevertheless, a more robust optogenetically controlled Cas9 system with less phototoxicity and better deep tissue penetration for in vivo applications is still needed.

Blue-Light-Controlled Devices for Endogenous Gene Activation

Genome engineering technologies have enabled activation or repression of endogenous genes in mammalian cells with synthetic transactivators or transrepressors targeting almost any DNA sequence (Beerli et al. 2000; Zhang et al. 2011; Gilbert et al. 2013; Konermann et al. 2013; Maeder et al. 2013; Qi et al. 2013). Very recently, researchers have engineered the CRISPR-dCas9 (catalytically dead Cas9 variant) system that lacks endonucleolytic activity but retains the capacity to interact with DNA to be functionalized with transcriptional activation domains as a hybrid transcription factor, enabling dCas9 to serve as a tool for cellular programming at the transcriptional level (Gilbert et al. 2013; Maeder et al. 2013; Perez-Pinera et al. 2013; Qi et al. 2013). The CRISPR-dCas9 activators have proven to be a powerful tool for the manipulation of targeted endogenous gene expression (Dominguez et al. 2016). These dCas9 activators can be further fused with inducible dimerization domains to spatiotemporally control endogenous gene expression (Maeder et al. 2013; Perez-Pinera et al. 2013). For example, Polstein and Gersbach (2015) have developed a CRISPR-dCas9-based photoactivatable transcription system based on the CRY2-CIB1 system (Fig. 5E). This device was made up of three parts, including CRY2 fused to a transcriptional activator (CRY2-activator), dCas9 fused with CIB1 (the binding partner of CRY2), and sgRNAs targeting specific DNA loci. Under blue-light illumination, CRY2 and CIB1 form a dipolymer to recruit the transcriptional activator to the promoter region of the target gene under the guidance of sgRNAs, thereby initiating transcription of user-defined endogenous genes (Nihongaki et al. 2015b). Similarly, Nihongaki et al. (2017) have also developed an improved CRISPR-Cas9-based photoactivatable transcription system Split-CPTS2.0. The amino-terminal and carboxy-terminal fragments of dCas9 are fused with two photoinducible dimerization domains, pMag and nMag, respectively. Blue-light irradiation induces heterodimerization between pMag and nMag, which enables the split dCas9 fragments to form a functional complex. The Split-CPTS2.0 enables blue-light-inducible activation of endogenous target genes in various mammalian lines. The authors further demonstrate that Split-CPTS2.0 can be used to up-regulate *NEUROD1* expression to induce neuronal differentiation from induced pluripotent stems cells (iPSCs) (Fig. 5F). To overcome the weakness of blue light, Shao et al. (2018) recently developed a far-red light-activated CRISPR-dCas9 effector (FACE) device that induces transcription of endogenous genes in the presence of fiber ring light (FRL) stimulation. This device has been shown to mediate targeted epigenetic modulation and utilized in the differentiation of iPSCs into functional neurons by FRL stimulation (Shao et al. 2018). These optogenetic platforms offer optogenetic control of CRISPR applications based on the recruitment of effectors, such as epigenetic modifications (Xu et al. 2016) and base editing (Hess et al. 2016).

OPTOGENETIC-BASED BIOELECTRONIC MEDICAL DEVICES

Mind-Controlled Transgene Expression System for Future Gene- and Cell-Based Therapies

Synthetic optogenetic devices for traceless remote control of transgene expression may provide new treatment opportunities for gene- and cell-based therapies. Currently available optogenetic devices that mediate the behaviors of implanted designer cells are controlled by percutaneous illumination using an extracorporeal light source or implanted optical fibers connected with a light source (Ye et al. 2011; Wang et al. 2012). Recently, Folcher et al. (2014) created a self-sufficient, removable wireless-powered optogenetic implant that provided high treatment compliance and host mobility (Fig. 6A). They developed a synthetic mind-controlled transgene expression platform that enabled wireless control of transgene expression in mammalian cells by human brain activities and mental states. This is a pioneering study, demonstrating that scientists can tap into human thought-specific brainwaves and wirelessly transfer them to a gene network to regulate transgene expression. Briefly, an electroencephalogram (EEG) headset captures mental-state-specific brain waves and delivers the signal to a brain-computer interface (BCI), which is programmed to turn on a NIR-LED in response to brain waves above a certain threshold. Thus, by capitalizing on both optoelectronics and optogenetics, the expression of proteins by a cell-based bioelectronics implant can be controlled by human thought. In their experimental system, the implant containing the optogenetic designer cells was placed under the skin of a mouse to produce secreted embryonic alkaline phosphatase (SEAP) under NIR light illumination controlled by a human's brain activity. This optogenetic device consists a light-dependent bacterial diguanylate cyclase (DGCL), which can convert GTP into cyclic diguanylate monophosphate (c-di-GMP). The signaling molecule c-di-GMP binds to and activates the stimulator of interferon genes (STING) at the endoplasmic reticulum, and this further activates the tank-binding kinase 1 (TBK1)-mediated phosphorylation of the transactivator IRF3. The phosphorylated IRF3 enters the nucleus and binds to a synthetic promoter to drive downstream transgene expression (Folcher et al. 2014). The mind–transgene interface is a breakthrough toward next-generation smart drug delivery systems that fine-tune the release of therapeutic drugs by synthetic designer optogenetic cells under the remote control of brain activities. Such mind-controlled bioelectronic medical devices might one day help to address neurological diseases, such as chronic headache, epilepsy, and back pain by monitoring specific brain waves and coordinating the transgene-based expression of an appropriate drug in the implant at the right time.

Smartphone-Controlled Optogenetic Device for Semiautomatic Diabetes Therapy

The traditional treatment of diseases based on inquiries by the doctor, diagnosis, and prescription of a suitable remedy is set to change with the emergence of cell-based therapies (Fischbach et al. 2013). Recently, Shao et al. (2017) made substantial progress toward smart designer cell-based therapies by creating a semiautonomous platform using smart electronics, software, and optogenetic cells that can respond to external commands. They constructed optogenetically engineered cells that could be photoactivated by far-red light to trigger transgene expression of the glucose-lowering hormone insulin (for type 1 diabetes therapy) or GLP-1 (for type 2 diabetes therapy) to maintain glucose homeostasis in diabetic mice (Gomelsky 2017; Shao et al. 2017). The optogenetic cells were then implanted into diabetic mice, and a light source was used to activate transgene expression. The illumination duration and intensity were determined on the basis of mouse blood glucose concentrations. The core technology of this optical system is a bacteriophytochrome-based optogenetic circuit that has three critical modules: BphS (a bacterial light-activated cyclic diguanylate monophosphate [c-di-GMP] synthase), YhjH (a c-di-GMP-specific phosphodiesterase to regulate intracellular c-di-GMP level), and BldD (a c-di-GMP-binding domain) fused to a

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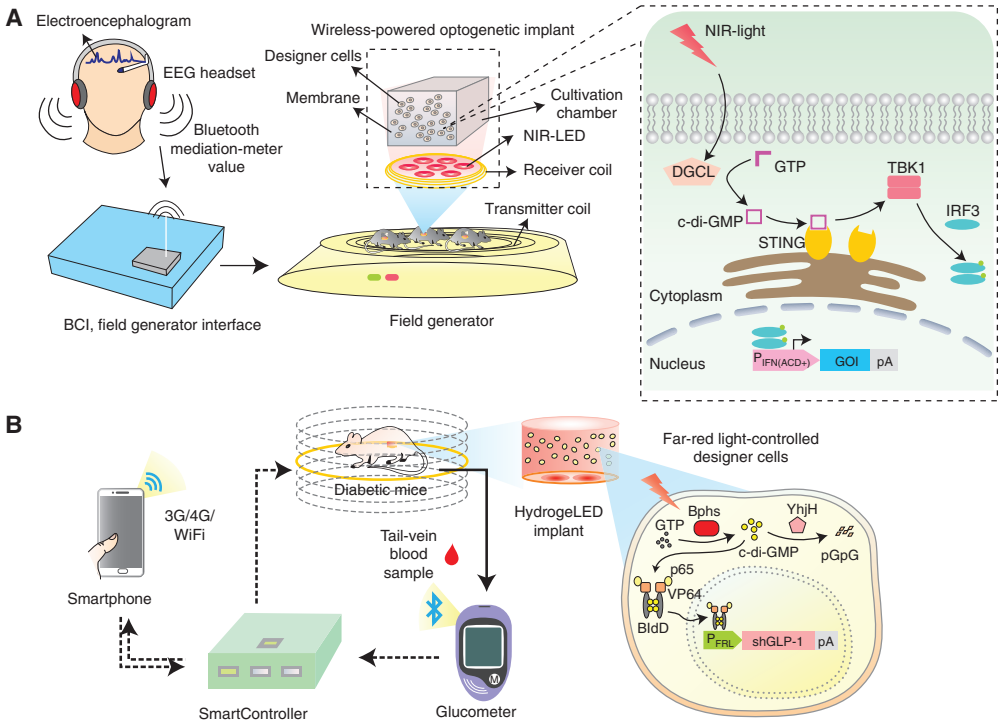

Figure 6. Schematic diagrams of optogenetic-based bioelectronic medicine devices. (*A*) A mind-controlled wireless-powered optogenetic implant device for transgene expression. The mind-controlled transgene expression device enables human brain activities to wirelessly control gene expression in human cells. The brain-wave activities are captured by an electroencephalography (EEG) headset and transmitted to the field generator interface via Bluetooth, which couples with a wireless-powered optogenetic implant. This brain–computer interface (BCI) can power the near-infrared (NIR) light-emitting diode (LED) and illuminate the culture chamber, thereby programming the designer cells to control the transgene expression. The synthetic optogenetic gene circuit contains NIR light-activated bacterial diguanylate cyclase (DGCL), converting GTP to the orthogonal second messenger cyclic diguanylate monophosphate (c-di-GMP), which activates the stimulator of interferon genes (STING)-dependent signal pathway to initiate gene expression. (*B*) Smartphone-controlled optogenetic designer cells for the treatment of diabetes. A Bluetooth glucometer can measure glucose in a drop of blood from a tail vein of mice and transmit the data to a SmartController or a smartphone. The SmartController sets different far-red light intensities either by modulating the electromagnetic field or an external far-red light source. Optogenetically engineered cells implanted into diabetic mice could be activated by far-red light and then trigger insulin/GLP-1 expression to control blood glucose homeostasis. The optogenetic designer cells carry a far-red light-inducible synthetic gene circuit containing a far-red light receptor BphS, phytochrome c-di-GMP synthase converting GTP into c-di-GMP, c-di-GMP phosphodiesterase YhjH controlling intracellular c-di-GMP homeostasis, c-di-GMP activated hybrid transactivator p65-VP64-BldD, and a far-red responsive promoter P_{FRL}. Far-red light activates the engineered BphS to produce c-di-GMP, which triggers p65-VP64-BldD dimerization followed by binding to the synthetic promoter P_{FRL} to drive transgene expression.

transactivation domain (p65-VP64) (Ryu and Gomelsky 2014; Tschowri et al. 2014) to form a hybrid transactivator BldD-p65-VP64. Under FRL illumination, the bacterial photoreceptor BphS converts intracellular guanylate triphosphate (GTP) into c-di-GMP. Increased c-di-GMP binds to the hybrid transactivator BldD-p65-VP64, which translocates into the nucleus and binds to its chimeric promoter to drive transgene expression (Fig. 6B).

Capitalizing on multidisciplinary design principles involving electrical engineering, soft-

ware development, and synthetic biology, these researchers have created a novel technological infrastructure that enables smartphones or Bluetooth-active point-of-care diagnostic devices to remote-control transgene expression of human designer cells with digital precision. In particular, an Android-based smartphone App "ECNU-TeleMed" was developed to regulate a custom-designed intelligent electronic home server box "SmartController" via the global GSM network. To translate this electronic controller circuit into biological responses, far-red light-responsive optogenetic designer cells were used as receiver units for smartphone-triggered, SmartController-driven, far-red light signals. To develop this wireless controller network for diabetes therapy, an implant architecture "HydrogeLED" capable of coharboring a designer-cell-carrying alginate hydrogel and wirelessly powered far-red LEDs was designed. In diabetic mice implanted with the HydrogeLED, in vivo expression of insulin or GLP-1 from the HydrogeLED implants could be controlled not only by preset ECNU-TeleMed programs, but also by a custom-engineered Bluetooth-active glucometer in a semiautomatic, glycemia-dependent, and self-sufficient manner (Fig. 6B) (Gomelsky 2017; Shao et al. 2017).

Collectively, this work combines the unique capacity of electronic devices to read and generate digital signals with the high theranostic precision of biological cells, and represents the first mobile healthcare system that unites the global markets of point-of-care technologies, mobile phone technology, and cellular medicines. Although current mobile healthcare systems are generally limited by a lack of automation between diagnosis and drug delivery, the modular design of the SmartController system should allow adaptation of this novel closed-loop telemedicine concept to any kind of disease, helping to bring cell-based precision medicines into the clinic.

CONCLUSIONS AND OUTLOOK

Synthetic biology provides a powerful platform to understand and harness biology (Way et al. 2014), and in this context, influenced by many scientific and engineering disciplines, scientists have developed a range of synthetic optogenetic devices using natural molecular modules (Boyden et al. 2005; Favory et al. 2009; Levskaya et al. 2009; Wu et al. 2009; Yazawa et al. 2009; Kennedy et al. 2010; Ye et al. 2011; Heyes et al. 2012; Wang et al. 2012; Zhou et al. 2012; Kaberniuk et al. 2016; Kainrath et al. 2017; Shao et al. 2017). Such optogenetic tools, activated by specific wavelengths of light, permit unprecedented spatiotemporal precision in the control of cellular activities without the need to employ chemical inducers, which might cause undesired effects, such as perturbing intracellular signaling pathways (Laplante and Sabatini 2012; Konermann et al. 2013). Furthermore, the remote-controllability, reversibility, and negligible toxicity of optical control systems provide a solid foundation for the application of optogenetics in biomedicine (News Staff 2010a; Editorial 2010b). Optogenetic approaches have already been applied for treating numerous diseases; for example, optically controlled gene switches have been developed for diabetes treatment (Ye et al. 2011; Wang et al. 2012), tumor immunotherapy (Xu et al. 2014; He et al. 2015), cardiac diseases (Kolossov et al. 2006; Jung et al. 2008; Bruegmann et al. 2010; Nussinovitch et al. 2014; Cohen et al. 2017), and genome activation and editing (Zoltowski et al. 2007; Nihongaki et al. 2015a; Schindler et al. 2015; Jain et al. 2016; Kawano et al. 2016). Notably, there are a few clinical trials with optogenetic treatment in patients that are ongoing. RetroSense therapeutics is the first ever for an optogenetic treatment and has reported that participants who are completely blind from retinal degenerative diseases have received injections of a human-engineered AAV to deliver the gene ChR2 to retinal ganglion cells in a phase I/II clinical trial. According to the reports, no adverse ocular events were observed and the treatment showed some biological activity. GenSight is also undergoing clinical trials of optogenetic therapies using red-light-sensitive opsins (ChrimsonR) to treat people who lost their vision. Circuit therapeutics is taking a different way with its pain therapy and has showed the ability to block neural circuits with yellow light. Obviously, these clinical trials will produce

large meaningful information about doing optogenetics studies in humans and will boost the practical application progress of optogenetic treatment.

A multidisciplinary design approach involving electrical engineering, software development, and optogenetics is providing novel technological infrastructures that enable electronic devices such as smartphones to remote-control transgene expression in optogenetic designer cells with digital precision. For example, Shao et al. (2017) recently developed a smartphone-controlled optogenetic device that combines the ability of electronic devices to read and generate digital signals with the high theranostic precision of biological cells to address diabetes. This closed-loop telemedicine concept should be applicable to any kind of disease, and should speed the progress of cell-based precision medicines into the clinic. This offers the long-term prospect that pills or injections will become unnecessary and, instead, implanted, custom-designed electronic pills containing smart designer cells to release therapeutic drugs will become commonplace. Such designer cells could monitor multiple blood markers and coordinate the release of therapeutic hormones or proteins to correct symptoms. Moreover, doctors or patients will be able to use their smartphones to monitor, adjust, or intervene in the functions of the implanted electronic pills at any time and from any geographic location.

With the rapid development of gene-editing tools, CRISPR-Cas9 technology has the potential to correct gene defects associated with various diseases (Wu et al. 2013; Long et al. 2014; Xie et al. 2014; Yin et al. 2014), but challenges remain, including immunogenicity and off-target effects (Fu et al. 2013; Hsu et al. 2013; Pattanayak et al. 2013). Therefore, tight spatiotemporal control of CRISPR-Cas9 activities will be critical. Although blue light has been used to modulate CRISPR-Cas9 activity in vitro (Nihongaki et al. 2015a), it will be necessary to develop optogenetic CRISPR-Cas9 systems controlled by red or far-red light to achieve deep tissue penetration capacity and negligible phototoxicity for in vivo applications. As a start along this line, a far-red light-controlled transcription device recently developed by Shao et al. (2018) was linked to CRISPR-Cas9 to construct a next-generation photoactivable genome or epigenome editing tool for in vivo applications.

In cancer treatment, immunotherapy is considered one of the most promising therapeutic strategies (Rosenberg et al. 2004; Rosenberg 2005; Tan et al. 2017), and optogenetic approaches have been extended to the immune system (Tan et al. 2017). For example, the opto-CRAC system was developed to phototrigger Ca^{2+} influx and thus to activate immune cells following light stimulation with UCNPs (He et al. 2015). However, the bottleneck here is currently the low efficiency of photoactivation within biological tissues or organs. However, our recently created far-red light-controlled transgene expression devices (Folcher et al. 2014; Shao et al. 2017) represent an important step toward the goal of translating optogenetic immunomodulation technology into routine clinic applications.

A further challenge facing clinical applications is to identify appropriate chassis cells to upload the synthetic optogenetic circuits into humans without side effects. Future clinical applications may require autologous parental cells such as the patients' own mesenchymal stem cells; this approach has already been clinically validated. Another challenge is to stably integrate the complex optogenetic devices into specific sites of the genome in human cells. Both CRISPR-Cas9 technology and the artificial chromosome technique (Mercy et al. 2017; Mitchell et al. 2017; Shen et al. 2017; Wu and Li 2017; Xie and Li 2017; Zhang and Zhao 2017) will be helpful in this respect. The continuing development of novel optogenetic tools should lead to rapid advances in basic research as well as biomedical applications. There is a real prospect that we will be able to treat diabetes, cancer, cardiac disease, etc. simply with a beam of light in the future.

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