Influence of Silica Matrix Composition and Functional Component Additives on the Bioactivity and Viability of Encapsulated Living Cells

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Supporting Information

ABSTRACT: The remarkable impact encapsulation matrix chemistry can have on the bioactivity and viability of integrated living cells is reported. Two silica chemistries (aqueous silicate and alkoxysilane), and a functional component additive (glycerol), are employed to generate three distinct silica matrices. These matrices are used to encapsulate living E. coli cells engineered with a synthetic riboswitch for cell-based biosensing. Following encapsulation, membrane integrity, reproductive capability, and riboswitch-based protein expression levels and rates are measured over a 5 week period. Striking differences in E. coli bioactivity, viability, and biosensing performance are observed for cells encapsulated within the different matrices. E. coli cells encapsulated for 35 days in aqueous silicate-based (AqS) matrices showed relatively low membrane integrity, but high reproductive capability in comparison to cells encapsulated in glycerol containing sodium silicate-based (AqS + g) and alkoxysilane-based (PGS) gels. Further, cells in sodium silicate-based matrices showed increasing fluorescence output over time, resulting in a 1.8-fold higher fluorescence level, and a faster expression rate, over cells free in solution. This unusual and unique combination of biological properties demonstrates that careful design of the encapsulation matrix chemistry can improve functionality of the biocomposite material, and result in new and unexpected physiological states.

KEYWORDS: living hybrid biomaterials, cell encapsulation, glycerol modified silanes, bioactivity, cell viability, whole-cell-based biosensors

INTRODUCTION

Development of composite materials that incorporate biomolecular components and living cells within the material matrix continues to be an expanding and challenging field of research. This research is driven by the potential of imparting unique functionalities to materials that are intrinsic to biomolecules. These include highly selective catalysis by enzymes, exceptionally specific recognition and binding by antibodies, storage and replication of information by nucleic acids, high-yield production of rare and difficult to synthesize molecules by metabolic pathways, and amplification of subtle signals by many orders of magnitude by cell signaling cascades. Such biofunctional materials show great promise for use in biocatalysis, bioelectronics, controlled delivery of therapeutics, tissue engineering, medical diagnostics, advanced prosthetics, environmental and industrial process monitoring, early warning of warfare agents, and energy conversion.

Arguably, the greatest challenge in developing biocomposite materials is stabilizing the biomolecule or cell in the ex vivo environment while maintaining long-term biological function.

Silica materials derived from the sol–gel processes have been used to encapsulate and stabilize a wide range of biological materials and living cells. Advantages of this approach include synthesis at room temperature and physiological pH, ability to retain water with negligible swelling, simple incorporation of additives, ready diffusion of small molecules, mechanical stability, chemical and biological inertness, controlled porosity, resistance to microbial attack, and the ease with which sol–gel chemistry can be varied.

The efficacy of the encapsulation matrix in stabilizing biological function and long-term activity is strongly dependent on the chemical/physical properties at the interface between the encapsulation matrix and the integrated biological material or cell. Several studies have shown that addition of ameliorants (e.g., gelatin, glycerol, poly(vinyl alcohol), lipids) to the silica matrix can significantly improve the long-term viability of

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encapsulated cells.$^{28-30}$ These additives have been shown to serve as a barrier between the inorganic matrix and the cell surface, providing greater fluidity around integrated cells while isolating membrane lipids from polar silanol groups that may irreversibly oxidize and damage membranes, resulting in lysis.$^{31,32}$

As the surface properties of biological materials vary widely, forces and interactions between different biological species and a given encapsulation material must also vary, influencing bioactivity. Thus, an encapsulation matrix chemistry that effectively stabilizes one enzyme or cell line may be less effective at stabilizing another. This effect is especially important when producing multifunctional materials that incorporate multiple biomolecules (e.g., enzyme cascades) or multiple biological material classes/species (e.g., diverse cellular communities). We recently reported the first coentrainment of bacterial and eukaryotic cells at intimate proximities within a single silica matrix.$^{33}$ These disparate cells remained responsive to stimuli over several weeks. However, viability and bioactivity over time within the silica matrix, and the impact of alternative chemistries, were not explored.

Further, characterizing the physiological state of cells encapsulated within a given matrix can be challenging. It has become increasingly common for researchers to report viability of encapsulated cells based on vital dye staining and fluorescence microscopy.$^{34}$ This allows for direct quantification of results, while avoiding the typically arduous task of freeing the cells from the encapsulation matrix required for viability measurement using the “gold-standard” of reproductive capability. However, it has been shown that vital dye staining does not always correlate with reproductive capability. Davey and Hexley reported that S. cerevisiae cells exposed to physical and chemical stresses can be stained by propidium iodide (PI), which is emblematic of cell death; however, some PI stained cells were able to recover and reproduce.$^{35}$ Thus, for cells encapsulated within silica matrices, which may experience chemical and physical stresses, vital dye staining may not be a sufficient measurement for accurately quantifying cell viability.

Herein we report the influence of two distinct silica matrix chemistries (aqueous silicate-based and alkoxysilane-based) and a functional component additive (glycerol) on the biofunctionality of living E. coli cells engineered with a synthetic riboswitch for cell-based biodetection. We explore bioactivity and viability over several weeks of encapsulation using membrane integrity (vital dye) assay, reproductive capacity measurements, and riboswitch-activated reporter protein expression. Clear distinctions between silica encapsulation matrix chemistries and bioactivity and viability were observed. Interestingly, long-term membrane integrity, reproductive capacity and protein expression were not all correlated. Further, fluorescent protein expression from cells within an aqueous silicate-based (AgS) matrix was significantly enhanced (1.83× over E. coli cells free in culture solution) following 35 days of encapsulation. Finally, we report the influence of silica encapsulation matrix on the biosensing properties of the riboswitch E. coli-based biocomposite. These results demonstrate the profound impact the silica matrix encapsulation chemistry can have on the functionality of the biocomposite, which can induce new and unexpected biological behaviors.

## EXPERIMENTAL SECTION

### Materials

Aqueous solutions were prepared using deionized water (DI, nanopure, 18 MΩ). Sodium chloride, sodium hydroxide, Dulbecco’s phosphate buffered saline (DPBS, 1×), ampicillin-sodium salt, sodium-silicate solution, tetraethoxyorthosilicate (TEOS), anhydrous glycerol, titanium(iv) isoproxide (97%), and theophylline were purchased from Sigma-Aldrich (St. Louis, MO). Sodium phosphate (dibasic, heptahydrate) and sodium phosphate (monobasic, monohydrate) were obtained from EM Science (Darmstadt, Germany). Bacto Yeast Extract, Bacto tryptone and agar were from Becton Dickinson and Company (Sparks, MD). The LIVE/DEAD BacLight Bacterial Viability Assay (L7012) was from Invitrogen (Carlsbad, CA). All reagents were used as received.

### Synthesis of Poly Glycerated Silicate (PGS).

Poly glycerated silicate (PGS) was synthesized as described previously.$^{36}$ Briefly, a 100 mL round-bottom flask was equipped with a stir bar and nitrogen-purge, connected to a reflux condenser, and charged with 63.4 g of anhydrous glycerol (688 mmol). The glycerol was heated to 60 °C with stirring, at which point a mixture of 10.21 g TEOS (49.0 mmol) and 1.02 g titanium(iv) isoproxide (3.0 mmol) was added, dropwise, with stirring, over a period of 15 min. After addition of TEOS and catalyst, the mixture was refluxed at 130 °C for 3 h. Ethanol coproduct was removed using vacuum distillation at 130 °C and ~10 mTorr for 2–3 min. The product was a viscous fluid with an opalescent white appearance. Typical yields were 92–96% of theoretical, which corresponded with the theoretical formula Si-(C$_2$H$_4$O$_2$)$_{10}$, however, it is certain that inadvertent side products and isomers are present in the final product.

### Preparation of PGS Derived Sol–Gels.

Stationary phase cells from overnight culture were pelleted, washed 3×, and resuspended in 0.1 M sodium phosphate buffer (NaPB, without saline), pH 6.0. Equal volumes of PGS and cells in NaPB were mixed with a 500 μL end volume. Final cell density was 1 × 10$^9$ to 1 × 10$^7$ cells/mL. Gelation occurred within 60–90 min. Gelation and storage of gels occurred under ambient conditions (21 ± 2 °C).

### Synthesis of Silicate-Based Sol Solution.

Stock silicate sol solution was prepared as described previously,$^{20}$ with slight modification. To 3.4 mL of DI water was added 827 μL of sodium silicate solution (26.5% SiO$_2$; 10.6% Na$_2$O). To this mixture was added 1.54 g of highly acidic H$^+$ cation-exchange resin (Dowex 50WX8–100) with mixing, bringing the solution pH near 4. The resin was removed via vacuum filtration, or centrifugation to pellet the resin followed by collection of the supernatant. Hydrochloric acid (2.0 M) was added to the sol solution (~7 μL/mL) to bring the solution pH near 2.0. This solution can be stored at 4 °C for up to 48 h.

### Preparation of Aqueous Silicate-Based (AgS) and Aqueous Silicate-Based with Glycerol Ameliorant (AgS + g) Sol–Gels.

Aqueous silicate-based (AgS) sol–gels were prepared by adding silicate sol stock solution with cells in 1.0 M NaPB, pH 7.0, in a 5:1 (sol-buffer) ratio. Stationary phase cells from overnight culture were pelleted, washed 3×, and resuspended in 1.0 M NaPB, pH 7.0 prior to mixing with silicate sol stock solution. Final cell density was identical to that described above for PGS-derived gels. Aqueous silicate based gels with added glycerol (AgS + g) were prepared as described above for AgS sol–gels, but with first addition of glycerol (20% v/v) to the silicate stock solution. This sol stock solution was added to the buffered biological suspension (5:1 ratio) resulting in a final glycerol concentration of 17% (v/v). Gelation of AgS derived gels occurred in 10–15 s. Gelation of AgS + g derived gels occurred in 25–30 s. Both gels were formed and stored under ambient conditions (21 ± 2 °C).

### Silica Matrix Characterization.

Small-angle X-ray scattering (SAXS) was performed using a Bruker Nanostar system equipped with a microfocus Cu kα source, high resolution pinhole set, and 2D VANTEC 2000 area detector. Gels were cast into 2.0 mm glass capillaries (sealed with household epoxy) for analysis, with scattering from an empty capillary used as the background.

Syneresis was measured by preparing silica matrices (PBS, AgS, AgS + g) with known initial volumes (3 mL) within sealed polystyrene tubes. Matrix shrinkage from condensation reactions over time resulted in expulsion of pore fluid from the matrices that was retained in the sealed tubes. Following storage under ambient conditions (21 ± 2 °C) for 35 days, the tubes were unsealed and the volume of the...
expelled pore fluid was used to calculate the percent loss in silica matrix volume.

**Cell Lines and Propagation.** BL21 (pSAL-GFPa1His) constant GFP expression, and riboswitch activated GFP expression (pSAL-RS12.1GFPa1His) BL21-BS, E. coli cell lines were inoculated into 50 mL of LB broth (10 g of bacto-tryptone, 5 g of yeast extract, and 10 g of NaCl in 1L of DI water, pH adjusted to 7.5 with NaOH) and incubated overnight at 37 °C. Directly prior to inoculation, 1 mL of ampicillin stock (50 mg/mL) was added to each flask.

**Cell Membrane Integrity and Reproductive Capacity Assays.** Silica matrix integrated cells were assayed for membrane integrity using the BacLight Viability Assay, which uses SYTO-9, a membrane-permeable nucleic acid stain, and propidium iodide (PI), a membrane impermeable nucleic acid stain. The assay was performed using the manufacturer’s protocol with slight modifications: 2.5 μL of SYTO-9 solution (3.34 mM in DMSO), and 1.5 μL of PI solution (20 mM in DMSO), was added to 1496 μL of DPBS (0.1×, pH 6.9). From this solution, 500 μL was pipetted on top of a silica encapsulated E. coli cell monolith and incubated at 37 °C for 30–40 min. Following incubation a portion from the center of the monolith was removed (20–25 mg) and mounted between two glass coverslips. Cells were then imaged with an inverted fluorescent microscope. Cells with intact membranes fluoresced green; cells with damaged membranes fluoresced red.

The ability of cells to reproduce on solid medium plates was also measured. Each week following encapsulation, a 16.5 μL portion of the silica gel monolith was removed and resuspended in 0.1 M NaPB (pH 7.0) with vortexing (30–40 s). Mechanical (friction) forces exerted on the sample upon vortexing removed E. coli cells from the aqueous silica matrix. The solution containing freed cells and silica matrix debris was then streaked onto an LB + ampicillin plate and incubated at 37 °C for 24 h.

**Riboswitch Activated GFPa1 Expression Measurements.** A nutrient limited solution was used for riboswitch activation to limit the concentrating cell metabolic activity during induction. This solution contained the aptamer target, theophylline (2.0 mM), 45 μL of LB broth, 150 μL of DPBS (1×, pH 6.9) and 15 mL of ampicillin stock solution (50 mg/mL) in 1.290 mL of DI water (1.5 mL of final volume). Cells in solution were washed and resuspended in riboswitch activation solution. Cells in silica matrices had riboswitch activation solution (500 μL) pipetted on top of the monolith. Following addition of riboswitch activation solution samples were incubated at 37 °C for 6 h.

Following incubation, a portion from the center of the monolith was removed and mounted for imaging via fluorescence microscopy (as described previously). Identical microscope and camera settings were used for all imaging. As fluorescence intensity from fluorescent proteins is a function of protein concentration, monitoring intensity over time provides quantitative information regarding the rate of expression and the expression level of the fluorescent protein.

## RESULTS AND DISCUSSION

The influence of three silica encapsulation matrices on the biofunctionality of living cells was evaluated, as outlined in Figure 1. Two distinct chemistries, and a functional component additive, were used to generate the silica matrices: aqueous silicate-based gels (AqS); alkoxysilane-based gels derived from tetraethyl orthosilicate (TEOS) subjected to alcohol exchange with glycerol (poly(glycerol silicate), PGS); and aqueous silicate-based gels prepared with addition of the ameliorant, glycerol (17% v/v, AqS + g). Living E. coli cells engineered with a synthetic riboswitch for cell-based biodetection of theophylline were encapsulated within each matrix. Following encapsulation the living cell containing biocomposites were sealed and stored under ambient conditions (21 ± 2 °C) over a 5 week period. The biofunctionality and viability of integrated E. coli cells was measured over this time period by assaying cell membrane integrity, reproductive capability, and small molecule activated reporter protein expression.

**Silica Matrix Structure and Morphology.** Small-angle X-ray scattering (SAXS) was used to examine the nanostructure of AqS, AqS + g and PGS gels in the size range of ca. 2 to 120 nm, both with and without the presence of added E. coli. SAXS data for these gels is presented in Figure 2. Between q of 0 and 0.4, all curves exhibit features that are consistent with a primary silica particle size of ca. 5–6 nm and a narrow size dispersity. At lower q, in the Porod scattering regime, the slope of each curve yields the fractal dimension, d_P, of the gel structure formed by primary particle aggregation. A chart of d_P for each data set is given as an inset in Figure 2, including slopes obtained between q of ~0.8 and ~0.4.

For AqS, a d_P of 1.9 is diagnostic for a gelation mechanism dominated by a mass transfer limited reaction between individual clusters (here the preformed silica particles). However, addition of glycerol (AqS + g) shifts the fractal dimension to a value suggestive of reaction-limited aggregation (2.06). We posit that the presence of this additive blocks condensation between silanol groups of colliding primary silica particles, reducing the "stickiness" of these events and shifting the overall rate limiting step of network formation. Another
difference between the scattering curves for these two gels is the presence of a rollover point for AqS below $q$ of about $-0.9$ (ca. 50 nm) that is not present (or present at even lower $q$) in the data for AqS + g matrices. This may be related to the size of secondary aggregates in AqS gels being limited by rapid gelation, with reduced interparticle reactivity arising from the presence of glycerol allowing further expansion of the microstructure beyond this point.

For PGS, the fractal dimension (2.29) is closer to the regime expected for gelation via a monomer-cluster growth process ($d_f \geq 2.5$), where network formation is predominately by reaction of individual silicate monomers rather than condensation of preformed clusters. This may reflect a mixed mode of gelation involving both preformed particles and unreacted precursor.

Addition of E. coli has no effect on gel structure in the AqS and AqS + g systems, as evidenced identical SAXS scattering curves and similar fractal dimensions. However, $d_f$ for PGS gels is reduced somewhat (to 2.17 from 2.28); the presence of E. coli may impact the reactivity of small silicate precursors, altering the importance of monomer-cluster growth relative to cluster–cluster network formation.

**Membrane Integrity.** Membrane integrity was monitored via fluorescence microscopy using the membrane permeant nucleic acid stain, SYTO-9, which emits green fluorescence, and propidium iodide (PI), a red fluorescence emitting nucleic acid stain that is excluded from intact membranes. This pair of fluorescent dyes is routinely used to measure viability of bacterial cells; however, herein the results are referred to as membrane integrity, not viability. Representative fluorescent microscopy images of the SYTO-9/PI stained cells within each silica matrix over 35 days are shown in Figure S1.

Membrane integrity for cells encapsulated in all three matrices over 5 weeks are presented in Figure 3. Initially, cells in all three matrices exhibit similar membrane integrity that decreased rapidly from approximately 90% to 65% over the first week. Similar behavior has been observed for silica matrix integrated yeast cells, which was attributed to stresses exerted on the cells by ensuing condensation reactions and the compressive stresses associated with gelation. Ten days postencapsulation, the membrane integrity of cells integrated in AqS and AqS + g based matrices diverged from those of cells in PGS-derived gels. This trend continued through the remainder of the five week experiment with E. coli cells within PGS-based matrices showing the greatest retention in membrane integrity (50% after 5 weeks), followed by cells in AqS + g derived gels (34% with intact membranes), and cells in AqS-based gels (29% with intact membranes). Although cells within AqS + g gels showed greater retention in membrane integrity than cells within AqS matrices, addition of glycerol to the aqueous silicate-based gel only slightly increased the membrane integrity. These data indicate that the presence of glycerol alone is not likely the source of the significantly higher membrane integrity of cells within PGS-derived matrices.

Retention of membrane integrity, however, may be a strong function of silica matrix syneresis. As condensation reactions of the hydroxylated silica gel framework continue, matrix shrinkage and the resultant compressive stresses are exerted on encapsulated biological components. Syneresis measured from AqS, AqS + g, and PGS derived gels over the same 35 day period was 21.8, 20.3, and 0%, respectively. Although addition of glycerol to the aqueous silicate-based matrix chemistry did slightly reduce syneresis, expulsion of pore fluid equal to approximately 20% of the total monolith volume was observed from both matrices. The concomitant stresses and shrinkage of the silica matrix may slowly damage cells and further expose them to polar silanol groups, leading to damage at the cell/matrix interface, eventually resulting in cell lysis. In contrast, the alcohol exchanged alkoxide-based chemistry showed no measurable syneresis, presumably due to near complete nucleophilic substitution of reactive silanols with glycerols. Thus, the silica network formed from PGS is inherently more stable, significantly reducing stresses that may result in damaged cell membranes.

**Reproductive Capacity.** As viability is ultimately defined as the ability of cells to reproduce, the ability of cells encapsulated in the differing silica matrix chemistries to form colonies on solid medium was assessed. Following encapsulation and storage for a given time period, cells were extracted from the silica matrix by physically removing a small portion of the monolith, resuspended in buffer, and then streaked onto a solid growth medium. Reproductive capacity of these extracted cells is reported in Table 1. Within the first 3 days of encapsulation, cells extracted from all matrices were capable of growth on solid medium. Reproductive capacity of these extracted cells is reported in Table 1. Within the first 3 days of encapsulation, cells extracted from all matrices were capable of

$^{*}$Reproductive capability of encapsulated BL21-RS cells determined by extracting 15–17 mg of silica matrix from a monolith sample followed by resuspension in buffer and plating on LB-agar (24 h incubation, 37 °C). $^{*}$Silica matrix encapsulated BL21-RS cells stored under ambient conditions (21 ± 2 °C).

![Figure 3. Membrane integrity of BL21-RS E. coli cells integrated within silica matrices derived from aqueous silicate sol (AqS, blue), aqueous silicate sol +17 vol % glycerol (AqS + g, red), or poly(glycerol) silicate (PGS, green). Encapsulated cells were stored under ambient conditions (21 ± 2 °C). Viability determined via SYTO-9/PI fluorescent dye assay. Error bars are the standard deviation of measurements from 10 regions of a given sample.](image-url)
culture on LB-agar medium. Significantly higher numbers of colonies were observed from cells integrated within AqS and AqS + g gels than from PGS gels. However, after the first week of encapsulation, few to no colonies were observed from cells encapsulated within AqS + g and PGS matrices, whereas the number of colonies counted from cells in AqS gels remained nearly constant over the 35 day experiment.

Interestingly, these results do not correlate well with the membrane integrity data which showed significantly greater retained membrane integrity for cells encapsulated in PGS matrices versus aqueous silicate-based matrices, and similar behavior between AqS and AqS + g gels. Discrepancy between cell growth assay and viability dye assay, which typically measures membrane integrity and/or metabolic activity, is a common and challenging issue for systems in which living cells are physically confined within the matrix, restricting growth and division. This discrepancy was recently attributed to silica matrix encapsulated cells entering a viable but nonculturable (VBNC) state. In this state the cells are viable, but are under conditions of extremely low metabolic activity, or have entered a nonreversible metabolic state that does not permit growth under standard culture conditions.48,39 Bacterial cells usually enter the VBNC state in response to stresses exerted on the cells.40 Cells encapsulated within these silica gels are subjected to osmotic, compressive and nutrient stresses, and are ultimately unable to divide. Thus, the low correlation between cell growth and viability dye assays may be due to a population of encapsulated cells entering a VBNC-like state. In this case, PGS and AqS + g matrices may have induced a greater population of E. coli cells to enter a VBNC-like state than the AqS-derived gels.

However, based on the reproductive capacity data presented in Table 1, it is also possible that only cells within AqS gels remain viable after 2 weeks of encapsulation. Cells integrated in PGS and AqS + g matrices, which do not replicate, have a greater population of cells with intact membranes (Figure 3). This may be due to a glycerol interface forming between the silica matrix and cells surface, preserving the membrane structure such that membrane impermeant dyes remain excluded from the nonviable cells.

**Metabolic Activity (Riboswitch Activated Reporter Protein Expression).** To further elucidate the induced physiological state of E. coli cells incorporated within each of the silica matrices, cells engineered with a synthetic riboswitch were encapsulated to assay metabolic activity through reporter protein expression. As shown in Figure 4, the riboswitch consists of an mRNA strand that is a fusion of an aptamer sequence against the respiratory drug, theophylline, and the sequence for a high quantum yield green fluorescent protein from Branchiostoma floridae (GFPa1).41,42 In the absence of theophylline, the mRNA forms a hairpin loop, preventing translation of the GFPa1 sequence. In the presence of theophylline, the aptamer sequence binds the drug, exposing the GFPa1 sequence for translation. (A, B) Fluorescent microscopy images of riboswitch E. coli cells (liquid culture) in the (A) absence or (B) presence of 3.5 mM theophylline.

Fluorescent microscopy images of riboswitch engineered E. coli cells (BL21-RS) exposed to theophylline solution (6 h) following encapsulation within AqS, AqS + g, and PGS matrices for 1, 14, or 35 days, are shown in Figure 5. Following initial encapsulation, cells entrapped in each of the silica matrices for 1 day are able to recognize theophylline, and express GFPa1 at similar levels. After 15 days of encapsulation, fewer cells
entraped within AqS + g and PGS matrices are observed to fluoresce and fluorescent intensities are roughly comparable, indicating similar gene expression levels and metabolic activity. Following 35 days of encapsulation, cells in AqS + g and PGS matrices continue to express GFPα1 in response to theophylline, but expression levels reduced further to only 68% (AqS + g), or 55% (PGS), of that observed on day 1.

The data for AqS + g and PGS matrices are consistent with the membrane integrity data (Figure 3), which showed gradual reduction over time. And, as RNA translation mechanisms require maintenance of several biochemical pathways, encapsulated cells that recognize and respond to the presence of theophylline by expressing GFPα1 are clearly metabolically active. Thus, the lack of growth from cells removed from AqS + g and PGS gels (Table 1) may be more appropriately attributed to cells entering a VBNC-like state, than to the cells being nonviable. However, these results still do not directly correspond to bacteria viability. The encapsulated cells could be nonviable, but with all cellular components required for protein expression functional and intact, much like cell-free protein expression systems.

In contrast, and interestingly, cells integrated within AqS matrices showed a 1.3-fold increase in fluorescence at 14 days post encapsulation, and a 2.8-fold increase in fluorescence at 35 days post encapsulation, over that observed on day 1. This also represents an increase of 1.83× over free E. coli cells in culture solution. Similar phenomena have been recently reported for silica-gel immobilized E. coli in which ATP production and GFP expression was nearly 2-fold greater than that of E. coli in solution. In these works, the authors did not provide a hypothesis for this observation. This significant enhancement in fluorescent signal from E. coli cells entrapped in AgS matrices, even over cells free in solution, may be unexpected based on the relatively poor performance of these cells in the membrane integrity assay (Figure 3). However, cellular contents and general 3D morphology may be held intact by the surrounding silica cage, keeping necessary cytoplasmic components preserved, localized, and accessible, perhaps resulting in improved protein expression rates.

**Rate of Small Molecule Induced Reporter Protein Expression.** To further explore this hypothesis, we measured the response time for cells within each of the three silica matrices (2–3 days post encapsulation) against the response of free cells in solution. As shown in Figure 6, cells within PGS matrices (solid triangles, green), which reported the greatest retention in membrane integrity, had a similar GFPα1 expression response profile to cells free in solution (open square, orange). However, cells within AgS (solid diamonds, blue) or AqS + g (solid square, red) gels showed substantially enhanced response rates compared to cells in PGS gels and cells free in solution. This observation supports the hypothesis that the lower membrane integrity of cells within these matrices may be correlated to the faster and higher yield protein expression. Additionally, encapsulated cells may sense and respond to the stresses of confinement, shifting metabolic load from growth and division to higher protein and metabolite synthesis. This behavior would be similar to that reported for plant cells encapsulated within an aqueous silica gel that showed a 10–100 fold increase in secondary metabolite production over cells in solution. Further, cells under conditions of stress and nutrient deprivation may also be “poised” to respond to the introduction of nutrients, reacting more quickly than cells in liquid culture under logarithmic growth.

For data presented in Figures 5 and 6, a nutrient restricted medium was used to introduce theophylline to the cells, reducing the impact of this possible effect. However, the “poised” response may still be a contributing factor in the higher expression rate and level for cells integrated within AqS matrices.

**Influence of Silica Encapsulation Matrix on Riboswitch E. coli Biosensing Properties.** In aggregate, these results hold significant implications for developing functional biocomposite materials and demonstrate that the silica matrix chemistry can improve upon the response time and signal level for encapsulated cell-based biosensors, even over that of cells free in solution. Previous work characterizing BL21-RS cells in liquid culture measured a theophylline detection limit of 10 μM. In this work, detection limits for cells encapsulated with each silica matrix were comparable to, or nearly an order of magnitude lower, than that reported for BL21-RS cells free in solution, as shown in Table 2. Also shown in Table 2 are the sensitivity and dynamic range of theophylline from cells within

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<tr>
<th>Table 2. Effect of Silica Matrix Composition and Functional Component Additives on Integrated BL21-RS Cells Response to Theophylline</th>
<th>detection limit (μM)</th>
<th>sensitivity (Fluor. (a.u.) · μM−1)</th>
<th>dynamic range (mM)</th>
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<td>AqS + g</td>
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<td>PGS</td>
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*aCalculated from a logarithmic fit of measurements within the dynamic range.*

![Figure 6. Response of BL21-RS E. coli cells in solution (open symbols), or encapsulated in silica matrices (closed symbols), to theophylline (2 mM), measured by GFPα1 fluorescence intensity over time. Solution samples: positive control BL21 cells (engineered constant GFPα1 expression, open circles, purple); negative control BL21-RS cells (not exposed to theophylline, open diamonds, black); BL21-RS cells free in solution exposed to 2 mM theophylline (open squares, orange). Silica matrix integrated BL21-RS cells: aqueous silicate sol-based (AqS, solid diamonds, blue); aqueous silicate sol +17% (vol) glycerol-based (AqS + g, solid squares, red); poly-(glycerol) silicate-based (PGS, solid triangles, green). Encapsulated cells were stored under ambient conditions (21 ± 2 °C) for 2–3 days prior to exposure to theophylline. Error bars are the standard deviation of measurements from 10 regions of a given sample.*
each matrix. These differences in response are attributed to matrix/thiophylline interactions (i.e., electrostatic, hydrophobic/hydrophilic) and the physiological state of the integrated cells induced by the specific matrix chemistry. These data show that careful design of the encapsulation matrix chemistry can improve the resultant cell-based biosensor’s detection characteristics by inducing a more desirable physiological state and improve analyte transport through the encapsulation matrix.

**CONCLUSIONS**

In summary, we have described the substantial impact silica matrix chemistry can have on the functionality of multiple types and classes of biologicals within the biocomposite material. We show the striking differences each encapsulation matrix had on long-term membrane integrity, reproductive capacity, and riboswitch-based protein expression rates and levels of encapsulated *E. coli* cells. These data are not all correlated, highlighting the complex relationship between the encapsulated biological and the inorganic matrix. *E. coli* cells encapsulated for 35 days in AqS-based silica matrices have a relatively greater population of cells with compromised membranes, but show greater reproductive capability than cells in AqS + g and PGS matrices. This provides a caution against relying solely on viability dye assays to quantify viability. Further, cells integrated within AqS matrices showed a 1.8-fold increase in fluorescence output, and a faster expression rate, over cells free in solution. We are unaware of other studies demonstrating this unusual and unique combination of properties for encapsulated living cells. This clearly demonstrates that design of the encapsulation matrix chemistry can substantially improve the functionality of the biocomposite, inducing more desirable, and even new or unexpected physiological states.

**ASSOCIATED CONTENT**

 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomater.5b00261.

Figure S1, representative fluorescent microscopy images of the vital dye stained cells within each silica matrix over time, as described in the text (PDF)

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Notes

The authors declare no competing financial interest.

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