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Two-Way Chemical Communication between Artificial and Natural Cells

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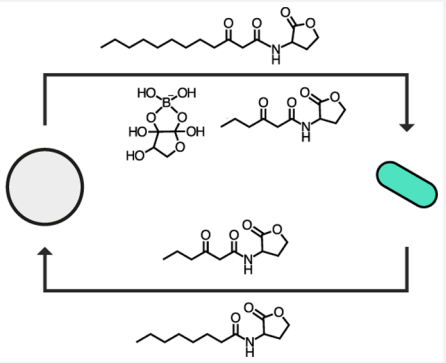
Supporting Information

ABSTRACT: Artificial cells capable of both sensing and sending chemical messages to bacteria have yet to be built. Here we show that artificial cells that are able to sense and synthesize quorum signaling molecules can chemically communicate with *V. fischeri*, *V. harveyi*, *E. coli*, and *P. aeruginosa*. Activity was assessed by fluorescence, luminescence, RT-qPCR, and RNA-seq. Two potential applications for this technology were demonstrated. First, the extent to which artificial cells could imitate natural cells was quantified by a type of cellular Turing test. Artificial cells capable of sensing and in response synthesizing and releasing *N*-3-(oxohexanoyl)homoserine lactone showed a high degree of likeness to natural *V. fischeri* under specific test conditions. Second, artificial cells that sensed *V. fischeri* and in response degraded a quorum signaling molecule of *P. aeruginosa* (*N*-(3-oxododecanoyl)homoserine lactone) were constructed, laying the foundation for future technologies that control complex networks of natural cells.



INTRODUCTION

Artificial cells are encapsulated chemical systems that mimic cellular life. Most attempts at making artificial cells have focused on building some type of self-replicating system.[1,2] Although self-replication is an important feature of life as we know it, self-replication alone is an insufficient criterion for assessing how lifelike a chemical system is.[3] For example, cross-catalytic ribozyme ligases are capable of self-replication[4] but do not alone constitute a living system. What is lacking is some sort of metric by which progress can be measured. One solution may be to describe chemical systems on a continuum where the typical binary categorization of alive and not alive is replaced by states that are increasingly lifelike. In this way, each iteration of constructing an artificial cell could be objectively and quantifiably evaluated in terms of likeness to a target natural cell. Such an approach is intuitive, because the emergence of life on Earth did not occur in a single event, but likely encompassed a series of steps, each bringing the chemical system closer to what is recognized as living today.[5,6]

It was previously suggested that a type of imitation game could be used to guide the construction of artificial cells in a way that bypasses the problems associated with a lack of a definition of life.[7] In the original imitation game (or Turing test), the ability of a machine to deceive a judge (or interrogator) through textual communication into believing that the machine is a person was used to circumvent the problem of defining intelligence.[8] In the cellular version, the ability of an artificial cell to deceive a natural cell is used to evaluate the artificial cell. Such a cellular Turing test is possible, because all cells communicate, from quorum sensing pathways in bacteria to pheromone responses in higher organisms.[9] Further, artificial cells containing DNA and/or transcription–translation machinery can express genes,[10,11] send chemical messages to bacteria,[12,13] and interact with each other.[14] Additionally, genetic constructs in water-in-oil emulsion droplets are able to either sense or send quorum molecules.[15] Therefore, it should be possible to build genetically encoded artificial cells that can chemically communicate with bacteria. Since chemical communication leads to measurable changes in gene expression, next generation sequencing technologies can be used to quantifiably evaluate the extent of mimicry in a manner that is neither subjective nor binary. In other words, the cellular Turing test allows for the quantification of how lifelike the artificial cells are in comparison to a target living cell in a stratified manner.

RESULTS AND DISCUSSION

Artificial Cells Can Sense Bacteria. To build artificial cells that mimic the ability of natural cells to chemically communicate, we attempted to reconstitute the well characterized quorum sensing pathways of *Vibrio fischeri*, *Pseudomonas aeruginosa*, and *Escherichia coli in vitro*. Genetic constructs were assembled with genes coding for the quorum responsive

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transcriptional activator or repressor plus additional accessory factors, as needed, and a transcriptional regulator binding site upstream of a gene encoding a fluorescent protein. In this way, the activity of each pathway could be assessed by the fluorescence arising from *in vitro* transcription−translation reactions. The *N*-3-(oxohexanoyl)homoserine lactone (3OC6 HSL) responsive system from *V. fischeri* was functional *in vitro* (Figure S1a,b). GFP expression in the presence of 10 μM 3OC6 HSL was 4-fold greater than in the absence of this quorum signal. Since the same transcriptional activator can sense another quorum molecule (*N*-octanoyl-L-homoserine lactone or C8 HSL) secreted from *V. fischeri*,[16] responsiveness to C8 HSL was assessed. Although the affinity of the transcriptional regulator LuxR for C8 HSL was low, a higher affinity mutant version of the protein (T33A S116A S135I LuxR or LuxR*)[17] activated cell-free expression 7-fold in the presence of C8 HSL and 6-fold in the presence of 3OC6 HSL (Figure S1a,b). The ability to sense 3OC6 HSL could be removed by introducing an additional M65R substitution, as previously reported.[17] Next, two *P. aeruginosa* quorum pathways were tested, including the *N*-(3-oxododecanoyl)-homoserine lactone (3OC12 HSL) responsive LuxR and the *N*-butanoylhomoserine lactone (C4 HSL) responsive RhlR pathways. As previously observed,[18] the genetic construct containing *lasR* was responsive to the quorum signal 3OC12 HSL *in vitro*, showing a 2-fold increase in protein expression (Figure S1c). However, the RhlR dependent system showed indistinguishable activity in the presence and absence of C4 HSL (Figure S1d). Finally, the autoinducer-2 (AI-2) system from *E. coli* was tested. While the expression of the transcriptional repressor LsrR fully inhibited protein expression, none of the tested constructs were derepressed by AI-2 (Figure S1e). The inclusion of the cAMP receptor protein (CRP) did not sufficiently improve derepression (Figure S1f). In summary, 3OC6 HSL, C8 HSL, and 3OC12 HSL were successfully detected by *in vitro* transcription−translation reactions.

Each functioning quorum sensing pathway was then encapsulated within cholesterol containing 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) phospholipid vesicles to determine whether quorum molecules could diffuse across the phospholipid membrane and activate gene expression of living cells. Here, activation resulted in the expression of firefly luciferase instead of GFP. Vesicles were incubated at 37 °C for 5 h in the presence and absence of the quorum molecule. The vesicles were then broken with Triton X-100 in the presence of luciferin and immediately measured for luminescence. Only in the presence of 3OC6 and C8 HSL was luminescence observed, indicating that the signaling molecules crossed the phospholipid membrane and activated gene expression (Figure S2a,b). 3OC6 HSL was previously shown to diffuse through the oil phase of water-in-oil emulsion droplets.[15] Together, these results suggested that artificial cells should be able to sense quorum molecules that are naturally secreted from bacteria. To demonstrate that the sensing mechanism of artificial cells was capable of responding to *V. fischeri*, the supernatant of a *V. fischeri* culture was added to the suspension of vesicles. After 4 h of incubation, 69-, 19-, and 8-fold more luminescence was observed for artificial cells expressing LuxR, LuxR*, and M65R LuxR*, respectively, in response to the supernatant of *V. fischeri* than in the absence of the supernatant (Figure 1). The use of the supernatant of a *V. fischeri* culture removed the confounding effects of the natural luminescent properties of the bacterium itself. The data

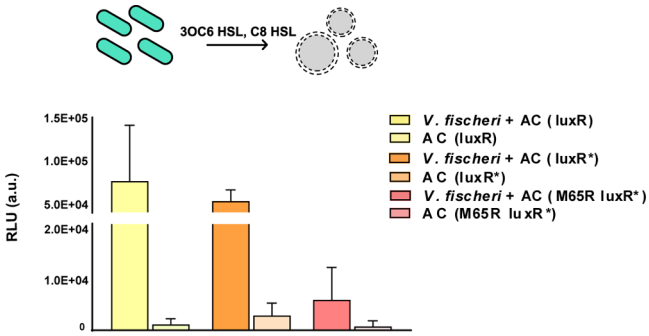

Figure 1. Artificial cells can sense quorum molecules released by natural cells. Artificial cells (AC) encoding either LuxR or LuxR* were able to sense the presence of *V. fischeri*. Negative control reactions were the artificial cells in the absence of the supernatant from *V. fischeri* (n = 3 biological replicates, mean ± SD). The schematic shows *V. fischeri* (teal, oblong) releasing quorum molecules that are sensed by artificial cells (gray, circle). RLU (relative luminescence units).

supported the ability of artificial cells made of phospholipid vesicles and transcription−translation machinery to sense molecules secreted from natural cells.

Artificial Cells Can Synthesize and Send Quorum Molecules to Natural Cells. Since communication requires the ability to both receive and send messages, we next probed whether it was possible to build artificial cells that could send chemical messages to bacteria in the form of quorum molecules. Genetic constructs encoding the synthesis machinery necessary to send chemical messages to *V. fischeri*, *P. aeruginosa*, and *E. coli* were assembled. *V. fischeri* synthesizes the *N*-acylhomoserine lactone 3OC6 HSL through the activity of LuxI, which uses *S*-adenosylmethionine and acyl chains donated from acyl carrier proteins as reactants.[19] Similarly, *P. aeruginosa* synthesizes 3OC12 HSL through the activity of the LuxI homologue LasI. Additionally, *P. aeruginosa* synthesizes C4 HSL through a similar pathway that uses RhlI in place of LasI.[20] The functionality of each genetic construct was assessed with reporter *E. coli* strains engineered to express GFP in response to a specific quorum molecule. After 6 h of transcription−translation at 37 °C of each genetic construct, an aliquot was added to the reporter strain and analyzed by flow cytometry. The 3OC6 HSL, 3OC12 HSL, and C4 HSL synthesis systems individually activated the expression of GFP of 90%, 50%, and 87% of the cells of the corresponding reporter bacterial strain, indicating that each genetically encoded quorum synthesis system was functional *in vitro* (Figure S3a). The AI-2 synthesis pathway used by *E. coli* is different and depends on the activity of three enzymes.[21] The SAM-dependent methyltransferase converts *S*-adenosylmethionine to *S*-adenosylhomocysteine, which is in turn converted to *S*-ribosylhomocysteine by the enzyme Pfs. Lastly, LuxS produces AI-2 and homocysteine in a 1:1 ratio from *S*-ribosylhomocysteine. Pfs and LuxS can be fused together to form a larger polypeptide that efficiently synthesizes AI-2 in the presence of *S*-ribosylhomocysteine.[22,23] We demonstrated that this fusion protein was active after *in vitro* transcription−translation by detecting synthesized AI-2 with the luminescent reporter *Vibrio harveyi* BB170 (Figure S3b).

To ensure that each synthesized quorum molecule could escape lipid vesicles, the transcription−translation reactions were placed inside of vesicles. The loaded vesicles were mixed with reporter bacterial strains at 37 °C and analyzed by flow

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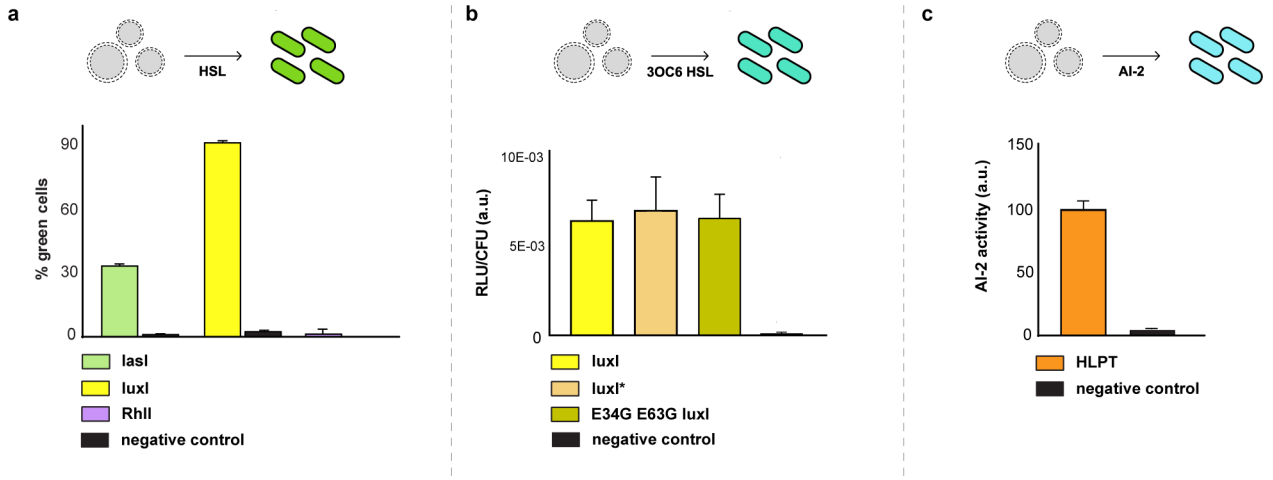

Figure 2. Artificial cells can synthesize and release quorum molecules to natural cells. (a) Artificial cells (AC) carrying genetic constructs for the synthesis of 3OC12 HSL, 3OC6 HSL, and C4 HSL were incubated with *E. coli* sensor strains and quantified by flow cytometry. (b) Artificial cells that expressed either LuxI, LuxI*, or E34G E63G LuxI for the synthesis of 3OC6 HSL successfully induced the production of luminescence in *V. fischeri*. (c) Artificial cells that expressed the AI-2 synthesizing fusion protein HLPT (His_6-LuxS-PfS-Tyr_5)[22] were incubated with *V. harveyi* and monitored by luminescence. For all the experiments, n = 3 biological replicates, mean ± SD. RLU/CFU (relative luminescence units per colony forming unit per milliliter).

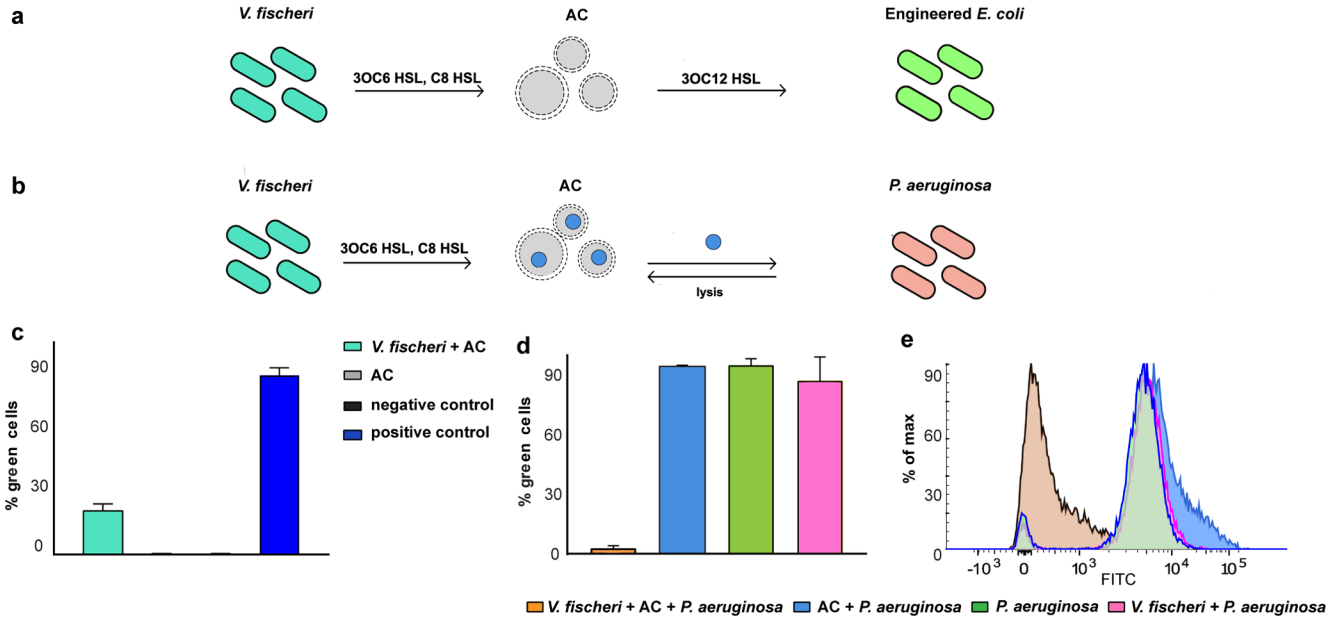

Figure 3. Artificial cells mediate communication between two different cell types. (a, b) A schematic of the experimental setup. (c) Communication between *V. fischeri* and engineered *E. coli* mediated by artificial cells was assessed by flow cytometry. (d, e) Artificial cells sense *V. fischeri* and in response degrade the 3OC12 HSL released by *P. aeruginosa*. Quantification was with an *E. coli* reporter strain by flow cytometry. For all the experiments, n = 3 biological replicates, mean ± SD. AC indicates artificial cells.

cytometry. Encapsulated genetic constructs for the synthesis of 3OC6 HSL and 3OC12 HSL resulted in approximately 90% and 35%, respectively, of fluorescent cells after 6 h of incubation, while the encapsulated C4 HSL synthesis system failed to induce detectable fluorescence of the reporter strain (Figure 2a). Two mutated versions of LuxI were also evaluated in an attempt to identify more active versions of this 3OC6 HSL synthesizing enzyme.[24] Vesicles containing DNA encoding wild type LuxI, E34G E63G LuxI, and E34G E40G E63G LuxI (hereafter referred to as LuxI*) were incubated with a dilute culture of *V. fischeri*, and the induced luminescence of *V. fischeri* was evaluated. All three of the tested versions of LuxI induced similar levels of luminescence from *V. fischeri* (Figure 2b). The encapsulation of the genetically encoded AI-2 synthesis system resulted in the induction of luminescence of the AI-2 reporter strain of *V. harveyi* (Figure 2c). Therefore, the data indicate that artificial cells can be built to synthesize and release 3OC6 HSL, 3OC12 HSL, and AI-2. To ensure that the vesicles used to build the artificial cells could withstand the presence of bacteria, the release of encapsulated fluorophore from vesicles incubated with different bacteria was monitored. *V. fischeri*, *V. harveyi*, and *E. coli* did not degrade the vesicles under the conditions used for chemical communication within 6 h, whereas the presence of the opportunistic pathogen *P. aeruginosa* resulted in the degradation of the vesicles (Figure S4a,b).

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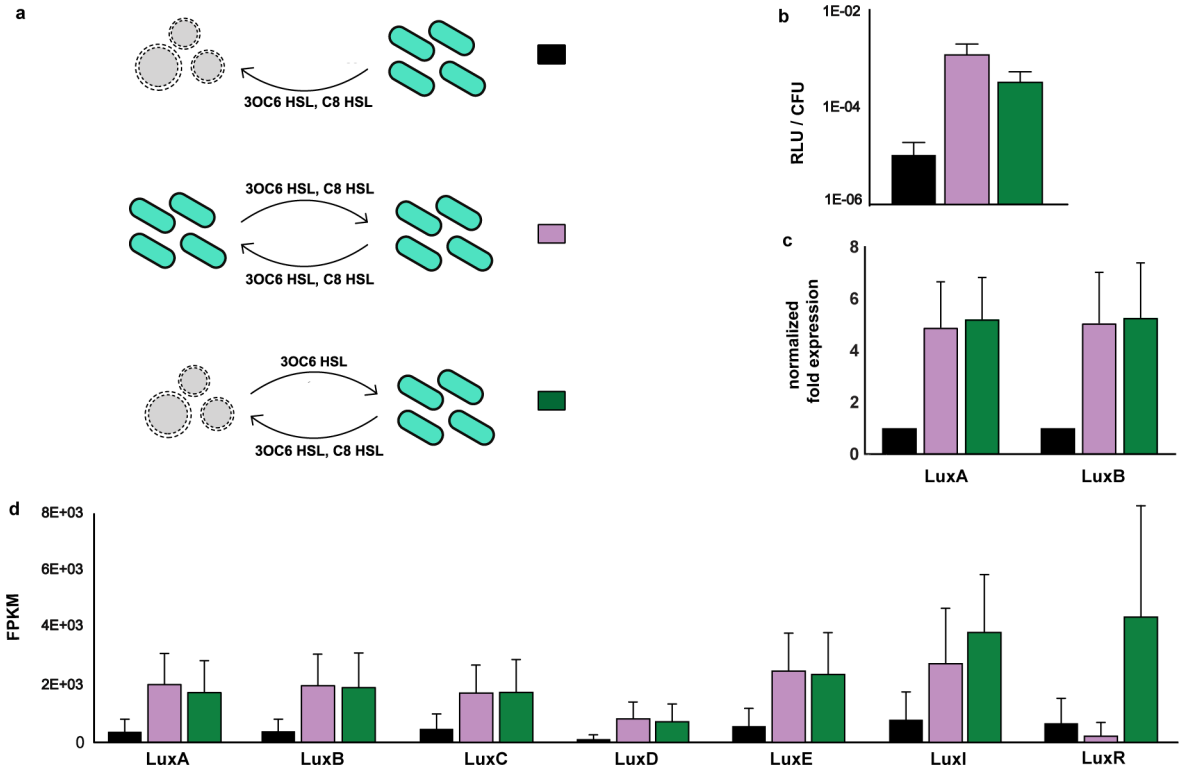

Figure 4. Two-way chemical communication for a cellular Turing test. (a) A schematic of the experimental setup showing chemical communication between *V. fischeri* and functional artificial cells (top, green), nonfunctional artificial cells (middle, black), and *V. fischeri* (bottom, magenta). Nonfunctional artificial cells could sense the presence of the quorum molecules released by *V. fischeri* and in response express T7 RNA polymerase, i.e., a response that had no bearing on *V. fischeri*. (b) Luminescence of *V. fischeri* in response to functional and nonfunctional artificial cells. (c) The activation of *luxAB* was assessed by RT-qPCR. Gene expression with respect to the negative control (*V. fischeri* in the presence of nonfunctional artificial cells) is shown. (d) RNA-seq analysis of the *lux* operon for communication between *V. fischeri* and nonfunctional artificial cells, *V. fischeri*, and functional artificial cells. For all the experiments, $n = 6$ biological replicates; mean ± SD. AC (artificial cells), FPKM (fragments per kilobase of transcript per million mapped reads), RLU/CFU (relative luminescence units per colony forming unit per milliliter).

Artificial Cells Can Establish New Communication Networks between Natural Cells. After demonstrating that the sensing and sending modules were functional inside of lipid vesicles, we next constructed artificial cells that were able to sense a quorum molecule and in response synthesize and release another quorum molecule. When properly engineered, such artificial cells would be able to mediate communication between two organisms that do not naturally communicate with each other. Further, the activity of the artificial cell would be easy to evaluate since the confounding influences of natural quorum pathways would be diminished. A genetic device that allowed for the synthesis of 3OC12 HSL in response to the presence of 3OC6 HSL was constructed. An engineered *E. coli* sensor strain for 3OC12 HSL was used as the receiver cell to avoid the cytotoxic effects of *P. aeruginosa*. The supernatant of *V. fischeri* was mixed with artificial cells and the *E. coli* reporter strain for 3OC12 HSL. 20% of the reporter strain expressed GFP, indicating that *E. coli* received a chemical message from the artificial cells in response to the 3OC6 HSL secreted by *V. fischeri* (Figure 3a,c). In the absence of the supernatant of *V. fischeri*, the artificial cells showed no activity. When the gene coding for the enzyme that synthesizes 3OC12 HSL was replaced by the fusion protein that produces AI-2, the resulting genetic circuit did not mediate communication with *V. harveyi* (Figure S5a,b).

Artificial cells can be designed to disrupt the natural quorum pathways of *P. aeruginosa*. Acylhomoserine lactones are degraded by the *Bacillus thuringiensis* enzyme AiiA.[25] After confirming that *in vitro* expressed AiiA was functional (Figure S6a), artificial cells were built to constitutively express AiiA so that the quorum molecules secreted by *P. aeruginosa* would be degraded. The LasR sensor for 3OC12 HSL was not encoded within the genetic content of the artificial cells since the membrane itself could serve as the sensor, that is, the membrane was disrupted by *P. aeruginosa*. When artificial cells expressing AiiA were incubated with *P. aeruginosa*, the extracellular levels of 3OC12 HSL were significantly reduced. In fact, in the absence of artificial cells, 90% of the *E. coli* reporter strain sensed 3OC12 HSL, whereas, in the presence of the artificial cells, only 18% of the reporter cells were activated (Figure S6b). Next, a 3OC6 HSL and C8 HSL responsive version of the artificial cells was prepared so that the signaling from one type of cell could result in the quenching of communication of another type of cell. A genetic construct expressing AiiA under the control of LuxR* allowed the artificial cells to decrease extracellular 3OC12 HSL by 95% in the presence of *V. fischeri* (Figure 3b,d,e). Although more work would be needed to convert such artificial cells into a useful technology, including the development of a membrane that can withstand *P. aeruginosa*, the data show that artificial cells could be built to interfere with biofilm formation in response to chemical signaling from another natural cell, since biofilm formation is strongly influenced by quorum signaling. However, more is possible. Engineered living cells have already been embedded in the gut microbiota[26] and developed to treat inflammatory bowel disease[27] and psoriasis,[28] and to suppress

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appetite.[29] Such technologies avoid flooding the organism with drug molecules, since therapeutic agents are only synthesized and released when and where needed. Artificial cells could do the same but within a more controllable chassis that does not replicate nor evolve.[13]

Artificial Cells Capable of Two-Way Communication Can Be Quantified by a Cellular Turing Test. Having established that artificial cells can sense quorum molecules that are naturally secreted from bacteria, send chemical messages to natural bacteria, and mediate communication between two different bacterial species, we next sought to evaluate how lifelike such artificial cells are through a cellular Turing test. Therefore, artificial cells were constructed that could chemically communicate in a manner similar to *V. fischeri*. Four different genetic constructs that included the wild type or mutant versions of the receptor LuxR and the synthase LuxI were tested (Figure S7a). Artificial cells were added to a low density culture of *V. fischeri* exhibiting low luminescence and incubated for 3 h at 30 °C. The artificial cells containing DNA encoding LuxR* and LuxI* induced the greatest luminescent response per colony forming unit (CFU) and thus were best able to chemically communicate with *V. fischeri* (Figure S7b,c). Since the artificial cells could not replicate, the CFU solely reflected the number of viable natural cells. The extent of communication was influenced by the lipid composition of the membrane of the artificial cells, consistent with the diffusion of molecules across intact membranes (Figure S8). Further, identical reactions that were not encapsulated in vesicles were not able to engage in chemical communication with *V. fischeri* under the experimental conditions employed (Figure S8). The experiment was then repeated with the optimized genetic sequence so that the same samples could be evaluated by luminescence, RT-qPCR, and RNA sequencing. The luminescence data (Figure 4b) was confirmed by RT-qPCR (Figure 4c), which showed that the expression of *luxA* and *luxB* was similarly upregulated 5-fold both for communication mediated by artificial cells and for natural *V. fischeri*-*V. fischeri* communication. *luxA* and *luxB* were previously shown to be upregulated by 3OC6 HSL.[30]

RNA-seq can be used to quantify the extent to which artificial cells mimic natural cells. Although the luminescence and RT-qPCR data demonstrated that the artificial cells behaved at some level as natural cells, such data were clearly not sufficient to determine if the artificial cells were alive or not. To more quantitatively assess the performance of the artificial cells, the gene expression profile of natural cells in response to the activity of artificial cells was evaluated. Six replicates of the cellular Turing test were subjected to RNA-seq analysis. Incubation of *V. fischeri* with nonfunctional artificial cells resulted in 175 differently expressed coding sequences with respect to the undiluted, *V. fischeri*−*V. fischeri* communicating sample (Tables S1 and S2). Nonfunctional artificial cells contained transcription−translation machinery plus DNA encoding LuxR and T7 RNA polymerase under the control of a LuxR-responsive promoter. That is, nonfunctional artificial cells could sense quorum molecules but could not respond by synthesizing quorum molecules. The same experiment in the presence of functional artificial cells containing DNA encoding LuxR* and LuxI* showed 107 differently expressed coding sequences (Tables S1 and S3), meaning that the functional artificial cells better mimicked the influence of natural *V. fischeri* on *V. fischeri* than nonfunctional artificial cells. Although the RNA sequencing analysis, after false discovery rate (FDR) p value adjustment, did not identify statistically significant differences in the expression of the *lux* operon in response to functional and nonfunctional artificial cells, the increase in the number of reads from the six RNA-seq samples (Figure 4d) was similar to the activation measured by RT-qPCR (Figure 4c). In other words, although all of the comparisons had a FDR adjusted p value >0.05, the data were consistent with RT-qPCR data with p values of 0.0001 and 0.0006 for *luxA* and *luxB*, respectively. Further, the expression over the entire *lux* operon, with the exception of *luxI* and *luxR*, was more similar between natural *V. fischeri* and functional artificial cells than with nonfunctional artificial cells (Figure 4d). The *luxI* and *luxR* data were more difficult to interpret since these two genes were present in both *V. fischeri* and the functional artificial cells. A correlation between the gene expression profile of *V. fischeri* in response to nonfunctional and functional artificial cells showed that six of the seven genes of the *lux* operon fell off the correlation trend (Figure S9a), suggesting that the critical difference between the two types of artificial cells was their effect on quorum signaling, as expected. Additionally, the difference in the number of reads between *V. fischeri*−*V. fischeri* compared with *V. fischeri*−nonfunctional artificial cell and *V. fischeri*−functional artificial cell samples showed that the functional artificial cells better mimicked the effect on gene expression across the entire genome than the nonfunctional artificial cells (Figure S9b).

It is possible to calculate how lifelike the artificial cells are from the RNA-seq data. The nonfunctional artificial cells changed the expression of 175 coding sequences differently than *V. fischeri*. An artificial cell that functioned identically to *V. fischeri* would have induced zero differences in gene expression. If we consider the nonfunctional artificial cells as having 0% likeness to *V. fischeri*, then any reduction in the number of differences in gene expression would increase the degree of likeness of the artificial cell to *V. fischeri*. Such a calculation would indicate that the artificial cells here were 39% lifelike or *V. fischeri*-like ($[(175 - 107)/175] \times 100$), but this value is clearly an overestimation because only two of the necessary components of the artificial cell were genetically encoded (LuxR* and LuxI*). The remaining components came from an extract of *E. coli* that was used to mediate transcription and translation. Engineered and naturally reduced bacterial genomes require over 100 genes to produce their transcription−translation machinery. In fact, the percentage of reduced genomes dedicated to gene expression is similar to the 39% lifelike value calculated here. For example, 41% of the synthetically produced, reduced *Mycoplasma mycoides* genome (i.e., JCVI-syn3.0) is necessary for gene expression.[31] Similarly, one-third of the naturally reduced genomes of parasitic microorganisms, including *Sulcia muelleri*, *Carsonella ruddii*, and *Buchnera aphidicola*, are retained for gene expression.[32−34] In other words, the data only make sense when put into the context of the entire genetic system required to support the synthesis of RNA, protein, and the products of protein enzymes, in this case quorum molecules. It thus follows that, even if it were possible to assemble an artificial cell containing a genome that can make its own transcription−translation machinery[35] plus additional genes for quorum signaling, this artificial cell would still not pass the 50% mark with respect to *V. fischeri*. That is, it is more accurate to say that if the artificial cells used here were completely genetically encoded, then these artificial cells would be 39% *V. fischeri*-like, according to the described cellular Turing test.

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As the complexity of artificial cells increases, more stringent versions of a cellular Turing test that better capture lifelike activity can be built. Here, the artificial cells were mixed with natural *V. fischeri* at an OD of 0.2−0.3 and incubated for 3 h at 30 °C before analysis. Under these conditions, replication was not required and the artificial cells did not need to survive for very long. A more stringent version of the cellular Turing would mix artificial cells with more dilute cultures of *V. fischeri*, or another target cell type, and would be assessed for activity after longer lengths of time. Such artificial cells would be capable of replication, which would also lead to daughter vesicles containing a greater fraction of machinery encoded within its own genome, as opposed to components purified from bacteria. It should be emphasized that such cellular Turing tests are not meant to function as a definition of life, but rather as a way to circumvent the problems associated with defining life. The choice of quorum signaling may appear arbitrary, particularly since not all organisms engage in quorum signaling, but all organisms do sense and respond to their chemical environment and interact with each other in some way that is processed on a chemical level. A version of the cellular Turing test described here may not be applicable to all organisms, but this test does provide an objective metric that does not emerge from qualitative lists of lifelike properties.

CONCLUSIONS

Our incomplete understanding of basic biochemical processes limits what can be built. Although we succeeded in assembling several different quorum pathways, the cycle of sensing and responding was only fully reconstituted for *V. fischeri*. One critical difficulty was the reconstitution of active sensing systems, even if the sensing mechanisms of the transcriptional activators and repressors were thought to be known.[9] Conversely, every cell-free, quorum molecule synthesis pathway tested was functional. Although *in vivo* experiments are indispensable to the study of biology, *in vivo* experiments alone are often not sufficient to identify all of the molecular components needed for activity. Only by reconstituting a fully functional system *in vitro* can we begin to understand how the pieces fit together.[36−39] Such an approach can extend beyond the characterization of individual biomolecules and pathways to our understanding of cellular life. In other words, we likely will not understand what is needed to make something alive until we can build a living cell from individual component parts. This requires an identification of the necessary genes and cytoplasmic components needed to synthesize a functioning cell from DNA.[40] Impressive progress has been made in synthetic genomics,[31,41] but the resulting living systems still depend on many genes with unknown function and many unidentified factors present in the living cell that receives the synthetic genome. The artificial cells described here suffer from similar complications; extract compositions are not fully known, and it is not currently possible to express *in vitro* functioning translation machinery.[35] Removing these unknowns is necessary to build artificial cells that more fully break from the concept of *vivum ex vivo*. Building a fully defined artificial cell from scratch would lead to a much deeper understanding of life. A cellular Turing test can help guide progress toward such a goal.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscentsci.6b00330.

Experimental details and supporting figures and tables (PDF)

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Notes

The authors declare no competing financial interest.

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