Supplementary Data

Cardiac Differentiation

Cardiomyocytes are differentiated from human-induced pluripotent stem cells (hiPSCs) following the methods established by Palecek's and Wu's groups.[49,50] Briefly, hiPSCs are treated with 6 μM Chiron 99021 (Tocris), a glycogen synthase kinase 3 (GSK3) inhibitor at day 1, followed by IWP2 (Tocris), a chemical Wnt inhibitor at day 3. Cardiac phenotype, expressed by beating cells, is visible between days 8 and 12. Cardiomyocytes differentiated from hiPSCs are used for the production of engineered constructs between days 14 and 18 of differentiation. Cardiac purity is measured by FACS analysis on every cell batch used for the preparation of cardiac constructs.

Neonatal Rat Cardiomyocyte Harvest

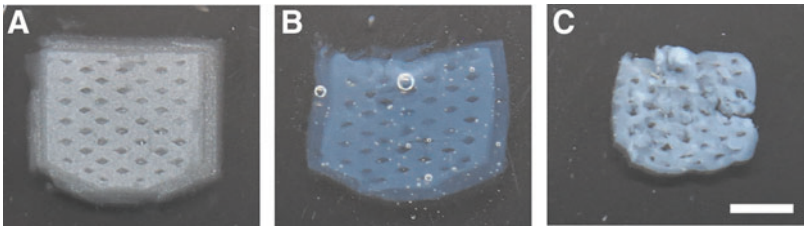
Primary cardiomyocytes were isolated from Sprague-Dawley neonatal rats (P2) via serial digestion with trypsin and collagenase followed by Percoll gradient centrifugation according to a protocol routinely used by our collaborators[51] that leads to highly pure cell fractions.

Isolation of Mouse Embryonic Fibroblasts

Mouse embryonic fibroblasts (MEFs) were harvested from two-time pregnant female mice (Charles River) on the 13th day after pregnancy. Animals were sacrificed via CO_2 gas asphyxiation, and embryos were dissected from their uteri. Head, heart, and liver were removed from the embryos, and the remaining material was mixed with ''MEF medium'': Dulbecco's modified Eagle's medium (DMEM; Invitrogen) containing 10% fetal bovine serum (FBS; Life Technologies) and 1% penicillin/streptomycin (Sigma P0781) and extruded twice from syringes with 18G and 25G needles to break up cell clumps. The cells were then centrifuged and washed in MEF medium before being plated in 10-cm dishes (3–4 embryos per dish) for expansion. Cells were cultured at 37°C, 5% CO_2 with MEF medium (changed 3× per week), frozen at passage 2 in 10% dimethyl sulfoxide (DMSO), and used between passages 3 and 7.

Optical Mapping of Action Potential *In Vitro*

For the *in vitro* experiment, the engineered tissue was placed in a temperature-controlled chamber and perfused with Tyrode's solution (in mmol/L:130 NaCl, 24 NaHCO3, 1.0 MgCl2, 5.0 KCl, 1.2 NaH2PO4, 5 dextrose, and 1 CaCl2, at pH 7.3, gassed with 95% O_2 and 5% CO_2). The engineered tissues were stained with a voltage-sensitive dye, di-4-ANEPPS (Thermo Fisher Scientific), and stimulated using a bipolar concentric electrode (small stimulation electrode set; Harvard Apparatus). Fluorescence images (Ex/Em = 530/580 nm) from the engineered tissues were captured using a CMOS camera (100 × 100 pixels, Ultima-L; SciMedia) with a 50 mm Nikon f/1.2 lens, which results in a field of view measuring $10 \times 10\,mm^2$ and achieves a spatial resolution of $100 \times 100\,\mu m^2$. The sampling rate was set to 1000 frames s^{-1}, and data were captured and analyzed with a custom-built software program developed in Interactive Data Language (Harris Geospatial Solutions).[52,53]



SUPPLEMENTARY FIG. S1. Alginate **(A)**, collagen **(B)**, and fibrin **(C)** acellular hydrogels produced in the *diamond*-shaped PDMS mold after 1 h of incubation at 37°C (scale bar, 1 cm). The visually different morphologies and mechanical properties of the hydrogels depend on the different materials: alginate hydrogel was crosslinked with calcium ions, by mixing 1% alginate with 10 mg $CaCO_3$ and 10 mg GDL, gelling time = 10 min. Collagen hydrogel was formed by the pH- and temperature-dependent gelation of a 1.25 mg/mL collagen solution (with pH adjusted to 7.0, gelling temperature = 37°C, and gelling time = 1 h). Fibrin gel was obtained mixing 1 mL fibrinogen (10 mg/mL) with 25 μL of 200 U/mL thrombin, gelling time = 5 min. GDL, glucono-delta-lactone; PDMS, poly(dimethylsiloxane).