

ADVANCED BIOSYSTEMS

Supporting Information

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3D In Vitro Neuron on a Chip for Probing Calcium
Mechanostimulation

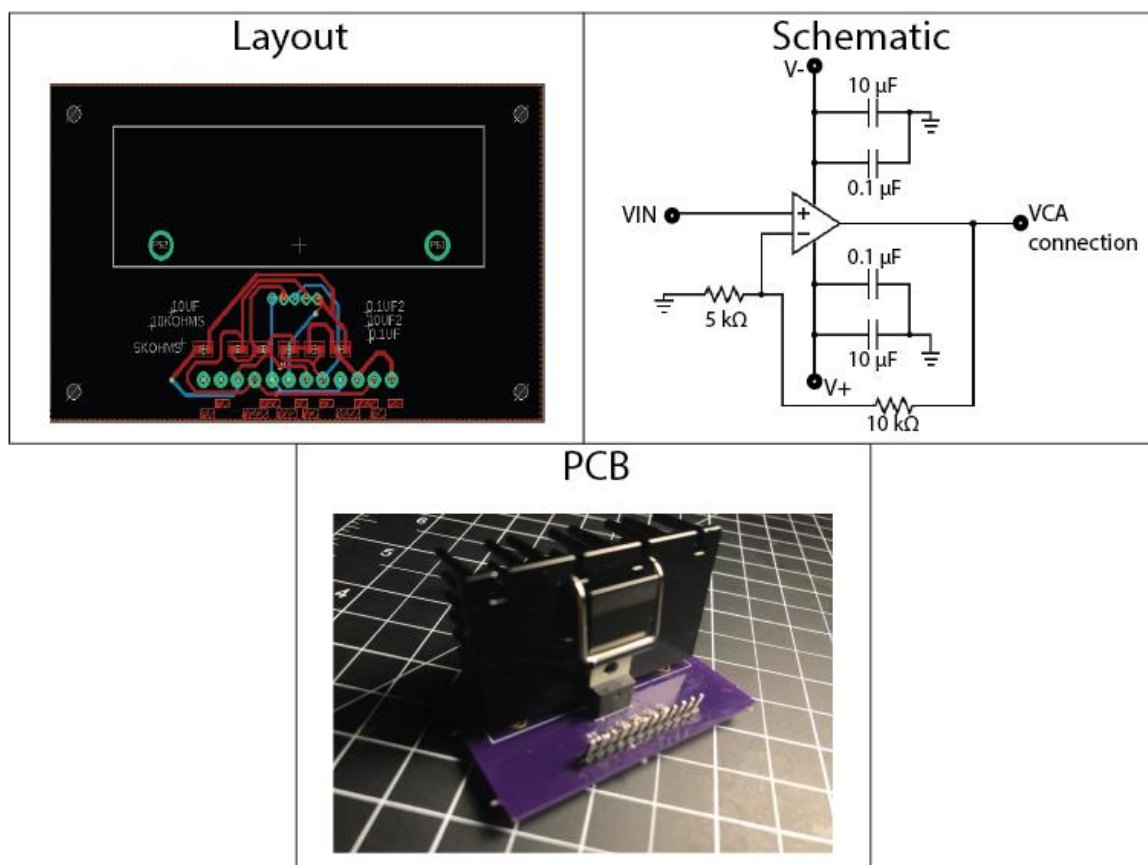
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3D in vitro neuron on a chip for probing calcium mechanostimulation

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Figure S1. Electrical circuit diagram layout and Printed Circuit Board (PCB). The PCB incorporates an operational amplifier (Mouser: OPA544T) coupled with a single pin fin heat sink (Mouser: 588-PA-T21-38E) to transmit a smooth current SNR to the VCA.



Equation 1-4. Theoretical modeling of Mechanical Excitation Testbed (MET) System. The spring coil in the VCA and the cellularized 3D collagen are modeled as springs and the precision force applicator as a mass. For one dimensional undamped motion and both springs relaxed at equilibrium, the total energy of the system is zero (Equation 1) (Figure 1A, B). Using the law of conservation of energy (Equation 2), we derive an expression relating potential and kinetic energy. From this equation, we are able to simplify the expression to velocity as a function of displacement (Equation 3). Taking a derivative of the velocity equation, we now have a theoretical expression of displacement (Equation 4).

$$E_T = E_0 = \frac{1}{2}k_{coil}A^2 + \frac{1}{2}k_{cellularized\ collagen}A^2(1)$$

$$\begin{aligned} E = E_0 = PE + KE &= \frac{1}{2}k_{coil}x^2 + \frac{1}{2}k_{cellularized\ collagen}x^2 + mv^2 \\ &= \frac{1}{2}k_{coil}A^2 + \frac{1}{2}k_{cellularized\ collagen}A^2(2) \end{aligned}$$

$$v(x) = \sqrt{\frac{k_{coil}+k_{cellularized\ collagen}}{m}(A^2 - x^2)}(3)$$

$$x(t) = A\sin\left(\sqrt{\frac{k_{coil}+k_{cellularized\ collagen}}{m}}t\right)(4)$$

Table T1. 3D collagen hydrogels prepared to a final concentration of 5 mg/mL.

Nomenclature

 V_{tot} = Volume total V_{col} = Volume of collagen V_{PNC} = Sum of the Volumes of 10X PBS, NaOH, Collagen I

3D Collagen Concentration	10X PBS	NaOH	Cell Media	Collagen I
5 mg/mL	$\frac{V_{tot}}{10}$	$0.023(V_{col})$	$V_{tot} - V_{PNC}$	$\frac{5}{Col.Con.} V_{tot}$

Figure S2. Zoom image add-on for $t = 1, 64, 125$ s. Ca^{2+} response during No-Contact Control phase, Primary phase, and Secondary phase mechanical stimulation in 3D NITC system.

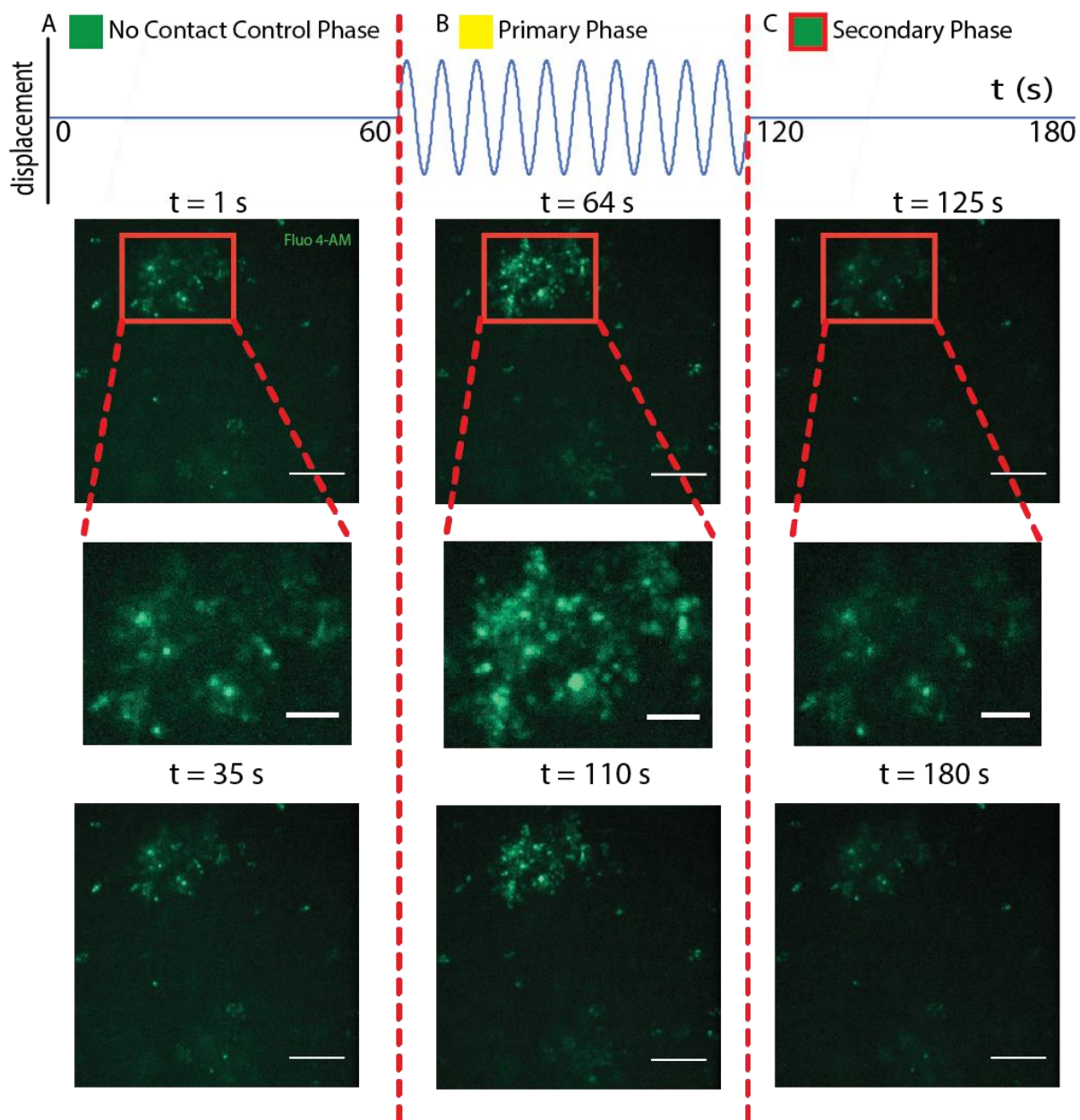


Figure S3. Primary Phase Imaging: In Plane/Out of Plane. Differentiated PC12 cell gels have increased Ca^{2+} with force application in MET device. Compression forces cells out of the focal plane.

