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Three dimensional bioelectronic interfaces to small-scale biological systems

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Recent advances in bio-interface technologies establish a rich range of electronic, optoelectronic, thermal, and chemical options for probing and modulating the behaviors of small-scale three dimensional (3D) biological constructs (e.g. organoids, spheroids, and assembloids). These approaches represent qualitative advances over traditional alternatives due to their ability to extend broadly into volumetric spaces and/or to wrap tightly curved surfaces of natural or artificial tissues. Thin deformable sheets, filamentary penetrating pins, open mesh structures and 3D interconnected networks represent some of the most effective design strategies in this emerging field of bioelectronics. This review focuses on recent developments, with an emphasis on multimodal interfaces in the form of tissue-embedding scaffolds and tissue-surrounding frameworks.

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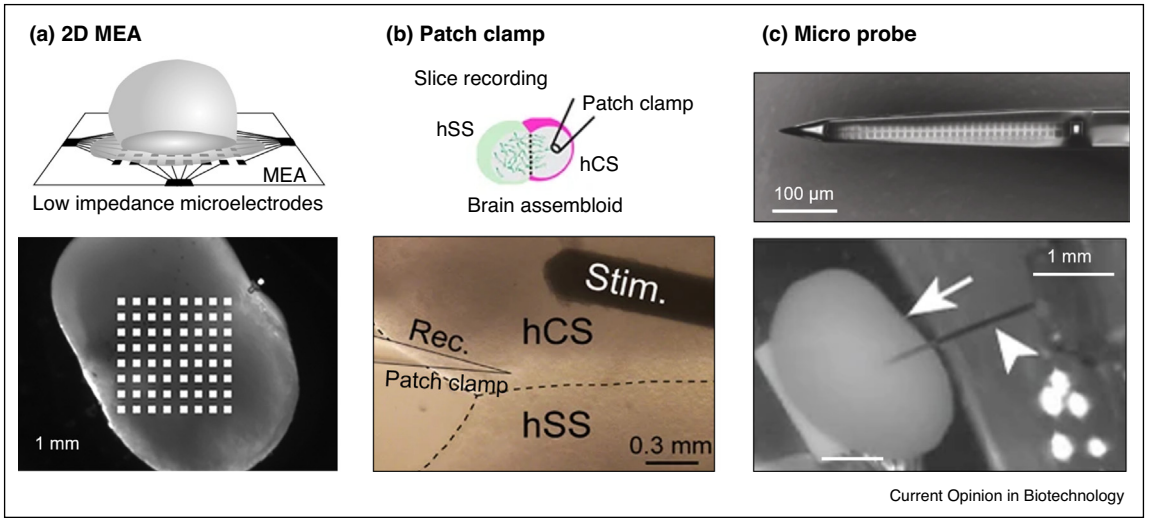
Introduction

Two-dimensional (2D) cell cultures represent powerful *in vitro* platforms for fundamental research into biological phenomena, ranging from mechanisms of disease evolution to processes of cellular differentiation and tissue formation. A specific example of interest is in various stages of development, degeneration and regeneration of neural tissues, with initial work that began in the early 1900s [1]. The most common scheme exploits thin, planar cell cultures grown on flat plates for studies using various imaging and probing techniques. A key deficiency of this 2D configuration is that it is morphologically and functionally distinct from the 3D layouts that characterize all naturally occurring tissues. Furthermore, 2D confinement effects associated with diffusive transport can lead to cellular and extracellular environments that induce cell death. These and other considerations limit the utility of 2D cultures as model systems for living organisms, including the human body.

Recent advances in techniques for tissue growth and stem cell differentiation establish routes to millimeter-scale biological constructs that resemble real organs, with features and forms that resemble those of the human neural [2–5], pulmonary [6], kidney [7], retinal [8,9], and intestinal [10] systems. Such types of organoids have vast potential applications in personal medicine (drug screening and discovery), disease research (infectious diseases, inheritable genetic disorders, and cancer) and genetic engineering. Brain organoids in particular have utility in studies of the development [11–13] and degeneration [14,15] of the human brain. Anatomical and transcriptional changes in these systems can be investigated using various optical methods, but functional activity at the cellular or network levels cannot be captured with high spatiotemporal resolution. Sensing and modulation based on thermal, chemical, and electrical effects also lie generally outside of the scope of optical methods, with few exceptions.

Conventional neural interfaces such as patch clamps [16,17], probe shanks [4,18], and multielectrode arrays (MEAs) [19,20••] are also not directly applicable. For instance, traditional MEAs adopt planar, 2D layouts that can effectively capture electrophysiological activity and deliver electrical stimulation [20••] (Figure 1a) but only across the base regions of 3D tissue constructs such as organoids. Patch clamps can record action potentials associated with individual neurons (Figure 1b) in cortical-thalamic organoids [21], including those combined into fused units known as assembloids and in dorsal and ventral forebrain assembloids [22]. Such measurements, however, apply only at isolated locations due to geometric constraints of the cell-sensing interface.

Figure 1



Established classes of devices for measurements of 3D biological systems.
(a) Schematic illustration and picture of a cortical organoid on a 2D multielectrode array (MEA) [20••,55]. **(b)** Schematic illustration and picture of a slice of a brain assembloid probed with a glass pipette patch clamp [22]. **(c)** Optical micrograph of the tip of a multi-electrode shank for extracellular recordings and picture of a brain organoid with such a shank inserted [4].

Collections of electrodes integrated onto penetrating probes can capture intercellular interactions within organoids, as demonstrated in observations of suppressed neural activity of photosensitive neurons during illumination [4] (Figure 1c). This technique reveals high resolution information from a localized area but induces damage to cell cultures during insertion, thereby limiting its potential for long-term experiments. A well-recognized need, then, is in technologies that can support direct physical interfaces between sensors and actuators across the surfaces and into the depths of 3D tissues.

Tissue-embedding 3D electronic scaffolds

One class of advanced platform for such purposes involves cells cultured within and around electronic networks, prefabricated in open geometries, as scaffolds. Recent studies describe complex systems of this type, with multiple interface points based on nanowire-based field-effect transistors [23–26], noble metal electrodes [27,28•,29], and conducting polymers [30••], for collecting high quality electrophysiological data, including intracellular recordings. Other related work demonstrates that cardiac cells can be grown around folded electronic structures for similar purposes in monitoring and stimulation (Figure 2a,b). The results demonstrate monitoring of electrical activity of cardiomyocytes with high spatiotemporal resolution, where applying electrical stimulation in the direction opposite to signal propagation illustrates an ability to manipulate behaviors of the tissue (Figure 2c) [26].

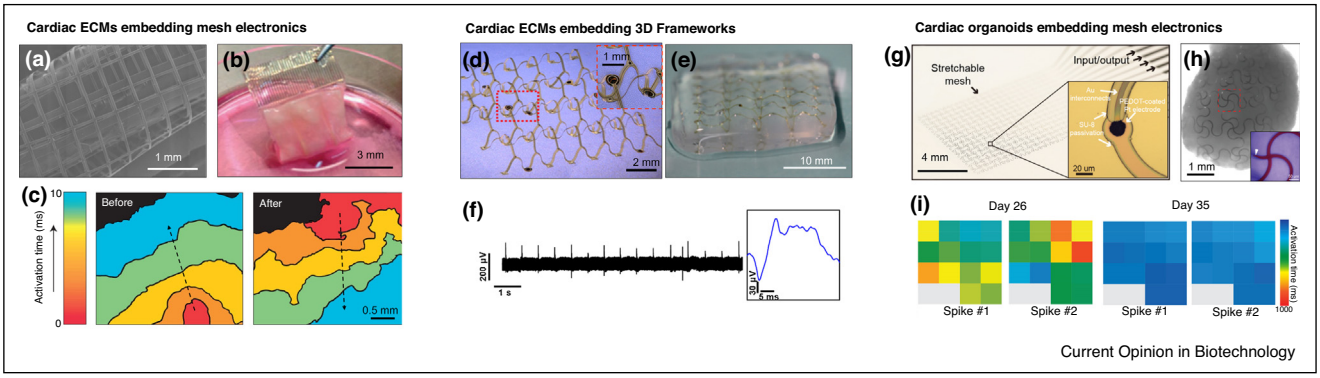
The most sophisticated examples use 3D electronic scaffolds created in a process of mechanically guided assembly that transforms 2D electronic systems into 3D architectures (Figure 2d,e). Deterministic access to diverse 3D formats with integrated electrodes, electronic components and sensors creates many functional possibilities at the bio-interface. In one example, hybrid 3D electronic-tissue systems measure electrical activity of the engineered tissues (Figure 2f) and provide control over its function through electrical stimulation and controlled drug release [31].

Other examples use stretchable mesh electronics delivered to the surfaces of growing cellular systems that begin in 2D layered formats and then merge into final 3D structures of organoids by natural mechanisms of organogenesis (Figure 2g,h). Experimental demonstrations indicate feasibility as chronic, multiplexed, tissue-wide electrical interfaces to cardiac organoids for electrophysiological monitoring. Activation mapping indicates functional maturation from day 26 to day 35, as evidenced by monitoring of synchronized local field potential propagation across the biological system (Figure 2i) [30••]. Further research will define the extent to which the mesh structure affects the growth and differentiation processes.

Tissue-surrounding 3D electronic frameworks

As an alternative to 3D interfaces that form following the growth of 3D tissues, those that gently envelop these tissues after growth are also of recent interest. Such

Figure 2

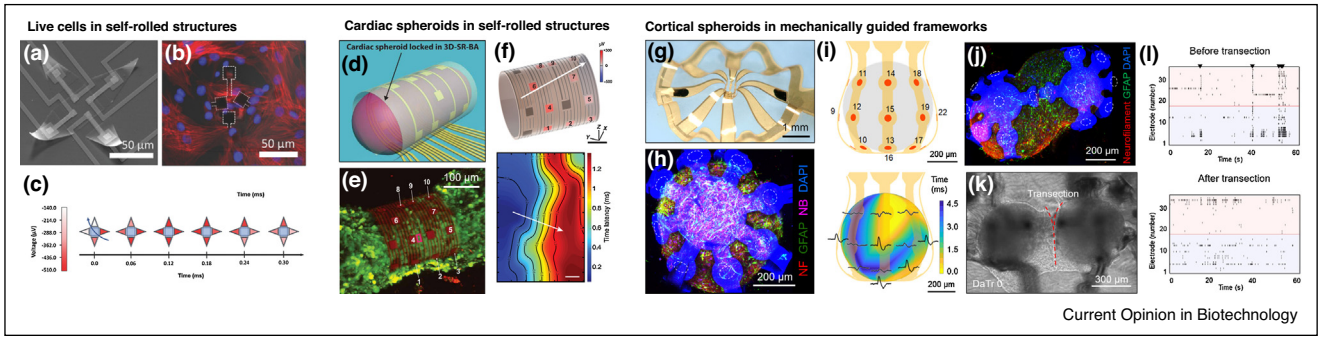


Tissue-embedding 3D electronic scaffolds.
(a-c) Mesh electronics embedded into cardiac ECM hydrogels. Scanning electron microscope (SEM) image of nanowire electronics folded into a multilayer structure (a) and photograph after seven days of culture with cardiomyocytes (b). (c) Heat maps presenting normal (left) and reversal (right) of electrical signal propagation of the identical engineered cardiac tissue as a result of electrical stimulation [26]. **(d–f)** 3D frameworks embedded into cardiac ECM hydrogels. (d) An optical micrograph of a 3D electronic scaffold formed by a mechanical assembly process. (e) A photograph after embedding in an ECM hydrogel. (f) Representative field potential trace recorded from an electrode within the ECM (left) and magnified view of a single recorded spike from a trace (right) [28•]. **(g–i)** Cardiac organoids embedded with mesh electronics. Images of a system of electronics in the form of a stretchable mesh (g) and after integration into a growing cardiac organoid (h). (i) Isochronal mapping of local field potentials at day 26 and day 35 of differentiation [30••].

approaches allow accurate recording and modulation of global networked activity for long-term studies of unaltered tissues. These 3D device architectures can open and softly enclose delicate tissues without damage or deformation. 3D assembly techniques based on self-rolling or self-folding [32–35], mechanical manipulation [29] or mechanically guided assembly [36–40] serve as powerful routes to the necessary 3D frameworks. Self-rolling/folding approaches exploit out-of-plane deformations in thin film materials that arise from capillary forces [41,42] or from residual stresses. These effects transform 2D electronic structures into simple 3D shapes [43,44], typically triggered by the removal of underlying sacrificial layers [45,46]. In one example, this type of framework rolls into its 3D structure to enclose a collection of neonatal rat ventricular cardiomyocytes cultured on a flat surface. Specifically, upon dissolution of a sacrificial layer after immersion in culture media, intrinsic stresses associated with a bilayer of SiO/SiO_2 cause four panels integrated with electrodes to fold over the top of the layer of cells to yield a structure that can be used to track the propagation of action potentials (Figure 3a–c) [46]. Examples of advanced forms of 3D frameworks formed by the action of residual stresses include self-rolled arrays of 12 electrodes that enclose 3D cardiac spheroids (about 300 μm in diameter) formed from embryonic stem cells. This system captures 3D electrophysiological behaviors by monitoring the propagation of field potentials over the surfaces of submillimeter scale spheroids, with negligible adverse effects, as evidenced by viability assays (Figure 3d–f) [47•].

Greatly expanded classes of 3D frameworks can be realized using methods in mechanically guided 3D assembly, similar to those used for certain of the scaffolds described in the previous section. Here, stresses imposed at strategic locations across a 2D structure (i.e. precursor) initiate a coordinated set of in-of-plane and out-of-plane translational and rotational motions to yield a desired 3D architecture. Planar processing methods can form precursors with a wide range of thin film active components, spanning from those with electronic and optoelectronic function to those in chemical, mechanical, and thermal devices for sensing and actuation. Bonding these structures at strategic sites to a pre-stretched elastomeric substrate followed by release of this pre-stretch induces compressive forces at these sites to form an engineered 3D configuration by mechanical buckling [40,48–51]. The results are 3D multifunctional frameworks that can be reversibly opened and closed by stretching and releasing the elastomer substrates. With tailored geometric features to match those of targeted 3D tissues, these compliant 3D frameworks form gentle, enveloping interfaces, as first demonstrated with cortical spheroids formed from human induced pluripotent stem cells (hiPSCs) (Figure 3g,h). In one example, this type of device yields data on spatiotemporal patterns of neural activity through recordings from 25 low impedance microelectrodes distributed across the 3D surfaces of spheroids for periods of up to several weeks (Figure 3i). Finite element analysis serves as a versatile design tool for creating tailored 3D geometries not only for individual spheroids, but also for interconnected collections of them, known as assembloids.

Figure 3



Tissue-surrounding 3D electronic frameworks.
(a–c) Live cells in self-rolled structures (a) SEM image of a planar multielectrode platform after release of a collection of four non-planar wing structures designed to envelop a biological tissue. (b) confocal fluorescence microscope image of cardiomyocytes integrated with this platform; actin filaments (red) and cell nuclei (blue) with four electrodes wrapped around the cells (dashed white lines). (c) Recordings of spatiotemporal patterns of action potentials of cells captured with multielectrode shells [46]. **(d–f)** Cardiac spheroids in self-rolled structures. (d) Schematic illustration of a cardiac spheroid enclosed in a cylindrical electronic structure formed by a stress-driving rolling process. (e) Confocal fluorescence microscope image of this system, with spheroid labeled using a Ca^{2+} indicator dye (Fluo-4, green). (f) Propagation of electrical activity across the surface of a cardiac spheroid rendered in 3D (top) and 2D (bottom) [47•]. **(g–l)** Cortical spheroids in mechanically guided frameworks. (g) A microscope image of a multifunctional 3D framework formed by a mechanical assembly process. (h) A confocal fluorescence microscope image of a cortical spheroid labeled with neurofilament (red), glial fibrillary acidic protein (green), Nissl bodies 797 (magenta), DAPI nuclear stain (blue) enclosed in a framework labeled with an autofluorescence of the parylene-C (blue). (i) An illustration of the positions of microelectrodes across the surface of a spheroid (top) and electrical activity across the surface of cortical spheroid rendered in 3D (bottom). (j) A confocal microscope image of a related framework designed for an assembloid of two such spheroids. (k) A transmission mode optical microscope image of this assembloid after transecting the neurite bridge (red dashed line). (l) Raster plots presenting neural activity associated with transection and recovery of a neurite bridge in an assembloid [52••].

Initial work demonstrates methods for using such frameworks for electrophysiological studies of neural injury and recovery using a pair of spheroids (Figure 3j–l) [52••]. Large arrays of such 3D frameworks can be formed in a parallel fashion, with a range of geometries, layouts, and functional purposes.

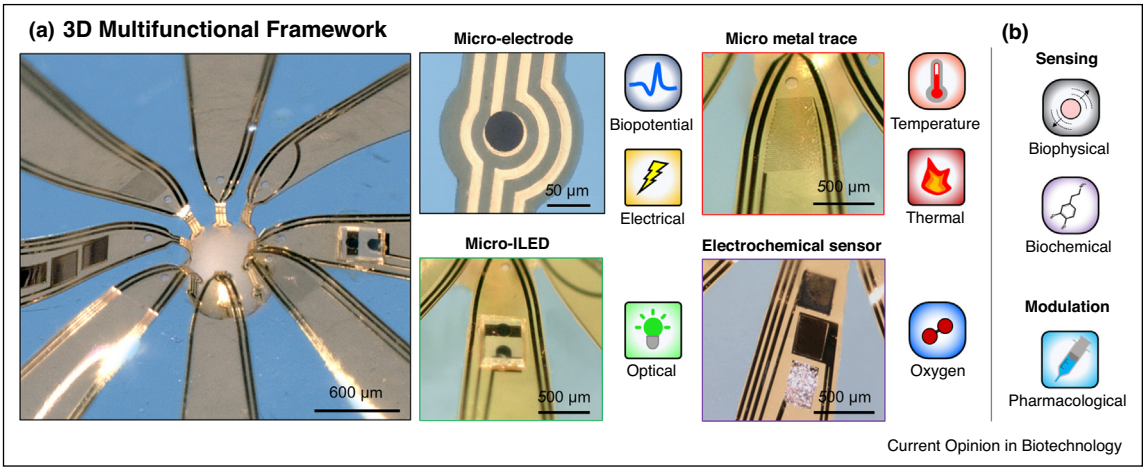
Interface technologies with multimodal function in sensing and modulation

Routes to 3D interfaces that begin with 2D structures are, in many cases, naturally compatible with a broad range of conventional planar microsystems technologies with function not only in multimodal sensing but also in various forms of stimulation and neuromodulation. Such capabilities have broad utility in the study of 3D tissue systems, with consequences that may lead to insights into fundamental questions in biology as well as practical issues in implantable devices. Functions in biopotential, biochemical, and biophysical sensing as well as electrical, optical, pharmacological, thermal, and mechanical modulation are of interest. A recent example in neuromodulation exploits 3D electronic scaffolds embedded in an engineered extracellular matrix (ECM), where the devices generate electrically evoked responses and record their propagation [26,53]. Another focuses on pharmacological stimulation through release from electroactive polymers [26,28•]. Other studies describe expanded modes of operation in 3D frameworks formed by mechanically guided assembly, in which integrated optoelectronic devices optogenetically stimulate activity in genetically modified cortical spheroids and independently controlled thermal actuators induce local thermal stresses. In both cases, microelectrode arrays across the 3D culture quantify electrophysiological network phenotypes (Figure 4a) [52••]. Additional classes of 3D frameworks include biophysical strain sensors for monitoring contractile activity associated with engineered muscle tissues [54]. Biochemical sensors specific for neurotransmitters such as dopamine and glutamate of 3D cultures represent additional possibilities demonstrated in feasibility experiments (Figure 4b).

Conclusion and outlook

The development of technologies that enable direct, physical coupling onto the surfaces and throughout the volumes of 3D biological tissues represents a frontier area for research in bioengineering. A key goal is to realize capabilities in sensing and modulation that qualitatively extend beyond those supported by traditional methods based on patch clamp techniques [16,17], penetrating probes [4,18], and 2D MEAs [19,20••]. Successful outcomes exploiting advances in materials engineering and 3D assembly methods could yield powerful biotechnology platforms for fundamental investigations into the mysteries of living systems, particularly those of relevance to the human body. Some of the most compelling

Figure 4



3D frameworks with multimodal function in sensing and modulation.
(a) Optical micrograph of a 3D multifunctional framework designed as an interface to an isolated cortical spheroid (white) (left), with magnified views of various components: microelectrodes for biopotential sensing and electrical stimulation (upper left), serpentine traces for temperature sensing and thermal modulation (upper right), microscale light emitting diode for optogenetic stimulation (bottom left) and three-electrode system as an electrochemical sensor (bottom right) [52••]. **(b)** Schematic depiction of additional possibilities in bio-interfaces.

opportunities are in studies of miniature organ-like systems that replicate essential features of natural biology using advanced methods in stem cell science and tissue engineering. Interfaces to neural organoids are of particular interest for non-destructive and long-term studies of the development of the brain, as well as the evolution and origins of aberrant behaviors and disease states, including those performed on an individual basis using stem cells harvested directly from the patient.

Current work on 3D interfaces falls broadly into two categories, each with pros and cons. The first involves growth of tissues in and around pre-fabricated mesh-type electronic scaffolds (i.e. tissue-embedding 3D electronic scaffold). An appealing feature of this approach is that it yields distributed interfaces throughout volumetric spaces, allowing monitoring of intracellular information. Uncertainties are in the potential for adverse effects on the growing biological system. Several impressive examples of this scheme appear in recently published work, mainly conducted with cardiac organoids and cell cultures, with an emphasis on electrical and simple pharmacological functions [26,28•,30••,46]. In addition to compliant mesh and folded electronic platforms, open 3D architectures offer significant promise in functional and geometrical engineering options. The second category of 3D interfaces (i.e. tissue-surrounding 3D electronic frameworks) exploits thin, deformable frameworks that engage across the 3D surfaces of fully formed biological tissues allowing for monitoring of superficial electrophysiology, excluding intracellular information [46,47•,52••]. These platforms can open and close on demand, to enclose organoids or small collections of cells, without adverse perturbation. Wide ranging components for optical, electrical, chemical, and thermal actuation and sensing can be delivered to soft biological surfaces in this way.

Progress in these areas could expand capabilities in biological and medical research, via stable, precision multimodal engineering interfaces to complex, evolving biosystems. Additional opportunities are in bio-hybrid robotic systems, where these same capabilities can be used to control and command, rather than study, small-scale biological tissues, with potential applications in biomedical and medical technologies of the future.

Conflict of interest statement

Nothing declared.

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