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REVIEW

Flexible and stretchable bioelectronics for organoids

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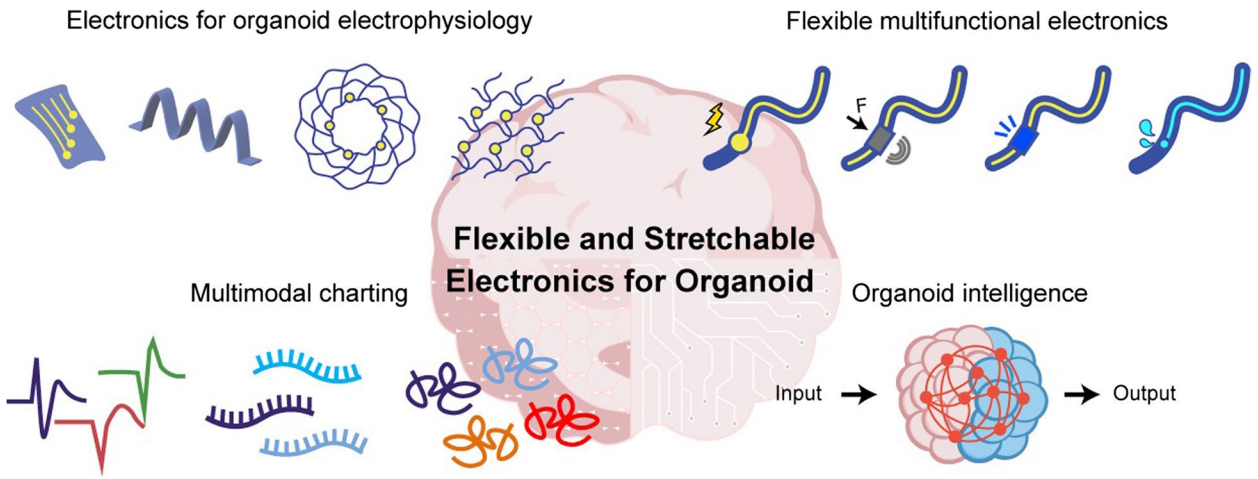
Abstract

Organoids have gained significant interest due to their ability to recapitulate the structural, molecular, and functional complexity of corresponding organs. While methods have been developed to characterize and benchmark organoid structural and molecular properties, capturing the functional development and maturation of organoids remains challenging. To address this, the development of multifunctional bioelectronics for interfacing with organoids has been actively pursued. However, conventional electronics face limitations in achieving multifunctional recording and control across the entire three-dimensional (3D) volume of organoids in a long-term stable manner due to the large morphological and cellular composition changes during development. In this review, we first discuss the application of conventional electronics for organoid interfacing. We then focus on the development of flexible and stretchable electronics designed to create organoid/electronics hybrids for chronically stable interfaces. We also review recent advancements in flexible multifunctional electronics for charting multimodal cell activities throughout development. Furthermore, we explore the integration of flexible bioelectronics with other characterization modalities for comprehensive multimodal charting of cells within 3D tissues. Finally, we discuss the potential of integrating artificial intelligence into the organoid system through embedded electronics, harnessing organoid intelligence for biosymbiotic computational systems. These advancements could provide valuable tools for characterizing organoid functional development and maturation, establishing patient-specific models, developing therapeutic opportunities, and exploring novel computational strategies.

Graphical abstract



Highlights

- Comprehensive review of bioelectronics for 3D interfacing with organoids.
- Recent advancements in flexible multifunctional electronics for multimodal charting organoid functional properties.
- Discussion of emerging fields; multimodal profiling of single cells in organoids and organoid intelligence.

Extended author information available on the last page of the article

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Keywords Flexible electronics · Multifunctional electronics · Bioelectronics · Organoid · Electrophysiology

Introduction

Organoids are three-dimensional (3D) tissues that mimic the key structures and functions of their in vivo counterparts [1–3]. Through self-organization, stem cells can differentiate into diverse organoids that resemble various organs, such as the heart [4–9], pancreas [10–12], and brain [13–17]. In addition to cellular composition and architecture, organoids can also mimic organ functions, including cell electrophysiological activities, mechanical contraction, and metabolism. For example, brain organoids can exhibit brain-like electrical features, such as increased firing rate and synchrony over time course of development, resembling evolving neural networks in vivo [18]. Cardiomyocytes in human heart organoids display beating rates comparable to early human embryos and cell-type specific action potentials, such as ventricular, atrial, and nodal-like waveforms [9]. The ability to electrically record organoids allows for non-invasive, real-time analysis, making these models promising for long-term studies of functional phenotypes throughout development, disease, and therapeutic interventions [19, 20]. To achieve this, the development of tools capable of interfacing with organoids at high spatiotemporal resolution has been actively pursued.

Here, we first review conventional electronics technologies for organoid interfacing, from Two Dimensional (2D) to 3D microelectrode arrays (MEAs). Next, we discuss the development of flexible and stretchable electronics to create long-term stable organoid/electronics interfaces. We also discuss recent advances in flexible multifunctional electronics for monitoring multimodal functional phenotypes of organoids. Furthermore, we explore the integration of flexible bioelectronics with single-cell molecular characterization techniques to achieve a spatially resolved, comprehensive, multimodal understanding of cell properties within 3D tissues. Finally, we introduce the emerging field of combining artificial intelligence with organoid/electronics hybrids, harnessing organoid intelligence for biocomputing (Fig. 1).

Traditional MEA

2D MEAs

Traditional MEAs consist of electrodes on a 2D substrate designed to record extracellular field or action potentials from cells. Due to their non-invasive nature, MEAs have been widely used to record electrophysiological activities in organoids over time [18, 21, 22]. For example, Negraes et al. generated cortical organoids from human induced pluripotent stem cells (hiPSCs) from individuals with CDKL5 deficiency disorder (CDD), a condition associated with early-onset seizures and epilepsy. Weeks of electrophysiological recordings using MEAs revealed hyperexcitability and overly synchronized networks during the development of CDD cortical organoids compared to controls, implying the influence of the gene expression variations on functional phenotypes (Figs. 2a, b) [22].

The integration of complementary metal–oxide–semiconductor (CMOS) technology has led to the development of high-density 2D MEAs (HD-MEAs) with increased electrode density. Integrated circuit and multiplexing technology have reduced the number of interconnects needed, allowing for the creation of subcellular-scale single electrodes with densities exceeding 1,000 electrodes per MEA [23, 25–28]. These advancements have enabled high-resolution electrophysiological mapping. For example, Schröter et al. monitored action potential propagation in a single neuron within slices of cultured human cerebral organoid (Fig. 2c) [23]. Single-unit action potentials were recorded by groups of nearby electrodes in HD-MEAs, and subsequent spike sorting and electrical footprints analysis enabled quantification of axonal action potential propagation velocity in the recorded neurons (Fig. 2d).

3D MEAs

However, traditional 2D MEAs are limited to only measuring the outermost surface of organoids, making it challenging to capture and reconstruct complex cellular networks throughout the 3D tissue. To address these limitations, 3D MEAs have been developed with electrodes capable of penetrating the organoids, thereby enabling the measurement of signals from cells deep within the tissue [24, 29–32]. For example, 3D MEAs with platinum-tipped micro-electrode pillars could penetrate 50–70 μm into 3D rodent neuron-astrocyte co-cultures and record extracellular field potentials from cells embedded within the tissue [30]. Phouphetlinthong et al. demonstrated protruding cantilever MEAs that utilize accumulated stress in multilayer films, causing the electrodes to bend into sharp cantilevers after release (Fig. 2e). These protruding MEAs penetrated and reached cells inside human embryonic stem cell (hESC)-derived cerebral organoids, extending hundreds of micrometers from the surface (Figs. 2f, g) [24]. Similarly, CMOS-based high-density 3D MEAs, consisting of 64 × 64 microneedle

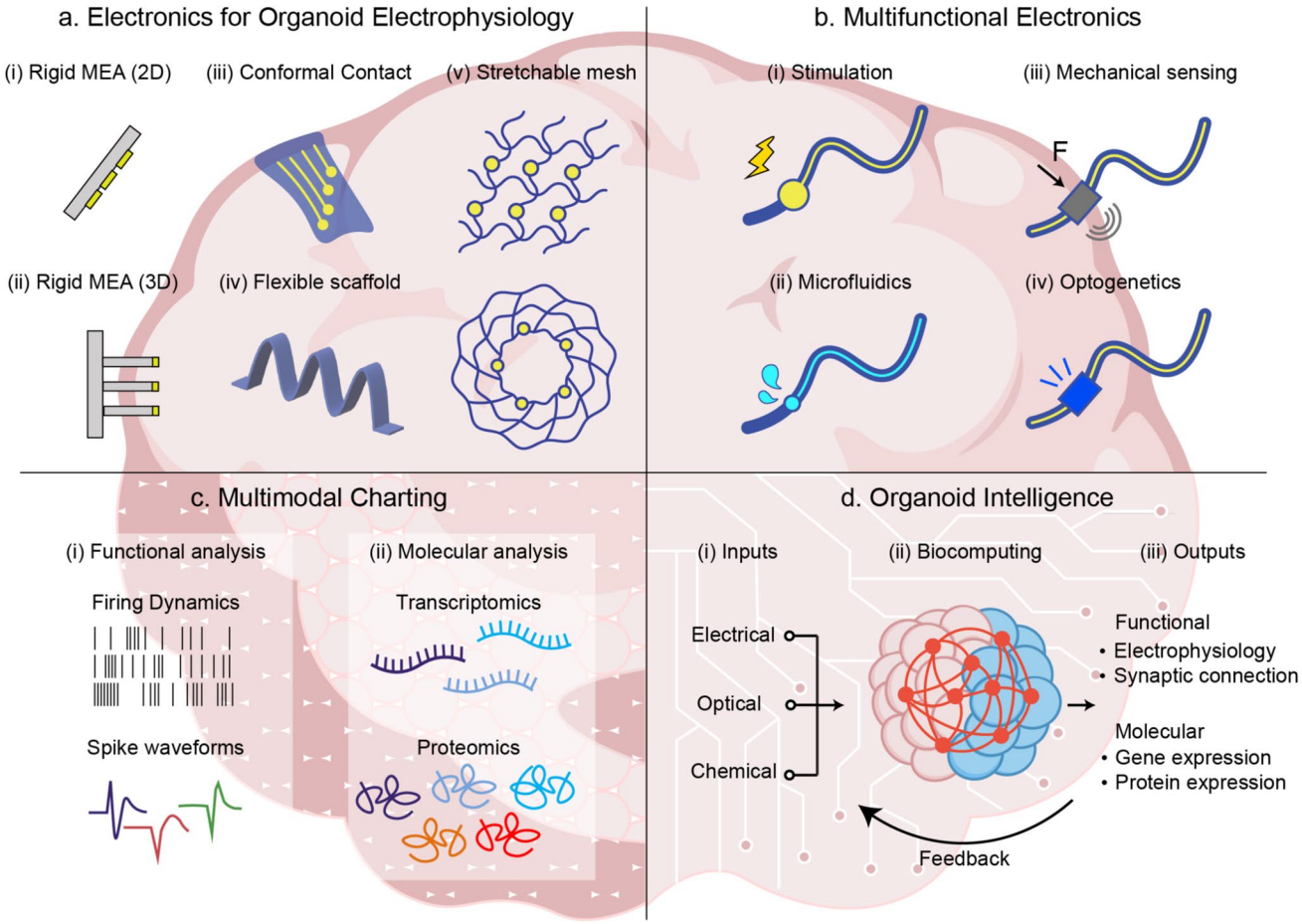

Fig. 1 Overview of this review. **a** Electronics technologies for organoid interfacing, from conventional 2D and 3D electronics to flexible and stretchable electronics. **b** Multifunctional electronics for multimodal functional characterizations. **c** Multimodal charting of cells within 3D organoids by integrating functional and molecular analysis. **d** Embedding artificial intelligence with organoid/electronics hybrids to leverage organoid intelligence for biocomputing

electrode arrays with heights of 65 to 90 μm, have been reported to efficiently penetrate and record signals from brain spheroids [31].

In addition to extracellular recording, various 3D MEAs incorporating nanostructured electrode arrays have been introduced for 'in-cell' [33–35], or intracellular recording of cultured cells [36–45]. Chemically functionalized gold-spine structured electrodes induced neurons to engulf the electrode, enhancing electrical coupling and enabling in-cell recording with a high signal-to-noise ratio [35]. Nanopillar, nanowire, and other 1D nanostructured electrode arrays penetrated through cell membrane via optical or electrical poration, enabling intracellular signal recordings. Although these nanoelectrodes have been primarily demonstrated with 2D cell cultures, they hold great potential for 3D organoid applications.

Flexible MEA

Rigid electrodes in conventional 2D and 3D MEAs often face challenges due to mechanical mismatches with soft tissue, which can lead to cell dislocation and loss of recorded signals during tissue development. Additionally, the protrusion of 3D MEAs may damage the structures and cellular networks of these soft tissues. Flexible electronics offer a promising solution to reduce the mechanical mismatch between electronics and organoids.

Mechanical flexibility in flexible electronics generally refers to the ability of the structure to undergo bending deformation [46, 47]. According to plate theory, the bending stiffness (flexural rigidity) of a thin film is determined by the equation:

$$D = \frac{Eh^3}{12(1-\nu^2)}$$

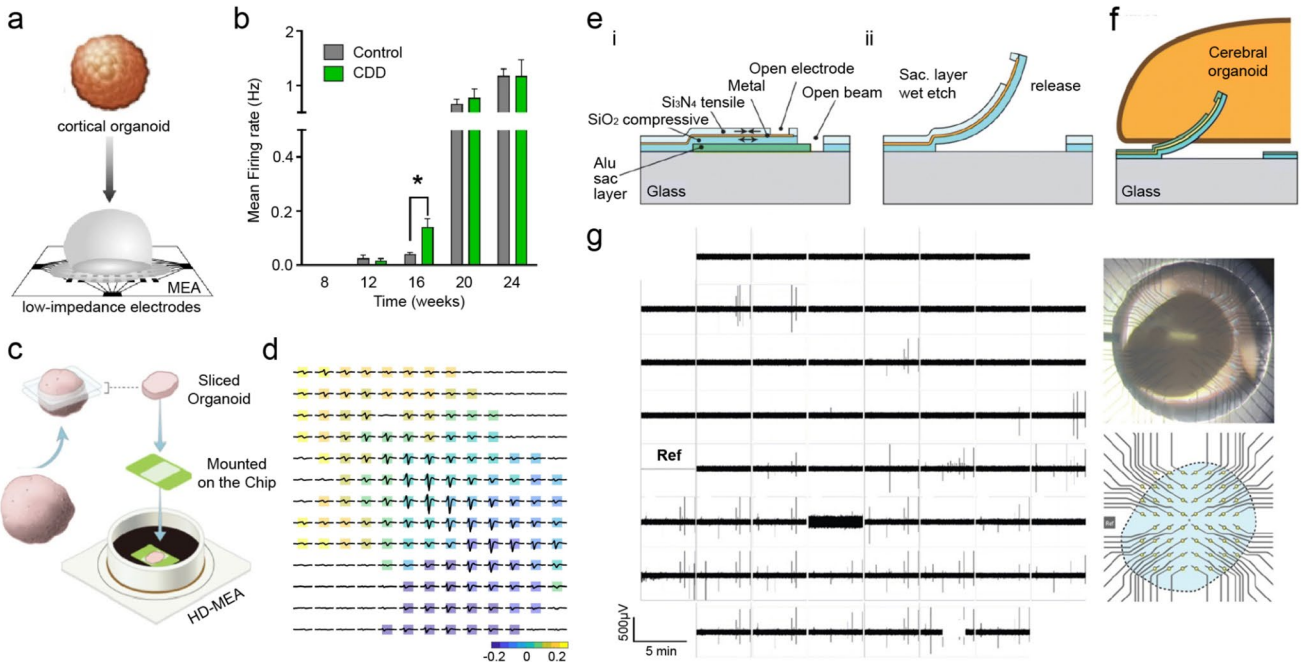

Fig. 2 Planar MEAs for organoid electrical mapping. **a** Schematics of organoid on microelectrode array (MEA). **b** Mean firing rates of cortical organoids derived from CDKL5 deficiency disorder (CDD) patients compared to controls, recorded via MEA. Adapted from [22] under a Creative Commons CC BY license. Copyright 2021, Springer Nature. **c** Schematic of a sliced cerebral organoid on high-density microelectrode array (HD-MEA). **d** Representative recording of action potential amplitude and latency recorded from HD-MEA. Adapted from [23] under a Creative Commons CC BY license. Copyright 2022, Springer Nature. **e** Schematics of a protruding electrode before (left) and after (right) release. **f** Schematic of the organoid inserted on a protruding microelectrode. **g** Representative recordings from protruding MEA (left) and photograph and schematic of the protruding MEA superposed to the organoid position (right). Adapted from [24] under Creative Commons CC BY-NC license. Copyright 2023, The Royal Society of Chemistry

where E, h, and ν indicate the Young's modulus of the material, film thickness, and poisson's ratio, respectively. Since the effective bending stiffness is proportional to the cube of its thickness, flexible electronics minimize bending stiffness by using thin films with reduced thickness. Structural designs that convert in-plane stress into out-of-plane buckling have also been employed to enable the electronics to endure strain.

Advances in nanofabrication techniques have accelerated the development of thin-film flexible and stretchable electronics that are mechanically compatible with biological tissues [48]. Micrometer-thick flexible substrates, made from polymers such as SU-8, polyimide, and parylene, are patterned on top of sacrificial layers like nickel or germanium through methods such as spin coating, photolithography, and reactive-ion etching. Sub-micrometer thick, single-cell scale active layers of electronics, including electrodes, diodes, and interconnects, can be deposited using physical or chemical vapor deposition methods. Additionally, lift-off techniques for epitaxial thin-film structures [49–52], combined with transfer printing [53, 54], have enabled the integration of functional 2D materials or 3D thin-film devices onto flexible substrates [55, 56], expanding functionalities of these systems. These advances in materials science and micro/nanoengineering have broadened the applications of flexible and stretchable electronics, which are now widely used in both in vitro and in vivo studies [48, 57–60]. In this review, we focus on the use of flexible and stretchable electronics for interfacing with stem cell-derived 3D microtissues or organoids. Table 1 summarizes the materials and features of flexible and stretchable electronics for organoid electrophysiology.

Flexible electronics for organoids

One of the key advantages of flexible electronics is their ability to achieve conformal contact with the curved surfaces of organoids. Flexible, self-folding structures have demonstrated the capacity to establish intimate contact with organoids [71–73]. Kalmykov et al. fabricated self-rolled biosensor arrays using a prestressed metal/polymer multilayer structure (Fig. 3a) [61]. High tensile stresses generated in metal layers (chromium [Cr] and Palladium [Pd]), compared to the negligible internal stress built in the SU-8 support layer, resulted in self-rolling when the device was released from the substrate. The residual stress and the resulting bending radius were determined by metal deposition parameters and the thickness of the metal and polymer

Table 1 Materials and features of flexible and stretchable bioelectronics for organoid electrophysiology

Type	Design	Substrate	Electrode materials	Number of Electrodes	Applications	Ref
On surface	Self-rolled	SU-8	Au/PEDOT:PSS Graphene	16	hESC-cardiac spheroid	[61]
On surface	Self-folding	SU-8	Au/PEDOT:PSS	3	hiPSC-brain organoid	[62]
On surface	Compressive buckling	Polyimide, PDMS	Au/Pt Black	25/34	hiPSC-cortical spheroids/assembloids	[63]
Embedded	Hammock-like mesh	Polyimide	TiN	61	hiPSC-neural organoid	[64]
Embedded	Kirigami	SU-8	Au	32	hiPSC-brain organoids/assembloids	[65]
Embedded	Serpentine mesh	SU-8	Pt/PEDOT:PSS	16	hiPSC-cardiac organoids	[66]
Embedded	Serpentine mesh	SU-8	Pt/Pt Black	16	hiPSC-brain organoids	[67]
Embedded	Serpentine mesh	SU-8	Pt/Pt Black	64	hESC-islet organoids	[68]
Embedded	Serpentine mesh	SU-8	Graphene	16	hESC-cardiac organoids	[69]
Embedded	Stretchable mesh	SEBS	PEDOT:PSS hydrogel	16	hiPSC-cortical organoids	[70]

hESC: human-embryonic stem cells

hiPSC: human-induced pluripotent stem cells

PEDOT:PSS: poly(3,4-ethylenedioxythiophene) polystyrene sulfonate

PDMS: poly(dimethylsiloxane)

SEBS: poly(styrene-ethylene-butylene-styrene)

layers. These self-rolled MEAs encased hESC-derived cardiac spheroids (Fig. 3b) and recorded cardiac field potentials (Fig. 3c). Similarly, Huang et al. demonstrated self-folding shell microelectrode arrays by varying cross-linking levels in bottom and top SU-8 layers [62]. By exposing the layers to varying UV doses, distinct cross-linking levels were achieved, causing differential contraction upon release and chemical treatment, leading to self-folding (Figs. 3d, e). The folding level was controlled by adjusting the thickness and UV dose of the SU-8 bilayers, enabling intimate contact with hiPSC-derived brain organoids and the recording of their electrical activities (Figs. 3f–h).

Another approach involves building flexible 3D scaffolds using mechanically designed compressive buckling [63, 74–77]. In this method, 2D precursor layers are partially anchored to a pre-strained elastomer film. Upon releasing the pre-strain, the bonding sites experience high compressive forces, leading to the buckling of 2D films into 3D architectures. Various geometries can be achieved based on the design and bonding site placement. Park et al. developed a flexible scaffold that enclosed hiPSC-derived cortical spheroids (Fig. 3i) [63]. The authors attached 2D multilayer electronics, including a PI substrate, microelectrode arrays, micro-LEDs, and sensors, onto pre-strained PDMS elastomers. They designed the 2D electronics layer and bonding sites to create a pouch-shaped cage that can hold spheroids upon release of the pre-strain (Fig. 3j). The electrodes formed close contact with the spheroids, successfully recording field potentials through their surfaces (Figs. 3k, l).

To establish stable electronics/organoid interfaces, methods to maintain long-term viability of organoids should also be considered. Adequate perfusion of oxygen and nutrients is crucial for tissue growth and survival. In this regard, efforts have been made to integrate organoids with flexible electronics in a suspended solution. McDonald et al. demonstrated hammock-like mesh microelectrode arrays by attaching spider-web-like mesh electronics to polymer/glass wells [64]. The authors successfully integrated hiPSC-derived neural organoids suspended with electronics and measured electrophysiological signals from them. Yang et al. demonstrated kirigami electronics for the suspended growth of cortical spheroids and assembloids [65]. They employed the kirigami concept to transform electronics from 2D to suspended 3D structures. Upon release, the devices were transferred to the culture chamber to form basket-like structures and then integrated with organoids. The authors recorded long-term electrophysiological signals from hiPSC-derived cortical organoids and corticostriatal assembloids.

Stretchable electronics for organoids

Flexible electronics have been effective in conforming to the 3D surface of organoids. However, achieving long-term stable electrical interfacing with 3D organoids remains challenging, as the electronics must adapt to the volume changes of the organoids during development while minimally interrupting the developmental process. To address this, stretchable electronics that can accommodate the morphological changes of organoids during development have been developed. These devices can integrate with organoids in 3D throughout the organogenesis or morphogenesis process, enabling signal recording across the 3D organoid

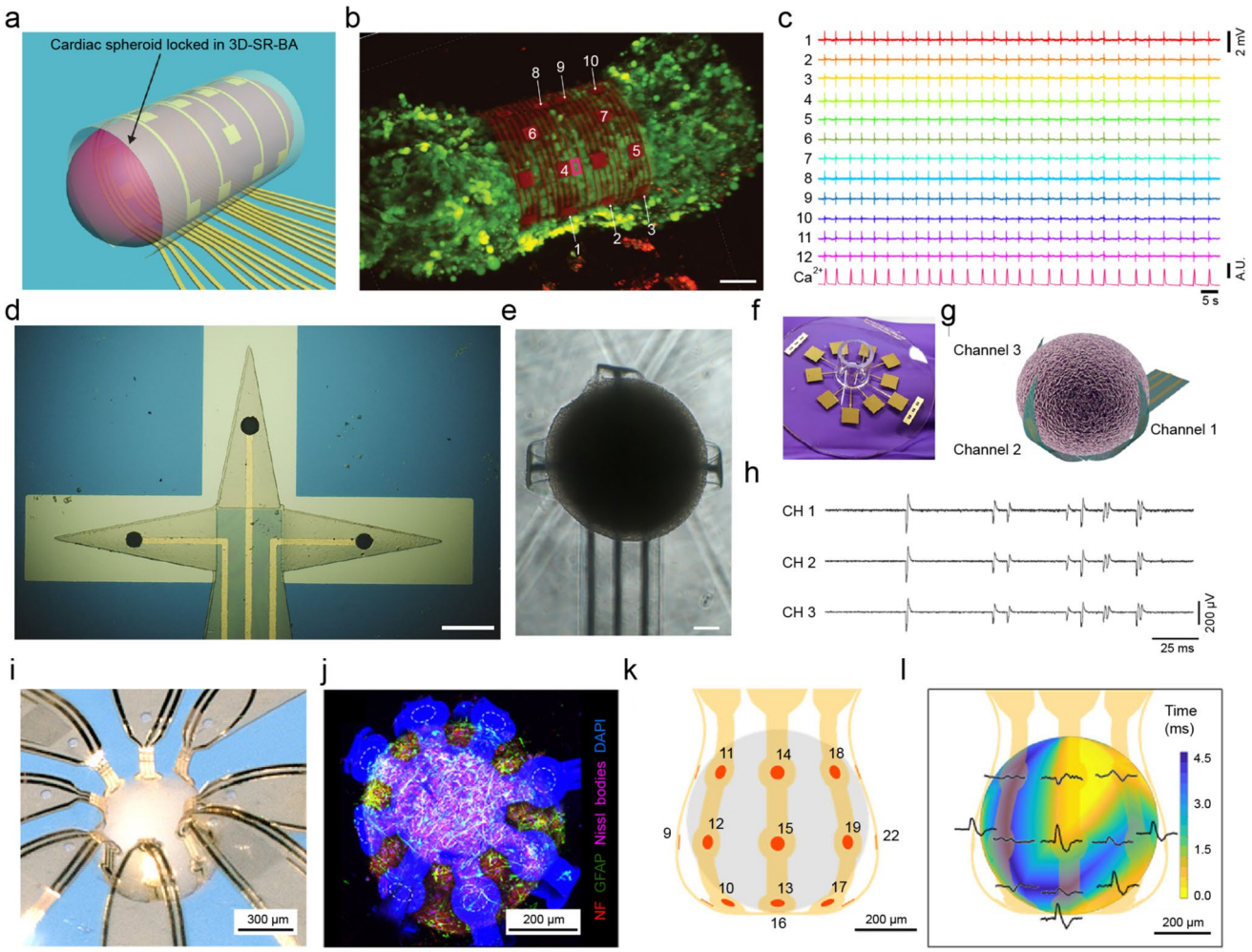

Fig. 3 Flexible electronics for 3D surface mapping of organoid activity. **a**, **b** Schematics (a) and confocal image (b) of a 3D self-rolled biosensor array encapsulating cardiac spheroids. Scale bar: 50 μm. **c** Representative field potential (FP) recordings from the electrodes marked in (b). Adapted from [61]. Copyright 2019, AAAS. **d** Optical image of the shell MEA. Scale bar: 200 μm. **e** Bright-field image of the organoid enclosed in a 3D shell MEA. Scale bar: 100 μm. **f** Image of a 3D shell MEA integrated onto a quartz wafer. **g** Schematics illustrating electrode distribution of the 3D shell MEA surrounding the brain organoid. **h** Representative field potentials recorded from a 3D shell MEA. Adapted from [62]. Copyright 2022, AAAS. **i** Optical image of 3D electronics enclosing a cortical spheroid. **j** Confocal image of the spheroid enclosed in a similar 3D mesostructure made with transparent polymer. **k** Schematic illustration of the electrode positions across the surface of the spheroid. **l** 3D plot of time latency. Adapted from [63]. Copyright 2021, AAAS

during its entire development [66–68]. The devices feature cellular scale electrodes and submicrometer thickness to minimize the bending stiffness, while a serpentine mesh design endows stretchability, allowing the devices to deform with cell migration (Fig. 4a). Li et al. integrated stretchable mesh electronics with 2D hiPSC-derived cardiac cell plates (Fig. 4b) [66]. The organogenetic process stretched, folded, and integrated the devices within 3D organoids (Fig. 4c). The tissue-embedded electronics enabled electrical mapping of cardiac signals throughout the 3D cardiac organoid during its developmental process (Figs. 4d, e). Le Floch et al. demonstrated stretchable mesh electronics 3D integrated with hiPSC-derived brain organoids throughout organogenesis and differentiation (Figs. 4f, g) [67]. These mesh electronics embedded in brain organoids enabled chronically stable monitoring of electrical activity at the single-cell and single-spike spatiotemporal resolution throughout the 3D brain organoids over extended periods. This revealed how the single-cell electrical activity emerges and evolves in early-stage brain organoids, as well as how the electrical activity evolves during in vitro brain organoid development (Figs. 4h, i). In a recent study, stretchable mesh electronics have been integrated with hESC-derived pancreatic islets, enabling the tracing of islet maturation-related electrical activity of α and β-like cells (Figs. 4j, k) [68] and demonstrating the universal applicability of mesh electronics for long-term, single-cell, millisecond scaled electrophysiology across various types of organoid.

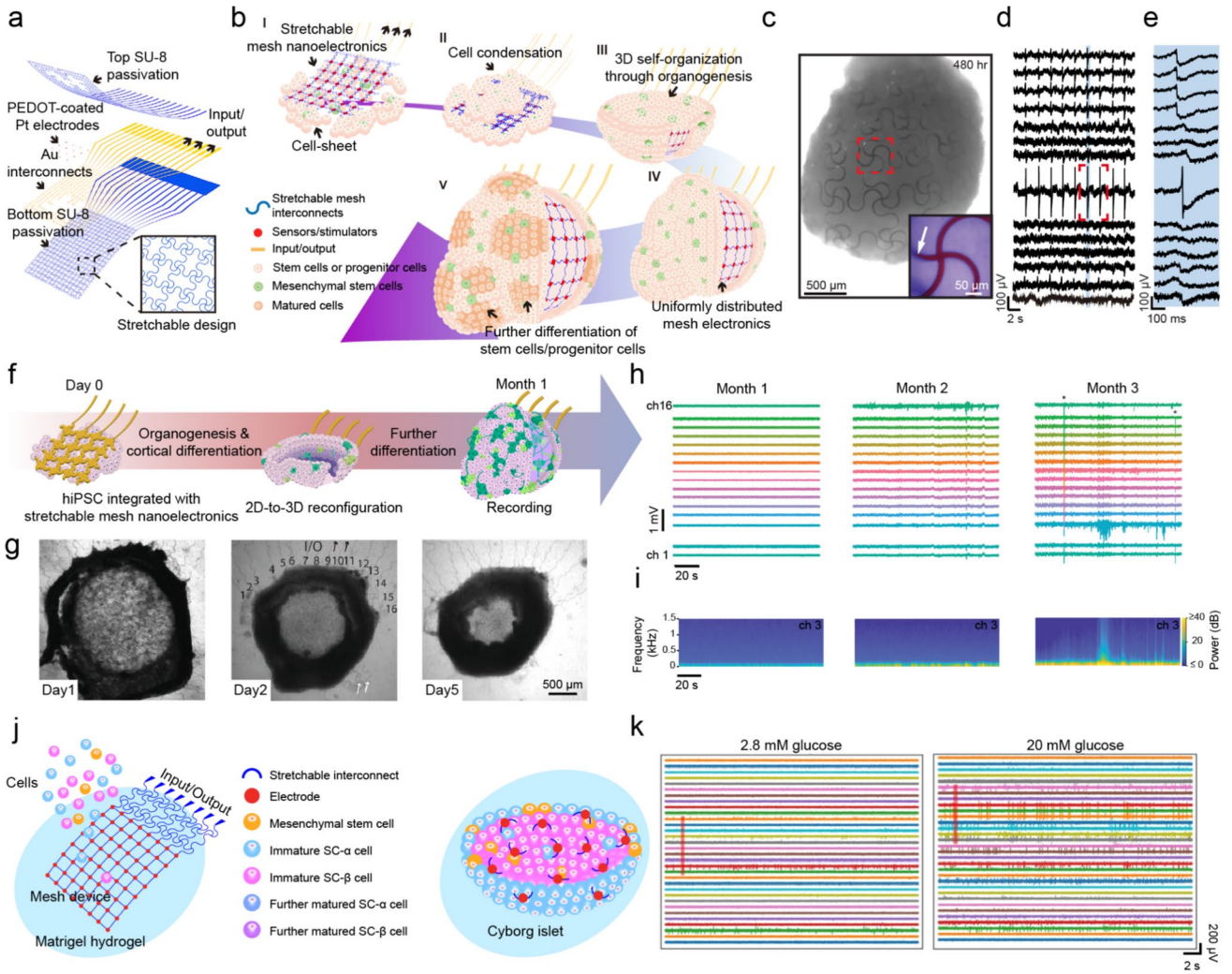

Fig. 4 Stretchable electronics for 3D interfacing throughout organoids. **a** Structure of stretchable mesh nanoelectronics. **b** Schematics of integrating stretchable mesh nanoelectronics within organoids through organogenesis. **c** Bright-field image showing the distribution of the device over the organoid. Inset shows a false-colored zoom-in image. **d** Representative recording from the cardiac cyborg organoid. **e** Zoom-in view of signals highlighted in (d). Adapted with permission from [66]. Copyright 2019, American Chemical Society. **f** Schematic illustration of the stepwise integration of mesh electronics with hiPSC-derived neurons. **g** Optical images of stretchable mesh nanoelectronics integrated with hiPSC-derived neurons. **h**, **i** Representative voltage traces (h) and spectrograms (i) at 1, 2, and 3 months after cortical differentiation. Adapted with permission from [67]. Copyright 2022, Wiley–VCH GmbH. **j** Schematic illustration of the integration of mesh electronics with hiPSC-derived islets. **k** Representative voltage traces recorded from a cyborg islet in response to 2.8 mM and 20 mM glucose exposure. Adapted from [68]. Copyright 2024, the Authors

Flexible multifunctional electronics

Multifunctional electronics for organoid interfaces

Integrating different types of sensing and stimulation modalities with electrophysiology tools can offer valuable insights into organoid studies. For example, the simultaneous measurement of electrophysiological and mechanical characteristics of cardiac tissue could enhance our understanding of excitation–contraction (EC) coupling, a process closely related to cardiac function [69, 78].

Flexible multifunctional electronics have been developed to simultaneously record electrophysiology and mechanophysiology in cardiac organoids. Kim et al. integrated 3D liquid metal electrodes with pressure-sensitive field-effect transistor arrays to measure electrophysiological signals and compressive pressures in hiPSC-derived cardiac organoids (Figs. 5a, b) [79]. The low mechanical modulus of soft eutectic gallium-indium (EGaIn) electrodes ensured biocompatibility with soft organoids. Spontaneous and electrically stimulated beating activity of a cardiac organoid could be simultaneously monitored via electrical recordings, pressure mapping, and calcium imaging (Fig. 5c). Although

electrical recordings experienced interference from electric stimulation signals, pressure mapping remained unaffected, demonstrating the potential of multifunctional electronics for studies involving stimulation and response monitoring, such as cardiac pacing.

Gao et al. demonstrated a 2-in-1 sensing approach using graphene transistors integrated into mesh electronics [69]. Electrical signals and mechanical strain were detected by field-effect and piezoresistive effect (Fig. 5d) of graphene transistors, respectively (Figs. 5e, f). The authors embedded mesh electronics with hESC-derived cardiomyocytes, where the device was folded with the tissue during development (Fig. 5g). Each graphene transistor could continuously and simultaneously detect electrical and mechanical characteristics throughout the 3D organoid (Figs. 5h, i), enabling tracking of tissue maturation, drug testing, and disease modeling.

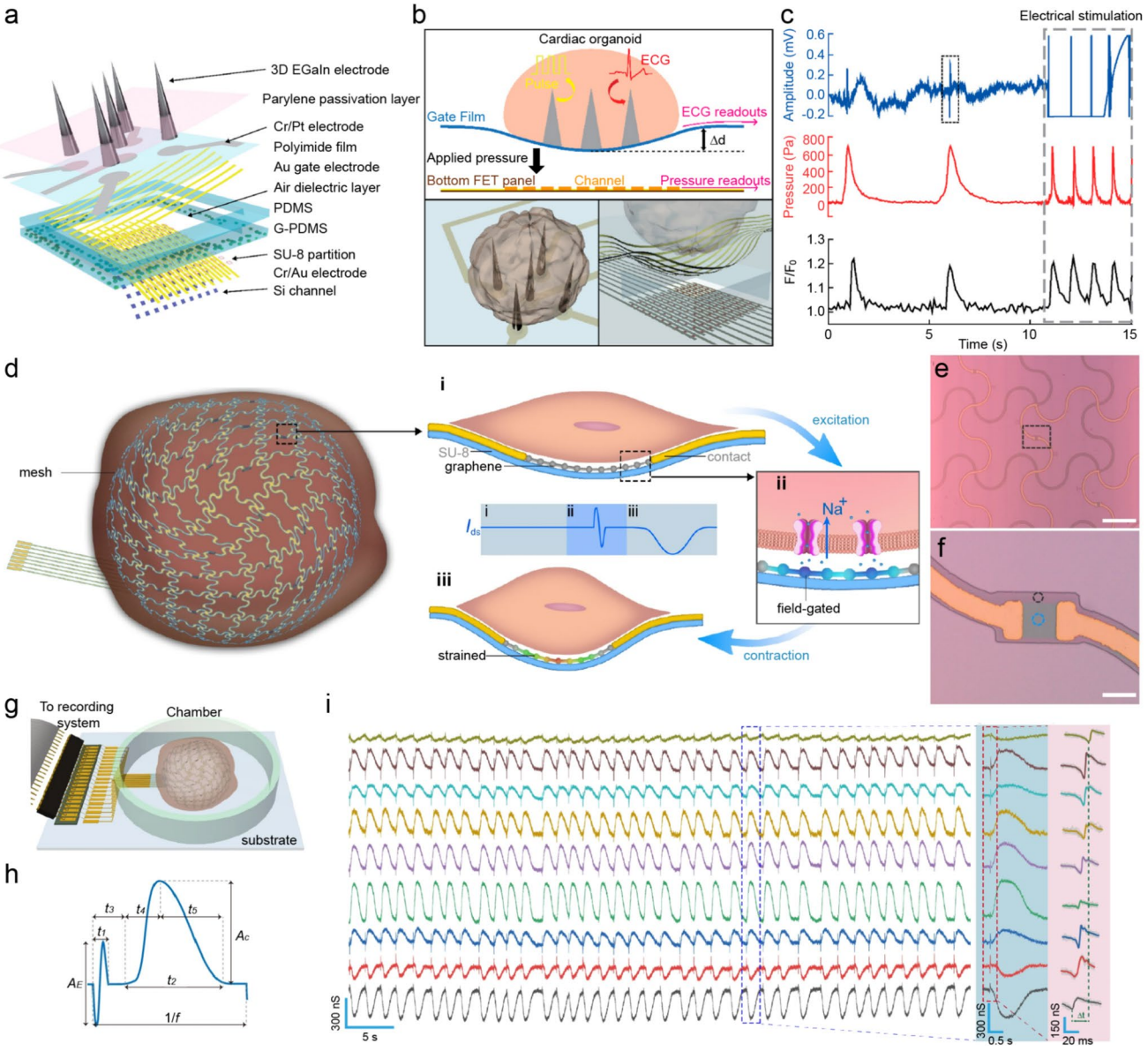

Fig. 5 Multifunctional electronics for multimodal mapping of organoid activities. **a**, **b** Schematic illustration of the structures (a) and sensing mechanisms (b) of the multimodal sensor composed of 3D liquid–metal electrodes on top of a pressure-sensitive transistor array. **c** Real-time monitoring of ECG (blue), compressive pressure (red), and normalized fluorescence intensity (black) in the hiPSC-derived cardiac organoid, observed before and during electrical stimulation (gray dotted line, 0.8 V/mm). Adapted from [79] under a Creative Commons CC-BY-NC-ND license. Copyright 2022, American Chemical Society. **d** Schematic of cardiac microtissue innervated with graphene-integrated mesh electronics. Schematics of cell-device interface (i), where the graphene device detects the action potential through the field effect (ii) and mechanical strain via the piezoresistive effect (iii). **e**, **f** Optical image of the serpentine feature (e) and graphene transistor (f) marked in (e). Scale bars: 200 μm (e), 20 μm (f). **g** Schematic of recording setup. **h** Schematic of the parameter sets of the recorded signal. **i** Representative recordings from 9 graphene sensors. Adapted from [69] under a Creative Commons CC BY license. Copyright 2024, Springer Nature

Optogenetics and chemogenetics, which use light or chemical delivery to modulate cells in a cell type- and region-specific manner, provide precise control and mechanistic studies of cellular activity in organoids. When combined with electrophysiological recording using MEAs, optogenetic inhibition and stimulation improved our understanding of bidirectional axonal connectivity between two hiPSC-derived cerebral organoids connected by reciprocally extended axons [80]. Optogenetic stimulation combined with long-term electrophysiological recordings using kirigami electronics revealed the formation of unilateral cortico-striatal connectivity in assembloids of human cortical and striatal organoids, resembling their in vivo connections [65]. Cvetkovic et al. developed multicellular neural organoids by differentiating hiPSC into chemogenetic astrocytes and optogenetic neurons [81]. By modulating chemogenic astrocytes with chemical compounds and optogenetic neurons with light while recording their activities using MEAs, they uncovered the dynamics of astrocyte reactivity and its impact on downstream neuron activity. These multifunctional stimulation/recording platforms provide interesting models for studying cell dynamics within organoids.

Incorporating flexible electronics with microfluidic channels and miniaturized light sources, such as micro-LEDs, will further enable localized, precise delivery of optical and chemical modulation throughout the organoid. Park et al. showed that micro-LEDs could be embedded with microelectrode arrays and deformed into a 3D flexible scaffold that covers cortical spheroids, providing more localized light delivery to cells [63]. Flexible multifunctional electronics combining recording electrodes with light sources or microfluidic channels have already been developed for in vivo applications [82–85]. Engineering these multifunctional electronics to establish chronically stable interfaces with organoids will enable more precise mechanistic studies using organoids.

Applications of flexible electronics/organoid hybrids

Multimodal profiling

The development of flexible bioelectronics has paved the way for studying cell functional phenotypes within organoids. A promising direction for expanding and applying these technologies is to investigate the molecular basis behind functional activities and disorders. Various levels of molecular analysis techniques, such as transcriptomics and proteomics, have been utilized to investigate the relationship between genes and functions [86]. Notably, single-cell transcriptomics, which assays RNA transcripts within individual cells, has been widely used to classify cell types and states within tissues due to its comprehensiveness and high scalability [87]. Detailed summaries of these molecular analysis technologies can be found in other reviews [88–92]. The integration of transcriptomics and electrophysiology has further enhanced the precision of cell type classification and facilitated the study of the relationship between cell types, states, and functional phenotypes [93–95]. For example, applying Patch-seq – which combines whole-cell patch-clamp recording from individual cells with morphologic reconstruction and single-cell RNA sequencing of the recorded cells [96] – to hESC or hiPSC-derived neurons has revealed genes associated with neuronal maturation determined by electrophysiological properties, and has enabled the development of machine learning-based classifiers to predict electrophysiological functional states from transcriptome features [97, 98].

Studies have also utilized MEAs capable of long-term, network-level electrophysiological recordings in combination with single-cell RNA sequencing. Fair et al. tracked the maturation of hiPSC-derived cerebral organoids, observing a progression from weak, immature electrical activities to more complex network bursting using 2D MEAs. Immunohistochemistry and single-cell RNA sequencing of cerebral organoids at different time points revealed morphological and transcriptomic features correlated with electrophysiological maturation [99]. Lin et al. explored the effect of hiPSC-derived endothelial cells on the electrical maturation of hiPSC-derived cardiomyocytes using flexible mesh electronics combined with single-cell RNA sequencing [100]. The authors embedded tissue-like mesh electronics into 3D cardiac microtissues cultured with or without endothelial cells. The long-term tracking of electrical activity in these cardiac tissues revealed accelerated electrical maturation of the cardiomyocytes co-cultured with endothelial cells. Single-cell RNA sequencing further identified differential gene expression and multiple intercellular interactions that potentially contributed to the accelerated electrical maturation. In this study, stretchable mesh electronics enabled tissue-wide, long-term stable tracking of single-cell activity during their maturation. However, single-cell RNA sequencing cannot provide a transcriptome of the electrically recorded cells.

Recently, Li et al. demonstrated in situ electro-sequencing technology capable of tissue-wide multimodal charting of electrophysiology and gene expression at the single-cell level (Fig. 6a) [101]. Electrophysiological recording was conducted by tissue-like mesh electronics integrated with the cardiac microtissues (Fig. 6b). After electrophysiological recording, the tissues were cleared and underwent in situ RNA sequencing to obtain gene expression profiles. Fluorescent E-barcodes fabricated next to the electrodes allowed for the identification of electrode locations during multiple imaging cycles of RNA sequencing, enabling the back-mapping of RNA transcripts to the recorded electrophysiological

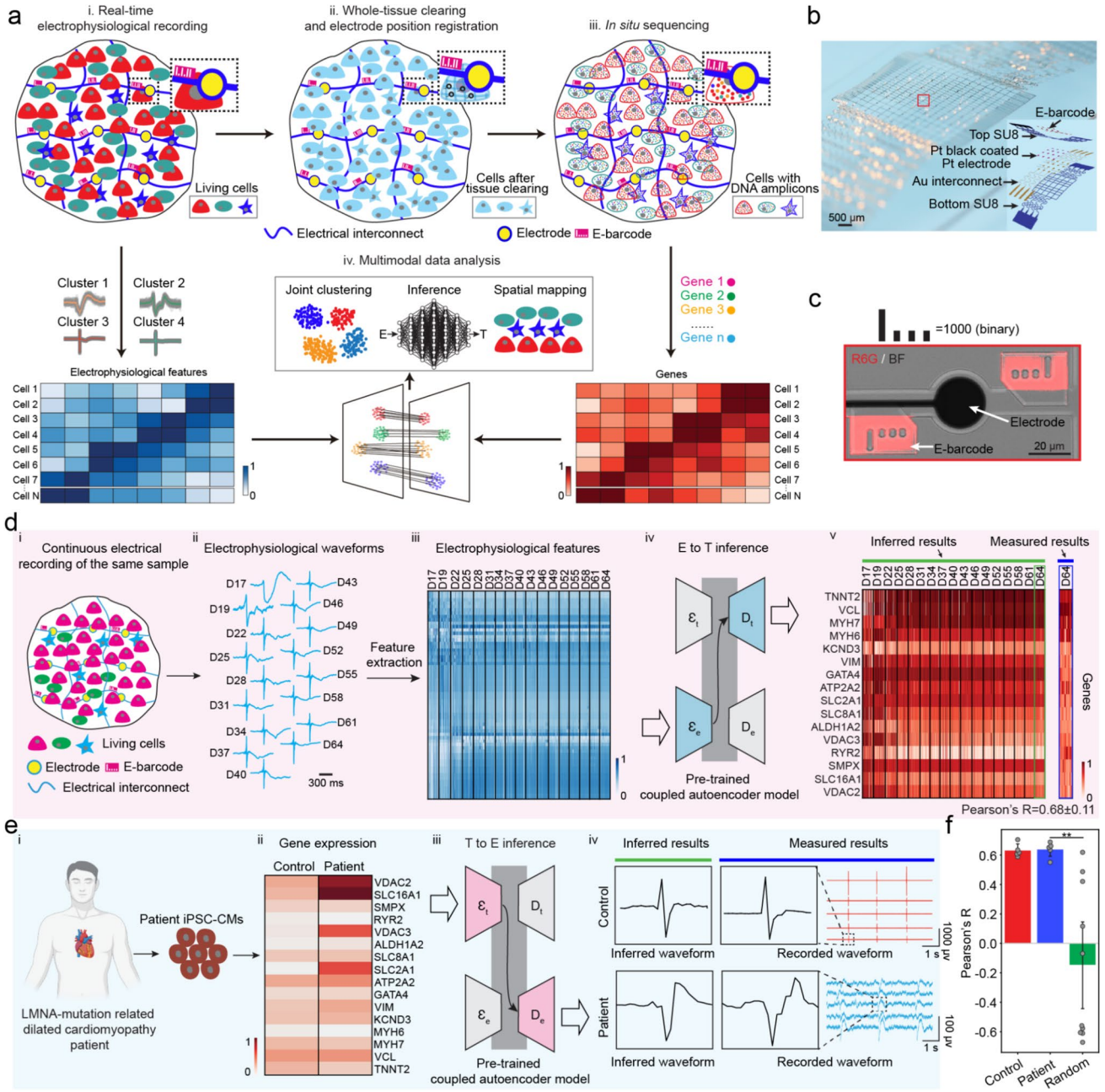

Fig. 6 Flexible electronics-enabled integrative multimodal charting of organoids. **a** Schematic overview of in situ electro-sequencing. **b** Photograph of flexible mesh electronics. Inset shows a schematic of the electronics structure. **c** Image of binary fluorescent E-barcodes next to the electrode, highlighted in red box in (b). **d**, **e** Application of a pre-trained coupled autoencoder model, inferring dynamics of gene expression from long-term electrophysiological recordings (d) and inferring electrophysiology of LMNA-mutation patient-derived iPSC-CMs (e). **f** Performance of gene expression to electrophysiology inference using control, patient-derived, and randomly shuffled iPSC-CM gene expression profiles. Adapted with permission from [101]. Copyright 2023, Elsevier Inc

signals (Fig. 6c). With data collected from multiple developmental stages, machine-learning-based cross-modal inference models were built to infer gene-to-electrophysiology relationships. The model could predict gene expression profiles from long-term stable electrophysiological recordings throughout the development of cardiomyocytes (Fig. 6d), and abnormal electrophysiological characteristics based on the gene expression of dilated cardiomyopathy patient-derived LMNA (lamin A/C protein) mutant cardiomyocytes (Figs. 6e, f). In situ electro-sequencing can provide high-throughput single-cell multimodal charting by stable tracking of electrophysiological features at high spatiotemporal

resolution and analyzing transcriptomics of the recorded cells at high spatial resolution. Future directions may include integrating flexible multifunctional electronics for investigating multimodal functional phenotypes with in situ sequencing of multiple biomolecules, such as RNA and proteins, for more detailed molecular analysis.

Organoid intelligence

Organoid intelligence is an emerging field that aims to harness cognitive functions of brain organoids, such as learning and memory, for biocomputing, which can be enabled through organoid-electronics interfaces [102, 103]. Biocomputing has the potential to surpass traditional silicon-based computing by consuming less energy, enabling parallel processing, and learning from incomplete or ambiguous data [103]. The key components of organoid intelligence include electronic systems for interfacing with 3D organoids, advances in brain organoid engineering, and algorithms for interpreting and decoding data from biological neural networks.

MEAs have been utilized to interface with organoids for this purpose. Kagan et al. cultured neurons on 2D HD-MEAs and demonstrated that cultured neurons in a dish can exhibit learning capabilities [104]. They designed a closed-loop system in which neurons were embedded into a simulated game of 'Pong', where electrical signals represented the ball, and neuron responses controlled the paddle's movement. The neural networks adapted over time, showing signs of reinforcement learning.

Cai et al. introduced Brainoware, a reservoir computing approach that utilizes the unsupervised learning capability of brain organoids (Fig. 7a) [105]. This method interfaces with the brain organoids via 2D HD-MEAs, converting inputs into spatiotemporally patterned electrical stimulation. The organoids functioned as an adaptive reservoir layer, projecting received inputs into high-dimensional computational spaces (Fig. 7b). The output signals, electrophysiological signals from MEAs, were used for tasks such as speech recognition and nonlinear equation prediction. Brainoware exhibited properties similar to conventional reservoir computing, such as nonlinearity and short-term memory (Fig. 7c). Since organoid intelligence directly studies input–output functions by stimulating and recording organoids, providing stable and reproducible environments is important. Jordan et al. developed an automated, remotely accessible platform to control the organoid environment [106]. This platform placed MEAs on top of a permeable membrane with culture medium flowing beneath (Fig. 7d) and a thin layer of medium covering the organoids (Fig. 7e). A closed-loop microfluidic system controlled medium circulation, while cameras monitored the organoids. Environmental conditions were displayed through a graphical user interface (Fig. 7f).

However, all the current platforms rely on 2D planar MEAs. Flexible electronics offer the potential for precise stimulation and recording within 3D organoids, enabling cell-specific interactions over extended periods. Additionally, flexible multifunctional electronics could provide localized multimodal stimulation using light and chemical compounds, further enhancing the communication with biological neural networks. Building machine-learning based models to infer gene expression from continuous electrophysiological recording during organoid development might help evaluating the reproducible production of organoid models for computing. Other critical considerations also remain, such as engineering organoids or assembloids to model different brain regions, designing algorithms to delineate input and output, and addressing ethical issues surrounding the use of brain organoids in biocomputing. These details are beyond the scope of this paper, and readers are referred to Smirnova et al. [102, 103] for further information.

Conclusions and outlook

Long-term electrophysiological recording of organoids offers promising models for studying development, disease, and drug discovery. Flexible electronics have revolutionized this field by enabling electrophysiological recordings from 3D organoids. The development of organoid/electronics hybrids, or cyborg organoids, has enabled long-term, stable electrophysiological recording at the single-cell level across 3D tissues throughout development. When combined with in situ sequencing, organoid/electronics hybrids have provided novel opportunities for multimodal profiling of single cells throughout 3D organoids, potentially uncovering the molecular basis of functionally specific types of cells. Flexible electronics interfacing with brain organoid can further advance organoid intelligence by enabling cognitive function training through stimulation and recording of biological neural networks. Additionally, the development of flexible, multifunctional electronics for precise cell stimulation with light or chemicals will enhance the characterization and study of organoid models.

To advance this field, several key areas require attention. Increasing electrode density in flexible electronics is crucial, as current systems have limited electrode densities compared to traditional HD-MEAs. However, increasing electrode density may increase system rigidity, potentially causing additional mechanical interruptions to organoids. Utilizing intrinsically soft materials, such as elastomers and viscoelastic materials, can further reduce mechanical mismatch between the electronics and the soft organoid tissues [70]. Extensive research has been conducted on intrinsically soft electronics [107, 108]. Stretchable

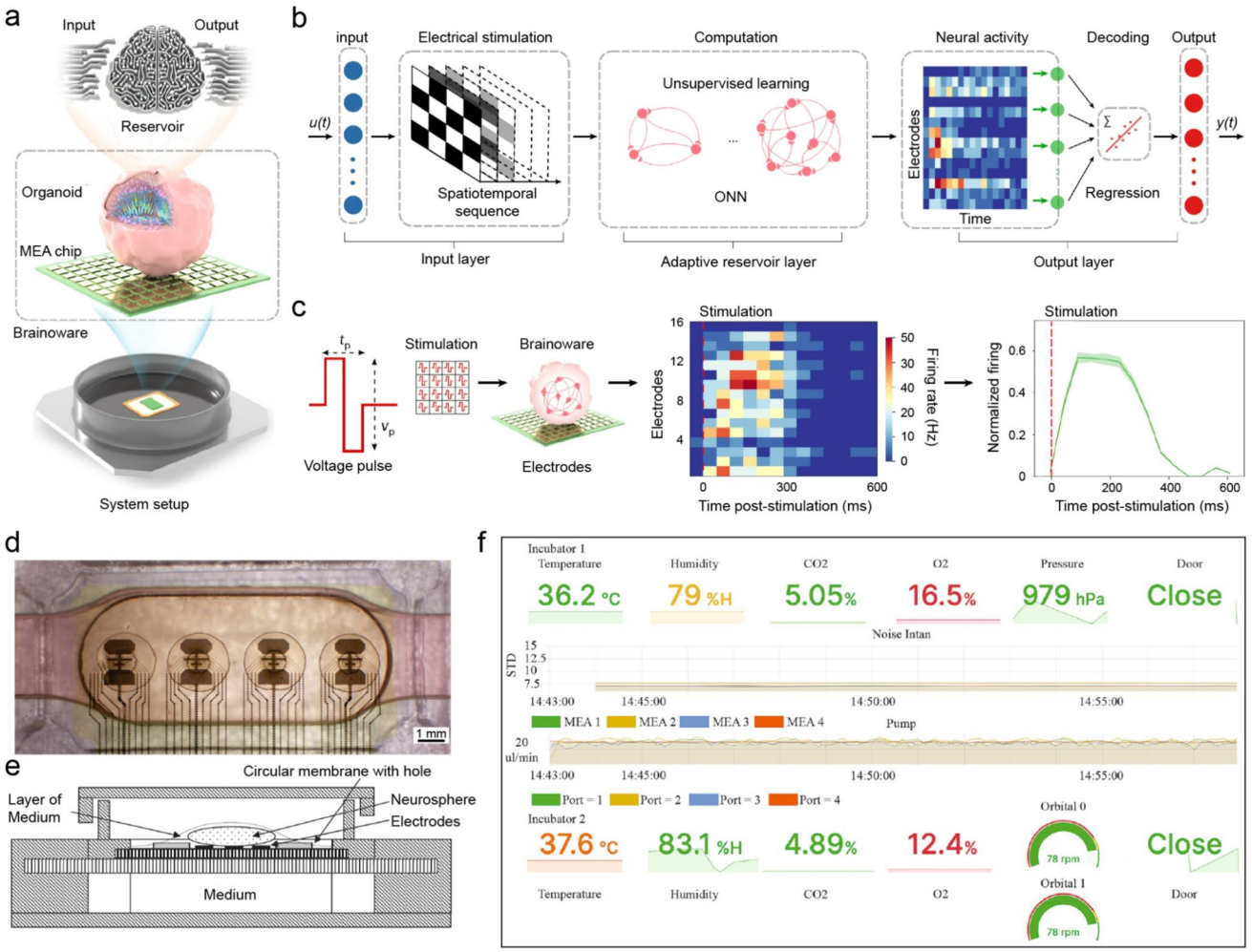

Fig. 7 Organoid intelligence. **a** Schematics of the Brainoware setup; a brain organoid mounted on a high-density MEAs. **b** Schematic overview of an adaptive reservoir computing framework utilizing the Brainoware setup. **c** Response of Brainoware following a bipolar voltage pulse stimulation. Adapted with permission from [105]. Copyright 2023, Springer Nature. **d** Optical image of forebrain organoids on top MEA. Scale bar: 1 mm **e** Cross-sectional schematic of the MEA setup. **f** Graphical User Interface showing environmental conditions. Adapted from [106] under Creative Commons CC BY license. Copyright 2024, Frontiers Media S.A

conductors have been developed by combining soft materials with conductive nanomaterials, including nanoparticles, 1D nanowires and carbon nanotubes, and 2D materials like graphene and MXene [109, 110]. Organic conductive polymers, ionic conductive hydrogels, and liquid metals have also been employed as conductive layers [111, 112]. In addition, intrinsically stretchable semiconductor devices, such as transistors and LEDs, have been actively explored [113–115]. Advances in manufacturing techniques, including 3D printing and inkjet printing, have enabled precise micro-patterning of these soft materials [116, 117], facilitating the development of intrinsically soft electronics that may fulfill mechanical and dimensional compatibility with organoids.

In addition to mechanical compatibility, biocompatibility should be considered when designing bioelectronics. Biocompatibility generally refers to the ability of materials and devices to perform their intended functions without negatively impacting surrounding tissues or overall health [46, 118]. Several strategies have been developed to improve biocompatibility of implantable devices, including surface chemistry modifications, anti-biofouling coatings, and the incorporation of bioactive molecules [119–121]. It is important to note that the effects of a material can vary depending on the application site and the specific tissues involved [118].

Current organoid models, especially those derived from pluripotent stem-cells, have yet to achieve the full complexity of actual organs, as they typically consist of fewer cell types and show limited maturation [122, 123]. Most 3D organoid models demonstrated with bioelectronic devices are devoid of immune cells, making it difficult to assess

immune responses – one of the important factors for evaluating biocompatibility of devices in vivo. To test biocompatibility, studies have shown that integrating devices into organoids does not inhibit essential biological processes, such as cell proliferation, differentiation, or gene expression, when compared to control organoids without device integration. Researchers have evaluated the expression of developmental stage-specific biomarkers through immunohistostaining [66, 101] or marker gene expressions using single-cell RNA sequencing [65, 67]. Efforts to incorporate immune components into organoid systems are ongoing, aiming to create more physiologically relevant models [124]. As organoid models become more and more sophisticated, comprehensive biocompatibility assessments will be necessary, encompassing local cellular to whole tissue responses, while ensuring that the electronics function properly within organoids.

Integrating flexible electronics with microfluidic systems to ensure stable nutrient delivery and waste removal will further support the viability of organoids. This can also be achieved by incorporating engineered vascularization systems, as suggested by previous research [125–127]. One of the challenges in using organoids is their intrinsic variability, including batch-to-batch variation, which can impact the reproducibility of results. Integrating multifunctional electronics with organoids to monitor the organoid culture and standardize the protocol will pave the way for more reliable and sophisticated models for biological research. Additionally, ethical guidelines need to be developed to clarify the potential and limitations of organoid models, facilitating the continued advancement of this exciting interdisciplinary field.

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Authors' contributions J.Lee drafted, wrote, and revised the manuscript. J.Liu drafted, reviewed, and revised the manuscript. All authors have read and approved the final version.

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Data availability Not applicable.

Declarations

Competing interests J.Liu is a co-founder of Axoft, Inc.

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