

SUPPORTING INFORMATION

Muscle-on-a-chip with on-site multiplexed biosensing system for in situ-monitoring of secreted IL-6 and TNF- α

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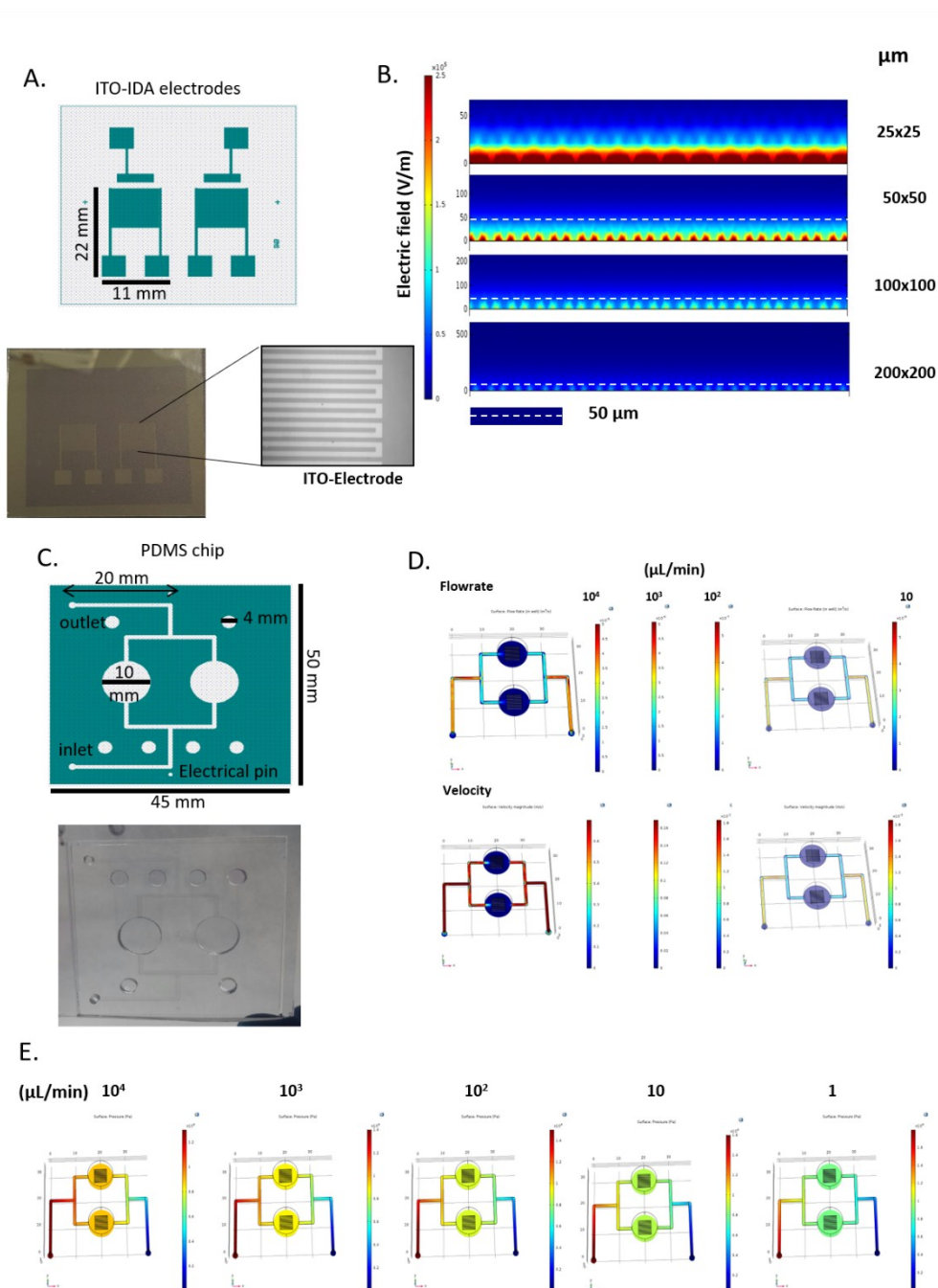


Fig S1. (A) Dimensions of custom-made ITO-IDA electrodes. Real image of electrodes substrate with zoomed view of the interdigitated array. B) COMSOL Multiphysics® simulations of the electrical field behavior at various interelectrode distance. (C) PDMS microfluidic chip dimensions. (D) and (E) COMSOL Multiphysics® simulations of flow rate, velocity and pressure throughout the device respectively.

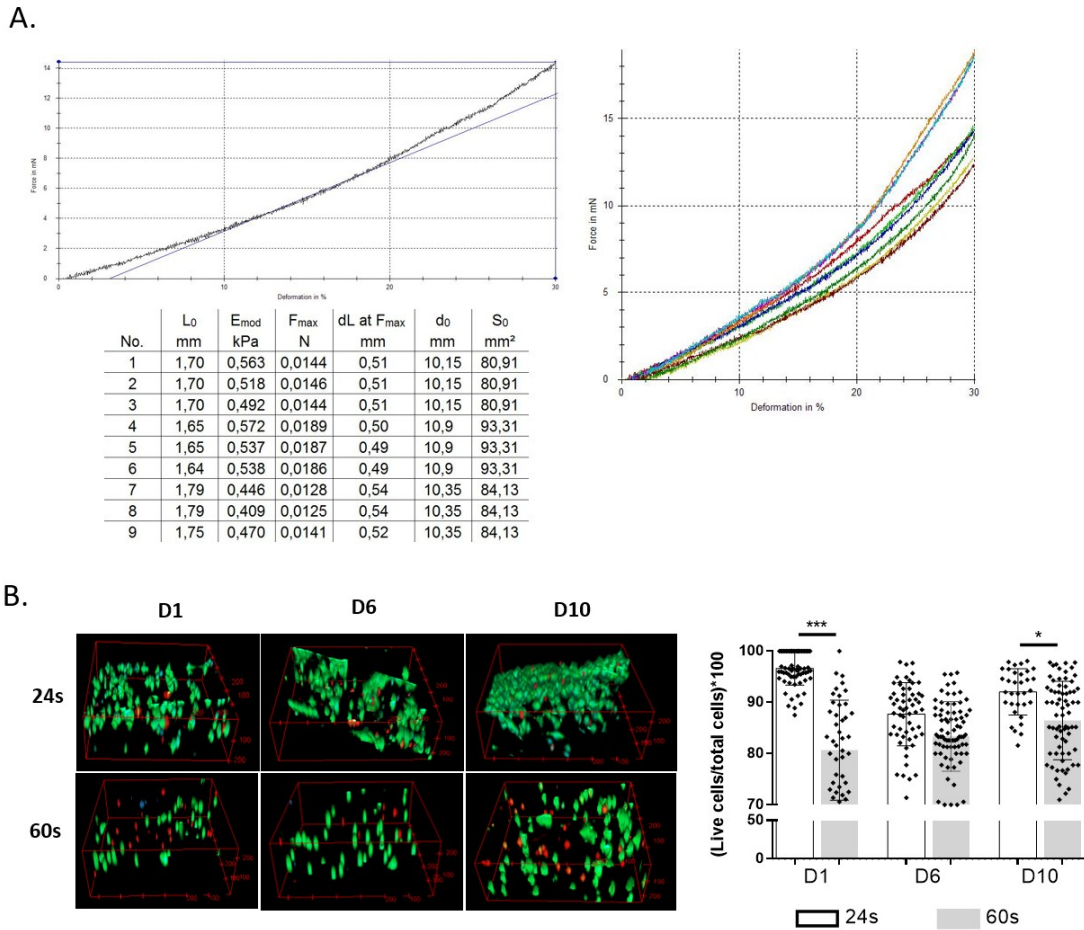


Fig. S2. (A) Force deformation curves and data for uniaxial compression test of GeIMA-CMCMA hydrogels photocrosslinked at 24 sec. (B) Live/dead staining to assess cell viability in composite hydrogels fabricated at 24 s and 60 s of UV exposure (live cells in green and dead cells in red) Data from three different experiments (mean, SD): ** $p < 0.01$; *** $p < 0.001$.

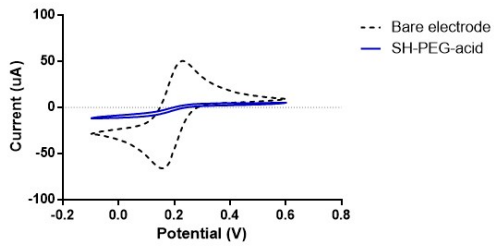
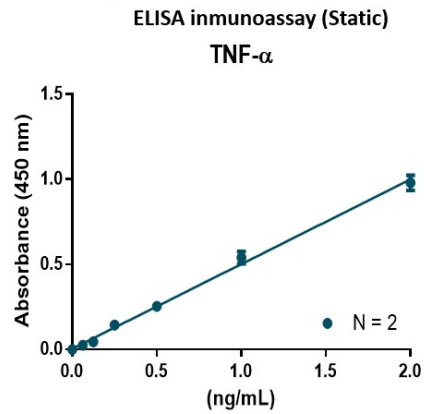
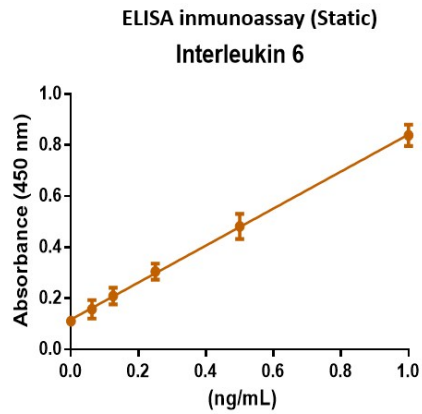
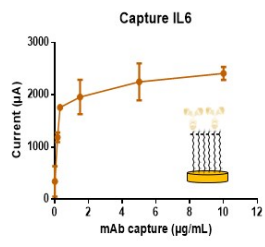
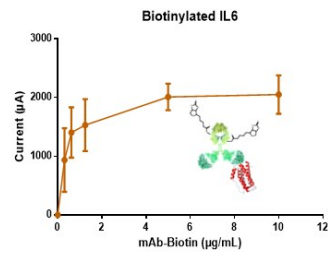
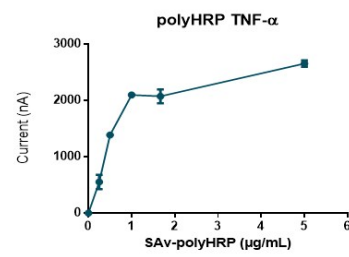
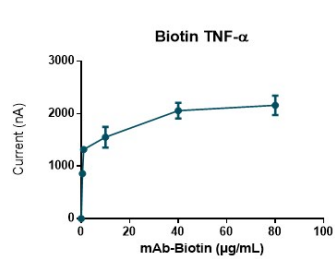
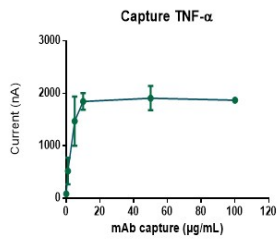
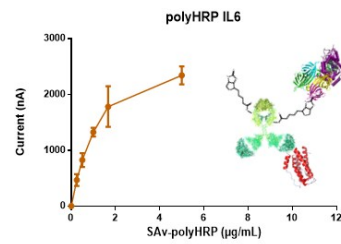
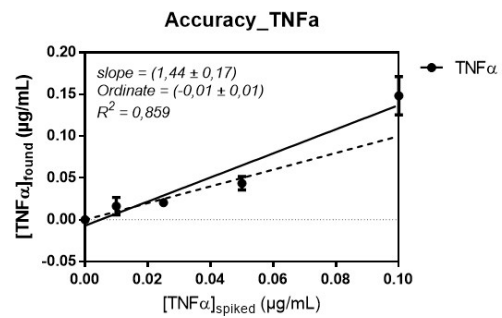
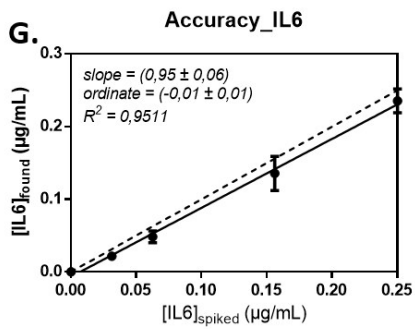
A.**B.****C.****D.****E.****F.****G.**

Fig. S3. (A) Cyclic voltammetry applied at 100 mV/s to bare electrode (dashed curve) versus functionalized with SH-PEG-acid (blue line). Changes in signal indicates the coverage of SAM on SPGE surface. (B) In-house PMMA static cells used for SPGEs functionalization protocol. (C) ELISA calibration curves for IL-6 and TNF- α respectively. Optimization of immunoassay on SPGEs surface using 1 $\mu\text{g mL}^{-1}$ of IL-6 and TNF- α during detection step. Saturation levels were reached at 1.5, 5, and 1 $\mu\text{g mL}^{-1}$ for IL6 and 10, 40 and 1 $\mu\text{g mL}^{-1}$ for TNF- α for (D) capture antibody, (E) secondary biotinylated antibody and (F) SAV-polyHRP conjugate, respectively. (G) Accuracy studies made by IL6 and TNF- α , performed using blind spiked samples prepared in differentiation media and used directly in the immunosensor without previous dilution. The data shown correspond to the average of at least two replicates. All data shown by mean \pm SD

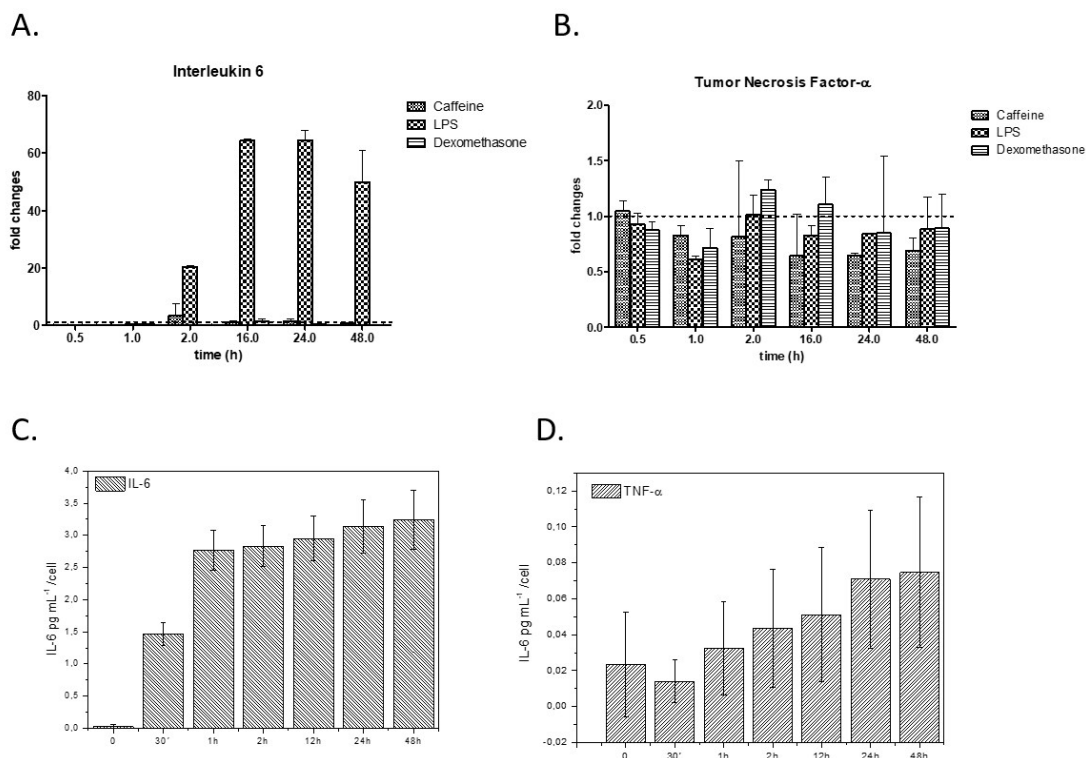


Fig. S4. (A) 2D drug screening assays evaluating IL-6 and (B) TNF- α release induced by caffeine (100 μM), dexamethasone (100 μM) and LPS (10 $\mu\text{g mL}^{-1}$). 2D myotubes were incubated for 0.5, 1, 2, 16, 24 and 48 h, in DM supplemented by the drug. Three replicates for each condition were analyzed. The screening of the cytokine levels was carried out by ELISA technique. (C) and (D) Amount of secreted IL-6 and TNF- α (pg mL $^{-1}$) per cell upon LPS-stimulation inside the microdevice. Calculations were made counting cell nuclei (DAPI immunostaining) inside 3D SM microtissue, with an estimation of 840.000 encapsulated cells.