5-6-2019

Wearable Devices for Single-Cell Sensing and Transfection

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Published in *Trends in Biotechnology,* 2019
doi 10.1016/j.tibtech.2019.04.001

Published May 6, 2019.

Abstract
Wearable healthcare devices are mainly used for biosensing and transdermal delivery. Recent advances in wearable biosensors allow for long-term and real-time monitoring of physiological conditions at a cellular resolution. Transdermal drug delivery systems have been further scaled down, enabling wide selections of cargo, from natural molecules (e.g., insulin and glucose) to bioengineered molecules (e.g., nanoparticles). Some emerging nanopatches show promise for precise single-cell gene transfection in vivo and have advantages over conventional tools in terms of delivery efficiency, safety, and controllability of delivered dose. In this review, we discuss recent technical advances in wearable micro/nano devices with unique capabilities or potential for single-cell biosensing and transfection in the skin or other organs, and suggest future directions for these fields.

Highlights
- Current wearable sensors have allowed for long-term, real-time detection of specific biomarkers directly from patients.
- Miniaturized wearable biosensors with sensing elements interacting with skin or organs can capture target molecules from single cells, which results in significantly increased sensitivity, responding time, and precision.
- Emerging wearable devices based on novel nanomaterials or nanofabrication show potential for single-cell detection in cancer cell screening, cardiomyocyte detection, and optogenetics.
- Transdermal delivery devices have been scaled down to the micro- and/or nanoscale, and their applications have extended to wide selections of natural molecules and bioengineered molecules.
- Emerging nanodevices show unique capabilities in precise single-cell gene transfection *in vivo*, with improved delivery efficiency, safety, and dose controllability.

Introduction

The field of **wearable electronics** (see **Glossary**) and devices has advanced quickly over the past decade, with a focus on sensing physical and chemical properties and delivering stimulation and/or substances via direct contact with the skin [1–4]. With the integration of nanomaterials and rapid advancement of fabrication technologies, new devices functioning at the cellular level will lead to improved efficiency, safety, and non-invasiveness [5]. Miniaturizing these new devices to the scale comparable to a single cell could significantly increase the precision of cellular diagnosis and treatment that could not be achieved within bulk environments [6–8]. By miniaturizing the sensitive module to the submicron or nanoscale, a wearable biosensor interacting with skin or organs enables the capture of target molecules

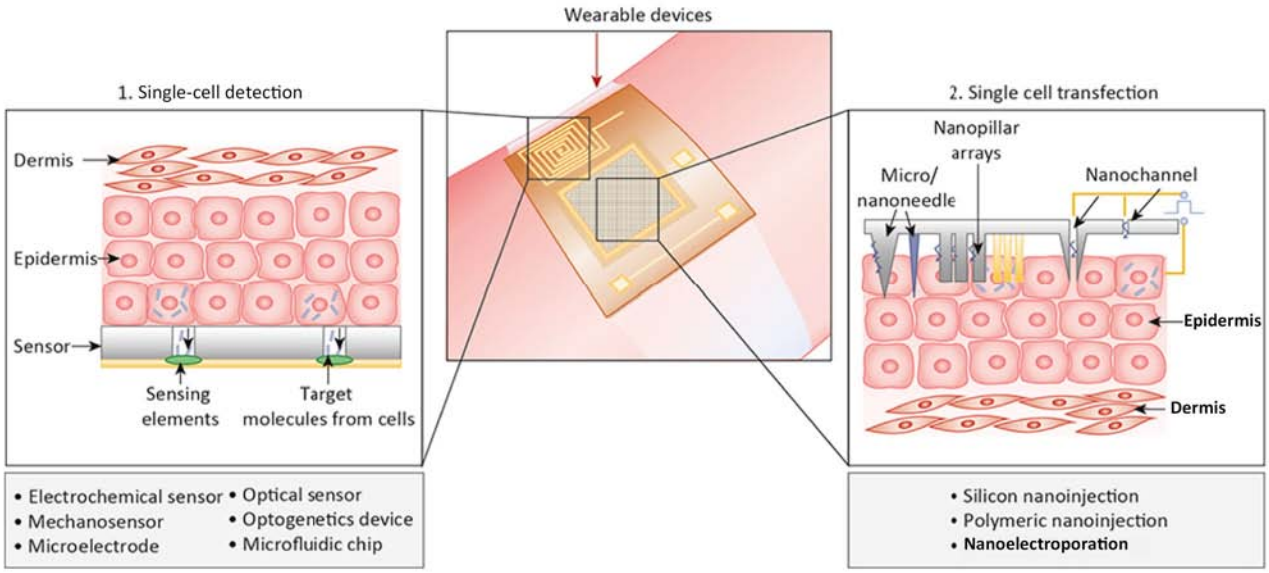


Figure 1. Overview of (A) Wearable Single-Cell Sensors and (B) Single-Cell Transfection Devices.

from single cells, which results in significantly increased sensitivity, shorter response times, and precision for spatiotemporal measurement. Skin-patch devices with micro and/or nano features accurately guide external forces (e.g., penetration, electroporation, etc.) onto the cell membrane while actively injecting **cargo** into cells. Wearable healthcare devices have been widely discussed over the past few years [9–13].

Here, we primarily review the latest developments in unique wearable biosensors and delivery nanodevices aiming for single cell-level interactions. These developments include device designs, fabrication techniques, working principles, and comparisons between these wearable devices and conventional devices. We first review wearable devices for single-cell sensing, followed by wearable devices for **single-cell transfection**, as summarized in **Figure 1**, and end by offering concluding remarks and future perspectives.

Wearable Devices for Single-Cell Sensing

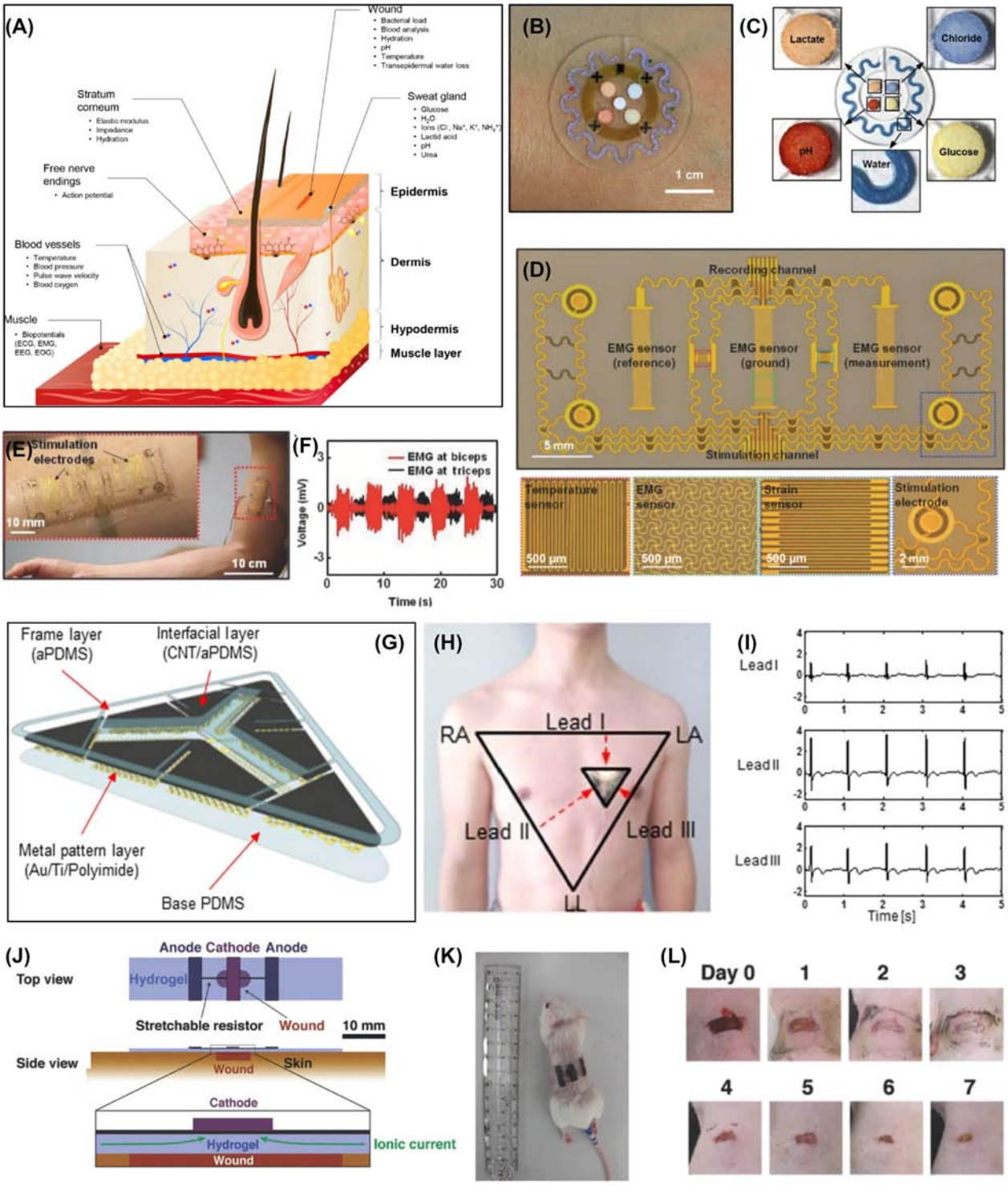
Here, we describe various parameters from the body that can be measured by soft, wearable micro- and/or nanosensors, from the organ

and tissue levels down to the single-cell scale. Some emerging devices based on novel nanomaterials or nanofabrication have shown potential for wearable single-cell detection in scenarios including cancer cell screening, cardiomyocyte detection, and **optogenetics**.

Current Flexible and Wearable Sensors

Wearable sensors with mechanical flexibility provide advantages for measuring physiological parameters and monitoring therapeutic responses in patients. These wearable sensors are likely to offer excellent sensitivities for detecting biomolecules that are low in abundance or have a short half-life in the body [14]. Through **exocytosis**, cells release numerous molecules, including hormones, peptides, and metabolites, that mediate cell–cell interactions. These molecules frequently indicate cellular states and provide clinicians with ample information about the patient's health [14,15]. Many flexible and wearable sensors have been developed to enable the detection and measurement of biomarkers at the interface of the skin and internal organs. For instance, the content of water, glucose, inorganic ions, lactic acid, and urea in sweat can be detected using skin-patch sensors (**Figure 2**A) [2]. Certain sensors can detect vital signals generated by the nervous system, blood vessels, and muscle tissues under a skin barrier (Figure 2B,C) [16]. These sensors have been used for monitoring the normality of cardiovascular functions, brain activities, rehabilitation, wound healing, sleep conditions, blood pressure, and metabolism [16–30].

Figure 2. Wearable Biosensors on the Skin. (A) Representative signals measured from the surface and below the skin. (B) A wearable microfluidic device for monitoring sweat. (C) Detection of lactate, chloride, glucose, water, and pH with a microfluidic sensor using colorimetric reservoirs. (D) Multifunctional device for prosthetic control showing a color-coded electromyography (EMG), temperature, strain sensors, and stimulation electrodes. (E), (F) EMG activity at the biceps and triceps, corresponding to extension and flexion of robotic arm. (G) Electrocardiogram (ECG) electrodes based on carbon nanotube (CNT)/adhesive polydimethylsiloxane (aPDMS) electrode structure. (H) Electrode on the chest of a subject showing a conventional method of ECG measurement. (I) Signals recorded from Lead I, II, and III with the ECG electrodes. (J) Schematic of a wearable wound-healing device. (K) Device on the skin of living mouse. (L) Wound healing over 7 days with the application of the device. Reproduced, with permission, from [2] (A), [16] (B,C), [35] (D–F), [36] (G–I), [37] (J–L).


(A)
Wound
Bacterial load
Blood analysis
Hydration
pH
Temperature
Transepidermal water loss
Stratum corneum
Elastic modulus
Impedance
Hydration
Sweat gland
Glucose
Free nerve endings
Action potential
Epidermis
Blood vessels
Temperature
Blood pressure
Pulse wave velocity
Blood oxygen
Dermis
Muscle
Biopotentials (ECG, EMG, EEG, EOG)
Hypodermis
Muscle layer
(B)
1 cm
(C)
Lactate
Chloride
pH
Water
Glucose
(D)
Recording channel
EMG sensor (reference)
EMG sensor (ground)
EMG sensor (measurement)
5 mm
Stimulation channel
Temperature sensor
EMG sensor
Strain sensor
Stimulation electrode
500 μm
500 μm
500 μm
2 mm
(E)
Stimulation electrodes
10 mm
10 cm
(F)
EMG at biceps
EMG at triceps
Voltage (mV)
Time (s)
(G)
Frame layer (aPDMS)
Interfacial layer (CNT/aPDMS)
Metal pattern layer (Au/Ti/Polyimide)
Base PDMS
(H)
Lead I
RA
LA
Lead II
Lead III
LL
(I)
Lead I
Lead II
Lead III
Time [s]
(J)
Top view
Anode Cathode Anode
Hydrogel
Stretchable resistor
Wound
10 mm
Side view
Wound
Skin
Cathode
Hydrogel
Wound
Ionic current
(K)
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Day 0
1
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In addition to those sensors, integrated wearable systems have been developed; these systems can communicate wirelessly via near-field communication (NFC), utilize optoelectronics for simultaneous monitoring of vascular disease and ultraviolet (UV) exposure, and/or perform multiplexed sweat analysis of various biomarkers [19,31]. Devices that can offer both sensing and therapeutic functions are also of great interest [32,33]. However, most of these sensors and systems barely have the capability to enable detection and measurement at a single-cell resolution.

For amputees, the nerve endings at the remaining portion of an amputated limb continue to be electrically active and are often used as the source of signals to control robotic actuators. Typically, electrode patches are placed on muscle and skin sites at which the nerves can be re-innervated [34]. However, conventional electrodes have large dimensions, which compromise their function during simultaneous recording and stimulation and hinder their capability to detect at the single-cell level. Xu and coworkers recently developed a multifunctional epidermal sensor (Figure 2D) for recording electromyogram (EMG) signals from muscles while stimulating them [35]. The device was fabricated using a lithographic process to achieve an appropriate dimension for the stimulation of biceps and triceps muscles. Although this device can stimulate flexor and/or extensor muscle groups in a human to operate a gripper (Figure 2E,F), the electrical stimulation induced by this device may not be specifically directed to a single cell. In addition, the EMG sensor, which is 24mm2 in size, was too large to specifically detect the response of a single cell to the stimulation, and instead detected signals from a population of cells. In another study, wearable devices also demonstrate the ease for daily monitoring of cardiac functions of patients with **arrhythmias** and atrial fibrillation [36]. For instance, a carbon nanotube (CNT)- and adhesive polydimethylsiloxane (aPDMS)-based sensor for electrocardiogram (ECG) measurements was reported (Figure 2G,H). This sensor functions wirelessly while permitting repeated adhesion and normal operation under water (Figure 2I) [36]. Yet, the electrodes are, again, too large to detect signals at the single-cell resolution. In both of the aforementioned studies, signals are acquired from the surface of the skin, which primarily contains epithelial cells. For the cells at the skin level, it is preferable for the sensor to be designed in a low-modulus format for compliant interfacing. Although some existing wearable

technologies lack the specifications required to sense at the single-cell level, they have the potential to realize this capability.

The skin forms a physical barrier between internal organs and the environment. It has many important functions in maintaining the normal physiology of the human body. Thus, proper wound healing is crucial for restoring the integrity of the skin when it is damaged. The quantitative measurement of wound healing often requires invasive procedures. To overcome this challenge, Hattori and coworkers developed a wearable device that can be used to monitor skin wound healing (Figure 2J) [37]. This device aimed to discriminate wounded from normal skin in humans based on the temperature and thermal conductivities. Results showed increased temperature and thermal conductivity near the wounded skin of mouse. Moreover, stimulating the wounded mouse skin with stretchable electrodes fabricated on a hydrogel substrate remarkably accelerated healing (Figure 2K,L). This technology showcases the potential for single-cell therapy from a wearable electronic device because the stimulation mobilizes many individual epithelial cells and fibroblasts to close the wound [38]. As the relevant theories and technologies continue to advance, more devices capable of sensing biomolecules and/or offering therapy at a single-cell resolution could be developed to improve disease diagnosis and treatment.

Wearable Sensing Devices at Single-Cell Resolution

The dimensions of a sensor or sensing elements primarily determine whether it can perform detection at the single-cell level [15]. By positioning the **sensing element** of a device at a submicron to micron distance away from individual cells, molecules released by cells can be effectively captured by a sensor positioned in a limited extracellular volume, which essentially allows for precise measurement of cells at a single-cell resolution (as shown in Figure 1) [15]. This tiny interface is analogous to the **artificial synaptic cleft**. By miniaturizing the size of the biosensing element to the size of a cell (10–100 μm in diameter, depending on the cell type), a greater surface area of the sensor can be in contact with a cell [14]. The enhanced contact between the sensor and cells results in an increased signal noise ratio (SNR) and is critical for determining the limits of detection and response time for the

sensor [15]. Numerous investigations of single-cell sensors are ongoing, some of which are summarized in **Table 1**. The existing single-cell

Table 1. Single-Cell Sensing Devices

Sensing/stimulation material	*Device features and capabilities*	*Cell type*	*Observed property*	*Results*	*Refs*
Au	CMOS device fabricated on Si Wafer for ECG detection with Au electrodes and optical detection of cardiomyocytes using photodiodes	Rat cardiomyocytes	Contraction and relaxation of cardiomyocytes	Real-time action potential and light intensity detection from cardiomyocytes	[40]
Au	Ion-sensitive field effect transistor (ISFET) fabricated on glass with gate electrode dimensions on a micron scale for cellular adhesion impedance sensing	Human lung adenocarcinoma epithelial cells and human embryonic kidney cells	Impedance of cells attached to substrate of ISFET	Impedance spectra and membrane capacitance of single cells adhered to substrate were obtained	[41]
Au	Optofluidic system comprising Au nanohole array for detecting cytokines and pneumatic valves for controlling flow of cells through isolated microchamber	EL4 lymphoma cells	Label-free IL-2 secretion detection	Captured IL-2 cytokine secretion to show real-time functional state of single cells	[42]
Au	Au nanodisk electrodes dip coated in Nafion for detecting neurotransmitters inside single cells	PC12 cells	Concentration of dopamine	Nanodisks can detect dopamine inside vesicles of chemically stimulated cells	[43]
Carbon fiber	Carbon-fiber microelectrodes in form of conical nanotips for detecting catecholamine neurotransmitters	PC12 cells	Concentration of catecholamines	Nanotips can detect concentration of catecholamine neurotransmitters inside vesicles	[44]
Fe-Porphyrin functionalized rGO	NO monitoring using a FET fabricated on Si wafer	Human umbilical vein endothelial cells (HUVECs)	Concentration of NO	Monitoring of NO in cultured HUVECs stimulated with L-Arg can be performed in real time	[45]
High-temperature rGO	Optical flow cytometer utilizing laser and PDMS microfluidic chip	Jurkat (leukemia) cells	Identification of cancer cells	Successful detection of a few cancer cells among healthy cells	[46]
InP nanowires (NWs)	InP NW array on InP substrate for *ex vivo* force measurements of single bacterial cells	*Xylella fastidiosa* cells	Horizontal and vertical direction force mapping of attached cells on NWs	Increased cell adhesion strength on surfaces coated with adhesin XadA1	[47]
Polypyrrole (PPy)	FET based on carbon nanotube electrodes and PPy as channel material functionalized with various biological receptor molecules	Melanocytes and cardiomyocytes	pH changes from melanocytes and ATP concentration for cardiomyocytes	Drain-source current controlled by pH value and hexokinase-modified FET sensors successfully detect ATP	[48]

sensors can be improved for: (i) higher sensitivity/ultra-low detection limit; (ii) high throughput (e.g., array of sensors); and (iii) better mechanical compatibility with the tissues [14,15,39]. When the devices are placed on the body, these benefits will maintain the reliability and prolong the working time of the device. For example, implantable electrodes with improved mechanical compatibilities with surrounding tissues effectively reduced the immune responses for **mechanosensing** individual glial cells [7,8,39].

The substrate material is another important factor to consider when building single-cell sensors. Conventionally, the **sensitive modules** are functionalized on rigid substrates that are not comfortable to wear [45]. However, it is possible to adapt the sensing modalities on soft flexible substrates to improve their wearability [49]. Xie and coworkers fabricated a field-effect transistor (FET) based on a composite of **reduced graphene oxide** (rGO) and a metalloporphyrin (Fe (III) mesotetra(4-carboxyphenyl) porphyrin, FeTCP) (**Figure 3**A) [45]. This composite material is capable of detecting nitric oxide (NO), an important neurotransmitter, with a detection limit as low as 1 pM in PBS. The response time was found to be close to 500 ms, sufficient for monitoring NO released by growing single human umbilical vein endothelial cells on the FE (Figure 3B) [45]. Further modification of the sensitive modules onto soft substrates would allow these single-cell sensors to be interfaced with internal organs and skin [50].

So far, wearable devices showing clear proof of single-cell sensing are limited. However, the integration of multiple sensors on a wearable device has shown the feasibility of sensing a single or a few cells, as evidenced in the diagnosis of cancer cells, monitoring the metabolism of cardiomyocytes, and optogenetics [46]. Beside the transistor mentioned earlier, other techniques, including microfluidic chips and **multimodal sensors**, could also provide promising detection limits, **delivery specificity**, and a breadth of information. For example, on-chip flow cytometry analysis has been frequently applied for 'sensing' cancer cells from normal cells [46]. Xing and coworkers developed an ultrasensitive high-temperature, reduced graphene oxide (h-rGO) optical sensor for the detection of cancerous cells at single-cell resolution based on a microfluidic chip (Figure 3C) [46]. A flow-sensing set-up comprised h-rGO sandwiched between a PDMS chip and a quartz layer on top of a prism. The variation of the refractive index of the flowing cells caused changes in the light reflected from

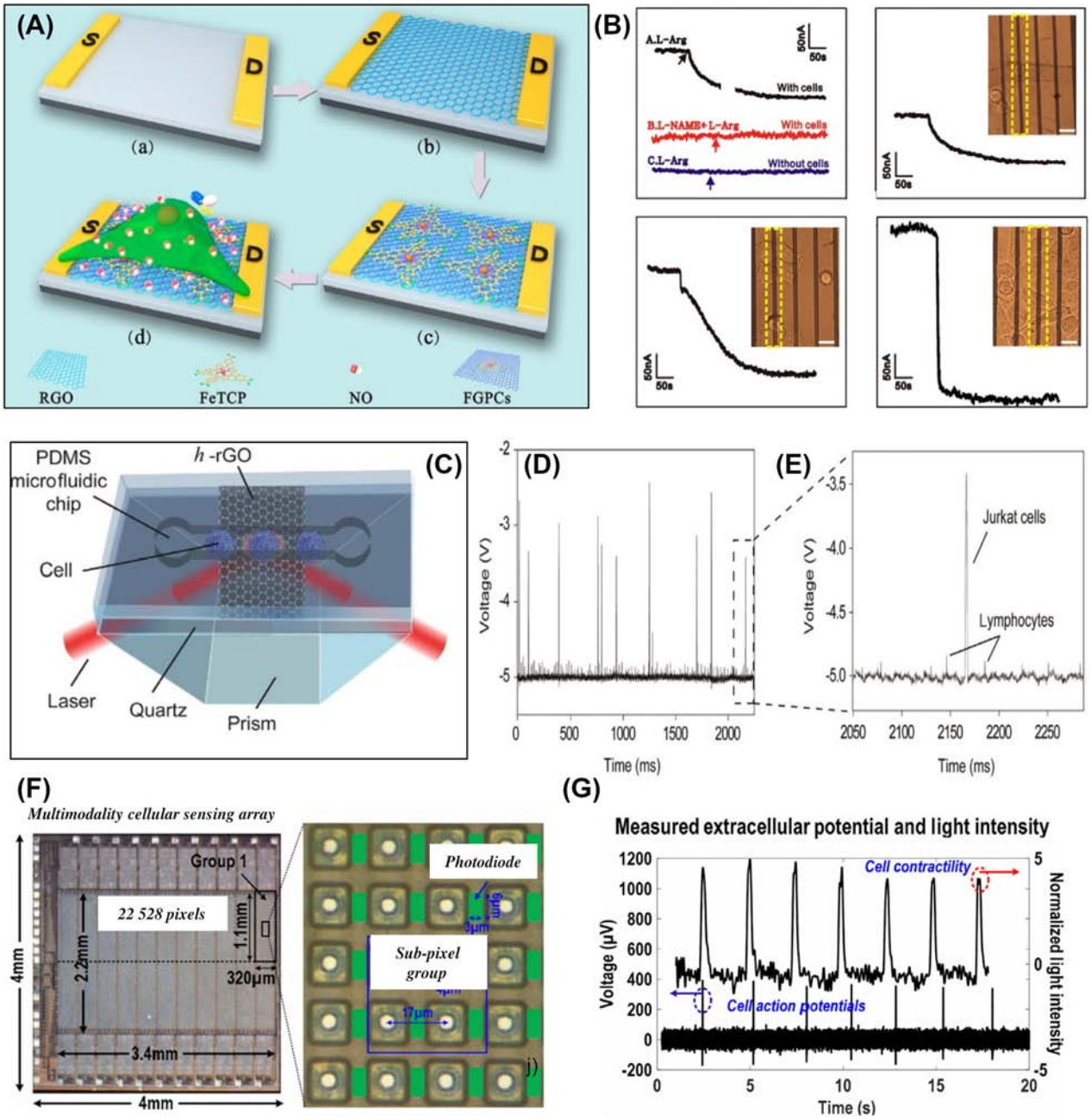


Figure 3. Single-Cell Sensors in Wearable Format. (A) Diagram of a reduced graphene oxide (rGO) and metalloporphyrin (Fe(III)meso-tetra(4-carboxyphenyl) porphyrin) (FeTCP) composite field-effect transistor (FET) biosensor for nitric oxide (NO) monitoring. (B) Signals of the FET under different conditions with detection in a single cell, three cells, and five cells. (C) Flow sensing device for single cells based on h-rGO layer between quartz and PDMS microfluidic chip. (D) Single-cell detection using the flow-sensing device. (E) Voltage difference between detected Jurkat (leukemia) cell and normal lymphocyte. (F) Multimodality sensing array for potential recording and optical detection. (G) Measurement of light intensity and action potentials in real time. Reproduced, with permission, from [45] (A,B), [46] (C–E) and [40] (F,G).

the h-rGO layer. As a proof-of-concept application, Jurkat cells (a leukemia cell line) were successfully distinguished from normal lymphocytes due to a voltage change on the sensor (Figure 3D,E) [46]. Miniaturizing these optical sensors on flexible and stretchable substrates is likely to enable implantable microdevices for the real-time detection of **hematopoietic malignancies** in the body. A 22-k-pixel sensing array was reported for both action potential recording and optical detection of individual cardiomyocytes (Figure 3F) [40]. The pixels in the sensing array comprised transmission gate switches, a gold-plated electrode, a photodiode, and a buffer for the photodiode. Two sets of 2×2 groups of pixels were used to record the extracellular potential of the cells. The optical detection of cell activities (contraction and relaxation) and the recording of action potentials in each cardiomyocyte were simultaneously achieved on one device (Figure 3G) [40]. In addition, wearable devices for optogenetics also provide precise manipulation (i.e., activation and/or inhibition) and sensing of single neurons [51]. Park and coworkers developed a soft and stretchable microdevice for wireless optogenetic intervention [52]. The device comprises two elastomeric substrates that sandwich a stretchable antenna, capacitors, Schottky diodes, a LED, and an inductor. It showed efficient cellular stimulation at the sciatic nerve and caused rats to exhibit reactionary behavior when pain pathways were activated. These devices have shown the potential to realize multimodal sensors for simultaneous single-cell stimulation and action potential sensing. In practice, they have inspired wearable products for vision restoration with single-cell resolution based on optogenetics [53]. The single-cell sensors covered in this review have varied device architectures, operate with different sensing mechanisms, and interface with multiple cell types. Thus, the sensing platforms from single cells varies from application to application.

Wearable Devices for Single-Cell Transfection

The use of skin and/or organ drug delivery devices is often limited to small molecules that can diffuse or be actively transported across the cell membrane, due to many challenges associated with macromolecules, including peptides and nucleic acids, to effectively penetrate the cell membrane to enter cells in both in vitro and in vivo

settings. Over the past few years, multiple advances have been made with the micro- and/or nano devices that enable *in vivo* transfection of macromolecules at the single-cell level. Each one generally belongs to one of two types of design that perform either electroporation-based transfection or micro- and/or nanoneedle-based transfection via physical penetration.

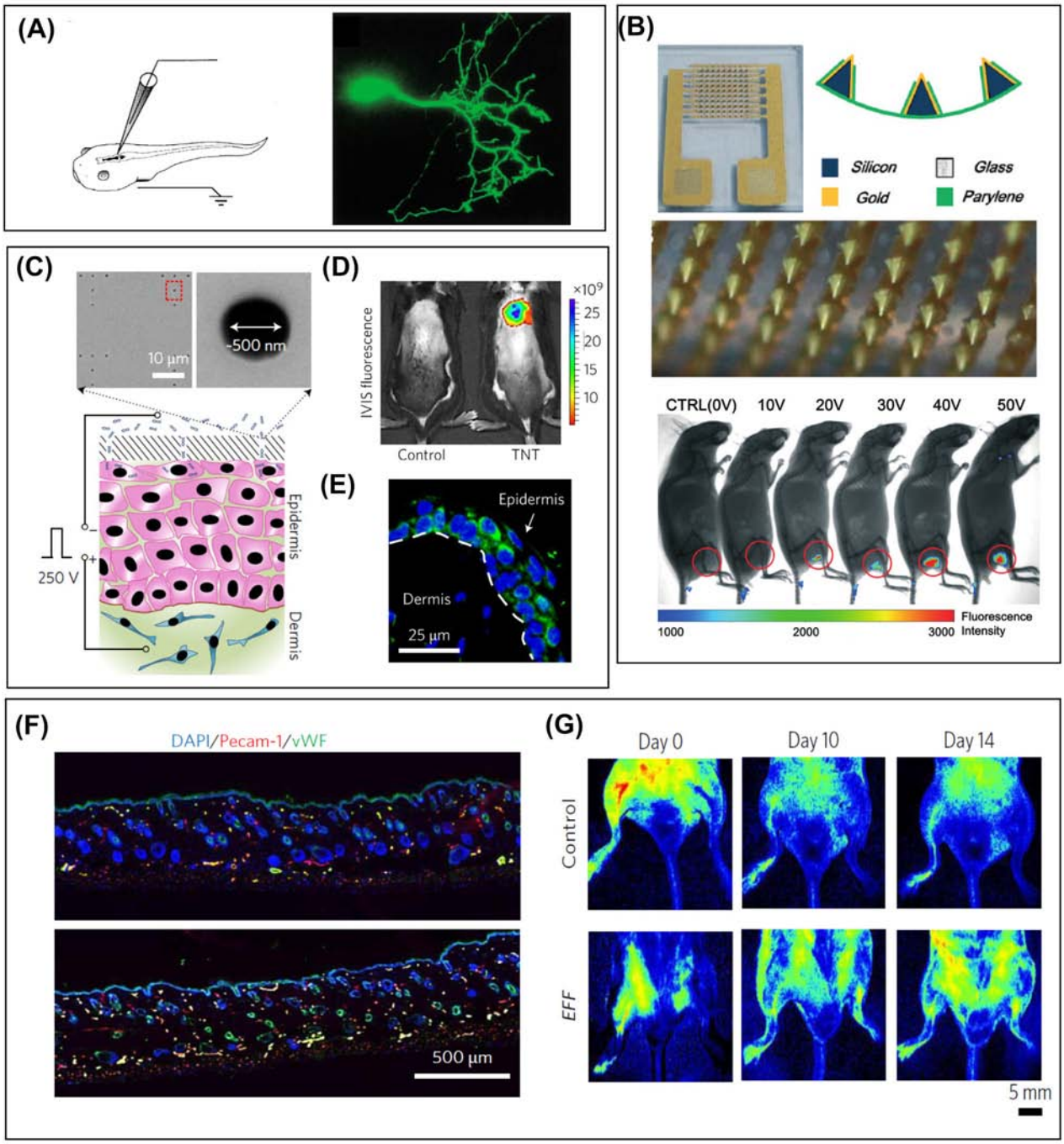
In transdermal delivery or cell transfection, microneedles and electroporation have been applied for decades [3]. However, i*n vivo* techniques still face challenges for delivering DNA plasmids among other macromolecular cargo with high efficiency and specificity. Single-cell electroporation has been studied for many years in *in vitro* settings [54,55]. Recently, novel designs and fabrication techniques have been attempted to enable single-cell transfection *in vivo*. For instance, a nanochannel array-based, single-cell electroporation system was recently fabricated on a skin-patchable substrate [56]. A high efficiency of transfecting biomolecules into animal cells in the skin was achieved with minimum invasiveness. This method is in sharp contrast to microneedle devices that require physical penetration. To date, the nanochannel and micro- and/or nanoneedle-based single-cell electroporation represents the closest technique for wearable single-cell delivery, although further research is needed to develop integrated chips with drug reservoirs, microfluidic transportation, and electronics.

Wearable Electroporation for Single-Cell Transfection

The intrinsic heterogeneity existed in almost any given cell population is a well-known confounding factor with important biological meanings. To properly address this variable and take the cellular heterogeneity into account in research, many platforms that allow biological samples to be analyzed at the single-cell level have been developed and have increasingly significant roles in the studies of **genomics**, **transcriptomics**, **epigenomics**, and **proteomics** relevant to different fields [57–59]. The heterogeneity of cells in a pathological lesion (e.g., a tumor) is likely to determine the therapeutic outcome and long-term prognosis in patients. While having analysis at the single-cell level provides unprecedented capacity to understand biology and possibly disease progression in patients, it is also important to establish approaches that enable precise manipulation on

specific cells to target rare cell populations in organs or pathological lesions for therapeutic purposes [60]. Many technological advances have enabled genomic manipulation at a single-cell resolution and with high throughput [61–63]. The development and use of single-cell transfection techniques allows us to quantitatively deliver macromolecules, such as DNA or RNA, into individual cells [64,65]. By temporarily cracking the cell membrane, cell permeability to macromolecules is increased [66]. This **permeabilization** can be achieved by several means, including viral vectors, chemical vectors, and physical methods [3,67]. In *in vitro* conditions, multiple physical approaches, including cell squeezing [68], **sonoporation** [69], **microinjection** [70], and **optical transfection** [71], have been reported. Physical methods show less tendency than carrier-mediated methods to cause mutagenesis in cellular genomes, thus proving to be safer for *in situ* transfections [72]. For *in vivo* application, however, most reported physical methods have been limited to human organs.

Electroporation is a transfection process where molecules are delivered assisted by applying a voltage across the cell membrane. This produces a transmembrane potential difference (~0.5 V) and induces nanoscale pore formation on the lipid bilayer of the membrane [73]. Bulk electroporation *in vivo* has been performed in muscle tissues by inserting a pair of electrode needles that create a gap of a few millimeters into the tissue to inject DNA plasmids before electroporation [74, 75]. Despite its feasibility, the application of bulk electroporation *in vivo* remains limited, because a large electric potential required to generate sufficient transmembrane potential leads to significant cell death and low efficiency [72]. By contrast, single-cell electroporation shows superior performance in terms of specificity, **dosage control**, **cell viability**, and **transfection efficiency** [64, 76]. In single-cell electroporation, a nanochannel connected to a microfluidic reservoir and filled with electrolytes deploys the electrical potential to a small patch of the cell membrane. The area of the membrane that is subjected to poration depends on the opening of the nanochannel, which is normally b500 nm in diameter [64]. This nanochannel can be fabricated in an array [77], can be the opening of the apex of an atomic force microscopy (AFM) probe [64], or other features [78,79]. These different forms of set-up ensure the poration of a small patch of the cell membrane while electrophoretically forcing polarized macromolecules through the nanoscale pores into the



individual cell. The first *in vivo* single-cell electroporation was performed on individual neurons using a micropipette constructed on a wearable patch [80] (**Figure 4**A).

By miniaturization and integration of microneedle electrodes or microchannels, these devices are capable of patching the skin and

Figure 4. Single-Cell Transfection *in vivo* by Electroporation. (A) *In vivo* single-cell transfection of neuron cells in *Xenopus*. (B) *In vivo* transfection of skin cells by a patchable device using a flexible microneedle arrays with hollow tip for electroporation. Electroporation on the leg of mice induces dosage-depend expression of fluorescence markers. (C) A nanochannel-based patch for transfection of epidermis cells *in vivo* with resolution at a single-cell level. The reference electrode is inserted under the dermis of the mouse skin, and the silicon substrate with nanochannels (500 nm in diameter) is adhered onto the top of the skin. (D) After single-cell electroporation on mouse, the *in vivo* imaging system (IVIS) shows high levels of Ascl1/Brn2/Myt1l (ABM) expression. (E) Cellular level transfection confirmation with confocal imaging of the dermis and epidermis layers. (F) Increased angiogenesis of the skin tissue observed with a one-time treatment of Etv2/Foxc2/Fli1 (EFF) transfection. (G) Single-cell electroporation-based patch on a mouse limb rescues the limb from necrotizing ischemia, as indicated by the increased blood flow for the EFF-treated limb compared with the control. Reproduced, with permission, from [80] (A), [82] (B), and [56] (C–G).

performing single-cell electroporation [81]. The first such example used a silicon microneedle coated with a layer of gold (6 μm thick) and passivated with a layer of parylene on a parylene substrate (8 μm) (Figure 4B) [82]. The interdigitated arrangements of the microneedle arrays formed opposing rows of cathodes and anodes to deliver the electrical potential. The parylene substrate with a designed size of 20 mm^2 ensured the flexibility of the device to conform on mouse skin. DNA transfection was achieved by an application of 20 V voltage or above. On-chip single cell electroporation was also demonstrated using a silicon-based nanochannel [56]. This paradigm shift in how to deliver the electrical field also brings the convenient transformation from an *in vitro* testing to a wearable patch format. Figure 4C illustrates the set-up of the study with the silicon wafer on top of the epidermis patterned with nanochannel arrays. The array of nanochannels is ~500 nm in diameter and ~10 μm in depth. To deliver the electrical potential, one reference electrode is inserted into the dermis and the other is placed above the silicon wafer. An electrical pulse of 250 V at 10ms intervals delivers DNA plasmids into epidermal cells. Compared with bulk delivery, this nanochannel offers a 50- to 250-fold increase in gene expression. Figure 4D,E show mouse skin cells expressing Ascl1/Brn2/Myt1l (ABM) factors in *in vivo*, and demonstrate cellular reprogramming by the delivery of three-gene cocktails

for direct on-skin reprogramming of fibroblasts into induced endothelial cells. Figure 4F shows the increased vascularization after reprograming factor Etv2/Foxc2/Fli1 (EFF) transfection, which leads to enhanced blood flow and whole limb rescue (Figure 4G). These animal studies demonstrate the potential of wearable single-cell electroporation for therapeutic options for wound healing and regeneration [83]. Similar to nanochannel-based electroporation, the issue of accessibility remains. Studies are required to investigate the contact between the substrates with the skin to ensure that a good seal is formed between the channel and the cells on the epidermis; without this seal, the electrical field strength would not be sufficient for the delivery of large molecules.

Micro/Nano Needle for Single-Cell Gene Transfection

Hypodermic needle injection has always been the most common method for drug or gene delivery *in vivo* [84]. Miniaturization of the conventional metal needles has the potential to eliminate a host of drawbacks inert to the method, such as pain and risk of infection [85]. This has resulted in micro- and/or nanoneedle designs tailored for delivering macromolecules for *in vivo* transfection. A host of these designs has brought new developments in DNA vaccination and gene editing [85,86]. By using biocompatible materials and novel fabrication techniques, the new micro and/or nano devices improve delivery efficiency in a non-invasive and safe manner. Furthermore, the use of thin and flexible polymer substrates that have the appropriate Young's moduli offers ideal conformity with the skin. Considering the dimension of the functional units of needles, they can be categorized for use in **local delivery** or **intracellular delivery**. They also use distinct methods of delivering the cargo: as part of a multilayer coating on the needle [85], encapsulation within a dissolvable polymer needle [86], or diffusion through a hollow opening of the needles [82]. Conventional microneedle arrays fabricated using different materials have been studied for DNA vaccination applications [87]. Vaccine viruses or plasmid DNAs (pDNA) delivered into the vicinity of cells in the epidermis are subjected to cellular uptake. Therefore, delivery efficiency is dependent on capsizes of cargo molecules. Most of the microneedles fabricated out of polymer materials

have a tip apex of a few tens of micrometers, which is unlikely to precisely pinpoint to the membrane of a single cell. Thus, delivery of plasmids or other molecules will most likely be local rather than intracellular, while needles with a nanometer apex can perform intracellular deliver without cell damage.

With respect to single cells, nanoneedle arrays are capable of offering higher precision due to a smaller functional area. Their effectiveness as a vehicle for macromolecule delivery in *in vivo* transfection lies in the small size of the needle diameter (b100 nm). At this scale, direct penetration with the help of cell gravity is deemed possible [88], although **endocytosis** is also suspected to assist the transport of large molecules [89]. Cargo can be supplied by either a hollow nanofluidic channel or direct grafting. Several nanoneedle arrays have been fabricated from nondegradable materials, such as nanowires [90], carbon nanotubes [91], and nanofibers [92], or from degradable porous silicon. Recently, nanostraws, with an inner diameter of b100 nm, were fabricated on top of a compliant polycarbonate membrane (**Figure 5**A) [90]. Direct access to the cell cytosol was achieved through the nanofluidic channel within the nanostraw. This was demonstrated by expression of a **GFP plasmid** reporter within 72 h after plasmid delivery (Figure 5B). Since the arrays were fabricated on a mechanically flexible substrate, there should be no compatibility issue for a wearable format. In addition, the size of the nanostraw also presents a better alternative for penetration of individual cells, but the device may require a protective outer shell to penetrate the outer layer of the skin [90]. Furthermore, a recent study by the same group demonstrated the ability of the nanostraw to perform intracellular sampling of proteins and mRNAs on live cells [93]. The same cells can be repeated sample for 5 days for longitudinal cell monitoring. This presents a platform where the dual functionality of sensing and delivery is integrated into one device. Similar designs have also been reported with the hollow nanoneedle array on top of silicon membranes [94].

Nanoneedle-featured electrodes can also be used in combination with *in situ* electroporation [78]. Due to the sharp apex of a nanopillar electrode (Figure 5C), a strong electrical field induces local permeability of the cell membrane and delivers target molecules into the cytosol (Figure 5D). Biodegradable nanoneedles were fabricated from porous silicon with a 50-nm-wide apex (Figure 5E) [95]. This sharp design facilitated cell membrane penetration and cargo injection. Delivery of

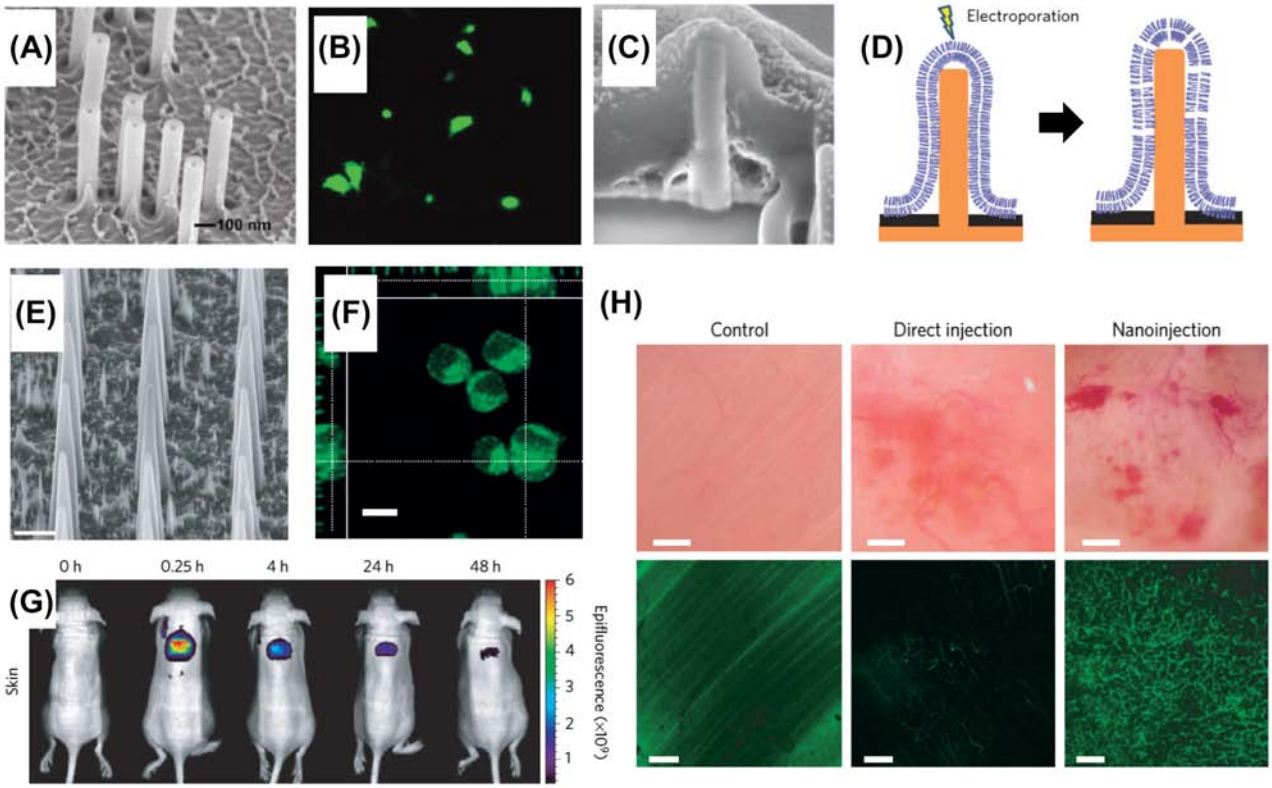


Figure 5. Micro- and/or Nanoneedle-Based Intracellular Delivery and Transfection. (A) An array of nanostraws growing on a polycarbonate substrate with the outer diameters of 100 nm. Scale bar: 100 nm. (B) Single-cell transfection was achieved with fluidic intracellular delivery of plasmids, and uniform expression levels of fluorescence markers are exhibited. (C) Nanopillar arrays used for the direct penetration of the cell membrane and for single-cell electroporation. The nanopillar (150 nm in diameter, 1.5 μm in height) was fabricated from platinum on a silicon nitride substrate. Scale bar: 200 nm. (D) The aspect ratio of the nanopillar enables either direct penetration. Moreover, the conductive platinum nanopillar can also apply a localized electrical field for single-cell electroporation (E) Biodegradable silicon nanoneedles for deliver and transfection. An array of nanoneedles fabricated out of porous silicon with a tip apex of b100 nm. Scale bar: 2 μm. A similar design was also reported in [96] for the delivery of nanoparticles. (F) Single-cell transfection is demonstrated with delivery of fluorescence markers. (G) The potential for a wearable patch format is shown by an *in vivo* experiment in mice. (H) Significant neovascularization was achieved by a nanoneedle-based single-cell transfection compared with direct injection. Reproduced, with permission, from [90] (A,B), [78] (C,D), and [95] (E–H).

plasmids can be achieved by simply growing cells on top of the bed of nanoneedle arrays (Figure 5F). When tested on the skin and muscle tissues on mice, the localized nanoinjection method using nanoneedle arrays provided a more uniform delivery profile compared with

direct injection (Figure 5G). Furthermore, delivering vascular endothelial growth factor (VEGF) plasmids to the mouse muscle tissue induced **neovascularization** and increase vessel connectivity (Figure 5H). The success of these animal studies demonstrates the potential of nanoneedles for *in vivo* studies as a patchable single-cell transfection platform.

Concluding Remarks and Future Perspectives

Advancements in nanotechnology have made it feasible to design nanodevices with applications for skin or organ sensing and therapy. Existing soft and wearable electronics provide a basis for transforming the currently rigid single-cell sensors into formats that are compliant with the curved interfaces of the human body. Greater effort is envisioned to establish precise and definitive diagnosis for wearable single-cell sensing, which may directly indicate the conditions of cells from patients. Currently, various single-cell sensors have been observed for multiple cell types, including endothelial, epithelial, cardiomyocytes, lymphoma, and neurons, but these sensors have yet to be realized in wearable formats. The key challenges include how to design sensing elements with single-cell precision (see Outstanding Questions), and how to ensure connection of the physical interface between the wearable devices and single cells during wearing or positioning. Furthermore, wearable single-cell sensors still need to be developed for other cell types, such as pancreatic cells, osteocytes, chondrocytes, and other stem cells, to better understand their behavior in pathophysiological or normal physiological states.

Currently, most single-cell transfection platforms are still only used for *in vitro* experimentations. This is due to the difficulty in transforming these microfabricated devices into wearable formats and the challenge in delivering sufficient voltage with compact wearable electronic units. Although some micro- and/or nanoneedles have been fabricated on soft polymeric substrates, the wearable system goes beyond mechanical conformity, and requires the integration of drug reservoirs, microfluidic transports, and electronics with the micro- and/or nano-needles. This is also true for nanochannel-based electroporation. To achieve single-cell transfection, key questions include how to miniaturize the system so that it can achieve precise single-cell gene

or drug delivery topically, and how to achieve therapeutic treatment in wider and deeper layers of skin or organs. In terms of the electronics involved, single electroporation requires an input voltage ranging from a few tens of volts to a few hundred volts; thus, wearable electronics units will have to include amplification and a power supply. Thus, to tailor these microfabricated device to be completely wearable, research efforts need to develop integrated microchips that contain all elements for transfection.

Targeted delivery with a single-cell resolution will increase accuracy and lessen the risk of disturbing cells that do not require any remedy *in vivo*. In the future, translational technologies will witness increasing nanodevices in wearable formats, such as **nanoelectroporation** and nanoneedles, with promises for precise drug or gene delivery in a variety of applications, from on-skin gene therapy and wound healing to regenerative medicine and beyond.

Outstanding Questions

- How can we design wearable sensors with sensing elements with single-cell precision?
- How can we ensure that physical interfaces are maintained between wearable devices and single cells during wearing or positioning?
- How can we miniaturize a drug delivery system so that it can achieve precise single-cell gene or drug delivery topically?
- How can micro and/or nano delivery devices achieve therapeutic treatment in wider and deeper layers of skin or organs with limited loading space?
- How can we tailor microfabricated devices to be completely wearable by integrating microchips with elements for transfection?

Glossary

Arrhythmias: also known as irregular heartbeat or cardiac dysrhythmia; a group of conditions where the heartbeat is irregular.

Artificial synaptic cleft: here, the gap or distance between the sensing element of the sensor and the cell to be detected.

Cargo: here, the substance or molecules to be delivered into the cells.

Cell viability: ability of a cell to remain viable in the presence of a foreign material.

Delivery specificity: ability of cargo delivered to its destination rather than delivered to unwanted regions.
Dosage control: amount or doses of molecules, or copy numbers of DNA or RNA delivered into cells can be controlled by delivery methods.
Endocytosis: taking in of matter by a living cell via invagination of its membrane to form a vacuole.
Epigenomics: study of the complete set of epigenetic modifications of the genetic material of a cell.
Exocytosis: process by which the contents of a cell vacuole are released to the exterior through fusion of the vacuole membrane with the cell membrane.
Genomics: branch of molecular biology concerned with the structure, function, evolution, and mapping of genomes.
GFP plasmids: DNA plasmid that expresses GFP after being expressed in cells.
Hematopoietic malignancies: tumors of the hematopoietic and lymphoid tissues that affect the blood, bone marrow, lymph, and lymphatic systems.
Intracellular delivery: cargo delivered into cells across the cell membrane. In vitro **techniques:** tools or devices that can be applied to cells outside of the body.
***In vivo* techniques:** tools or devices that can be applied to cells directly in the skin or organ of an organisms.
Local delivery: cargo delivered into the proximity of cells and taken up by them.
Mechanosensing: responsivity to mechanical stimuli, especially at the cellular level.
Microinjection: method that delivers cargo into cells by directly penetrating the cell membrane and transporting cargo through its hollow structure.
Multimodal sensors: sensor system that has multiple functions or modules.
Nanoelectroporation: precise single-cell electroporation using a device that focuses electric field and/or electroporation on cells via a nanostructure.
Neovascularization: natural formation of new blood vessels, usually in the form of functional microvascular networks, capable of perfusion by red blood cells, that form to serve as a collateral circulation in response to local poor perfusion or ischemia.
Optical transfection: membrane breakdown and delivery of cargo into cells using laser beams focused on the cell membrane.
Optogenetics: biological technique that involves the use of light to control cells in living tissues, typically neurons, which have been genetically modified to express light-sensitive ion channels.
Permeabilization: cell membrane permeabilities are significantly reduced so that extracellular molecules enter cell.
Proteomics: large-scale study of proteins.
Reduced graphene oxide (rGO): product of reduction of graphene oxide.
Sensing element: element or area where the sensitive materials are functionalized on the sensor.
Sensitive modules: membrane or area where the sensitive materials are functionalized on the sensor.
Single-cell transfection: technique that can deliver external substances into single cells for specific purposes of cellular altering. The substances include drugs, DNAs, RNAs, and so on.
Sonoporation: technique that can transfect cells based on ultrasound.

Transcriptomics: study of the transcriptome (the complete set of RNA transcripts produced by the genome, under specific circumstances or in a specific cell) using high-throughput methods, such as microarray analysis.

Transfection efficiency: among all cells processed by a technique, the percentage of the cells successfully are delivered with targeted cargo while being tracked in the cytosol.

Wearable electronics: electronic devices that can be worn, patched, or implanted by the consumer, relevant mainly for determining health and fitness measurements.

Acknowledgments — L.C. and Y.F. acknowledge funding from INSCA of Beijing Advanced Innovation Center for Biomedical Engineering and Beihang University. L.C. acknowledges start-up funding from University of North Texas. R.Y. acknowledges funding from the Nebraska Center for Integrated Biomolecular Communication (NCIBC) (NIH National Institutes of General Medical Sciences P20GM113126), from the Nebraska Center for Nanomedicine (P30GM127200) and from the NSF (Award #1826135). Y.C.W. is supported by the UNTHSC Start-up Fund for Stem Cell Laboratory and Faculty Pilot Grant FY15 (RI6182). C.Y. would like to acknowledge NIH Grant (R21EB026175) and a Doctoral New Investigator Grant from the American Chemical Society Petroleum Research Fund (56840-DNI7).

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