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Development and Characterization of 3D, Nano-Confined Multicellular Constructs for Advanced Biohybrid Devices

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Abstract

This is the final report for the President Harry S. Truman Fellowship in National Security Science and Engineering (LDRD project 130813) awarded to Dr. Bryan Kaehr from 2008-2011.

Biological chemistries, cells, and integrated systems (e.g., organisms, ecologies, etc.) offer important lessons for the design of synthetic strategies and materials. The desire to both understand and ultimately improve upon biological processes has been a driving force for considerable scientific efforts worldwide. However, to impart the useful properties of biological systems into modern devices and materials requires new ideas and technologies. The research herein addresses aspects of these issues through the development of 1) a rapid-prototyping methodology to build 3D bio-interfaces and catalytic architectures, 2) a quantitative method to measure cell/material mechanical interactions *in situ* and at the microscale, and 3) a breakthrough approach to generate functional biocomposites from bacteria and cultured cells.

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1. Introduction

Biological systems and structures are prioritized on the molecular, supramolecular, and macroscopic scales to achieve superior specificity and functionality, optimized over the course of evolution. The development of artificial materials and synthetic strategies that bridge to, or otherwise incorporate biological systems and living cells provides great opportunities to advance materials science and technology and synergistically couple multiple scientific disciplines.

As an example, the integration of biological components, such as live cells, into devices is an increasingly attractive route to address the engineering shortcomings of materials and fabrication methods currently employed in the production of MEMs (microelectromechanical) devices and nanotechnologies. Cells detect and report analytes in “noisy” environments such as soil and wastewater, power molecular and nanoscale motors using highly efficient energy conversion and can synthesize inorganic materials under mild conditions. However, implementation of biological strategies in the design of new materials and/or integration of cells into devices requires new ideas and the development of new technologies.

To these ends, we describe work that addresses fundamental issues of the cell/material interface—with the ultimate goal of exploiting living systems in the construction of materials and devices. We developed a system by which to microfabricate complex, 3D structures in the presence of living cells, while subsequently maintaining cell viability and allowing propagation of populations derived from single cells. Here we employed the technique of multiphoton lithography (MPL), a method by which to direct-write micro- and nanostructures with precise 3D architectures. This new technology provides great opportunities in the study of, for instance, cell heterogeneity and cellular communication, and offers a means to bridge cells to device platforms with high resolution and using biocompatible chemistries.

A key aspect of our strategy is the use of biomolecules such as proteins to build (artificial) microstructures using MPL. Proteins are the archetypal building blocks for biological systems, programmed for chemical functionality and dynamic molecular interactions to achieve functional, hierarchical architectures. MPL-biomaterials are chemically accessible hydrogels while providing physical barriers between neighboring cells (or other microscale objects) and therefore bare resemblance to natural structures such as extracellular matrix. We wished to understand the mechanical properties of MPL-biomaterials and particularly their interaction with developing cells. Thus we developed a method to measure the modulus of these materials *in situ*, and found them to be highly tunable based on fabrication parameters and dynamic—determined by the chemical (e.g. pH) and physical (e.g. temperature) environment. Further, we demonstrated this technique by directly measuring the pressure exerted by growing bacterial colony confined in a microfabricated chamber. This work demonstrates an ability to quantify mechanical properties under both chemically and biologically dynamic microenvironments and will enable the development of a robust platform to

investigate cell/microenvironmental interactions with high spatial resolution, in three dimensions, using mechanically tunable biological materials.

These microfabricated protein structures are, to some extent, analogous to the soft scaffolds built by cells and microbes—including silica condensing microorganisms (e.g., diatoms). Given that diatoms are able to create intricate mineral structures using protein scaffolds, we hypothesized that MPL-protein structures may have similar capabilities. Indeed, these crosslinked protein hydrogels were able to controllably direct silica condensation resulting in a structurally sound, nanoporous material with user defined features. These protein hydrogels are highly concentrated, producing a locally crowded molecular environment which acts to capture and concentrate silica precursors via hydrogen bonding and other non-covalent interactions. Therefore, we wondered if naturally crowded molecular environments, such as cells, could direct silica condensation under similar conditions. This question led to the development of a fundamentally new biohybrid material: the cell silica composite. This discovery has the potential to impact a broad range of areas spanning biocatalysis, bio-preservation, and bioinorganic chemistry and provides a fundamentally new pathway for the design and synthesis of hierarchical materials with chemically and genetically engineered functions.

2. Experimental and Results

2.1 Biocompatible microfabrication for targeted confinement of single cells and their progeny

To advance both our understanding of cell behavior, as well as exploit these cellular processes for engineering purposes requires strict control over the cellular microenvironment. Cells naturally exist in complex 3D environments. Therefore, presentation of cues from mechanical and chemical gradients in 3D is essential for optimal function of artificial cell culture systems and biohybrid materials. The development of approaches to pattern cells and biological components (e.g., proteins) has been enabled by advances in microtechnologies using, for instance, hard and soft lithography and ink-jet cell printing. Currently, these methods provide only rudimentary control over the 3D morphology of cellular assemblies. Additionally, methods to target distinct cell phenotypes and contain their progeny would provide great opportunities for both single cell analysis, and exploitation of rare phenotypes in sensors and biochemical synthesis (e.g., antibiotics).

To these ends, we developed a means for the isolation of single and small populations of cells using biocompatible 3D microfabrication. In this approach, cells in a solution containing a high concentration of a protein (e.g., serum albumin) and bio-friendly sensitizer (e.g., methylene blue) are contained in a 3D microchamber using multiphoton excitation driven process induced photocrosslinking of proteins at site specific locations. This results in a solid, hydrated matrix (hydrogel) physically sequestering the contained cell(s) from the surrounding population while preserving access to the fluidic environment. This process permits targeting of both pro- and eukaryotic cells, allowing subsequent monitoring of their behavior, as well as their progeny (Figure 1). As an example, aspects of cell to cell communication can be interrogated under ‘real world’ conditions by engineering the diffusivity of hydrogel microchambers (Figure 2). The ability to direct write biocompatible microenvironments with high resolution enables single and multicellular communities to be interrogated with high precision—facilitating optical, electrical, and electrochemical interfaces to viable and engineered cell populations.

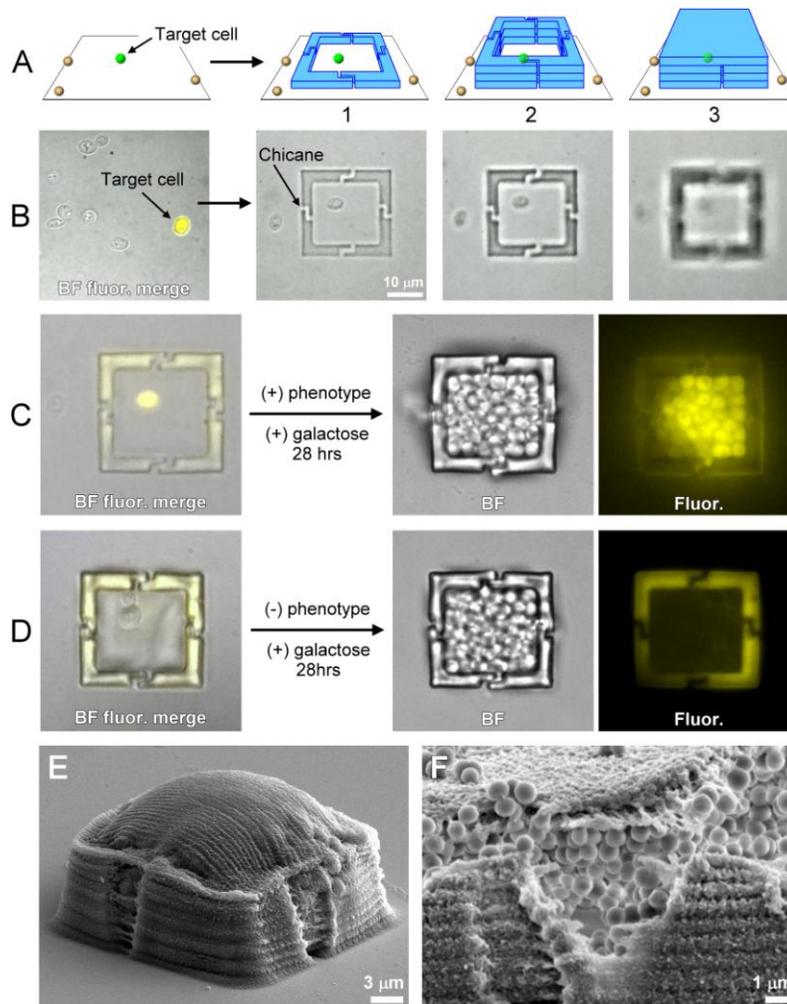


Figure 1. Biocompatible fabrication of 3D protein microstructures allows physical isolation and confinement of a target cell within a mixed population. (A) Schematic of hydrogel fabrication to isolate a target cell. (B) Isolation of a *S. cerevisiae* cell expressing YFP from a population of non-expressing cells. (C, D) Isolation of targeted phenotypes and confinement of their progeny (E) SEM image of a protein structure containing *S. cerevisiae* cells. (F) SEM image of a BSA protein structure with roof retracted revealing confined *S. aureus* cells.

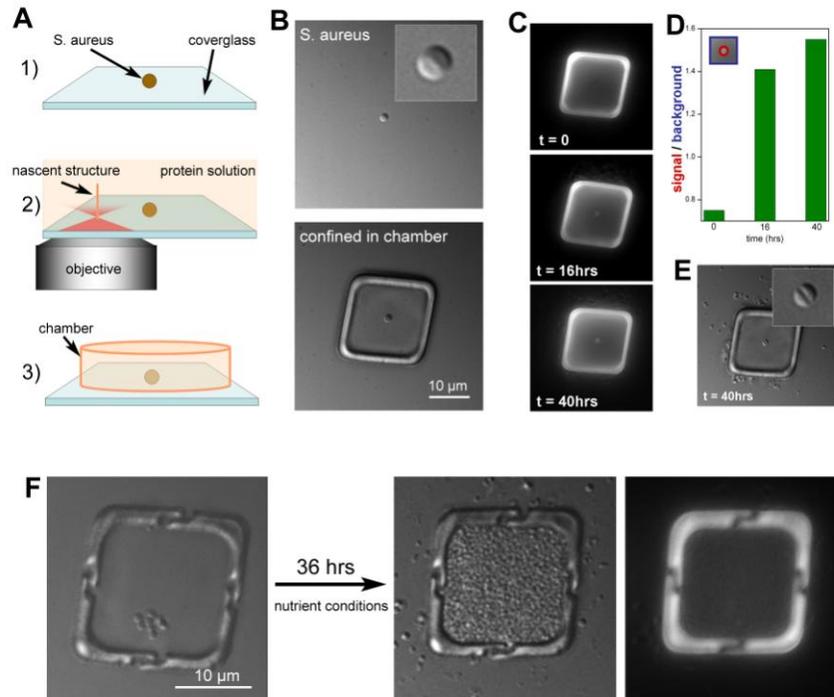


Figure 2. *In situ* confinement of single cells and groups leads to outcomes dependent on the diffusivity of the microchamber. (A) Schematic of entrapment. *S. aureus* carrying the QS reporter agr: P3-gfp (1) are confined in a protein microchamber fabricated from a biocompatible protein solution (2, 3). (B) Optical microscopy images of a single cell confined in a low diffusivity chamber. (C, D) At 16 hrs and 40 hrs the cell reports QS via fluorescence and forms a cell-division septum (E). (F) A group of ~7 reporter *S. aureus* cells entrapped in a high diffusivity chamber (left panel, achieved via chicane ports on the sidewalls), replicates to fill the chamber over time (center panel) but does not report QS (right panel).

Reference: Harper J, Brozik S, Brinker CJ, Kaehr B, 2011. Biocompatible microfabrication for targeted confinement of single cells and their progeny. *Submitted*.

2.2 A strategy to measure the mechanical dynamics of cell/material interactions.

The chemical and physical interactions with the surrounding microenvironment encountered by developing cells play a crucial role in determining cell fate. For instance, it is increasingly evident under *in vitro* cell culture conditions that the elasticity of the substrate can alter the outcome of cell development for a wide range of cell types. Although the bulk mechanical properties of native biological tissues such as cartilage have been well studied, there is a strong interest in determining microscale properties of biological tissues with the potential to model these properties *in vitro*. Thus, in addition to biocompatibility and chemical functionality, *in vitro* cell culture substrates should be capable of mirroring the mechanical properties of the biogenic tissue microenvironments

they are intended to represent—potentially in three dimensions—in order to successfully replicate *in vivo* development.

In this work, we demonstrated multiphoton lithography (MPL) of protein microcantilevers whose mechanical properties were determined using a cantilever beam model (Figure 3). We showed that, depending on laser dwell time which governs the extent of protein crosslinking, the modulus of these cantilevers can be varied over several orders of magnitude, across a range relevant toward mirroring many types of biological tissues. The ability to mimic the modulus of biological microenvironments is crucial towards understanding, and ultimately directing cell fate in artificial (i.e., *in vitro*) settings. Thus, MPL protein microstructures show great potential to serve as a highly tunable platform for studying the interaction of cells with tissue microenvironments.

An interesting question raised in this study is how the modulus of protein hydrogels is related to the modulus of constituent proteins. Our observations of protein hydrogel modulus as a function of pH, temperature, and ligand binding correlate well with known structural transitions of the protein molecules. Transitions such as the N-E and N-B transitions of albumin, as well as the compaction avidin undergoes after binding biotin, strongly influence the elastic modulus of the microcantilevers derived from these constituent proteins. Thus, it should be feasible to investigate structural transitions of protein molecules using the system described here—provided the establishment of an appropriate theoretical framework.

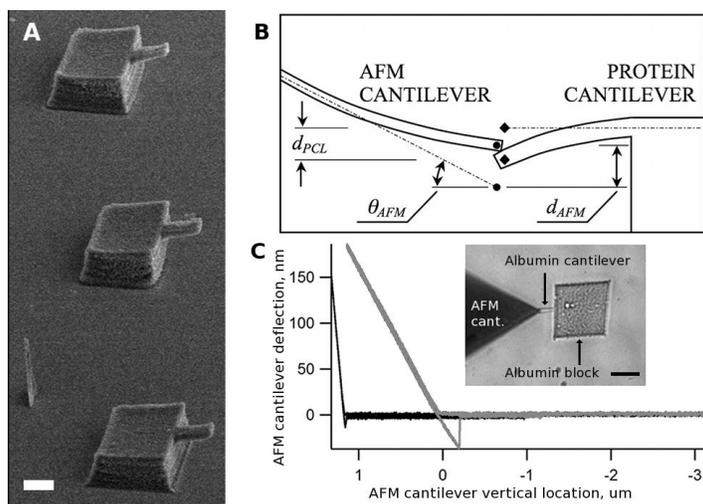


Figure 3. Multiphoton fabricated protein microcantilevers investigated using AFM. Protein microcantilevers (A; SEM image, scale bar, 5 μm) are tested in aqueous media by pressing with an AFM cantilever (B). An AFM force curve with the recorded cantilever deflection on the ordinate and the AFM z position on the abscissa is shown in C. The black force curve was collected by pressing the AFM tip on the glass substrate, and consequently the slope = 1. The grey curve was collected by pressing the AFM tip upon the tip of the protein microcantilever (insert) and the slope < 1 due to the additional compliance of the protein microcantilever. The insert shows an optical microscope image of the AFM cantilever (dark triangle) positioned at the tip of a protein cantilever (scale bar, 10 μm).

Finally, we measured the pressure exerted on a hydrogel membrane of known modulus by a colony of *E. coli* confined in a microchamber (Figure 4). The accuracy of this technique relied on an *in situ* measurement of the modulus of the microchamber roof using cantilevers built into the structure. This demonstrates the possibility of using 3D protein hydrogels to create cell/material interfaces with precise mechanical properties and should facilitate development of *in vitro* cell models aimed at understanding cell confinement-effects found, for instance, during stages of infection and tumor development.

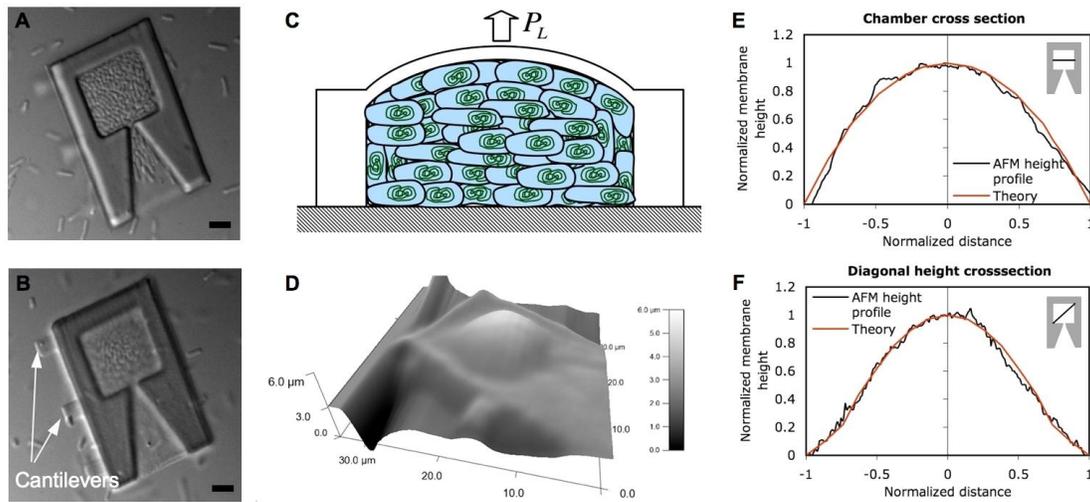


Figure 4. Motile bacteria are captured from the surrounding environment and confined in a protein microchamber (A, B, images show the glass substrate/chamber interface and the chamber ceiling respectively; scale bars, 10 μm); bacterial cells reproduce, filling the chamber and deforming the chamber ceiling (B, C, D). The deflection of the membrane, measured by AFM, is compared to a theoretical model for the lateral (E) and diagonal (F) cross-sections. Cantilevers fabricated in conjunction with the ceiling layer are used to measure the modulus of the ceiling material, which in turn is used to calculate the pressure inside the chamber.

Reference: Khripin CY, Brinker CJ, Kaehr B. 2010. Mechanically tunable multiphoton fabricated protein hydrogels investigated using atomic force microscopy *Soft Matter*: 2842-8

2.3 Using Protein Architectures to Generate Nanoporous Composites

Natural bioinorganic composites as found in bone, shell, and diatoms have long been heralded as model functional materials that evolved over billions of years to optimize properties and property combinations. Often functionality derives from hierarchical architectures composed of hard and soft components organized according to multiple prioritized length scales. To date it has been difficult to mimic these multiscale designs in synthetic manmade materials. In this work, we explored synthetic strategies that mimic features of the natural silica depositing system of diatoms, single celled organisms that are known to construct exquisite and elaborate silica composite exoskeletons. Although there has been significant progress towards an understanding of the molecular components involved in biogenic silica formation, the whole picture remains vague, as evidenced by a current inability to reproduce diatom-like silica features *in vitro* using synthetic or native silica-associated biomolecules. Diatom silica biosynthesis is clearly a process by which the chemical microenvironment is tightly controlled through compartmentalization and transport. Taking lessons from nature concerning silica morphogenesis within the acidic diatom silica deposition vesicle (SDV), we wondered if a mildly acidic and highly crowded and confined macromolecular scaffold would prove sufficient for silica deposition.

To test this hypothesis, we used multiphoton lithography (MPL) to fabricate protein hydrogel scaffolds. As demonstrated in previous sections, this technique enables microstructures comprised of proteins of choice to be fabricated with arbitrary 3D geometries. We observed that under dilute acidic conditions these scaffolds direct their precise replication to form silica/protein biocomposites (Figure 5). Importantly, proteins of diverse properties (e.g., isoelectric point; pI) directed silica condensation under identical solution conditions, which is to some extent contrary to the existing paradigm that cationic species (e.g., proteins with pI > 7) are necessary for *in vitro* silica condensation/ deposition. These protein hydrogels are highly concentrated (> 40% protein by wt/vol) producing a locally crowded molecular environment, which acts to capture and concentrate silica precursors (mono-, oligo-silicic acid, and nanoparticles) via hydrogen bonding and other non-covalent interactions.

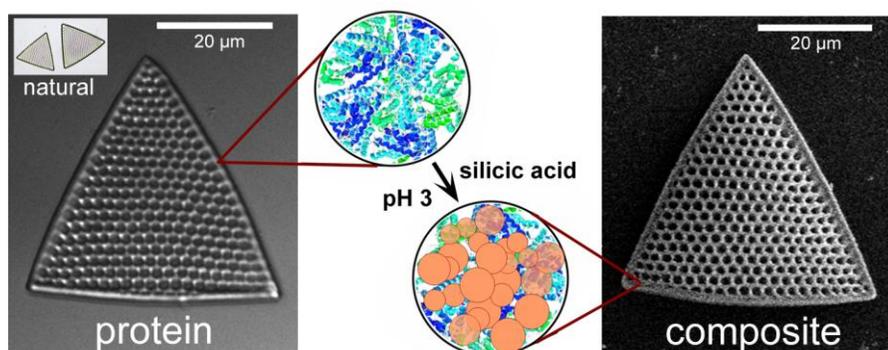


Figure 5. Protein hydrogels direct silica condensation. Protein hydrogels comprised of cross-linked proteins were microfabricated using MPL. Incubation in silicic acid resulted in a composite that retained template features following calcination.

Following silica deposition, the protein template can be removed using mild thermal processing yielding high surface area ($625 \text{ m}^2/\text{g}$) porous silica replicas that do not undergo significant volume change compared to the starting template. This approach demonstrates a simple route to create bio-inspired silica microstructures displaying hierarchical features over broad length scales. Further, infiltration/functionalization capabilities of the nanoporous silica matrix were demonstrated by laser printing a 3D gold image within a 3D silica matrix (Figure 6). This work is an important stepping stone toward understanding and mimicking biogenic silica condensation under the constraints of user-defined bio-templates, and further should enable a wide range of complex inorganic architectures to be explored using silica transformational chemistries.

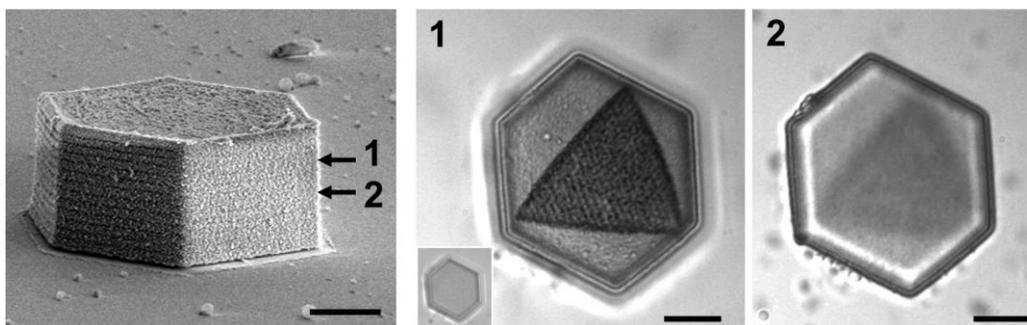


Figure 6. Photographic reproduction within a 3D porous silica hexagonal prism. BSA-templated, silica hexagonal prism containing an internal diatom-image produced *via* two-photon photo-reduction of gold salts. Arrows indicate the position of DIC images in panels 1 and 2. Panel 1 lower left inset shows the image plane before exposure to Ti:S laser light. Scale bars, $10 \mu\text{m}$.

Reference: Khripin C, Pristinski D, Dunphy D, Brinker CJ, Kaehr B. 2011. Protein-directed assembly of arbitrary three-dimensional nanoporous silica architectures, *ACS Nano*, 1401-09.

2.4 Using Bacterial Cell Growth to Template Catalytic Asymmetry

The use of biological structures to template inorganic materials has become an increasingly widespread strategy to develop otherwise synthetically inaccessible hierarchical structures under mild chemical conditions, for use as catalytic and device materials. For example, proteins, peptides, DNA, viruses, cells and multicellular structures have been employed as templates to develop inorganic particles, wires, and optical devices. While most of these approaches have treated the biological structure as a static template, the development of strategies to recruit biological processes (e.g., cell growth and differentiation) to direct active templating/positioning would greatly expand the pool of synthetic outcomes and potential applications for a given biotemplate. Here, we report an approach to 1) metalize the bacterial cell envelope and 2) segregate metallic regions by exploiting the polarity of the cell envelope undergoing growth and division. This strategy is applied to the synthesis of asymmetric catalytic micro particles that are capable of ‘bacterial-like’ swimming behavior in the presence of chemical fuel.

Figure 7 describes the approach used to generate catalytic asymmetry using bacterial growth. Gold nanoparticle (AuNP) seeded cells deposited at a nutrient agarose/glass interface were imaged over time and nanoparticles were developed following growth and formation of the septum. Figure 7A shows a representative time-lapse sequence of a NP-seeded bacterium and indicates AuNP development, following elongation and septum formation, is confined primarily to the poles of the bacterium (Figure 7A, panel 4, arrows).

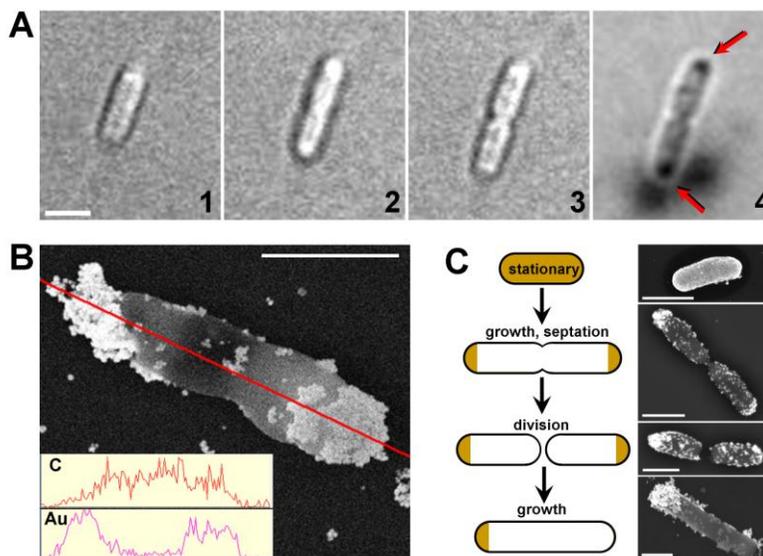


Figure 7. Analysis of AuNP coverage on single cells following incubation in growth conditions and development with gold solutions. (A) A single *E. coli* seeded with AuNPs (as in Fig. 1B top left panel) is monitored over time (panels 1-4 span ~2.5 hrs at 23°C). Following formation of the septum (panel 3), the cell is bathed in gold development solution. Panel 4 shows staining is largely confined to the poles of the dividing bacterium (arrows). (B) Backscatter SEM image shows gold deposition following development of AuNPs is primarily confined to the cell poles (B; inset) An energy dispersive X-ray spectrograph (EDS) across the red line shows the carbon trace of the bacterium and the gold trace concentrated at the cell poles. (C) Stages of murein segregation (left, adapted from ref. 5) show that the location of the metabolically inert pole (gold) corresponds to regions of metallization of AuNP seeded cells undergoing growth and division (right, SEM images). Scale bars, 2 μm .

This straightforward approach to segregate catalytic regions on a biotemplate using cell growth provides an alternative route to explore micro/nano-scale device applications that require asymmetric distribution of electrical contacts, electrostatic surface charge, plasmonic and/or catalytic regions using bio-templating (mild, inherently “green”) reaction conditions. Applications for asymmetric materials include energy storage, constituents for self assembly, and nano-propulsion. As an example of the latter, several proposed designs for chemically driven, catalytic nanomotors require asymmetric arrangement of metallic components (e.g., platinum and gold) in order to develop propulsion along forward, orbital, and rotational trajectories.

We investigated whether *E. coli* cell templates, metalized asymmetrically via cell growth, could undergo a second metallization reaction. Using platinum as the second metallic component produced outgrowths of high Pt concentration extending asymmetrically from the cell body (Figure 8). Pt-Au particles were immersed in a solution of 1% H_2O_2 . Where nodules on single cells could be resolved, propulsion, due to the Pt catalyzed reaction $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$, was observed in the direction of nodule outgrowth, corresponding to high Pt concentration and consistent with previous observations and proposed mechanisms of bi-segmented Pt-Au particle motility. Further, particles undergoing left

and right turns, small and large radii orbital and rotational trajectories were observed with velocities ranging from 3 – 6 $\mu\text{m/s}$.

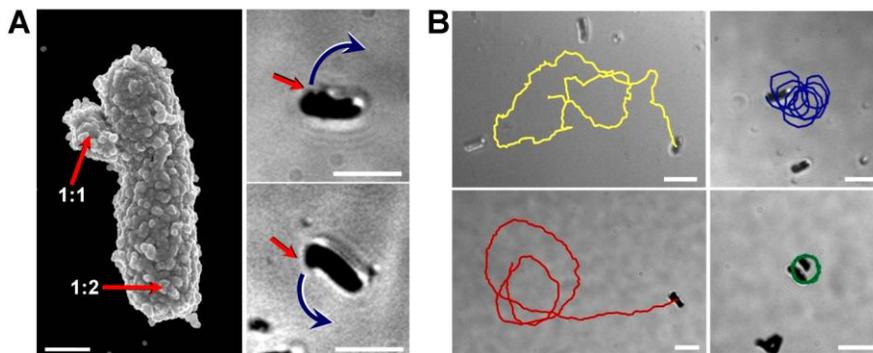


Figure 8. Motion of Pt-Au microparticles templated from *E. coli*. (A, scale bar 0.5 μm) SEM of a Pt-Au microparticle displaying an outgrowth formed after second metallization step (reduction of Pt). Numbers indicate the atomic weight ratio of Pt:Au measured using an EDS spot (diameter, 500 nm) centered at the arrow point. Observed motion in 1.0% hydrogen peroxide is in the direction of outgrowths corresponding to high Pt concentration. (B; scale bars, 5 μm) Left and right turns (upper left panel), small (upper right panel) and large (lower left panel) radii orbital and rotational (lower right panel) trajectories (colored lines) were observed with velocities ranging from 3 – 6 $\mu\text{m/s}$.

We have shown that segregation of catalytic regions on a biotemplate can be achieved by exploiting the polar development of *E. coli* cells undergoing growth and division. These results provide a foundation to further investigate dynamic bio-templating using other cell types and cell-cycle dependent behaviors (e.g., sporulation, pili/flagella, expression, etc.) and develop more complex materials and architectures using chemical, genetic, and confinement-induced modification of bacterial morphology and surface properties.

Reference: Kaehr B, Brinker CJ. 2010. Using Bacterial Cell Growth to Template Catalytic Asymmetry. *Chemical Communications*, 5268-70.

2.5 A generalized approach to biomorphic nano-composites derived from cultured cells.

This last section describes studies that were conducted following consideration of the work described in previous sections (broadly regarding the cell/material interface) which lead to a breakthrough. In particular, work described in Section 2.3 showed that the locally crowded molecular environment of microfabricated protein hydrogels can act to capture and concentrate silica precursors via hydrogen bonding and other non-covalent interactions. Based on these results, we considered that cells are composed of elaborate and functional protein scaffolds and surfaces that are organized over multiple length scales. Could these natural protein architectures also direct silica deposition under similar chemical conditions? To address this question we fixed cells (in this case a mammalian

cancer cell line, but any cells can be used) and treated them with dilute acidic silica solutions as for proteins. We observed that all cellular features are preserved with very high fidelity under what appears to be a self-limiting process (Fig. 9).

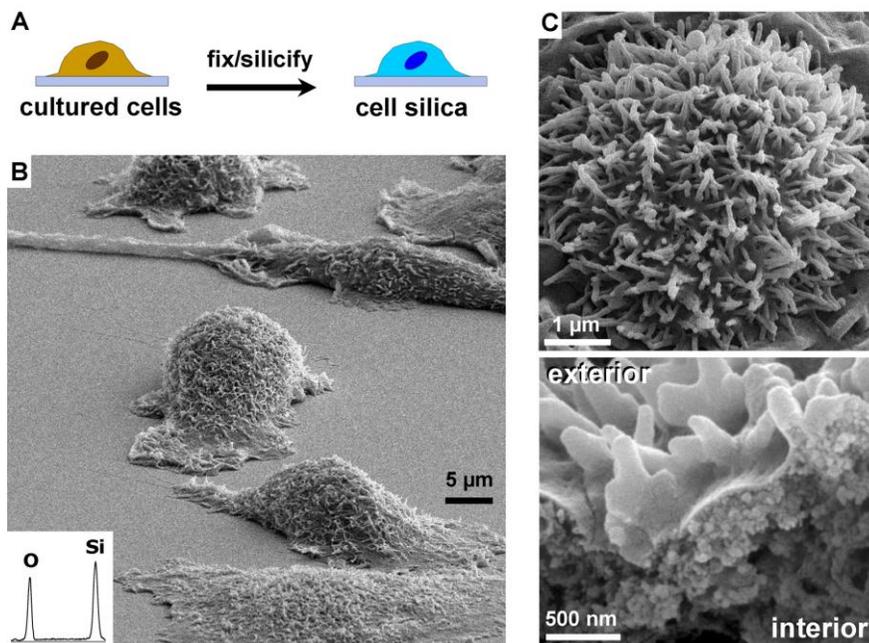


Figure 9. (A, B) Cultured mammalian cells (ASPC1 pancreas cells) were found to undergo (intracellular) silicification (C) using methods similar to those used for protein hydrogels. Cell/silica composites (CSCs) retained cellular features even following calcination.

The cell/silica composites (CSCs) preserve the original cellular structure upon evaporation (and calcination), whereas the parent cells without silica lose their shape and surface features upon drying (Fig. 10). Since cells when detached from the substrate are approximately spherical and monosized, silica cell composites can be prepared as nearly monodisperse powders in large scale batch operations (Fig. 11).

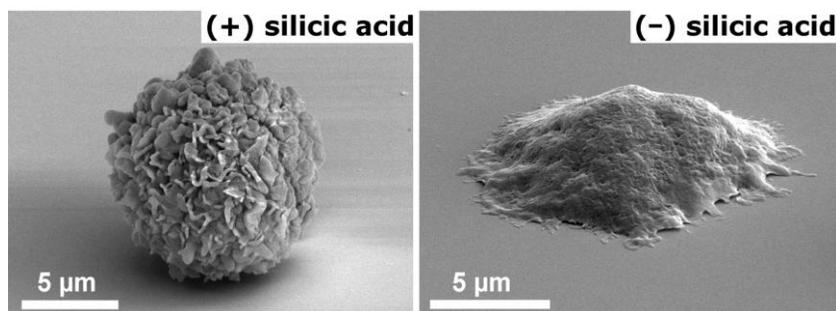


Figure 10. Cell silicification (HTB77 ovarian cells) produces a mechanically stable composite resistant to deformation upon drying.

The silica condensation in CSCs is directed via hydrogen bonding and other non-covalent interactions with proteins organized throughout the cell. Thus the resultant biocomposite does not perturb the protein secondary and tertiary structure. This allows at least a subset of biocatalytic enzymatic activity to be preserved in CSCs. Additionally the silica cell surface can be reconstituted with a lipid bilayer membrane. Fig. 4B shows enzymatic esterase activity can be monitored by fluorescence via a standard ‘viability’ probe.

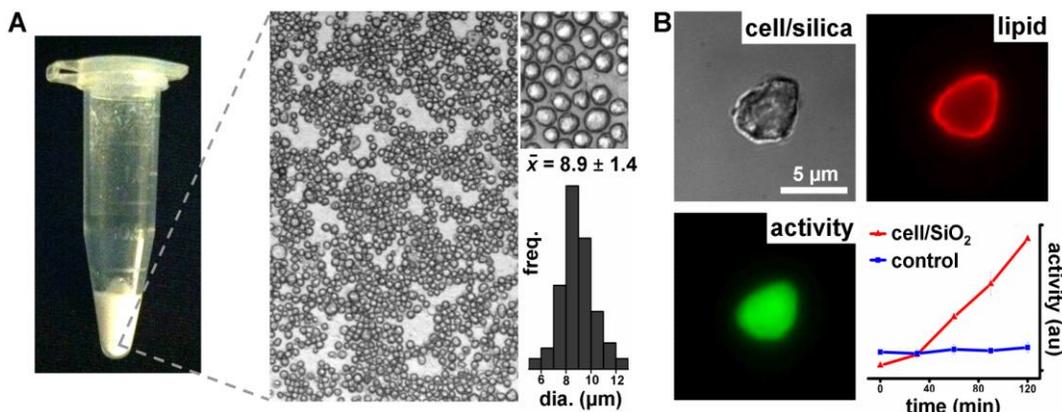


Figure 11. (A) Silicification of cells (4T1 breast cells) in solution leads to monosized particles through a scalable process. (B) A lipid bilayer is reconstituted on a cell/silica particle and shows accumulation of esterase fluorogenic products (red line). The blue line shows activity of calcined CSCs supporting lipid bilayers.

We have shown both a manmade (Section 2.3) and a cell-directed approach to form hierarchical 3D bioinorganic composites. These approaches can be adapted to other chemistries (e.g. titania), and silica structures can be transformed to semiconductors (e.g. silicon). As in diatoms, the silica serves to protect the internal biomolecular components. Preserved and possibly enhanced enzymatic activity could be useful for sensing, catalysis, decontamination, and energy conversion, and the composite may exhibit combined toughness, hardness, and light weight associated with natural biocomposites like nacre.

The high fidelity silica templating of the complete heterogeneity of surface and intracellular features via a self-limiting process shown here, to some extent, contradicts a general theme amongst the biomolecular community that molecular specificity is a key requirement for both directed in vitro and natural biomineralization processes. Based on our findings, we feel that the contribution of non-covalent (scaffolding) interactions governing silica biomineralization deserves greater attention.

CSCs represent a new and exciting discovery with implications for a spectrum of advanced materials as well as biopreservation. They should provide a foundation for biomineralization studies using tractable models. Multiple options of cell choice and silicification procedures remain to be explored in order to achieve maximum CSC functionality, environmental stability, and broad application. Customization of CSCs using genetic and chemical engineering will be explored to develop robust materials for desired applications. In an effort to extend preservation to multicellular systems (tissues,

organs) and ultimately organisms, we will investigate SCR using model organisms (e.g., *C. elegans*) to understand the effects of precursor diffusion and cell-cell interactions on preservation and reactivation.

Reference: Kaehr B, Townson J, Kalinich R, Dunphy D, Brinker CJ. 2011. Biomorphic Nanocomposites Derived from Cultured Cells. *Submitted*.

Summary

This work has generated a valuable toolset to both better understand and exploit the cell/material interface. We demonstrated a biocompatible microfabrication strategy that allows single cells and their progeny to be contained and monitored in arbitrary 3D microenvironments. We developed a method to study, *in situ*, the mechanical interactions of developing cells confined in microfabricated tissue analogues. We have shown that microfabricated biomaterials can form hierarchical 3D bioinorganic composites using mild chemistries inspired by natural systems (e.g., diatoms), and built from them (cultured cells).

Specifically we achieved the following major milestones:

- 1) We developed a microfabrication approach that allows biocompatible confinement of a broad range of cell types (and their subsequent progeny) within (bio)relevant materials.
- 2) We developed a quantitative methodology to study the mechanical properties of microfabricated protein hydrogels under dynamic chemical and physical conditions and applied this method to measure the pressure of developing cells confined in engineered microenvironments.
- 3) We demonstrated a protein-directed approach to template nanoporous silica frameworks into arbitrary 3D architectures by employing crosslinked protein hydrogels to controllably direct silica condensation. We further showed conversion of these porous structures into other device materials such as silicon.
- 4) We pioneered a new strategy that employs cell growth to direct formation of functional inorganic/cell interfaces. This discovery demonstrates a new route for Janus (asymmetric) metallic particle synthesis using biotemplates for the synthesis of microscale catalytic motors and pumps.
- 5) We developed a generalized methodology by which to construct biomorphic nanocomposites from any cell type. We showed that this simple procedure can be used to make conductive (carbonized) replicas of the interior cellular architecture.

Overall, this work bridges a number of areas spanning biology and materials science and provides a foundation to both understand and integrate biological systems into materials and devices. The new technologies described here to prototype 3D inorganic and cell-based device materials can be used as a platform to explore a broad range of applications including photonics, plasmonics, metamaterials, biocatalysts, sensors and decontamination systems.

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