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Engineered hybrid cardiac patches with multifunctional electronics for online monitoring and regulation of tissue function

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Abstract

In cardiac tissue engineering approaches to treat myocardial infarction, cardiac cells are seeded within three-dimensional porous scaffolds to create functional cardiac patches. However, current cardiac patches do not allow for online monitoring and reporting of engineered-tissue performance, and do not interfere to deliver signals for patch activation or to enable its integration with the host. Here, we report an engineered cardiac patch that integrates cardiac cells with flexible, free-standing electronics and a 3D nanocomposite scaffold. The patch exhibited robust electronic properties, enabling the recording of cellular electrical activities and the on-demand provision of electrical stimulation for synchronizing cell contraction. We also show that electroactive polymers containing biological factors can be deposited on designated electrodes to release drugs in the patch microenvironment on-demand. We expect that the integration of complex electronics within cardiac patches will eventually provide therapeutic control and regulation of cardiac function.

Cardiac patches for treating heart disease are created by seeding contracting cells in or onto 3D biomaterials prior to transplantation. These materials serve as temporary scaffolds, supporting the cells and promoting their reorganization into functional tissues. Following implantation and full integration within the host, the scaffold degrades, leaving a functional

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Author contribution

R.F. and T.D. conceived the idea and designed the experiments. R.F. performed all experiments. L.E. assisted in microfabrication and characterization of the electronics. S.F. and A.S. performed cell culture work. M.M. and I.G. assisted in drug release experiments. Y.S.D. analyzed data. R.F. and T.D. wrote the manuscript. The study was directed by T.D.

cardiac patch on the defected organ¹. In recent years it has been recognized that effective organization of cells into cardiac tissues with morphological and physiological features resembling those *in vivo* requires a 3D scaffold that precisely mimics the structural, biochemical and mechanical properties of the natural heart's extracellular matrix (ECM). Thus, researchers initially focused on developing technological tools to recapitulate aspects of this specialized microenvironment^{2–7}. It was previously shown that engineered cardiac tissues exhibit elongated and aligned morphology, synchronous contraction, and anisotropic transfer of the electrical signal when grown on various scaffolds^{5,8–10}. Furthermore, cardiac patches have been used to significantly improve heart function after infarction^{11–14}. However, once the 3D cardiac patches have been engineered, *in vitro* assessment of their quality in terms of electrical activity without affecting their performance is limited. This situation might lead to implantation of cardiac patches with limited or no potential to regenerate the infarcted heart. The cells engineered within the 3D biomaterial may be electrophysiologically inactive, jeopardizing the efficacy of the treatment. More importantly, the ability to monitor the performance of these patches and control their function following implantation is completely lost.

Recently, Tian and colleagues pioneered an integrated sensory system based on a nanowire field-effect transistor array to monitor the local electrical activity within 3D cardiomyocyte constructs¹⁵. Despite this groundbreaking progress, several central needs remain unmet. For example, questions remain regarding how to utilize the received data, which provide information about cell function, for interfering and encouraging tissue assembly and ensuring proper function.

To overcome this challenge, we introduce a conceptually new approach, where a dedicated free-standing electronic network is built within an engineered tissue and used to collect data from its surroundings. When required, the electronics can be remotely manipulated to activate the growing tissue, by providing electrical stimulation, and/or by controlling the release of drugs within the 3D microenvironment to affect the engineered tissue or the host.

Our approach (Fig. 1) involved the fabrication of a complex, free-standing, porous electronic mesh with multiple electrodes designated for recording tissue function, for providing electrical stimulation and for spatially releasing biochemical factors to affect the engineered tissue or its surroundings. The electronic network was designed to be thin and porous (>99%)¹⁵ to have minimal interference with the engineered tissue, allowing tissue growth in-between the electrodes (detailed technical aspects of the electronics can be found in the Methods section). Electroactive polymers were deposited on designated electrodes to allow for efficient, on demand, controlled release of drugs. Then, a 3D biomaterial scaffold composed of a dense nanofiber network was integrated with the electronics and cardiac cells were seeded into the hybrid to complete the 3D cardiac tissue. Finally, the engineered tissue was folded to create a thick, stand-alone, microelectronic cardiac patch (microECP) comprising embedded elements for sensing, stimulation and regulation.

Nanowire-based field effect transistors allow for a very high signal to noise ratio and the option of intracellular recordings¹⁶. However, they may be limited in supplying current to their surroundings, and therefore may not be effective for actuating cells or triggering drug

release¹⁷. To overcome impedance limitations arising from small electrode size¹⁸ and to enable the supply of sufficient current, we designed our device to include larger gold electrodes. The electronic chip was fabricated on a nickel sacrificial layer, which was subsequently removed to yield completely free-standing, flexible electronics (Methods and Supplementary Figs S1). This will allow future *in vivo* work, when the microECP could be easily transplanted on the infarcted heart. To ensure exclusive electrical interaction at the end of the gold electrodes, they were deposited on a thin, patterned SU-8 substrate, onto which an additional thin SU-8 layer was deposited and patterned to provide passivation (Methods and Supplementary Fig. S2). The chip (Fig. 2a and Supplementary Fig. S3) included 32 gold electrodes (width= 10 μm and thickness= 200 nm) terminating with exposed square pads. Twenty eight pads were designated for recording and stimulation (50x50 μm^2 ; Fig. 2b), and four larger pads (150x150 μm^2) were designed to accommodate controlled release systems (Fig. 2c). A rough nanoscale layer of titanium nitride (TiN) was deposited on the gold pads to increase their surface area and thus improve cell adherence¹⁹ (Fig. 2d and e, and Supplementary Fig. S4 and S5). The electronic network was designed to be flexible to enable rolling or folding together with the biomaterial scaffold to create a thick 3D structure (Fig. 2f and g and Supplementary Fig. S6). Thus, distant electrodes within the electronics are re-organized in space to form a denser electrode network and enable 3D monitoring of tissue function. As the electronics were designed to be thin, and must withstand mechanical forces for long periods of time, such manipulation could result in degradation of the metal electrode network, compromising device performance. Therefore, we ensured that rolling or folding of the fabricated device did not alter electrode conductance. (Fig. 2h).

To complete the fabrication process of the chip and incorporate on-demand controlled release systems that can influence tissue growth and function, electroactive polymers were deposited on designated pads. Such polymers are ideal for designing efficient drug delivery systems, where an “on/off” drug release mechanism is required or for releasing the entire payload at once^{20,21}. Here, we chose to focus on two types of polymers; one can store and release positively charged proteins, and the other can release negatively charged small molecules (Fig. 3). For protein release, chondroitin 4-sulfate (CS) was cross-linked with ethylene glycol diglycidyl ether (EGDGE) and deposited on the designated electrodes. As a proof-of-concept, lysozyme was selected as a model protein and loaded into the tortuous pores of the hydrogel by equilibrium swelling²⁰. Owing to electrostatic interactions with the negatively charged polymeric backbone, the positively charged protein could be stored within the hydrogel matrix. Upon application of electrical stimulation the gel was protonated and microscopically shrunk²⁰, and the protein could be released into the hydrogel’s microenvironment or surroundings in a voltage-dependent manner (Fig. 3a, b). Alternating the electrical stimulation showed an on/off release profile of the protein from the devices into the cellular compartment (Fig. 3c). Furthermore, long-term repetitive applications of electrical stimuli resulted in protein release for at least 28 days (Supplementary Fig. S7), without affecting gel stability (Supplementary Fig. S8).

Next, to evaluate the ability of the system to release physiologically-relevant factors we focused on stromal cell-derived factor-1 (SDF-1), a stem cell chemoattractant^{22,23}. The cytokine could be loaded and stored within the hydrogel matrix, as a function of its initial concentration in the solution (Supplementary Fig. S9) and released upon application of an

electric field (Fig. 3d). As SDF-1 has the ability to recruit bone marrow-derived stem cells and endothelial progenitor cells through strong interactions with CXCR4 receptors, the released cytokine promoted cell migration *in vitro* (Fig. 3e). *In vivo* release of SDF-1 from the patch may recruit stem cells into the engineered tissue, leading to enhanced vascularization²³. As SDF-1 is also known to activate ERK1/2 and Akt signalling pathways, which promote cardioprotection¹¹, its release into the cellular microenvironment may also have beneficial effects on the cardiac cells comprising the patch. Moreover, as in principle the system can store and release other positively charged growth factors, cell viability can be maintained by releasing insulin-like growth factor 1 (IGF-1). By releasing vascular endothelial growth factor (VEGF) the system may promote enhanced vascularization¹¹. Overall, these physiological processes can improve integration of the engineered tissue with the healthy part of the heart by prolonging cell survival until blood vessel networks can infiltrate into the porous patch to nourish it²⁴.

To store and release negatively charged drugs, polypyrrole (PPy), loaded with the model drug, dexamethasone (DEX), was deposited onto the electrodes by electropolymerization (Fig. 3f, g). As shown, the drug-loaded polymer covered the electrode designated for controlled release (Fig. 3g and Supplementary Fig. S10). Polymer volume could be adjusted by increasing the amount of charge inserted in the electropolymerization process (Supplementary Fig. S10), which leads to higher loading of the drug²⁵ for extended treatment periods. Similar to the release of the positively charged protein, the PPy released its cargo according to the degree of the applied stimulation (Fig. 3h). No change in electrode conductance could be observed following voltage application (Supplementary Fig. S11). As DEX is an anti-inflammatory drug²⁶, its release into the graft's surroundings after transplantation may reduce inflammation caused by the graft itself, or that occurring as a consequence of the pathology²⁷. In either case, DEX release may encourage better integration with the host tissue and improve the efficacy of the treatment²⁸. To evaluate the potential of the released DEX to decrease inflammation, macrophages, activated with interferon γ were added to the culture medium containing the released drug. As shown, the released DEX attenuated the activation of the macrophages as determined by their nitric oxide secretion²⁹ (Fig. 3i). As PPy is not limited to delivering DEX, other negatively-charged small molecules, such as glutamate, which has a protective effect on cardiac function during myocardial infarction,^{30,31} can be incorporated into the system to affect the cardiac cells within the patch.

Overall, the ability to remotely release drugs on demand within an engineered tissue through the built-in electronics, represents a significant improvement in tissue engineering, allowing better control over tissue growth or promoting better integration after transplantation.

Engineering a functional cardiac patch requires seeding cardiac cells within a 3D scaffold that mimics the natural ECM. This scaffold should encourage cell-matrix and cell-cell interactions and promote cell elongation, alignment and cardiac bundle formation³². Therefore, electrospinning was used to deposit nanocomposite polycaprolactone-gelatin fibers onto the electronics, creating a biomaterial-electronics hybrid (Fig. 4a). The fibers had an average diameter of 287 ± 67 nm, mimicking the endomysial fibers in the natural heart matrix (Supplementary Fig. S12). These fibers are a meshwork of fibrils with diameters of

tens to hundreds of nanometers, surrounding individual cardiomyocytes, connecting to their plasma membrane through integrins and forming interactions between the ECM and cytoskeleton proteins³³. The electrospun fiber scaffold had a porous structure to allow cell penetration to the recording/stimulation electrodes (Fig. 4b), and to enable drug diffusion from the electroactive polymers into the cellular microenvironment, while concomitantly preventing polymer delamination from the electrode (Fig. 4c). Next, cardiac cells (cardiomyocytes and fibroblasts), isolated from the left ventricles of neonatal rat hearts were seeded into the electronic scaffolds and the hybrid was folded to create a ~4 mm thick microECP (Fig. 4d and e). As the layers were not tightly packed, oxygen within the medium could easily diffuse to nourish the cells.

The potential of the hybrid system to support cardiac tissue assembly was assessed. Confocal fluorescence microscopy of the engineered tissue revealed that the cells were homogeneously distributed throughout the hybrid scaffold (Fig. 5a), interacted with the nanofibers and were in close contact with the electrodes (Figure 5b-d). Higher magnification of the cells revealed that the cardiomyocytes were elongated, with a high aspect ratio and massive striation (Fig. 5b), providing information on the maturation of the cells and formation of tissue with hallmarks of the native myocardium³⁴. In its folded state, distant electrodes were brought into close proximity to provide sensing from different layers of the engineered tissue (Fig. 5c). Then, the *in vitro* viability of the cardiac cells was evaluated over 7 days by measuring their metabolic activity. As shown, the electronics did not have any effect on the viability of the cardiac cells (Fig. 5e), suggesting that in addition to a therapeutic approach, the system can be exploited for *in vitro* studies, such as drug screening assays in a 3D microenvironment³⁵.

The ability of the system to detect electrical signals, produced by cardiomyocytes in various locations in the 3D scaffold was demonstrated (Fig 5f, and Supplementary Fig. S13). The recorded spikes were regularly spaced with a frequency of ~1-2 Hz and exhibited a shape and width consistent with cardiomyocyte extracellular signals³⁶. The ability of the system to detect changes in the frequency of electrical signals in response to the addition of norepinephrine (NE) was investigated. Recording from the same electrode before and after perturbation revealed a twofold increase in signal frequency following drug supplement to the culture (Fig. 5g). To validate the reliability of the recorded extracellular signals calcium imaging was performed simultaneously with microECP sensing (Fig. 5g). Measuring the conduction velocity between electrodes revealed a signal propagation of ~17cm/s in accordance with reported *in-vitro* measurements of engineered cardiac tissues (Supplementary Fig. S14)³⁷.

Another essential feature within engineered cardiac patches is the ability to remotely interfere with cell function and, when needed, to provide spatiotemporal electrical stimulation. Such stimulation will pace the seeded cells, activate the non-contracting cells and synchronize cell contraction throughout the patch. To demonstrate the ability of the electronics to improve patch function, a non-synchronously-contracting patch was selected, and contractions throughout the engineered tissue were assessed. Calcium transient imaging in 10, randomly-chosen locations within the microECP revealed that some of the areas of the tissue did not contract, and others contracted at different time points (Fig. 6a and b, and

Supplementary movie M1). This phenomenon is recognized in cardiac tissue engineering and may jeopardize the therapeutic success of this approach³⁸. Remotely interfering by applying acute electrical stimulation (3 V, 50 ms) at different frequencies (1 and 2 Hz) activated the cells throughout the patch and synchronized their contractions (Fig. 6b, and Supplementary movies 2 and 3). Furthermore, by applying electrical stimulation in the opposite direction of the observed electrical signal propagation, tissue contraction could be manipulated and reversed (Supplementary Movies 4 and 5, Fig. 6c and Supplementary Fig. S15). Detailed experiments revealed that these electrical manipulations did not affect electrode conductivity (Supplementary Fig. S16), scaffold integrity (Supplementary Fig. S17), or cell viability (Supplementary Fig. S18).

Overall, these results provide information on the ability of the built-in electronics to control cell and tissue function. In addition to synchronizing cardiac patch contraction, spatiotemporally controlling tissue activity may also assist in integrating the patch with the healthy part of the heart, creating a new paradigm for repairing damaged cardiac conduction systems.

Integrating multifunctional electronics with engineered cardiac patches, as described here, represents a new direction in tissue engineering, where living cells and tissues interact with electronics to improve tissue function. We have demonstrated that the microECP technology enables on-line monitoring of the engineered tissue function, and provides, for the first time, on-demand, remote interference to regulate tissue performance. This set-up combined multiple electrodes located in the 3D space of the cardiac patch, designated to record electrical activity of cells. By using a flexible basis for our device we have shown that data could be gathered from distant locations that were brought together within the mesh to enable monitoring of tissue activity in a 3D configuration. Collecting data from multiple electrodes will enable to build a more comprehensive picture of tissue function, and to pinpoint the occurrence, timing and location of a failure, if and when it arises. Then, according to physiological needs, the system can control the engineered tissue by providing electrical stimulation, or affecting the cells or the patch surroundings by releasing soluble biofactors.

Future devices will benefit from design features that extend their mechanical stability by using stretchable electronics fabrication techniques³⁹. Additional sensing capabilities such as pH, heat and mechanical stress may be useful as well to provide a more comprehensive report on the condition of the engineered tissue^{40–42}.

Looking forward, the technology can be used in the future to notify physicians of a patient's health condition and for subsequently remotely triggering regenerative processes. As cardiac performance will be recorded over time, physicians could follow heart regeneration in real-time, providing new means for disease management. Moreover, the ability to integrate a feedback loop into the system will generate self-regulating cardiac patches, where physician assistance may not even be required.

Methods

Fabrication of microelectronics

Mechanical grade silicon wafers (300 μm thick, University wafers, Boston, MA) were used as a substrate onto which a 20 nm thick nickel relief layer was deposited using a VST e-beam evaporator (VST, Petah Tikva, Israel). A layer of SU-8 photoresist (2 μm , 2002, MicroChem Corp. Newton, MA) was deposited by spin-coat over the entire wafer, patterned by photolithography into a mesh structure, and cured (190°C, 35 minutes). Metallization was performed by lift-off. Briefly, the wafer was coated with a layer of AZ-5214E resist (MicroChem Corp., Newton, MA), exposed and developed. A blanket layer of Cr/Au (10/200 nm) metals were sequentially deposited by evaporation. The photoresist was then dissolved in n-methyl pyrrolidone (Avantor, Center Valley, PA) leaving behind defined the gold electrodes and connectors. Subsequently, titanium nitride was deposited in the same manner. A passivation layer of SU-8 (5-20 μm , 3005-3025) was then deposited onto the wafer, defined by photolithography, and cured. The device was then released from the substrate by etching the bottom nickel layer in a solution of 21% nitric acid overnight. The devices were readily collected and rinsed in water prior to use.

Drug release

Controlled release of the biomolecules was performed as previously described^{20,25} (see Supplementary information).

Nanofiber scaffold fabrication

Nanofibers were fabricated by electrospinning as previously described⁴³. Briefly, 10% gelatin and 10% PCL (Sigma, St. Louis, MO) were separately dissolved in 2,2,2-trifluoroethanol (Acros Organics, Geel, Belgium) overnight at room temperature. The following day, the solutions were mixed in a ratio of 1: 1 and the polymer solution was delivered through a stainless steel 20G capillary at a feeding rate of 0.5 mL/h using a syringe pump (Harvard apparatus, Holliston, MA). A high voltage power supply (Glassman high voltage Inc., High Bridge, NJ) was used to apply a 10 kV potential difference between the capillary tip and the collector, positioned 10 cm beneath the tip. The metallized SU-8 devices were positioned inside a PBS bath covered in tin foil used as a collector. Fibers were deposited on both sides of the devices.

Cardiac cell isolation and seeding

Neonatal Sprague-Dawley rat ventricular cardiomyocytes were isolated and cultured in the microECP using established protocols⁴⁴ (Supplementary information).

Device operation

A high-resolution microelectrode array recording system (Multichannel Systems, Reutlingen, Germany) was used to characterize the electrophysiological properties of the microECP. Devices were designed to conform to the Multichannel systems multi electrode array ADPT-FM-32 adapter. Data was acquired by connecting the day 4 microECP to a ME64-FAI-MPA-System (Multichannel systems, Germany). Data was visualized using MC

Rack software (Multichannel systems, Germany) and acquired at a sample rate of 10 kHz. One hundred nM norepinephrine bitartrate (Sigma-Aldrich) was used to increase cardiomyocyte beating rate.

Stimulation was performed using a Multichannel systems ADPT-EcoFlexMEA36-STIM adapter and an STG-4002 stimulus generator (Multichannel systems). Pacing was performed by applying 1-3 V, 50 ms long pulses at 1-2 Hz. Calcium imaging was used to visualize signal propagation.

Calcium imaging

Briefly, calcium transients were evaluated as previously described. Constructs were incubated with 10 μ M fluo-4 AM (Invitrogen, Waltham, MA) and 0.1% Pluronic F-127 for 45 minutes at 37°C. Constructs were then washed in medium once and imaged using an inverted fluorescent microscope (Nikon Eclipse TI). Videos were acquired with a Hamamatsu Orcaflash 4.0 (Hamamatsu, Japan) at 100 frames/s using NIS element software. Data collected from 10 ROIs, chosen at random, was analyzed using ImageJ software (NIH). Fluorescence was normalized by dividing by the basal cell fluorescence and the first derivative was created for each sample.

Statistical analysis

Statistical analysis data are presented as means $\pm$ SD. Differences between samples were assessed by a Student's t-test. All analyses were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software). $p < 0.05$ was considered significant. n.s. denotes not significant.

Other methods can be found in the Supplementary Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematics of the microelectronic cardiac patch concept.

The flexible, freestanding electronic mesh device is designed to include electrodes for sensing of tissue electrical activity, for cell and tissue electrical stimulation and for controlled release of biomolecules within the tissue microenvironment. The device is integrated with an electrospun nanofiber scaffold to provide a supporting 3D microenvironment for cardiac tissue growth and tissue assembly. The internal electronic device provides on-line monitoring of tissue function, and if needed can interfere to activate it. Incorporation of electroactive polymers onto designated electrodes enables control over

the release of proteins and small molecules that may promote tissue growth or integration with the host.

Figure 2. Free-standing electronic mesh device.

a, An image of a free-standing, flexible device consisting of 32 gold electrodes dispersed within a porous mesh of SU-8. **b,c**, Scanning electron micrographs (SEM) of a $50/50\ \mu\text{m}^2$ electrode pad designated for recording cellular electrical activities and cell and tissue stimulation (**b**) and a larger $150/150\ \mu\text{m}^2$ electrode pad, on which electroactive polymer is deposited for controlling the release of biomolecules (that is, growth factors and small molecules) (**c**). Scale bars, $50\ \mu\text{m}$. **d,e**, Atomic force microscopy images of a pristine gold electrode pad (**d**) and an electrode with a nanoscale layer of titanium nitride deposited to increase surface area (**e**). **f**, Schematic representation of electrode location in a rolled device (green halos represent drug release). **g**, SEM image of a rolled device as presented in the dashed square of the scheme. A sensing/stimulating electrode (yellow areas in panel f) can be seen in the upper layer, and an electrode designated for controlled release (brown areas in panel f) is in the underlying layer. Scale bar, $200\ \mu\text{m}$. **h**, Assessment of electrode conductance as a function of increasing radii of curvature before and after manipulation of

the device. No significant difference was detected. All error bars represent the standard deviation (n=3 devices).

Figure 3. Controlled release of biomolecules from the electronic device.

a, Schematic representation of a system designed to release positively charged proteins (blue circles). The protein is loaded by equilibrium swelling into the tortuous pores of a negatively charged chondroitin sulfate (CS) hydrogel. Upon application of an electric field, the hydrogel shrinks and the negative charge is neutralized leading to the release of the positively charged protein (blue circles). **b,c**, As a proof-of-concept lysozyme was chosen as a model protein. **b**, Lysozyme release from within the CS electroactive system as a function of applied voltage. **c**, On-Off protein release profile from a CS system. **d**, SDF-1 release

under an applied voltage. **e**, Cell migration under the influence of SDF-1 released from the microECP. **f**, Schematic representation of a system designed to release negatively charged drugs. The pyrrole monomers (black circles) are oxidized leading to the creation of a polypyrrole (PPy) film containing the negatively charged drug (blue circles). Upon PPy reduction the electrostatic bonds between the polymer and the drugs are broken leading to the release of the drug. **g**, SEM image of a PPy film containing the negatively charged small molecule DEX, deposited onto the controlled release electrode. Scale bar, 50 μm . **h**, DEX release from the PPy film as a function of applied voltage. **i**, Immune cell function as measured by NO secretion (measured as nitrite, a stable metabolite of NO) from activated macrophages, as a response to the dexamethasone released from the microECP. All error bars represent the standard deviation ($n = 3$).

Figure 4. Biomaterial-electronics hybrid.

a, A stitched optical image of nanocomposite fibers of polycaprolactone-gelatin deposited on the electronic mesh. Scale bar, 1.5 mm. Inset, a single sensing/stimulating electrode covered with the nanofiber scaffold. **b**, SEM image of a recording/stimulating electrode covered by electrospun fibers. Scale bar, 20 μm . **c**, The deposited electroactive polymer on the designated electrode was held by the nanocomposite fibers. Scale bar, 50 μm . **d**, Schematic representation of the process for cell seeding and scaffold folding to create a thick 3D microECP. The electronic mesh is shown in blue, electrospun fibers are in grey, drug

release systems are in brown, the released drug is in green and the cells appear in red. **e**, An image of the folded microECP after 7 days of cultivation with cardiac cells.

Figure 5. Tissue organization and function within the 3D electronic scaffold.

a, Confocal microscope image showing the assembled cardiac tissue within the biomaterial/electronics hybrid. A sensing/stimulating electrode can be seen in the middle. Scale bar, 50 μm . Sarcomeric actinin in pink, nuclei are in blue (Hoechst 33258). **b**, Zoomed-in image of cardiac cells revealing cell elongation and massive striation. Scale bar, 10 μm . **c**, Bright field microscope image of a folded device depicting distant electrodes brought into close proximity by folding. Scale bar, 30 μm . **d**, 3D reconstruction of the cardiac tissue enveloping the electronic mesh, and distributed both above and beneath the electrode and polymer mesh. Scale bar, 50 μm . **e**, Comparison of cell viability of cells cultured with (yellow) and without (blue) the electronic mesh. Viability was measured by a Prestobblue metabolic activity assay. n.s. denotes not significant. All error bars represent the standard deviation (n = 3). **f**, Electrophysiological data collected in parallel from 9 different electrodes throughout the microECP. **g**, Recordings of cellular electrical activity in parallel with quantification of

calcium transients (bottom row, quantified through fluorescence intensity), before and after the addition of norepinephrine.

Figure 6. Remote control over the microECP function.

a, Fluorescence micrograph showing the regions of interest used for data quantification of calcium imaging. Scale bar, 100 μm . **b**, Quantification of calcium transients (as quantified through normalized fluorescence intensity) without perturbation (**I**) and with a 3 V, 50 ms, 1 Hz (**II**), and 2 Hz (**III**) pacing regime (separate regions of interest are represented in different colours). **c**, Heat maps showing reversal of signal propagation of the engineered cardiac tissue by electrical stimulation. Scale bar, 200 μm .