Electronic Supplementary Material (ESI) for Materials Advances.

ELECTRONIC SUPPLEMENTARY INFORMATION

(ESI)

Mechanically tunable Elastomer and Cellulose Nanocrystal composites as Scaffolds for *In vitro* Cell Studies

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1. SEM

6A-PCL/CNC (6 Arm star block-copolymer/cellulose nanocrystal) composite films and foams were cryo-cracked in preparation for Scanning Electron Microscopy (SEM) imaging. Figure S1 shows cross sections of the composite with a) 0, b) 5 and c) 40 weight (wt.) % of CNC in 6A-PCL/CNC films respectively.

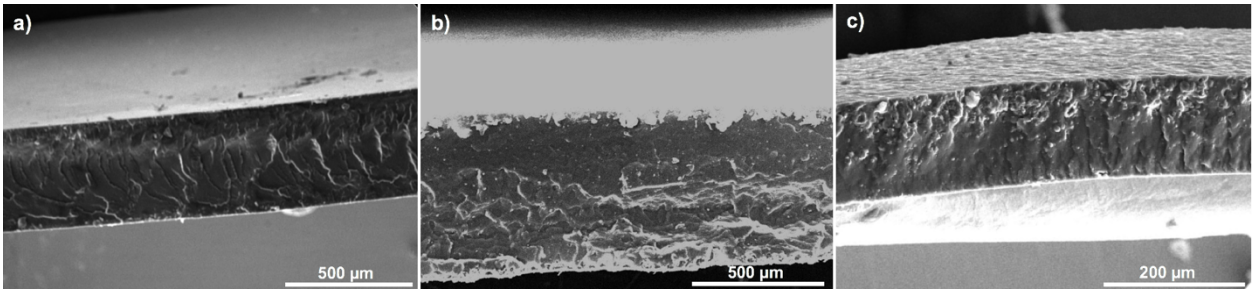

Figure S1. SEM cross section images of a) 0 wt.%, b) 5 wt.% and c) 40 wt.% of CNC in 6A-PCL/CNC film cross sections.

6A-PCL/CNC composite foams were prepared with a salt leaching method, and their porous structure was observed under SEM. **Figure S2** shows both 5 wt.% and 40 wt.% of CNC in 6A-PCL/CNC foam structures.

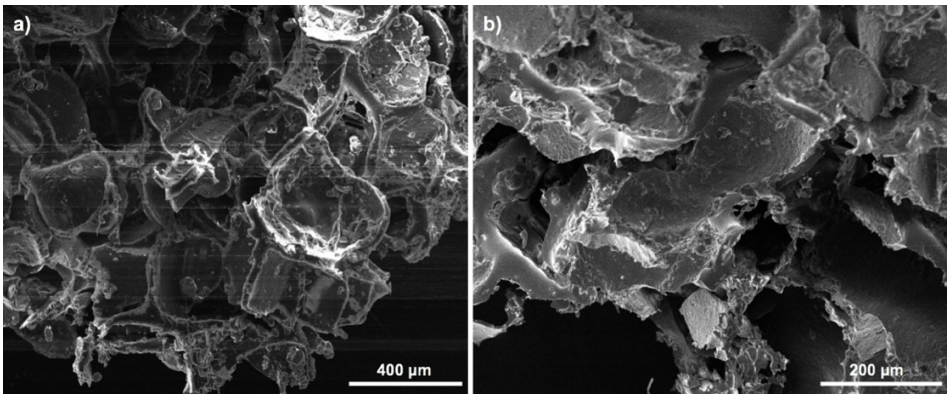

Figure S2. a) 5 wt.% b) 40 wt.% of CNC in 6A-PCL/CNC foam SEM images.

6A-PCL/CNC composite films were cryo-cracked in preparation for SEM imaging before and after *in vitro* degradation to observe the integrity of the films overtime.

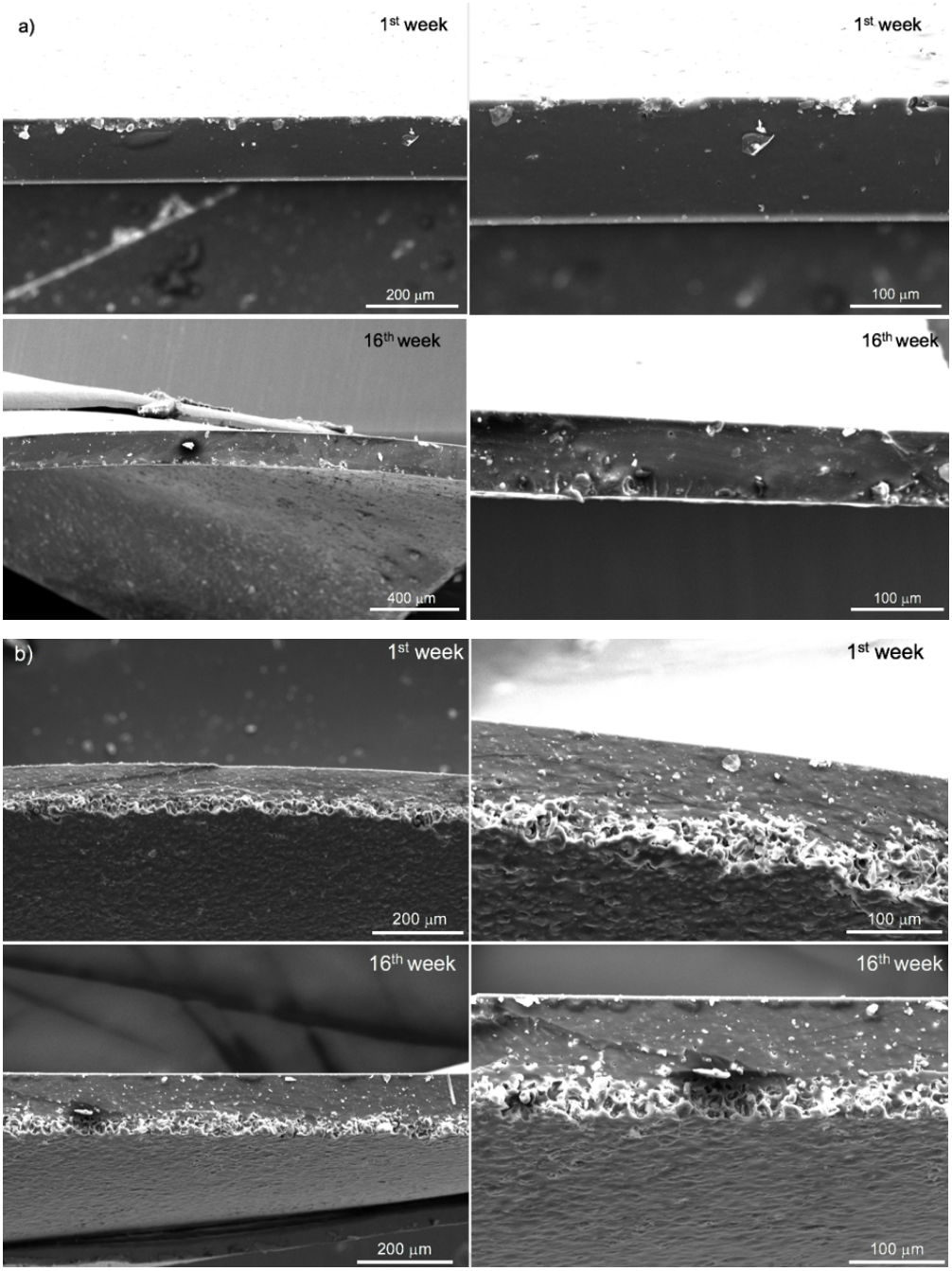

Figure S3. shows cross sections of the composite with a) 5 and b) 40 weight (wt.) % of CNC in 6A-PCL/CNC films before (week 1) and after degradation (week 16), respectively.

2. Confocal Imaging

SH-SY5Y and hDF cells were seeded on 5 wt.% and 40 wt.% of CNC in 6A – PCL/CNC composite foams and allowed to grow and proliferate for two weeks, after which they were compared using confocal microscopy (CM). CM images of SH-SY5Y and hDF cells seeded 5 wt.% and 40 wt.% of CNC in 6A – PCL/CNC composite foams are shown for comparison, with cell nuclei shown in red (Propidium Iodide staining).

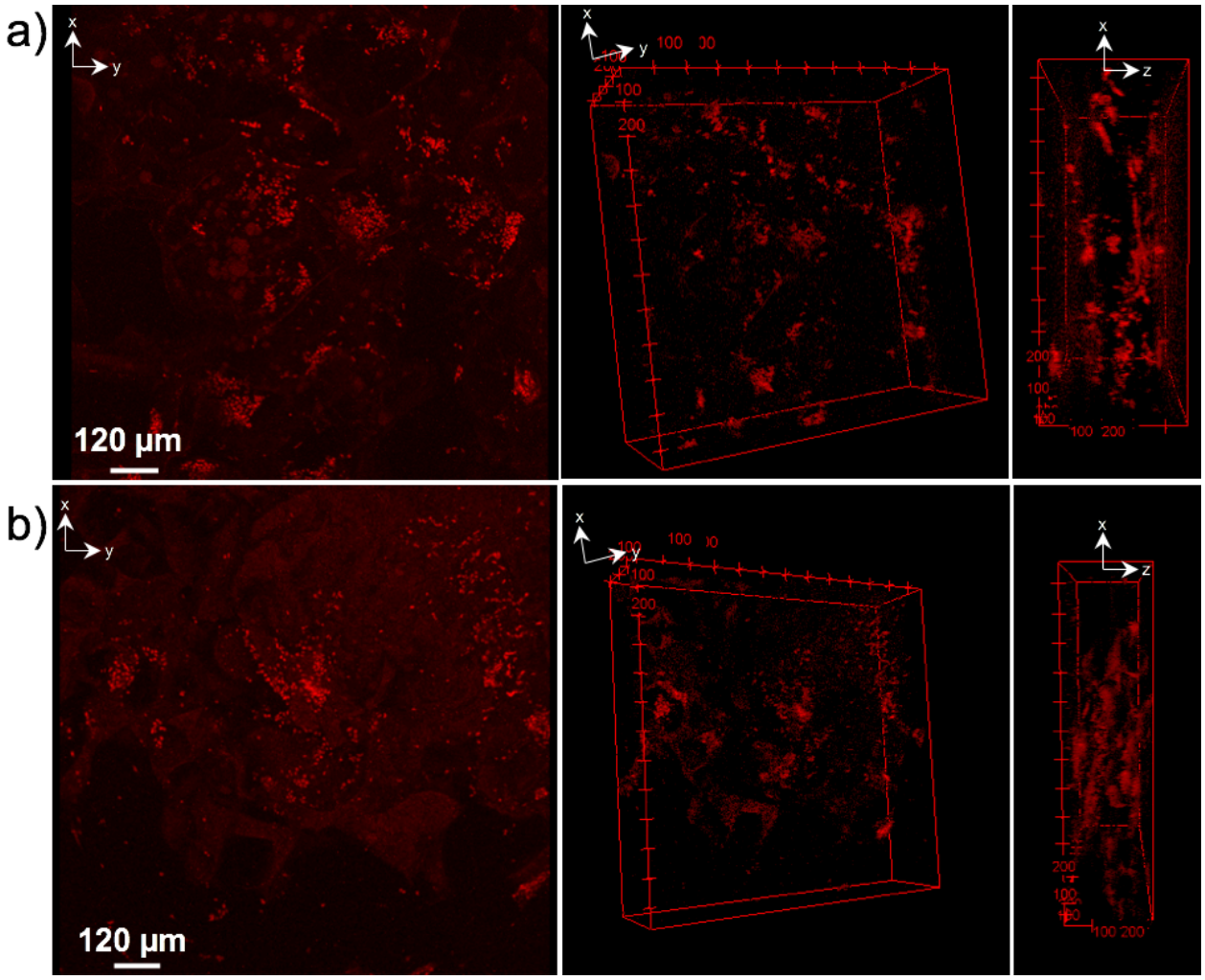

Figure S4. Supplemental confocal images of a) 5 and b) 40 wt.% of CNC in 6A – PCL/CNC composite foams with SH-SY5Y cells seeded after two weeks of proliferation. Cell nuclei are shown in red; approximate counted number of nuclei are 797 and 388 for 5 wt.% and 40 wt.% of CNC in 6A – PCL/CNC, respectively.

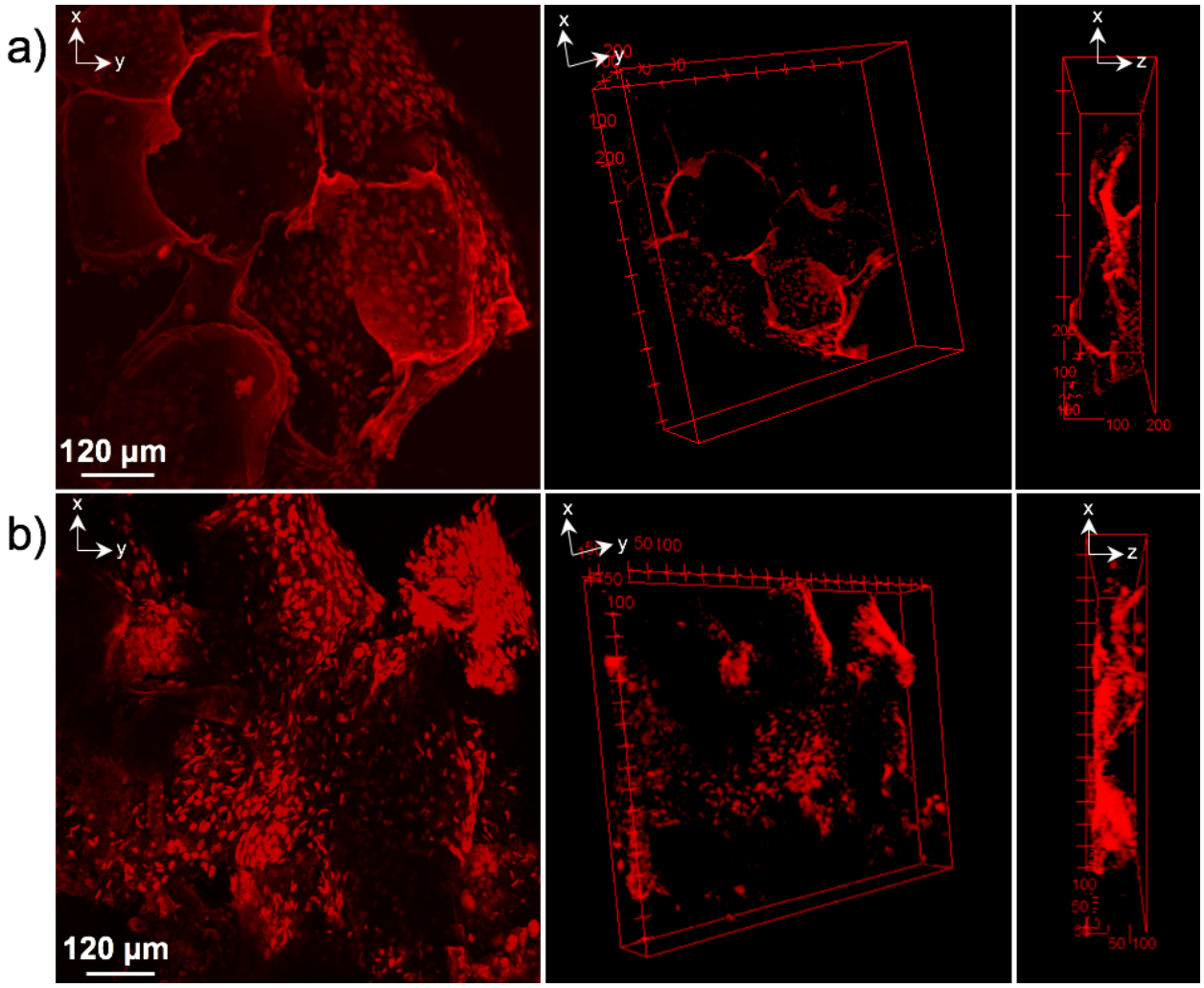

Figure S5. Supplemental confocal images of a) 5 and b) 40 wt.% of CNC in 6A – PCL/CNC composite foams with hDF cells seeded after two weeks of proliferation. Cell nuclei are shown in red; approximate counted number of nuclei are 401 and 901 for 5 wt.% and 40 wt.% of CNC in 6A – PCL/CNC, respectively.

CM images of hDF cells seeded in 40 wt.% of CNC in 6A – PCL/CNC composite foams are shown for comparison, after two weeks of proliferation. Cell nuclei were stained and are shown in red (Propidium Iodide staining).

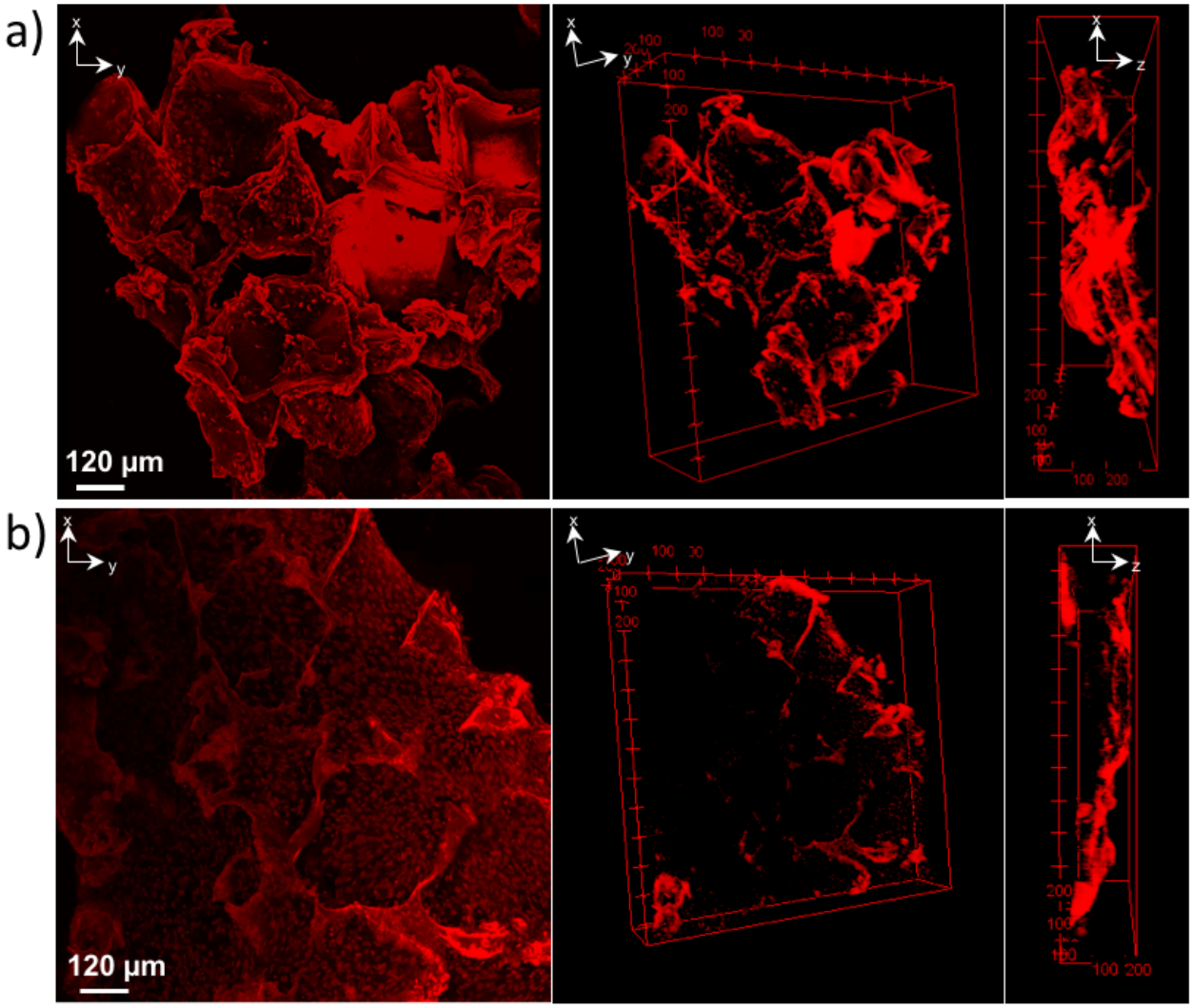

Figure S6. 3D rendered confocal micrographs of a) SHSY-5Y cells seeded after 8 weeks proliferation on a 5 wt.% of CNC in 6A – PCL/CNC composite foam, and b) hDF cells seeded after 8 weeks proliferation on a 40 wt.% of CNC in 6A – PCL/CNC composite foam.

CM images of hDF cells seeded in 40 wt.% of CNC in 6A – PCL/CNC composite foams are shown after eight weeks of proliferation. Cell nuclei was stained using 4',6-Diamidino-2-phenylindole (DAPI) (purchased from Invitrogen) and cell body was stained with primary anti-Tubulin β 3 (TUBB3) Antibody.

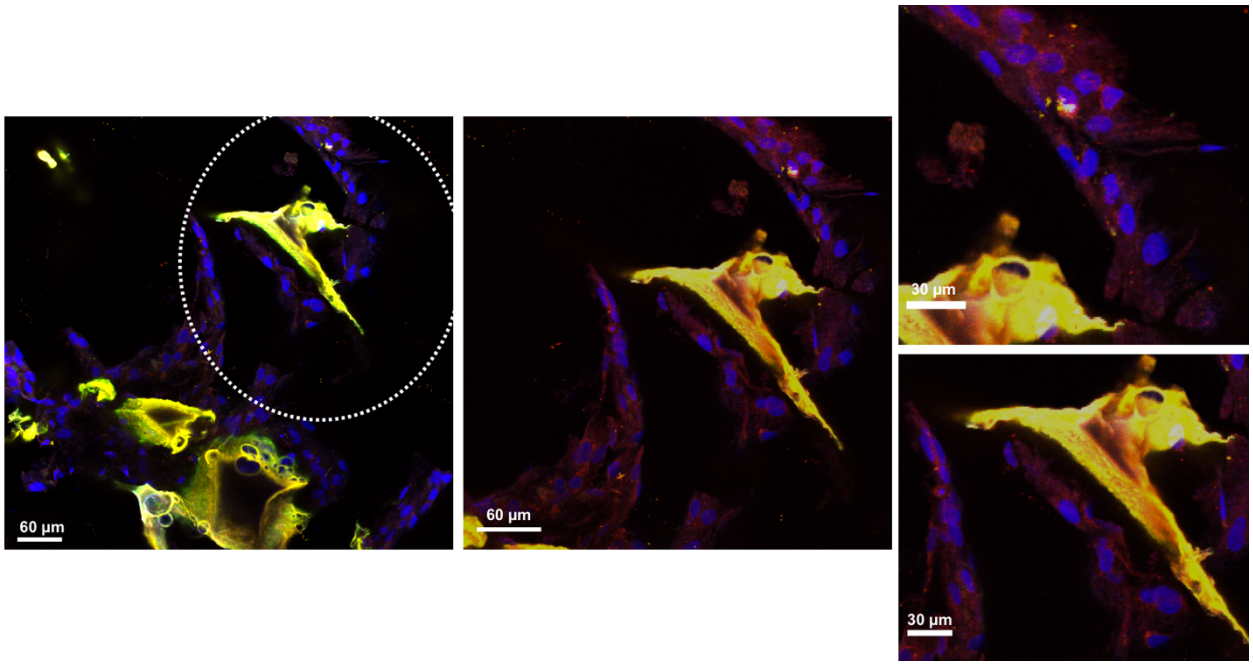

Figure S7. Confocal micrographs of hDF cells seeded after 8 weeks proliferation on a 40 wt.% of CNC in 6A – PCL/CNC composite foam.

3. SAXS Data

We have previously reported the use of side-chain liquid crystal elastomer-based scaffolds (LCE). Small Angle X-ray Scattering (SAXS) studies have shown the characteristic[1,2,3] features of a smectic-A (Sm-A) phase with interdigitated cholesterol moieties by the presence of two sharp scattering peaks at 0.16 $Å^{-1}$ and 0.32 $Å^{-1}$, respectively. To study the effect of CNC addition to a Sm-A LCE, we compared (see Figure S9) SAXS data for 0, 5 and 40 wt.% CNC addition in 6A-PCL-LCE-based material. No disruption of the Sm-A phase was observed within the LCE material as we can see both first and second order peaks at 0.16 $Å^{-1}$ and 0.32 $Å^{-1}$ for 0, and 5 wt.% CNC. In the case of the 40 wt.% CNC at 0.16 $Å^{-1}$, only the first order peak could be observed.

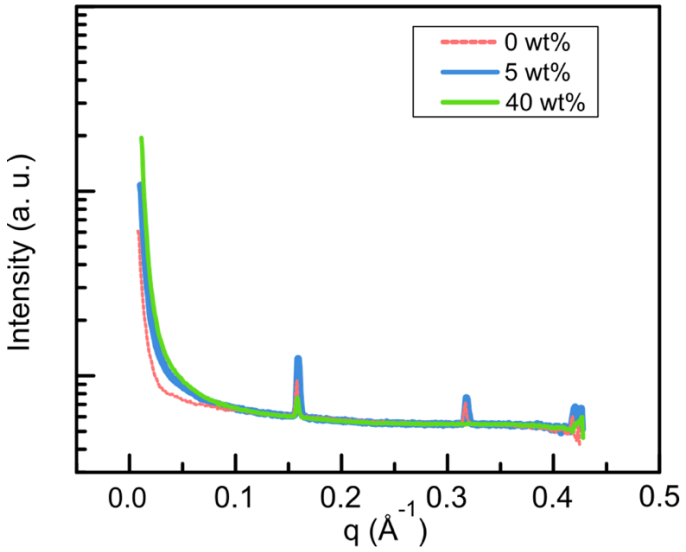

Figure S8. SAXS data on 6A-PCL-LCE matrix containing 0 wt.% CNC (dashed red curve), 5 wt.% CNC (blue curve) and 50 wt.% CNC (green curve).

4. Contact angle measurements

Contact angle measurements were obtained from all composites prepared (from 0 wt.% to 40 wt.% content of CNCs in 6A – PCLs).

wt.% of CNC content	Contact angle θ_c (degrees)
0	77.5 ± 0.1
5	73.3 ± 0.1
15	72.2 ± 0.5
25	68.9 ± 0.5
35	66.8 ± 0.5
40	64.5 ± 0.1

Table S1. Contact angle measurements data on 6A-PCL-LCE matrix containing 0 wt.% to 40 wt.% content of CNCs. Only the composites with 5 wt.% of CNC content and 40 wt.% of CNC content (grey cells) were used for cell studies.

4. REFERENCES

1. A. Sharma, A. Neshat, C.J. Mahnen, A.d. Nielsen, J. Snyder, T.L. Stankovich, B.G. Daum, E.M. LaSpina, G. Beltrano, 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.
2. 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.
3. M.E. Prévôt, H. Andro, S.L.M. Alexander, S. Ustunel, C. Zhu, Z. Nikolov, S.T. Rafferty, M.T. Brannum, B. Kinsel, L.T.J. Korley, E.J. Freeman, J.A. McDonough, R.J. Clements, and E. Hegmann, *Soft Matter* 2018, **14**, 354-360.