Mechanical Characterization of Human Brain Tissue and Soft Dynamic Gels Exhibiting Electromechanical Neuro-Mimicry

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S.1 Materials and Methods

Chemicals and Reagents

Hyaluronic acid sodium salt from Streptococcus zooepidemicus (1.5-1.8 MDa), vinyl imidazole, benzyl bromide, poly(ethylene glycol) methacrylate (PEGMa; 500 Da), poly(ethylene glycol) dimethacrylate (PEGDMa; 550 Da), and 2-Hydroxy-4'-(2-hydroxyethoxy)-2- methylpropiophenone (98%) were purchased from Sigma Aldrich (Milwaukee, WI, USA) and used as received unless stated otherwise. DMEM/F12 cell culture media and other culture reagents were purchased from Invitrogen and sterile filtered prior to use.

Synthesis of 1-Benzyl-3-Vinylimidazolium Bromide

The imidazolium monomer was synthesised as previously reported.[22] Briefly, benzyl bromide (0.05 M) was added drop wise into a solution of vinyl imidazole (0.05 M) in diethyl ether at 0 °C, and after 10 minutes, the reaction flask was brought to room temperature and left for 16 hours. The crude was filtered, washed with diethyl ether, and dried under vacuum. Yield: 99%. ^{1}H NMR (400 MHz, D_2O, 298 K, δ, ppm): 10.68 (s, 1H, -N$^+$-C**H**-N-), 7.17–7.91 (m, 7H, aromatics), 7.22 (dd, 1H,-N-C**H**=CH_2), 5.89 (dd, 1H,-N-CH=CH-H_{trans}), 5.52 (s, 2H, Ph-CH_2-N$^+$-), 5.16 (dd, 1H, -N-CH=CH-H_{cis}).

Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III HD NMR spectrometer (400 MHz) and are reported as follows: chemical shift δ (ppm) (multiplicity, coupling constant J (Hz), number of protons, assignment). D_2O ($\delta_H = 4.79$ ppm) and dimethylsulfoxide (DMSO, $\delta_H = 2.50$ ppm) were used as an internal standard. Chemical shifts are reported in ppm to the nearest 0.01 ppm for ^{1}H and the nearest 0.1 ppm for ^{13}C. Photoirradiation was performed in an LZC LED/VIS Photoreactor (Luzchem, Canada) using 10 UV lamps (350 nm, power density of 4.8 mW$\cdot$ cm^{-2}). All rheological sweeps were conducted on an AR-G2 Rheometer (TA Instruments, New Castle, DE, USA) with a 8 or 20 mm parallel plate geometry. Temperature was recorded using the built in platinum resistance thermocouple in standard AR-Series Peltier lower plates. Statistical significance and p-values were determined using ANOVA with Tukey post-test.

General procedure for the synthesis of supramolecular hydrogels

PEGMA (105.5 mg, 0.211 mmol), VIm (2.8 mg, 10.55 μmol) and CB[8] (7.0 mg, 5.28 μmol) were mixed with 1 mL of reaction medium (10.55 wt%) in a seal-capped glass vial at room temperature. In darkness, the reaction vial was bubbled with N_2 for 30 min. Then, photoinitiator (54 μL of a 1.5 mg/mL slt, 0.36 μmol) were added and the reaction vial was bubbled with N_2 for additional 5 min. Next, the sample was stirred in a photoreactor ($\lambda =$ 360 nm) at room temperature for 6 h. The reaction medium varied between MilliQ water and HA-containing aqueous solutions.

General procedure for the synthesis of covalent hydrogels and blends

PEGMA (105.5 mg, 0.211 mmol), PEGDMA (5.8 mg, 10.55 μmol), VIm (2.8 mg, 10.55 μmol) and CB[8] (7.0 mg, 5.28 μmol) were mixed with 1 mL of reaction medium (10.55 wt%) in a seal-capped glass vial at room temperature. In darkness, the reaction vial was bubbled with N_2 for 30 min. Then, photoinitiator (54 μL of a 1.5 mg/mL slt, 0.36 μmol) were added and

the reaction vial was bubbled with N_2 for additional 5 min. Next, the sample was stirred in a photoreactor (λ = 360 nm) at room temperature for 6 h. The reaction medium varied between MilliQ water and HA-containing solutions (c = 0.1, 0.2 and 5 mg/mL).

Gel Rheology

All rheological sweeps on hydrogels were conducted on an AR-G2 Rheometer (TA Instruments, New Castle, DE, USA) with either 8 or 20 mm parallel plate geometries. Zero gap, rotational mapping (precision bearing mapping; 2 iterations), geometrical inertia, and friction calibrations were done prior to each use of the rheometer. Hydrogel samples were loaded onto the rheometer with a 600-1000 μm loading gap. A moist drape was placed over the rheometer chamber to create a moist environment to prevent dehydration. Amplitude sweeps were conducted to ensure the probing conditions were within the linear viscoelastic region of the sample.

Impedance Spectroscopy

Hydrogels were cast between two copper plate electrodes separated by a Teflon spacer (0.5 mm), where the active area was fixed at 0.85 cm^2. Potentiostatic electrochemical impedance spectroscopy (PEIS) spectra were recorded using a BioLogic VSP Potentiostat from 1 MHz to 100 mHz with an applied amplitude of 20 mV_{rms} relative to the open circuit potential. Raw Nyquist plots were fit using the EC-Lab ZSim Software (BioLogic) to a modified Randles circuit model, $R_s + Q/R_{ct}$, where R_s is the solution resistance, Q is a constant phase element acting as a non-ideal capacitor, and R_{ct} is the charge transfer resistance. Conductivity was calculated from the modeled R_s values using the cell geometry according to Eq. 3.1, where K is the cell constant, l is the gel thickness and A is the active area.

$$\sigma = \frac{l}{R_s A} = \frac{K}{R_s} = \frac{0.06cm^{-l}}{R_s} \tag{1}$$

Biological Assays

Cytotoxicity of gels was screened against normal adult mouse neural stem cells (ANS) derived from the subventricular zone of adult black 6 mice. Cells were cultured in DMEM/HAMS-F12 (500 mL; Sigma D8347) with glucose (7.25 mL; Sigma G8644), MEM NEAA 100x (5 mL; LifeTech/Gibco 11140-035), penicillin/streptomycin (5 mL; LifeTech/Gibco 15140-122), bovine serum albumin solution (800 μL; LifeTech/Gibco 15260-037), bMercETOH 50 mM solution (1 mL; LifeTech/Gibco 31350-010), B27 Supplement 50x (5 mL; LifeTech/Gibco 17504-044), N2 Supplement 100x (2.5 mL; LifeTech/Gibco 17502-048), with mouseEGF (peprotech) at 10 ng/ml, humanFGF (peprotech) at 10 ng/ml, and Trevigen Cultrex 3D Laminin1 1 μg/ml. Cells were cultured and passaged on laminin coated T25 flasks. Cells were plated onto laminin coated 96-well plates to generate confluence curves and for immunohistochemistry staining. The dual network gel, which has a covalent network, was not observed to dissolve in cell culture media over time, while the purely supramolecular gel did. To incubate cells with the dual network gel, the samples were granularized into small pieces with a stainless steel spatula for 5 min before infusing into the media. As a control, the supramolecular gel was dissolved in cell culture media at the noted concentrations and screened with these cells to account for potential heterogeneity or concentration gradients in the covalent gel/media solution. Confluence curves were generated using an IncuCyte® S3 Live Cell Analysis System. Percent confluence was calculated using Standard Analysis (n=4/well). Cells were fixed in 4% paraformaldehyde solution for immunohistochemistry staining. Cells were blocked in donkey serum. Rabbit primary and donkey anti-rabbit secondary antibodies were used for cas3; goat primary and donkey anti-goat antibodies were used for sox2. Nuclei were stained with DAPI. Confocal images were taken on an Operetta CLS High Content Analysis Instrument (Perkin Elmer). Harmony software package was used for identification and quantification of cell expression.

Ethical Approval

The analysis of resected tissue and brain MRI acquisition was approved by the Health Research Authority (England) and the Cambridge University Hospitals NHS Foundation Trust (IRAS ref: 238683). Written, informed consent from patients was obtained.

Tissue Resection

Freshly resected tissue was taken immediately from the operating room, sliced into disk shapes 8 mm in diameter, and transported to the rheometer on ice. The entire process took less than 30 min to complete.

Tissue Rheology

All rheological sweeps were conducted on an AR-G2 Rheometer (TA Instruments, New Castle, DE, USA) with a 8 mm parallel plate geometry. Zero gap, rotational mapping (precision bearing mapping; 2 iterations), geometrical inertia, friction, UV intensity, and gap temperature compensation calibrations were done prior to each use of the rheometer and its UV accessory. Tissue samples were loaded onto the rheometer with a 400–800 μm loading gap. A water trap was placed to prevent dehydration and as a safety precaution against splatter. It was observed that with the water trap, samples could be analysed up to 1 h before dehydration noticeably affected the sample. Tissue samples were stored with 1 mL of DMEM/F12 with L-Glutamine medium containing glucose (0.3%), penicillin/streptomycin (50μg/mL), Apo-Transferrin (0.1 mg/mL), Progesterone (20 nM), sodium selenite (30 nM), putrescine (60 μM), insulin (25 μg/mL), EGF (20 ng/mL), and laminin (20 ng/mL) at 4 °C until analysis. For the time-resolved mechanical analysis, measurements were all taken at 20 °C because it was observed the tissue rapidly dehydrated at temperatures above 25 °C after 24 h. Such observations were also made for tissue samples that were frozen then thawed, independent of time (data not shown).

Statistical Analysis

Harmony software package was used for identification and quantification of cell expression. DAPI-stained nuclei were identified for pre-processing images to discrete cells. Data presented was mean $\pm$ standard deviation. Three replicates were used for immunohistochemistry and PEIS measurements. Three replicates and four images per replicate were used for brightfield image confluence quantification.

S.2 Supporting Figures

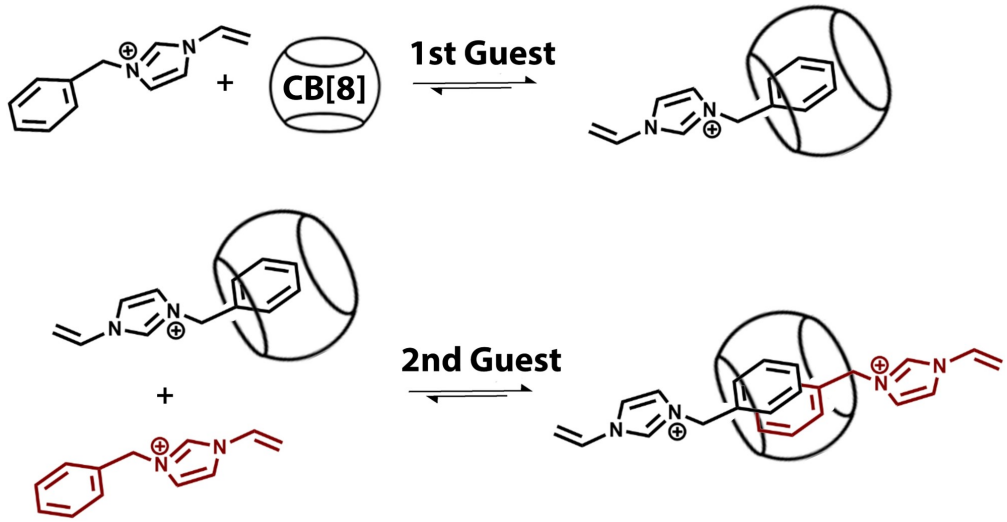

Figure S1: Sequential binding of two benzyl vinyl imidazolium guests with cucurbit[8]uril to form a 2:1 homoternary host-guest complex.

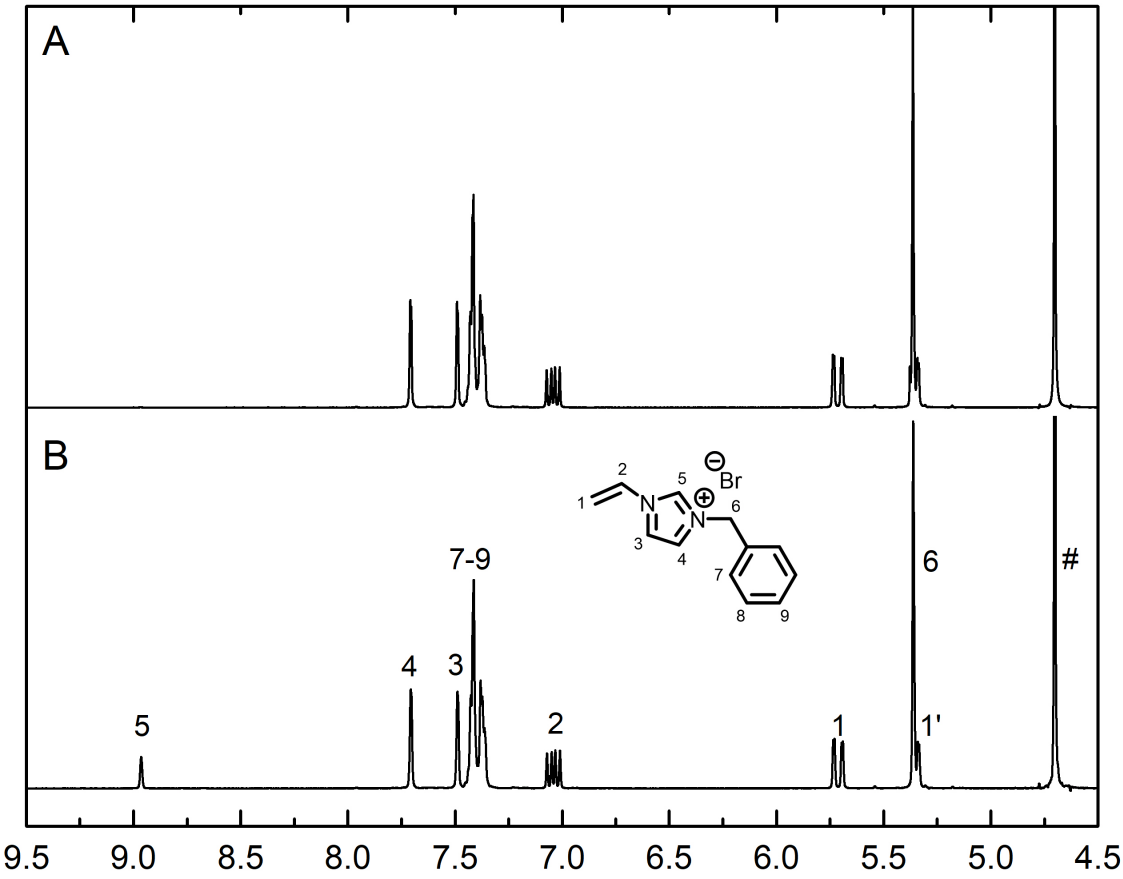

Figure S2: ^{1}H NMR data of benzyl vinyl imidazolium (VIm) monomer solution (A) and after 6 h of photopolymerization (B). Residual solvent peaks: # H_2O.

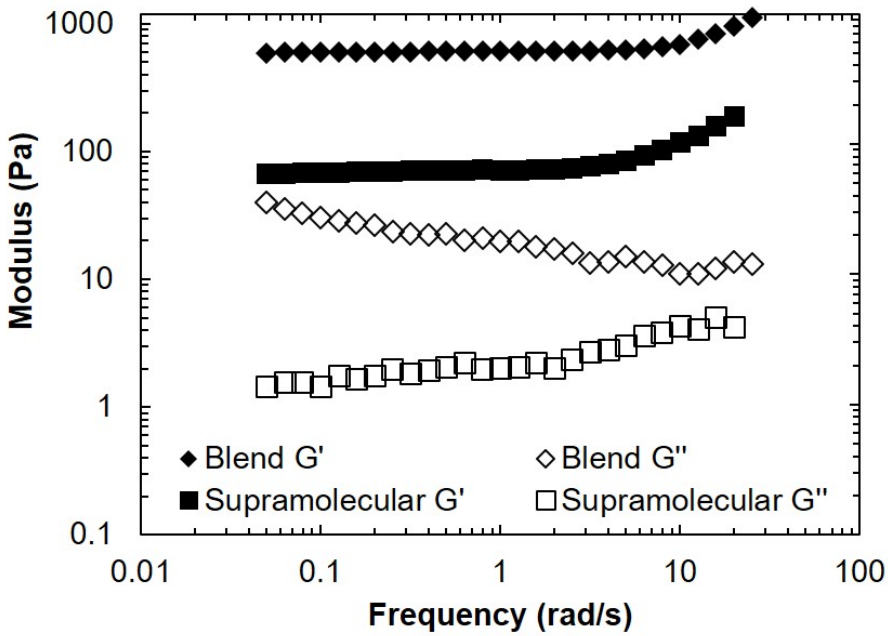

Figure S3: Oscillatory frequency sweeps on supramolecular network and dual network blended with hyaluronic acid.

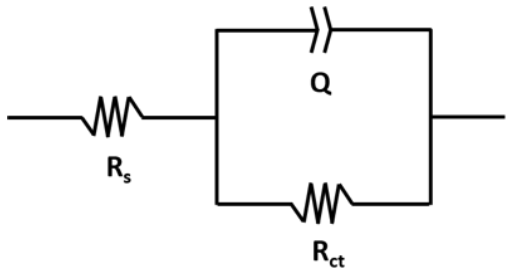

Figure S4: PEIS equivalent circuit containing a resistor R_S in series with a parallel circuit comprising a constant phase element Q and resistor R_{ct}.

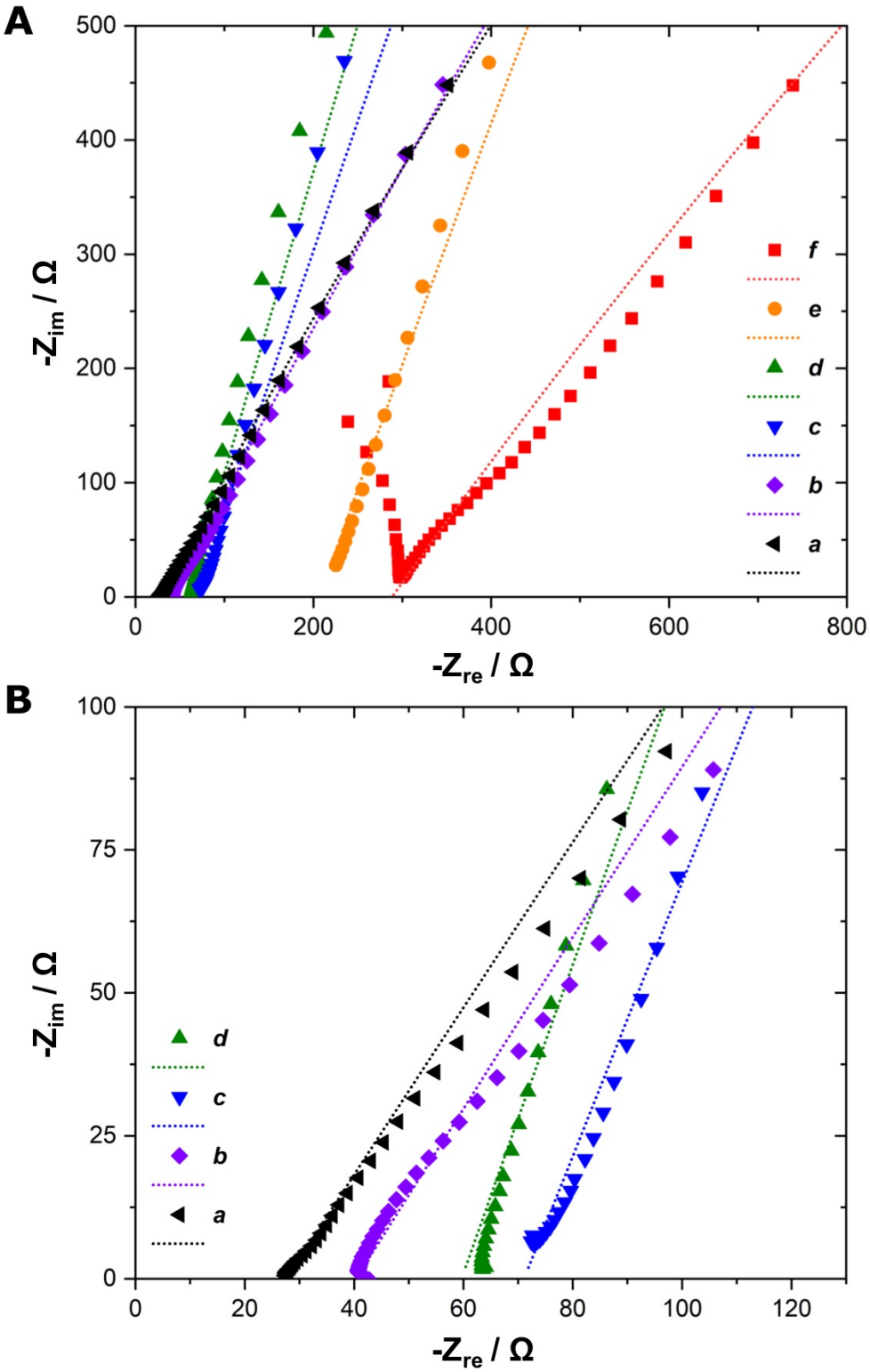

Figure S5: Spectra from PEIS measurements. (A) shows all samples from Fig. 1F: ***a*** Milli-Q water, ***b*** covalent OEG network with no ionic species, ***c*** VIm/OEG system with no CB[8], ***d*** OEG system with HA, ***e*** VIm/OEG system with CB[8], ***f*** VIm/OEG system with HA. (B) shows samples ***a***-***d***.

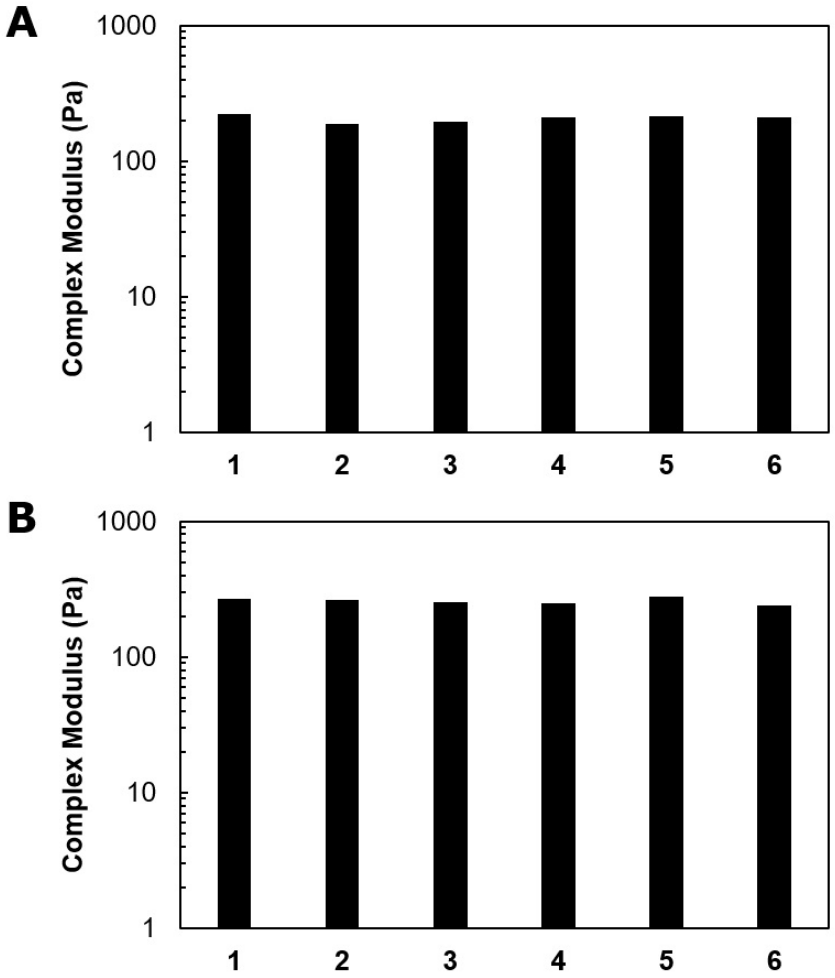

Figure S6: Shear resistance of (A) healthy tissue and (B) dual network gel. Plotted values **1-6** are probing conditions (1% and 1 rad/s). **2-6** after cycles of high shear and strain (100% and 100 rad/s).

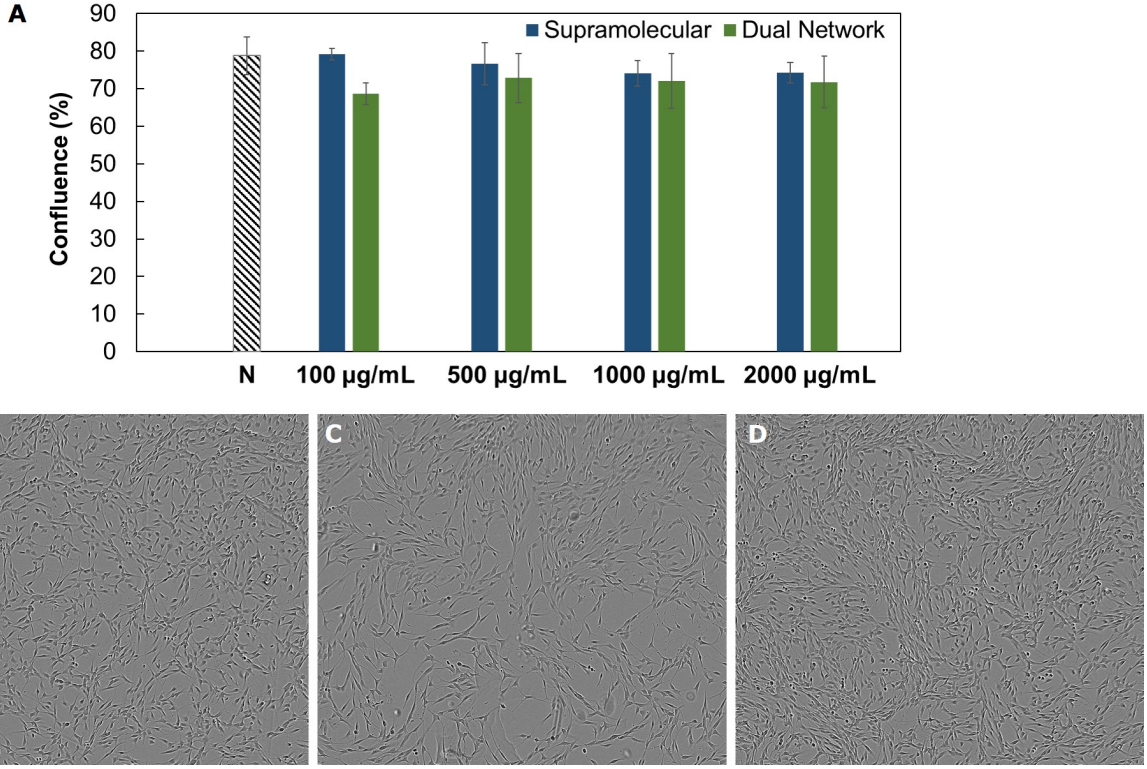

Figure S7: Adult mouse neural stem cell confluence at 48 h. (A) Terminal confluence of cells with the specified material and concentration. No reduction the rate of growth or the terminal confluence was observed. (B-D) Brightfield images of (B) no material, (C) supramolecular gel, and (D) dual network gel at 2000 μg/mL. Cell morphology for (C-D) are qualitatively normal for adult mouse neural stem cells. Scale bar = 200 μm. $n = 3$; mean $\pm$ standard deviation.

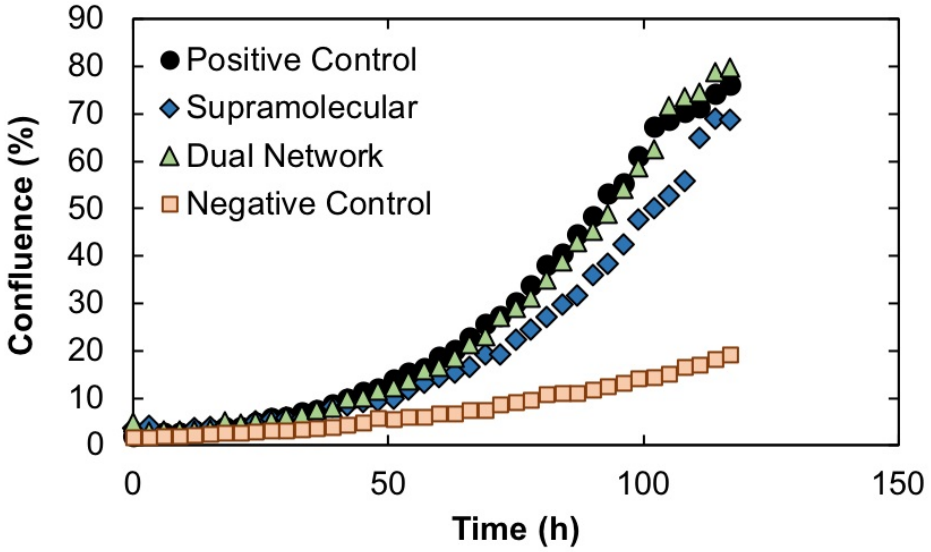

Figure S8: Adult mouse neural stem cell confluence over time. The rate of growth for the control N was unchanged after addition of 2000 μg/mL of supramolecular or dual network gels. Hydroxyethyl cellulose-dibenzofurany^{l4} was used as a negative control.

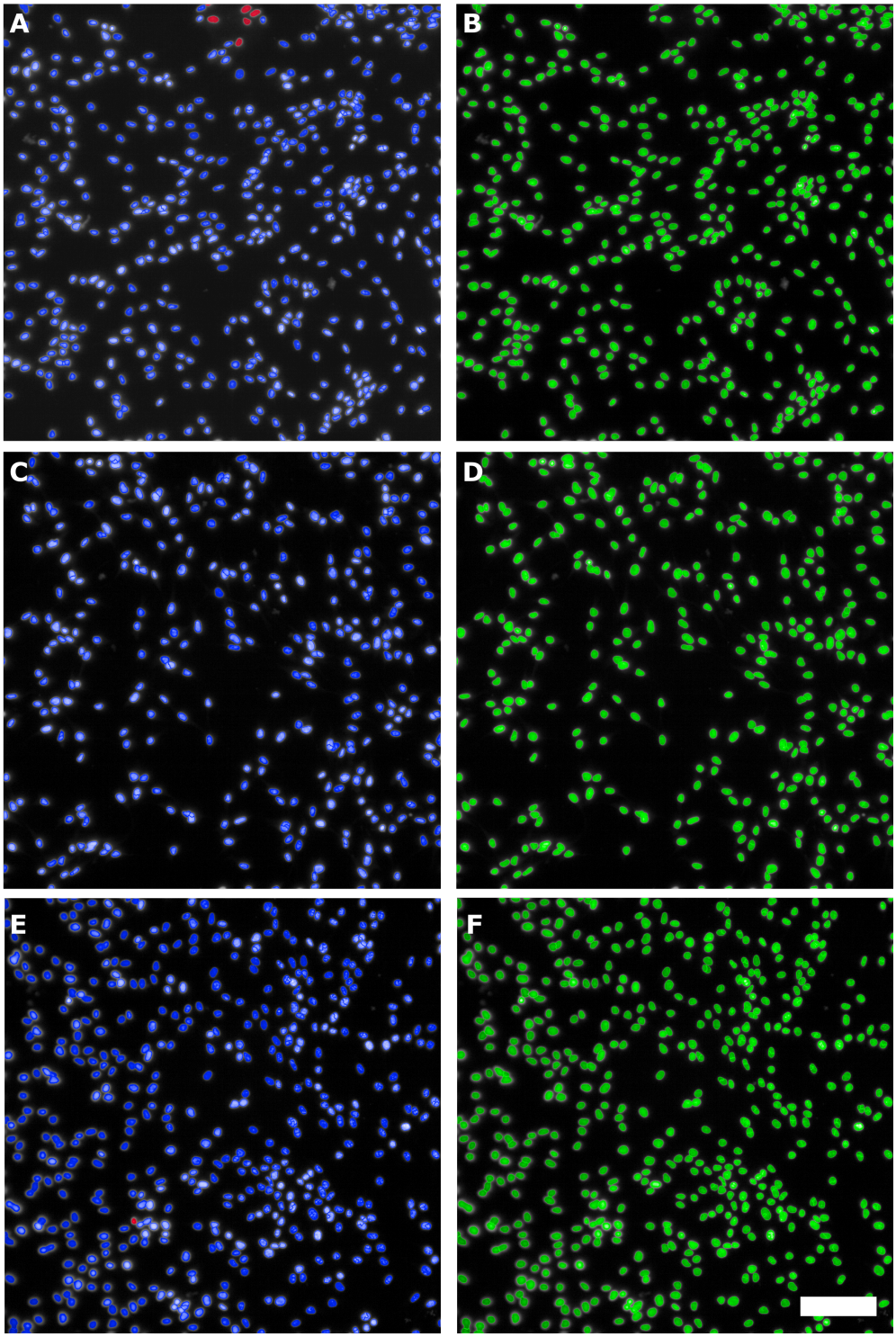

Figure S9: Immunohistochemistry of adult mouse neural stem cells; blue = DAPI, green = sox2, red = cas3. A-B: control, C-D: supramolecular gel, E-F dual network gel at 2000 μg/mL. Scale bar = 100 μm.

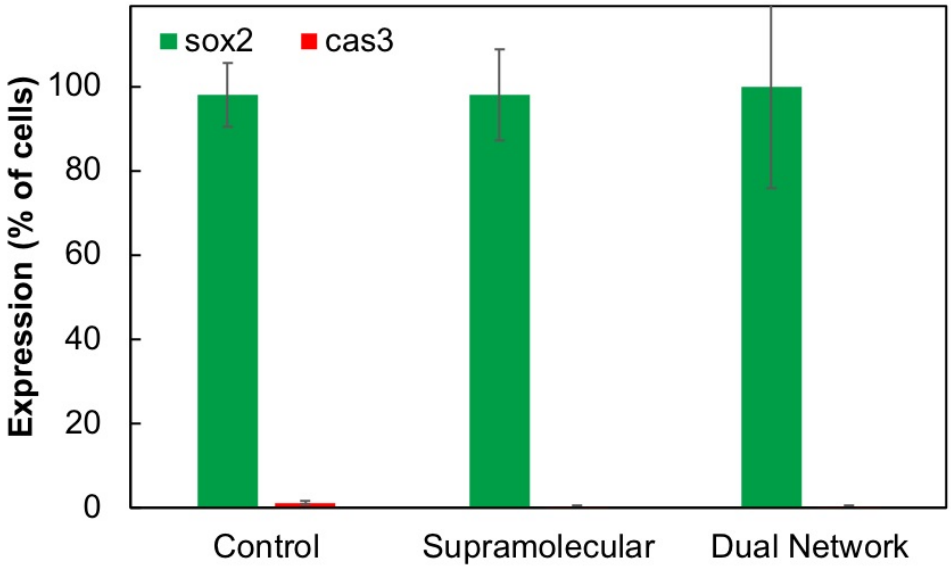

Figure S10: (F) Quantitative analysis of sox2/cas3 stains showin in Fig. S9. Incubation occured with 2000 μg/mL of material. $n = 3$; mean $\pm$ standard deviation.

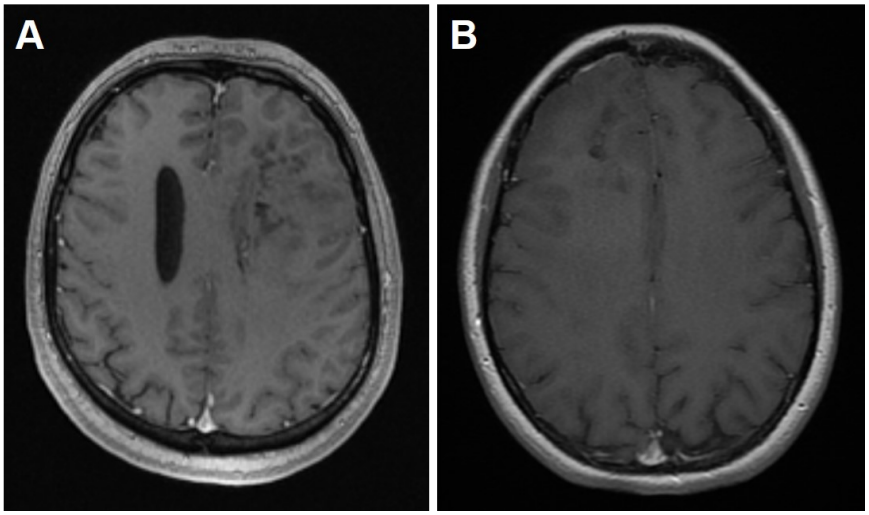

Figure S11: Magnetic resonance imaging of patients' brains analysed in this study. (A) 44 year old male Oligodendroglioma, IDH-mutant, WHO-2016 grade II. (B) Anaplastic oligodendroglioma, IDH-mutant, WHO grade III.