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Implantable Optofluidic Systems for Wireless In Vivo Photopharmacology

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Photopharmacology, which uses chemical photoswitches for the optical manipulation of biological process, holds immense potential for neuroscience and clinical medicine due to its high specificity, fast response, and versatility. However, enabling photopharmacology in living subjects has been an arduous undertaking mainly because of limitations of the available tools. Conventional approaches to drug delivery and photostimulation involve the use of bulky, rigid, and tethered implants in the form of metal cannulas and optical fibers. These prevent highly precise, spatially matching stimulation with drugs and light, aggravates adverse tissue responses, and causes undue stress in the freely-moving subject. Recent advances in materials science and device engineering have led to the development of miniaturized, standalone multimodal implants referred to as "optofluidic" devices, which allow wireless delivery of both light and drugs. Herein, we review state-of-the-art wireless optofluidic systems, which can open up new horizons for in vivo photopharmacology, and discuss future directions for further technology developments.

1. Introduction

Exploring and understanding the key signaling elements and pathways within a convoluted mesh of billions of neurons is among the biggest challenges in the field of neuroscience. Although neuromodulation techniques, such as optogenetics and pharmacology, have helped precisely isolate and dissect neural circuits,[1,2] they suffer from inherent limitations. Optogenetics offers relatively rapid and reversible cell-type selective excitation or inhibition compared to other contemporary or pharmacological methods, but requiresgenetic modification of target cells to express light-sensitive proteins.[3] Pharmacology provides high selectivity but is limited because of the low temporal resolution in control.[4] The need for high spatiotemporal selectivity without necessitating expression of exogenous microbial opsins has led to the development of a new technique called 'photopharmacology', which uses light to activate chemical compounds to offer rapid, potentially reversible, and highly precise neuromodulation.[4,5] This novel multimodal biological technique not only helps target the same cells using chemical photo-switches or ligands, allowing extremely versatile, temporally-precise and rapid biological control, but also enables receptor-subtype level control (an arguably higher level of specificity than that provided by cell-type selective optogenetics) reducing limitations on pharmacokinetics and pharmacodynamics.

Photopharmacology, also referred to as optopharmacology, sits firmly at the intersection of photochemistry, the chemical effects of light, and pharmacology, the study of how drugs work. This multidisciplinary field encompasses many diverging pursuits that include the development of therapeutic agents, chemical probes, and photosensitive approaches that require a genetic modification to a receptor and/or ligand. For this minireview, we focus on photopharmacology as it pertains to photosensitive chemical entities aimed at receptors on neurons. These compounds can be broadly partitioned into three distinct strategies. First, "caged compounds" are prototypical receptor ligands altered to contain a photocleavable domain rendering the compound inert until presented with the optimal wavelength and intensity necessary to activate the cleavage site. Though countless examples of caged compounds exist, caged glutamate has perhaps been the most ubiquitously used of these compounds.[6–8] Secondly, "photoswitchable" compounds are similar in concept to caged compounds, but these ligands have reversible photosensitive domains that can trigger conformational changes that lead to activity or inactivity of the compound. Unlike with caged compounds, the light-induced activation of photoswitchable compounds is reversible either with another wavelength of light or removal of the light source.[9,10] Finally, a third class of ligands known as photolabels covalently bond their receptors in the presence of light.[11] The chemical details and application of these and more photopharmacology approaches have been extensively reviewed elsewhere by pioneers in this field.[5,12,13] Critical for this review

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is that in vivo application of these highly-selective tools has remained challenging even while in vivo light delivery has made substantial advances. These challenges arise from many issues inherent to many photoactivatable compounds, including the frequent need for high energy ultraviolet light to uncage, the opacity of mammalian brain tissue, the metabolic stability of photopharmacological ligands, and any potential toxicity they may present. However, many of these limitations can be mitigated by devices that enable spatially-matched ligand and light delivery, which is the focus of this mini-review. While many of these devices have been used to demonstrated combined optogenetics and pharmacology, they also show clear promise for more traditional photopharmacological applications.

Although immense progress has been made in photopharmacology, enabling in vivo photopharmacology still remains challenging. There are significant limitations due to the lack of advanced in vivo tools that can provide simultaneous drug delivery and photostimulation in a minimally invasive manner. Traditionally, metal cannulas[14] and optical fibers[15] are used for drug delivery and photostimulation, respectively. However, combined use of these tools is not ideal for reliable long-term in vivo experiments for the following reasons. First of all, conventional tools are too rigid to remain for a long enough time inside soft brain tissues (~73 GPa for optical fibers[16] and ~200 GPa for metal cannulas[17] vs. 0.1–6 kPa for soft neural tissue[18]). The substantial mechanical mismatch between the implanted structures and soft tissue causes adverse glial scarring and inflammatory response, thus hindering chronic implantation.[19] Additionally, integration of the two bulky tools – that is, a cannula (~500 μm in diameter) and an optical fiber (~200 μm in diameter) – for simultaneous drug delivery and photostimulation, either increases the dimensions of the implant, thereby causing significant tissue displacement and damage or increases the chance of failure in either modality. This also makes highly precise, spatially-matched stimulation with drugs and light an arduous undertaking. Furthermore, conventional tools are tethered to their light or fluid source, limiting the naturalistic movement of freely moving animals and causing undue stress. Recent advances in materials science and device engineering have tried to overcome these limitations by allowing researchers to develop soft, miniaturized multimodal devices referred to as "optofluidic" devices, which when integrated with wireless technologies, can provide unleashed drug delivery and photostimulation with minimal invasiveness.[20–24] Possessing tremendous potential for chronic in vivo photopharmacology, these emerging tools can enable not only spatiotemporal manipulation of drug and light delivery into precise, spatially matching locations in tissue but also seamless long-term implantation based on their soft constructs complying with biological tissue. In this mini-review article, we introduce and discuss state-of-the-art implantable optofluidic devices, which may open new opportunities for in vivo photopharmacology with easy-to-use, versatile, and untethered access to various space-critical locations inside soft neural tissues. We first discuss key requirements and design considerations for implantable optofluidic devices. The subsequent section reviews recently developed wireless optofluidic devices, highlighting their design and key features. We will then present a discussion of challenges and future development directions for this emerging technology, which can possibly lead to the realization of the full potential of in vivo photopharmacology.

2. Key Requirements and Design Considerations for Implantable Optofluidic Devices

2.1. Key Requirements

Figure 1 highlights the key requirements for an ideal optofluidic device, compared to conventional tools used for drug delivery and photostimulation. First, the features for drug delivery and photostimulation in the probe must be compactly integrated to achieve an ultrathin form factor to minimize tissue damage both during and post-surgery. This will significantly increase its chronic biocompatibility and minimize inflammatory response in tissue as shown in several studies.[20,21] It will further reduce the surgical stress on the subject and speed recovery. These miniaturized optofluidic probes can be constructed using microfabrication techniques by integrating optical and chemical pathways in co-centric[25,26] or sandwiched[20–24] configurations. Second, the implanted multimodal probe of the optofluidic device must mechanically match the soft neural tissue to minimize adverse inflammation and glial scarring effects. This will help increase its biocompatibility for conducting long-term in vivo studies. The biomechanical compatibility of the probes can be achieved by employing soft and flexible materials (e.g., polydimethylsiloxane (PDMS), polyimide (PI), polyethylene terephthalate (PET), and parylene C) for construction of the implants so that they can easily accommodate the micro-motions of tissue occurring because of cardio-respiratory functions.[14] Finally, entirely self-contained optofluidic systems integrating light sources, fluidic pumps, power sources, and wireless control, need to be implemented for a tether-free operation to remove any restriction and the associated stress on the behavior of freely moving animals. Apart from helping minimize 'perceived weight' on the subject, it also allows remote control of subjects, thus eliminating any 'observer-effect' caveats in experiments. Furthermore, wireless control technologies, such as Bluetooth and WiFi, can enable unmatched scalability and selective control among a cohort of a large group of simultaneously running experiments with minimal cost, time, and effort, thereby opening opportunities for high-throughput in vivo studies. With all of these features integrated and enabled, an implantable wireless optofluidic system will offer spatiotemporal control of drug delivery and photostimulation in freely moving animals with unprecedented versatility, chronic biocompatibility, and ease-of-use. Thus, it will be able to help speed unraveling of various unsolved challenges towards in vivo photopharmacology.

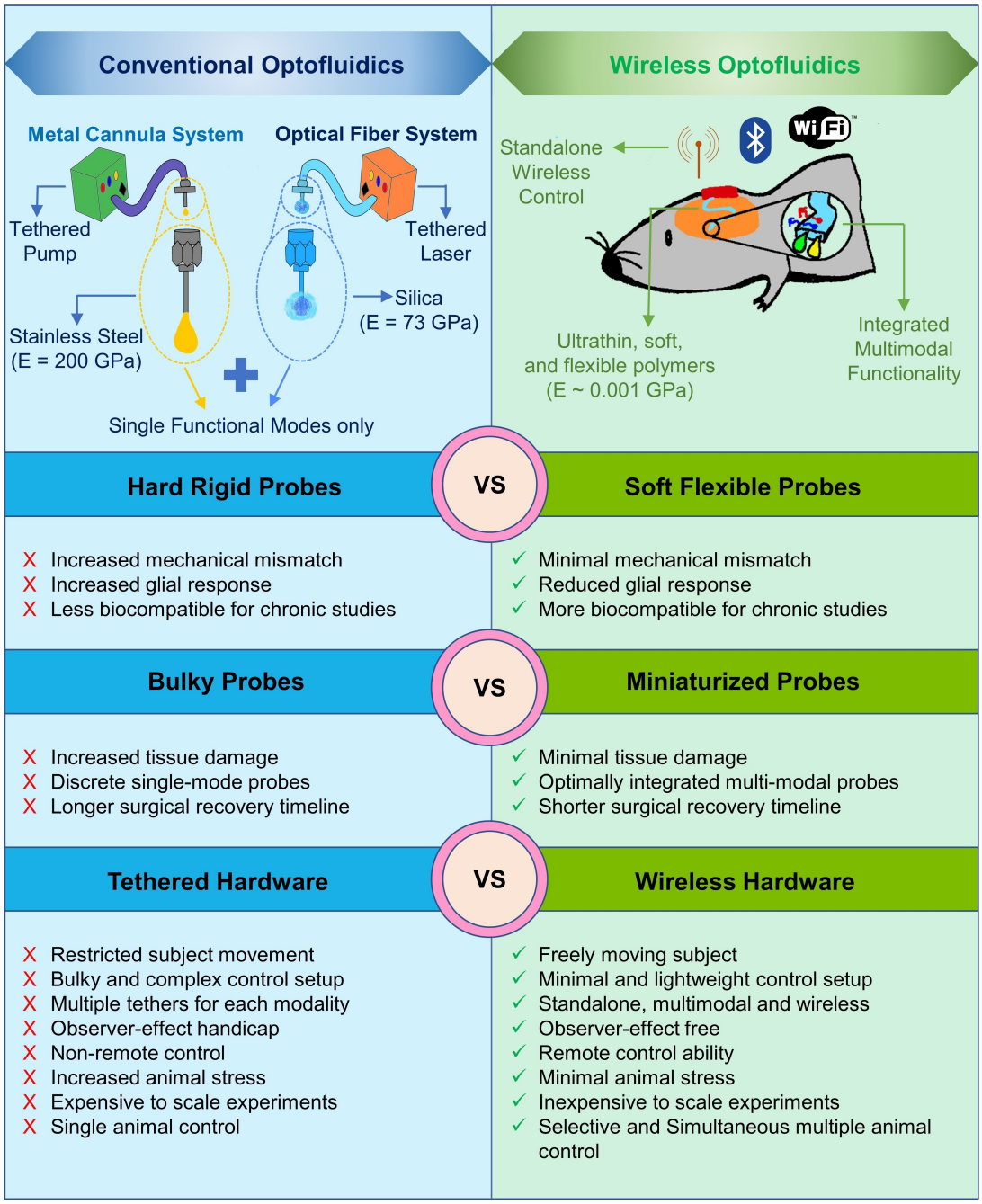

Figure 1. Comparison of conventional and wireless optofluidic devices for in vivo photopharmacology. Flexible, miniaturized probes and wireless hardware are key requirements for chronic drug delivery and photostimulation.

2.2. Design Considerations

To construct implantable optofluidic systems that satisfy the aforementioned key requirements, three main components, namely, a light source, a drug infusion system, and a wireless control module, must be optimized in both size and performance for in vivo photostimulation and drug delivery.

For photostimulation, micro-scale inorganic light-emitting diodes (μ-ILEDs) are considered one of the most attractive options as a light source.[27] μ-ILEDs can be mounted directly on soft flexible probes built with biocompatible polymers (e.g., PDMS,[20–24,28,29] PI,[22,24,30,31] PET,[20,21,29] etc.) to achieve chronic and minimally invasive light delivery.[27] Their miniaturized size (equivalent to a grain of salt) not only helps reduce tissue inflammation and damage but also allows μ-ILEDs to be directly inserted into tissue and access near the target area, thereby maximizing photostimulation efficiency while minimizing optical power loss, which is common in optical waveguide-based methods.[32] It also enables highly versatile designs by allowing the integration of tiny light sources with different wavelengths in any configuration of probes. These μ-ILEDs have been shown to achieve reliable in vivo photostimulation (>1 mW/mm^2)[27]

even when operated at minimum required power levels, thus making them easy to power using a small battery or a wireless energy harvester module. Despite some potential heating concerns related to in vivo operation of μ-ILEDs, recent studies have demonstrated their biothermal safety by limiting the input current and driving signal pulse width to μ-ILEDs to suppress the temperature increase in brain tissue below ~1 °C.[20–24,28–31,33–36]

To enable untethered drug delivery in freely moving animals, an implantable optofluidic system also must be integrated with micro-pumps (i.e., drug infusion systems) that can convert an electrical signal into the actuation of the drug fluid. Like light sources, the size of the integrated drug infusion system is a critical factor for implantation, especially for small animal applications. Depending on the application, the micro-pump part can be designed to be fully-implantable or placed outside the body (for example, on the surface of the skull[20,21]). For long-term drug delivery, fully-implantable drug infusion systems can be designed to have refillable drug reservoirs,[24] while surface-mounted systems can be constructed to have either refillable[22] or replaceable drug cartridges.[21] An approach in which drugs are refilled is economical, but it requires a slow and technical process, and its actuation pump may degrade over time.[22,24] On the other hand, replaceable drug cartridges offer rapid and easy-to-use steps but may suffer from mechanical damage of devices mounted on the body surface.[21] Depending on the application needs, the drug pumps need to be carefully designed, considering the reservoir volume (relatively larger reservoirs are preferred for studies in large animals), the number of drug storage units (multiple reservoirs to deliver more than one type of drugs), the desired flow rate, and flow rate variability (for versatility). Moreover, the volume of infused drug needs to be optimized based on the pharmacokinetics of the ligands involved and the area of tissue that needs to be perfused with the ligand. In practice, for rodent studies, most volumes are less than 500 nL, but permissible volumes are dependent on the rate and location of delivery as well. For example, circulation of cerebrospinal fluid is nearly 20x greater within the ventricles of the brain than within the brain parenchyma itself.[37] In rats, this micro-circulation appears to be near 100 nL/minute[38] – a rate that should ideally not be exceeded for most intracranial drug deliveries. Among various types of fluid pumps,[19] the two most feasible drug actuator types for standalone, lightweight, wireless optofluidic devices are thermal[20,21,23] and electrochemical pumps.[22,24] Thermally-actuated pumps offer small and light form factors with simpler designs and minimum performance variability due to ambient conditions, but they lack compatibility with heat-sensitive drugs, require relatively high power (on the order of 100 mW), and are not reusable (this drawback can be mitigated by making replaceable cartridges[21]). On the other hand, electrochemical pumps can be reusable (using reversible chemical reactions and refilling procedures), power-efficient (i.e., power consumption on the order of 0.1 mW), and more compatible with a wide variety of drug types due to low heat generation (<0.2 °C) during operation. However, electrochemical pumps require relatively complex fabrication and hermetic sealing process because of the need for additional storage for chemical agents (e.g., water) for electrolytic actuation.

Last but not least, a wireless control module is another crucial component that can improve the overall usability and scalability of optofluidic technology. One of the key considerations in choosing a wireless control technology is accessibility to neuroscientists, which makes infrared (IR),[20,29,39] radio-frequency (RF),[22–24,28,30,31,33–35] and Bluetooth low energy[21,36,40] among the top contenders to be integrated. Ideally, wireless control should be simple and easy-to-use while offering a long working distance, omnidirectionality, and no line-of-sight (LOS) handicaps. Moreover, for tether-free operation, the power consumption must be minimized in wireless control because the power supply can either be limited (e.g., battery-powered system) or unstable (e.g., wirelessly powered system). Furthermore, it should provide the ability to control multiple animals within a large cohort simultaneously and/or selectively, not only to increase experimental throughputs but also to allow complex behavioral studies (such as social interactions under different stimulation profiles in each animal). Finally, the setup costs and effort should be minimal to enable seamless deployment of these devices within biomedical research laboratories across the globe. Comparing wireless technologies, IR-based devices[20,29,39] offer a more economical and simpler control setup, but suffer from LOS handicap and directional sensitivity. RF-controlled devices[22–24,28,30,31,33–35] offer opportunities to provide both wireless controls and wireless power transfer (WPT), which can eliminate the requirement for on-board energy storage units, such as batteries and thus help achieve lighter and highly miniaturized form factors (critical for fully-implantable systems). However, this approach is not easily accessible due to the need for expensive, bulky RF equipment, and it is limited by its short working distance (10–20 cm), relatively large susceptibility to orientation, and inability to provide selective control of a specific device in a group of multiple devices. Bluetooth low energy is an attractive option for wireless optofluidics to enable fully-automated, versatile, and scalable studies.[21] It provides ease-of-access with minimalistic setup (i.e., smartphone-controlled), power-efficient communication, a long reliable control range (10–100 m) with bidirectional data communication capabilities, higher degree of selectivity among a large group of animals, and ability for automated closed-loop experiments. Integrated with a miniaturized, large-capacity power supply, this wireless technology can broaden the practical utility of implantable optofluidic devices.

3. State-of-the-Art Wireless Optofluidic Device Technologies

The device community has tried to realize a variety of wireless optofluidic devices by taking into account the aforementioned requirements and design considerations. State-of-the-art wireless optofluidic devices can be categorized into two groups, namely, (i) head-mounted[20–22] and (ii) fully-implantable

systems,[23,24] as shown in Figure 2 and Table 1. Although both technologies offer attractive solutions for in vivo photopharmacology while overcoming various limitations of tethered optofluidic systems, each approach has its strengths and weaknesses. Head-mounted methods have relaxed dimensional restrictions that allow them to integrate off-the-shelf energy sources (such as rechargeable batteries), which can provide stable and ample power to the standalone systems. Their exposure to the outer body also facilitates straightforward replacement or refilling of the exposed drug reservoirs. However, apart from being heavier and larger, head-mounted devices are vulnerable to external collision during animal movements, which likely cause damage to both devices and tissues. On the other hand, unwanted damage to the device and tissue can be prevented with fully-implantable approaches offering minimalistic form factors. However, using off-the-shelf energy sources is not an ideal option in fully-implantable devices targeted for small animal models (i.e., mouse) due to their bulky size. WPT is hence preferred to minimize the overall device dimensions, but the downside of this approach is that implanted devices must be in close vicinity to a special cage installed with a wireless power transmitting antenna. Also, full implantation of the whole devices (including drug reservoirs) inside the animal body might have an adverse effect on the stability of stored drugs when exposed to the body temperature during chronic in vivo studies, thereby restricting their long-term use. In this section, we introduce exemplary devices recently invented and engineered to achieve wireless drug delivery and photostimulation for chronic in vivo use in freely moving animals

3.1. Head-Mounted Solutions

3.1.1. Battery-Powered, Infrared-Controlled, Programmable Wireless Optofluidic Systems[20,29]

Researchers developed a head-mounted wireless optofluidic device (~1.8 g), which consisted of an implantable, flexible optofluidic probe (~80 μm thick) integrating μ-ILEDs (450 nm in wavelength) and microfluidic channels and a head-mounted system incorporating thermally-actuated micro-pumps, drug reservoirs, a wireless module, and a power supply. Two rechargeable lithium-polymer (LiPo) batteries (~0.3 g, 8 mAh

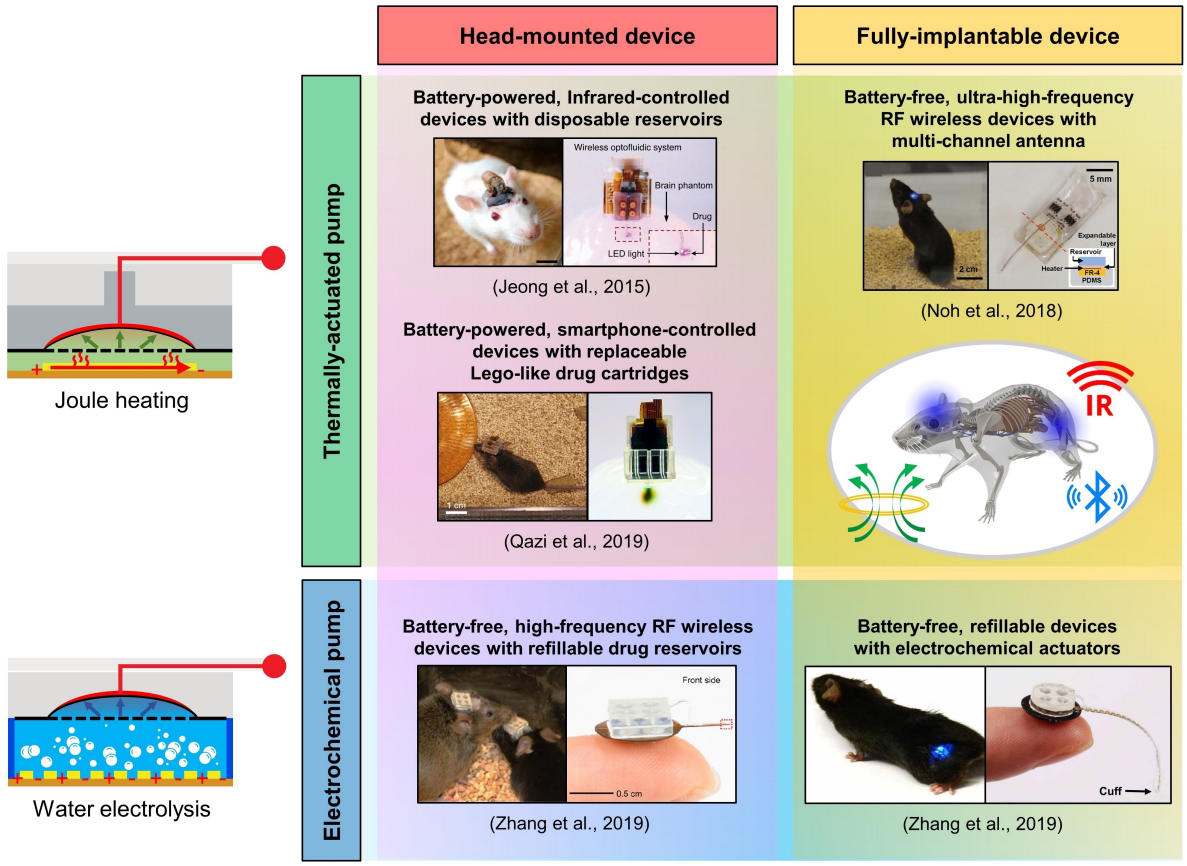

Figure 2. State-of-the-art wireless optofluidic systems, which are classified in terms of the implantation scheme (fully-implantable vs. head-mounted) and the fluid pumping principle (thermally-actuated vs. electrochemical). Image top, left: Reproduced with permission from Ref. [20]. Copyright (2015) Elsevier. Image middle left: Reproduced with permission from Ref. [21]. Copyright (2019) Springer Nature. Image bottom left: Reproduced with permission from Ref. [22]. Copyright (2019) National Academy of Sciences. Images top right, middle right: Republished with permission of John Wiley & Sons (2018) from Ref. [23], permission conveyed through Copyright Clearance Center, Inc. Image bottom right: Reprinted from Ref. [24] Science Advances. © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. Distributed under a Creative Commons Attribution NonCommercial License 4.0 (CC-BY-NC) http://creativecommons.org/licenses/by-nc/4.0/

Table 1. Summary of key characteristics of cutting-edge wireless optofluidic systems.

Property	Head-mounted			Fully-implantable	
Reference	**[20]**	**[21]**	**[22]**	**[23]**	**[24]**
Probe (Thickness)	PET + PDMS (80 μm)		PI + PDMS (100 μm)	PDMS (80 μm)	PI + PDMS (200 μm)
Power (Replaceability)	LiPo batteries (Replaceable)		Magnetic resonant coupling	UHF capacitive coupling	Magnetic resonant coupling
Control type (Frequency)	Infrared (38 kHz)	Bluetooth low energy (2.45 GHz)	RF power source switching (13.56 MHz)	Resonant Frequency Selective (1.8-3.2 GHz)	RF power source switching (13.56 MHz)
Control range	~2 m	10-100 m	~25 cm (Same with powering range)	~10 cm (Same with powering range)	~25 cm (Same with powering range)
Weight	1.8 g	2 g	0.29 g	0.22 g	0.3 g
Fluid pumping principle (Power consumption & Temperature increase in reservoirs)	Thermally-actuated pump (>100 mW & <60 °C)		Electrochemical pump (<1 mW & <0.2 °C)	Thermally-actuated pump (>100 mW & <60 °C)	Electrochemical pump (< 1 mW & <0.2 °C)
Fluid replenishability (Method)	No	Yes (Replaceable cartridge)	Yes (Refillable)	No	Yes (Refillable)

each) ensured reliable power supply to independently and/or simultaneously operate μ-ILEDs and one of the four micro-pumps to infuse 0.5 μL of drug through the associated micro-fluidic channel. This optofluidic system was remotely operated through modulated infrared waves (IR; 38 kHz; 950 nm wave-length) triggered by a programmable microcontroller for customizing operation parameters. Although IR technology enabled remote control up to 2 m, it suffered from LOS handicap and user directionality, thus limiting its ability for selective or simultaneous control within an animal group. The batteries were rechargeable allowing chronic optogenetic studies, but it still required experimental intervention to recharge, unlike in wirelessly powered systems.[22–24] Also, the drug reservoirs were neither refillable nor replaceable, hence limiting the lifetime of this optofluidic device to only four drug infusions. The optofluidic capability of this device was verified through a real-time place preference test where optogenetic stimulation with μ-ILEDs was used to activate ventral tegmental area dopaminergic neurons to drive a place behavior in mice. In a counter-balanced experiment, researchers wirelessly released a dopamine receptor antagonist (SCH23390, 400 ng, Tocris) to prevent the place preference behavior by blocking the effect of ventral tegmental area dopaminergic neuron excitation. This was the first wireless optofluidic device, which offered neuroscientists a compact engineered system to enable programmable in vivo pharmacology and optogenetics with minimal invasiveness in soft brain tissue.

3.1.2. Battery-Powered, Smartphone-Controlled, Programmable Optofluidic Systems with Replaceable Lego-like Drug Cartridges[21]

To enable chronic in vivo pharmacology and photostimulation, scientists and engineers designed special Lego-like, plug-n-play drug-cartridges that could be replaced whenever the drug reservoirs were exhausted. The implantable optofluidic probe constituted of a soft, compliant optofluidic probe (~80 μm thick) mounted with two independently controlled μ-ILEDs (470 nm and 589 nm in wavelength) to achieve multi-wave-length photostimulation. The head-mounted structure con-sisted of replaceable Lego drug cartridges (with four distinct drug pumps, reservoirs, and pillars that plugged into exposed microfluidic channel openings), a wireless module, and two rechargeable LiPo batteries (8 mAh each). The device was shown to deliver multiple wavelengths of light and distinct drugs simultaneously and/or independently, thus offering extreme customization in combinatorial photopharmacological studies. This novel device was controlled through a readily available and user-friendly custom smartphone app through

Bluetooth low energy (2.4 GHz) communication, thus minimizing setup time and costs for an experiment. Furthermore, it not only eliminated LOS handicap and helped achieve omnidirectional control but also allowed through-wall control (limiting any possible "observer-effect" caveats) and long-range operation (10–100 m). Finally, it also allowed precisely selective and/or simultaneous manipulation of both multimodal outputs within a device as well as multiple animals within a large cohort, which opened opportunities for complex neuroscience studies as well as scalable control and increased experimental throughput. To demonstrate its chronic in vivo capabilities, photo-pharmacological stimulants were wirelessly delivered over multiple days via smartphone into the bed nucleus of the stria terminalis GABAergic terminals in the lateral hypothalamus, modulating reward-like behavior in the animal and driving real-time place preference test behavior as a result. This helped demonstrate the powerful ability of this optofluidic device to perform reliably throughout multiple drug cartridge replacements over a month. Although it requires experimental intervention to recharge batteries or replace drug cartridges, it offers a simplistic, economical, and compact solution for chronic drug delivery and photostimulation. Further developments in making the device smaller will make this technology more appealing to the neuroscience community.

3.1.3. Battery-Free, High-Frequency RF Wireless Optofluidic Systems with Refillable Drug Reservoirs[22]

A WPT system based on magnetic resonant coupling at a near-field communication band (13.56 MHz) integrated with power-efficient electrochemical drug pumps helped eliminate the need for a battery in this wireless optofluidic system and significantly reduced the device form-factor (0.29 g) accordingly. This optofluidic system consisted of a μ-ILED and a microfluidic probe (~100 μm thick; PDMS), which penetrated brain tissue while the WPT circuit, refillable drug reservoirs, and electrochemical micropumps remained outside the head of the animal. When the voltage was applied to the interdigitated electrodes, liquid water chemically broke down into hydrogen and oxygen gas through electrolysis, which expanded the micropump chamber and mechanically deformed the flexible membrane (~150 μm thick; poly(styrene-butadiene-styrene)) to push out the drugs from the reservoirs. This electrochemical approach required significantly less power consumption and minimized heat generation in comparison to thermally-actuated pumps (Table 1). Once exhausted, drugs could be refilled by direct injection into the refilling ports of reservoirs using a syringe, followed by sealing the ports with silicone elastomer. Due to the exposed reservoir of this head-mounted device, drug refilling is straightforward, and the stability of the drug inside the reservoir is less affected by the body temperature of the animal, especially in comparison with the other fully-implantable approach with a similar pump activation method.[24] In vivo photopharmacological manipulation ability was validated through optogenetic stimulation in the dorsal hippocampus region to increase locomotor activity, followed by blocking of the effect by delivering a receptor antagonist drug (APV). This wireless battery-free optofluidic device might be desirable for studies involving freely moving animals such as mice. As a trade-off, it requires (i) consistent vicinity to a special bulky cage setup for wireless power transmission, (ii) a short control range (~25 cm), (iii) angular dependency, and (iv) limited operation selectivity.

3.2. Fully-Implantable Solutions

3.2.1. Battery-Free, Ultra-High-Frequency RF Wireless Optofluidic Systems with Stretchable Multi-Channel Antenna[23]

By removing bulky batteries from the device, researchers were able to develop miniaturized (125 mm^3), lightweight (0.22 g), and fully implantable optofluidic systems powered through ultra-high frequency (UHF) RF WPT technology. They were encapsulated with soft silicone rubber (2 mm thick; PDMS), which not only prevented biofluid invasion into the devices, but also helped provide biothermomechanical compatibility with tissue during the operation. An ultrathin and flexible optofluidic probe (~80 μm thick) mounted with power-efficient μ-ILEDs (450 nm) penetrated the brain to access the target area, while the remaining device (comprising a thermally-actuated drug pump and associated WPT circuitry) conformally adhered to the surface of the skull. The implementation of a capacitively-coupled stretchable UHF RF (GHz range) antenna for WPT not only enabled miniaturization of the device but also enhanced its adaptability to various mechanical strains ($<20\%$) while maintaining reliable wireless energy harvesting. Each adjacent antenna trace pair offered distinct non-overlapping energy harvesting channels, enabling independent and/or simultaneous operation of μ-ILEDs and a drug pump. However, this fully implantable optofluidic device could operate only within a 10-cm distance from the transmitting antenna and suffered from LOS handicap. Although it did not require physical intervention as needed in head-mounted devices (to recharge batteries), drug infusion was limited to only one per device, thus significantly limiting its long-term use. This technology offers a minimalistic footprint for photopharmacology and can be improved further by enabling a method to replenish the drug supply, which can open opportunities for chronic fully-implantable in vivo optofluidics.

3.2.2. Battery-Free, Refillable Optofluidic Systems with Electrochemical Actuators for Peripheral Nerves[24]

To enable chronic drug administration for long-term experiments on freely behaving animals, researchers designed fully-implantable optofluidic systems (~0.3 g) with a μ-ILED (470 nm) and four distinct, refillable and electrochemically-actuated drug pumps. The optofluidic probe was thicker (~200 μm) than those used in other technologies,[20–23] but the electrochemical actuation based on electrolysis not only offered better thermal compatibility with various drugs (i.e., almost no

temperature increase in drug reservoirs during fluid pumping), but also required less power for actuation. To provide a longer operating distance, these devices were powered through magnetic resonant coupling at the near-field communication band (13.56 MHz). Because of the lower power consumption of drug pumps and higher transmitting power (12 W), the device could be operated stably and reliably in behaving animals, which moved freely inside a 10 cm by 33 cm maze. In vivo multimodal functionality was validated through opsin excitation (ChR2) to stimulate aversive behavior and temporally-defined fluid deliveries (saline vs. bupivacaine) to control thermal sensitivity in freely moving mice. For chronic operation, although the device offered refilling ports at each chamber, they could only be accessed after an incision due to the limitation of port visibility from outside the skin and also due to the need to cover and uncover the port sealants before and after replenishing of the drug. This limited the true potential of this refillable and fully implantable optofluidic device for application in uninterrupted chronic photopharmacological studies.

4. Challenges and Future Developments

Photopharmacology can help solve various complicated problems in neuroscience and medicine due to its high selectivity, rapid onset, and versatile applications. In this regard, standalone implantable devices capable of light and drug delivery offer powerful tools that can bring unprecedented opportunities for in vivo photopharmacology. State-of-the-art wireless optofluidic devices,[20–24] highlighted in this review, enable minimally invasive photopharmacological manipulation in untethered, freely moving animals with small implant footprints and minimal associated cost along with biocompatibility, customizability, and functionality. The use of power-efficient optical sources (e.g., μ-ILEDs) and fluidic pumps (e.g., thermally-actuated pumps and electrochemical pumps) further helps improve their integration with low-power wireless control schemes.

Despite innovations brought by cutting-edge wireless optofluidic devices, further development and breakthroughs need to be made to address challenges to realize the full potential of in vivo pharmacology. Specifically, ideal optofluidic devices should provide four key features, namely, a) an ultraminiaturized footprint, b) chronic biocompatibility, c) modular functionality, and d) wireless scalability, as illustrated in Figure 3. An ultraminiaturized footprint is important to realize fully-implantable devices with minimal invasiveness capable of delivering multiple distinct drugs and wavelengths of light. Such small form factors with lightweight will allow seamless subdermal implantation, enabling non-disruptive operation in freely moving animals. Advanced micro/nano-manufacturing technology along with the incorporation of an application-specific integrated circuit[41,42] and innovative energy harvesting units[22–24,28,30,31,33–35,43] can significantly miniaturize optofluidic devices, thereby not only helping minimize tissue damage but also allowing applications in any space-critical regions of the body. Chronic biocompatibility is another key aspect that should be achieved for biologically safe operation of implantable optofluidic devices within the body. Long-term in vivo operation ideally requires ultrathin and ultrasoft implants built with biocompatible materials, which are well-matched with the mechanical property of soft tissue. One such solution would be the development of ultrafine nano-mesh-type devices[44,45] that integrate light sources and microfluidic systems. Such tissue-like, mechanically invisible devices would substantially mitigate inflammatory glial responses, making them suitable for long-term in vivo use.[46] Moreover, beyond optofluidic capability, innovative modular multi-functionality is desired to facilitate complex biomedical studies. Designing a modular device would ease restructuring an implant in functionality based on experimental demands. By incorporating optofluidics with various sensors, such as electrophysiological sensors,[47] photometric sensors,[48] and/or temperature sensors,[49] sophisticated closed-loop control[21,35] can be enabled to achieve fully automated neuroscience and various other biomedical research. For example, it may allow complicated studies with real-time modulation of light and drug dosage based on the animal behavioral response (recorded from electrophysiological and/or optical sensors[50]) to preceding stimulations. Finally, wireless device control should aim for scalable, large-range, and automated system control networks. In particular, the development of the Internet of Things network, which can provide high-speed bidirectional communication, would assist in conducting high-throughput automated studies across large cohorts of animals simultaneously and selectively across hundreds of experiments at a time. This can significantly help neuroscientists to control, monitor, and share experiments and resources (both animals and technology) across the globe. In summary, with all these future developments, advanced optofluidic implants would offer unique capabilities that are necessary to enable photopharmacology in live animals to have a practical impact on neuroscience research and clinical medicine.

While this review has focused almost exclusively on applications in neuronal modulation, we note that many photopharmacological approaches would be well suited for other cell-types in the brain. Indeed photopharmacological modulation of glia has been demonstrated using a genetically modified light-gated glutamate receptor on astrocytes[51] and any number of photopharmacological ligands could be targeted to endogenous cell-surface receptors on glial cells. Beyond the brain, however, these types of devices are certainly well positioned to modulate peripheral targets. Particularly compelling in vivo examples are the control of glucose homeostasis in the pancreas,[52] photodynamic therapy to reduce bladder cancer cells in mice and pigs,[53] and photoactivation of a metabotropic glutamate receptor negative allosteric modulator in the hindpaws of mice to inhibit pain.[54] Applications in the periphery will no doubt expand as fully implantable devices[23,24] advance for rodent models and human clinical applications[55] alike.

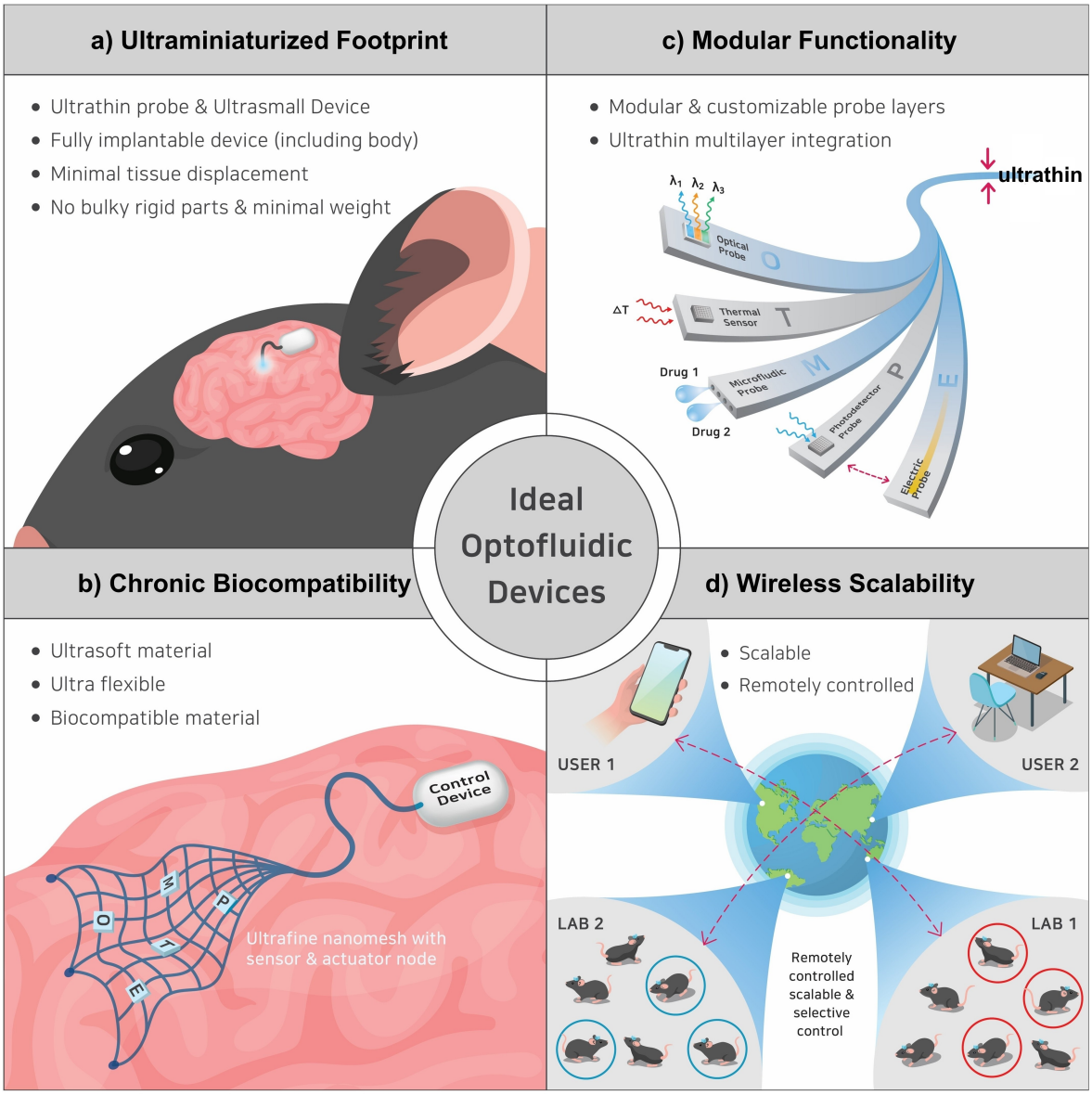

Figure 3. Future development directions for the next-generation optofluidic devices to support chronic photopharmacological studies. The four key features that are desired for future optofluidic devices are an ultra-miniaturized footprint, chronic biocompatibility, modular functionality, and wireless scalability.

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Conflict of Interest

The authors declare no conflict of interest.

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