Isolated Reporter Bacteria in Supramolecular Hydrogel Microwell Arrays

Ping Li,† Xiaqiu Dou,*‡ Chuanliang Feng,‡ Mareike Müller,‡ Matthew Wook Chang,§ Martin Frettlöh,∥ and Holger Schönherr‡,*

†Physical Chemistry I and Research Center of Micro and Nanochemistry and Engineering (Cμ), Department of Chemistry and Biology, University of Siegen, Adolf-Reichwein-Strasse 2, 57076, Siegen, Germany
‡State Key Lab of Metal Matrix Composites, School of Materials Science and Engineering, Shanghai Jiaotong University, 800 Dongchuan Road, 200240, Shanghai, People’s Republic of China
§Department of Biochemistry, Yong Loo Lin School of Medicine, and NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI), Life Sciences Institute, National University of Singapore, 28 Medical Drive, Singapore 117456, Singapore
∥Quh-Lab Food Safety, Siegener Strasse 29, 57080, Siegen, Germany

ABSTRACT: The combination of supramolecular hydrogels formed by low molecular weight gelator self-assembly via noncovalent interactions within a scaffold derived from polyethylene glycol (PEG) affords an interesting approach to immobilize fully functional, isolated reporter bacteria in novel microwell arrays. The PEG-based scaffold serves as a stabilizing element and provides physical support for the self-assembly of the C2-phenyl-derived gelator on the micrometer scale. Supramolecular hydrogel microwell arrays with various shapes and sizes were used to isolate single or small numbers of Escherichia coli TOP10 pTetR-LasR-pLuxR-GFP. In the presence of the autoinducer N-[(3-oxododecanoyl)] homoserine lactone, the entrapped E. coli in the hydrogel microwell arrays showed an increased GFP expression. The shape and size of microwell arrays did not influence the fluorescence intensity and the projected size of the bacteria markedly, while the population density of seeded bacteria affected the number of bacteria expressing GFP per well. The hydrogel microwell arrays can be further used to investigate quorum sensing, reflecting communication in inter- and intraspecies bacterial communities for biology applications in the field of biosensors. In the future, these self-assembled hydrogel microwell arrays can also be used as a substrate to detect bacteria via secreted autoinducers.

1. INTRODUCTION

Conventional physiological assays of bacteria in single and multispecies cultures are traditionally carried out in comparatively large fluid volumes or on solid media without addressing the influence of spatial organization and environmental heterogeneity on the community development and function.1 Among the formats reported, we can differentiate microfluidic approaches, which rely on the formation and manipulation of small liquid droplets with controllable sizes, shapes, and morphologies,8,9 and microwell-based formats. Microwell arrays offer, in addition to compatibility with established analysis techniques in plate reader or automated fluorescence microscopy setups, defined and controllable microenvironments for bacteria investigation on various length scales. Microwell arrays have emerged as robust and versatile physical and chemical barriers for protein adsorption and cell adhesion on the micro- and nanoscale level.10,11

Standard microfabrication techniques, including photolithography,12 micromolding,13 embossing,14 and colloidal lithography15 have been used for microwell fabrication. For instance, polyethylene glycol (PEG)-based microwell arrays fabricated
using micromolding in capillaries, polystyrene microwell arrays obtained by hot embossing, or poly(vinyl alcohol) (PVA) microwell arrays prepared using photolithography are illustrative examples. Microwell arrays can be adapted to facilitate the acquisition of detailed information about cell–cell and cell–environment interactions. Recent advances in micro-well-based environments (e.g., poly(ethylene glycol) diacrylate (PEGDA) microwell arrays, protein-coated silicon microwell assays, SU-8 microwells) have provided convenient routes to research on cell–cell communication by effective isolation of cells in predefined volumes. In addition, in situ tracking of single bacteria may become possible. However, challenges that are to date not adequately met also in these systems refer, e.g., to the lack of a sustained level of nutritional supply or the need to apply agar cover slides to confine bacteria inside the wells, which limits widespread application also in the fields of biosensing or biomedicine. The confinement of individual or few bacteria in each microwell should not interfere with cell viability and natural behavior, including mobility, proliferation, and interbacterial communication.

Herein, a new approach is introduced, in which designed microwells are filled with a tailored hydrogel of low molar mass precursors (Figure 1). The amino acid-based molecular hydrogel self-assembles from 1,4-bi(phenylalanine-diglycol)-benzene (PDB) and engulfs the to be studied bacteria in predefined positions defined by individual wells within a PEGDA microwell array. Distinct from conventional chemically cross-linked hydrogels, self-assembled hydrogels are formed through noncovalent interactions, such as hydrogen bonds, π−π stacking, hydrophobic, electrostatic, or van der Waals interactions, between hydrogelators and do not require any additional cross-linking reagents or initiator species. Typically, small molecule hydrogels possess an extraordinary capability to trap water because the nanosized hydrophilic cavities of the gel efficiently accommodate small clusters of water molecules. The high water content (>99%) leads to excellent permeability for nutrition, waste, as well as signaling molecules, thereby facilitating interbacterial communication and corresponding gene expression. In particular, bacteria are embedded into the hydrogel under physiological conditions during self-assembly, i.e., without addition of any chemical or physical stimulus (e.g., cross-linking agent or UV light, radicals, or other reactive species), which may impair bacterial viability and activity.

In addition to the fabrication of novel hydrogel-filled microwells and their detailed characterization, the central aim of this study was the investigation of bacteria viability and the dependence of the sensing capability targeting intra- and interbacterial communication molecules (autoinducers), relevant for quorum sensing-based biosensing, as a function of microwell size and geometry. By analyzing the production of green fluorescent protein (GFP) by Escherichia coli TOP10 pTetR-LasR-pLuxR-GFP that is triggered by an autoinducer, which belongs to the class of N-Acyl homoserin lactones (AHLs), on the single cell level, the feasibility of novel biosensor microarrays was shown, and new insights into the function of spatially immobilized reporter bacteria were obtained.

2. EXPERIMENTAL SECTION

Materials. All chemicals were purchased from Sigma-Aldrich and used without further purification unless otherwise noted. Poly(ethylene glycol) diacrylate (Mn = 250 g/mol) was purchased from Sigma-Aldrich and purified using aluminum oxide 90 active neutral (0.063–0.200 mm, Merck KGaA). Luria–Bertani medium (LB broth) and LB agar (Luria/Miller) were purchased from Carl Roth. Throughout the whole study, Milli-Q water was drawn from a Millipore Direct Q8 system (Millipore Advantage A10 system, Schwalbach, with MilliMark Express 40 filter, Merck, Germany) with a resistivity 18.2 MΩ cm.

Gelator Synthesis. The gelator PDB was synthesized with high yields (59%) through a conventional liquid phase reaction according to refs 26 and 27 (see Scheme S-1, Supporting Information).

Hydrogel Preparation. The gelator was dissolved in dimethyl sulfoxide (DMSO) to form a solution with concentrations of 100, 150, and 200 mg/mL, respectively. H2O or LB medium was then mixed with the gelator solution at a volume ratio of 49:1 (H2O: gelator) under continuous vortexing (RS-VA10, Phoenix instrument, Germany) for 3 min. Gelation was considered to have occurred when the mixture was left quiescent for 2–3 min. Gelation was considered to have occurred when the substance exhibited no gravitational flow upon inversion (compare also rheology data; see below).

Fourier Transform Infrared (FT-IR) Spectroscopy. Transmission FT-IR spectra of PDB and PEGDA gels were taken using a Bruker EQUINOX55 Instrument. The KBr disk technique was used for the solid-state measurement. The samples were scanned between wavenumbers of 4000 and 400 cm−1 with a resolution of 2 cm−1.

Circular Dichroism (CD) and Ultraviolet–Visible (UV–vis) Spectroscopy. CD and UV–vis spectra were measured using a Chirascan CD-spectrometer (Applied Photochemistry, UK). A quartz cuvette (Hellma Analytics, Germany) with 0.1 mm path length was used for gel measurements and a quartz cuvette (Hellma Analytics, Germany) with 1 mm path length was used for gelator solution.
measurements. Data between wavelengths of 190 and 350 nm were collected at 25 °C.

**Small Angle X-ray Scattering (SAXS) Measurements.** The SAXS pattern for PDB xerogels was recorded using an Anton Paar SAXSess Instrument.

**Transmission Electron Microscopy (TEM).** TEM images were obtained using a 120 kV Biology Transmission Electron Microscope (Tecnai G2 spirit Biotwin, FEI, USA). The samples were prepared by placing drops of the diluted aqueous suspension (gelator concentration around 0.2 mg/mL) onto a TEM copper grid, which was dried under ambient conditions.

**Scanning Electron Microscopy (SEM).** The morphologies of the PDMS mold, PEGDA microwell arrays, PDB xerogels, and PEGDA microwells filled with PDB hydrogel were imaged using an FEI QUANTA 250 SEM (USA). Samples were dried under vacuum and coated with 8–10 nm of gold on a sputtering coater (S 150B, Edwards, UK).

**Rheological Measurements.** The rheological properties of the hydrogels were measured using a Rotovis Rotor Rheometer (Gemini HR nano, Malvern, UK) with a 20 mm diameter plate–plate geometry (steel). The measurement cell was surrounded by a solvent trap to minimize evaporation. Dynamic strain scans (0.05–65%) were performed at 1 Hz frequency at 25 °C to determine the linear viscoelastic region. Dynamic frequency sweep tests were performed, in which a sinusoidal shear strain of constant peak amplitude (1%) was applied over a range of frequencies (0.01–10 Hz) at 25 °C. Dynamic temperature scan were done from 70 to 20 °C with a cooling rate of 2 K/min at 1 Hz and 1% strain.

**Contact Angle (CA) Measurements.** Static water contact angles were measured at ambient conditions with an OCA-15 instrument (Dataphysics, Germany). A 2 μL drop of Milli-Q water was applied to the sample surface via a flat-end stainless steel needle. The contact angle was measured with minimal delay.

**PEGDA Gel Swelling Ratio.** The equilibrium swelling ratios of bulk PEGDA gels were measured by using the classical gravimetric method. The weight of the vial, the weight of the formed PEGDA gel, and the weight of the xerogels (PEGDA gels were dried in a vacuum oven at 37 °C) were recorded. Milli-Q water was added into the vials, and gels were allowed to swell at 37 °C for 48 h. Then the residual water on the swollen gel surface was removed by blotting with filter paper, and the weight of the samples in the swollen state was measured. The value of the arithmetic mean and the standard deviation of at least three measurements for each sample were calculated. The weight equilibrium swelling ratio (ESR, $Q_{eq}$) is defined as follows:

$$Q_{eq} = \frac{(W_f - W_0)}{W_0} \times 100\%$$

(1)

$$Q_{eq} = \frac{(W_f - W_0)}{W_0} \times 100\%$$

(2)

where $W_f$ is the weight of the gel after gelation, $W_0$ is the weight of the gel after equilibrium swelling, and $W_f$ is the weight of the xerogel.

**Fabrication of PEGDA Microwells.** Thoroughly cleaned silicon wafers were first coated with 5 nm titanium, followed by a layer of negative photoresist (AZ EXP 125nXT-10A). After baking at 140 °C for 10 min, the photoresist was exposed to ultraviolet light in a mask aligner (Karl Suss MJB 3) through a chromium glass mask. The photoresist was developed using photoresist developer (AZ 300 MIF) followed by rinsing in Milli-Q water. A thin layer of polyfluorodecyltrichlorosilane was deposited on the sample exposing the cured resist before pouring polydimethylsiloxane (PDMS) prepolymer (Sylgard 184, Dow Corning, USA) on the structured sample. A mixture of 10:1 silicon elastomer and curing agent was mixed and degassed for 30 min, applied to the structured sample, followed by curing at 70 °C for 1 h. The cured PDMS was peeled from a photoresist master and cut with a scalpel to form PDMS molds with two open ends.

These PDMS molds were pressed on polystyrene Petri dishes to form conformal contact (as shown in Scheme S-2). Then a drop of PEGDA precursor with 2 mg/mL 2-hydroxy-1-[4-(1-hydroxyethoxy)-phenyl]-2-methyl-1-propanone (Irgacure2959) was placed at one open end of the mold, and the precursor filled the space between mold and substrate due to capillary forces. After 15 min of UV irradiation (CL-1000 series UV cross-linker, with CL-1000L Model 365 nm UV tubes, S × 8 W), the PDMS mold was peeled off, and the formed hydrogel was rinsed with excess ethanol and Milli-Q water. In several samples, 0.1 mg/mL Nile red (kindly provided by Dr. Simon Haas; the synthesis is shown in the Supporting Information, part 10) was mixed with the PEGDA precursor to enhance the details of microwell arrays for fluorescence microscopy images. The microwell assays were viewed under an inverted fluorescence microscope (Axiovert 135, Carl Zeiss, Germany) with excitation filters of 546 nm and long-pass emission filters.

**3D Laser Scanning Microscope Studies.** The morphologies of the PEGDA microwell arrays were investigated using a LEXT OLS4000 Industrial Laser Confocal Microscope (Olympus, Japan). Before the measurements, the samples were sputter coated with 8–10 nm of gold.

**PDB Hydrogel Permeability Tests.** The permeability of PDB hydrogels for autoinducers was studied by measuring the diffusion of N-dodecanoyl-L-Homoserine lactone-3-hydrazono-fluorescein (FITC-AHL, purchased from Biomol, Germany) into the gel over time. One hundred microliter, 3 mg/mL PDB gels were formed in a 96 well plate, followed by adding 100 μL 1 × 10^{-3} mol/L FITC-AHL solution on the top of the gel for each well. After immersion for 3, 6, 9, 12, 15, 30, 45, 60, or 120 min, 50 μL of the supernatant was withdrawn and pipetted into a black flat-bottom 96 well plate (Greiner Bio-one, Germany). The fluorescence intensities of 1 × 10^{-3} mol/L FITC-AHL solution and the xerogel supernatant were measured using a microplate reader (Tecan SAFIRE, Tecan, Switzerland).

**Bacteria Growth.** Unless mentioned otherwise, Luria–Bertani (LB) medium was used. The bacterial glycerol stock was stored at −80 °C. E. coli TOP10 pTetR-LasR-pLuxR-GFP was grown overnight from a single colony in 5 mL of LB broth supplemented with ampicillin (100 μg/mL) for 16 h in a shaking incubator (MaxQ 4000 benchtop orbital shaker, Thermo Scientific, USA) at 37 °C and 200 rpm. Then the bacteria suspension was diluted to 1:100 and was allowed to incubate further (3 h) to reach the middle exponential growth phase ($OD_{500}$ ≈ 0, measured by a Tecan SAFIRE microplate reader).

**Bacteria Encapsulation in Bulk Gels.** One milliliter of cell suspension was concentrated in a sterilized tube for 10 min at 5000 × g (microcentrifuge, Micro Star 17, VWR, USA), and the supernatant was discarded. The cells were resuspended in fresh sterile LB media and mixed with PDB-DMSO solution to form a 3 mg/mL PDB bulk hydrogel with bacteria encapsulated inside. E. coli proliferation (growth curves) in the PDB bulk gel over time was recorded by measuring the absorbance of bacteria suspension at 600 nm wavelength ($OD_{600}$) using the microplate reader. The absorbance of culture media (PDB hydrogels formed using LB media without bacteria) was subtracted for each time point. The green fluorescence due to GFP expression of E. coli in bulk PDB gel and LB media was monitored. 50 μL Planktonic bacteria and bacteria encapsulated inside PDB gel were transferred into a transparent, flat-bottom 96-well plate in triplicate aliquots and 150 μL LB media was added to each well for incubation with N-(3-oxododecanoyl) homoserine lactone (3OC12HSL) at varying molar concentration (at zero, 1 × 10^{-5}, 1 × 10^{-4}, 1 × 10^{-3}, 1 × 10^{-2}, 10^{-1} mol/L 3OC12HSL; serial dilutions from 1 × 10^{-3} mol/L 3OC12HSL in DMSO stock solution). The plate was incubated in a shaking incubator at 37 °C and 200 rpm. After 6 h, $OD_{600}$ and fluorescence intensity were recorded using a microplate reader. The result was zeroed with culture media to remove background fluorescence of LB medium. The arithmetic mean and the standard deviation were calculated from at least three biological replicates.

**Characterization of E. coli in Microwell Arrays.** Single colonies of E. coli were inoculated into 5 mL of LB broth supplemented with ampicillin (100 μg/mL) for 16 h in a shaking incubator at 37 °C and 200 rpm, followed by 100 folds dilution and incubation to reach the exponential growth phase ($OD_{500}$ ≈ 0.5). One mL cell solution was concentrated in a sterilized tube for 10 min at 5000 × g and the supernatant was discarded. The cells were resuspended in fresh sterile LB media to reach the desired $OD_{500}$ of 0.05, 0.25, 0.5. Ten microliters of gelator in DMSO (150 mg/mL) was mixed with 490 μL of bacteria.
LB suspension under vortexing, as shown in Scheme S-3. Subsequently, a 5 μL drop of this mixture was pipetted onto PEGDA microwells and covered by a clean PDMS (75% ethanol sterilized) cover slide. After 5 min, the PDMS cover slide was gently removed. Then 500 μL of fresh LB media with or without 3OC12HSL was applied to cover the microwells completely. The samples were analyzed under an inverted fluorescence microscope (Axiovert 135, Carl Zeiss, Germany) with excitation filters of 450−490 and 515 nm long-pass emission filters. Microwell arrays in square, circle, and triangle shapes were used. Fluorescence emission images were quantitatively analyzed using ImageJ software. The fluorescence intensity of single bacteria cell was measured across a population of bacteria cells, allowing for the determination of average fluorescent intensity per bacteria cell.

Figure 2. (a) FT-IR spectra of PDB xerogel and PEGDA; (b) circular dichroism (CD) spectra of PDB hydrogel (2 mg mL\(^{-1}\)) and diluted PDB solution in H\(_2\)O (0.2 mg mL\(^{-1}\)).

Figure 3. Dynamic rheological investigations of PDB hydrogels: (a) frequency sweep test of 2 mg/mL, 3 mg/mL, 4 mg/mL PDB hydrogels from 0.01 to 10 Hz, at 1% strain and 25 °C, elastic modulus \(G'\) (solid symbol), viscous modulus \(G''\) (open symbol); (b) thermal stability sweep test of 3 mg/mL PDB hydrogel from 70 to 20 °C, at 1 Hz and 1% strain, \(G'\) (solid square), \(G''\) (open square), phase angle (solid star); (c) dynamic frequency sweep test of 3 mg/mL PDB hydrogel indicative of shear thinning material, viscosity (solid circle), shear stress (solid triangle); (d) dynamic strain sweep test to determine the linear viscoelastic range and yielding point, \(G'\) (solid square), \(G''\) (open square), and shear stress (solid triangle).

### 3. RESULTS AND DISCUSSION

The PEGDA microwell arrays, fabricated according to Lee et al., provide physical separation between different bacteria and enable facile investigation, while the novel PDB hydrogel confines the bacteria and prevents bacteria escape from the microwells. At the same time, the hydrogel must not prevent the diffusion of nutrients as well as autoinducers, which requires that the hydrogel properties are thoroughly investigated to allow for optimization.

C\(_2\)-phenyl-derived gelators possess self-assembly abilities in aqueous media and thereby form hydrogels through the combination of hydrogen bonds, hydrophobic, and \(\pi−\pi\) interactions, which has been demonstrated in previous studies. The FT-IR spectra of the PDB xerogel, shown...
in Figure 2a, exhibits the characteristic band at 1735 cm\(^{-1}\), which is attributed to \(-\text{C}=\text{O}\) stretching vibration, and bands at 1641, 1545 cm\(^{-1}\), which are assigned to the amide I and amide II bands of typical secondary amides. The broad peaks at 3442 and 3296 cm\(^{-1}\) indicated the inter- and intramolecular hydrogen bonding.\(^{20}\)

An absorption peak at the wavelength of 248 nm was observed for PDB in the UV–vis spectrum (Figure S-2), which corresponds to the benzene group’s \(\pi-\pi^*\) transitions. CD spectrum was used for assessing the superstructure of self-assembled gelators. The measured CD bands of PDB in solution (black line in Figure 2b) with \(\lambda_{\text{max}}\) values at 217 nm (peak) and 240 nm (trough) were due to the amide carbonyl groups and phenyl group in the 1,4-benzenedicarboxamide, respectively.\(^{32,33}\) Compared to the PDB solution, the PDB hydrogel showed a significantly increased CD intensity and the spectral peaks shifted to 226 and 266 nm (blue line in Figure 2b), indicating the self-assembly in the hydrogel.\(^{34}\)

The small angle x-ray scattering (SAXS) pattern of the PDB xerogel (Figure S-3) suggested the crystalline nature and polymorphic arrangement of self-assembled gelator.\(^{35}\) The SAXS peak at 3.3 nm\(^{-1}\), corresponding to a \(d\)-spacing of 19.2 Å, is comparable with the calculated molecular length of 20.5 Å. Thus, it is assumed that the gelators adopt a conformation, in which the phenyl moieties fold inward, shielding the amide moieties from the aqueous environment. Thereby the hydrogen-bonding interactions provide strong and uniaxial inter-molecular interactions, which enforce one-dimensional (1D) self-assembly.\(^{20,31,36,37}\)

From empirical observation, the PDB hydrogels possessed poor mechanical properties, which led to instability under external mechanical forces, e.g., vortexing or agitation. To investigate the mechanical properties of the PDB hydrogels, dynamic oscillatory rheology experiments were carried out. A shear strain amplitude sweep was performed first to determine the linear viscoelastic range.\(^{38}\) The linear regime, in which the elastic modulus \((G')\) and the viscous modulus \((G'')\) were independent of applied strain, stretched up to 3% strain (Figure 3d). The shear stress went through a maximum (yield point, 135 Pa) at 3.2% strain. At larger amplitude strain, the moduli decreased dramatically, and \(G'\) became smaller than \(G''\), which indicates that the gel structure was deformed and the material flowed.\(^{39}\) The onset of complete fluidization or the transition from a solid-like to a liquid-like response, determined at \(G' = G''\), was at 5.5% strain. The following experiments were performed at 1% strain.

Nondestructive frequency sweep tests within the linear viscoelastic range from 0.01 to 10 Hz were performed for PDB hydrogels with different gelator concentrations. Here the elastic modulus \((G')\) and the viscous modulus \((G'')\) were independent of applied strain, stretched up to 3% strain (Figure 3d). The shear stress went through a maximum (yield point, 135 Pa) at 3.2% strain. At larger amplitude strain, the moduli decreased dramatically, and \(G'\) became smaller than \(G''\), which indicates that the gel structure was deformed and the material flowed.\(^{39}\) The onset of complete fluidization or the transition from a solid-like to a liquid-like response, determined at \(G' = G''\), was at 5.5% strain. The following experiments were performed at 1% strain.

Nondestructive frequency sweep tests within the linear viscoelastic range from 0.01 to 10 Hz were performed for PDB hydrogels with different gelator concentrations. Here the elastic modulus dominated the viscous modulus and the moduli exhibited frequency-independent behavior over the investigated oscillating frequency, which indicates the formation of solid gels,\(^{40}\) as shown in Figure 3a. In addition, the elastic modulus, as an indicator for hydrogel stiffness, showed a dependence on gelator concentration. With increasing gelator concentration,
the modulus of hydrogels increased and the elastic modulus was about 5 times higher than the viscous modulus. It is hence possible to tune the hydrogel stiffness by changing the gelator concentration.

To investigate the thermal stability, thermal rheological scans from 70 to 25 °C were recorded. Figure 3b shows that $G'$ remained substantially greater than $G''$ upon increasing the temperature, which indicates that the PDB hydrogels kept the gel state in the test temperature range. For 3 mg/mL PDB hydrogels, $G'$ increased from $6.1 \times 10^9$ Pa to $6.3 \times 10^9$ Pa upon cooling down from 37 to 25 °C. Above 50 °C, the modulus decreased rapidly, which may be attributed to a thermal response of the PDB hydrogel; the gel-to-sol transition temperature was approximately 85 °C (Figure S-4).

The complex viscosity measured as a function of frequency is shown in Figure 3c. The viscosity decreased significantly with increasing shear rate, which implies that the self-assembled PDB hydrogels were shear thinning, which may be because of the noncovalent cross-links and/or entanglements among fibers that created the three-dimensional networks for trapping water and imparting rigidity. At low frequency, the viscosity was about 5 times higher than the noncovalent cross-links and/or entanglements among fibers and microcavities. The investigation of hydrogels of various gelator concentrations with SEM showed that the gelator concentration had an influence on the observed fiber diameters. For low concentration hydrogels ($0.2 \text{ mg/mL}$), fibers with diameter $110 \pm 37 \text{ nm}$ intertwined into fiber networks (Figure 4b). With increasing gelator concentration (1 mg/mL), fibers tended to bundle with each other, and the fiber diameters increased to around $160 \pm 67 \text{ nm}$ (Figure 4c). Those nanofibers formed networks containing nanoscale size hydrophilic cavities, which can accommodate small clusters of water molecules and thus entrap more water compared to microfibers and microcavities.

For the formation of the hydrogel-filled microwell, PEGDA microwell arrays with desirable size and geometry were patterned on polystyrene (PS) by micromolding in capillaries and in situ photochemical cross-linking using ultraviolet radiation. In brief, a PDMS mold with two open ends was pressed into conformal contact with tissue culture polystyrene, and then the liquid PEGDA precursor was added to one side of PDMS. The precursor filled the void spaces between the posts of the PDMS mold. After UV exposure, PDMS was peeled away and the PEGDA microwell arrays with a polystyrene bottom floor were obtained (Figure 4d,e). Since the bottom of the formed microwell arrays was the surface of the substrate on which the conformal contact with PDMS was formed, it is easy to adjust the floor properties by using different substrates, including polyethylene terephthalate glycol-modified film, and 3-trimethoxysilylpropyl methacrylate (TPM)-treated glass. The flexibility of fabricating PEGDA microwell arrays on a substrate of choice, e.g., glass, made PEGDA microwell arrays more suitable for microscopy studies than, e.g., PDMS microwell arrays. The formed microwell arrays did not detach from the supporting substrates after immersion in aqueous media. The tight contact prevents cells from migrating under the PEGDA barriers. Low molar mass PEGDA was used for microwell array fabrication due to the low swelling ratio and high precise pattern replication. Choosing 2 mg/mL Irgacure 2959 as photo initiator, after 1 min UV irradiation, the formed PEGDA hydrogel had a swelling ratio around 10% (Figure S-8). The ease of microwell arrays preparation made it possible to fabricate the desired configuration of shape, size, depth/aspect ratio and spacing of arrays used as the controllable spatial confinement for bacteria communities. The microwell features are summarized in Table S-2. As shown in Figure 4f, Nile Red could be mixed with PEGDA precursor before photopolymerization to indicate the boundaries of microwell arrays.

The microwell arrays thus fabricated provided a controlled and uniform growth environment for bacteria in a massively parallel and reproducible format. Due to the hydrophilic properties of PEGDA gel (the contact angle of water was $46° \pm 2°$, Figure S-9a), the PDB solution could wet the microwell arrays, and imparting rigidity.41 At low frequency, the viscosity was that created the three-dimensional networks for trapping water and imparting rigidity.
arrays. The nonwetting PDMS cover slide (the contact angle of water was 115° ± 2°, Figure S-9b) effectively reduced the formation of a residual interconnecting gel layer on top of wells. As is shown in Figure 4g, the PDB gel mixed with FITC was successfully filled into the void space of the PEGDA microwell and the intensity of wells’ interior was higher than that of the PEGDA barriers. Some fibers of the PDB gel were detectable on the top surface of the microwell arrays in SEM images of PEGDA wells filled with PDB gel (Figure 4h,i). Due to the low critical gel concentration (0.3 mg/mL PDB), only a small amount of residual gel could form visible fibers. This thin layer did not interfere with the isolation of bacteria in the designated well (see below).

Before the bacteria were loaded into the microwells filled with PDB hydrogel, the diffusion of autoinducers into the hydrogel and the response of genetically modified E. coli cultivated in bulk PDB hydrogels to added autoinducers was investigated. The diffusion of 3OC12HSL into PDB bulk hydrogel was assessed by measuring the diffusion of the fluorescently labeled autoinducer (FITC-AHL) over time (Figure 5a). The FITC-AHL concentration in the hydrogel increased rapidly in the first 3 min and reached a plateau within 15 min under static conditions. In addition, the final concentration of FITC-AHL inside the gel equals that of the solution. The results imply that FITC-AHL permeated the PDB hydrogels rapidly.

E. coli TOP10 pTetR-LasR-pLuxR-GFP was used as model bacteria to test the hydrogel-microwell system for the investigation of small number of bacteria. The genetic modification of E. coli was based on the Type I quorum sensing mechanism of Pseudomonas aeruginosa. The tetR promoter controls the expression of the transcriptional regulator LasR, which binds to the autoinducer N-(3-oxododecanoyl) homoserine lactone (3OC12HSL). The LuxR promoter, to which the LasR-3OC12HSL activator complex binds, was adopted as the inducible promoter to activate the expression of GFP. Green fluorescence indicated the level of LuxR induction, which has been shown to correlate closely with the extracellular concentration of the autoinducer 3OC12HSL. Since E. coli does not encode signal-generating enzymes for producing autoinducers used in the LuxR family, the interference of quorum sensing of E. coli itself can be neglected. To investigate the effect of 3OC12HSL on E. coli viability and proliferation, the growth curves of E. coli in PDB hydrogels with 0 and 0.5 × 10^{-6} mol/L 3OC12HSL in 96 well plates were recorded using a microplate reader. The three phases of the growth curve, including lag phase, exponential phase, and stationary phase, were recorded. The final concentration of DMSO in the gels was 2 v/v%, which is below the minimal inhibitory concentration (10%) for E. coli. As shown in Figure S-12, independent of the presence of 3OC12HSL, E. coli in PDB gel had the same generation time of 35 ± 5 min, suggesting that 3OC12HSL had no effect on the growth rate of E. coli under our test conditions, which is in agreement with reported results.

In addition, for E. coli incubated in PDB hydrogels, the density of cells in the stationary phase was not affected by the presence of 3OC12HSL.

The influence of 3OC12HSL on E. coli GFP expression was first studied via monitoring the fluorescence intensity and OD_{600} in bulk LB and in bulk PDB hydrogels (Figure 5b). The fluorescence intensity as a relative measure for GFP expression per cell (fluorescence intensity divided by OD_{600}) was normalized to control cultures (without exposing to 3OC12HSL). In the presence of 1 × 10^{-6} to 1 × 10^{-8} mol/L 3OC12HSL, the GFP expression per cell was around 12 times higher than in the absence of AHL. For bacteria in both LB media and bulk hydrogels, no significant difference in relative GFP expression was observed for all AHL concentrations tested. For the following experiments with isolated E. coli, 1 × 10^{-6} mol/L 3OC12HSL was used.

E. coli were seeded into microwell arrays by pipetting a drop of PDB hydrogel to fill the well arrays and subsequently establishing conformal contact with a PDMS slide that was applied from the top. Due to the good wetting of water on PEGDA and the nonwetting of the PDMS covers, the residual hydrogel on top of wells was effectively reduced (see above). PEGDA microwell arrays labeled with Nile Red exhibited green color under the fluorescence microscope upon 485 nm excitation due to the long pass emission filter used in the microscope (Figure 6; the fluorescence spectrum of PEGDA-

![Figure 6](image)

Nile Red precursor is shown in Figure S-10). The successful entrapment of motile bacteria was demonstrated using E. coli, which showed GFP expression in the presence of 3OC12HSL. Mobile bacteria were clearly confocal within well arrays during time lapse monitoring (Figure S-15 and Video-SI.1) under fluorescence microscopy. The microbes were observed to move back and forth within the PDB hydrogel area enclosed by the PEGDA well arrays. The projected size and fluorescence intensity of the same bacteria changed over time, which revealed that the bacteria were cultured in a three-dimensional environment that provided sufficient space for different orientation. When cultured without 3OC12HSL, E. coli exhibited weak fluorescence intensity, which is likely due to the leaky expression in LB medium and in agreement with the basal GFP expression level detected in bulk culture bacteria. As shown in Figure 8, the fluorescence intensity and projected area of individual cells varies significantly across the microwell array. Thus, a large number of bacteria was analyzed, and the size and fluorescence intensity of the bacteria were averaged.

It is well known that the initial cell occupancy in the wells can be tuned by adjusting the size and the aspect ratio of the...
microwell arrays and cell seeding concentration.\textsuperscript{1,47} For the square shape well arrays with well areas of 36, 81, 225, and 1600 μm\textsuperscript{2}, the number of bacteria expressing GFP was 1.0 ± 0.2, 1.1 ± 0.3, 1.4 ± 0.7, and 1.5 ± 1.4, respectively (Figure 7a).

With increasing well area, the standard deviation increased, while no significant influence of the well area on the average fluorescence intensity and the projected bacteria size was observed (Figure 7b). Here, the influence of the initial bacteria population density on the distribution of GFP expressed bacteria was studied. Bacteria populations of different OD\textsubscript{600} were investigated by counting the colony forming unit (CFU) on agar plates (Figure S-11). The bacteria number per well was estimated by calculating the well volumes and number of bacteria per volume. For OD\textsubscript{600} values of 0.05, 0.25, 0.5, the estimated bacteria numbers per well were 1, 4, 11, respectively. The number of bacteria that express GFP (exhibited green fluorescence under the microscope) after 2 h incubation with 3OC\textsubscript{12}HSL for bacteria seeded inside (15 μm)\textsuperscript{2} square shaped microwell arrays were 1.1 ± 0.4 at OD\textsubscript{600} = 0.05, 1.4 ± 0.7 at OD\textsubscript{600} = 0.25, and 2.0 ± 1.1 at OD\textsubscript{600} = 0.5 (Figure 7c). These results suggest that the response of \textit{E. coli} cells to AHL is heterogeneous in time. For bacteria grown on the surface of PDB hydrogel for 2 h, demonstrated in Figure S-14, the overlay of the brightfield and fluorescent images pointed out not every cell initiated GFP expression. Because the autoinducer concentration was expected to be uniform, the heterogeneous expression of GFP may be explained by other reasons, e.g., the inhomogeneous distribution of dead/nonreactive organisms, and only partial bacteria expressed GFP when exposed to 3OC\textsubscript{12}HSL. Extending the incubation time to 4 h, the number of bacteria expressed GFP per well increased from 1.4 ± 0.7 to 1.9 ± 0.9, likely due to the proliferation of bacteria. As shown in Figure 7d, there was no significant difference in the average fluorescence intensity and projected bacteria size among bacteria seeded with various OD\textsubscript{600} values.

The average fluorescence intensity of single bacteria cell showed no significant difference during the first 4 h incubation. In addition, no evidence for \textit{E. coli} migration between
neighboring wells was found, implying adequate immobilization of reporter bacteria within a given well. Thus, the entrapped bacteria were able to sense the added autoinducer and expressed GFP, which is a proof that at least a fraction of the entrapped cells remained viable and reactive for a reliable bioassay performance.

The influence of shape and size of microwell arrays on *E. coli* GFP expression, as a necessary consistency check, was further investigated. The fluorescence of the produced GFP could be detected with high signal-to-noise ratio and was found to be independent from the well shape and size (Figure 8 and 9). When exposed to media that contained 3OC12HSL, GFP expression of *E. coli* was induced. There was no significant difference in fluorescence intensity and projected size among bacteria cultured in microwell arrays with different size and shape. The large standard deviations for area and perimeter are likely caused by various orientations of bacteria in the wells.

The use of PDB hydrogel to entrap bacteria into microwell arrays reduces the distribution bias of bacteria population in microwell arrays caused by differences in microbial traits, such as motility or cell membrane composition, which may affect the cell-surface and cell–cell affinities. Thus, the PDB supramolecular hydrogel filled microwell platforms offer control on spatial structures, which will be useful for future quorum sensing studies.

4. CONCLUSION

In conclusion, the low molar mass gelator PDB was shown to form hydrogels with controllable shape and size defined by a PEG-based microwell scaffold. The formed PDB hydrogels consisted of a fibrous network with numerous nanosized hydrophilic cavities for trapping water, which rendered the gels suitable for three-dimensional cell culture. *E. coli* TOP10 pTetR-LasR-pLuxR-GFP exhibited similar autoinducer-triggered GFP production in PDB bulk gels as in LB medium. A fluorescently labeled autoinducer diffused into PDB bulk gels and reached equilibrium in half an hour. These PDB hydrogels have been successfully utilized to physically isolate single or few *E. coli* in the microwell arrays, while retaining *E. coli* viability and functionality in particular in sensing N-(3-oxododecanoyl) homoserine lactone by triggered GFP expression. The fluorescence intensity of the produced GFP and the projected cell sizes were not affected by the initial bacteria culture density or the microwell arrays’ dimensions. Since N-(3-oxododecanoyl) homoserine lactone is one of the autoinducers used by *P. aeruginosa*, it is conceivable that the microwell arrays with the encapsulated reporter *E. coli* can in principle be used as biosensor for the detection of *P. aeruginosa*.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b00749.

Synthesis of gelator PDB, 1H NMR spectra of PDB and PEGDA, FT-IR peak assignments, UV–vis and SAXS spectra of PDB, gel-to-sol transition temperature, schematic of both PEGDA and PDB–PEGDA microwell arrays fabrication, 3D laser scanning microscopy images of microwell arrays and PDB microwell arrays, parameters of microwell arrays, swelling ratio of PEGDA gel, contact angle of PEDGA gel, PDMS, and PDB xerogel film, fluorescent spectrum of PEGDA with nile red solution, *E. coli* OD600–CFU curve, *E. coli* growth curve in PDB hydrogel, OD600 and fluorescence intensity of *E. coli* with/without exposure to 3OC12HSL, GFP expression on bulk PDB hydrogel, *E. coli* in microwells at different times, and SEM image of PDB in DMSO (PDF).
Time-lapse video of E. coli entrapped in PDB–PEGDA microwell arrays (AVI)

■ AUTHOR INFORMATION

Corresponding Authors
*(X.D.) E-mail: dou@chemie-bio.uni-siegen.de; Fax: +49(0) 271 740 2805. Physical Chemistry I and Research Center of Micro and Nanochemistry and Engineering (Cμ), Department of Chemistry and Biology, University of Siegen, Adolf-Reichwein-Str. 2, 57076, Siegen, Germany.
*(H.S.) E-mail: schoenherr@chemie.uni-siegen.de.

ORCID

Holger Schönerr: 0000-0002-5836-5569

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

The authors would like to acknowledge funding received for the project from the funding program of the Federal Ministry of Economics and Technology in Germany, “Central Innovation Programme SME” (ZIM, KF2589507SK4), that is carried out together with our cooperation partner Quh-Lab (Siegen), financial support from the Alexander von Humboldt Foundation (postdoc stipend to X. Q. Dou), the EU (ERC project ASMIDIAS, Grant no. 279202) and the University of Siegen.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Dipl.-Ing. Gregor Schulte, M.Sc. Marc Steuber, and Dr. Inga Lilge for technical support concerning photolithography, Dr. Yvonne Voss for help with the initial experiments, Dr. Simon Haas for the dye sample, and Prof. Christ (Department of Mechanical Engineering, University of Siegen, Germany), who kindly provided access to the 3D Laser Scanning Microscope for surface characterization.

■ REFERENCES

(1) Hansen, R. H.; Timm, A. C.; Timm, C. M.; Bike, A. N.; Morrell-Falvey, J. L.; Pelletier, D. A.; Simpson, M. L.; Doktycz, M. J.; Retterer, S. T. Stochastic Assembly of Bacteria in Microwell Arrays Reveals the Importance of Confinement in Community Development. PLoS One 2016, 11, e0155080.
(2) Hol, F. J. H.; Dekker, C. Zooming in to See the Bigger Picture: Microfluidic and Nanofabrication Tools to Study Bacteria. Science 2014, 346, 1251821.
(3) Ge, Z.; Girguis, P. R.; Buie, C. R. Nanoporous Microscale Microporous Incubators. Lab Chip 2016, 16, 480–488.
(4) Zhang, Q.; Lambert, G.; Liao, D.; Kim, H.; Robin, K.; Tung, C. K.; Pourmand, N.; Austin, R. H. Acceleration of Emergence of Bacterial Antibiotic Resistance in Connected Microenvironments. Science 2013, 333, 1764–1767.
(5) Drescher, K.; Shen, Y.; Bassler, B. L.; Stone, H. A. Biofilm Streamers Cause Catastrophic Disruption of Flow with Consequences for Environmental and Medical Systems. Proc. Natl. Acad. Sci. U. S. A. 2013, 110, 4345–4350.
(6) Renner, L. D.; Weibel, D. B. Physicochemical Regulation of Biofilm Formation. MRS Bull. 2011, 36, 347–355.
(7) Vincent, M. E.; Liu, W.; Haney, E. B.; Ismagilov, R. F. Microfluidic Stochastic Confinement Enhances Analysis of Rare Cells by Isolating Cells and Creating High Density Environments for Control of Diffusible Signals. Chem. Soc. Rev. 2010, 39, 974–984.
(8) Dong, L.; Chen, D. W.; Liu, S. J.; Du, W. Automated Chemotactic Sorting and Single-Cell Cultivation of Microbes using Droplet Microfluidics. Sci. Rep. 2016, 6, 24192.
(9) Rakszewska, A.; Tel, J.; Chokkalingam, V.; Huck, W. T. S. One Drop at a Time: Toward Droplet Microfluidics as a Versatile Tool for Single-Cell Analysis. NPG Asia Mater. 2014, 6, e133.
(10) Prewitz, M.; Seib, F. P.; Pompe, T.; Werner, C. Polymeric Biomaterials for Stem Cell Bioengineering. Macromol. Rapid Commun. 2012, 33, 1420–1431.
(11) Charnley, M.; Textor, M.; Khademhosseini, A.; Lutolf, M. P. Integration Column: Microwell Arrays for Mammalian Cell Culture. Integr. Biol. (Camb) 2009, 1, 625–634.
(12) Wang, Y.; Salazar, G. T.; Pai, J. H.; Shadpour, H.; Sims, C. E.; Allbritton, N. L. Micropallet Arrays with Poly(ethylene glycol) Walls. Lab Chip 2008, 8, 734–40.
(13) Shim, H. W.; Lee, J. H.; Hwang, T. S.; Rhee, Y. W.; Bae, Y. M.; Choi, J.; Han, J.; Lee, C. S. Patterned Proteins and Cells on Functionalized Surfaces Prepared by Polyelectrolyte Multilayers and Micromolding in Capillaries. Biosens. Bioelectron. 2007, 22, 3188–3195.
(14) Kobel, S.; Limachet, M.; Gobaa, S.; Laroche, T.; Lutolf, M. P. Micropatterning of Hydrogels by Soft Embossing. Langmuir 2009, 25, 8774–8779.
(15) Liu, C.; Liu, J.; Gao, D.; Ding, M.; Lin, J.-M. Fabrication of Microwell Arrays Based on Two-Dimensional Ordered Polystyrene Microspheres for High-Throughput Single-Cell Analysis. Anal. Chem. 2010, 82, 9418–9424.
(16) Sridhar, A.; de Boer, H. L.; van den Berg, A.; Le Gac, S. Microstamped Petri Dishes for Scanning Electrochemical Microscopy Analysis of Arrays of Microtissues. PLoS One 2014, 9, e93618.
(17) Song, W.; Lu, H.; Kawazoe, N.; Chen, G. Aliphatic Differentiation of Individual Mesenchymal Stem Cell on Different Geometric Micropatterns. Langmuir 2011, 27, 6155–6162.
(18) Flickinger, S. T.; Copeland, M. F.; Downes, E. M.; Brasch, A. T.; Tuson, H. H.; Eun, Y. J.; Weibel, D. B. Quorum Sensing between Pseudomonas aeruginosa Biofilms Accelerates Cell Growth. J. Am. Chem. Soc. 2011, 133, 5966–5975.
(19) Boedicker, J. Q.; Vincent, M. E.; Ismagilov, R. F. Microfluidic Confinement of Single Cells of Bacteria in Small Volumes Initiates High-Density Behavior of Quorum Sensing and Growth and Reveals Its Variability. Angew. Chem., Int. Ed. 2009, 48, 5908–5911.
(20) van Bommel, K. J.; van der Pol, C.; Muizebelt, I.; Friggeri, A.; Heeres, A.; Meetsma, A.; Feringa, B. L.; van Esch, J. Responsive Cyclohexane-Based Low-Molecular-Weight Hydrogelators with Modular Architecture. Angew. Chem., Int. Ed. 2004, 43, 1663–1667.
(21) Du, X.; Zhou, J.; Shi, J.; Xu, B. Supramolecular Hydrogelators and Hydrogels: From Soft Matter to Molecular Biomaterials. Chem. Rev. 2015, 115, 13165–13307.
(22) Zhang, S. Hydrogels: Wet or Let Die. Nat. Mater. 2004, 3, 7–8.
(23) Hawser, L. A.; Jung, S. A.; Ng, W. L. Specificity and complexity in bacterial quorum-sensing systems. FEBS Microbiol. Rev. 2016, 40 (5), 738–752.
(24) Papenfort, K.; Bassler, B. L. Quorum sensing signal-response systems in Gram-negative bacteria. Nat. Rev. Microbiol. 2016, 14 (9), 576–588.
(25) Saedt, N.; Wong, C. K.; Lo, T. M.; Nguyen, H. X.; Ling, H.; Leong, S. S.; Poh, C. L.; Chang, M. W. Engineering Microbes to Sense and Eradicate Pseudomonas Aeruginosa, a Human Pathogen. Mol. Syst. Biol. 2011, 7, 521.
(26) Dou, X. Q.; Li, P.; Zhang, D.; Feng, C. L. C2-Symmetric Benzene-Based Hydrogels with Unique Layered Structures for Controllable Organic Dye Adsorption. Soft Matter 2012, 8, 3231–3238.
(27) Li, P.; Dou, X. Q.; Feng, C. L.; Zhang, D. Mechanical Reinforcement of C2-Phenyl-Derived Hydrogels for Controlled Cell Adhesion. Soft Matter 2013, 9, 3750–3757.
(28) Li, P.; Dou, X. Q.; Tang, Y. T.; Zhu, S.; Gu, J.; Feng, C. L.; Zhang, D. Gelator-Polysaccharide Hybrid Hydrogel for Selective and Controllable Dye Release. *J. Colloid Interface Sci.* 2012, 387, 115–122.

(29) Li, P.; Yin, Z.; Dou, X. Q.; Zhou, G.; Feng, C. L. Convenient Three-Dimensional Cell Culture in Supramolecular Hydrogels. *ACS Appl. Mater. Interfaces* 2014, 6, 7948–7952.

(30) Dou, X. Q.; Li, P.; Zhang, D.; Feng, C. L. RGD anchored C2-Benzene Based PEG-Like Hydrogels as Scaffolds for Two and Three Dimensional Cell Cultures. *J. Mater. Chem. B* 2013, 1, 3562–3568.

(31) Dou, X. Q.; Zhang, D.; Feng, C.; Jiang, L. Bioinspired Hierarchical Surface Structures with Tunable Wettability for Regulating Bacteria Adhesion. *ACS Nano* 2015, 9, 10664–10672.

(32) Edwards, W.; Smith, D. K. Enantioselective Component Selection in Multicomponent Supramolecular Gels. *J. Am. Chem. Soc.* 2014, 136, 1116–1124.

(33) Hirst, A. R.; Smith, D. K.; Feiters, M. C.; Geurts, H. P. M. Two-Component Dendritic Gel: Effect of Stereochemistry on the Supramolecular Chiral Assembly. *Chem. - Eur. J.* 2004, 10, 5901–5910.

(34) Liu, G. F.; Zhang, D.; Feng, C. L. Control of Three-Dimensional Cell Adhesion by the Chirality of Nanofibers in Hydrogels. *Angew. Chem., Int. Ed.* 2014, 53, 7789–7793.

(35) Adhikari, B.; Palui, G.; Banerjee, A. Self-Assembling Tripeptide Based Hydrogels and Their Use in Removal of Dyes from Waste-Water. *Soft Matter* 2009, 5, 3452–3460.

(36) Moyer, T. J.; Cui, H.; Stupp, S. I. Tuning Nanostructure Dimensions with Supramolecular Twisting. *J. Phys. Chem. B* 2013, 117, 4604–4610.

(37) Aida, T.; Meijer, E. W.; Stupp, S. I. Functional Supramolecular Polymers, *Science* 2012, 335, 813–817.

(38) Sathaye, S.; Mbi, A.; Sonmez, C.; Chen, Y.; Blair, D. L.; Schneider, J. P.; Pochan, D. J. Rheology of peptide- and protein-based physical hydrogels: Are everyday measurements just scratching the surface? *WIREs: Nanomed. Nanobi.* 2015, 7, 34–68.

(39) Frohm, B.; DeNizio, J. E.; Lee, D. S. M.; Gentile, L.; Olsson, U.; Malm, J.; Akerfeldt, K. S.; Linse, S. A Peptide from Human Semenogelin 1 Self-Assembles into a pH-Responsive Hydrogel. *Soft Matter* 2015, 11, 414–421.

(40) Song, F.; Zhang, L. M.; Shi, J. F.; Li, N. N.; Yang, C. A.; Yan, L. Using Hydrophilic Polysaccharide to Modify Supramolecular Hydrogel from a Low-Molecular-Mass Gelator. *Mater. Sci. Eng., C* 2010, 30, 804–811.

(41) Menger, F. M.; Caran, K. L. Anatomy of a Gel. Amino Acid Derivatives That Rigidify Water at Submillimolar Concentrations. *J. Am. Chem. Soc.* 2000, 122, 11679–11691.

(42) Moeller, H. C.; Mian, M. K.; Shrivastava, S.; Chung, B. G.; Khademhosseini, A. A Microwell Array System for Stem Cell Culture. *Biomaterials* 2008, 29, 752–763.

(43) Rogers, C. L.; Pagaduan, J. V.; Nordin, G. P.; Woolley, A. T. Single-Monomer Formulation of Polymerized Polyethylene Glycol Diacylate as a Nonadsorptive Material for Microfluidics. *Anal. Chem.* 2011, 83, 6418–6425.

(44) Rolland, J. P.; Maynor, B. W.; Euliss, L. E.; Exner, A. E.; Denison, G. M.; DeSimone, J. M. Direct Fabrication and Harvesting of Monodisperse, Shape-Specific Nanobiomaterials. *J. Am. Chem. Soc.* 2005, 127, 10096–10100.

(45) Ahmer, B. M. M. Cell-to-Cell Signalling in Escherichia Coli and Salmonella Enterica. *Mol. Microbiol.* 2004, 52, 933–945.

(46) Basch, H.; Gadebusch, H. H. In Vitro Antimicrobial Activity of Dimethylsulfoxide. *Appl. Microbiol.* 1968, 16, 1953–1954.

(47) Rettig, J. R.; Folch, A. Large-Scale Single-Cell Trapping And Imaging Using Microwell Arrays. *Anal. Chem.* 2005, 77, 5628–5634.