Supporting Information

Encapsulation of Autoinducer Sensing Reporter Bacteria in Reinforced Alginate-based Microbeads

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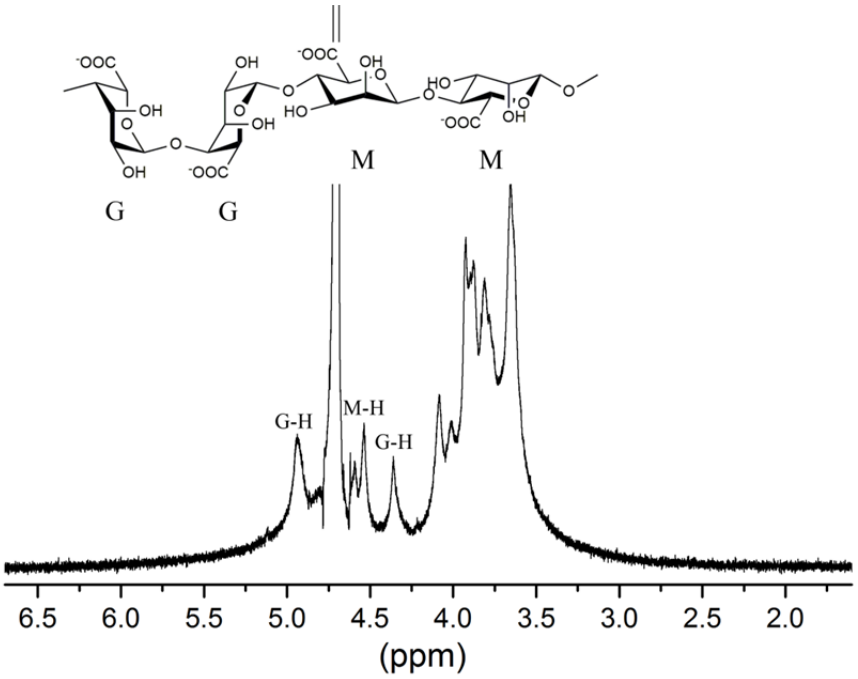
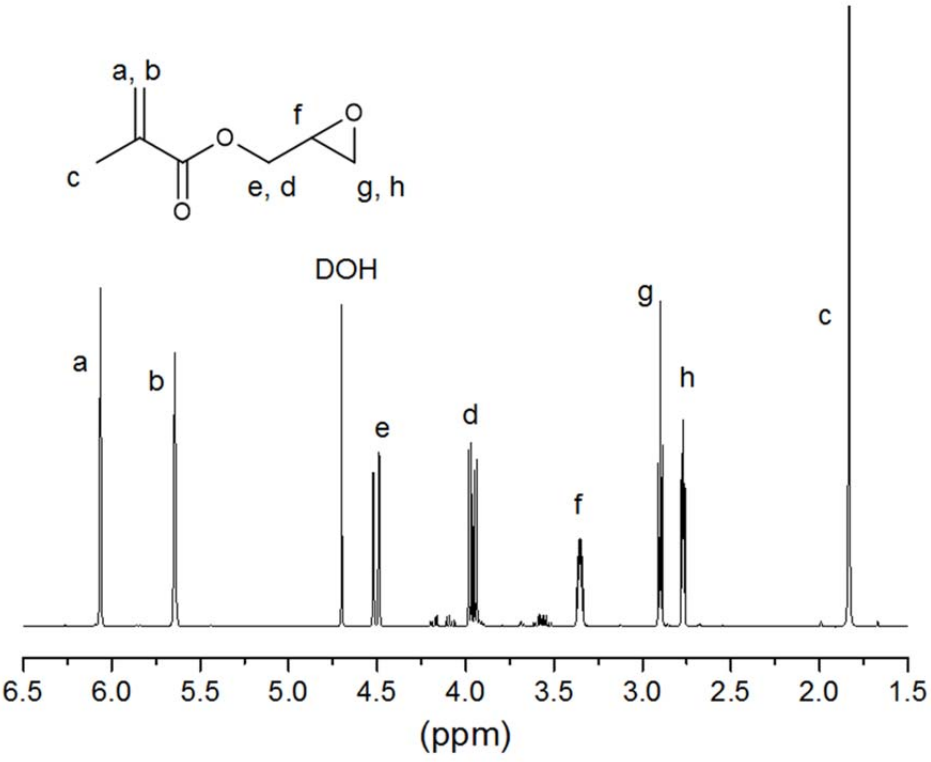
1. ^{1}H NMR spectra

a) ^{1}H NMR (400 MHz, D_2O, δ): δ = 4.95 (s, G-H), 4.55 (s, M-H), 4.36 (s, G-H) ppm;



b) ^{1}H NMR (400 MHz, D_2O, δ): δ = 6.07 (dd, 1H, -CH=CH2), 5.64 (dd, 1H, -CH=CH2), 4.49 (m, H, CH2), 3.95 (m, H, CH2), 3.35 (m, H, CH), 2.90 (dd, H, CH2), 2.77 (dd, H, CH2), 1.83 (s, 3H, CH3) ppm;



c) ^{1}H NMR (400 MHz, D_2O, δ): δ = 6.09 (dd, 1H, -CH=CH_2), 5.65 (dd, 1H, -CH=CH_2), δ=4.94 (s, 10H, G-H), 4.54 (s, M-H), 4.34 (s, 10H, G-H), 4.49 (m, CH_2), 3.95 (m, CH_2), 3.35 (m, CH), 1.83 (s, 3H, CH_3) ppm;

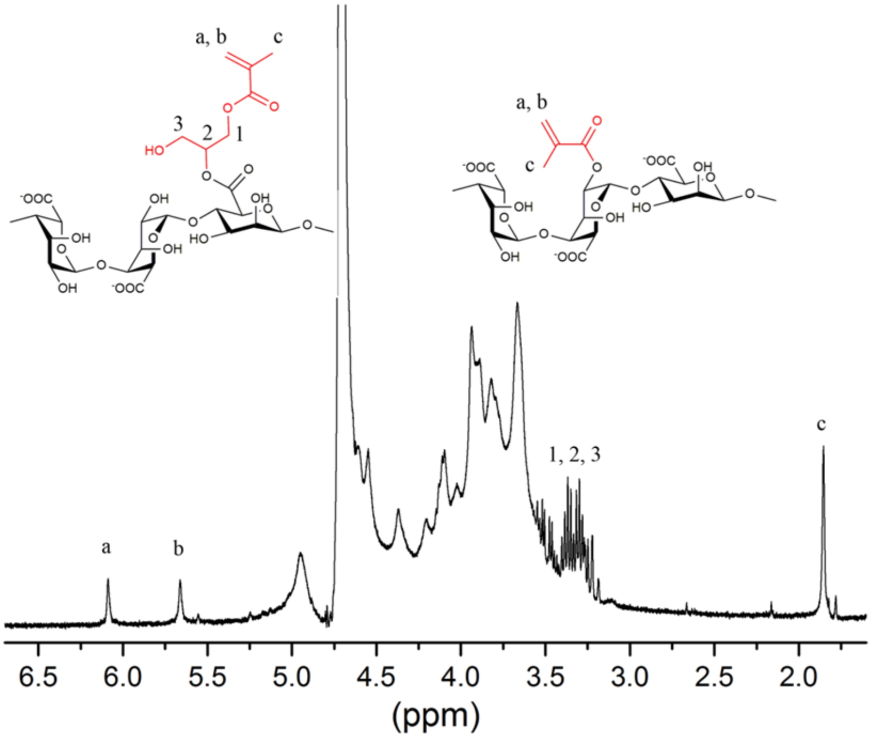


Figure S1. ^{1}H-NMR spectra of a) alginate, b) glycidyl methacrylate, and c) alginate-MA.

2. Alginate-MA beads obtained at different electric potential

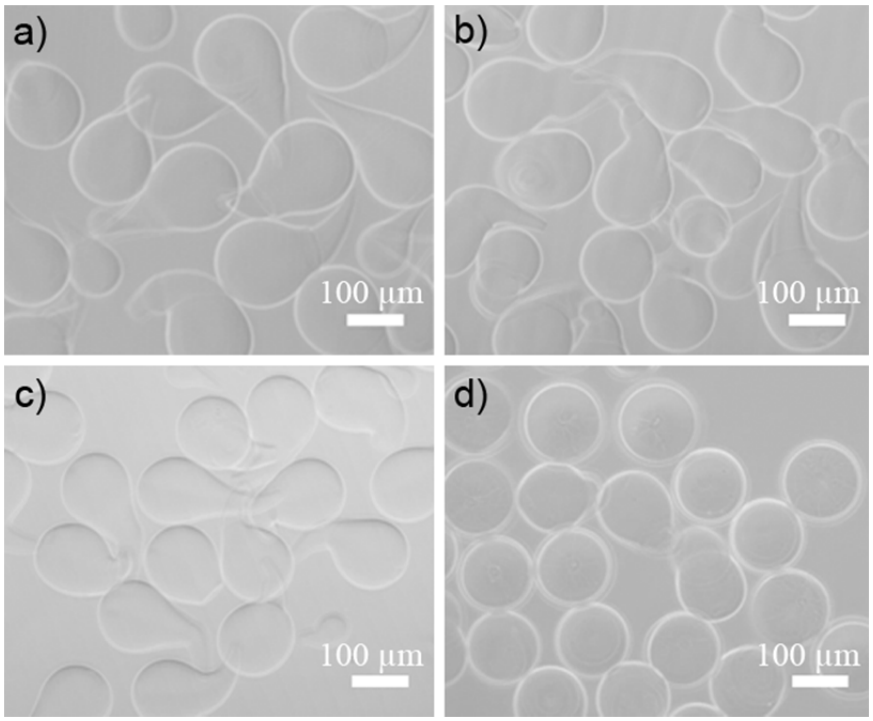


Figure S2. Optical microscopy images of 2 wt% alginate-MA beads formed under electric potentials of: a) 4.0 kV; b) 5.0 kV; c) 5.5 kV; and d) 6.0 kV, respectively, the beads were illuminated for 5 min with UV and were hardened 15 min in $CaCl_2$ gelling solution.

3. Characterization of fluorescein-labelled Alginate-MA beads

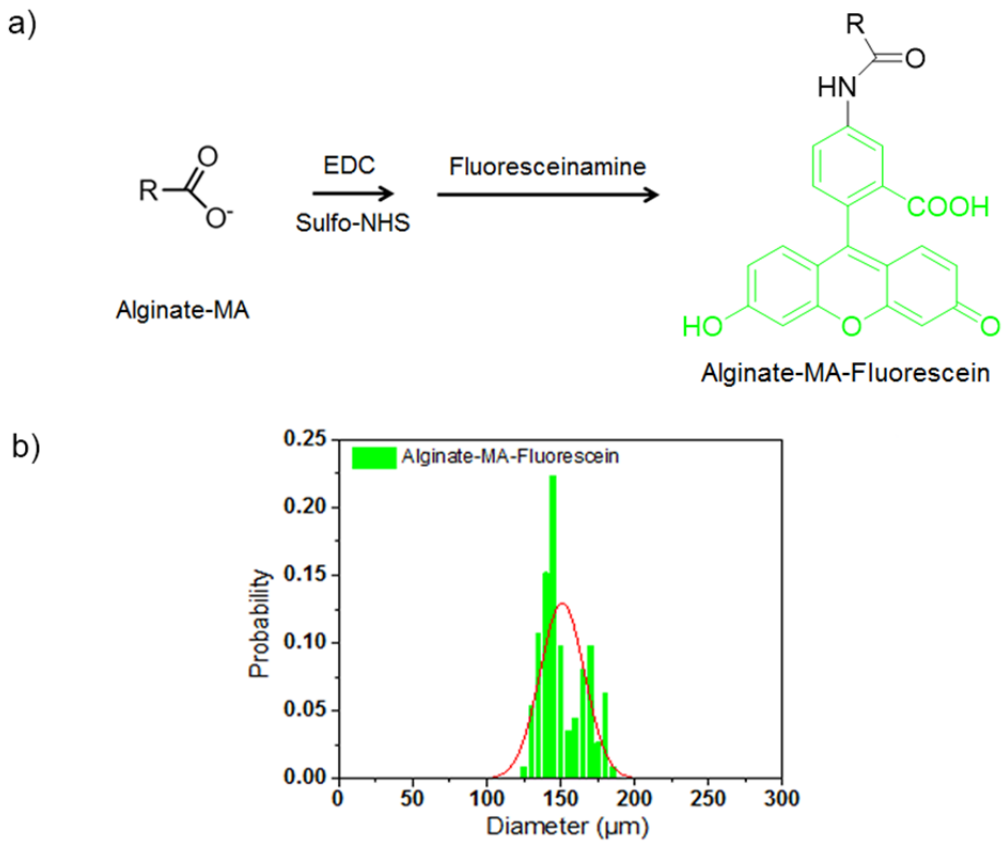


Figure S3. a) Scheme of conjugation of alginate-MA conjugated with fluoresceinamine; b) Diameter distribution of fluorescein-labelled alginate-MA beads formed under the following conditions: 2 wt% fluorescein-labelled alginate-MA, 6.0 kV.

4. Polymer distribution inside Fluorescein-labelled Alginate-MA beads

The concentration of fluorescein-labelled alginate-MA in the beads was investigated using a confocal laser scanning microscope (PicoQuant, Germany), comprising an OLYMPUS IX-71 frame (Olympus, Germany) and a commercial main optical unit (Microtime 200, PicoQuant, Germany). The measurements were done with a water immersion objective (60× magnification, Olympus, Germany). A pulsed diode laser (LDH-D-C-485, PicoQuant, Germany) with an excitation wavelength of 485 nm was used. The images (80 μm × 80 μm) were recorded with a resolution of 512 pixels × 512 pixels and the data were analyzed using software SymPhoTime (PicoQuant, Germany).

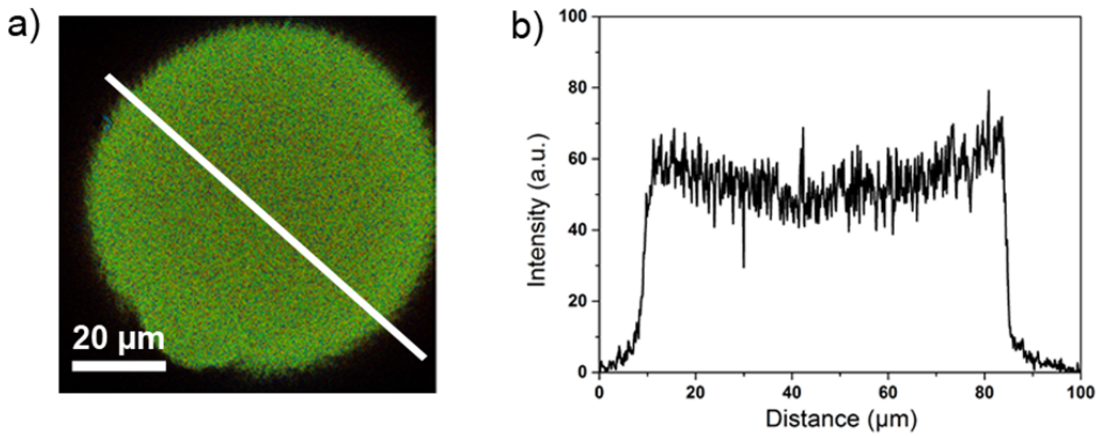


Figure S4. a) Confocal laser scanning microscopy image of 2 wt% alginate-MA beads formed at 6.0 kV, 5 min UV irradiation and 15 min hardening in $CaCl_2$ gelling solution; b) Corresponding cross sectional fluorescence intensity plot of Figure S4a).

5. Scanning electron microscopy (SEM) image

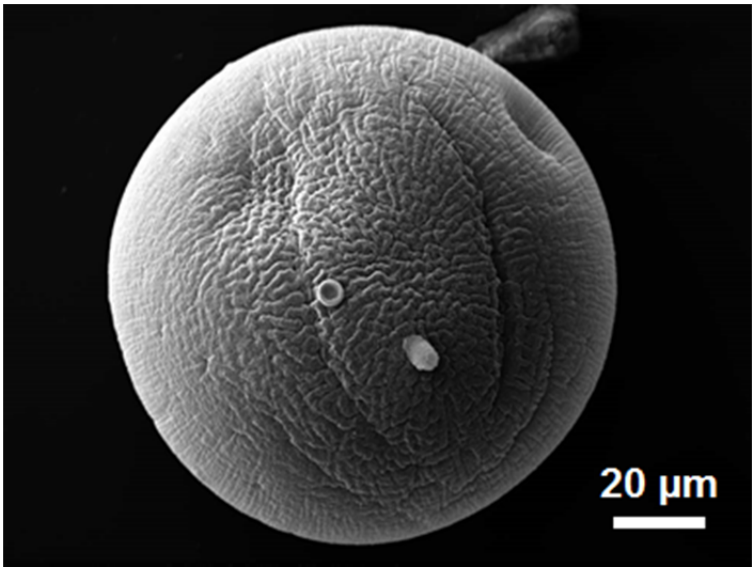


Figure S5. SEM image of an alginate-MA bead (diameter: 100 µm), 2 wt% alginate-MA beads were formed at 6.0 kV, followed by 5 min UV irradiation and 15 min hardening. The size of beads in the hydrated state (before water/ethanol exchange) was 184 ± 6 µm.

6. Hydrogel swelling

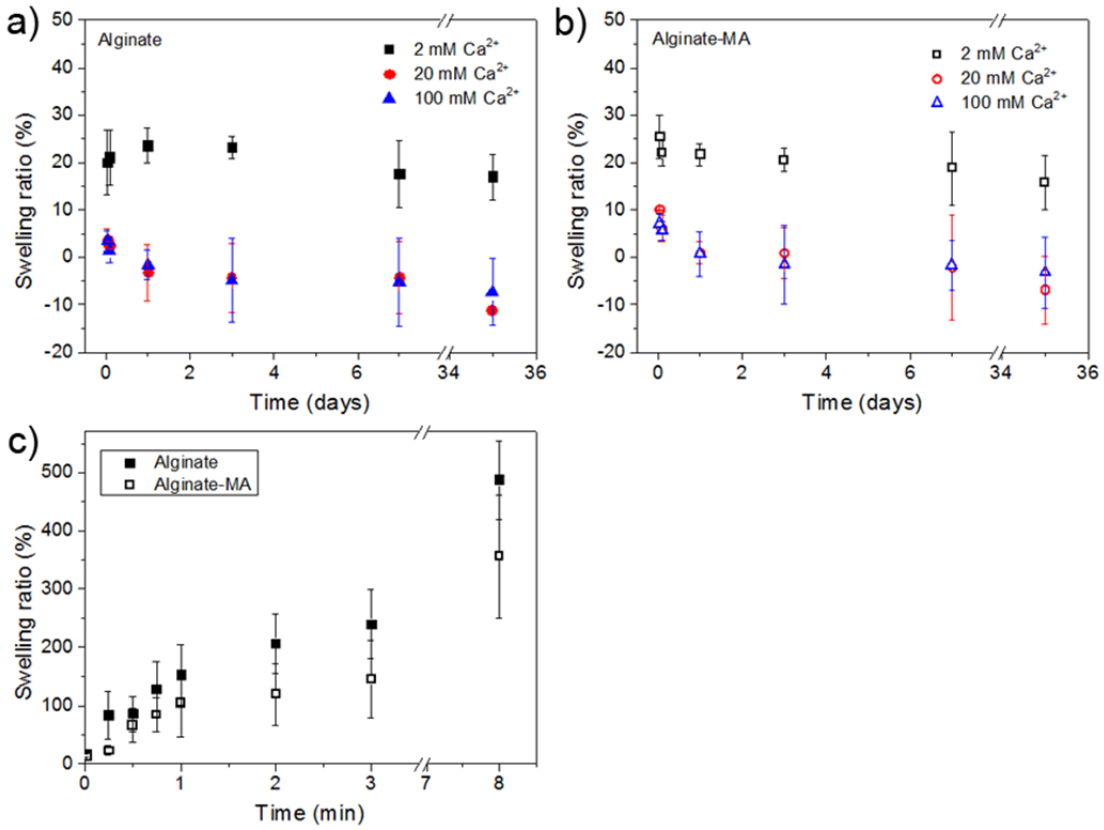


Figure S6. a) Swelling ratio of 2 wt% alginate beads in 2 mM, 20 mM, 100 mM $CaCl_2$ solution vs. time; b) Swelling ratio of 2 wt% alginate-MA beads in 2 mM, 20 mM, 100 mM $CaCl_2$ solution vs. time; c) Swelling ratio of 2 wt% alginate and 2 wt% alginate-MA beads in 50 mM sodium citrate solution vs. time; 2 wt% alginate beads were hardened in 100 mM $CaCl_2$ solution for 15 min, 2 wt% alginate-MA beads were exposed to 5 min UV irradiation and hardened for 15 min.

7. FITC-AHL standard curves

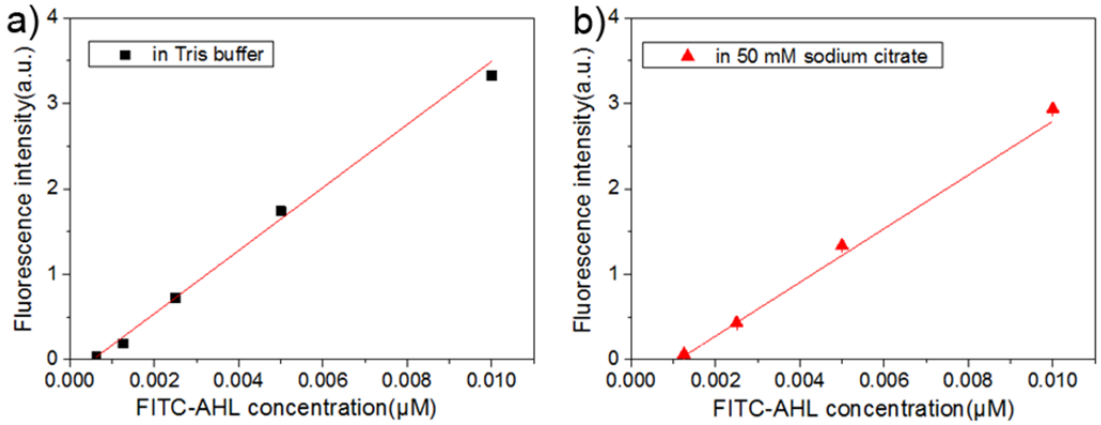


Figure S7. Standard curves and linear regression fit of fluorescence intensity *vs.* concentration of FITC-AHL in a) Tris buffer (10 mM, pH = 8.5) and b) 50 mM sodium citrate solution.

8. Bacteria growth curve and fitting

To investigate the influence of alginate, alginate-MA, initiator Irgacure 2959, and UV irradiation time on the bacteria viability, the proliferation of *Escherichia coli* TOP10 pTetR-LasR-pLuxR-GFP (*E. coli* pLuxR-GFP) in each solution was analyzed by measuring the absorbance at a wavelength of 600 nm (OD_{600}) using a microplate reader. The absorbance of the culture media (without bacteria) was subtracted for each time point. The growth curves were characterized by plotting $\ln(N_t/N_0)$ vs. time t and fitting the data using the Gompertz equation[1]:

$$\ln(N_t/N_0) = a\ exp[-exp(\mathrm{b} - \mathrm{ct})] \qquad (1)$$

From equation 1, the stationary phase bacteria population, lag time and maximum growth rate, generation time were calculated (according to Zwietering, M. H.; Jongenburger, I.; Rombouts, F. M.; van 't Riet, K., Modeling of the Bacterial Growth Curve. *Applied and Environmental Microbiology* **1990**, *56 (6*), 1875-1881): the asymptotic value of a growth curve in the logarithmic form equals a; the lag time $\lambda = (b\text{-}1)/c$; the maximum specific growth rate $\mu_m = ac/e$, e is Euler number; and the generation time equals $(\ln 2)/\mu_m$.

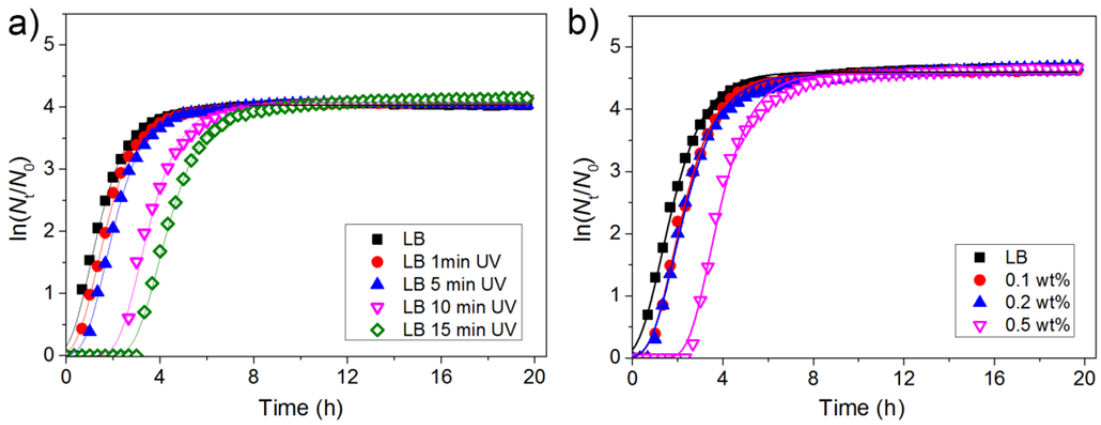


Figure S8. Plots of the logarithm of normalized bacteria number vs. time: a) *E. coli* pLuxR-GFP proliferation in LB medium after 0, 1, 5, 10, 15 min UV irradiation; b) *E. coli* pLuxR-GFP proliferation in LB medium after 5 min UV irradiation in presence of 0.1, 0.2, 0.5 wt% Irgacure 2959.

Table S1: Parameters of growth curves

	Asymptotic value	Lag time (min)	Generation time (min)
LB medium	4.7 ± 0.6	11± 9	25 ± 3
2% Alginate-MA/LB solution	4.7 ± 0.8	10 ± 9	26 ± 4
LB, 1 min UV	4.6 ± 0.7	11 ± 8	25 ± 4
LB, 5 min UV	4.5 ± 0.6	26 ± 11	27 ± 5
LB, 10 min UV	4.6 ± 0.8	132 ± 9	26 ± 4
LB, 15 min UV	4.6 ± 0.5	174 ± 10	26 ± 4
LB, 5 min UV, 0.1 wt% Irgacure 2959	4.6 ± 0.6	47 ± 15	25 ± 5
LB, 5 min UV, 0.2 wt% Irgacure 2959	4.5 ± 0.8	47 ± 17	26 ± 4
LB, 5 min UV, 0.5 wt% Irgacure 2959	4.7 ± 0.7	147 ± 14	27 ± 4

9. Encapsulation efficiency

Table S2. Encapsulation efficiency of *E. coli* pLuxR-GFP

Bacteria count in LB broth (CFU/mL)	Bacteria count in hydrogel beads (CFU/cm^3)	Encapsulation efficiency
7.0×10^7	5.3×10^7	76%
1.3×10^8	1.1×10^8	82%
1.5×10^8	1.3×10^8	89%
1.9×10^8	1.1×10^8	56%

10. Bacteria proliferation inside beads

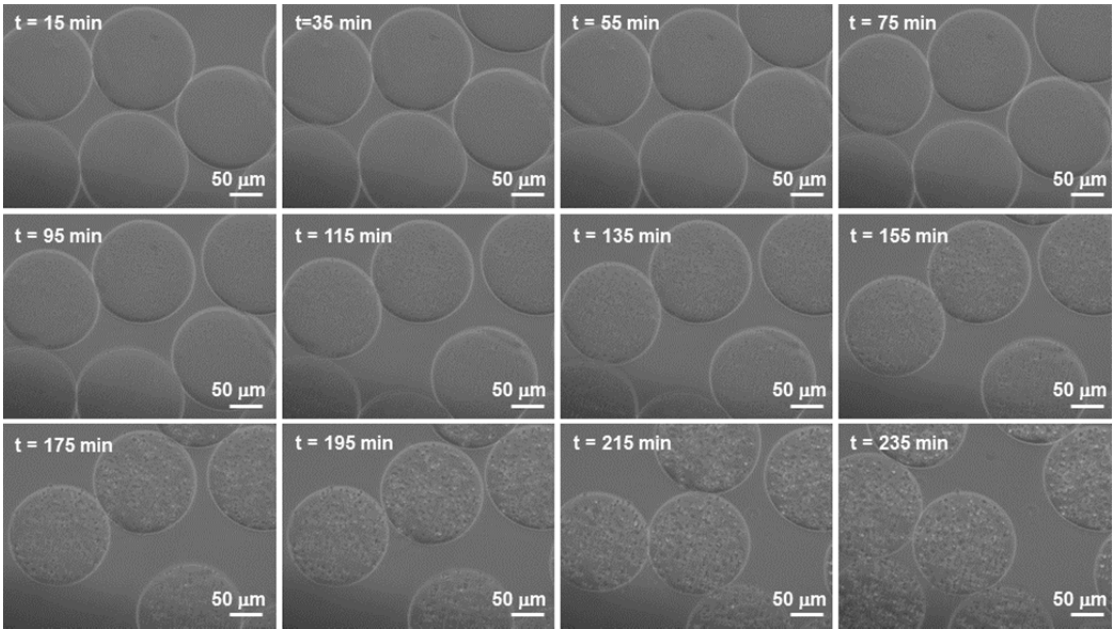
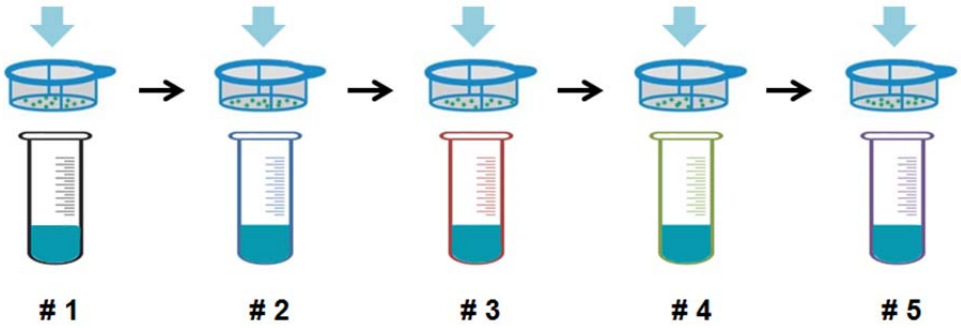


Figure S10. Time-lapse optical microscopy images of alginate-MA beads incubated in LB medium at 37°C. The increase amount of black dots indicates the proliferation of the entrapped *E. coli* pLuxR-GFP, the time interval between subsequent images is 20 min and the last picture was taken after around 240 min incubation.

11. Bacteria leakage test

Before bacteria leakage experiment, the beads were thoroughly washed with an excess volume (25 mL each time) of $CaCl_2$ solution to remove free bacteria attached on the bead surface, as shown in Scheme S1.



Scheme S1. Removal of free bacteria attached on the bead surface.

The number of bacteria in the washing solution is summarized in Table S3.

Table S3. The number of *E. coli* pLuxR-GFP in the washing solution.

Sample name / Washing time	Alginate beads washing solution CFU/mL	Alginate-MA beads washing solution CFU/mL
# 1	22	17
# 2	9	8
# 3	4	2
# 4	0	0
# 5	0	0

12. Fluorescence intensity and OD_{600}

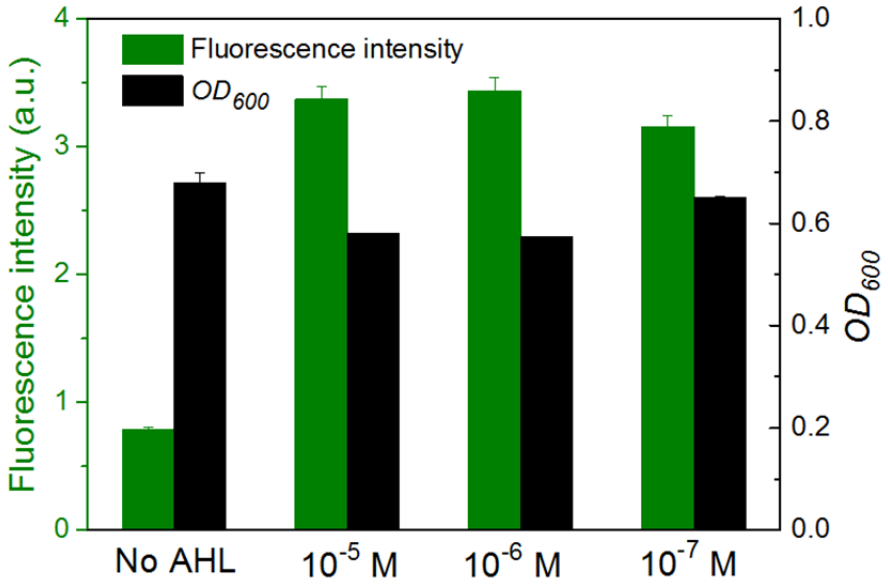


Figure S11. Fluorescence intensity and OD_{600} of *E. coli* pLuxR-GFP encapsulated in alginate-MA beads at 1.0×10^{-5}, 1.0×10^{-6}, 1.0×10^{-7} mol/L $3OC_{12}HSL$ after 4 h incubation. Error bars: standard deviation of three technical replicates from one exemplary data set out of a three times repeated experiment (biological replicate).

Reference:

1 *Zwietering, M. H.; Jongenburger, I.; Rombouts, F. M.; van 't Riet, K. Modeling of the Bacterial Growth Curve. Appl. Environ. Microbiol.* **1990**, *56*, 1875-1881.