Supporting Information

Poly (L-lysine)-Coated Liquid Crystal Droplets for Cell-Based Sensing Applications

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Experimental Section

Materials

Poly-L-lysine (PLL) solution 0.1 % (w/v) in H2O, poly(4-styrenesulfonic acid) sodium salt (PSS), Dulbecco's modified Eagle's medium (DMEM), propidium iodide, Annexin V, FITC-Annexin, hexadecyltrimethylammonium bromide (HTAB), tris (hydroxyl methyl) amino-methane, N,N-dimethyl-N-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP), hydrochloric acid, sodium hydroxide, and calcium chloride were purchased from Sigma-Aldrich (St. Louis, MO). Opti-MEM cell culture medium, phosphate-buffered saline (PBS), fetal bovine

serum (FBS), trypan blue and calcein AM were purchased from Invitrogen (Carlsbad, CA). The thermotropic liquid crystal (LC) E7, sulfuric acid, chloroform and hydrogen peroxide (30% w/v) were purchased from Merck (Mumbai, India). Ethanol was obtained from Jebsen & Jenssen GmbH and Co., Germany (s d. fine–chem limited). 4-cyano-4'-pentylbiphenyl (5CB) was obtained from Sigma-Aldrich (St. Louis, MO). Deionization of a distilled water (DI water) source was performed using a Milli-Q-system (Millipore, bedford, MA). All materials were used as received without further purification.

Formation of PLL laden 5CB films on Micro-pillars for PM-IRRAS Measurements

The gold films with thicknesses of ≥ 2000 Å were deposited onto micro-fabricated supporting surface mounted on rotating planetaries (no preferred direction or angle of incidence) by using thermal evaporator (Excel Instruments, India). This supporting surface is an array of nickel (Ni) micro-pillars electroplated on a glass substrate fabricated as described in detail elsewhere. A layer of titanium (thickness~ 100 Å) was used to promote adhesion between the micro-pillars and the film of gold. The rates of deposition of gold and titanium were ~1Å/s. The pressure in the evaporator was close to 9×10^{-7}torr before and during each deposition.

These gold coated micro-pillars were dipped into 0.1% (v/v) DMOAP solution in DI water for 5 min at room temperature and were then rinsed with DI water to remove unreacted DMOAP from the surface. The DMOAP coated micro-pillars were dried under a stream of nitrogen gas and kept in oven at 100 °C. Then, 5CB was dispersed onto the micro-pillars. These LC filled micro-pillars were then incubated in PLL solution (0.5 mg/mL) for 4-5 h. The PLL laden 5CB films onto DMOAP supported on a uniformly deposited film of gold coated micro-pillars were kept under vacuum for PM-IRRAS study.

Vibrational Circular Dichroism (VCD) and Circular Dichroism (CD) Measurements

The VCD spectra were measured in the 1800-800 cm^{-1} range using a Bruker FT-IR spectrometer equipped with the Bruker polarization modulation accessory PMA 50. In the PMA 50 module, the light beam is focused onto the sample passing through an optical low pass filter (blocking wavenumbers >1800 cm^{-1}), a KRS-5 wire grid polarizer, and a ZnSe Photoelastic Modulator (PEM) with an oscillation frequency of 42 kHz. The light is focused by a ZnSe lens to a MCT detector. The detector signal comprises two components: a low-frequency modulation which corresponds to the IR absorption bands, i.e., the A signal, and a high-frequency modulated signal (42 kHz) corresponding to the dichroic absorptions, i.e., the ΔA signal. Additionally, the reference signal direct from the PEM (42 kHz) is mixed to the high-frequency modulated detector signal. These two high-frequency modulated signals are demodulated by an internal synchronous demodulator integrated in the electronic units of the TENSOR series FTIR spectrometers. The VCD spectra were recorded with 1 h data collection time at 4 cm^{-1} resolution. The sample was held in demountable cell with CaF_2 windows and a 25 μm spacer. Spectra were measured in D_2O solvent at a concentration of 10 mg/mL. For each measurement, the intensity calibration factor was obtained using a multiple-wave retardation plate combined with the second wire grid polarizer, whereby the system tuning was exactly the same as for the sample measurement. The spectra were corrected by subtracting the absorption (or VCD) of the corresponding solvent and were plotted in Origin 8 software.

The CD spectra were acquired using a Chirascan Spectrophotometer (Applied Photophysics, UK) in a 1 mm path length quartz cell with a scan range of 190–260 nm and 1 nm as step size. The spectra were corrected for the buffer signal.

Zeta Potential Measurements

A Malvern Zetasizer Nano ZS90 instrument (Malvern Instruments, Southborough, Massachusetts) was used to measure zeta potential at 25 °C for LC emulsions. The DLS instrument was operated under the following conditions: temperature: 25 °C, detector angle: 90 incident laser wavelength: 632 nm, and laser power: 4 mW. Samples prepared were loaded into a pre-rinsed zeta potential cell for measurements. Approximately 5 μL of 1 vol. % emulsions was added to 2.5 mL of millipore water. The quoted values were calculated by taking the average of 5 successive measurements.

Preparation of Hollow Capsules

The dried $(PSS/PLL)_5/PSS$ capsules were obtained after removing the E7 core, according to the method reported in the literature.[1-2] Briefly, 1 mL of ethanol was added to 1 mL of $(PLL/PSS)_5/PSS$- coated E7 emultion. The mixture was agitated with a vortex mixer and allowed to stand for 15 min. The mixture was then centrifuged, the supernatant removed, and fresh ethanol was added. This was repeated three more times. After the last ethanol addition, the samples were allowed to stand overnight to ensure complete dissolution and removal of 5CB. Before characterization, the hollow capsules were washed three times with water. Atomic force microscopy was performed with an Innova AFM (Bruker, Santa Barbara, CA). The images were acquired with a nanodrive 8.03 software.

Polarized and Fluorescence Microscopy

The PLL coated LC droplets treated cells were examined with bright field, plane–polarized in transmission mode on a (Zeiss, Scope A1, Germany) polarizing optical microscope using an

objective power of 200X and 500X. The orientation of the LC within droplets was examined with plane-polarized light in transmission mode with crossed polarizers. Cells were characterized with bright field, phase contrast and fluorescence microscopy. Fluorescence imaging was performed with (Zeiss, Axio Scope A1, Germany) fluorescence microscope equipped with a 100 W mercury lamp. The samples were viewed using a fluorescence filter cube with a 460 nm excitation filter and a 534 nm emission filter. Images were obtained with Axio cam camera.

Trypan Blue Exclusion Assay

Viability of T84 cells exposed to PLL-coated LC droplets was determined by trypan blue dye exclusion assay. Briefly $1x10^6$ cells/mL were plated and treated with different concentrations (0, $1x10^5$/mL, $2x10^5$/mL and $3x10^5$/mL) of PLL-coated LC droplets and incubated for 45 minutes 37 °C. Exposed cells were harvested, washed with PBS, and mixed with equal volume of 0.25 % trypan blue dye solution and incubated for 5 minutes at room temperature. 10 µL of this suspension was loaded onto a hemocytometer (Invitrogen, UK) and viable cells were counted manually. Loss in viability was expressed as percent dead cells.

Table S1. Vibrational frequency (cm^{-1}) assignments for PLL and 5CB-laden PLL monolayers using PM-IRRAS.

Frequency (cm^{-1})	Vibrational assignment	Frequency (cm^{-1})	Vibrational assignment
	PLL Monolayer		**Monolayer of PLL-Modified 5CB**
3037, 3030	ν (N-H) mode of protonated NH_3^+ side chain group	**3037, 3030, 3025**	ν (N-H) mode of protonated NH_3^+ side chain group
2964, 2924	ν (C−H) symmetric and asymmetric stretching	**2964, 2924**	ν (C−H) symmetric and asymmetric stretching
1653	ν (C=O) amide I (carbonyl stretch)	**2224**	ν (C≡N) stretch
1541	ν (C-N) + amide II (CN stretch and NH bend) ν (N-H)	**1653**	ν (C=O) amide I (carbonyl stretch)
1398	ν_s (COO^-) symmetric carboxylate stretch	**1605**	ν (C–C) stretch
		1558, 1541	ν (C-N) + amide II (CN stretch and NH bend) ν (N-H)
		1398	ν_s (COO^-) symmetric carboxylate stretch

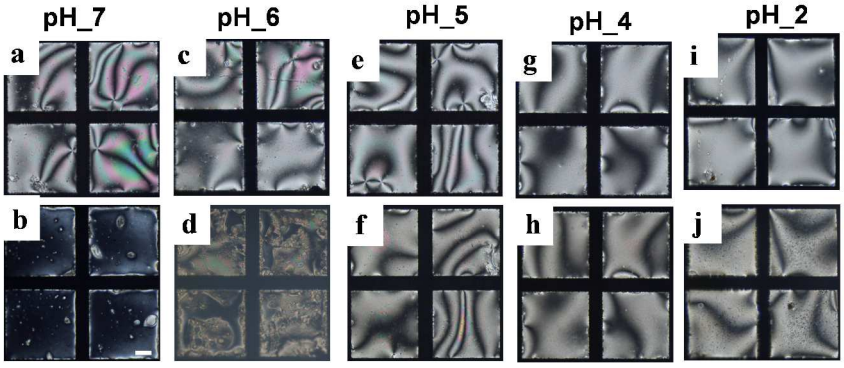

Figure S1: Polarized light micrographs (a,b) confined to a gold grid on DMOAP-coated surfaces in contact with different pH solutions (a,c, e, g,i) and after addition of 0.5 mg/mL of PLL (225 kDa) solution (b,d, f, h, j). Scale bar = 40 μm.

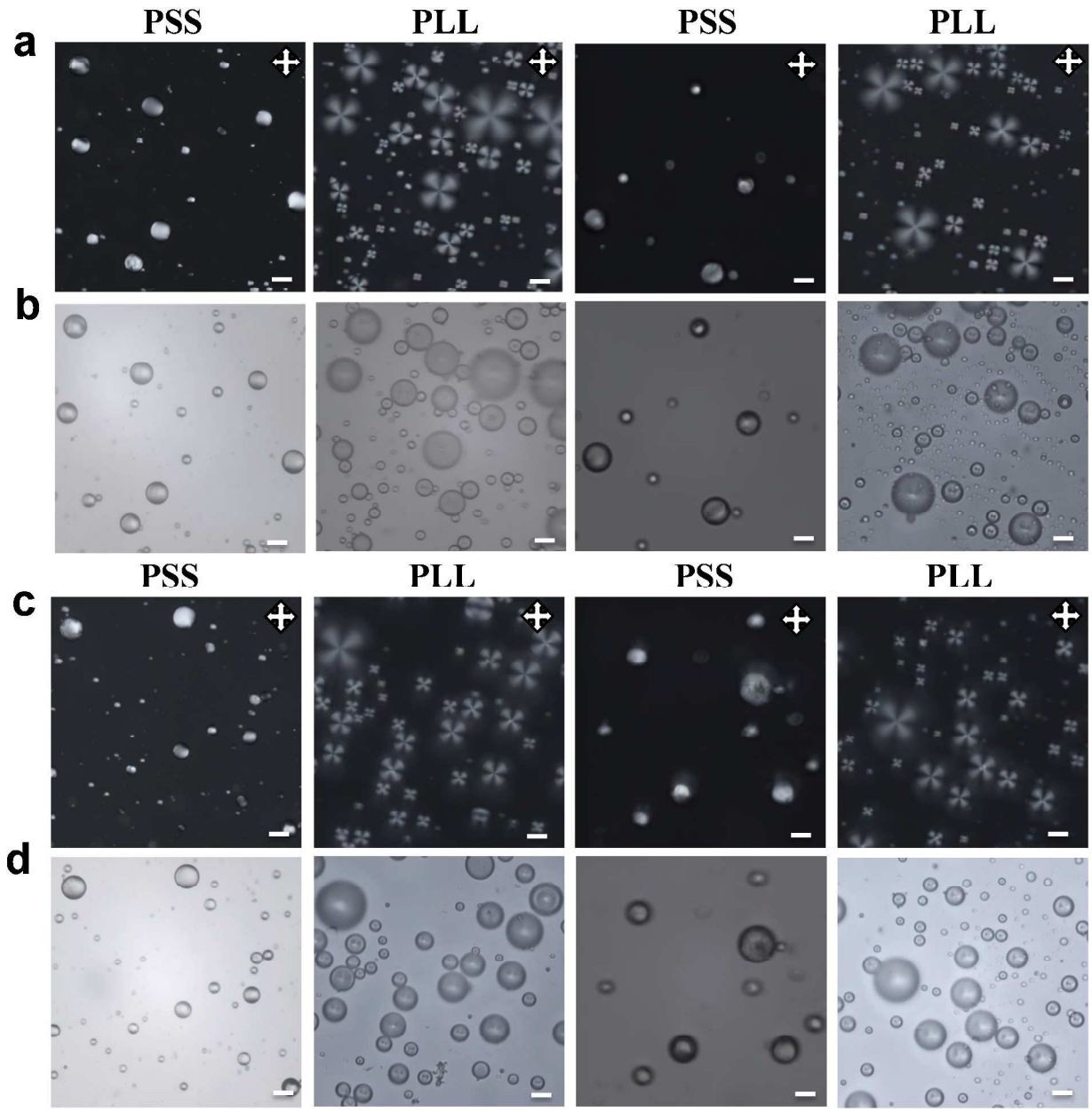

Figure S2. Polarized optical microscopic characterization of polyelectrolyte multilayer of LC droplets build up. (a,c) Polarized optical micrographs of PSS-coated LC droplets as a function of the number of deposition steps. (b,d) Corresponding bright field micrographs. The first deposition is PLL followed by PSS. [PLL], [PSS] = 1mg/mL. The scale bar= 40 μm.

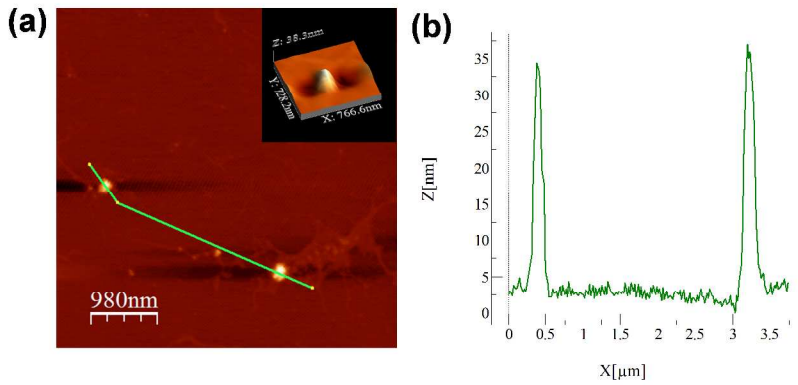

Figure S3. (a) AFM image of a hollow capsule obtained from (PLL/PSS)5 /PSS E7 emulsion and its b) height profile.

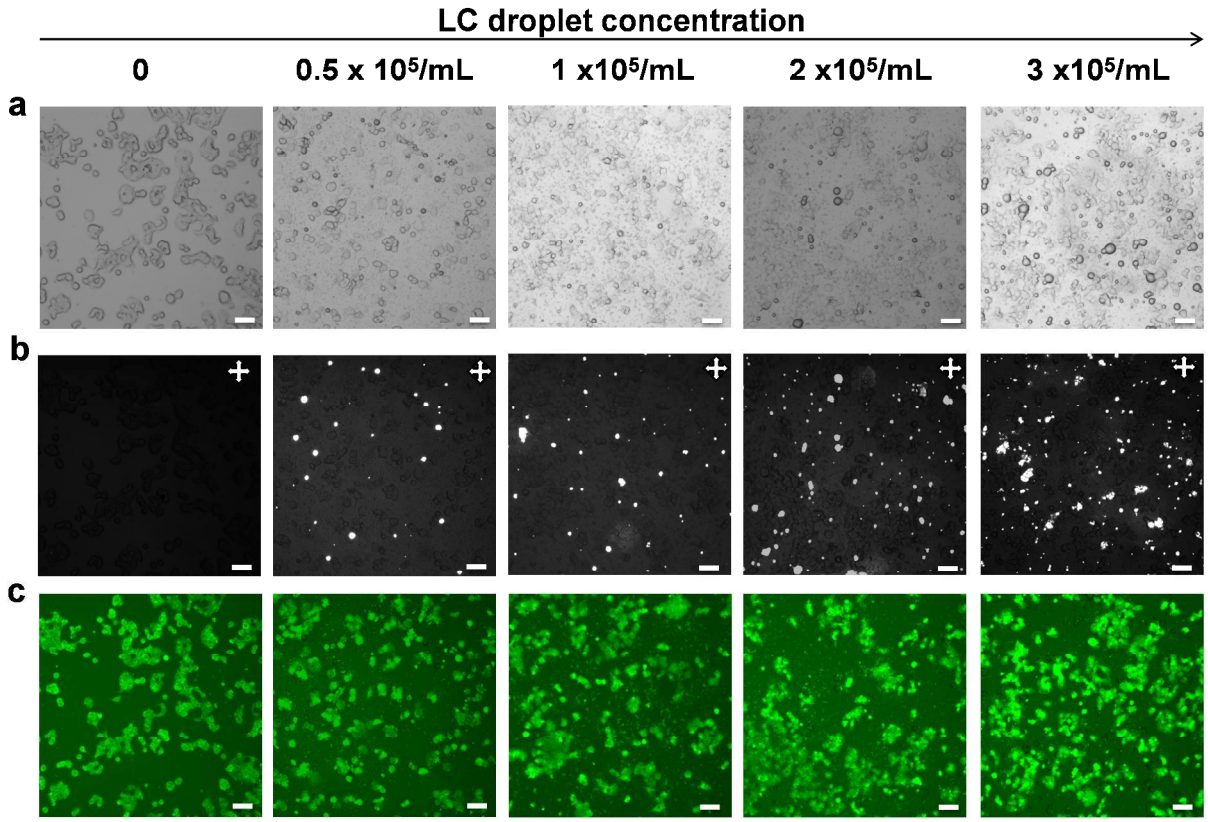

Figure S4. Optimization of PLL-coated LC droplet concentration for cell adherence. a) bright field, b) crossed polars and c) epi-fluorescence (cells stain with calcein–AM) microscopic images of cell adhered PLL coated LC droplets on incubation of 0, 10^4/mL, 1 x 10^5/mL, 2 x 10^5/mL and 3 x 10^5/mL concentration of PLL-coated LC droplets in contact with cells. The increasing concentration of droplets resulting more number of droplets attached to cells without affect the cell viability. The green fluorescence corresponds to the cells stain with calcein–AM as a marker of cell viability. Scale bar = 40 μm.

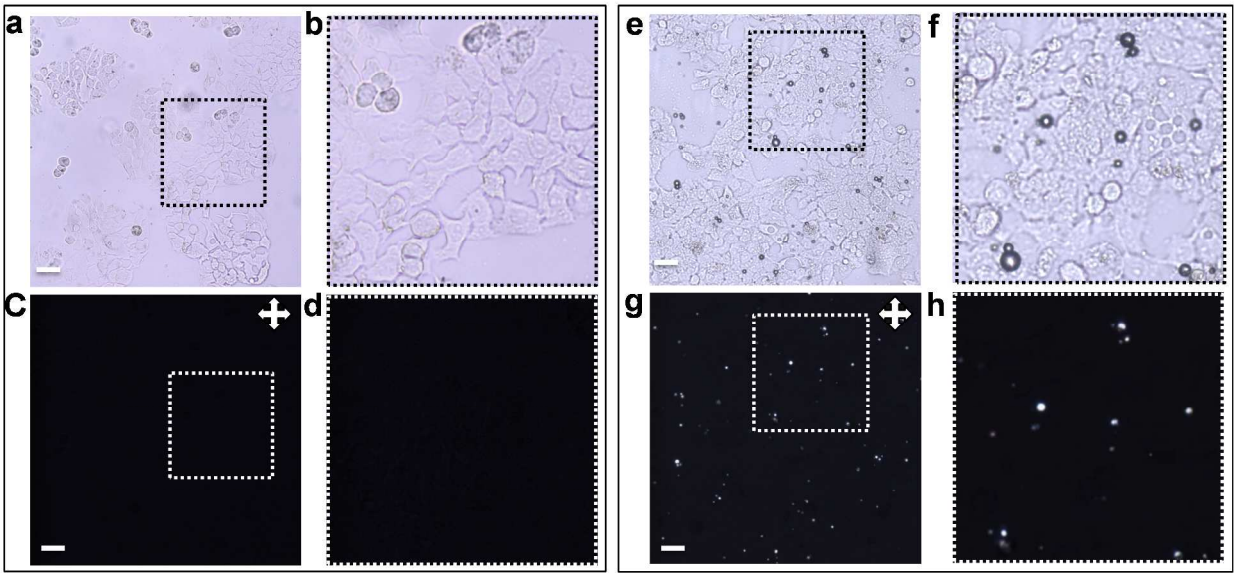

Figure S5. Cell adherence towards PLL-coated/uncoated LC droplets. (a,e) Bright field and (c,g) corresponding polarized light microscopy images of bare and PLL-coated LC droplets in contact with cells for 1 h incubation, respectively. (b,d,f,h) are the zoomed portions of the insets shown in images (a,e,c,g). The PLL-coated LC droplets adhered strongly to the surface of cells while bare droplets are completely removed in washing step. Scale bar = 40 μm.

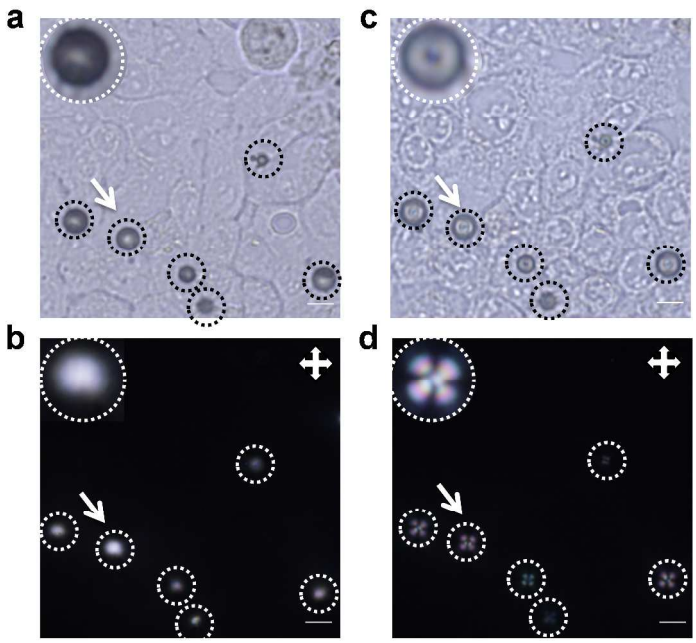

Figure S6. Ordering response of cell anchored PLL-coated LC droplets in addition to cytotoxic agents such as HTAB. (a,b) Bright-field and polarized microscopic images of PLL-coated LC droplets anchored on cells. (c,d) corresponding bright and polarized microscopic images after addition of HTAB (100 μM). The director configuration of PLL-coated LC droplets attached with cells change from bipolar to radial after adsorption of HTAB through PLL layer and then interacts with LC. The insets show the magnified version of configuration transition of arrow marked PLL-coated LC droplet anchored on cells before and after addition of HTAB. Scale bar = 10 μm.

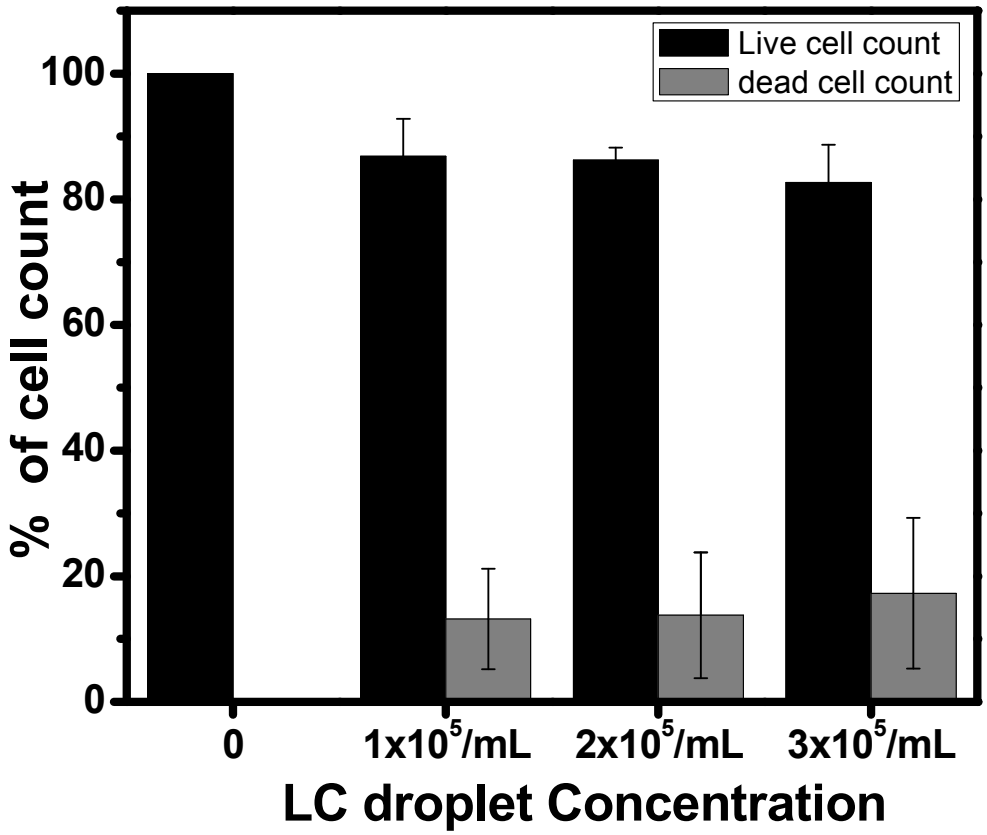

Figure S7. Quantification of PLL-coated LC droplets anchored on T-84 cell survival using Trypan blue exclusion assay. The graph represents the live and dead cell count percentage with increasing concentration of PLL-coated LC droplets. The relative change in the number of live and dead cell count after PLL coated LC droplet treatments was slight even at $3x10^5$/mL concentration in contrast to relative change observed using flow cytometry staining assay. These results also exemplify that the PLL coated droplets are biocompatible to cells upto 10^5/mL level, consistent to the optimum level of droplet concentration used for cell based study.

References

(1) Tjipto, E.; Cadwell, K. D.; Quinn, J. F.; Johnston, A. P. R.; Abbott, N. L.; Caruso, F. Tailoring the Interfaces between Nematic Liquid Crystal Emulsions and Aqueous Phases via Layer-by-Layer Assembly. *Nano Lett.* **2006**, *6*, 2243-2248.

(2) Caruso, F.; Yang, W.; Trau, D.; Renneberg, R. Microencapsulation of Uncharged Low Molecular Weight Organic Materials by Polyelectrolyte Multilayer Self-Assembly. *Langmuir* **2000**, *16,* 8932-8936.