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A Microfluidic Bioreactor Based on Hydrogel-Entrapped *E. coli*: Cell Viability, Lysis, and Intracellular Enzyme Reactions

Jinseok Heo, K. Joseph Thomas, Gi Hun Seong, and Richard M. Crooks*

Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, Texas 77842-3012

Viable *E. coli* cells were entrapped in hydrogel micropatches photopolymerized within microfluidic systems. The microfluidic channels and the micropatches have sizes on the order of 100−500 μm. Small molecules, such as dyes and surfactants, present in the solution surrounding the hydrogel, are able to diffuse into the gel and encounter the cells, but the cells are sufficiently large to be retained. For example, sodium dodecyl sulfate is a lysis agent that is able to penetrate the hydrogel and disrupt the cellular membrane. Entrapment of viable cells within hydrogels, followed by lysis, could provide a convenient means for preparing biocatalysts without the need for enzyme extraction and purification. Hydrogel-immobilized cells are able to carry out chemical reactions within microfluidic channels. Specifically, a nonfluorescent dye, BCECF-AM, is able to penetrate both the hydrogel and the bacterial membrane and be converted into a fluorescent form (BCECF) by the interior cellular machinery. These results suggest that cells immobilized within microfluidic channels can act as sensors for small molecules and as bioreactors for carrying out reactions.

In this report, we describe the fabrication of hydrogel micropatches containing *Escherichia coli* cells within a microfluidic channel, cell lysis, and intracellular enzymatic reactions. Integration of viable bacteria into microfluidic channels followed by subsequent cellular manipulation, such as lysis or poration, provides a new means for fabricating biosensors and using cells as microbioreactors for biochemical synthesis.

Recently, we have been developing microfluidic devices based on biomaterials, such as enzymes,[1,2] DNA,[3] and cells, that can perform multistep reactions or multitarget sensing. The approach involves fabrication of well-defined microreactor zones that are connected by microfluidic channels containing the necessary biomaterials for the required application. For example, we reported that a linear array of microreactors, each of which is filled with different sequences of DNA-immobilized beads, could be used for separating and sensing DNA mixtures.[3] This same basic approach can be used for studying enzymatic reaction kinetics.[2] In addition to beads, hydrogel micropatches photopolymerized within microfluidic channels can also host active, accessible enzymes.[1] Here, we extend this idea by demonstrating that viable bacteria immobilized within a microfluidic channel by photo-cross-linked hydrogel micropatches can also function as microbioreactors.

Cell-based systems have been receiving attention recently because of potential applications to biochemical sensing, fermentation, waste treatment, and drug screening.[4−9] Although cell-based systems often exhibit a long response time, because of slow diffusion through the cell wall, and poor selectivity for substrates,[7] they have three advantages compared to enzyme-based systems: (1) no enzyme extraction and purification is required; (2) depending on the environmental conditions, cells may have a longer lifetime than enzymes;[9] and (3) individual cells provide a contained volume in the picoliter range that may reduce the necessity of microfabricating ultrasmall reaction vessels.[10,11] Moreover, the poor selectivity that is often associated with cell-based systems can often be overcome by inactivating particular enzymes or leaking cofactor molecules through permeabilized cell membranes.[9]

Cells can be immobilized onto surfaces by nonspecific adsorption, covalent bonding, biospecific affinity, or entrapment within a polymer.[7] Methods for patterning cells onto surfaces include microcontact printing,[12−14] membrane-based patterning,[15] flow-based methods,[14,16] and hydrogel-based photolithography.[17] Photo-

* To whom correspondence should be addressed. Voice: 979-845-5629. Fax: 979-845-1399. E-mail: crooks@tamu.edu.

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cross-linked hydrogel polymers are an especially attractive matrix for cell immobilization within a microfluidic system, because cells or enzymes within hydrogels do not generally suffer severe loss of cell viability[18,19] or enzyme activity.[20] Moreover, hydrogels can be easily photopolymerized in a wide variety of geometries within channels after the microfluidic device is fully assembled.[1,21] Photo-cross-linkable hydrogels have previously been used to immobilize DNA,[22] enzymes,[1,20] and cells.[19] Hydrogel immobilization of biological materials is effective because pores within the gel are on the order of 1−10 nm in diameter.[23−25] This permits ingress of small molecules, such as enzyme substrates, but it prevents egress of relatively large entities such as enzymes and cells. Mass transport of small molecules into hydrogels can occur by diffusion or migration, but not by convection.[3]

Here, we fabricated hydrogel micropatches containing *E. coli* cells by in situ UV photopolymerization in microfluidic devices composed of PDMS molds and glass channel covers. *E. coli* was chosen for this study, because it can be easily engineered to express enzymes having desirable properties using recombinant DNA technology.[26−28] Such enzymes have applications for sensing and drug discovery.[4,26−28] The hydrogel micropatches were fabricated to have a smaller volume than the microchannel so that they would not block solution flow in the channel. The viability of gel-entrapped *E. coli* was tested using dyes that selectively bind to nucleic acids. Lysis was carried out to demonstrate that small molecules, a detergent in this case, could penetrate the hydrogel and perform a chemical transformation on the gel-entrapped bacteria. The ability of enzymes within entrapped *E. coli* to carry out reactions was demonstrated by allowing intracellular esterase enzymes to hydrolyze fluorogenic substrates. Fluorescence microscopy confirmed that the enzyme reaction occurred.

EXPERIMENTAL SECTION

Chemicals. PDMS prepolymer (Sylgard 184) was purchased from Dow Corning Co. (Midland, MI). Positive photoresist (AZ P4620) was purchased from Clariant Co. (Somerville, NJ). Poly(ethylene glycol) diacrylate (PEG-DA, MW 575), 2-hydroxy-2-methylpropiophenone, NaCl, KCl, and sodium dodecyl sulfate (SDS) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Tryptone and yeast extract were obtained from Becton Dickinson (Sparks, MD). Phosphate-buffered saline (PBS) solution was prepared by dissolving 130 mmol of NaCl, 2.7 mmol of KCl, 10 mmol of Na_2HPO_4 (Sigma Chemical Co., St. Louis, MO), and 1.8 mmol of KH_2PO_4 (Sigma) in 1 L of deionized water and adjusting its pH to 7.2 with HCl or NaOH. All aqueous solutions were prepared using 18 MΩ·cm water (Milli-Q reagent water system, Bedford, MA). The Live/Dead *Bac*light Bacterial Viability test kit, propidium iodide (PI), 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF), and 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluoroescein acetoxymethyl ester (BCECF-AM) were purchased from Molecular Probes Inc. (Eugene, OR). The bacteria Gram-stain kit was purchased from Sigma.

***E. coli* Immobilization in Microfluidic Channels.** Fabrication of the PDMS microfluidic device followed a literature procedure.[29] Briefly, PDMS polymer molds were prepared by casting a PDMS prepolymer against a positive photoresist master and curing at 65 °C for 2 h. The PDMS mold was irreversibly sealed to a clean microscope cover glass (thickness 0.17 mm) after both were treated with an oxygen plasma (60 W, model PDC-32G, Harrick Scientific, Ossining, NY) for 30 s. The height and width of the fluidic channels were about 20 and 500 μm, respectively. *E. coli* BL21 cells were grown in Luria-Bertani (LB) medium, which is prepared from 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl, in an incubator at 37 °C for 12−16 h. After centrifuging the cell solution, the supernatant was removed, and the resulting cell pellets were washed with deionized water. Washing and centrifugation were repeated three times. The resulting cell pellets were resuspended in the hydrogel precursor solution consisting of 50% (w/w) PEG-DA and 0.5% (w/w) 2-hydroxy-2-methylpropiophenone photoinitiator in deionized water. The microfluidic channel was filled with the hydrogel precursor solution, containing ∼10^9 cells/mL, and photopolymerized for 3 s through a photomask using a fiber-optic-equipped UV light source (365 nm, 300 mW/cm^2, EFOS Lite E3000, Ontario, Canada). The width of the photomask was 100 or 250 μm, which defined the size of hydrogel micropatches. Unreacted hydrogel precursor solution was removed using ∼0.5 mL of PBS solution introduced into the channel at 10−500 μL/min with a syringe pump. We have previously used a silane monolayer to enhance adhesion between the hydrogel and the interior of the microfluidic channel,[1,3] but detachment of the micropatches was rarely observed in these experiments and therefore this procedure was not adopted.

Cell staining was carried out by flowing a solution of crystal violet into the microfluidic channel, stopping the flow for 1 min, washing the channel with deionized water, and then repeating the same procedures with KI. After staining, *E. coli* cells within the hydrogel micropatches were observed with an optical microscope having a 10×, 20×, or 40× objective lens.

Cell Viability Test and Lysis. A bacterial cell Live/Dead test kit composed of SYTO 9 (6.7 μM in deionized water) and PI (40 μM in deionized water) solution or PI solution alone was used to test the viability of *E. coli* entrapped within hydrogel micropatches (Caution: propidium iodide is a potential mutagen. Handling and disposal of the chemical should follow procedures provided by the manufacturer). The viability stains were introduced to the cells by flowing them into the microfluidic channel and then stopping the flow for 20 min.

Cell lysis was performed by flowing 1% (w/v) SDS aqueous solution through the channel for 20 min at a rate of 5 μL/min.

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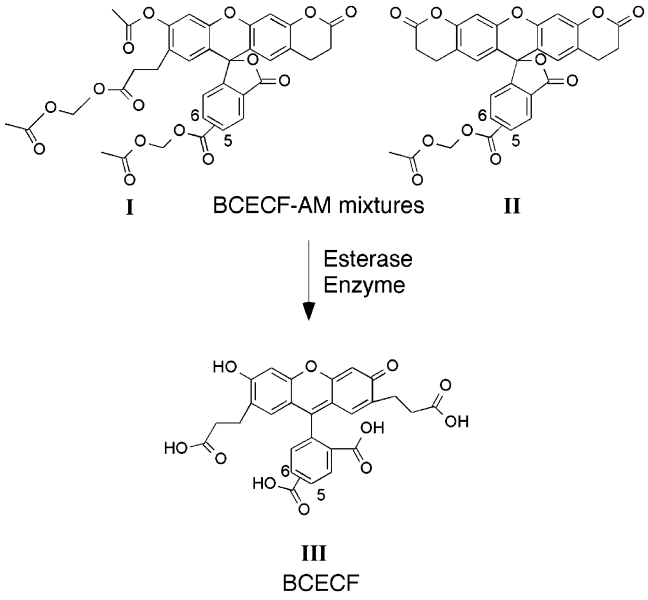
Scheme 1



After the channel was rinsed with deionized water, the cells were incubated with PI solution so that they could be visualized. Images of cells incubated with PI or the cell viability test solution were obtained using an inverted epifluorescence microscope (Nikon Eclipse TE 300, Nikon Co., Tokyo, Japan) equipped with a 100-W mercury lamp, a cube-type XF 115-2 filter set (Omega optical, Inc., Brattleboro, VT). The cell images were collected with 24-bit color CCD camera (D1H, Nikon Co.).

Intracellular Reactions in Entrapped *E. coli*. The dye BCECF-AM was used to demonstrate that the hydrogel-entrapped *E. coli* cells are able to carry out enzyme reactions. BCECF-AM is a nonfluorescent mixture consisting of two major components (**I** and **II** in Scheme 1). The ester groups of BCECF-AM are hydrolyzed by esterase enzymes contained within *E. coli* cells to yield BCECF (**III** in Scheme 1), which is a pH-sensitive fluorescent dye. In every experiment, 10 μM BCECF-AM solution was freshly prepared by diluting 160 μM BCECF-AM stock solution dissolved in anhydrous DMSO with PBS. The hydrogel-entrapped *E. coli* cells were incubated with this solution for 1 h, and then the cells were observed by epifluorescence microscopy. Images were acquired using a 16-bit gray scale CCD camera (Photometrics Ltd., Tucson, AZ) and V++ precision digital imaging software (Digital Optics, Auckland, New Zealand). False color images were prepared using the V++ software.

RESULTS AND DISCUSSION

***E. coli* Cell Immobilization in Microfluidic Channels.** Bacteria-containing hydrogel micropatches were prepared in microfluidic channels by UV photopolymerization of the cell-containing hydrogel precursor solution through a photomask. Details are given in the Experimental Section. An optical micrograph of a typical cell-containing micropatch is shown in Figure 1A. This micropatch is in contact with the PDMS mold and cover glass. It is about 100 μm wide and 400 μm long, and it is centered within the channel, which is 500 μm wide and 20 μm in height (the walls of the channel are indicated by the dashed white line in Figure 1A). We and others previously observed that hydrogel

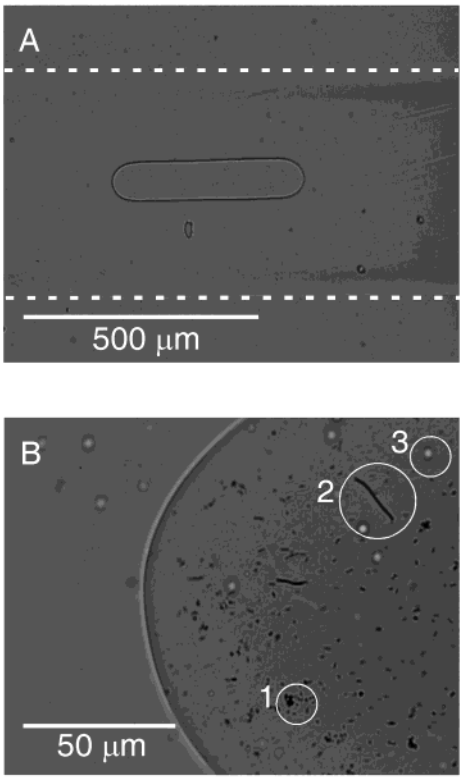


Figure 1. (A) Optical micrograph of a hydrogel micropatch containing bacteria and photolithographically defined within a microfluidic channel. The micropatch extends from the top to the bottom of the channel, but there is space on either side for fluid to flow. Small molecules are able to enter the hydrogel by diffusion, but the much larger cells are trapped. The dashed white line indicates the location of the walls of the channel. (B) A high-resolution optical micrograph of the same hydrogel micropatch after staining the entrapped cells with crystal violet and KI. Circles 1−3 are examples of cell aggregates, an unknown contaminant, and a bubble trapped during PDMS channel fabrication, respectively.

micropatches are prone to delamination from glass substrates unless an intermediate adhesion layer is used to convalently link the hydrogel to the glass slide.[3,19] However, detachment was rarely observed in these experiments. This is probably because the hydrogel micropatch occupies only a small fraction of the channel width, and therefore, solution can be easily pumped through the channel without exerting too much force on the hydrogel. Additionally, because the hydrogel was swollen with water during fabrication, mechanical changes caused by swelling, which could cause delamination during experiments, should not be significant.[19]

After staining, individual *E. coli* cells are clearly observed within the hydrogel by optical microscopy (Figure 1B). Different features within the hydrogel are labeled as circles 1, 2, and 3; these correspond to cell aggregates, an unknown contaminant, and a bubble trapped in the PDMS during fabrication, respectively. The cell stain is composed of crystal violet, which easily penetrates through cell membranes to stain interior organelles, and KI, which forms a crystalline complex with crystal violet.[30] We estimated the number of cells present in the hydrogel micropatch to be in the range of 10^2-10^4 by considering the concentration of cells

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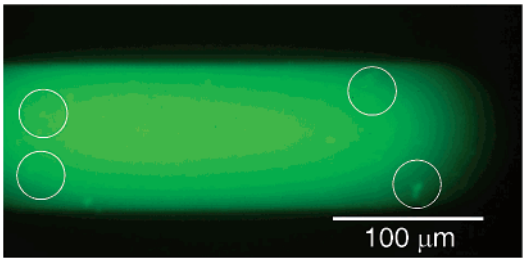

Figure 2. Fluorescence micrograph of *E. coli* cells within a hydrogel micropatch. The cells were incubated with SYTO 9 and PI. Although some cells (indicated by circles) were observed to fluoresce above background, strong background fluorescence caused by activation of the dye in the hydrogel makes it difficult to determine the extent of cell viability using this common approach.

suspended in hydrogel precursor solution prior to polymerization and the final volume of the hydrogel micropatch. The extent of cell aggregation depends on the solvent used to prepare the hydrogel prepolymer/cell precursor solution. The micropatch shown in Figure 1B was prepared using deionized water, and it is clear that the cells do not extensively aggregate. However, when the precursor solution was prepared in PBS solution, cells aggregated in the precursor solution and as a result few were entrapped within hydrogel micropatches. Because exposure of the cells to deionized water for short periods of time did not affect their viability, it was used for making all cell-containing hydrogel precursor solutions.

Cell Viability and Lysis. It is important to know whether the hydrogel-entrapped cells are viable, because cell membranes can be damaged during UV photopolymerization. The viability of hydrogel-entrapped *E. coli* was tested with solutions composed of SYTO 9 and PI. SYTO 9 penetrates through intact membranes, and therefore, viable cells fluoresce green. PI is able to enter cells having compromised membranes, and therefore, dead cells fluoresce red. Unfortunately, Figure 2 shows that the SYTO 9 dye also fluoresces strongly when it is entrapped within the hydrogel micropatch, which makes it difficult to determine cell viability with certainty. However, cells highlighted by the white circles do appear more green than the background, suggesting they may be viable. The strong background fluorescence may be due to a high local concentration of the dye in the hydrogel or differences in fluorescence quantum efficiency for the hydrogel-entrapped dye and the free dye in solution.

In contrast to SYTO 9, PI does not display a strong background fluorescence when it is present within hydrogel micropatches. Accordingly, PI alone can be used to test the viability of hydrogel-entrapped *E. coli* cells: live cells show no fluorescence in the presence of PI, while dead cells fluoresce red. Panels A and B of Figure 3 are optical and fluorescence micrographs, respectively, of hydrogel-entrapped *E. coli* cells. The optical micrograph reveals that many *E. coli* cells are entrapped in the hydrogel micropatch (Figure 3A), but except for very weak background fluorescence, they do not exhibit the characteristic red color of dead cells (Figure 3B). We conclude that photopolymerization does not cause serious defects in the cell membranes. This observation is consistent with previous reports for hydrogel-entrapped mammalian cells.[19] Indeed, we observed that *E. coli* cell membranes were intact even after several minutes of UV exposure.

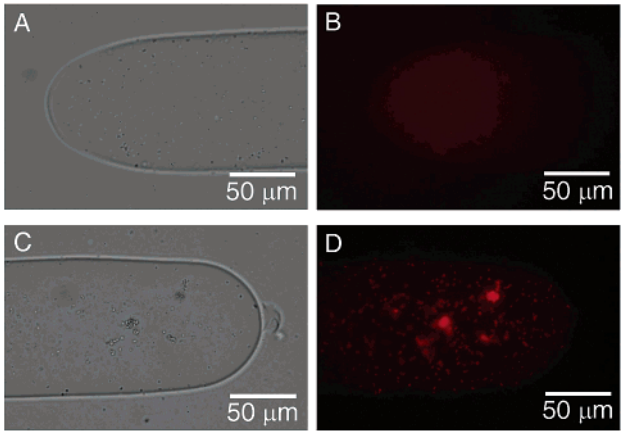

Figure 3. (A) Optical micrograph of a hydrogel micropatch containing bacteria and photolithographically defined within a microfluidic channel. (B) Fluorescence micrograph of the same micropatch after incubating with PI. (C) Optical micrograph of a hydrogel micropatch analogous to the one shown in (A), but after cell lysis with 1% (w/v) SDS solution. (D) Fluorescence micrograph of the micropatch containing the lysed cells after incubating with PI.

Lysis agents porate cellular membranes, and this in turn should permit dyes that are unable to cross the intact cell wall, such as PI, to permeate into the cell interior. Therefore, to confirm that the hydrogel-entrapped cells do not have compromised membranes, they were lysed with SDS and the resulting micrographs were compared with those just discussed. The optical micrograph in Figure 3C and the corresponding strong fluorescence observed in Figure 3D indicate that SDS lysis occurs within the hydrogel. This finding is useful for two reasons. First, it shows that agents present within a surrounding solution can penetrate the hydrogel and perform a chemical operation on the cells residing therein. Second, if lysis agents that do not cause severe loss of enzyme activity are selected,[31] the resulting cell poration will enhance the rate of mass transfer of small molecules across the cellular membrane. This means that the contents of lysed hydrogel-entrapped *E. coli* cells can be used as biocatalysts or sensing agents.

Intracellular Reactions in Entrapped *E. coli*. Although enzyme activity in membrane-compromised cells can be significant,[9] cellular membranes protect enzymes resulting in higher activity. To test the enzyme activity of live, hydrogel-entrapped *E. coli* cells, we relied on an esterase-catalyzed hydrolysis reaction. The dye BCECF-AM (a combination of **I** and **II** in Scheme 1) exhibits fluorescence when hydrolyzed to BCECF (**III** in Scheme 1) by intracellular esterases.[32,33] Conveniently, hydrophobic BCECF-AM easily penetrates cell membranes, but the hydrolysis product, BCECF, is retained within the cells.[32,33] The fluorescence micrograph shown in Figure 4A was obtained after entrapping *E. coli* cells within a hydrogel micropatch and then flowing BCECF-AM through the microfluidic channel surrounding the micropatch. As indicated by the intense fluorescence, BCECF-AM is able to diffuse through 1−5-nm hydrogel pores[23−25] and the cell mem-

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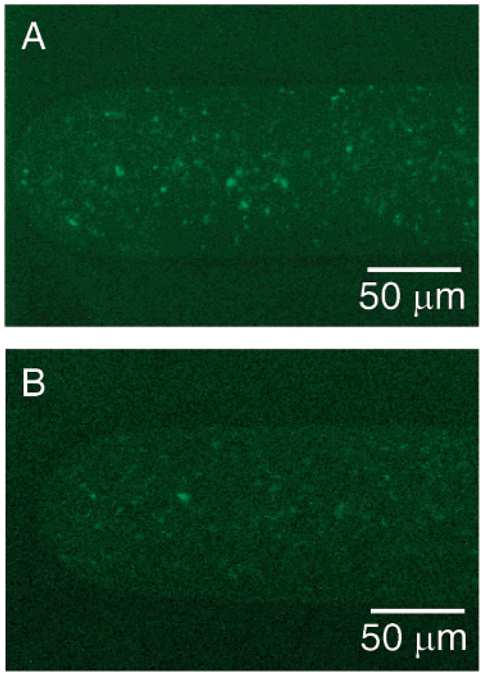

Figure 4. Fluorescence micrographs of hydrogel micropatches containing bacteria and photolithographically defined within a microfluidic channel. (A) After incubating with BCECF-AM for 1 h. (B) Same experiment as in (A), but after cell lysis with SDS.

branes, where it encounters intracellular esterase enzymes that convert it to the highly fluorescent BCECF form.

The weak green fluorescence of the hydrogel and the solution inside the microfluidic channel arises from slow BCECF-AM hydrolysis by the pH 7.2 PBS solution. When *E. coli* cells suspended in PBS solution or entrapped in hydrogel were incubated with 10 μM BCECF for 1 h, the cells showed very weak fluorescence. This result indicates that the fluorescence of *E. coli* cells mainly arises from the enzyme hydrolysis reaction occurring within the cells, rather than from diffusion of BCECF (derived from slow solution hydrolysis of BCECF-AM) from the solution into the cells.

Lysed hydrogel-entrapped *E. coli* cells were also incubated with BCECF-AM. Because SDS usually severely damages enzymes,[31] only very weak intracellular fluorescence is observed after lysis (Figure 4B). In this case, fluorescence probably does result from transport of BCECF from solution directly into the membrane-compromised cells.

SUMMARY AND CONCLUSIONS

In this article, we showed that viable *E. coli* cells can be entrapped in hydrogels photopolymerized within microfluidic systems. Small molecules, such as dyes and SDS, present in the solution surrounding the hydrogel, are able to diffuse into the gel and encounter the cells. For example, we showed that SDS is an active lysis agent. Entrapment of viable cells within hydrogels, followed by lysis, could provide a convenient means for preparing biocatalysts without the need for enzyme extraction and purification. We also showed that the immobilized cells are able to carry out chemical reactions. Specifically, a nonfluorescent dye, BCECF-AM, is able to penetrate both the hydrogel and the bacterial membrane and be converted into a fluorescent form (BCECF) by the cellular machinery. These results suggest that cells immobilized within microfluidic channels can act as sensors for both small molecules and bioreactors. Future studies will highlight more complex functions of bacteria immobilized within microfluidic systems.

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