Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary information

Bacterial growth sensing in microgels using pH-dependent fluorescence emission

Anil Chandra[a] and Neetu Singh [a,b]

[a] Centre for Biomedical Engineering, Indian Institute of Technology Delhi, Hauz Khas, New Delhi-110016, India

[b] Biomedical Engineering Unit, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029, India

*E-mail: sneetu@iitd.ac.in Fax: + 91-11-26582037 Tel: + 91-11-2659-1422

Materials and reagents

Dulbecco's Modified Eagle's medium (DMEM-high glucose) and SnakeSkin® dialysis tubing (3500 kDa) were procured from Thermo Scientific. Cuvettes for fluorescence and absorption spectroscopy were obtained from BioTek (USA). Calcium chloride and sodium chloride was procured from Merck. Sodium alginate was purchased from Sigma Aldrich. *E.coli* DH5α was borrowed from Department of Biochemical Engineering & Biotechnology at IIT Delhi. E.coli DH5α-pET 32a vector with resistance towards ampicillin was received as a generous gift from Department of Biosciences and Bioengineering Indian Institute of Technology Guwahati, India. Low-liter Boost Step-up Power Module was purchased online from amazon.com. 30 Gauge hypodermic needle was procured from BD India. PMO line tubing was procured from Romsons. Ampicillin was purchased from Himedia, India.

Production of alginate microgels

Sodium alginate gelation using divalent cation like Ca^{2+} is a common technique for producing hydrogels. We produced alginate microgels by first producing micron size droplets of sodium alginate followed by its crosslinking in a solution of $CaCl_2$. Production of micron scale droplets was achieved by using high voltage droplet generator which was assembled in the lab itself. The high voltage was generated using Low-liter Boost Step-up Power Module, which produced ~10,000 volt DC using 3.5 volts DC as an input voltage. For microdroplet production 1% sodium alginate solution was prepared in 0.9% NaCl solution and made to come out from a needle of small diameter using syringe pump at a defined flow rate. The voltage of the needle is maintained at ~10,000 volt by connecting it to one of the output terminals of the power module. The charged sodium alginate solution coming out of the hypodermic needle gets pulled towards calcium chloride solution (1.1 wt%) in Petri plate with a small magnetic bead for stirring. The Petri plate was maintained at a charge of opposite polarity to that of charge on the needle by connecting the $CaCl_2$ solution to the other output terminal of the Power Module. In this way, the monomer solution was pulled as soon as it comes out of the needle opening to generate microdroplets due to electrostatic attraction, followed by their crosslinking in $CaCl_2$ solution. The drops instantly crosslink in $CaCl_2$ followed by further incubation of 30 minutes for proper completion of crosslinking.

The size of the microgel was modified by varying the needle gauge, dropping distance, and flow rate or combination of all of these parameters. The dropping distance which is the distance between needle tip and surface of the $CaCl_2$ solution affects the downward pulling force being experienced by the droplets. Thus changing the dropping distance can result in smaller or larger droplets due to variation in overall resultant attractive force. Use of a needle with smaller inner diameter produced microgels of a smaller size when other parameters were fixed.

Encapsulation of carbon dots and *E.coli* was done by preparing their suspension in the sodium alginate solution before droplet generation. After crosslinking the microgels were washed with DMEM media without phenol red (high glucose). The microgels were them placed in 96 well plate after resuspending them in media (DMEM and LB in 1:1 ratio) followed by incubation at 37°C. The number of microgels per well was controlled to get a defined number of bacteria in each well of the 96 well plate.

Estimation of number of *E.coli*

The *E.coli* cell density in the broth culture was estimated by taking absorbance at 600 nm (OD600 of 1 = 8 x 10^8 cells/mL). As the absorbance of the broth reached a value of 0.5, a volume from the broth is added to the sodium alginate solution to yield the desired final bacterial concentration. In this study, the final bacterial concentration in sodium alginate was chosen to be $2x10^7$ cells/mL. The number of encapsulated E.coli in microgel per well was fixed to be 10^6 estimated by putting equal volumes of gels in each well of the 96 well plate.

Synthesis of pH-sensitive carbon dots

Carbon dots were synthesized using simple hydrothermal reaction. *Agaricus bisporus* (0.6 gram) was lyophilized, ground and dispersed in 10 mL deionized water along with 3 mL (1M) ethylenediamine. The solution was ultra-sonicated for 30 minutes followed by heating for 12 hours at 160°C in Teflon lined stainless steel autoclave. After completion of the reaction, the autoclave was cooled slowly at room temperature. The dark yellow-brown solution containing carbon dots was centrifugation at 12000 rpm for 30 minutes to separate the supernatant containing carbon dots from large non-fluorescent debris. The solution containing carbon dots was further dialyzed using 3.5 kDa cut-off dialysis tubing against deionized water for 48 hours to

remove unreacted small organic molecules. After dialysis, the solution was characterized and used without further modification.

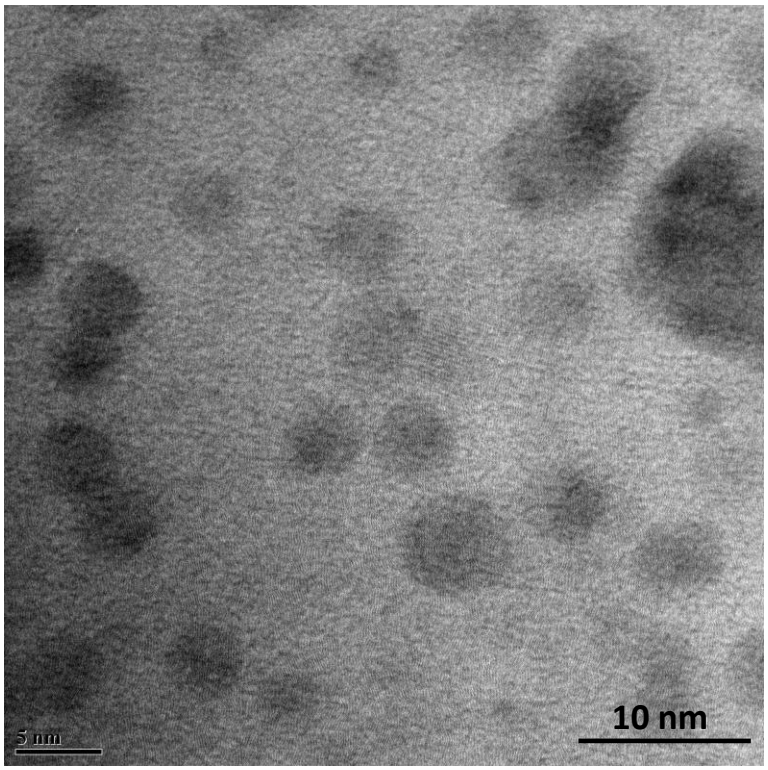

Figure S1: High-resolution transmission electron microscopy image showing monodispersed carbon dots.

Absorption and Fluorescence spectroscopy

Absorption and fluorescence spectra were taken using Take3 Micro-Volume Plate with Synergy H1 multiplate reader (BioTek USA) with monochromator-based optics. Fluorescence emissions were collected from microgels being cultured in 96 well flat bottom black plates. Absorbance at 600 nm was collected from microgels incubated in transparent 96 well flat bottom plates. Absorbance of the E.coli stock solution was assessed by using quartz cuvette of path length 10 mm.

Fluorescence microscopy

Widefield fluorescence microscopy was done using Olympus IX 73 fluorescence microscope with DAPI, FITC and TRITC filter cubes and color camera with Peltier cooling.

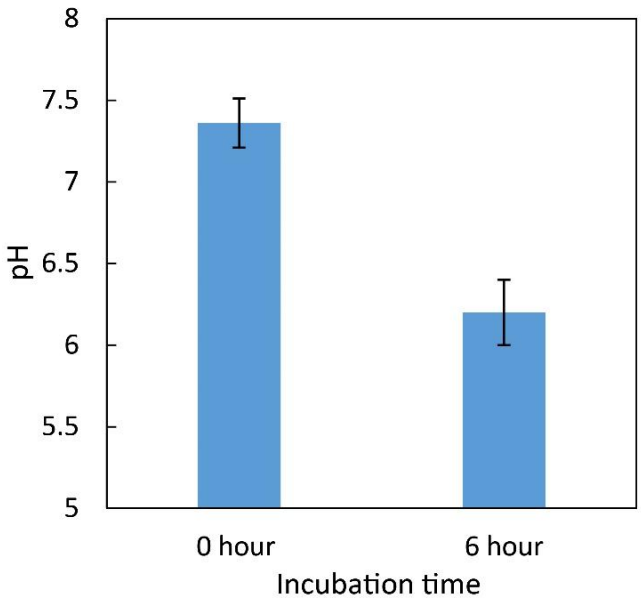

Figure S2. Effect of bacterial culture duration on pH of the broth. Error bar indicates standard deviation between triplicates

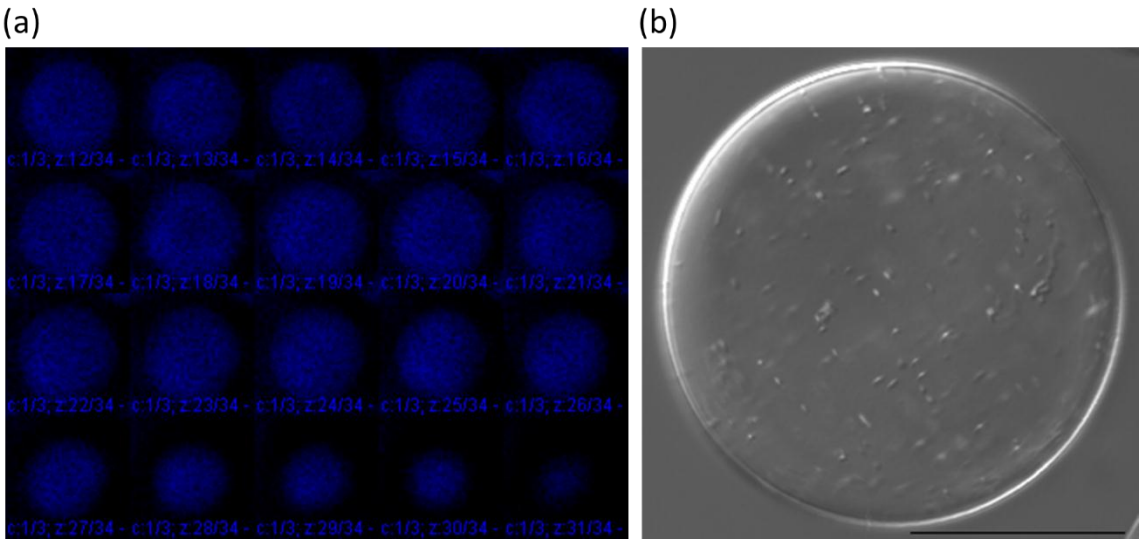

Figure S3. (a) Confocal Z-sectioning fluorescence images of microgel encapsulating *E.coli* and carbon dots showing a uniform distribution of carbon dots inside the microgels. (b) Brightfield image of the microgel showing encapsulated *E.coli* in the microgel. (scale bar: 50 µm)

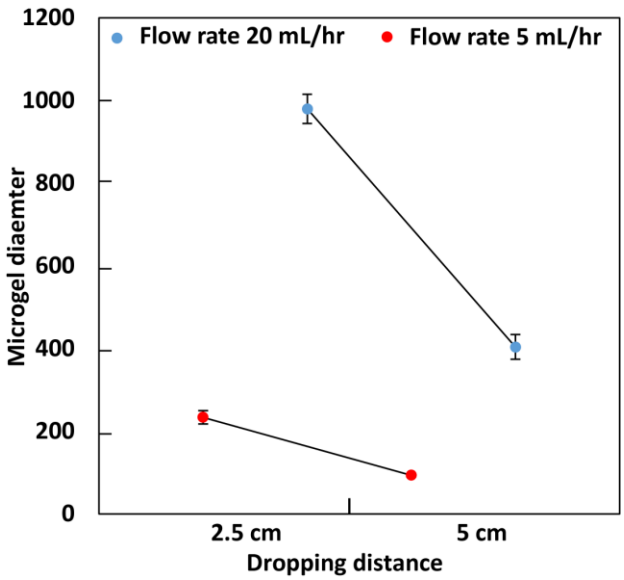

Figure S4. Combination of parameters used for generation of differently sized microgels. Error bar indicates standard deviation between triplicates

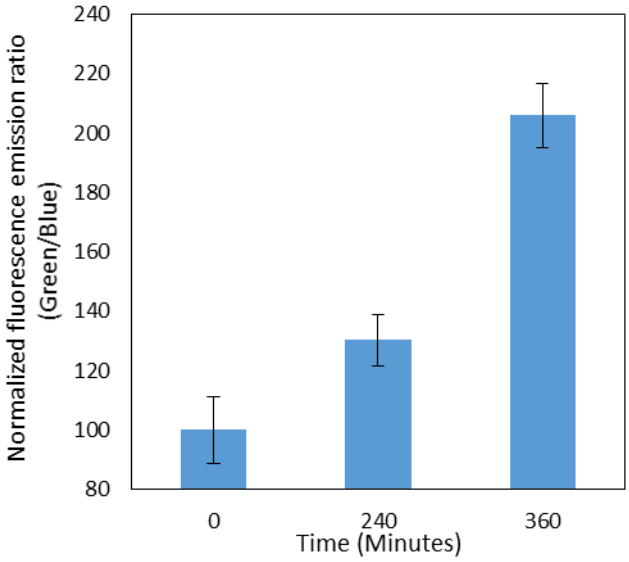

Figure S5: Sensitivity of the developed microgel-carbon-dot system in detecting E.coli growth in 240 and 360 minutes. The increase in the intensity ratio is ~0.3 fold after 240 minutes compared to ~2.1 fold after 360 minutes. Thus, the growth can be detected in as less as 240 minutes (4 hours) that becomes pronounced in 360 minutes (6 hours). Error bar indicates standard deviation between triplicate.

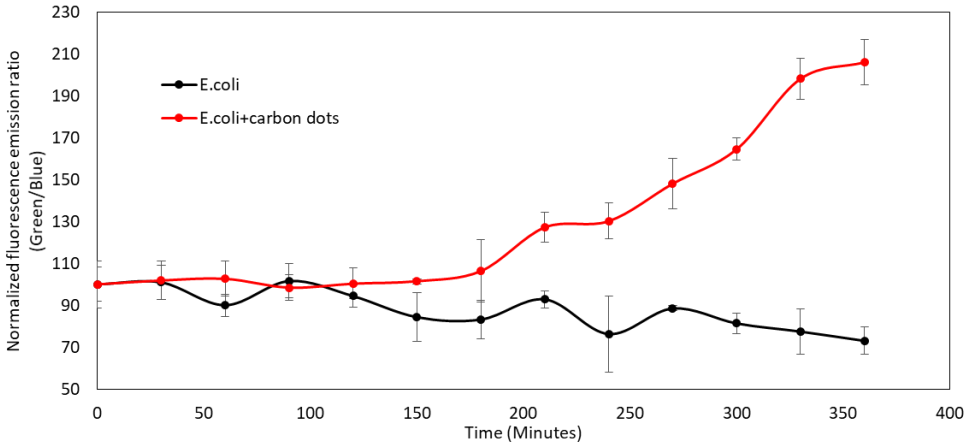

Figure S6: Difference between normalized fluorescence emission ratios of microgels containing only E.coli and E.coli in presence of carbon dots. A difference in the fluorescence emission ratios is observed from as early as 150 minutes (2.5 hour). Error bar indicates standard deviation between triplicate.

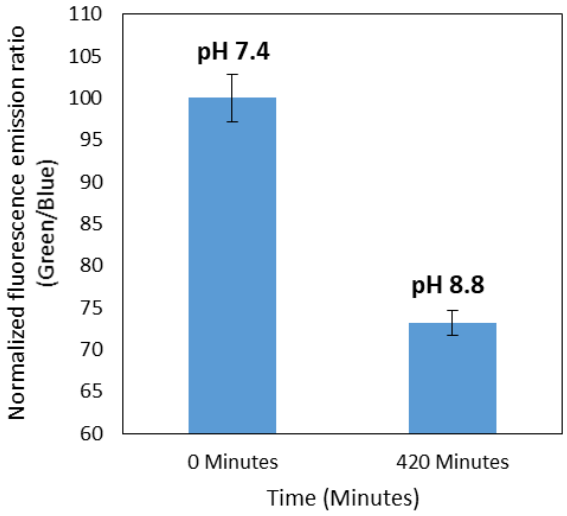

Figure S7: Estimation of S.aureus growth by assessing normalized fluorescence emission ratio variation over time. The emission ratio is decreasing over time, which indicates an increase in pH, which was also confirmed by pH measurement using pH electrode. Thus, our approach can not only sense decrease in pH associated with growth but it can also register an increase in pH, which many times occur during bacterial growth involving amino acid catabolism and conversion of generated urea finally into ammonia, which increases the pH of the medium.[1,2] Error bar indicates standard deviation between triplicate.

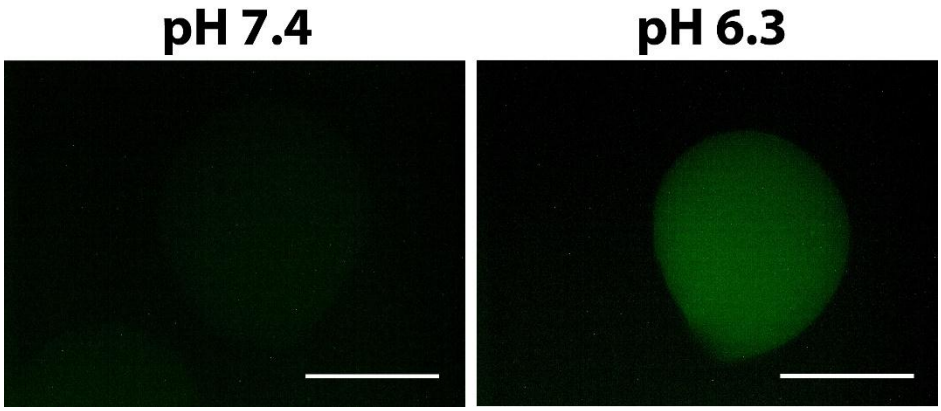

Figure S8. Fluorescence microscopy showing an increase in emission from microgels encapsulating E.coli and carbon dots due to a decrease in pH. (Scale bar: 100 µm)

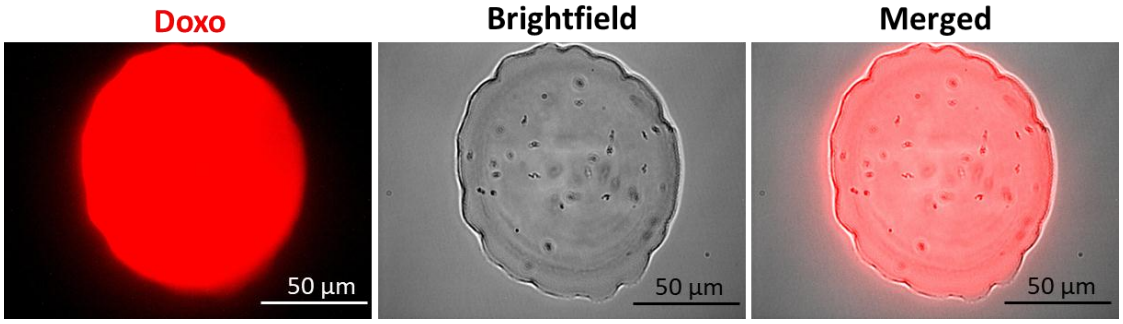

Figure S9: Fluorescence micrograph showing diffusion of fluorescent drug doxorubicin in the alginate microgel encapsulating E.coli.

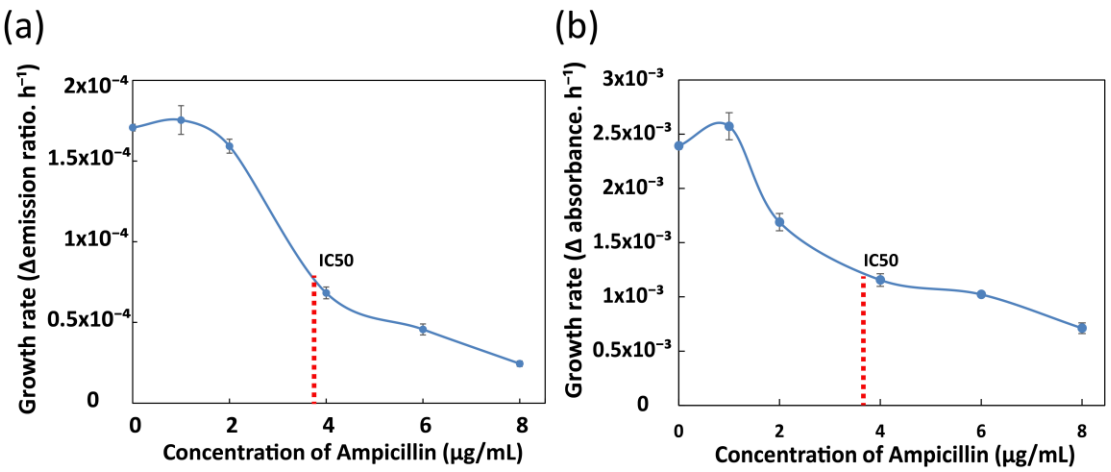

Figure S10. (a) Variation in the growth rate of E.coli, assessed from fluorescence emission ratio during exponential phase using a different concentration of ampicillin in the medium. (b) Comparison of the maximum growth rate of E.coli during exponential phase using absorbance at 600 nm at a different concentration of ampicillin in the medium. Error bar indicates standard deviation between triplicate.

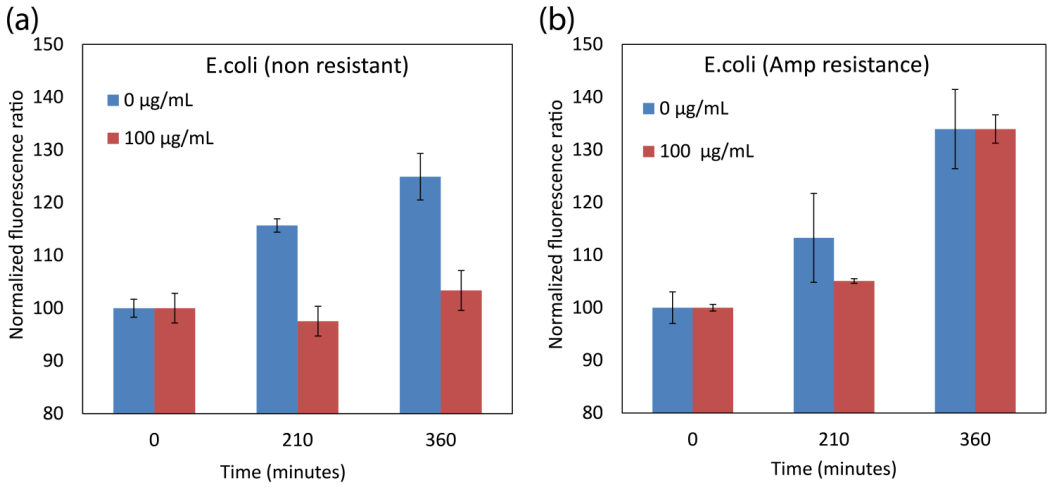

Figure S11. Effect of Ampicillin concentration on growth of E.coli and Ampicillin resistant E.coli at different time points. (a) Ampicillin concentration dependent difference in fluorescence emission ratio, which indicates growth, is clearly visible in case of non-resistant E.coli after 210 minutes and 360 minutes. (b) Growth of ampicillin resistant E.coli is affected to a very small extent in presence of ampicillin as observed in early time point of 210 minutes. The resistance towards ampicillin becomes more pronounced after 360 minutes. Error bar indicates standard deviation between triplicate.

References

1. C. R. Halsey, S. Lei, J. K. Wax, M. K. Lehman, A. S. Nuxoll, L. Steinke, M. Sadykov, R. Powers and P. D. Fey, *mBio*, , DOI:10.1128/mBio.01434-16.
2. A. G. Lone, E. Atci, R. Renslow, H. Beyenal, S. Noh, B. Fransson, N. Abu-Lail, J.-J. Park, D. R. Gang and D. R. Call, *Infect. Immun.*, 2015, **83**, 2531–2541.