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Functionalizing Soft Matter for Molecular Communication

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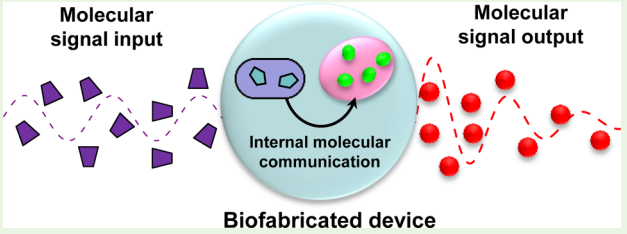
Supporting Information

ABSTRACT: The information age was enabled by advances in microfabrication and communication theory that allowed information to be processed by electrons and transmitted by electromagnetic radiation. Despite immense capabilities, microelectronics has limited abilities to access and participate in the molecular-based communication that characterizes our biological world. Here, we use biological materials and methods to create components and fabricate devices to perform simple molecular communication functions based on bacterial quorum sensing (QS). Components were created by protein engineering to generate a multidomain fusion protein capable of sending a molecular QS signal, and by synthetic biology to engineer *E. coli* to receive and report this QS signal. The device matrix was formed using stimuli-responsive hydrogel-forming biopolymers (alginate and gelatin). Assembly of the components within the device matrix was achieved by physically entrapping the cell-based components, and covalently conjugating the protein-based components using the enzyme microbial transglutaminase. We demonstrate simple devices that can send or receive a molecular QS signal to/from the surrounding medium, and a two-component device in which one component generates the signal (i.e., issues a command) that is acted upon by the second component. These studies illustrate the broad potential of biofabrication to generate molecular communication devices.

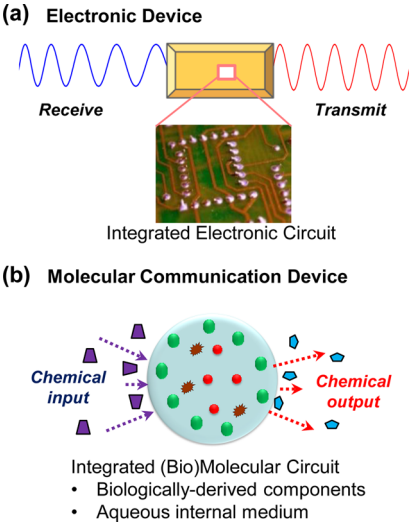


KEYWORDS: *alginate, biofabrication, gelatin, molecular communication, quorum sensing*

INTRODUCTION

Traditional information processing devices receive input in the form of electromagnetic (EM) radiation, process the information using electrons, and then transmit EM outputs as illustrated in Scheme 1a. The remarkable advances in this traditional communication have enabled autonomous systems to be deployed to explore and report from distant planets (e.g., Mars). Recently, there have been efforts to extend traditional communication theory to biological information processing with the dual goals of obtaining a broader understanding of biological signal processing and building systems that can communicate with biology.[1−6] The possibility of building autonomous devices capable of communicating with biology would have exciting applications in medicine (e.g., theranostics), but could also provide transformational capabilities for remote monitoring for environmental protection, food safety, and national security. However, as illustrated in Scheme 1b, biological communication is fundamentally different from traditional communication in that inputs, outputs, and processing is typically performed using molecules and ions (not EM and electrons).[2] Currently, the study of molecular communication is at its infancy with minimal capabilities to build robust autonomous devices.[7,8] Thus, although traditional communication devices can report on activities from distant planets, molecular communication devices are currently

Scheme 1. (a) Traditional and (b) Molecular Communication Devices



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unavailable to probe the exchange of biochemical information that routinely occurs in our gut.

Biology is the obvious source of "components" for molecular communication devices with proteins and cells being two options for receiving and transmitting molecular information. Proteins offer molecular recognition properties that allow chemical messages to be selectively received, processed (i.e., interconverted) and sent.[9] Further, protein engineering and directed evolution should enable protein-based components to be designed or evolved with tailored communication properties. Cells allow more sophisticated computational and communication capabilities since their genetic machinery can be engineered to process molecular inputs into a wider array of chemical outputs. In fact, synthetic biology[10−12] is envisioned as an enabling technology for molecular communication[13] with current activities focusing on multicellular consortia as a means of performing complex and distributed communication functions.[14−17]

A unique feature of molecular communication devices is that they must be open systems to allow the exchange of information-carrying molecules. This chemical exchange will likely involve an aqueous medium because many of the envisioned applications for molecular communication devices involve the medical or life sciences. The medium within the device will also likely be aqueous (especially if the device is fabricated from biological components) and thus internal chemical signals will need to propagate through an aqueous medium. As a result, autonomous molecular communication devices will not likely be built from a traditional silicon-based platform, but rather will be built from capsules, vesicles and hydrogels. While there has been some effort to create the individual components for molecular communication (gates and switches),[18,19] to perform logic functions,[20] and to integrate molecular subsytems for biocomputing applications,[21] there has been much less effort in creating the platform technologies necessary to assemble and integrate these biological components into complex systems.[22]

We suggest that biofabrication provides a unique opportunity to build molecular communication systems by allowing functional components to be generated and then organized over a hierarchy of length scales. For our purposes, we define biofabrication as the use of biological materials and mechanisms to create structure and function.[23−25] Here, we use bacterial quorum sensing (QS) as our biomolecular communication system.[26−29] QS is a well-known mode of bacterial communication that guides population-level actions (often pathogenic actions).[30−33] Thus, QS serves as a convenient model of biomolecular communication as well as an important modality for understanding homeostasis and pathogenicity in complex biological environments (e.g., the gut). Here, we demonstrate the biofabrication of simple molecular communication devices in which: (i) a protein-based device transmits a chemical signal; (ii) a cell-based device receives and reports such a chemical signal; and (iii) an integrated system in which a protein-based component transmits a molecular "command" that is acted upon by a cell-based component.

EXPERIMENTAL SECTION

Materials. The following materials were purchased from Sigma-Aldrich; alginic acid sodium salt from brown algae (medium viscosity), gelatin from porcine skin (type A and type B), *S*-adenosylhomocysteine (SAH), phosphate buffered saline (PBS) tablets, and calcium chloride anhydrous beads. Microbial transglutaminase (mTG; Activa TI; 100 U/g as reported by the manufacturer) was obtained from Ajinomoto (Japan). Fluorescent FITC labeled polystyrene microparticles (0.84 μm, 0.1% w/v) were purchased from Spherotech Inc. (Lake Forest, IL). Water was deionized (DI) with Millipore SUPER-Q water system until final resistivity >18 MΩ·cm was reached. Buffer and calcium chloride solutions were filtered with a Fisherbrand sterilized syringe filter (0.22 μm) before use.

Biofabrication Methods. An alginate stock solution (1.5% w/v) was prepared by dissolving alginate powder in DI water. A gelatin stock solution (15% w/v) was prepared by dissolving equal amount of type A and type B gelatin in 37 °C PBS buffer (20 mM, pH 7.0). Typically, we prepared our bead devices from a warm (37 °C) prebead mixture containing: gelatin (5%), alginate (1%), mTG (1 U/ml), fusion tagged protein (for protein-based device), and/or bacterial cells (for cell-based device). The prebead mixture was dropped into a stirring $CaCl_2$ (0.1 M) solution using a syringe with a 27 Gauge needle. The formed beads were incubated in $CaCl_2$ solution at room temperature for 2 h. To prepare cell-based beads, the bacterial cells were initially cultured in LB medium to an OD_{600} of 4.0, then 0.3 mL of this cell suspension was centrifuged, the pellet was resuspended with 25 μL of PBS buffer and mixed with 1.5 mL of warm solution containing other components. For bacteria cells related experiments, all incubation solutions contain antibiotics (Kanamycin 50 μg/mL; ampicillin 50 μg/mL) and 3 mM $CaCl_2$. Experimental details are provided in the text.

Instrumentation. Raman spectra were obtained from a Jobin Yvon LabRamHR Raman microscope. Fluorescence images of beads were obtained using an Olympus MVX10 MacroView microscope, and cell-containing samples were examined using an Olympus BX60 microscope. Images were obtained using an Olympus DP72 digital camera connected to the microscope.

Molecular Biology. The target proteins used in this study were engineered with glutamine fusion tags to facilitate mTG-catalyzed conjugation to the gelatin matrix. Specifically, we engineered the red fluorescent mCherry protein to have a C terminal tag with 5 added glutamine residues (Gln-mCherry). We also engineered the fusion enzyme (Pfs-LuxS) to have a C terminal tag (5 Gln). Two types of reporter cells are used in this study. The CT104(pCT6+pETEGFP-T5DsRedExpresss2)[34,35] report cells were engineered to constitutively express a red fluorescent protein (DsRed) and to conditionally express the green fluorescent protein (EGFP) in the presence of autoinducer 2 (AI-2).[36] The CT104(pCT6+pET200-DsRed)[32,34,37,38] reporter cells (without constitutively expressing fluorescent protein) were engineered to express the red fluorescent protein (DsRed) in the presence of AI-2. Methods to engineer the target proteins and cells used in this study are provided in the Supporting Information.

RESULTS AND DISCUSSION

Biofabrication to Build Structure and Confer Biofunction. Biology provides a variety of stimuli-responsive self-assembling biopolymers that can be used to create structure. Here, we generated molecular communication devices using hydrogel-based matrices constructed from two stimuli-responsive biological polymers. One biopolymer is the Ca^{2+}-responsive polysaccharide alginate which can readily form hydrogel beads.[39] Considerable recent efforts are enlisting alginate's stimuli-responsive responsive properties for biocomputing and delivery applications.[40−44] As illustrated in Figure 1a, Ca^{2+}-alginate beads were typically prepared by dropping an alginate solution (1%) into a stirring solution of $CaCl_2$ (0.1 M). Importantly, Figure 1a illustrates that components added to the prebead alginate mixture (e.g., FITC-labeled microparticles; μP-FITC, 0.01%) can be incorporated into the gelled beads. The second biopolymer is the thermally responsive protein gelatin.

To prepare gelatin-alginate beads, a warm solution (~37 °C) containing gelatin (5%)[45,46] and alginate (1%)[47−49] was

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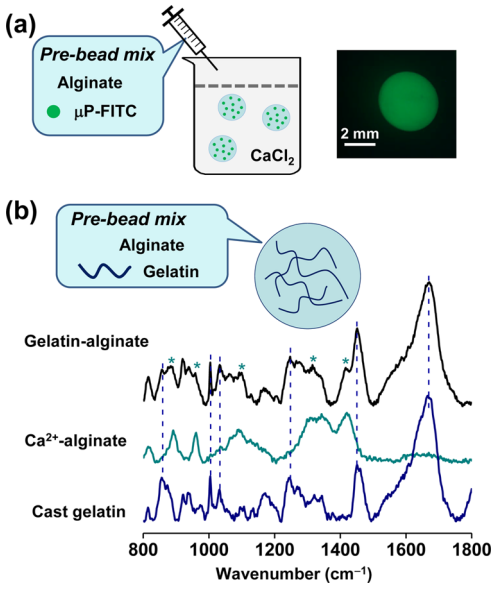

Figure 1. Fabrication of the device (i.e., bead) matrix. (a) Schematic illustrates Ca^{2+}-alginate bead formation and the fluorescence image shows that FITC-labeled microparticles (μP-FITC) can be entrapped within the matrix. (b) Raman spectra provide evidence for gelatin-alginate bead formation. The bottom two spectra are controls that show characteristic peaks for cast gelatin film and Ca^{2+}-alginate beads.

transferred to a syringe with a 27 Gauge needle and dropped into a stirring $CaCl_2$ (0.1 M) solution. The beads were incubated in the $CaCl_2$ solution (2 h), rinsed with water, vacuum-dried (4 h), and then examined using Raman spectroscopy. Figure 1b compares peaks for the gelatin-alginate beads against controls of a cast gelatin film and Ca^{2+}-alginate beads. The beads prepared with a mixture of gelatin and alginate show Raman peaks characteristic of both biopolymers providing chemical evidence that alginate can incorporate gelatin into the hydrogel matrix.

Enzymes provide a selective means for introducing covalent bonds to build structure and confer function to materials. Here, we incorporate gelatin into the bead matrix to allow protein-based components to be covalently conjugated to the matrix using the enzyme microbial transglutaminase (mTG).[50−52] Figure 2a shows that mTG catalyzes the grafting of proteins to gelatin through a glutamine (Gln) residue of one protein and a lysine (Lys) residue of the other protein.[45,53] Often, the residues (Gln or Lys) of target proteins with globular structures are inaccessible for mTG catalysis and thus target proteins are commonly engineered with short fusion tags that provide accessible amino acid residues.[54,55]

To illustrate enzymatic-assembly of a protein, we prepared a warm prebead mixture containing: gelatin (2.5%), alginate (1%), a glutamine tagged model red fluorescence protein Gln-mCherry (20 μg/mL);[56] mTG (1 U/ml); and μP-FITC (0.01%, for visualization). This solution was dropped into a $CaCl_2$ (0.1 M) solution to form multicomponent beads and these beads were incubated in the $CaCl_2$ solution for 2 h to allow protein conjugation. These beads were rinsed with water and incubated in 50 mM $CaCl_2$ for 2 h to remove unreacted proteins, and then examined using a fluorescence microscope. As shown in Figure 2b, green fluorescence (from entrapped microparticles) is observed in gelatin-alginate beads prepared with mTG and also in control beads in which mTG was deleted from the prebead mixture. Red fluorescence was only observed

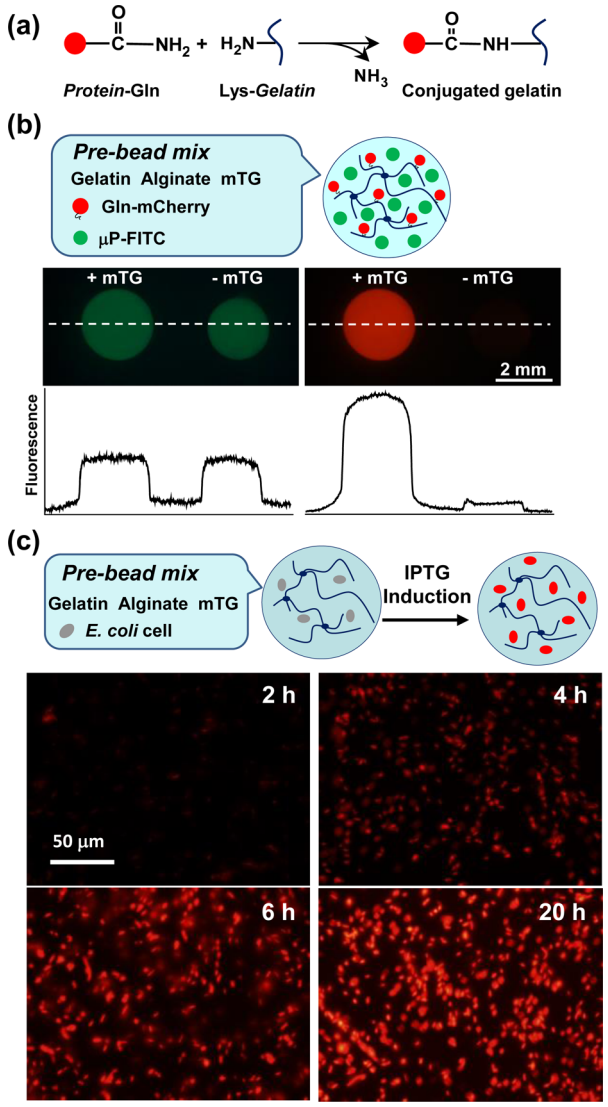

Figure 2. Conferring protein and cellular functionalities. (a) Schematic illustrates the protein conjugation reaction catalyzed by microbial transglutaminase (mTG). (b) Fusion tagged mCherry protein (Gln-mCherry) was conjugated to the gelatin-alginate beads while fluorescent microparticles (μP-FITC) were entrapped within the matrix. Control beads were prepared by deleting mTG from the prebead mixture. (c) Entrapped *E. coli* cells in mTG-cross-linked gelatin-alginate beads can proliferate and express red fluorescent protein after IPTG induction.

in the gelatin-alginate beads prepared in the presence of mTG, while control beads (lacking mTG) showed no red fluorescence. This result indicates that mTG-catalyzed the conjugation of the mCherry protein to the gelatin-alginate beads. It should be noted that gelatin molecules in the gelatin-alginate beads are also cross-linked by the mTG-catalyzed reaction and the gelatin matrix becomes stable at warm temperatures (e.g., 37 °C).[45,46,57,58]

Next, we illustrate the incorporation of cellular functionality into the gelatin-alginate beads. For this we prepared a warm prebead mixture containing: *E. coli* BL21DE3(pET-DsRed) that serve as model reporter cells that can express a red fluorescence protein (DsRed) upon IPTG induction; gelatin (5%); alginate (1%), and mTG (1 U/ml). Beads prepared from this prebead mix were transferred to an LB medium containing

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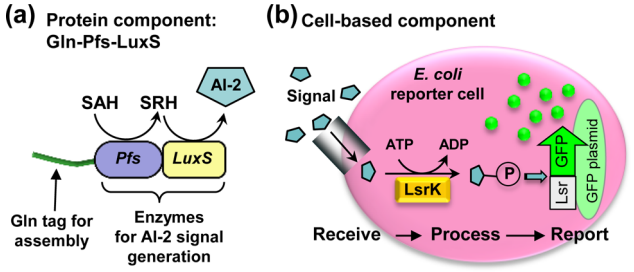
1 mM IPTG at 37 °C, and the fluorescence was measured intermittently. Fluorescence images in Figure 2c show a progressive increase in fluorescence as the entrapped *E. coli* cells both proliferate and express florescence.[46] It should be noted that the florescence observed in Figure 2c corresponds to colonies and not individual cells. Presumably the mobility of these microscale bacteria is restricted within the gelatin-alginate network.[59]

The results in Figures 1 and 2 with fluorescent models (of microparticles, proteins and cells) provide visual illustrations of important features of biofabrication. First, stimuli-responsive biopolymers (e.g., alginate) provide the self-assembling systems that can generate hydrogel structure. These hydrogel networks allow microscale components to be entrapped (e.g., bacteria) to confer functionality.[60] Second, the mTG enzyme allows the covalent conjugation of smaller components (e.g., proteins) onto the gelatin-component of the matrix. Finally, advanced methods in biology can be enlisted to facilitate assembly and confer functionality: proteins can be engineered with fusion tags to permit enzymatic-assembly and cells can be engineered to receive and respond to information in their environment.

Components of Bacterial Quorum Sensing-Based Communication. Here, we use bacterial quorum sensing (QS) based on the autoinducer 2 (AI-2) signaling molecule as our model for molecular communication and we engineered two types of components based on either proteins or bacterial cells. As illustrated in Scheme 2a, AI-2 is synthesized in a two-step pathway involving the enzymes Pfs and LuxS. Our protein-based molecular communication component is an engineered fusion protein with two functional domains and an assembly tag. The Pfs and LuxS domains provide the catalytic function necessary for AI-2 signal generation.[36] An "assembly-tag" composed of a short sequence of 5 glutamine (Gln) residues was added to the Pfs-LuxS fusion protein (Gln-Pfs-LuxS) to facilitate mTG-catalyzed conjugation of this protein component to the gelatin matrix.[36,61]

Scheme 2b shows that AI-2 is taken up by *E. coli* and phosphorylated, and the Phospho-AI-2 activates Lsr promoter for gene expression. Our cell-based communication components are *E. coli* strains (W3110) engineered to express various proteins (e.g., fluorescent reporter proteins) under the cascaded control of Lsr operon system. Thus, these cell-based communication components can receive AI-2 as a chemical input, process this input through an appropriate genetic circuit, and report an output. For this study our cell-based components are reporter cells[32,37,38] that are engineered to express fluorescent proteins[34] upon exposure to AI-2 (details of the creation of these protein and cell components are provided in Supporting Information).

Scheme 2. Schematic Illustrating the Basis for Engineering Components (Protein and Cell-Based) for Molecular Communication via Bacterial Quorum Sensing (QS): (a) Protein-Based Components Are Fusions of the Two Biosynthetic Enzymes (Pfs and LuxS) Required for AI-2 Signal Generation; (b) Cell-based Components Are Reporter Cells That Receive the AI-2 Signal, Process This Input Genetically, and Respond by Expressing a Protein (e.g., Fluorescent Reporter Protein)



Biofabricated Protein-Based Signal Sender. Figure 3a illustrates that we prepared a protein-based sender device by the mTG-catalyzed conjugation of the Gln-Pfs-LuxS to the gelatin-alginate matrix. Specifically, we prepared our bead devices from a warm prebead mixture containing: gelatin (5%), alginate (1%), Gln-Pfs-LuxS (50 μg/mL), and mTG (1 U/mL). To assess the ability of these devices to transmit the AI-2 molecular signal, the beads were transferred to a 50 mM tris buffer solution (with 10% v/v LB medium) containing the precursor SAH (0.5 mM) and the AI-2 reporter cells CT104(pCT6+pETEGFP-T5DsRedExpress2)[34,35] that had been engineered to constitutively express a red fluorescent protein (DsRed) and to conditionally express the green fluorescent protein (EGFP) in the presence of AI-2.[36] After incubation at 37 °C for 20 h, the surrounding medium was sampled and observed by fluorescence microscopy. The fluorescence images in Figure 3b show that when beads containing the Gln-Pfs-LuxS were present, the reporter cells in the surrounding medium expressed both red and green

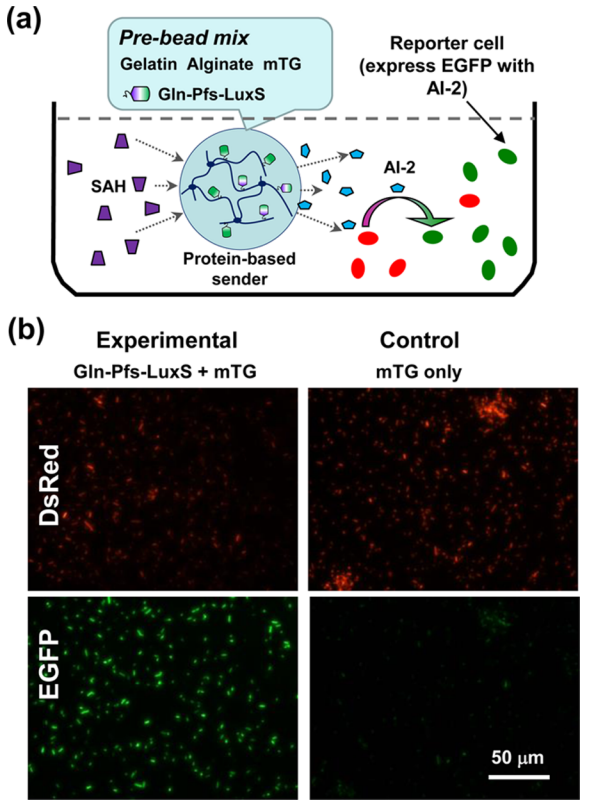


Figure 3. Protein-based sender device. (a) Schematic illustrates that mTG-catalyzed conjugation of Gln-Pfs-LuxS to gelatin allows AI-2 signal generation that can be detected by reporter cells in the surrounding environment. (b) Fluorescence images of *E. coli* reporter cells from the surrounding environment: red fluorescence (DsRed) is constitutively expressed by both experimental and controls, while green florescence (EGFP) is only observed in the experimental samples.

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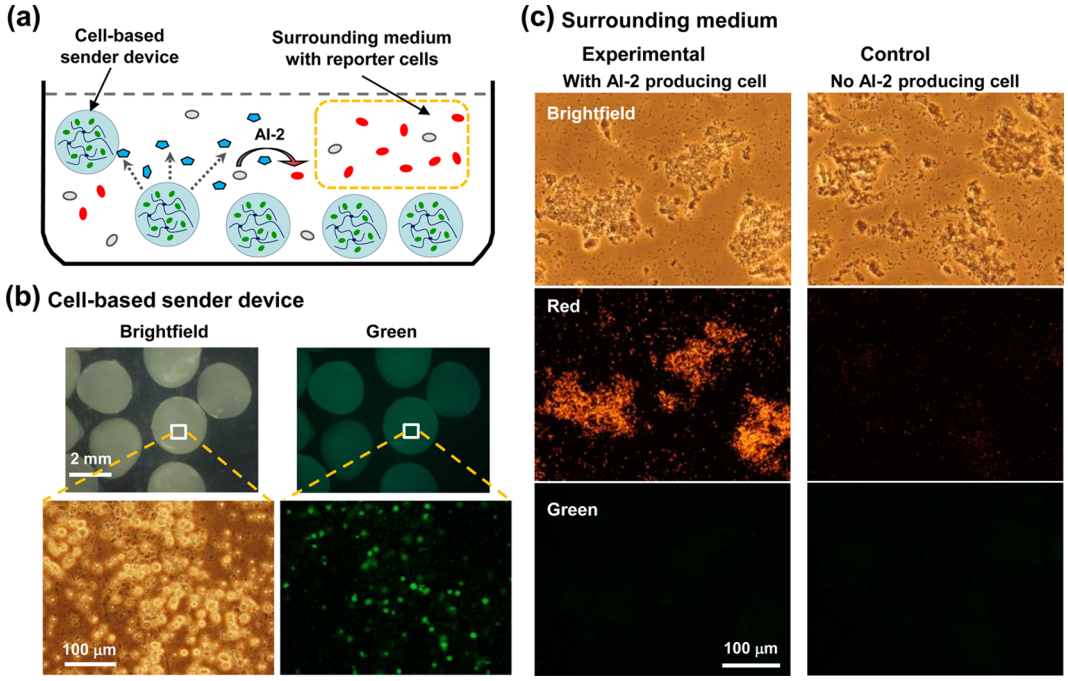

Figure 4. Cell-based sender device. (a) Schematic illustrates that AI-2 producing bacteria are entrapped in gelatin-alginate beads to allow AI-2 signal generation that can be detected by reporter cells in the surrounding environment. (b) After 18 h of incubation, bright-field and green fluorescence images show the sender beads with the AI-2 producing cells (green). (c) Bright-field, red, and green fluorescence images of the surrounding medium: reporter cells express red fluorescence protein only in the presence of sender devices, whereas the limited green fluorescence indicates that few sender cells have escaped from the device into the surrounding medium.

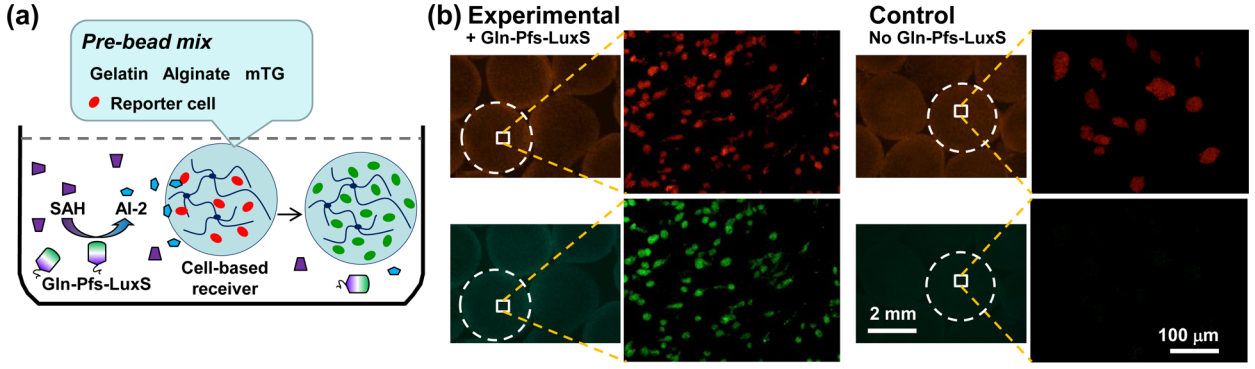

Figure 5. Cell-based receiver device. (a) Schematic illustrates that reporter cells entrapped in cross-linked gelatin-alginate beads respond to signaling molecule AI-2 that is in situ generated in the surrounding environment. (b) Fluorescence images of *E. coli* reporter cells show green fluorescence (EGFP) in the presence of AI-2 signal generation (with Gln-Pfs-LuxS in the surrounding medium) and no green fluorescence in the absence of AI-2 generation (without Gln-Pfs-LuxS).

fluorescent proteins. Controls in which these reporter cells were incubated with beads that lacked Gln-Pfs-LuxS (Gln-Pfs-LuxS was deleted from the prebead mixture) showed red but not green fluorescence. The results in Figure 3 demonstrate that a biofabricated gelatin-alginate bead can act as a protein-based sender of an AI-2 signal that is recognized by bacteria in the surrounding environment.

Biofabricated Cell-Based Signal Sender. A cell-based device capable of sending AI-2 signals was biofabricated by entrapping AI-2-producing cell BL21(pCT5+pET-GFP) within the gelatin-alginate beads. In addition to producing AI-2, these sender cells have been engineered to constitutively express GFP. To prepare these cell based sender devices, we generated beads from a warm prebead mixture containing: these *E. coli* sender cells, gelatin (5%), alginate (1%), and mTG (1 U/mL). Beads prepared with these sender cells were transferred to a solution (50% v/v of PBS and LB medium) containing reporter cells CT104(pCT6 + pET200-DsRed),[32,34,37,38] and incubated at 30 °C for 18 h.

As illustrated in Figure 4a, sender cells entrapped within the gelatin-alginate device can generate AI-2 signaling molecules, which can be detected by the reporter cells in the surrounding medium. Figure 4b shows that the cell-based sender beads remain intact after 18 h incubation and the entrapped AI-2 producing cells (green) grew to form colonies within the matrix. After incubation, the surrounding medium was sampled and observed using fluorescence microscopy. The left column of Figure 4c shows images obtained for samples prepared in the presence of AI-2 sender beads. The red fluorescence indicates that the reporter cells in the surrounding medium detected the generation of the AI-2 signaling molecules while the absence of green florescence indicates that few of the sender cells escaped

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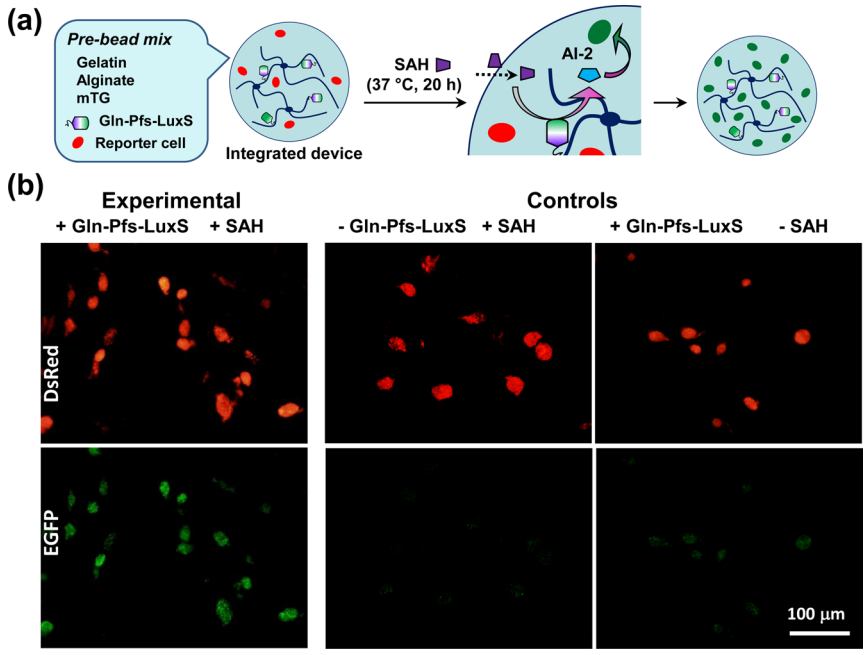

Figure 6. Device capable of internal molecular communication. (a) Schematic illustrates that protein-based component (Gln-Pfs-LuxS) can generate an AI-2 command that instructs the response of the cell-based component (reporter cells). (b) Fluorescence images of entrapped *E. coli* reporter cells show their presence (constitutive expression of red florescence; DsRed) and their response to an internally generated AI-2 command (conditional expression of green fluorescence; EGFP).

from the device and appeared in the surrounding medium. We should note that failure of the cell-based sender device could result from various causes (e.g., loss of viability or leakage of these cells from the beads) and such issues would need to be tailored to specific applications.

The right column of Figure 4c shows images of the surrounding medium for controls in which reporter cells were contacted with beads that had no AI-2 producing sender cells. No red florescence is observed for the reporter cells in this control. The results in Figure 4 demonstrate that gelatin-alginate bead with a cell-based sender component can generate the AI-2 signal that can communicate with bacteria in the surrounding medium.

Biofabricated Cell-Based Signal Receiver. Next, we biofabricated a cellular communication device capable of receiving QS molecular signals. For this experiment we used *E. coli* reporter cells CT104 (pCT6+ pETEGFP-T5DsRedExpress2) that constitutively express a red fluorescent protein (DsRed) but can be induced to express a green florescent protein (EGFP) in the presence of AI-2. This cell-based receiver device was generated from a prebead mixture containing: the *E. coli* reporter cells, gelatin (5%), alginate (1%), and mTG (1 U/mL). These receiver beads were transferred into a 50 mM tris buffer solution (with 10% v/v LB medium) containing Gln-Pfs-LuxS (4 μg/mL) plus precursor SAH (0.5 mM), and incubated at 37 °C for 20 h.

As illustrated in Figure 5a, the addition of AI-2 producing enzyme (Gln-Pfs-LuxS) and SAH to the surrounding medium allows the in situ generation of AI-2 signaling molecules that can be "received" by the entrapped reporter cells. The red fluorescence images in Figure 5b show that the entrapped reporter cells grew in colonies during the course of the experiment. The green fluorescence images at the bottom left in Figure 5b show very strong fluorescence, indicating that the entrapped reporter cells responded to the in situ generated AI-2. The control for this experiment is a cell-based receiver device incubated in a surrounding medium containing SAH but lacking the AI-2 producing enzymes. The images at the right in Figure 5b show red but not green fluorescence for this control, indicating that the reporter cells were present in the beads but not induced in the absence of the signal-generating Gln-Pfs-LuxS in the external medium. These results in Figure 5 demonstrate that a biofabricated gelatin-alginate bead with AI-2 reporter cells can act as a cell-based signal receiver.

Biofabricated Device for Internal Molecular Communication. In a final demonstration, we created a device that can engage in internal molecular communication such that one, protein-based, component transmits a molecular signal (i.e., a command) to a second, cell-based, component that receives and responds to this command. As illustrated in Figure 6a, this device was prepared from a prebead mixture containing: reporter cells CT104(pCT6+pETEGFP-T5DsRedExpress2), gelatin (5%), alginate (1%), Gln-Pfs-LuxS (50 μg/mL), and mTG (1 U/mL). The biofabricated beads were transferred to a 50 mM tris buffer solution (with 10% v/v LB medium) containing the precursor SAH (0.5 mM), and incubated at 37 °C for 20 h. Two controls were prepared by either deleting Gln-Pfs-LuxS from the prebead mixture used to prepare the beads, or by incubating Gln-Pfs-LuxS -containing beads in external solution lacking SAH.

The red fluorescence images in Figure 6b indicates that the entrapped reporter cells grew in colonies and constitutively expressed the DsRed protein in all the samples. The images at the bottom left in Figure 6b show considerable green fluorescence, indicating that the entrapped reporter cells received and responded to the AI-2 molecular signal. This result indicates that the covalently conjugated Gln-Pfs-LuxS can generate the internal AI-2 command necessary to induce expression of the green florescent protein by the reporter cells. For the controls, the images at the bottom right in Figure 6b

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show minimal green fluorescence, indicating that the entrapped reporter cells did not receive/respond to an AI-2 command. Results for these controls are consistent with the expectation that the internal AI-2 signal was not generated in the absence of the protein-based transmitter (Gln-Pfs-LuxS) or in the absence of the SAH substrate (i.e., the AI-2 command was never sent in these controls). Thus, the results in Figure 6 indicate that biofabrication can be enlisted to create molecular devices that are capable of generating and processing internal signals for molecular communication.

CONCLUSIONS

In this study, we demonstrate that materials and methods from biology can be used to biofabricate molecular communication devices. First, molecular and synthetic biology were used to generate the components: (i) a multidomain fusion protein capable of generating the quorum sensing molecular signal (AI-2); and (ii) reporter cells engineered to receive and respond to this AI-2 molecular signal. Second, stimuli-responsive biological polymers (alginate and gelatin) were used to create the structural matrix to contain these components. Third, an enzyme (microbial transglutaminase; mTG) was used to covalently assemble the protein-based nanocomponents to this matrix. Proof-of-concept demonstrations show sender and receiver devices could be biofabricated to communicate with the surrounding environment, while an integrated device used one component to transmit an internal molecular command to direct the response of a second component. Although these results demonstrate some possibilities for molecular communication devices, it is likely that real applications would require devices to be "tuned" to application-specific conditions (e.g., localized concentrations). Importantly, protein engineering and synthetic biology provide the means to create such tuned biological components. In addition to tailoring the individual biocomponents, we envision that improved capabilities for fabricating soft matter will enable their integration into hydrogel-based devices with increasingly complex structures and capable of performing increasingly complex functions. We anticipate that such complex functionality will require a detailed molecular level understanding of the various interactions (e.g., between signaling molecules and matrix) just as controlling electron flow is integral to the performance of electronic devices. In conclusion, advances in the biological science offer enabling materials science capabilities for the creation of devices that can communicate with our biosphere and such capabilities could have transformative impacts in numerous fields. Our sensors could become better "listeners" for threats to our health, environment, and security, whereas communication/biocomputing devices could make our foods, cosmetics, and medicines "wiser".

ASSOCIATED CONTENT

Supporting Information

The following file is available free of charge on the ACS Publications website at DOI: 10.1021/ab500160e.

Molecular biology details and Table S1 (PDF)

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Notes

The authors declare no competing financial interest.

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