Macroporous nanowire nanoelectronic scaffolds for synthetic tissues

Bozhi Tian\textsuperscript{1,2,3}, Jia Liu\textsuperscript{4}, Tal Dvir\textsuperscript{2,4,4}, Lihua Jin\textsuperscript{5}, Jonathan H. Tsui\textsuperscript{2}, Quan Qing\textsuperscript{1}, Zhigang Suo\textsuperscript{5}, Robert Langer\textsuperscript{3,4}, Daniel S. Kohane\textsuperscript{2} and Charles M. Lieber\textsuperscript{1,5}\textsuperscript{*}

The development of three-dimensional (3D) synthetic biomaterials as structural and bioactive scaffolds is central to fields ranging from cellular biophysics to regenerative medicine. As of yet, these scaffolds cannot electrically probe the physicochemical and biological micro-environments throughout their 3D and macroporous interior, although this capability could have a marked impact in both electronics and biomaterials. Here, we address this challenge using macroporous, flexible and free-standing nanowire nanoelectronic scaffolds (nanoES), and their hybrids with synthetic or natural biomaterials. 3D macroporous nanoES mimic the structure of natural tissue scaffolds, and they were formed by self-organization of coplanar reticular networks with built-in strain and by manipulation of 2D mesh matrices. NanoES exhibited robust electronic properties and have been used alone or combined with other biomaterials as biocompatible extracellular scaffolds for 3D culture of neurons, cardiomyocytes and smooth muscle cells. Furthermore, we show the integrated sensory capability of the nanoES by real-time monitoring of the local electrical activity within 3D nanoES/cardiomyocyte constructs, the response of 3D-nanoES-based neural and cardiac tissue models to drugs, and distinct pH changes inside and outside tubular vascular smooth muscle constructs.

The design and functionalization of porous materials have been actively pursued to enable new material properties and applications\textsuperscript{1,2,3}. In particular, the development of synthetic 3D macroporous biomaterials as extracellular matrices (ECMs) represents a key area because functionalized 3D biomaterials allow for studies of cell/tissue development in the presence of spatiotemporal biochemical stimuli\textsuperscript{3,4}, and the understanding of the pharmacological response of cells within synthetic tissues is expected to provide a more robust link to in vivo disease treatment than that from 2D cell cultures\textsuperscript{6,7,8}. Advancing further such biomaterials requires capabilities for monitoring cells throughout the 3D micro-environment\textsuperscript{6,8}. Although electrical sensors are attractive tools, it has not been possible to integrate such elements with porous 3D scaffolds for localized real-time monitoring of cellular activities and physicochemical change; such capability could lead to new lab-on-a-chip pharmacological platforms\textsuperscript{9,10} and hybrid 3D electronics–tissue materials for synthetic biology\textsuperscript{11,12}.

Recently, there have been several reports describing the coupling of electronics and tissues using cellular flexible and/or stretchable planar devices\textsuperscript{13–17} that conform to natural tissue surfaces. These planar devices have been used to probe electrical activities near surfaces of the heart\textsuperscript{15–17}, brain\textsuperscript{16} and skin\textsuperscript{17}. So far, seamless 3D integration of electronics with biomaterials and synthetic tissues has not been achieved. Key points that must be addressed to achieve this goal include: the electronic structures must be macroporous, not planar, to enable 3D interpretation with biomaterials; the electronic network should have nanometre to micrometre scale features comparable to biomaterial scaffolds; and the electronic network must have 3D interconnectivity and mechanical properties similar to biomaterials.

Here we introduce a conceptually new approach that meets this challenge by integrating nanoelectronics throughout biomaterials and synthetic tissues in three dimensions using macroporous nanoelectronic scaffolds. We use silicon nanowire field-effect transistor (FET)-based nanoelectronic biomaterials, given their capability for recording both extracellular and intracellular signals with subcellular resolution\textsuperscript{18–21}. FET detectors respond to variations in potential at the surface of the transistor channel region, and they are typically called active detectors\textsuperscript{21}. Metal–electrode\textsuperscript{22,23} or carbon nanotube/nanofibre\textsuperscript{24,25}-based passive detectors are not considered in our work because impedance limitations (that is, signal/noise and temporal resolution degrade as the area of the metal or carbon electrodes is decreased) make it difficult to reduce the size of individual electrodes to the subcellular level\textsuperscript{21–23}, a size regime necessary to achieve a non-invasive 3D interface of electronics with cells in tissue.

Our approach (Fig.1) involved stepwise incorporation of biomimetic and biological elements into nanoelectronic networks across nanometre to centimetre size scales. First, chemically synthesized kinked\textsuperscript{18} or uniform silicon nanowires were deposited either randomly or in regular patterns for single-nanowire FETs—the nanoelectronic sensor elements of the hybrid biomaterials (step A, Fig. 1). Second, individual nanowire FET devices were lithographically patterned and integrated into free-standing macroporous scaffolds (step B, Fig. 1), the nanoES. The nanoES were designed to mimic ECM structures, and specifically, to be 3D, to have nanometre to micrometre features with high (>99%) porosity

\textsuperscript{1}Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, USA, \textsuperscript{2}Department of Anesthesiology, Division of Critical Care Medicine, Children’s Hospital Boston, Harvard Medical School, Boston, Massachusetts 02115, USA, \textsuperscript{3}David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA, \textsuperscript{4}Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA, \textsuperscript{5}School of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts 02138, USA. \textsuperscript{*}These authors contributed equally to this work. *e-mail: Daniel.Kohane@childrens.harvard.edu; cml@cmllnr.is.harvard.edu.
network of 3D features that mimic the size scale and morphology of submicron ECM features, such as the fibrous meshwork of brain ECM (ref. 26). Open mesh nanoES were made by photolithography with a regular structure, similar to the ECM of the ventricular myocardium.27,28 3D scaffolds were then realized in a straightforward manner by directed mesh manipulation. The planar design and initial fabrication of these 3D nanoES use existing capabilities developed for conventional planar nanoelectronics, and could enable integration of additional device components (for example, memories and logic gates)29,30 and substantial increases in device number and overall scaffold size.

The 2D structure of the reticular scaffold was designed so that metal interconnects were stressed.31 Removal of the sacrificial layer prompted self-organization into three dimensions. Reconstructed 3D confocal fluorescence images of typical reticular scaffolds viewed along the y and x axes (Fig. 2b and II respectively) showed that the framework was 3D with a highly curvilinear and interconnected structure. The porosity (calculated from the initial planar device design and the final 3D construct volume) was >99.8%, comparable to that of hydrogel biomaterials.32,33 Nanowire FET devices (Fig. 2bII) within the scaffold spanned separations of 7.3–324 µm in three dimensions (Supplementary Fig. S3), and the reticular scaffold heights were less than ~300 µm for our present fabrication conditions. Devices can be made closer together (for example, < 0.5 µm) by depositing denser nanowires on the substrate to improve the spatial resolution of nanoelectronic sensors; the span of device separations and scaffold heights can be increased substantially using larger field lithography (see below).

Scanning electron microscopy (SEM) of the reticular nanoES (Fig. 2c) revealed kinked nanowires (about 80 nm diameter), and metallic interconnects (about 0.7 µm width) contained within the SU-8 backbone (about 1 µm width). The feature sizes are comparable to those of synthetic and natural ECMs (refs 3, 8), and are several orders of magnitude smaller than those for electronic structures penetrating tissue in three dimensions. The performance of devices was evaluated through water-gate measurements for the nanowire FET elements in the 3D scaffolds in aqueous medium (Supplementary Information). The results show device yields (~80%), conductances (1.52 ± 0.61 µS; mean ± s.d.) and sensitivities (8.07 ± 2.92 µS V⁻¹) comparable to measurements from planar devices using similar nanowires.

3D mesh nanoES were realized by folding and rolling free-standing device arrays. Mesh structures (Fig. 2aII) were fabricated such that the nanoES maintained an approximately planar configuration following relief from the substrate. A typical 3.5 cm × 1.5 cm × ~ 2 µm mesh nanoES, was approximately planar with 60 mesh nanowire FET devices in a regular array with a 2D open porosity of 75% (Fig. 2d). This mesh porosity is comparable to that of a honeycomb-like synthetic ECM engineered for cardiac tissue culture.34 In addition, the nanowires (Fig. 2dI), metal interconnects (Fig. 2dII) and SU-8 structural elements (Fig. 2dIII) had an areal mass density of <60 µg cm⁻², the lowest value reported so far for flexible electronics, which reflects our macroporous architecture. The mesh nanoES was flexible and can be manually rolled into tubular constructs with inner diameters at least as small as 1.5 mm (Fig. 2e), and folded. Macroporous structures of the open mesh nanoES were formed either by loosely stacking adjacent mesh layers (Fig. 2f) or by shaping it with other biomaterials (Fig. 4 and Supplementary Information). These capabilities were consistent with the estimated ultralow effective bending stiffness (Supplementary Information), which was tuned between 0.006 and 1.3 nN m⁻¹ for this mesh and is comparable to recent planar epithelial membranes.35,36

The electrical transport characteristics of the mesh nanoES were evaluated in phosphate buffered saline. The typical device yield is 90–97%, with average device conductance ~3 µS and sensitivity.
explained by the low estimated strains of metal (<0.005%) and SU-8 (<0.27%) in this tubular construct (Supplementary Information),

The stable performance during rolling can be
during the rolling process showed a
difference in conductance and sensitivity of 14 nanowire FETs evenly distributed throughout a fully rolled-up mesh device. Upper panel, schematic of the nanowire FET position (yellow dot) during the rolling process; 0–6 denote the number of

g) Histograms of nanowire FET conductance and sensitivity in one typical mesh nanoES. The conductance and sensitivity were measured in the water-gate

e) Photograph of a partially rolled-up mesh device. Scale bar, 5 mm. f) SEM image of a loosely packed mesh nanoES, showing the macroporous structure. Scale bar, 100 µm.

g, h) Relative change in conductance and sensitivity of 14 nanowire FETs evenly distributed throughout a fully rolled-up mesh device. Upper panel, schematic of the nanowire FET position (yellow dot) during the rolling process; 0–6 denote the number of

9% versus the unrolled state, demonstrating

0.17 µS conductance change change (∆G) or <2.3% total change for 6 revolutions. Device sensitivity (S) remained stable with a maximum change (∆S) of 0.031 µS V⁻¹, or 1.5% variation. The stable performance during rolling can be

explained by the low estimated strains of metal (<0.005%) and SU-8 (<0.27%) in this tubular construct (Supplementary Information),

and showed that the properties were approximately independent of

location. Furthermore, 14 devices evenly distributed on six layers of a rolled-up scaffold (Fig. 2i) showed maximum differences of

∆G = 6.8 and ∆S = 6.9% versus the unrolled state, demonstrating device robustness. Repetitive rolling and relaxation to the flat state did not degrade the nanowire FET performance. These findings suggest the potential for reliable sensing/recording of dynamic and
deformable systems.

Figure 2 | Macroporous and flexible nanowire nanoES. a, Device fabrication schematics. (I) Reticular nanowire FET devices. (II) Mesh nanowire FET devices. Light blue: silicon oxide substrates; blue: nickel sacrificial layers; green: nanoES; yellow dots: individual nanowire FETs. b, 3D reconstructed confocal fluorescence micrographs of reticular nanoES viewed along the y (I) and x (II) axes. The scaffold was labelled with rhodamine 6G. The overall size of the structure, x-y-z = 300–400–200 µm. Solid and dashed open magenta squares indicate two nanowire FET devices located on different planes along the x axis. Scale bars, 20 µm. c, SEM image of a single-kinked-nanowire FET within a reticular scaffold, showing (1) kinked nanowires, (2) metallic interconnects (dashed magenta lines) and (3) the SU-8 backbone. Scale bar, 2 µm. d, Photograph of a mesh device, showing (1) nanowires, (2) metal interconnects and (3) SU-8 structural elements. The circle indicates the position of a single-nanowire FET. Scale bar, 2 mm. e, Photograph of a partially rolled-up mesh device. Scale bar, 5 mm. f, SEM image of a loosely packed mesh nanoES, showing the macroporous structure. Scale bar, 100 µm.

Water-gate sensitivity and conductance of a nanowire FET device during the

rolling process in a mesh device. Upper panel, schematic of the nanowire FET position (yellow dot) during the rolling process; 0–6 denote the number of

turns. I, Relative change in conductance and sensitivity of 14 nanowire FETs evenly distributed throughout a fully rolled-up mesh device. Upper panel, schematic of the nanowire FET position (yellow dots). In h,i the thicknesses of the tubular structures have been exaggerated for schematic clarity.
We have carried out simulations of a subunit of the self-organizing reticular structure (Fig. 3a–c). Measurements of bending for the corresponding experimental structures (Fig. 3c, open red squares) are consistent with the simulation (Fig. 3c). Additionally, changes in structural parameters (for example, the total length of the subunit and thicknesses of SU-8 or metals) yield predictable changes in the bending angle of the subunit (Supplementary Fig. S4). In this way, ordered 3D nanowire FET arrays can be designed and fabricated using reticular- or mesh-like structures that incorporate multi-layer metal interconnects with built-in stress to self-organize (roll-up) the scaffold (Supplementary Fig. S4). Finally, we have designed reticular domains in mesh-like structures (Fig. 3d). Images of reticular domains (Fig. 3e,f) show that regular nanowire FET devices with distinct device positions can be realized by varying the structural parameters of individual elements. Overall, this approach yields hierarchical 3D nanoES with submicrometre to micrometre scale control in reticular domains and millimetre to centimetre scale in the mesh matrix by folding or rolling as shown above (Fig. 2).

The reticular and mesh nanoES were also merged with conventional macroporous biomaterials. Specifically, gel casting, lyophilization and electrospinning were used to deposit and construct macroporous collagen (Fig. 4a), alginate (Fig. 4b) and poly(lactic-co-glycolic acid) (PLGA; Fig. 4c), respectively, around nanoES. A confocal fluorescence micrograph of a hybrid reticular nanoES/collagen scaffold (Fig. 4a) shows clearly that the collagen nanofibres (green) are fully entangled with the nanoES, with no evidence of phase separation. SEM images of the open mesh nanoES/alginate hybrid scaffold produced by lyophilization (Fig. 4b) show that the flexible nanoES mesh is intimately anchored to the alginate framework, which has a similar pore structure as the pure alginate scaffold prepared under similar conditions. Finally, optical micrographs of a multilayered mesh nanoES/PLGA scaffold (Fig. 4c), which was prepared by electrospinning PLGA fibres on both sides of the nanoES and subsequent folding of the hybrid structure, highlight the intimate contact between nanoES mesh and PLGA fibres. The hybrid nanoES/biomaterial 3D scaffolds retain the original nanowire FET device characteristics. For example, measurements in 1 x phosphate buffered saline solution showed that ΔG/G and ΔS/S were less than ±9% for the mesh nanoES/PLGA composite versus bare nanoES. Hybrid nanoES were stable under cell culture conditions. For example, nanowire FET devices in the hybrid reticular nanoES/Matrigel scaffold in neuron culture media (Fig. 4d) had ΔS/S < ±11% over a nine-week period, suggesting a capability for long-term culture and monitoring with the nanoES. These results show that nanoES can be combined with conventional biomaterials to produce hybrid scaffolds that now provide nanoscale electrical sensory components distributed in three dimensions.
The hybrid nanoES were evaluated in 3D culture for several cell types. Embryonic rat hippocampal neurons were cultured in the reticular nanoES/Matrigel for 7–21 days (Supplementary Fig. S5). Reconstructed 3D confocal micrographs from a two-week culture (Fig. 5a,b and Supplementary Fig. S6) showed neurons with a high density of spatially interconnected neurites that penetrated the reticular nanoES (Fig. 5a), often passing through the ring structures supporting individual nanowire FETs (Fig. 5b and Supplementary Fig. S6). Notably, the widths of the scaffold elements (passivated metal interconnects and structural ribs) were similar to those of the neurite projections, demonstrating the combination of electronics with biological systems at an unprecedented similarity in scale. 3D nanoelectronic cardiac culture was achieved from hybrid mesh nanoES/PLGA scaffolds (Supplementary Figs S7–S9). Confocal fluorescence microscopy of a cardiac 3D culture (Fig. 5c) revealed a high density of cardiomyocytes in close contact with nanoES components. Epifluorescence micrographs of cardiac cells on the surface of the nanoES cardiac patch showed striations characteristic of cardiac tissue (Fig. 5d and Supplementary Figs S8 and S9). In addition, the in vitro cytotoxicity of nanoES in 3D neural and cardiac culture was evaluated (Fig. 5e,f). Differences between hippocampal neurons in reticular nanoES/Matrigel versus Matrigel over 21 days, assessed with a standard LIVE/DEAD cell assay (Fig. 5e), and between cardiac cells in hybrid mesh nanoES/Matrigel and Matrigel/PLGA from 2 to 12 days, measured with a metabolic activity assay (Fig. 5f), were minimal. These studies show that on the 2–3 week timescale, the nanoES component of the scaffolds has little effect on the cell viability, and thus can be exploited for a number of in vitro studies, including drug screening assays with these synthetic neural and cardiac tissues.
Figure 5 | 3D cell culture and electrical sensing in nanoES. a, b, 3D reconstructed confocal images of rat hippocampal neurons after a two-week culture in Matrigel on reticular nanoES. Red (Alexa Fluor 546): neuronal β-tubulin; yellow (rhodamine 6G): epoxy ribbons. The metal interconnects are false-coloured in blue, and are imaged in the reflected light mode. The white arrow highlights a neurite passing through a ring-like structure supporting a NFWET. Dimensions in a: x: 317 µm; y: 317 µm; z: 100 µm; in b: x: 127 µm; y: 127 µm; z: 68 µm. c, Confocal fluorescence micrographs of a synthetic cardiac patch. (II and III), Zoomed-in view of the upper and lower dashed regions in I, showing metal interconnects, the SU-8 scaffold (arrows in II) and electrospun PLGA fibres (arrows in III). Scale bar, 40 µm. d, Epifluorescence micrograph of the surface of the cardiac patch. Green (Alexa Fluor 488): α-actin; blue (Hoechst 34580): cell nuclei. The position of the source-drain electrodes is outlined with dashed lines. Scale bar, 40 µm. e, Percentage of viable hippocampal neurons cultured in nanoES/Matrigel versus Matrigel. Cell viability was evaluated with a LIVE/DEAD cytotoxicity assay. Cells were counted from 3D reconstructed confocal fluorescence micrographs. n = 6; data are means ±s.d. Differences between groups were very small although statistically significant (p < 0.05). f, MTS cytotoxicity assay of cardiomyocytes evaluated using the MTS assay. n = 6; data are means ±s.d. Differences between groups were very small although statistically significant (p < 0.05). g, Conductance versus time traces recorded from a single-nanowire FET before (black) and after (blue) applying noradrenaline. h, Multiplex electrical recording of extracellular field potentials from four nanowire FETs in a mesh nanoES. I. Data are conductance versus time traces of a single spike recorded at each nanowire FET.

1. of a coherently beating cardiac patch, with submillisecond time resolution. Our current device design yields relatively sparse device distribution with 60 devices over an area of about 3.5 × 1.5 cm². Increases in nanowire FET density, the use of cross-bar circuits and implementing multiplexing/demultiplexing for addressing could allow the nanoES scaffolds to map cardiac and other synthetic
Figure 6 | Synthetic vascular construct enabled for sensing. 

a, Schematic of the synthesis of smooth muscle nanoES. The upper panels are side views, and the lower ones are either top views (I and II) or a zoom-in view (III). Grey: mesh nanoES; blue fibres: collagenous matrix secreted by HASMCs; yellow dots: nanowire FETs; pink: HASMCs. 

b, (I) Photograph of a single HASMC sheet cultured with sodium L-ascorbate on a nanoES. (II) Zoomed-in view of the dashed area in I, showing metallic interconnects macroscopically integrated with cellular sheet. 

c, Photograph of the vascular construct after rolling into a tube and maturation in a culture chamber for three weeks. 

d (I) Micro-computed tomograph of a tubular construct segment. (II) Zoomed-in view of the area outlined in I. The arrows mark the individual nanowire FET-containing layers of the rolled construct. Scale bar, 1 mm. 

(e) Haematoxylin–eosin- (I) and Masson-Trichrome- (II; collagen is blue) stained sections (⇠6 µm thick) cut perpendicular to the tube axis; lumen regions are labelled. The arrows mark the positions of SU-8 ribbons of the nanoES. Scale bars, 50 µm. 

f, Changes in conductance over time for two nanowire FET devices located in the outermost (red) and innermost (blue) layers. The inset shows a schematic of the experimental set-up. Outer tubing delivered bathing solutions with varying pH (red dashed lines and arrows); inner tubing delivered solutions with fixed pH (blue dashed lines and arrows).
tissue electrical activities over the entire constructs at high density in three dimensions.

We have also extended our approach towards the development of artificial tissue with embedded nanoelectronic sensory capabilities. Specifically, vascular nanoeOS constructs were prepared by processes analogous to those used for tissue-engineered autologous blood vessels except for the addition of the nanoeOS (Fig. 6a and Supplementary Fig. S12). Human aortic smooth muscle cells (HASMCs) were cultured on 2D mesh nanoeOS with sodium ascorbate to promote the deposition of natural ECM. The hybrid nanoeOS/HASMC sheets (Fig. 6b) were rolled into multi-layer 3D tubular structures and matured (Supplementary Information) without macroscopic delamination or desquamation (Fig. 6c), and analyses showed that the cells expressed smooth muscle α-actin (Supplementary Fig. S13), the key contractile protein in smooth muscle.

The distribution of nanoeOS in the tubular construct was visualized by micro-computed tomography (μCT). A projection of the reconstructed 3D μCT data (Fig. 6d) revealed regularly spaced metal interconnects with at least four revolutions (arrows, Fig. 6dII), consistent with the nanowire FET mesh and tissue rolling. Analyses of haematoxylin–eosin-stained sections (Fig. 6e) revealed smooth muscle tissue ~200 μm thick, with elongated cells and collagenous nanofibres, and embedded SU-8 ribbons from the nanoeOS (Fig. 6e). These findings confirm the 3D integration of nanowire FET nanoelectronics with healthy smooth muscle.

The potential of this vascular construct to function as a biomedical device was demonstrated by 3D pH sensing (Fig. 6f, inset). As the extracellular pH was varied stepwise with luminal pH fixed, simultaneous recordings from nanowire FETs in the outermost layer showed stepwise conductance decreases with a sensitivity of ~32 mV per unit of pH. nanowire FETs in the innermost layer (closest to luminal) showed minor baseline fluctuations. This ability to sense extracellular pH changes makes possible the detection of inflammation, ischaemia, tumour micro-environments or other forms of metabolic acidosis due to overproduction of organic acids or impaired renal acidification, although we stress that the implantation of these nanoeOS-based vascular and other nanoeOS-embedded constructs for in vivo studies will require substantial future work.

The nanoeOS concept and implementations described here represent a new direction in merging nanoelectronics with biological systems because we have demonstrated a 3D macroporous material/device platform that is distinct from either engineered tissue or flexible electronics. Looking forward, there are several areas to develop. Cell interactions with nanoeOS could be tuned by modification of the nanoeOS with growth determinants. In addition, the elements in the nanoeOS could be expanded to incorporate nanoscale stimulators and stretchable designs to provide electrical and mechanical stimulation to enhance cell culture.

Methods

Kinked and uniform silicon nanowires were synthesized by the nanocluster-catalysed methods described previously. Devices were fabricated on silicon substrates (Novo Electronic Materials, n-type 0.005 V cm) with 600 nm SiO2 layer of SU-8 photoresist (2000.5, MicroChem, Newton) was deposited over silicon substrates (Nova Electronic Materials, n-type 0.005 V cm) with 600 nm SiO2 nanocluster-catalysed methods described previously for at least 20 min. The 3D nanoES/alginate scaffolds were prepared from the individual steps. A similar approach was used in the fabrication of the mesh nanoeOS and the reticular nanoeOS, including the interconnected kinked nanowire FET devices, were released from the substrate by etching of the nickel layer (Nickel Etchant TFR, Transene Company, Danvers) for 60–120 min at 25 °C. Last, the free-standing nanoeOS were defined using a critical point dryer (Autosamdri 815 Series A, Tousimis) and stored in the dry state before use in tissue culture. Each EBL step will take 10 min–2 h depending on the writing speed and area, feature size and complexity, and electron beam dosage (for example, the typical area dosages for SU-8 and poly(methyl methacrylate) EBL are 3–8 μC cm–2 and 500–1,000 μC cm–2 at 25 kV, respectively). The entire fabrication took 2–5 days depending on the duration of the individual steps. A similar approach was used in the fabrication of the mesh nanoeOS and the reticular nanoeOS, except that photolithography was used and the entire process took 2–3 days.

NanoES/collagen/Matrigel hybrid matrices were made by casting 50–2,000 μl collagen or Matrigel solution onto the edge of (reticular nanoeOS) or directly above (mesh nanoeOS) the nanoeOS scaffolds, and at ~4 °C. The solutions were allowed to form gels around nanoeOS under conditions of 37 °C and 5% CO2 for at least 20 min. The 3D nanoeOS/alginate scaffolds were prepared from pharmaceutical-grade alginate (Prostal FS050, FMC Biopolymers) by calcium gluconate crosslinking and subsequent lysophosphatidyl ethanolamine to produce a sponge-like scaffold (5–15 mm x 2–10 mm, d x h). To prepare NanoES/PLGA hybrid scaffolds, a sheet of PLLA fibres with diameters of ~1–3 μm was deposited on both sides of the mesh nanoeOS. The hybrid scaffold can be folded to increase the thickness.

Embryonic Sprague Dawley rat hippocampal cells, neonatal Sprague Dawley rat cardiomyocytes and HASMCs were cultured in nanoeOS using established protocols (Supplementary Information). Optical micrographs of immunohistochemically and histologically stained samples were recorded using either Olympus Fluoview FV1000 or Olympus FXX100 systems. The structures of nanoeOS were characterized with Zeiss Ultra55/Supra55VP field-emission SEMs or the HMXSTK multi-chip optical microscopy system (model: HMXXST225, X-Tek). The in vitro cytotoxicity of nanoeOS was evaluated using the standard LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes, Invitrogen) and the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI). Cardiomyocyte recordings were carried out in Tyrode solution with a 100 mV d.c. source for the nanowire FETs. The current was amplified with a multi-channel preamplifier, filtered with a 3 kHz low-pass filter (CyberAmp 380) and digitized at a 50 kHz sampling rate (Axon Digi1440A). In extravascular pH sensing experiments, a single polydimethylsiloxane microfluidic chamber was used to deliver two streams of phosphate buffer solution, where inner and outer tubings were used to deliver solutions with fixed and varied pH, respectively. The electrical measurements were conducted using a lock-in amplifier with a modulation frequency of 79 and 93 Hz, a time constant of 30 ms and an amplitude of 30 mV; the d.c. source–drain potential was zero. Ag/AgCl reference electrodes were used in all recording and calibration experiments. The calibrated potentials (in millivolts) recorded from nanowire FETs were obtained as the ratios of inner and outer streaming solutions over the entire constructs at high density in three dimensions.

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Fig. 6C for 2 and 4 min, respectively; then an isopropanol solution of

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**Author contributions**

B.T., J.L., D.S.K. and C.M.L. designed the experiments. B.T. and J.L. performed experiments. T.D., J.T. and Q.Q. assisted in the initial stage of the project. L.J. and Z.S. performed calculations and simulations. B.T., J.L., D.S.K. and C.M.L. wrote the paper. All authors discussed the results and commented on the manuscript.

**Additional information**

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to D.S.K. or C.M.L.

**Competing financial interests**

The authors declare no competing financial interests.
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