Electronic Supplementary Material (ESI) for Soft Matter.

Electronic Supplementary Information (ESI) for

Liquid crystal elastomer foams with elastic properties specifically engineered as biodegradable brain tissue scaffolds

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Contents:

All compounds were synthesized according to previously reported methods.[1-8] For detailed synthesis of α-chloro-ε-caprolactone and cholesteryl 5-hexynoate see references 5 and 6.

1. Synthesis of monomers and reaction intermediates

a. Synthesis of 6-arm-star-poly(ε-CL-co-α-Cl-ε-CL-co-*D,L*-LA), 6SBC-αCl[7]

The general procedure for the synthesis of star copolymer of ε-CL and *D,L*-LA, developed by Amsden *et al.*[1, 9] was followed and slightly modified as follows: In a dry, and silanized flask, dipentaerythritol (1.27 g, 0.005 mol) was mixed with ε-CL (4.56 g, 0.04 mol) and α-Cl-ε-CL (1.48 g, 0.001 mol) and mixed using a vortex mixer for about a minute, then *D,L*-LA (7.21 g, 0.05 mol) was added. The solution was mixed again using the vortex mixer; the flask was then flushed with nitrogen and placed in oven at 140 °C until *D,L*-LA was completely melted. The contents were mixed on a vortex mixer and tin(II) 2-ethylhexanoate (60 mL, 0.185 mmol) was added and mixed one last time. The ampoule was vacuum-sealed and was placed in oven for 24 hours at 140 °C under nitrogen atmosphere. A highly viscous brown colored liquid was obtained and dissolved in cold dichloromethane. The solution was then allowed to precipitate in cold methanol and finally centrifuged for 15 minutes at 10,000 rpm to remove the non-dissolved residue. The supernatant was collected, and solvent was removed under reduced pressure and dried under high vacuum before further characterization. The purified product was characterized using proton 1 H NMR and IR. ^{1}H NMR ($CDCl_3$, 400 MHz) δ[ppm]: 5.42–5.04 (m, COC*H*CH_3), 4.44–4.25 (m, C*H*Cl), 4.23–4.11 (m, CH_2O), 4.10–4.04 (t, *J* = 4.6 Hz, CH_2O), 3.69–3.57 (m, C*H*CH_2), 3.10-2.62 (broad, s, OH), 2.48-2.37 (t, *J* = 4.5 Hz, α–H), 2.37-2.26 (t, *J* = 5.1 Hz, α–H), 1.78–1.25 (m, CH_2, CH_3). FT-IR (KBr) 1/λ [cm^{-1}]: 2932 (s), 2860 (m), 1720 (s), 975 (s), 884, 787 (m).

b. Synthesis of 6-arm-star-poly(e-CL-co-α-N_3-ε-CL-co-*DL*-LA), (6-SBC-αN_3).[5, 6, 8]

In a round bottom flask 6SBC-αCl (3.8 g, 15 mmol) was dissolved in dry dimethylformamide (DMF) under nitrogen. Then, sodium azide (0.97 g, 15 mmol) was added and the contents were allowed to react for four days at 55 °C. DMF was removed under reduced pressure and the remaining mixture was dissolved in toluene. The solution was then centrifuged (10,000 rpm for 15 min) thrice to remove the salt formed. Then, the supernatant was collected and evaporated under reduced pressure to yield color polymer and dried under high vacuum before further characterization. The final product was characterized using ^{1}H NMR and FT-IR. ^{1}H NMR ($CDCl_3$, 400 MHz) δ[ppm]: 5.34–5.00 (m, COC*H*CH_3), 4.56–4.23 (m, C*H*CN_3), 4.23– 4.11 (m, CH_2O), 4.11–4.04 (t, *J* = 4.6 Hz, CH_2O), 3.67 (broad, s, azide-H), 3.67–3.51 (m, C*H*CH_2), 3.47– 3.33 (broad, s, OH), 1.76-1.61 (t, *J* = 4.5 Hz, α–H), 1.60-1.47 (t, *J* = 5.1 Hz, α–H), 1.46–1.32 (m, CH_2, CH_3). FT-IR (KBr) 1/λ [cm^{-1}]: 2932 (s), 2100 (s, azide), 1720 (s), 1450 (s), 954 (m), 861 (s), 733 (s), 696 (m).

c. Synthesis of 6-arm-star-poly(e-CL-co-α-cholesteryl 5-hexynoate-ε-CL-co-*DL*-LA), (6SBC-αCLC) by click reaction[5, 6, 8]

In a round bottom flask, 6SBC-αN_3 (1.93 g, 10 mmol) was dissolved in DMF: toluene (75:25). In a second step, cholesteryl 5-hexynoate (1.93 g, 4 mmol), copper iodide (0.095 g, 0.5 mmol) and triethylamine (0.10 g, 1 mmol) were added. The mixture stirred for three days at 40 °C under nitrogen. Then, the solvent was evaporated under reduced pressure. Cold methanol was added to precipitate out and remove unreacted materials and side products. The solution was centrifuged thrice at 10,000 rpm for 10 minutes and the supernatant was collected. This process of centrifugation was repeated three times and supernatant was collected at each time. The solvent was removed under reduced pressure and dried under high vacuum S9 before further characterization. The final product was characterized using ^{1}H NMR and FT-IR. ^{1}H NMR ($CDCl_3$, 400 MHz) d[ppm]: ^{1}H NMR ($CDCl_3$, 400 MHz) δ[ppm]: 7.31 (s,

C*H*≡C-triazole), 5.42–5.30 (m, C=C*H* cholesterol), 5.24–5.03 (m, COC*H*CH_3), 4.68–4.55 (m, O–C*H* cholesterol), 4.43– 4.22 (m, CH_2O), 4.21–4.09 (m, CH_2O), 4.08–4.00 (t, *J* = 4.4 Hz, CH_2O), 2.46–2.36 (m, COCH_2), 2.36– 2.25 (m, COCH_2), 2.06–1.91 (m, CH_2, CH_3), 1.89 (s, CH_3), 1.73–1.59 (d, *J* = 3.3 Hz, CH_2, CH_3), 1.61–1.44 (dd, J = 1.9, J = 1.8, CH_3), 0.70 (s, CH_3). FT-IR (KBr) 1/λ [cm^{-1}]: 3260 (s), 2920 (s), 1710 (s), 1460 (s), 1370 (s), 1240 (m), 1190 (s), 733 (s), 668 (s).

2. Thermal behavior: DSC and TGA of 6SBC-αCLC foam.

a. DSC

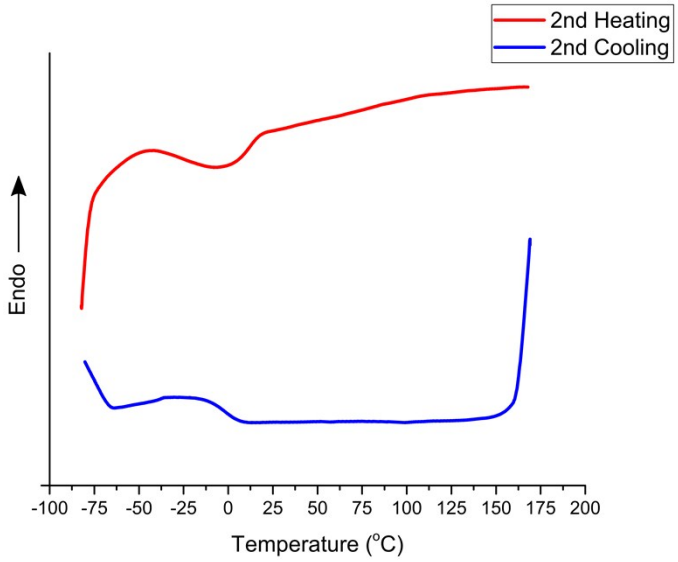

Figure S1. DSC thermogram of 6SBC-αCLC foam.

b. TGA

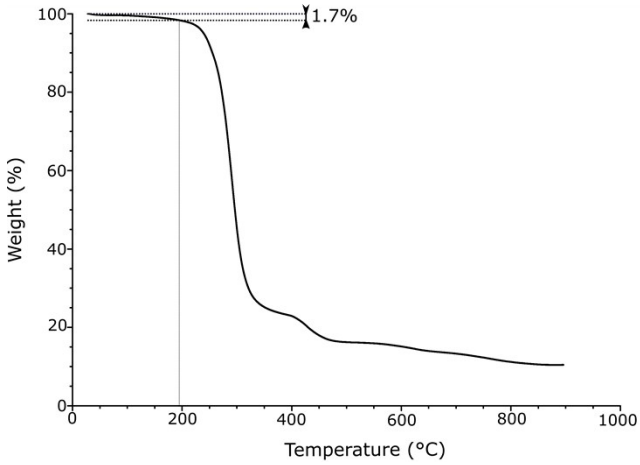

Figure S2. TGA of 6SBC-αCLC foam.

3. SEM pictures

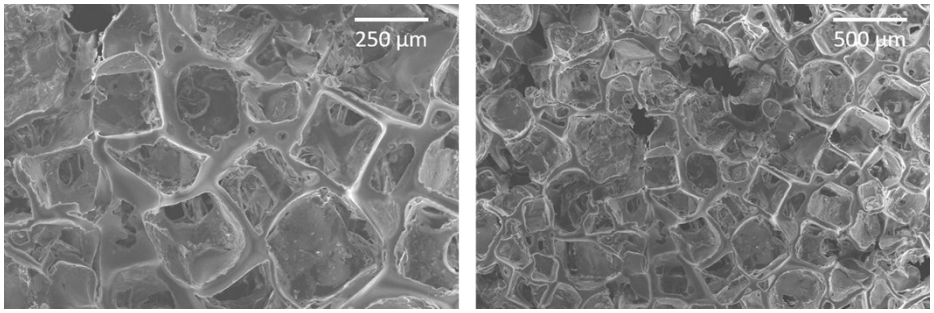

Figure S3. SEM images of 6LCE-α foam.

4. Porosity measurement

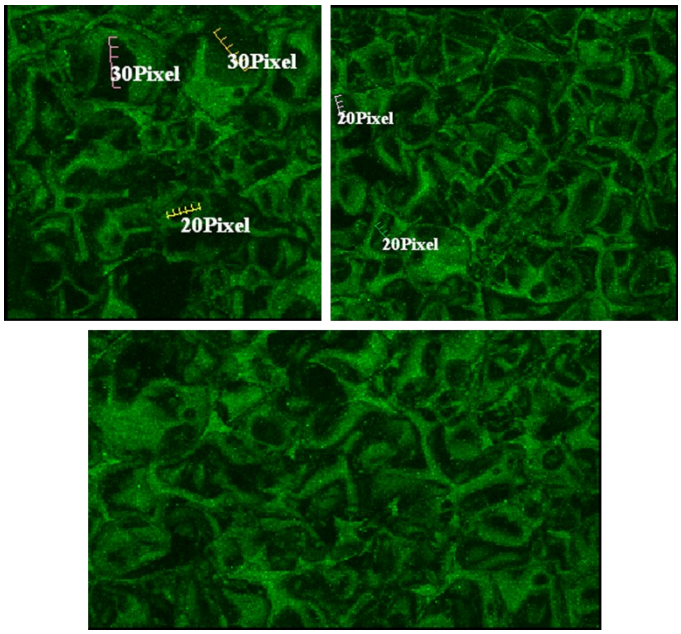

Figure S4. Example of projection of 3D confocal image used to determine the porosity of 6arm-αCLC foam. In this example, 1 pixel = 6.214 µm.

5. Cell studies

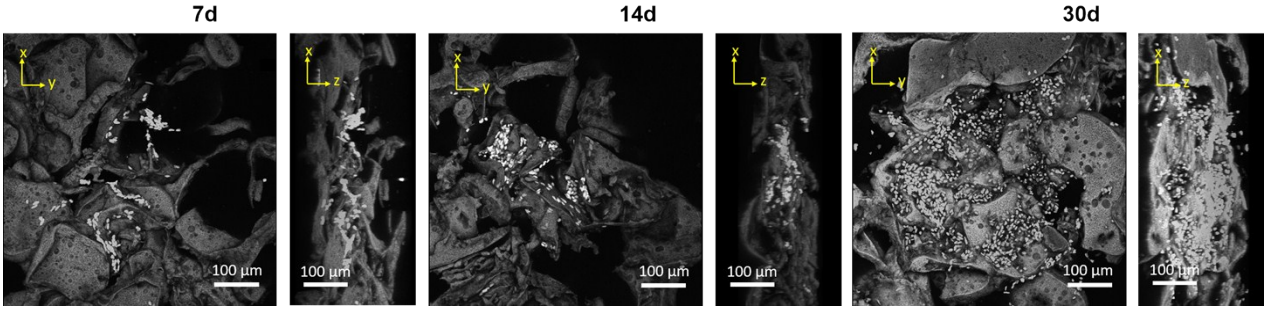

Figure S5. Confocal grey-scale images (x, y- and x, z-plane) obtained in LCE foams after 7 days (left), 14 days (middle), and 30 days (right) showing cell proliferation.

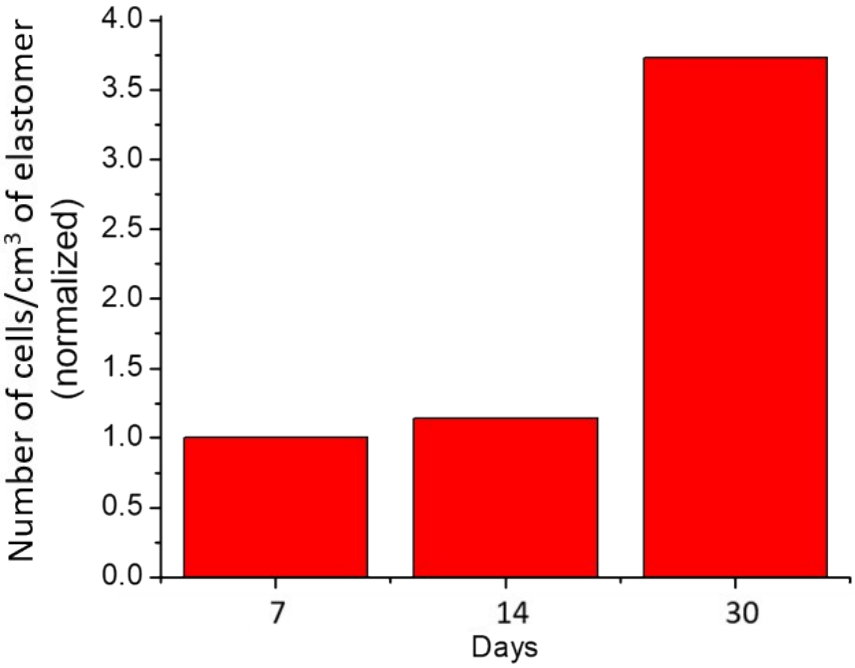

Figure S6. Normalized histograms cell count (proliferation) on LCE foams after 7 days (left), 14 days (middle), and 30days (right).

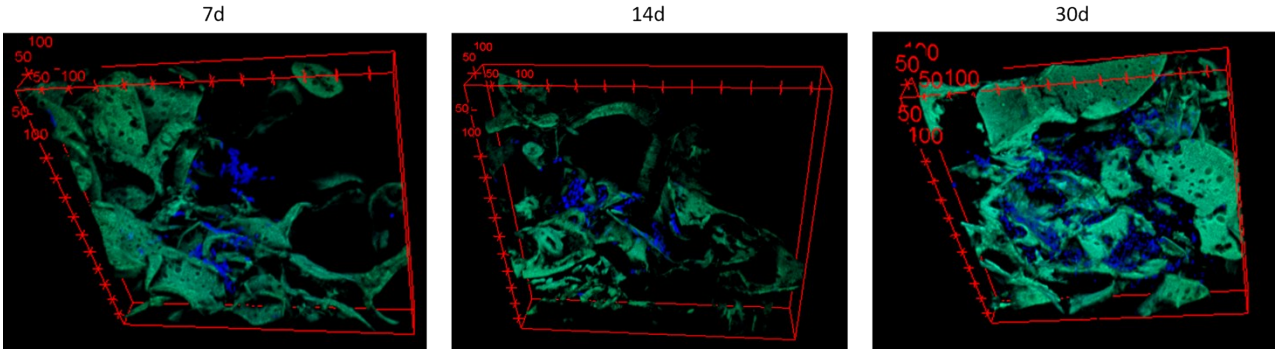

Figure S7. 3D reconstruction of confocal images (x, y- and x, z-plane) obtained in LCE foams after 7 days (left), 14 days (middle), and 30 days (right) showing cell proliferation.

Movie S1: Video of confocal images of cells obtained in LCE foams after 7 days.

Movie S2: Video of confocal images of cells obtained in LCE foams after 14 days.

Movie S3: Video of confocal images of cells obtained in LCE foams after 30 days.

References

1. H. M. Younes, E. Bravo-Grimaldo and B. G. Amsden, *Biomaterials*, 2004, **25**, 5261-5269.
2. D. S. Noyce, B. N. Bastian, P. T. S. Lau, R. S. Monson and B. Weinstein, *J. Org. Chem.*, 1969, **34**, 1247-1252.
3. C. Detrembleur, M. Mazza, O. Halleux, P. Lecomte, D. Mecerreyes, J. L. Hedrick and R. Jerome, *Macromolecules*, 2000, **33**, 14-18.
4. Y. Guindon, M. Therien, Y. Girard and C. Yoakim, *J. Org. Chem.*, 1987, **52**, 1680-1686.
5. A. Sharma, A. Neshat, C. J. Mahnen, A. D. Nielsen, J. Snyder, T. L. Stankovich, B. G. Daum, E. M. LaSpina, G. Beltrano, Y. Gao, S. Li, B.-W. Park, R. J. Clements, E. J. Freeman, C. Malcuit, J. A. McDonough, L. T. J. Korley, T. Hegmann and E. Hegmann, *Macromol. Biosci.*, 2015, **15**, 200-214.
6. Y. X. Gao, T. Mori, S. Manning, Y. Zhao, A. d. Nielsen, A. Neshat, A. Sharma, C. J. Mahnen, H. R. Everson, S. Crotty, R. J. Clements, C. Malcuit and E. Hegmann, *ACS Macro Lett.*, 2016, **5**, 14-19.
7. M. E. Prevot, S. Ustunel, L. E. Bergquist, R. Cukelj, Y. X. Gao, T. Mori, L. Pauline, R. J. Clements and E. Hegmann, *Jove-Journal of Visualized Experiments*, 2017, DOI: 10.3791/55452, 11.
8. A. Sharma, T. Mori, C. J. Mahnen, H. R. Everson, M. T. Leslie, A. d. Nielsen, L. Lussier, C. Zhu, C. Malcuit, T. Hegmann, J. A. McDonough, E. J. Freeman, L. T. J. Korley, R. J. Clements and E. Hegmann, *Macromol. Biosci.*, 2017, **17**, 1600278.
9. B. Amsden, *Soft Matter*, 2007, **3**, 1335-1348.