



3D Porous Liquid Crystal Elastomer Foams Supporting Long-term Neuronal Cultures

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3D liquid crystal elastomer (3D-LCE) foams are used to support long-term neuronal cultures for over 60 days. Sequential imaging shows that cell density remains relatively constant throughout the culture period while the number of cells per observational area increases. In a subset of samples, retinoic acid is used to stimulate extensive neuritic outgrowth and maturation of proliferated neurons within the LCEs, inducing a threefold increase in length with cells displaying morphologies indicative of mature neurons. Designed LCEs' micro-channels have a similar diameter to endogenous parenchymal arterioles, ensuring that neurons throughout the construct have constant access to growth media during extended experiments. Here it is shown that 3D-LCEs provide a unique environment and simple method to longitudinally study spatial neuronal function, not possible in conventional culture environments, with simplistic integration into existing methodological pipelines.

2D cell culture provides a repeatable system to study, test, and understand molecular and cellular phenomena associated with dynamic processes.^[1] In contrast, studying cells in 3D polymeric scaffolds is a promising strategy for growing cells in an environment that simulates the natural milieu and provides a realistic method for engineering tissues and in vitro experimentation.^[2] Abbot et al. reported one of the first examples of nematic liquid crystals as sensors for cell growth, and

orientational order.^[3–5] Thus combining liquid crystals intrinsic anisotropic properties with elastic properties of elastomers enable us to create liquid crystal elastomers (LCEs) with orientational ordering and mechanical properties. LCEs promote development of extracellular matrix (ECM), function as exceptional longitudinal multi-responsive cell scaffolds,^[6–14] and present mechanical properties associated with Young's moduli within the range of several tissues. Our successful approach to develop of LCE constructs for in vitro applications to support cell growth,^[15–20] satisfy requirements to be considered in tissue engineering constructs, such as biocompatibility, mechanical properties, fully interconnected porous architecture,^[21] and appropriate degradation times.^[6] Thus, 3D-LCEs have promise as scaffolds for neuronal cultures by mimicking features of endogenous tissue providing the ability to study more complex problems^[22] including spatial axonal growth^[23,24] and guidance,^[8] blood brain barrier function,^[25,26] high-throughput therapeutic response,^[27] and permits the creation of virtual networks.^[28] In addition to the use of spatial cultures that closely mimic living tissue, there is increasing interest in developing and applying

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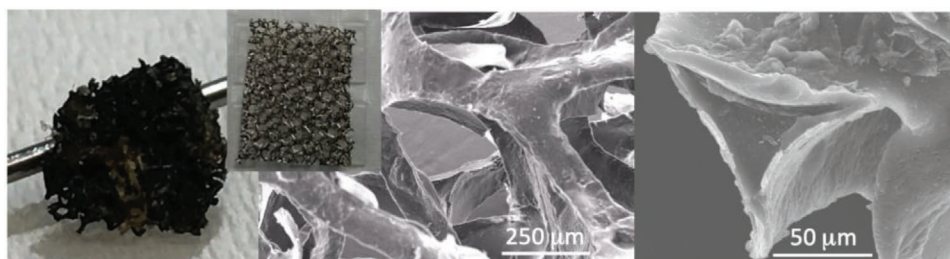


Figure 1. LCE Material. Optical images of LCE, Ni foam, and SEM images of the LCE foam. The dual morphology of the LCE foam is visible in the SEM images as larger pores (middle SEM image) and microchannel network cross section (far right SEM image).

long-term cultures^[29] for studying the dynamic nervous systems. Neuronal cultures that survive for multiple months permit developmental,^[30] neurodegenerative,^[31] aging,^[32,33] and multiphasic studies.^[34] Here we provide a proof-of-concept study of 3D-LCEs based neuronal scaffolds with features supporting spatial growth and long-term study of nervous cell types with simplistic integration into existing scientific pipelines.

We reported that LCEs promote cell penetration and attachment into the LCE scaffold successfully creating 3D-LCE foams capable of supporting long-term spatial growth of cells.^[17] To further refine and continue the development of our platform of LCE as growth constructs, we present and evaluate 3D-LCE foams as a platform that mimics endogenous nervous tissue and supports long-term cultures permitting growth of differentiated networks over multiple months. We present longitudinal cell counts as well as morphological data indicating that neurons survive on the LCEs growing throughout the material. Further, we validated our ability to chemically manipulate and evaluate neuronal function within the LCE by stimulating differentiation of cultured neurons resulting in a significant outgrowth of neuritic extensions evident throughout extended *in vitro* periods.

LCE foams were made as described in Figure S1, Supporting Information, using a sacrificial Ni metallic structure.^[17,20] The obtained LCE microstructure was verified using scanning electron microscopy (SEM) and included pore sizes ranging from 150–400 μm and secondary micro-channels with a diameter of $\approx 40 \mu\text{m}$ (Figure 1) clearly visible in the SEM images. Figure 1 shows the Ni metallic structure transferred to the LCE during crosslinking forming vessel-like interconnected channels ideal for cell culture applications. The process, allows for control of pore size, density, and morphology.^[17,20] Highly interconnected pores (>85%^[21]) provide suitable mass transport where cells have constant access to nutrients, gases, and waste management, reducing cell death and increasing viability,^[35] avoiding mass transfer restrictions,^[36] where diffusion of nutrients, and oxygen are promoted by the scaffold structure. The presence of LC moieties promote cell attachment and proliferation, as well as orientation and anisotropic cell growth,^[19] allowing the creation of neural networks in organized patterns to serve specific functions and that mimicking endogenous systems. As we reported^[19] before no cell anisotropy/orientation was observed on non-LC elastomers, in this study we focus solely on LC bearing elastomers (LCEs).

We observed that after week 1, seeded neurons adhered to LCEs, growing similar to published reports and survived for the extended duration of the study. Neurons grew throughout the LCE after seeding on top of constructs as indicated by sample

imaging from multiple orientations. This is a major benefit of our constructs' structure with layers of porosity that permits cells to infiltrate deep into the foam. Our microscopy equipment is limited to imaging ≈ 100 – 150 microns due working distance and limited laser penetration setting the limit to the depth of images analyzed. Micrographs were acquired every week for the two month long experiment from fixed and stained samples. Our LCEs have been previously assessed for cell proliferation, viability, and cytotoxicity indicating that our scaffolds promote cellular proliferation without inherent cytotoxicity.^[15,16,18] Cells expanded and grew throughout the material, eventually extending through the LCE creating multiple layers of neurons (Figure 2). Neurons fixed at the last time point (week 8) were distributed as multiple layers through over the 100 μm of LCE scanned (Figure 2, *xz* and *yz* plane images).

To quantify changes in the number and arrangement of cells within the foam, 4',6-diamino-2-phenylindole staining was analyzed to calculate cell number, volume, and density (Table S1, Supporting Information). Undifferentiated cell densities on the foam were calculated from the ratio between the number of the cells and V_{cell} . $\text{Cells}/V_{\text{unit}}$ is defined as the ratio between cell number and V_{unit} to normalize the number of cells on the foam.

Undifferentiated cell densities showed an almost constant value ($5.00 \times 10^{-5} \mu\text{m}^{-3}$, Figure 3a) to the end of the second month and the number of cells/ V_{unit} increased up to day 60 (Figure 3b). Cell cultures survived on the LCE foams until the experiment was terminated (over two months). We expect that the number of neurons on our foams will continue to increase after two months in the absence of differentiating factors.

Morphology of cells was also analyzed in a subset of samples throughout the two month period by immuno-fluorescence staining with an antibody toward neurofilament. Cells displayed multipolar morphologies with extensions indicative of healthy neuroblastoma cells as published^[37] (Figure 4, top row). To establish whether neurons can reliably mature in the developed constructs a subset of samples were treated with retinoic acid (RA) after four weeks to induce differentiation. RA results in a decrease in cell proliferation as well as maturation to the neuronal phenotype.^[38] RA induced growth of neuritic extensions that continued throughout the experimental protocol (Figure 4, lower two images) resulting in a threefold increase in length compared to untreated cultures (Figure 4, table).

Functioning nervous tissue is a complex interplay of interactions between many cell types and the ECM modulating development, plasticity, and when disrupted can cause disease related phenomena and developmental abnormalities. Simulating this *in vitro* for experimentation, manipulation, and growth of

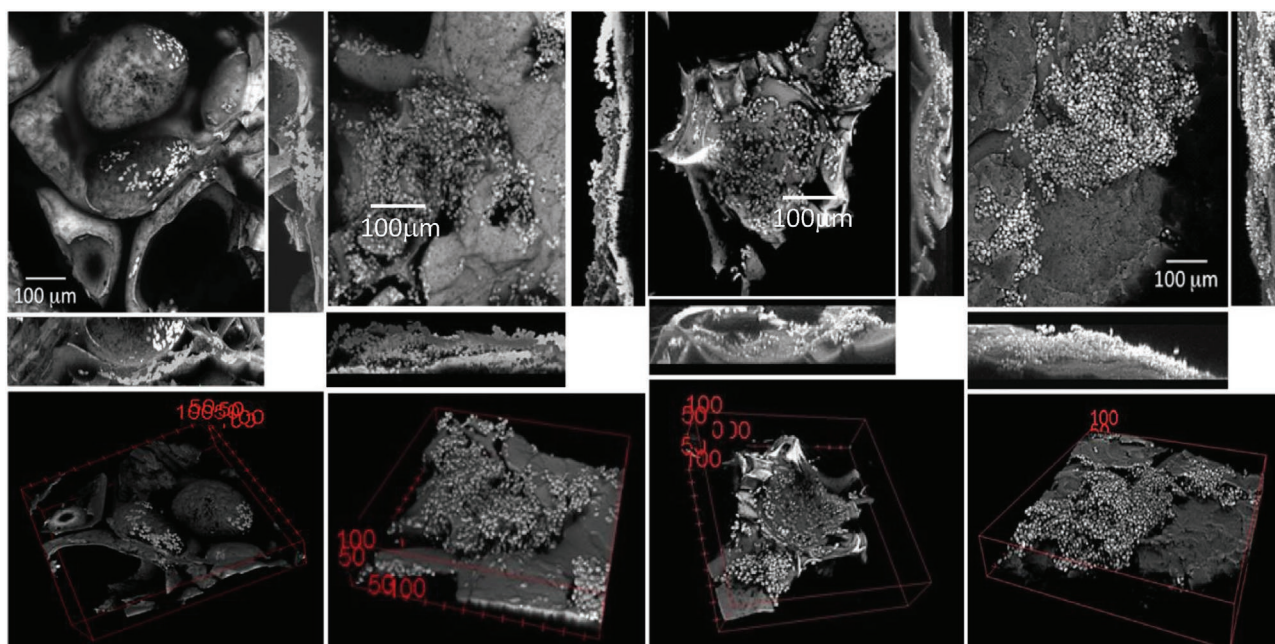


Figure 2. Confocal micrographs of seeded LCE weeks 2–9. Cells were stained with 4',6-diamino-2-phenylindole weekly throughout the duration of the experiment, in the above images elastomer samples from week 2, 4, 6, and 9 are displayed from left to right respectively. Cells adhered and grew spatially throughout the matrix as indicated by the different data projections. The stained nuclei were used to identify the number and distribution of SH-SY5Y cells in the elastomer. At the end of the two month experimental period, cells can be observed extending through the depth of the foam (xz/yz projections). In order to evaluate neuronal morphology and health a subset of samples were also counterstained and analyzed for neurite outgrowth.

implantable tissue is a tremendous benefit. Our approach to biocompatible elastomers allows for tunable porosity, elasticity, and biodegradation rate. As reported,^[26] caprolactone-lactide co-polymers have been known to degrade primarily via bulk acid-catalyzed hydrolysis, producing oligocarboxylic acids at cross-linking sites. We reported^[15] that our LCEs are stable for over 15 weeks before major biodegradation begins, via bulk acid-catalyzed hydrolysis producing oligocarboxylic acids anticipated to start at the cross-linking sites, a rate that can be tuned to a desired duration allowing for long-term studies. Another key property of our LCE is an intrinsic porosity that allows for efficient mass transport required for 3D cultures: a) oxygen transport (controlling metabolism rate), b) transport of nutri-

ents to cells, and c) waste transport (e.g., toxins that raise pH to toxic levels, among others).^[35] LCE's porosity also promotes spatial cell-scaffold interactions, space for ECM formation and the possibility of linking molecular entities to bind growth factors or other proteins to enhance adhesion. Recent techniques using stacked paper with Matrigel^[39] and 3D microenvironments using dielectrophoretically produced hydrogels^[40] suffer from cell clustering, are not biodegradable, and have non-homogenous distribution of oxygen and nutrients, all solved by our current design.

The secondary structure of our construct including microchannels with a diameter of ≈ 40 microns allows media to freely flow throughout the material enabling neurons deep within the LCE to be sustained. This helps provide surface area to

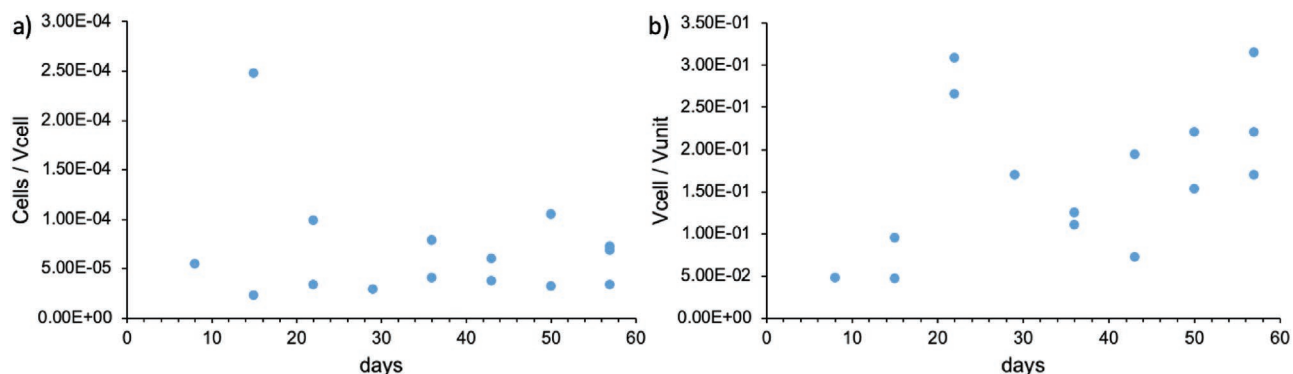


Figure 3. Neuronal Density on the LCE. a) The density of cells was analyzed by calculating the ratio of the number of cells per volume of cells. Cell density on the foam was relatively constant throughout the culture period. b) The volume of cells per V_{unit} was analyzed throughout the experiment to calculate how many cells were located per analyzed area of elastomer. Data indicated that the number of cells per analyzed area steadily increased during the experimental duration.

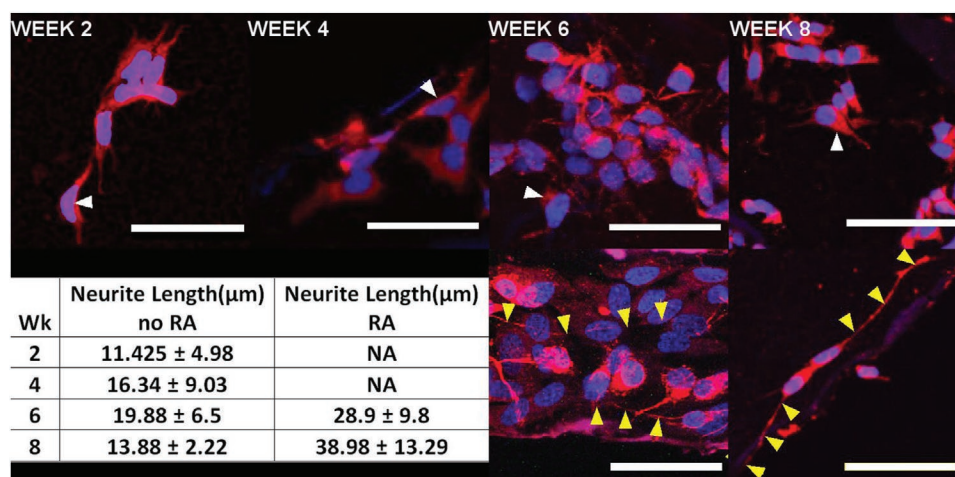


Figure 4. Neuronal Morphology Confocal micrographs of SH-SY5Y cells grown on the LCE foams for 2–8 weeks stained for nuclei (blue) and neurofilament (red). Unstimulated cells showed undifferentiated morphologies throughout the experiment and beyond the last time point tested with short neuritic extensions (white arrows top row). Subsets of samples were provided with retinoic acid (RA, bottom row) on week 4 to stimulate maturation of cells. Neuritic extensions could be clearly observed throughout the foam as neurons grew in multiple layers within the construct (yellow arrows bottom row). RA stimulated statistically significant growth of neuronal extensions (table inlay) compared to untreated cells (top row) and were viable for over two months. 20 neurons were analyzed per sample and a $p < 0.05$ considered significant.

support many cells for extended periods and facilitates quick media exchange for rapid pharmacological manipulations and homogenous nutrient distribution. Endogenously, the neurovascular system consists of conduits of varying diameter. The main arteries of the brain feed the smaller arteries and arterioles running along the surface of the brain in the leptomeninges (or astrocyte rich outer layer of the cortex, the glia limitans). Subsequently, penetrating arterioles link pial arteries with parenchymal arterioles deep in the brain within the Virchow–Robin space. Parenchymal arterioles are the major determinant of brain perfusion^[41] and critical to normal function. Resting luminal diameter of parenchymal arterioles is reported to be in the range of 30–70 microns in rat,^[42] mouse,^[43] and human.^[44] The secondary structure of our LCE (≈ 40 microns) is within the range of these parenchymal arteriole widths indicating that our construct emulates features of the endogenous neurovasculature.

Additional benefits this new approach includes the ability to initiate cellular alignment, ease of implementation and compatibility with common stains. We have previously reported that our LCEs (films and foams) promote spontaneous cellular alignment.^[28,30,31] Cellular alignment within our LCE's typically occurs without external ordering events (e.g., stretching) to initiate ordering. The current study was designed primarily evaluate the ability of neurons to populate the LCE and survive for extended periods, we intend to use external stimuli to initiate alignment in the future. Compared to existing systems this platform for long-term culture provides simplicity of integration with existing methodologies. Our culturing protocols are identical to growing neurons on a dish without the requirement to passage cells after seeding, which is frequently performed during long-term culture of neurones.^[45] The compatibility with existing methods is in contrast to many existing long-term culture systems that require specific setups such as sealed dishes with semi-permeable membranes^[46] or custom micro perfusion/fluidic systems.^[47,48] It should be noted that sterile techniques are an explicit requirement for any culturing environment, and

this is especially true for the maintenance of long-term cultures. Minimizing the number of media changes and ultrafiltration prior to feeding promotes longevity and prevents contamination. Unpublished data from our group indicates that LC-modified elastomers (LCEs) frequently suffer from auto-fluorescence or bind common fluorophores. The LCEs described herein displays background fluorescence well below the intensity of common fluorophores and did not appreciably bind any tested probes permitting the use of a wide array of imaging tools for assessing neural function. The ability to stain multiple channels within the construct with little background fluorescence, good antibody penetration via the mimicked microvascular network and the ability to image through over 200 microns with a standard confocal microscope provides an excellent platform to study 3D neuronal function. Further, the ability to support long term growth and differentiation of neurons permits multi-phase studies whereby cells are seeded, proliferate, and induced to differentiate via chemical cues (i.e. RA). Once differentiated, disease processes can be induced, and therapies designed to arrest and reverse these evaluated. It is our hope that these methods will permit the simulation of multiple disease phases for longitudinal therapeutic evaluation by emulating endogenous tissue.

In summary, the materials and methods described herein provide a new platform for long-term experimentation and neural tissue growth employing a LCE foam with a system of channels like the endogenous microvascular system. The extended in vitro lifespan permits the study of developmental and ongoing events allowing for in-depth, long-term study, and manipulation. An easily adjustable, modular design of these biocompatible LCEs will enable us to extend this work to medical implants and in vitro or in vivo tissue engineering for testing pharmacotherapies. The materials have been previously shown to support primary cell cultures^[19] and our future plans include the incorporation of oligodendrocytes with neurons into the material to study dynamic and dysfunctional processes associated with (re-)myelination.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

3D scaffolds, cell scaffolds, liquid crystals, longitudinal, neurons, neurovasculature

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- [1] J. W. Haycock, *3D Cell Culture: Methods and Protocols*, Methods in Molecular Biology series, Vol. 685, Humana Press, NJ **2011**, <https://doi.org/10.1007/978-1-60761-984-0>.
- [2] X. H. Liu, X. H. Jin, P. X. Ma, *Nat. Mater.* **2011**, *10*, 398.
- [3] N. A. Lockwood, J. C. Mohr, L. Ji, C. J. Murphy, S. P. Palecek, J. J. de Pablo, N. L. Abbott, *Adv. Funct. Mater.* **2006**, *16*, 618.
- [4] N. L. Abbott, J. J. de Pablo, S. P. Palecek, N. A. Lockwood, J. C. Mohr, C. J. Murphy, E. Huang, U.S. Patent 7,732,15, **2010**.
- [5] J. Fang, W. Ma, J. V. Selinger, R. Shashidhar, *Langmuir* **2003**, *19*, 2865.
- [6] M. E. Prévôt, E. Hegmann, *Advances in Bioinspired and Biomedical Materials*, Vol. 2, ACS, Washington, DC **2017**.
- [7] C. Ohm, M. Brehmer, R. Zentel, *Adv. Polym. Sci.* **2012**, *250*, 49.
- [8] C. E. Graves, R. G. McAllister, W. J. Rosoff, J. S. Urbach, *J. Neurosci. Methods* **2009**, *179*, 278.
- [9] *Handbook of Liquid Crystals* (Eds: H.R. Brand, H. Finkelmann, D. Demus, J.W. Goodby, C.W. Gray, H.W. Spiess, V. Vill), Vol. 3, Wiley, New York **2008**, p. 277.
- [10] W. H. de Jeu, *Liquid Crystal Elastomers: Materials and Applications*, Springer, New York **2012**.
- [11] A. H. Agrawal, H. Chen, H. Kim, B. Zhu, O. Adetiba, A. Miranda, A. C. Chipara, P. M. Ajayan, J. G. Jacot, R. Verduzco, *ACS Macro Lett.* **2016**, *5*, 1386.
- [12] A. Agrawal, O. Adetiba, H. Kim, H. Chen, J. G. Jacot, R. Verduzco, *J. Mater. Res.* **2015**, *30*, 453.
- [13] S. W. Ula, N. A. Traugott, R.H., V., R. R. Patel, K. Yu, C. M. Yakacki, *Liq. Cryst. Rev.* **2018**, *6*, 78.
- [14] D. Martella, P. Paoli, J. M. Pioner, L. Sacconi, R., C., L. Santini, M. Lulli, E. Cerbai, D. S. Wiersma, C. Poggesi, C. Ferrantini, C. Parmeggian, *Small* **2017**, *13*, 1702677.
- [15] A. Sharma, A. Neshat, C. J. Mahnen, A.d. Nielsen, J. Snyder, T. 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, E. Hegmann, *Macromol. Biosci.* **2015**, *15*, 200.
- [16] T. Bera, E. J. Freeman, J. A. McDonough, R. J. Clements, A. Aladlaan, D. W. Miller, C. Malcuit, T. Hegmann, E. Hegmann, *ACS Appl. Mater. Interfaces* **2015**, *7*, 14528.
- [17] Y. Gao, S. Manning, Y., Z., A.d. Nielsen, T. Mori, A. Neshat, A. Sharma, C. J. Mahnen, H. R. Everson, S. Crotty, R. J. Clements, C. Malcuit, E. Hegmann, *ACS Macro Lett.* **2016**, *5*, 4.
- [18] T. Bera, C. Malcuit, R. J. Clements, E. Hegmann, *Front. Mat.* **2016**, *3*, 31.
- [19] A. Sharma, T. Mori, C. J. Mahnen, H. R. Everson, T. Leslie, A.d. Nielsen, L. Lussier, C. Zhu, C. Malcuit, T. Hegmann, J. A. McDonough, E. Freeman, L. T. J. Korley, R. J. Clements, E. Hegmann, *Macromol. Biosci.* **2017**, *17*, 1600278.
- [20] M. E. Prévôt, S. Ustunel, L. E. Bergquist, R. Cukelj, Y. Gao, T. Mori, R. J. Clements, E. Hegmann, *J. Vis. Exp.* **2017**, *122*, e55452.
- [21] M. E. Prévôt, H. Andro, S. L. M. Alexander, S. Ustunel, C. Zhu, S. T. Rafferty, M. T. Brannum, L. T. J. Korley, E. Freeman, J. A. McDonough, R. J. Clements, E. Hegmann, *Soft Matter* **2018**, *14*, 354.
- [22] M. Ravi, V. Paramesh, S. R. Kaviya, E. Anuradha, F. D. P. Solomon, *J. Cell. Physiol.* **2015**, *230*, 16.
- [23] M. Ishihara, N. Mochizuki-Oda, K. Iwatsuki, H. Kishima, Y. Iwamoto, Y. Ohnishi, M. Umegaki, T. Yoshimine, *J. Neurosci. Methods* **2011**, *198*, 181.
- [24] Z. Pujica, G. J. Goodhilla, *J. Neurosci. Methods* **2013**, *215*, 53.
- [25] M. E. Boutin, L. L. Kramer, L. L. Livia, T. Brown, C. Moore, D. Hoffman-Kima, *J. Neurosci. Methods* **2017**, *299*, 55.
- [26] K. Hatherella, P. O. Couraud, I. A. Romero, B. Wekslerd, G. J. Pilkington, *J. Neurosci. Methods* **2011**, *199*, 223.
- [27] O. J. Klein, Y. K. Jung, C. L. Evans, *Ther. Screening Methods* **2014**, *66*, 299.
- [28] H. Braun, C. Bühnemann, J. Neumann, K. G. Reymanna, *J. Neurosci. Methods* **2006**, *157*, 32.
- [29] A. Kumar, B. N. Mallick, *J. Neurosci. Methods* **2016**, *263*, 123.
- [30] S. Kaech, C. F. Huang, G. Banker, *Cold Spring Harb. Protoc.* **2012**, *3*, 335.
- [31] M.-J. Kim, S.-J. Oh, S.-H. Park, H.-J. Kang, M. H. Won, T.-C. Kang, J.-B. Park, J. Kim, J. Kim, J. Y. Lee, *Exp. Mol. Med.* **2007**, *39*, 14.
- [32] C. Lesuisse, L. J. Martin, *J. Neurobiol.* **2002**, *51*, 9.
- [33] W. Dong, S. Cheng, F. Huang, W. Fan, Y. C. H. Shi, H. He, *Med. Sci. Monit.* **2011**, *17*, BR91.
- [34] A. Odawara, H. Katoh, N. Matsuda, I. Suzuki, *Sci. Rep.* **2016**, *6*, 26181.
- [35] Y. Martin, P. Vermette, *Biomaterials*, **2005**, *26*, 7481.
- [36] M. Lovett, K. Lee, A. Edwards, D. L. Kaplan, *Tissue Eng., Part B* **2009**, *15*, 353.
- [37] R. Constantinescu, A. T. Constantinescu, H. Reichmann, B. Janetzky, *J. Neural Transm., Suppl.* **2007**, *72*, 17.
- [38] H. Teppola, J. R. Sarkanen, T. O. Jalonen, M. L. Linne, *Neurochem. Res.* **2016**, *41*, 731.
- [39] R. Derda, A. Laromaine, A. Mammoto, S. K. Y. Tang, T. Mammoto, D. E. Ingber, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 18457.
- [40] D. R. Albrecht, G. H. Underhill, T. B. Wassermann, R. L. Sah, S. N. Bhatia, *Nat. Methods* **2006**, *3*, 369.
- [41] F. Dabertrand, M. T. Nelson, J. E. Brayden, *Circ. Res.* **2012**, *110*, 285.
- [42] P. W. Pires, W. F. Jackson, A. M. Dorrance, *Am. J. Physiol.: Heart Circ. Physiol.* **2015**, *309*, H127.
- [43] Y. Sekiguchi, K. Masamoto, H. Takuwa, H. Kawaguchi, I. Kanno, H. Ito, Y. Tomita, Y. Itoh, N. Suzuki, R. Sudo, K. Tanishita, *Adv. Exp. Med. Biol.* **2013**, *789*, 419.
- [44] F. Aydin, W. I. Rosenblum, J. T. Povlishock, *Stroke* **1991**, *22*, 1592.
- [45] J. Ray, D. A. Peterson, M. Schinstine, F. H. Gage, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3602.
- [46] S. M. Potter, T. B. DeMarse, *J. Neurosci. Methods* **2001**, *110*, 17.
- [47] K. Rambani, J. Vukasinovic, A. Glezer, S. M. Potter, *J. Neurosci. Methods* **2009**, *180*, 243.
- [48] A. Tourovskaia, X. Figueroa-Masot, A. Folch, *Lab Chip* **2005**, *5*, 14.