Supporting Information

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SI Text

SI Materials and Methods. ***PCL fibers.*** Fibers were electorspun from PCL (Mn 80,000, Aldrich) solutions as previously reported (1). The fibers were stored at 4 °C until cells were ready for seeding.

PLGA sponges. Biodegradable PLGA (a 50:50 copolymer of lactide:glycolide with molecular weight of 30,000–60,000, Aldrich) sponges were processed into wafers with sodium chloride crystals using a solvent casting technique (2). Briefly, sodium chloride salt (NaCl) was ground with a mortar and pestle, and 0.2 g was added to cylindrical molds. The polymer was dissolved in Hexafluoro-2-propanol (Fluka) (9 wt%), cast over the salt crystals, and allowed to dry overnight at room temperature. The sponges were removed from the molds, washed 3× in water, 1× in 70% ethanol, and 2× in sterile PBS. Each washing step was 10 min at room temperature on a shaker. PLGA-IPTG sponges were processed the same way as the PLGA sponges; however, PLGA was dissolved in Hexafluoro-2-propanol at 18 wt%. IPTG (Fermentas) was dissolved in Hexafluoro-2-propanol at a concentration of 3 M and was used to make various concentrations of sponges by mixing the IPTG with PLGA. This PLGA-IPTG polymer solution was cast over salt crystals and allowed to dry overnight at room temperature. The same washing steps were applied as with the PLGA sponges and used immediately for seeding cells.

Seeding cells on PLGA sponges and PCL fibers. Before cell seeding, sponges and fibers were UV sterilized and then soaked in FBS for 30 min. Sponges were transferred to a 24-well plate containing 2% agrose on the bottom of each well and 50 uL containing 250 K CHO cells stably transfected with LTRi_EGFP were seeded onto each side of the sponges four times (twice per side) for a total of 1×10^6 cells at 30-min intervals.

Cells and fibers were incubated at 37 °C incubator for 4 h. Sponges were then flooded with growth media, either with or without IPTG, and fibers were transferred to a 24-well plate with 2% agarose on the bottom of each well, and growth media either with or without IPTG was added to each well. After 24 h, sponges were moved to a plate without agrose coating.

Photo-polymerization. Prepolymerization solution was prepared by dissolving PEGDA (3400, SunBio) in PBS at 200 mg/mL. For the PEG α-CD gels, α-CD was prepared by dissolving 0.15 g of α-CD (Aldrich) in distilled water and filtered through a 0.22 um syringe filter. The α-CD solution was added to the PEGDA solution to obtain 1% (w/v) α-CD and 10% of PEGDA (w/v) gel. For the PEG-IPTG gels, IPTG-modified PEG was resuspended in PBS and added to the PEGDA solution to obtain various concentrations of IPTG in the final PEG-IPTG hydrogel. For the PEG-RGD gels, the RGD-modified PEG was prepared, according to previously described procedures (3–5). For the 1% RGD gels, 100 mg PEGDA, 10 mg PEG-RGD, and 40 mg PEGMA were mixed in sterile PBS. In all cases, the photo-initiator, Irgacure 2959 (CIBA Specialty Chemicals Corp.), was dissolved in 70% ethanol and added to the above solutions to make a final concentration of 0.05% (w/v). For cell encapsulation, CHO cells stably transfected with LTRi_EGFP were dispersed in the respective sterile polymer solution to yield a final cell density of 20×10^6 cells/ml, transferred to cylindrical molds, then exposed to 365 nm UV radiation for 5 min. The resulting constructs were removed from the molds and placed in CHO growth media with or without IPTG, and the cells were grown in a 37 °C, humidified incubator with 5% CO_2. Media changes were done every 2 to 3 d until harvesting.

To create the patterned PEG-IPTG hydrogel, 2×10^6 CHO cells stably transfected with LTRi_EGFP were resuspended in 100 uL of 10% PEG containing the photo-initiator. Additionally, 2×10^6 CHO cells stably transfected with LTRi_EGFP were resuspended in 10% PEG-IPTG with irgacure (IPTG concentration was equivalent to 250 uM). Polymerization steps started with 50 uL of the PEG solution with cells transferred to a cylindrical mold and UV polymerized for 1 min, followed by 50 uL of the PEG-IPTG solution with cells added to the top of the first layer, and UV polymerized for 1 min. This was repeated, and the final polymerization step was for 5 min. The constructs were transferred to a plate containing CHO growth media without IPTG. Microscopy was done 3, 5, 9, 12, and 16 d after the encapsulation.

Antibodies and immunolabeling. Constructs were fixed in 4% paraformaldehyde prepared in Sorensen phosphate buffer (pH 7.4). Constructs were permeabilized and blocked, using blocking solution (5% donkey serum, 1% BSA, PBS) and supplemented with Triton X-100 to 0.5%. GFP primary antibody (Invitrogen), DAPI (Invitrogen), and phalloidin AlexaFluor568 (Invitrogen) were diluted in blocking solution supplemented with Tween-20 to 0.1%, and incubated with the constructs overnight at 4 °C. The constructs were washed thoroughly with PBS-T (PBS, 0.05% Tween-20) followed by incubation with species-specific, Alexa Fluor (Jackson ImmunoResearch) conjugated secondary antibodies. The following commercially available antibodies were used in this study: GFP (Invitrogen, A6455); Alexa Fluor, 568 phalloidin (Invitrogen, A12380); DAPI (Invitrogen, 1306); and Donkey Anti-Rabbit 488 (Jackson ImmunoResearch, 711485152)

Microscopy. Confocal microcopy: The constructs were washed with PBS-T, mounted using Fluoro-Gel (Electron Microscopy Sciences), and imaged using a Zeiss LSM510 confocal microscope. Image frames were limited to 12 microns in the Y-dimension and a stack of 9–12 microns containing the cells was collected. A single image was generated by Z-projection using maximum or averaged pixel intensities. Stereomicroscope: At the specified time points, constructs were visualized on a Nikon SMZ1500 with a GFP fluorescent filter set.

HPLC. All solvents and water used were HPLC grade (Fisher Scientific). HPLC measurements were conducted using reversed-phase Nova-Pak C18 column (Waters, WAT086344, 4 um, 3.9×150 mm). All solvents were delivered at a flow rate of 1.0 mL/min and filtered, degassed, and kept under pressure. Mobile phase solvents (B: acetonitrile and D: 10 mM NaOAc pH 4.54) were injected into a HPLC system (Waters Delta 600 pump equipped with a PDA 2996, RI 2414 detection system, and an Autosampler 2707). Samples were prepared using deionized water and filtered using 0.2 um nylon filter units into Autosampler tubes and run on the HPLC with mobile phase solvents at 10B:90D and detected at a wavelength of 200 nm. A standard curve was generated by running various concentrations of IPTG (Fig. S2*A*) and calculating the area under the curve. These data were plotted, and the slope was taken for calculating the concentration of IPTG in materials using Beer's law.

RNA extraction and real-time quantitative PCR (Q-PCR). Constructs were homogenized, and total RNA was extracted using TRIzol (Invitrogen). cDNA was synthesized using SuperScript III (Invitrogen). Q-PCR was performed using Power SYBR Green (Applied Biosystems) and gene specific primer sets on a 7500 Real-Time PCR Detection System (Applied Biosystems). Each PCR reaction was carried out in triplicate, with at least three biological replicates. Relative gene expression was analyzed using the ΔΔCT method (6). A ribosomal gene (L19) and GAPDH were used as endogenous references. Gene-specific primer sets are listed in Table S1. Melt curves confirmed that each primer pair had one peak of amplification in the presence of template. The expression of EGFP was compare to 250 pM IPTG because the ΔΔCT method requires the comparison to some level of expression.

PEG-IPTG hydrogels. Synthesis of 2-azidoethyl amine. The 2-Bromoethyl amine hydrobromide salt (5.0 g, 24.4 mmol) was stirred with sodium azide in water at 75 °C for 22 h. After cooling to room temperature, 1.0 g of NaOH was added to the solution and stirred for 15 min. The product was extracted thrice with CH_2Cl_2 (50 mL each), followed by washing the organic phase with saturated aqueous NaCl (25 mL). After drying over anhydrous $MgSO_4$, filtration, and removing the solvent by rotary evaporation, 2-azidoethyl amine as a light yellow liquid (1.72 g, 81%) was obtained and used in further reactions without further purification. ^{1}H NMR ($DMSO_6$-2.54 ppm): 2.76 (CH_2N_3), 3.32 (CH_2NH_2). ^{13}C NMR (DMSO-D_6-40.4 ppm): 42.1 (CH_2NH_2), 54.7 (CH_2N_3).

Synthesis of acrylate-poly(ethylene glycol)-azide.
Acrylate-PEG-NHS (98.0 mg, Mw 3400 Da) was stirred with 2-azidoethyl amine (2.5 mg, 0.03 mmol) in $NaHCO_3$ aqueous solution (pH 8.2) for 2 h in dark, followed by dialysis against distilled water with MWCO-2000 Da. After lyophilization, 87.0 mg of a white powder with yield 85% was obtained. ^{1}H-NMR (DMSO-D_6): 2.36 (CH_2N_3), 3.30–3.60 (OCH_2 & CH_2NH), 3.92 (OCH_2CO), 4.25 (CH_2OCO), 5.98 (=CH trans to CO_2), 6.21 (dd, =CH gem to CO_2), 6.43 (=CH cis to CO_2).

Synthesis of pentynoyl chloride. Pentynoic acid (4.1 g, 42.0 mmol) was refluxed with excess of $SOCl_2$ (36.0 g, 0.30 mol) for 19 h. After removing unreacted $SOCl_2$ under reduced pressure at 75 °C, a dark residue was obtained. After vacuum distillation at 110 °C, 3.3 g of a clear liquid with 68% yield was obtained. ^{1}H NMR ($CDCl_3$-7.27 ppm): 2.04 (CC*H*), 2.57 (CH_2C), 3.13 (CH_2CO). ^{13}C NMR ($CDCl_3$-77.23 ppm): 14.86 (CH_2C), 45.8 ($OCCH_2$), 70.51 (*C*HC), 80.65 (CH*C*), 172.32 (C=O).

Esterification of pentachlorophenol with pentynoyl chloride. Pentynoyl chloride (9.0 g, 0.775 mol) in anhyd. CH_2Cl_2 (50 mL) was added dropwise to an ice-cold solution of pentachlorophenol (13.8 g, 0.06 mol) and pyridine (7.0 mL, 0.08 mol) in CH_2Cl_2 (100 mL). After overnight reaction, the solution was washed twice with dilute aqueous $NaHCO_3$ (50 mL each) and once with dilute aqueous HCl (50 mL). The organic solution was dried over anhyd. $MgSO_4$. After filtration, solvent was removed by rotary evaporation. The solid was recrystallized from warm hexanes (100 mL), followed by another recrystallization from acetone (100 mL) to yield 4.1 g (24%) as a white product. ^{1}H NMR ($CDCl_3$): 2.06 (C*H*C), 2.70 (CCH_2), 2.96 (CH_2CO). ^{13}C NMR ($CDCl_3$): 14.5 (CCH_2), 33.1 ($OCCH_2CH_2$), 70.1 (*C*CH), 81.6 (C*C*H), 167.8 (OC=O), 144.11 (O=C*O*C), 127.8–132.3 (*C*Cl).

Transesterification of pentachlorophenyl pent-4-ynoate with IPTG. A solution of pentachlorophenyl pent-4-ynoate (3.9 g, 0.01 mol) in anhyd. pyridine (10 mL) was added dropwise to a solution of IPTG (4.0 g, 0.17 mol) and imidazole (2.3 g, 0.03 mol) in pyridine (10 mL). After stirring for 48 h, the solution was concentrated by rotary evaporator and added with ethyl acetate (50 mL). The organic phase was extracted twice with aq. citric acid (50 mL, 10% w/v). The aqueous phase was extracted thrice with ethyl acetate (50 mL). The combined organic phase was dried over anhyd. $MgSO_4$. A pale yellow solid residue was obtained after filtration and solvent removal by rotary evaporator. After recrystallization in $CHCl_3$/Hexanes (3/7, v/v), 1.2 g (33%) of white crystals was obtained. ^{1}H NMR (D_2O): 1.35 (CH_3), 2.17 (CC*H*), 2.25 (CCH_2), 2.29 (CH_2C=O), 3.24 ($SCH(CH_3)_2$), 3.25–4.9 (OC*H*S, $OCHCH_2$ & C*H*OH x3). ^{13}C NMR (CD_3OD-49.9 ppm): 15.8 (CCH_2), 25.2 (CH_3), 35.2 ($SCCH_3$), 36.7 ($OCCH_2CH_2$), 63.1 (O=C*O*CH_2), 68.9 (CH*C*HCH), 69.8 ($CH_2CHCHOH$), 71.0 (*C*HC), 79.7 (S*C*HCH), 80.9 (CH_2CHO), 84.2 (O*C*HS), 88.0 (*C*CH), 173.9 (C=O).

Click chemistry of modified-IPTG with acrylate-PEG-azide. Acrylate-PEG-azide (150 mg, 0.042 mmol) in distilled water (0.5 mL) was added with modified-IPTG (15 mg, 0.05 mol) in dimethylformamide (0.3 mL). To this solution, $CuSO_4 \cdot 5H_2O$ (1.05 mg, 4 μmol) and sodium ascorbate (1.8 mg, 9.0 μmol) were added. After stirring for 21 h, this solution was added with EDTA disodium dihydrate (3.2 mg in 5 mL dist. water) to remove copper. After overnight stirring, the solution was dialyzed against dist. water with MWCO-1000 Da. A white fluffy powder (116.0 mg, 77%) of acrylate-PEG-conjugated IPTG was obtained after lyophilization. ^{1}H-NMR (DMSO-D_6): 1.35 (CH_3), 2.42 (CCH_2), 2.65 (CH_2C=O), 2.76 (CH_2C=N), 3.12 (CH_2NHCO & $SCH(CH_3)_2$), 3.25–5.0 (CH_2O of PEG, C*H* & CH_2 of IPTG), 5.97 (=CH trans to CO_2), 6.23 (dd, =CH gem to CO_2), 6.36 (=CH cis to CO_2), 7.89 (CON*H*).

1. Coburn JM, et al. (2011) Biomimedics of the extracellular matrix: An integrated three-dimensional fiber-hydrogel composite for cartilage tissue engineering. *Smart Struct Syst* 7:213–222.
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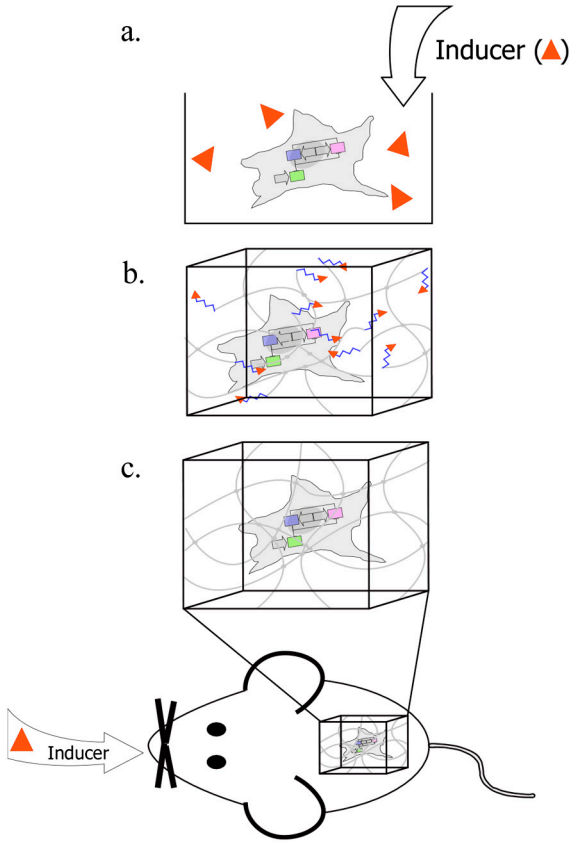

Fig. S1. Summary of various methods of activating and controlling genetic circuits in vitro and in vivo. (*A*) Adding inducer to the media allows for basic characterization of genetic circuits in 2D and in 3D. (*B*) Chemically linking the inducer to materials offers methods of patterning gene expression. These engineered biomaterials also allow for studies of cell-matrix studies, and the investigation of how signals from the ECM affect gene expression. (*C*) Potential clinical applications of interfacing synthetic biology and materials science offer mechanisms for translating synthetic biology.

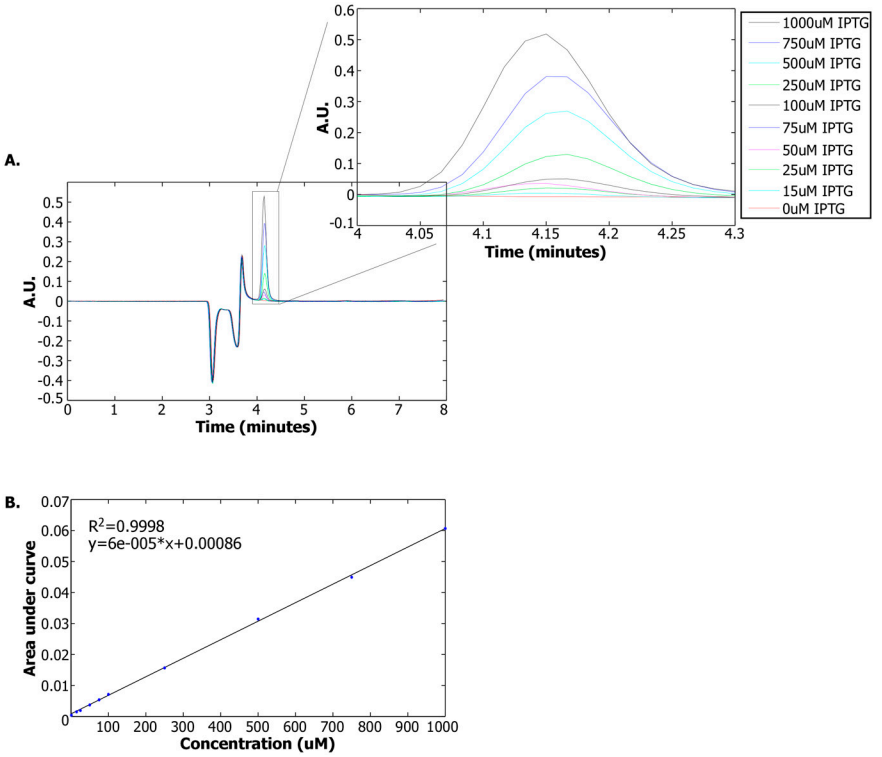

Fig. S2. IPTG loading in PLGA sponges. (*A*) HPLC of IPTG in a solution. HPLC offers a method for detecting and quantifying the amount of IPTG in a solution. (*B*) PLGA-IPTG sponges. IPTG was incorporated into the walls of PLGA scaffolds. As the PLGA scaffold degrades, the IPTG is released and becomes available to the cells. (*C*) Loading efficiency of IPTG into sponges. The amount of IPTG shown is the initial loading upon sponge fabrication.

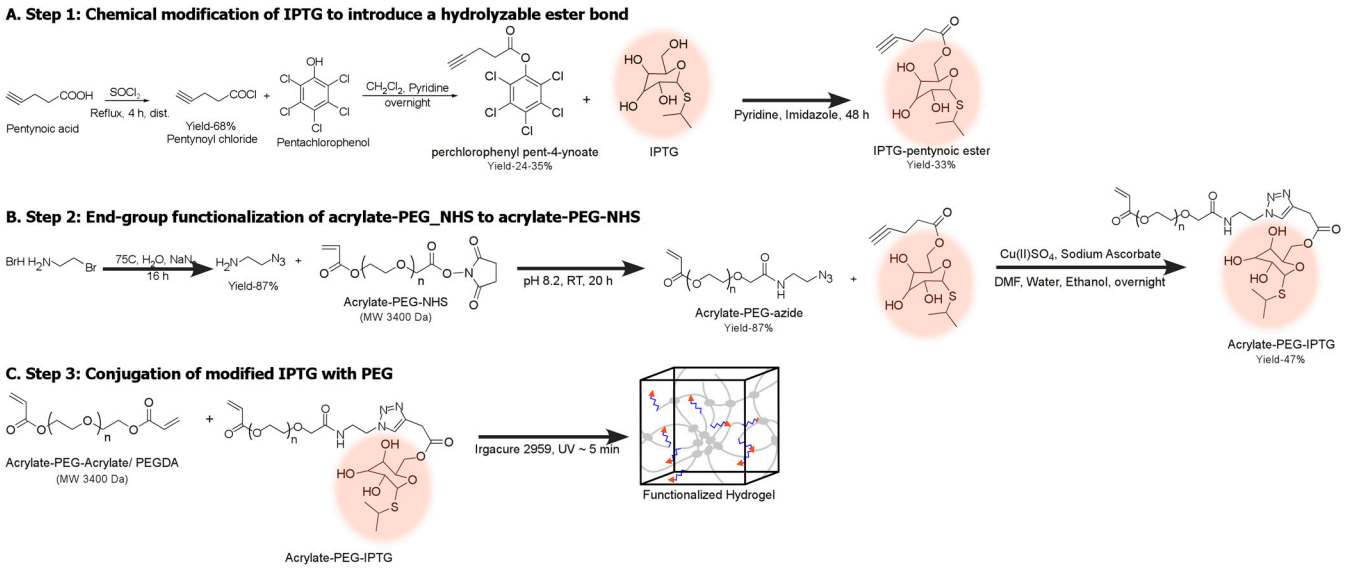

Fig. S3. Chemistry to functionalize PEG with IPTG. (*A*) An ester bond was added to IPTG (shaded orange). (*B*) IPTG containing the ester bond was added to acrylated PEG. (*C*) PEGDA and acrylate-PEG-IPTG were polymerized to form PEG-IPTG gels.

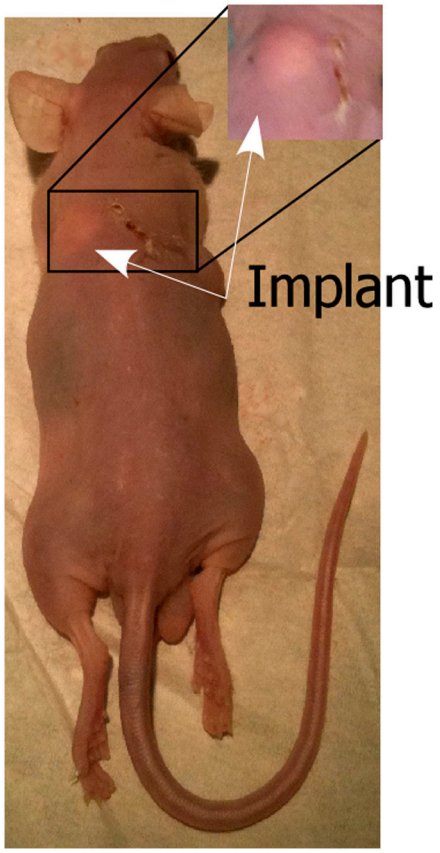

Fig. S4. In vivo implants. Implants were done subcutaneously or in the interperitoneum of athymic mice. These mice were chosen because they are immunodeficient so their immune system does not reject cells transplanted from humans or other species.

Table S1. Table showing gene-specific primers used for Q-PCR

Primer	Forward	Reverse
Bactin	5′-GCTCCTCCTGAGCGCAAGTAC-3′	5′-GGACTCGTCATACTCCTGCTTGC-3′
L19	5′-GGTCTGGTTGGATCCCAATG-3′	5′-CCCGGGAATGGACAGTCA-3′
GAPDH	5′-CACCCACTCCTCCACCTTTGA-3′	5′-TCCACCACCCTGTTGCTGTAG-3′
GFP	5′-CGTCTATATCATGGCCGACA-3′	5′-GGGGTGTTCTGCTGGTAGTG-3′