

Supporting Information

for

Biologically-Derived Soft Conducting Hydrogels Using Heparin-Doped Polymer Networks

Hangjun Ding^{a,b}, Mingjiang Zhong^{b,†}, Young Jo Kim^c, Pitirat Pholpabu^d, Aditya Balasubramanian^c, Chin Ming Hui^b, Hongkun He^b, Huai Yang^e, Krzysztof Matyjaszewski^{b,}, and Christopher John Bettinger^{c,d,*}*

Table S1. Chemical, physical, and mechanical properties of pristine HepMA primary hydrogel networks as a function of [Hep-MA].

Sample name	[Hep-MA] (wt%)	Gelation time (min)	Est. avg. pore diameter ^{a,b} $D_{pore,avg}$ (μm)	Swelling ratio ^a Q	Storage modulus ^a G' (kPa)	Compressive modulus ^a E (kPa)
Hep-MA ₀₂	2	60	10 ± 3	17.5 ± 2.5	0.3 ± 0.05	8.0 ± 1.5
Hep-MA ₀₅	5	42	40 ± 8	15.0 ± 1.5	0.8 ± 0.08	25 ± 2.0
Hep-MA ₀₈	8	25	30 ± 5	12.4 ± 1.2	1.4 ± 0.12	69 ± 5.4
Hep-MA ₁₀	10	25	20 ± 5	10.5 ± 0.8	2.8 ± 0.20	100 ± 9.8

^aValues are reported as mean \pm standard deviation.

^bValues estimated from SEM micrographs.

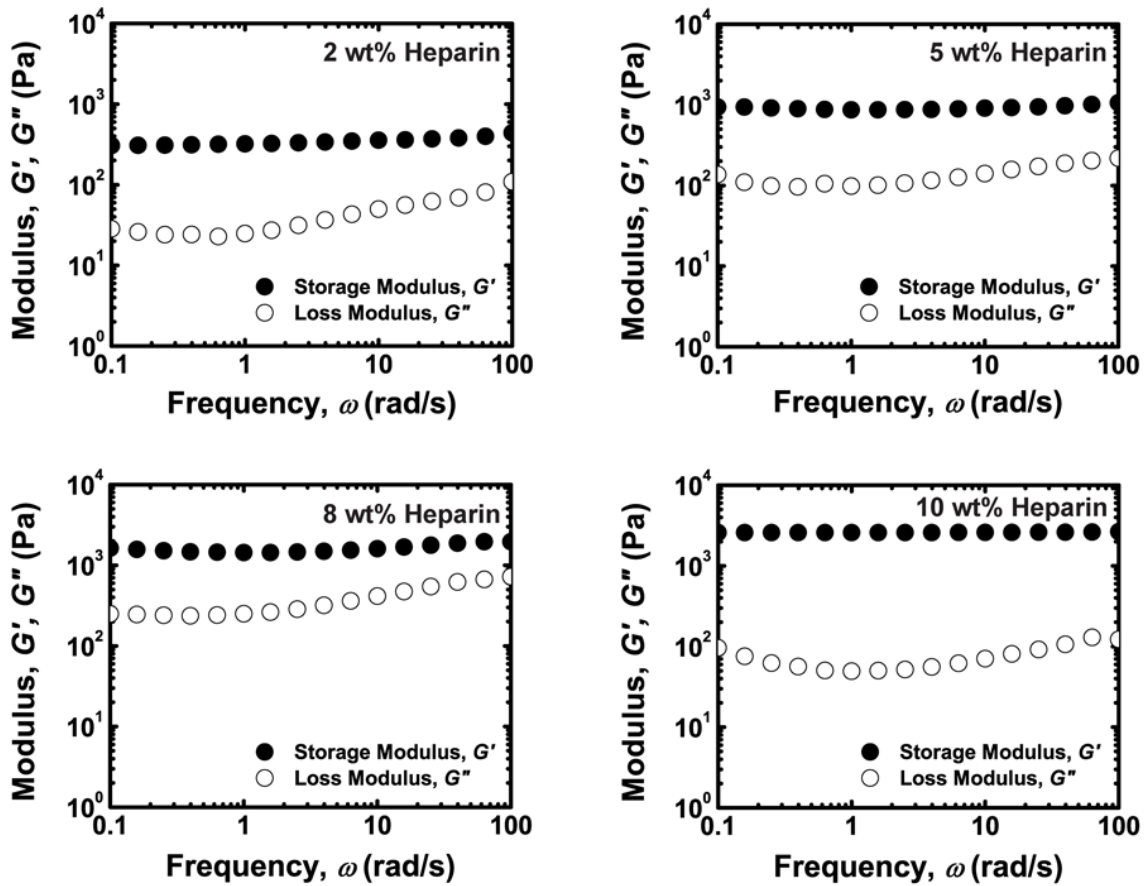


Figure S1. Representative rheological data show frequency sweeps of primary Hep-MA hydrogel networks for for ω between 0.1 and 100 rad s^{-1} . The values of [Hep-MA]₀ are varied between 2—10% (w/w).

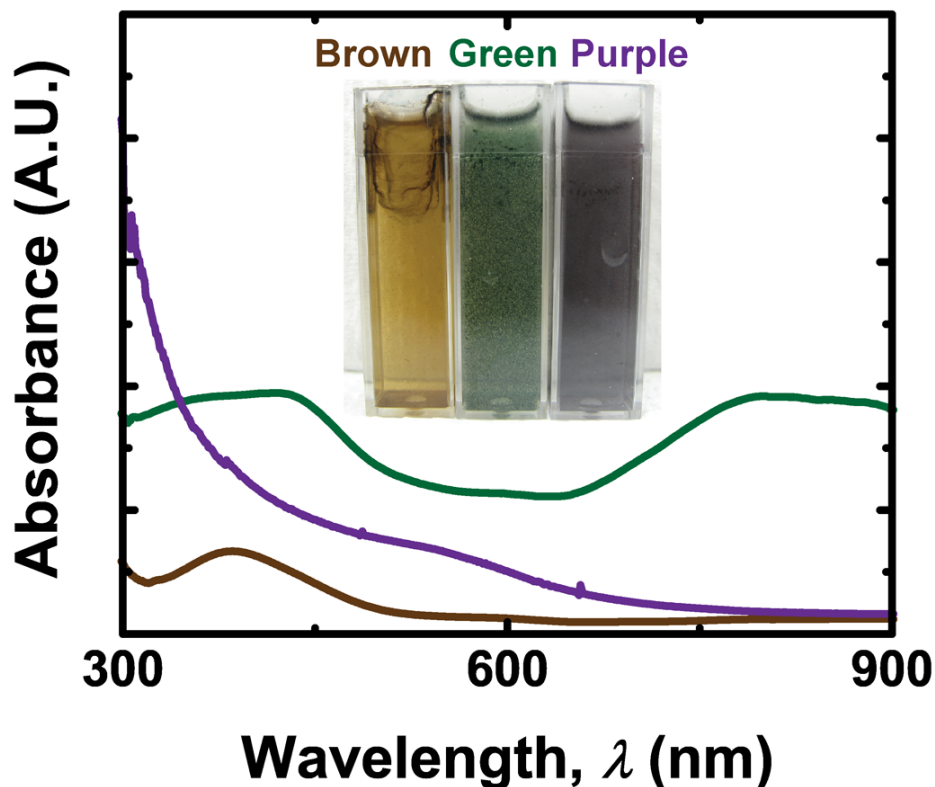


Figure S2. UV-vis spectra of pulverized suspensions of Hep-MA₀₅/PANI dual networks prepared using different ratios of [APS]₀ and [ANI]₀. The brown color in suspensions arises due to the presence of ANI oligomers that form when an insufficient oxidative driving force is present [ANI]:[APS] = 16:1. The green color observed in suspensions of Hep-MA₀₅/PANI hydrogel networks suggests the presence of electronically conductive emeraldine salt. This form of ANI is present for a proper balance between ANI precursors and oxidative potential is achieved for [ANI]:[APS] = 8:1. Lastly, pulverized suspensions of Hep-MA₀₅/PANI dual networks prepared using conditions of [ANI]:[APS] = 1:1 produce a purple hue. This is indicative of the electrically insulating over-oxidized pernigraniline formed during ANI polymerization. It should be noted that the primary role of APS is the generation of free radicals to initiate PANI polymerization. Homolytic bond dissociation of APS produces radical sulfate anions which have a reduction potential of -2.43 V.¹ This is much larger in magnitude compared to oxygen, which has a reduction potential of -0.13 V. Therefore, the APS concentration is the primary driving force for oxidative polymerization.

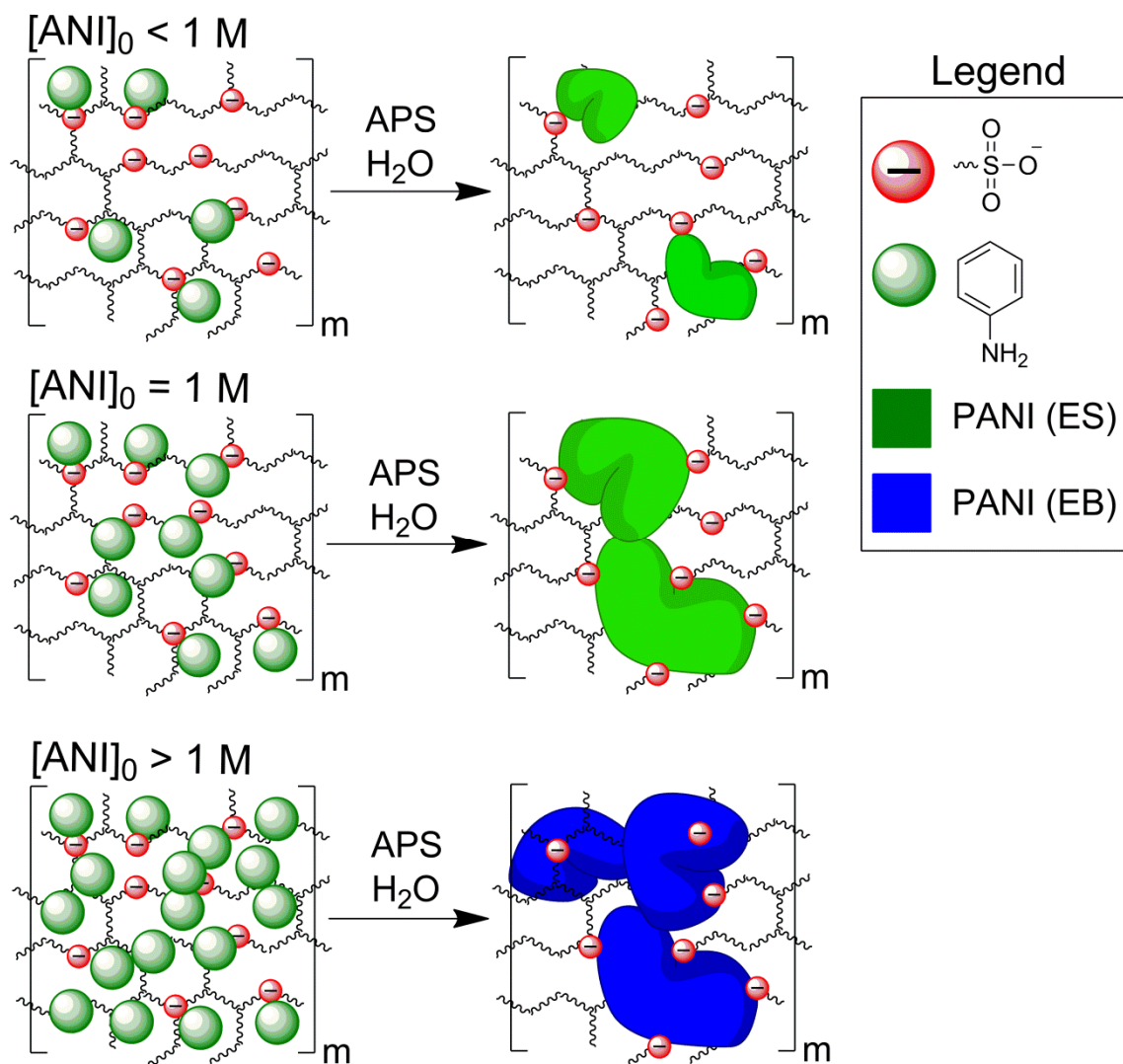


Figure S3. Proposed Model for Non-Monotonic Electrical Conductivity in Hep-MA/PANI Hydrogels. Hep-MA₀₅ gels loaded with ANI monomer ($[\text{ANI}]_0 = 1 \text{ M}$) and polymerized using APS at a ratio of $[\text{ANI}]_0:[\text{APS}]_0 = 8:1$ produced electrically conducting emeraldine salt (ES). Significant deviations from $[\text{ANI}]_0 = 1 \text{ M}$ form electronically insulating PANI networks. PANI structures formed using $[\text{ANI}]_0 < 1 \text{ M}$ lacked sufficient ANI to create percolating networks. PANI structures formed using $[\text{ANI}]_0 > 1 \text{ M}$ produced percolating PANI structures that are not sufficiently doped. The electrically insulating emeraldine base increases the impedance of the spectra as recorded by EIS. Altering the value of $[\text{ANI}]_0$ is known to impact the self-assembly of PANI structures during polymerization.²⁻⁴

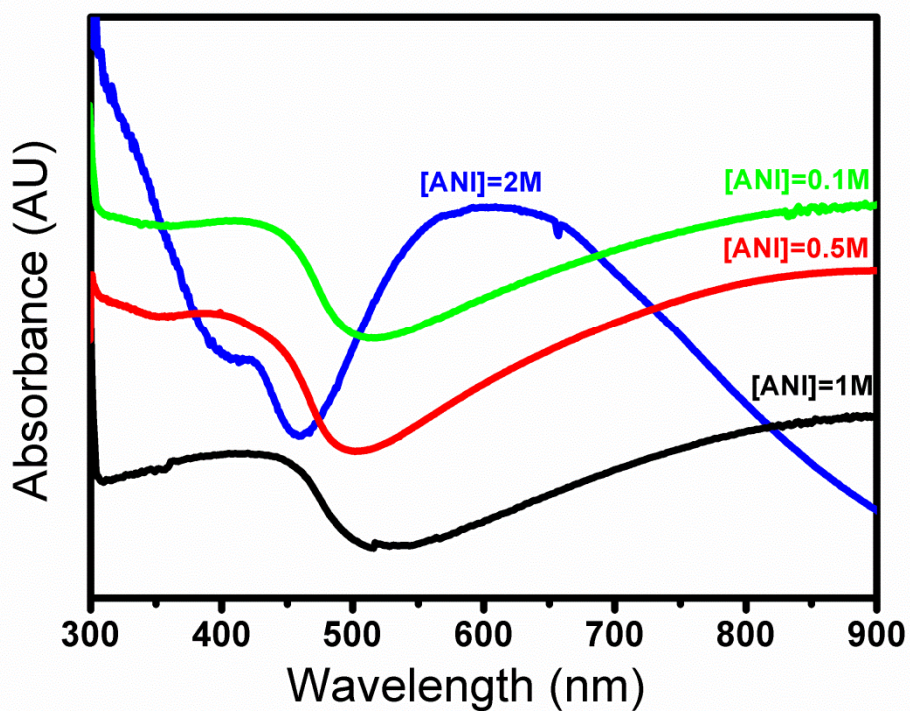


Figure S4. UV-vis Spectroscopy of secondary PANI networks formed within Hep-MA/PANI Hydrogels as a Function of $[ANI]_0$. PANI is maintained in the emeraldine salt state. The features of UV-vis spectra of Hep-MA/PANI networks formed using $[ANI]_0 < 2M$ are consistent with previous reports of PANI in the emeraldine salt.³

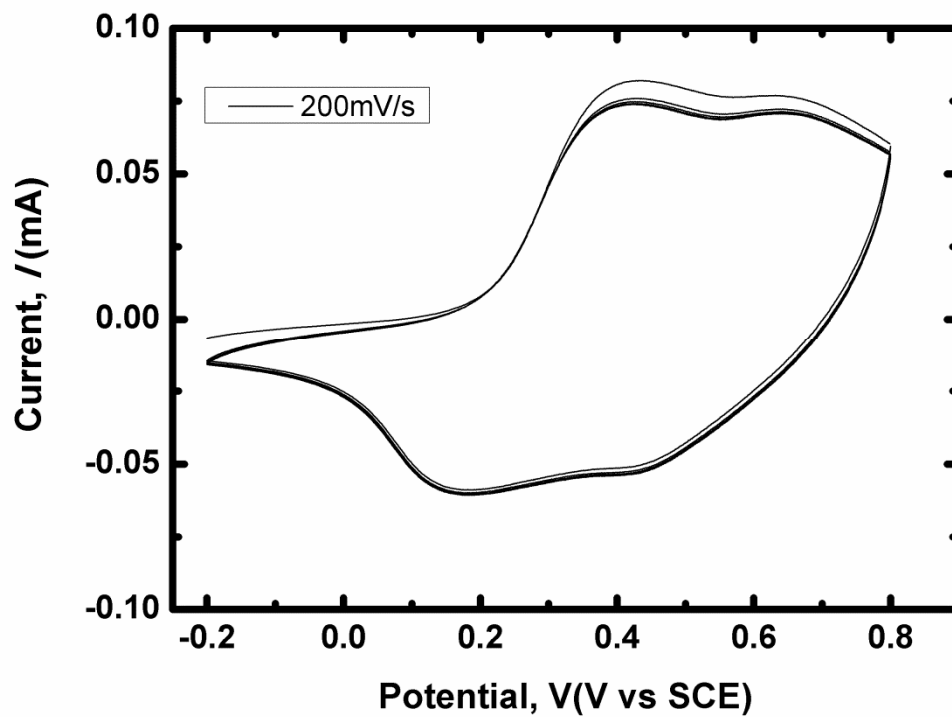


Figure S5. Cyclic voltammetry sweeps (>4 cycles) of Hep-MA₀₅/PANI hydrogel networks. These data indicate that redox properties of PANI in primary hydrogel networks are stable and repeatable.

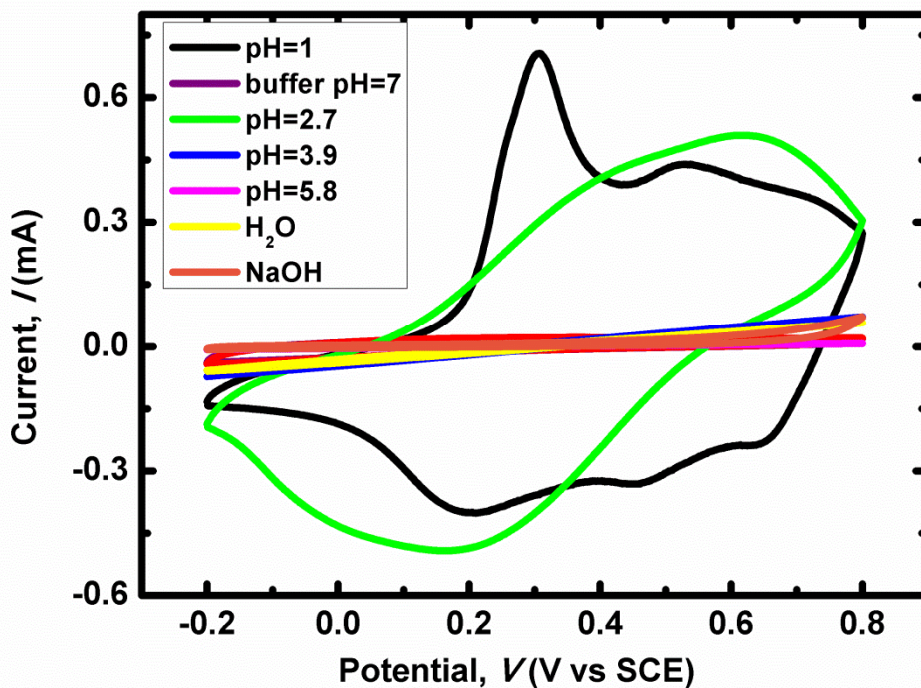


Figure S6. Cyclic voltammety sweeps (second cycle) of Hep-MA₀₅/PANI hydrogel networks in buffers of different pH values. The redox activity of PANI is dependent on the pH of the buffer. Two pairs of cathodic/anodic peaks are present at pH = 1. These features become attenuated as the pH of the buffer increases to 2.7. These features are abolished as the pH value of the buffer increases to >3.9.

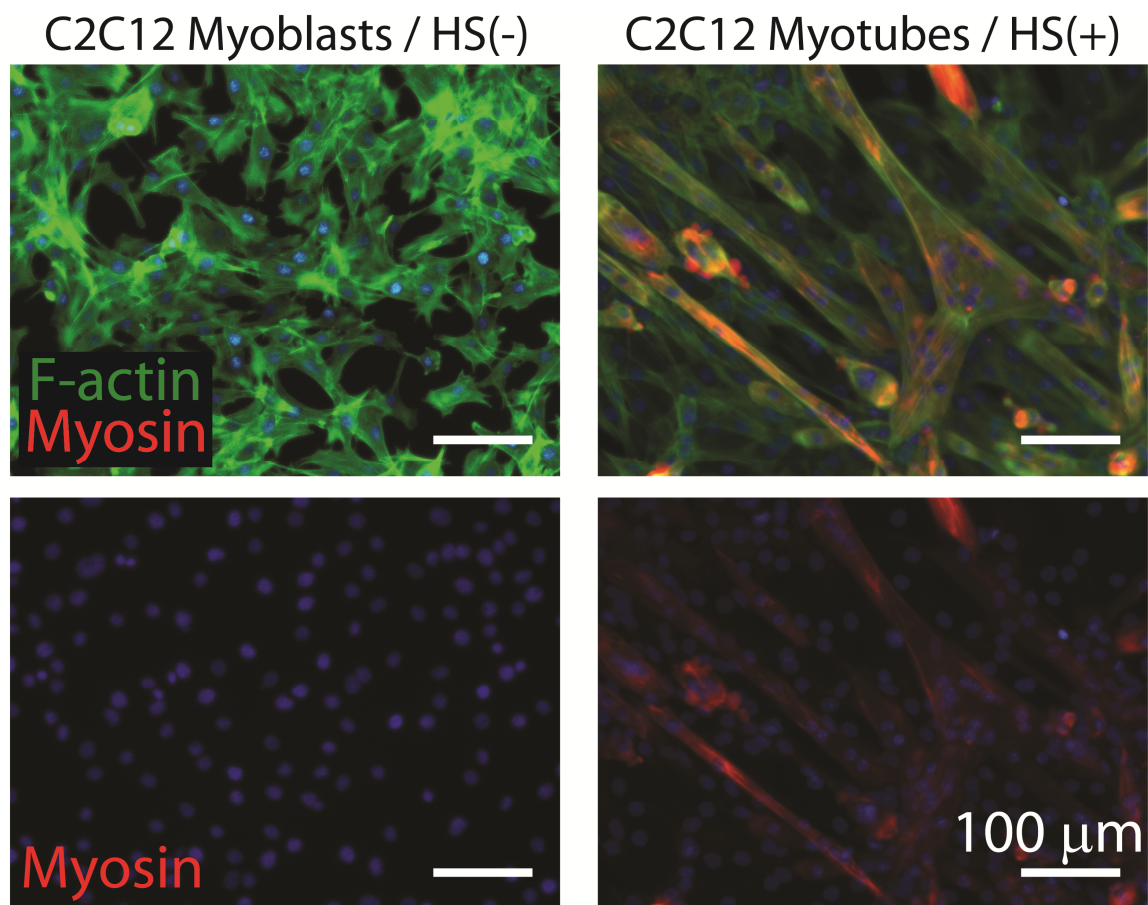


Figure S7. Morphology of C2C12 myoblasts and differentiated C2C12 myotubes on polystyrene substrates. C2C12 myoblasts cultured on rigid polystyrene substrates exhibit increased spreading compared to cells cultured on HepMA/PANI gels. A similar trend is also observed in myotubes. These trends are comparable to C2C12 cells cultured on various biomaterials substrates.⁵ Scale bars represent 100 μm in all panels.

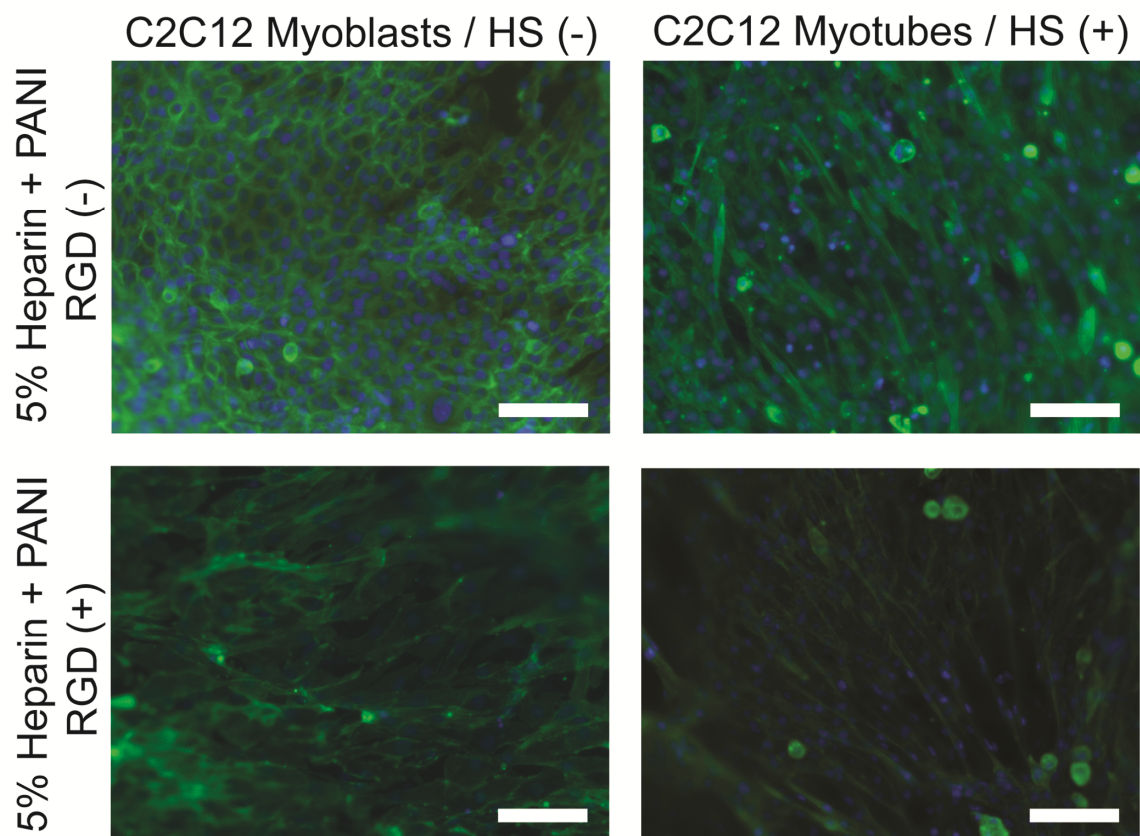


Figure S8. Hep-MA₀₅/PANI dual networks capable of promoting adhesion, proliferation, and differentiation of excitable cells. C2C12 myoblasts adhered to Hep-MA₀₅/PANI substrates without the need for surface treatments and (a) proliferated into confluent monolayers after 3 weeks in proliferation medium (HS (-)). Fluorescent micrographs (F-actin, FITC; DAPI) of C2C12 myotubes after 5 days in differentiation medium HS (+) suggest that Hep-MA₀₅/PANI substrates were permissive of C2C12 differentiation as assessed by tubular morphology. Scale bars represent 100 μ m in all panels.

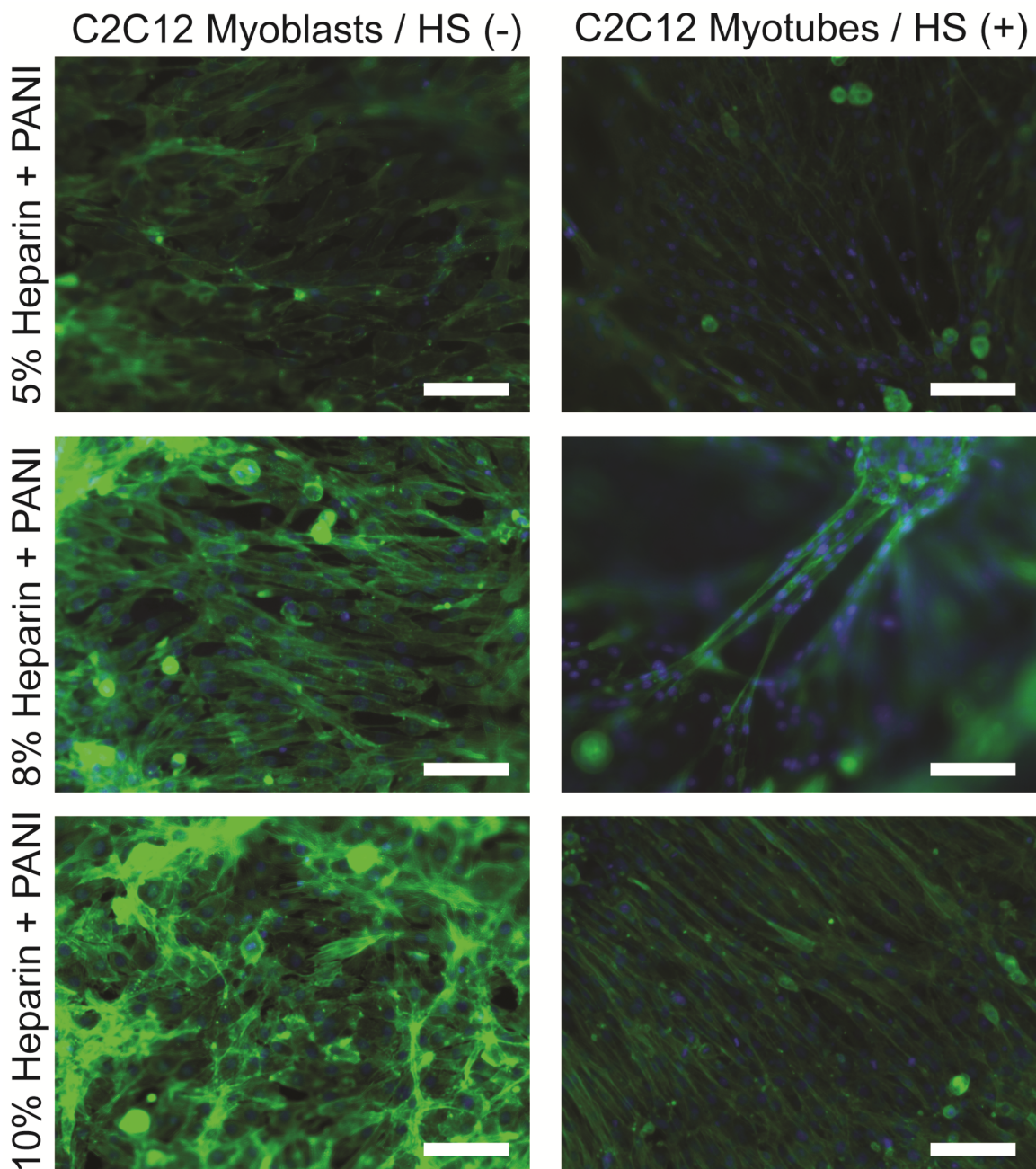


Figure S9. Fluorescent micrographs of C2C12 cells cultured on substrates composed of Hep-MA/PANI dual networks for Hep-MA₀₈ and Hep-MA₁₀. Populations of C2C12 cells cultured on these substrates exhibited a comparable morphology to those cultured on Hep-MA₀₅/PANI networks both before and after differentiation into mature myotubes. Scale bars represent 100 μ m in all panels.

Additional References

1. Gau, B. C.; Chen, H.; Zhang, Y.; Gross, M. L., Sulfate Radical Anion as a New Reagent for Fast Photochemical Oxidation of Proteins. *Anal. Chem.* **2010**, *82*, 7821-7827.
2. Park, M.-K.; Onishi, K.; Locklin, J.; Caruso, F.; Advincula, R. C., Self-Assembly and Characterization of Polyaniline and Sulfonated Polystyrene Multilayer-Coated Colloidal Particles and Hollow Shells. *Langmuir* **2003**, *19*, 8550-8554.
3. Wei, Z.; Wan, M.; Lin, T.; Dai, L., Polyaniline Nanotubes Doped with Sulfonated Carbon Nanotubes Made Via a Self-Assembly Process. *Adv. Mater.* **2003**, *15*, 136-139.
4. Long, Y.; Zhang, L.; Ma, Y.; Chen, Z.; Wang, N.; Zhang, Z.; Wan, M., Electrical Conductivity of an Individual Polyaniline Nanotube Synthesized by a Self-Assembly Method. *Macromol. Rapid Comm.* **2003**, *24*, 938-942.
5. Lan, M. A.; Gersbach, C. A.; Michael, K. E.; Keselowsky, B. G.; Garcia, A. J., Myoblast Proliferation and Differentiation on Fibronectin-coated Self-assembled Monolayers Presenting Different Surface Chemistries. *Biomaterials* **2005**, *26*, 4523-4531.