Biochimica et Biophysica Acta 1810 (2011) 259–267

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Cell-directed-assembly: Directing the formation of nano/bio interfaces and architectures with living cells☆

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ARTICLE INFO

Article history:
Received 10 December 2009
Received in revised form 27 September 2010
Accepted 29 September 2010
Available online 8 October 2010

Keywords:
Self-assembly
Yeast
E. coli
Silica
Encapsulation
Biosensors

ABSTRACT

Background: The desire to immobilize, encapsulate, or entrap viable cells for use in a variety of applications has been explored for decades. Traditionally, the approach is to immobilize cells to utilize a specific functionality of the cell in the system.
Scope of review: This review describes our recent discovery that living cells can organize extended nanostructures and nano-objects to create a highly biocompatible nano//bio interface [1].
Major conclusions: We find that short chain phospholipids direct the formation of thin film silica mesophases during evaporation-induced self-assembly (EISA) [2], and that the introduction of cells alter the self-assembly pathway. Cells organize an ordered lipid-membrane that forms a coherent interface with the silica mesophase that is unique in that it withstands drying—yet it maintains accessibility to molecules introduced into the 3D silica host. Cell viability is preserved in the absence of buffer, making these constructs useful as standalone cell-based sensors. In response to hyperosmotic stress, the cells release water, creating a pH gradient which is maintained within the nanostructured host and serves to localize lipids, proteins, plasmids, lipidized nanocrystals, and other components at the cellular surface. This active organization of the bio/nano interface can be accomplished during ink-jet printing or selective wetting–processes allowing patterning of cellular arrays–and even spatially-defined genetic modification.
General significance: Recent advances in the understanding of nanotechnology and cell biology encourage the pursuit of more complex endeavors where the dynamic interactions of the cell and host material act symbiotically to obtain new, useful functions.
This article is part of a Special Issue entitled Nanotechnologies - Emerging Applications in Biomedicine.

1. Introduction

The integration of living cells into an inorganic matrix through cell-directed assembly (CDA) represents a facile route to develop complex functionalities within an engineered material for applications such as sensors and biomedical studies [3]. Nature has provided complex machinery for sensing, communication, and biomolecular production in the living cell. Hybrid materials that incorporate the cell as one component of a biocompatible system that supplies protection and stability, fluidic support, and a tunable interface to the cellular module can deliver a range of biomimetic modalities, including collective and even transformative functions, all powered by the cell. Living cells encapsulated in porous silica have been used to deliver insulin, produce anti-cancer agents, sense environmental changes, and provide scaffolds for tissue growth [1,4–7]. The inorganic host matrix provides a biocompatible environment, a mechanically and chemically robust protective layer, and soft processing and storage conditions conducive to extended cell viability. Integration of cells with the silica host typically employs a synthetic route that adds cells to an acidic silicate sol that rapidly gels, immobilizing the cell. However, cell encapsulation with alkoxide precursors produces alcohols as toxic byproducts and gel shrinkage accompanying continual condensation reactions and drying imposes stresses harmful to cells. Recent attempts to optimize the encapsulation of living cells include adapting the sol–gel route to yield benign hydrolysis products, adding protective polymers to the encapsulation process, and using aerosol deposition after coating the cell with alginates [4,8–11] or directly on cells where hydrolysis and condensation of silica is promoted by water present on the cell surface [12]. However these approaches inevitably create random porous matrices that must remain immersed in water or maintained at high humidity to avoid drying stresses and loss of fluidic connectivity to the cell surface. To our knowledge only in cell directed assembly does the cell actively modulate the formation of a coherent bio/nano interface between

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doi:10.1016/j.bbagen.2010.09.005

the cell and a surrounding ordered silica nanostructure that resists drying and imposition of drying stresses, supplies fluidic connectivity, and maintains an accessible and recognizable cell surface.

CDA adapts so-called evaporation-induced self-assembly [2] to biocompatible conditions and exploits a living cell's ability to modify its environment to create a graded cell-specific biotic/abiotic interface that maintains a fluidic architecture of water-filled pores around the cell. The CDA process utilizes a short chain phospholipid that serves as a structure directing agent and forms a coherent interface between the abiotic and biotic components of the composite system. This uniformly nanostructured interface, which maintains important chemical and physical gradients around the cell, is a crucial part of the encapsulated cell platform. Encapsulation of cells, particularly for biomedical applications, must allow control of interfacial properties around the cell and dictates a 3-dimensional scaffold topography for maintenance of cellular function. Mechanical forces in the extra-cellular environment are sensed by integrins, which transduce the stimuli to downstream chemical signals that affect cellular processes and viability. Disruption of cellular adhesion can affect cell proliferation, alter gene expression and lead to anoikis, a form of apoptosis resulting from loss of cellular anchorage. In addition to satisfying cellular adhesion requirements, the interface formed by CDA is important in a systems biology approach to studying the interaction between components of a system that give rise to its collective properties. The unique nature of the abiotic-biotic interface allows localization of a variety of nanocomponents at the cellular surface, facilitating the use of the immobilized cell as a sensing and interrogation device, promoting cellular transformation and non-native functions, and providing a platform for characterization of the nano–bio interface and cell isolation behaviors (Fig. 1).

2. The bio–nano interface

While other cell integration methods either ignore the cell's interaction with its host matrix, or provide a barrier between the inorganic and organic components, CDA forms a fluid, lipidic transitional phase around the cell. Amphiphilic surfactant molecules will spontaneously self-assemble into periodic mesophases and can organize oligosilicic acids at the interface between hydrophilic surfactant head groups and water. Rapid evaporation of solvent in the system gives a condensed hybrid mesophase whose curvature is dictated by surfactant size, shape and concentration [13]. To promote biocompatibility with living cells during the self-assembly process, typical surfactants are replaced by phosphatidylcholines with zwitterionic headgroups and double acyl tails. Dipoles on the headgroups of these short-chain lipids interact with the amphoteric cell surface and through further electrostatic and Van der waals interactions, a lamellar structure of multiple lipid bilayers develops [1]. The lipid interface forms a coherent bridge between the cell and the inorganic phase and the fluid, minimal hydrophobic portion of the lipid molecular structure preserves both osmotic and pH gradients established by the cell in response to stressful environmental conditions during CDA. This artificial fluidic membrane surrounding the cell protects it from mechanical stresses generated during condensation of the surrounding silica host matrix, and in a manner similar to bacteria with naturally extended cell boundaries [14], the lipid interface of multiple thin bilayer sheets imparts chemical resistance and structural integrity while retaining membrane accessibility through high trafficking potential at the cell surface.

Comparison studies of these self-assembled composite materials with colloidal cell surrogate models (e.g. latex beads) demonstrate the cell's unique role in CDA. Only living cells, as opposed to similarly sized beads with charged surfaces or apoptotic cells, can actively participate in CDA, influencing silica nanostructure and forming the fluid lipid interface. During immobilization, the cell responds to osmotic stress by releasing water, helping to maintain a localized pH gradient which promotes the formation of the lipid interface and excludes silica oligomers from the vicinity of the cell [3].

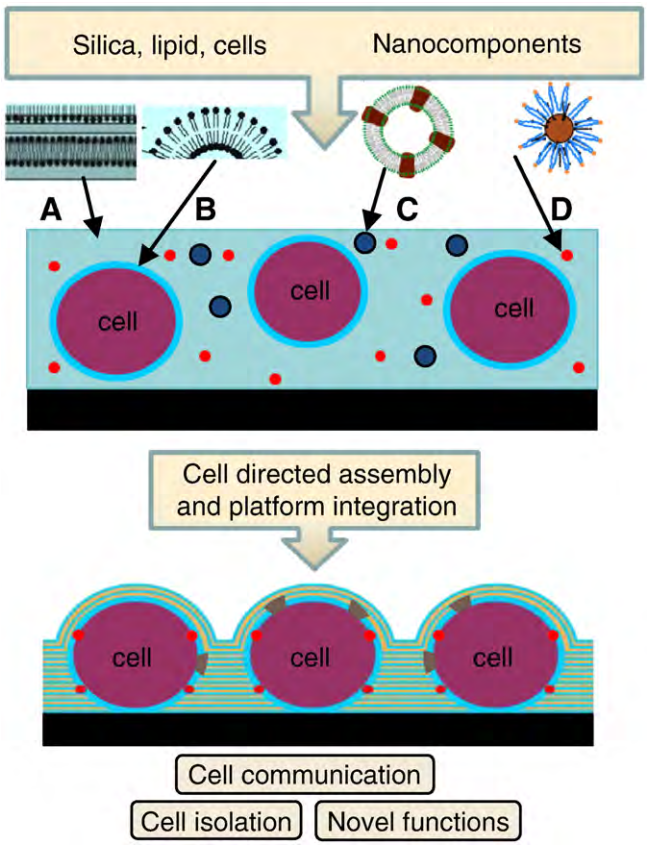

Fig. 1. During CDA, living cells are encapsulated in a nanostructured silica host (A) and protected by a lipid interface (B). Added nanocomponents [proteo-liposome (C) and nanocrystals (D)] are localized in the fluid lipid interface. Hybrid, patternable platforms support cell isolation, cell signaling and integrative functions. Adapted from [17].

The potential for utilizing the unique lipid phase formed during CDA to build integrated platforms depends on developing predictive relationships to describe the inhomogeneous and dynamic environment of the lipid interface and its abiotic and biotic boundaries. Influences on the nano–bio interface and its borders include size, shape, surface chemistry and electrostatic properties of the cell wall or membrane and the inorganic mesophase. The molecular composition of the interface dictates its chemical and physical properties, complicated by the presence of an actively responding cellular component that introduces a temporal dependency of these properties.

2.1. Interface fluidity and tailorability

An optically transparent silica matrix hosting immobilized cells allows the system to be probed in multiple regions for assessment of its different components. Fluorescence recovery after photobleaching (FRAP) experiments on the lipid interface indicate it maintains a fluidity around the cell that is between that of typical lipidic fluid phases and that of the surrounding matrix distant from the cell. Lipid fluidity is important in retaining protein conformation, particularly when incorporating foreign functions through transmembrane proteins, and can be modulated by changing tail length and incorporating molecules such as cholesterol into the bilayers. Elemental mapping across the interface shows that silica is excluded near the cellular side of the interface. Changes in the composition of the silica precursor sol can affect the condensation rate of the polymeric matrix, which in turn determines how far silica oligomers can diffuse across the interface before condensation occurs. Further fine-tuning of interface fluidity can be achieved by adding non-water soluble, longer chain lipids in the form of liposomes, which can preferentially enrich areas near the cell [15].

The wide range of lipid headgroups and tail lengths, resulting in bilayers of varying curvature and conformability, provides the flexibility needed to interact with different cellular organisms. Cell surfaces are widely inhomogeneous and vary among different species. By organizing lipids and creating local pH gradients, living cells create cell-specific interfaces with the surrounding lipid-templated nanostructure. Initial localization of the lipid interface may be promoted or hindered by electrostatic interactions, spatial hindrances, and curvature matching requirements. When system components include *Saccharomyces cerevisae* and a phosphatidylcholine lipid, localization at the cell surface occurs rapidly, but replacement of the lipid with a phosphatidylethanolamine does not result in a coherent interface with the yeast cell [16]. Similarly, the type of headgroup influences the strength of interaction with the silica framework and may need to be chosen carefully when framework physical properties such as strength, porosity and stiffness need to be adjusted for specific platform applications while preserving biocompatibility.

The silica–cell interface formed from highly fluid short chain lipids during CDA helps maintain cellular surface characteristics and accessibility. Fluorescent antibodies introduced after cellular encapsulation are able to recognize and bind to cell surface receptors, indicating that surface proteins remain intact and accessible throughout CDA. When FITC-tagged anti-*Escherichia coli* IgG antibodies are added to *E. coli* immobilized using CDA and allowed to dry for 24 hours, the antibodies are still able to recognize and bind to their receptors (Fig. 2), establishing a gradient of antibodies at the surface of the immobilized cells [17]. Spatio-temporal signals are important regulators of a variety of cellular behaviors and this ability to maintain molecular gradients within the film could have several biomedical applications, such as understanding the role of cytokine gradients that form after tissue injury, guiding peripheral cells into a wound for repair.

2.2. Interfacing functional proteins

Membrane receptor proteins are important in cellular communication through signal transduction and amplification; immobilization of these non-soluble molecules in accessible platforms will be important to a wide range of medical applications, including therapeutic drug-discovery through rapid screening. While soluble proteins can be accommodated through several aqueous sol–gel processing methods [18], membrane-bound proteins reside either partially or completely in an amphiphilic lipid bilayer, whose critical chemical and fluidic properties must be retained to prevent protein unfolding and loss of function [19]. Membrane bound protein encapsulation for incorporation into functional platforms has focused mainly on the photoactive protein-retinal complex, bacteriorhodopsin (bR). bR is a light-activated proton pump in the purple membrane (PM) of halophilic bacteria and relies on a net charge separation across the lipid membrane. Not only must the proton pump functionality of bR be maintained during encapsulation, the immobilized proteins must be spatially oriented in one direction to produce a proton gradient. Early attempts at encapsulating bR while retaining protein function used extracted fragments or intercalated sheets of the purple membrane [20], but the native bilayer is fragile, making it hard to maintain and difficult to manipulate. CDA allows incorporation of the protein into the lipid interface at the cell surface while retaining orientation and functionality (Fig. 3) [15]. By adding bR to a dimyristoylphosphatidylcholine (DMPC) liposome and then including the proteo-liposome in the CDA process, the bR is preferentially localized, along with the longer chained liposomal lipids, near the cellular boundary of the interface. The cell-supported layers of bR in liposomal lipid preserve the native functionality of the membrane protein and maintain it in a preferred orientation with respect to the yeast cellular surface. The mild detergent nature of the short chain lipids in the abiotic/biotic interface is likely important in orienting the protein by facilitating destabilization of the bR-proteoliposomes and subsequent fusion with other liposomal pieces to form multiple lipid layers with consistent directional conformation. The interfaced and oriented membrane bound proteins are able to modulate the pH gradient near the immobilized cell in a versatile platform configuration, and serve as a prototype for fundamental studies of membrane protein function. Recently photosynthetic sub-cellular plant structures have been immobilized in a transparent silica matrix, maintaining bioactivity for extended periods [21]. The sensitivity of thylkaloids to ionic and osmotic gradients, and their need for a suitable 3-d environment, suggests that the controlled interface formed during CDA would be helpful in the biocompatible immobilization of these structures for use as artificial photobioreactors.

3. Cell isolation platforms

Both the heterogeneity inherent in cellular populations and communication between cells such as that postulated to occur during quorum sensing, make single-cell analysis an important tool in understanding essential cellular processes. The characteristics of individual cells that show different physiological responses to signaling, or cell-to-cell variation in gene expression and growth rate, will be hidden in an averaged population [22]. The specific mechanism of action for a new drug, a bacterial population's means of persistence, or the kinetics of a metabolic response may all be masked unless response at the single-cell level can be monitored. Microfluidic systems [23], and seeded microwell arrays [24] have been used to study single-cell response, but the reductionist platform system provided by cell-directed assembly allows a systems biology approach to individual cellular behavior. A single viable cell can be encapsulated, interfaced with only the desired extra-cellular components, and confined in a matrix which can be optically probed. Physical and chemical gradients, transport to the cell, and cellular cross-talk can all be controlled while analyzing physiological measurements of a single living cell or a defined cellular sub-population.

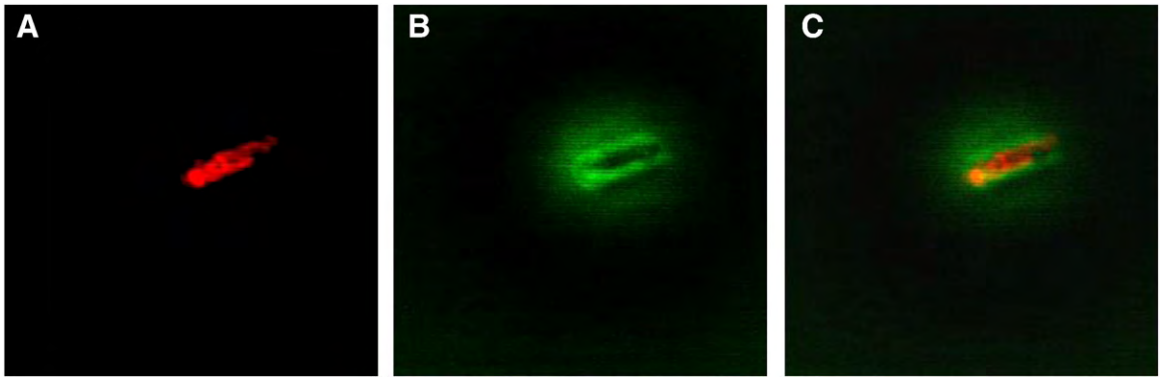

Fig. 2. The cell surface of immobilized (A) *E. coli* (red) is accessible and recognizable to (B) IgG antibodies (green) 24 h after immobilization (C). Adapted from [3].

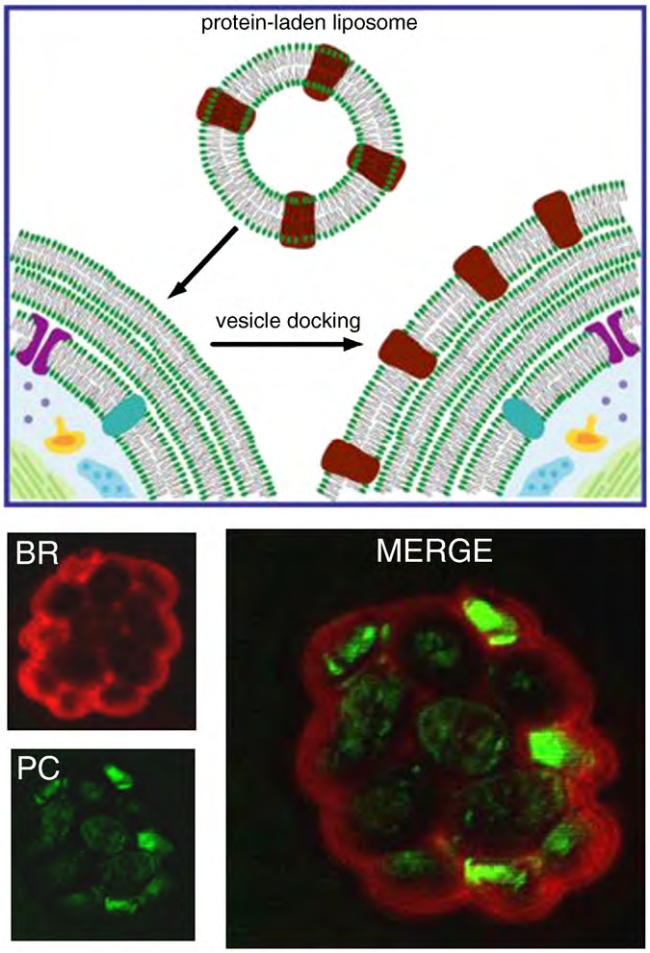

Fig. 3. Bacteriorhodopsin in DMPC liposome is integrated at the surface of *S. cerevisiae* in multiple lipid layers, retaining directional conformation. Adapted from [15].

3.1. Cell to matrix adhesion

Adhesive interactions between cells and their local microenvironment can have a critical effect on many cellular processes [25,26]. Cellular anchorage dependence is a key controlling factor in cellular homeostasis, the differentiation and growth of complex tissues, and cellular stress through mechanical insult. Cell-adhesion receptors, such as integrins, are multifunctional proteins that link cells to the extracellular matrix and form adhesion complexes at the cellular boundary. These adhesive molecular structures are available as anchor points for constructing and organizing the cellular cytoskeleton and shape. Receptor proteins are also instrumental in influencing cell proliferation and apoptosis; failure to maintain adequate extracellular matrix cues can be fatal to cells designed for therapeutic *in vivo* applications or for cell lines used in studying anchorage independent growth in pathogenic and metastatic cells. Matrix-molecule enhanced, engineered capsules formed from immobilized human marrow stromal cells, supplemented with exogenous adhesion molecules, are able to compensate for missing cellular matrix cues and improve the retention of the stromal cells implanted in rats [27]. When fibrinogen and fibronectin are supplied to single cells encapsulated in agarose, cell–matrix interactions are re-introduced and rescue the cells from anchorage dependent apoptosis.

Anchorage-independent growth, where cells no longer require adhesion signals for survival, is important in the development of metastatic cancers; identifying the mechanisms that govern anoikis will contribute to understanding how these cells can elude the natural suicidal response that a cell disrupted from its natural environment cell should have [28]. Other diseases may be due to the untimely induction of anoikis, which likely has a role in cardiovascular degenerative diseases, plaque rupture in atherosclerosis and chronic vascular disease in diabetes. For cells that require attachment to a very specific extra-cellular matrix (ECM), an inappropriate surrounding ECM can have the same detrimental effects as absence of an ECM. Whether the goal is to determine how a migrating cancer cell can survive outside its own microenvironmental niche to grow in an inappropriate environment or to identify the factors that must be added to an isolated cell's ECM, the interface generated through CDA provides a straightforward means of incorporating and delivering exogenous proteins to the cellular surface.

3.2. Cell therapy

Prevention and treatment of disease using cells that have been pharmacologically altered to repair damaged tissues or release therapeutic drugs presents two major challenges. Immunological rejection of *in vivo* foreign cells and biocompatibility of the therapeutic cellular delivery system are challenges that can both be addressed through cellular encapsulation [29]. Delivery of macromolecules through liposomes or a crossed-linked polymer such as alginate offers inadequate protection from degradation in host biological fluids and does not take advantage of a living cell's ability to serve as a micro-reactor, continuing to produce the required biomolecule *in vivo*. Encapsulating cells in a robust matrix with controlled porosity, however, can protect them against the host immune system and in turn defend the host from the engineered cell. Islets of Langerhans encapsulated in a porous matrix via silica coated alginate capsules and transplanted to diabetic dogs can successfully deliver insulin for more than 5 weeks [5].

Advances in cell therapy with encapsulated cells can be expected by harnessing the ability of cell-directed assembly to incorporate multiple functionalities in platforms through placement of individual components. A porous silica matrix and lipid interface surrounding an agent cell will be a valuable host platform for including nano-machines designed to control molecular transport. Molecules that react to a stimulus with large amplitude motion or nano-machines such as valves and snap-tops are useful in controlled release applications, discharging guest molecules on command through responses to light, pH, and enzymatic or competitive binding stimuli [30,31]. Released signal molecules could be chosen to either instigate or stop production in the cellular micro-reactor. The lipid interface will help localize the nano-objects in useful proximity for signaling the cell, and the silica matrix can be derivatized for improved immune response or targeted delivery in the host. Additionally, cell proliferation beyond the drug delivery system, common with encapsulation matrices subject to cracking or dissolution, can be avoided through the robust CDA platform.

3.3. Disease dormancy model

The ability of cells to enter a dormant, persistent state may be responsible for a number of distinct microbiological events, including multidrug tolerance of infectious diseases, antimicrobial tolerance of biofilms, and chronic diseases such as tuberculosis [32]. For a disease such as tuberculosis, with prolonged asymptomatic latent stages, a sub-population of non-dividing, specialized survivor cells may form in response to fluctuations in levels of persister proteins. These cells have an unusual number of hydrophobic lipids and fatty acids in their cells wall, and can reside clustered in lung granulomas for years before emerging in a virulent form. It is likely that a metabolic shift to an anaerobic state induced by gradual apoxia resulting from formation of the granulomas occurs. Understanding the mechanism that triggers latency and reemergence of TB should lead to new drug therapies for a disease that affects nearly a third of the world's population. Cell-

directed assembly allows cells to be encapsulated in surroundings that mimic the granuloma cluster, providing a phospholipid rich environment at the cells periphery, excluding outside nutrients, and extending cell viability. Cells from the vaccine strain of *Mycobacterium tuberculosis*, BCG, were encapsulated in a lipid–silica nanostructure and allowed to incubate for 16 months at body temperature, with over 50% of cells remaining viable [17]. This reduced physical model of a complex biological system will allow further studies on the metabolic state of these cells, their infectiousness, and their susceptibility to drugs following prolonged incubation, and can be extended to study numerous bacterial species that are viable but uncultivable *in vitro*.

4. Cellular communication

Intercellular communication relies on information transmitted through cell signaling networks and may occur through direct contact, or over varying distances. By decoupling communication that occurs through cellular contact from molecular signaling, cell-directed assembly can give information on the spatio-temporal dependence of cellular communication and can contribute to understanding coordinated cellular behaviors. Cellular isolation, as discussed above, allows signals arising from the local micro-environment, such as adhesive cues, to be investigated, and is also useful in regulating intercellular cross talk that can complicate understanding of local molecular concentration fluctuations and gradients.

4.1. Quorum sensing

Quorum sensing has been described as a decision-making process of a collective system that relies on recognition of changes in the population of individual components, coupled with a standard response to reaching a threshold density of the system. In bacterial populations, a variety of sensing, transport, and targeting functions exist but they all rely on the ability of the cells to produce, secrete, detect, and respond to small molecular signals [17,33,34]. When an individual cell senses an increased concentration of signaling molecules, further production of autoinducer signals can be activated. As concentration of the autoinducer increases, a positive feedback loop is established and behaviors that are of benefit to the population, such as coordinated gene transcription, can be activated. The implication that quorum sensing is a cooperative behavior, where a decision to alter behavior is made by a group of cells, doesn't take into account factors such as diffusion, spatial distribution of cells and clusters, and environmental cross-talk. The efficiency model of bacterial collective behavior acknowledges the cell's inability to separate the effects of mass transfer, signal decay, and confinement and proposes that the role of autoinducers is to signal when secretion of extracellular effectors will be efficient [35].

When *Staphylococcus aureus* cells are isolated and confined in a diffusion controlled matrix that eliminates cross-talk using an extension of CDA, individual cells will respond to an accumulation of extracellular auto inducing cyclic peptides (AIP) and activate a response regulatory system [36] Using an aerosol CDA process, individual cells can be physically and chemically isolated within an endosome like compartment surrounded by a nanostructured silica matrix, which can serve as a reservoir for buffer and media Fig. 4.Upon confinement, export and accumulation of AIP at the cell surface activates quorum sensing pathways in individual *S. auereus*, resulting in the up-regulation of toxins (e.g. α-hemolysin), degradatory enzymes, and metabolic pathways. The viability of isolated cells is increased compared to mutant cells that are unable to initiate quorum sensing, indicating that quorum sensing poises the cell to access and utilize environmental nutrients. By demonstrating that isolated, individual cells undergo quorum sensing and genetic reprogramming, it is unnecessary to invoke complex social interactions to explain how QS evolved. Cell–cell and cell–host communication are potential targets for novel anti-bacterial drugs and inhibition of infection near its onset may be accomplished by employing strategies that interrupt quorum sensing at the individual cell level [37,38].

4.2. Biofilms

The question of whether collective behavior is operating in a densely populated community also has important implications in the study of biofilms, communities of bacteria that grow on a variety of abiotic and biotic surfaces, including heart valves, bone, drinking water systems and medical implants. Cells in a biofilm are frequently highly resistant to antibiotics and the body's immune defenses, and are responsible for both acute and chronic infections and a variety of biofouling problems. The complex community of microbial cells secretes polysacchharides, forming a hydrated, fibrous extra-cellular matrix that provides structural integrity and adhesion cues, protects against host immune responses and antibacterial drugs, and concentrates exogenous nutrients near the cells. Under biofilm growth conditions, as compared to planktonic growth, some microbial species may differentially express up to 10% of their genome, influencing adhesion, secretion, motility and metabolism [39]. Cell-to-cell signaling regulates changes in the collective bacterial behavior, with whole-group coordination through altruistic individual cell cooperation postulated to underlie aggregate community properties. However, as seen in quorum sensing of *S. aureus*, complex biofilm behaviors may also result from the uncoordinated behavior of cells responding to their local microenvironments. A newly developed enzymatic approach to manufacturing large quantities of AIP has allowed biofilm dispersal experiments to be conducted, suggesting that by triggering quorum sensing with exogenous signaling molecules, the biofilm can be destroyed. Individual bacteria released from the film are once again susceptible to antibiotics, potentially providing a route to healing chronic infections such as methicillin resistant *S. aureus* by triggering quorum sensing [40].

Cell directed assembly allows cells capable of biofilm formation to be isolated within a defined local environment and supported by an inorganic matrix whose degree of condensation and structural integrity can be designed to direct cellular growth. The porous silica host has a high surface area and can be modified with additives for selective dissolution in biological or industrial fluids, allowing study of the hydrodynamic, physicochemical and biological processes involved in biofilm formation. A hierarchy of structural levels exists in biofilms, and CDA platforms can control a variety of factors influencing these levels, including topological and geometric characteristics, chemical composition, molecular signaling and the importance of shear flow and diffusivity around the first anchored cells. Using CDA to look at collective versus competitive behaviors, the adaptability of biofilms faced with mechanical or chemical insult, or the behavior of *Bacillus subtilis*, which can differentiate into multiple cell types in the absence of nutrient gradients without invoking quorum sensing [41], could contribute insight to the behavior of individual cells and the community.

The multi-functional potential of the CDA platform will also be important in discovering new antimicrobial agents, including tunable anti-bacterial nanoparticles which may have unique and targetable film penetrating properties [42]. An interesting potential extension of biofilm behavior studied through cell isolation or controlled growth of biofilms, may be the engineering of nanostructures by the biofilm itself [43]. Biofilms can be used to organize and immobilize nanoparticles, using the film's selective permeability to isolate nanocomponents of various sizes and construct hybrid materials with hierarchical structures.

5. Multi-functional cell immobilization platforms

Biologically inspired materials systems arising from innovative approaches to synthetic biology and protocell modeling are capable of

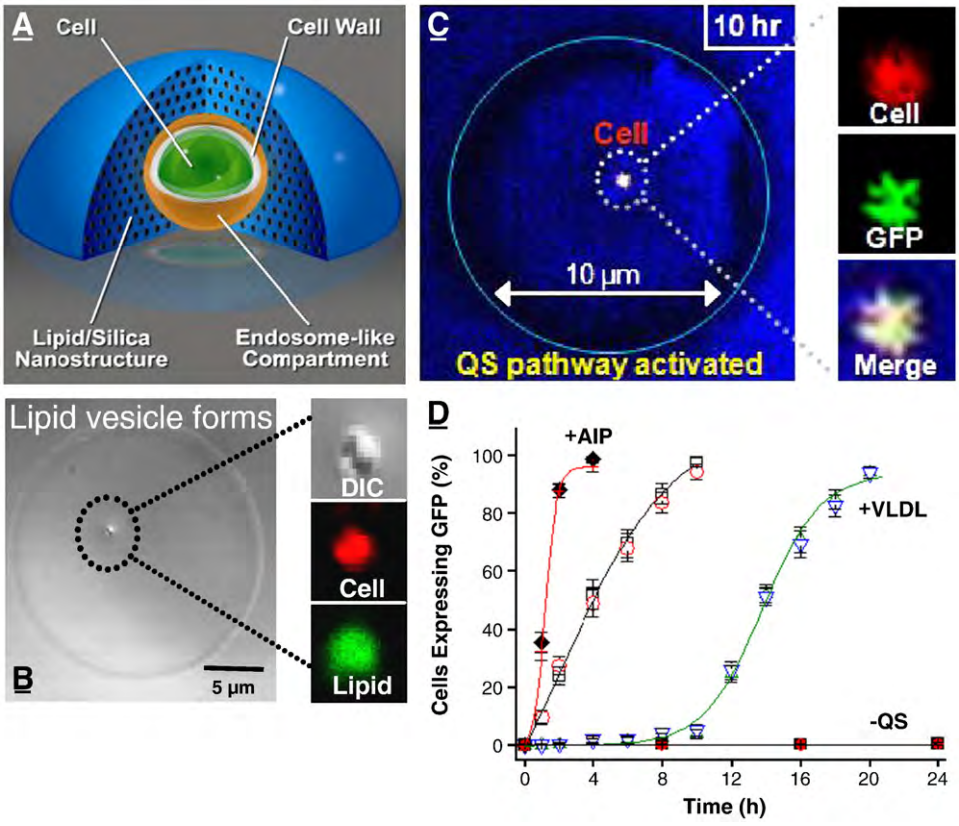

Fig. 4. (A) schematic of cell directed nanostructured silica droplet, (B) localization of lipid at cell membrane, (C) GFP reporting of quorum sensing, (D) GFP expression kinetics reporting QS and effect of added exogenous AIP or VLDL inhibitor. Adapted from [36].

revolutionizing many areas of medicine and technology. Medically relevant original, hybrid materials, however, will probably remain dependent on biological machinery, with completely artificial systems unable to provide the complexity inherent in biological components. The evolution of life itself likely depended on the development and coupling of multiple nanostructures that eventually linked information flow and metabolism within a defined boundary [44]. The assembly of a complex structure from modular building blocks can collect intrinsic operations in a reduced system with expanded functionality. The size scale of nanomaterials makes them suitable for medical and biotechnological applications, and they can be tailored with unique physical and chemical properties for biocompatibility with specific targets. Nano building blocks can be designed for simultaneous multiple functions and will be important in areas such as cancer treatments where one hybrid material can provide optical or magnetic probing coupled with therapeutic drug delivery [30]. One of the challenges, however, to building an integrated, collective system is to achieve spatial separation, ordering and interfacing of the individual components. When biotic and abiotic nanocomponents are introduced into a system by CDA, the fluidic lipid interface and molecular gradients surrounding the cell localize the components in the interfacial region. Nanoparticles, receptor proteins, transformation plasmids and instructive extracellular environments for a particular cell line can be incorporated into hierarchical structures and platforms for cellular interrogation, sensing or novel functions.

5.1. Integrative assembly and patterning

Optically directed patterning of porous silica immobilizes and localizes cells through selective wetting [16,45]. If a photoacid generator (PAG) is added to the system, along with a structure directing amphiphile, UV exposure of the film through a mask produces hydrophobic areas of the film where organic byproducts of the PAG decomposition accumulate. When cells are added, they localize to the unexposed, hydrophilic regions where they are incorporated into the nanostructured silica host. Alternatively, cells can be localized in patterned regions of structured silica by using UV exposure to rapidly condense unmasked areas on a silica–lipid host film [17]. Cells will then integrate only in the unexposed, fluid portions of the cell–silica–lipid platform, maintaining both the silica nanostructure and lipid interface upon eventual silica condensation (Fig. 5).

Integrative platform patterning can also be accomplished through controlled condensation of a silica–lipid host matrix combined with ink-jet printing. Cells are separated from silica precursors and deposited during a second printing pass. Cells will then center in previously printed sol droplets and can integrate into partially condensed areas of silica on the platform. By printing layers of cells or silica–lipid precursors, complex structures to investigate the proximity of different cell types and its effect on cell-to-cell communication in tissue formation or disease development could be formed [3,46,47]. Combined with the ability to tailor the lipid interface with adhesive cues, as described above, patterned cells could be used to investigate the coupled effects of cell density and matrix-mediated differentiation.

Control of cellular behavior depends on chemical signals that can have space and time dependent behaviors. While the cell construct formed by CDA can be used to reduce and determine the required adhesive cues in a cell's extracellular environment, as described above, integrative assembly can also be helpful in analyzing spatio-temporal signals. For progenitor cells to differentiate into motor neurons, they must seek a pathway to muscle targets through adhesive interactions, and react to gradients of soluble guidance factors [45]. When studying highly dynamic cellular events such as

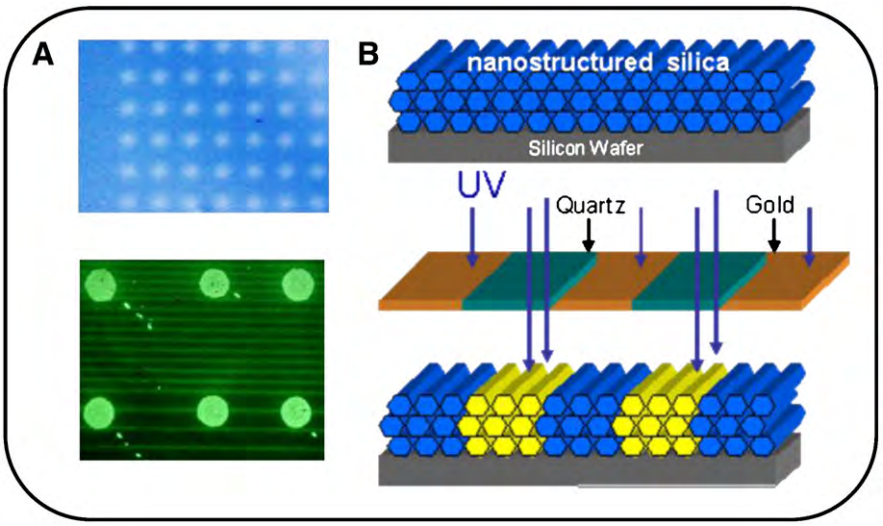

Fig. 5. (A) Cells added to a partially condensed lipid–silica film form a lipid rich interface and fully integrate into the film. Added nanocomponents localize at the cell surface. (B) Optically directed patterning and silica condensation provides spatial control during platform integration and supports fluidic connectivity through patterned hydrophilic regions. Adapted from [17].

tissue differentiation, host fluidic properties that allow fluctuations in the cells microenvironment must be incorporated. Integrative cellular assembly of platforms can maintain host porosity and nanostructure designed for controlled transport, and if combined with microfluidic inlets and outlets for fluid delivery, should give important opportunities for modulating the cell's environment.

5.2. Novel functions and behaviors

The living cell's ability to organize assemblies of nano-objects, mediate its local chemical and physical environment, and form a coherent nano–bio interface allows it to be used as a tool for developing both new material properties and cellular functions. A multiplicity of novel functions can be obtained by choice of cell during integrative assembly of a living organism into a robust, hybrid platform. Several model systems [17], including the eukaryote *Saccharomyces cerevisiae*, a Gram-negative bacterium, *E. coli*, and a Gram-positive bacterium *Staphylococcus epidermidis*, have been established. In addition an anthrax analogue for potential biodefense applications, the tuberculosis analogue *Mycobacterium smegmatis* for infectious disease studies, and extremophiles like *Thermus aquaticus*, which lives in high-temperature environments, and *Deinococcus radiodurans*, which withstands exposure to DNA damaging radiation, demonstrate the flexibility of the interfaced cell–inorganic host construct. During cell-directed assembly, each organism uniquely modifies its local and distant environments, creating distinct gradients and lipid interfaces, while influencing the silica nanostructure to different extents. The nanostructure of the host matrix can be controlled independently of the encapsulated organism through integrative processes, where hexagonal, cubic and modified-orthorhombic (GMO) structures with 3D pore connectivity [48] can be generated and retained while cells integrate with the silica host.

New functionality has also been introduced through cellular behavior modification, either by introduction of non-native proteins at the cellular surface, as described above for bacteriorhodopsin, or by plasmid transformation [17]. Genetic modification is typically accomplished by transforming bacterial cells with plasmids, pieces of extrachromosomal DNA that encode non-native genes. Heat-shock or electroporation may be used to make the host cells competent, allowing passive diffusion of plasmids across the cell membrane. Cell-directed assembly allows high-efficiency genetic transformation, overcoming current low transformation efficiencies that require the use of high plasmid concentrations. pGLO plasmids (for introducing D-arabinose dependent GFP expression) introduced during CDA of *E. coli* are immediately localized at the cell surface, creating a high effective concentration gradient. Combined with the fluid nature of the bio–nano interface and mild permeabilization of the cell membrane by biocompatible short-chained phospholipids, plasmids are able to enter the cell under ambient conditions during the evaporation-induced assembly process. With CDA, cell transformation efficiency is more than double that achieved with a standard heat shock protocol, allowing *in-situ* genetic modification of immobilized cells, which will have important applications in production and expression of medically useful recombinant proteins. By combining plasmid transformation with integrative patterning techniques, where cells and plasmids are added to pre-formed nanostructured films it should be possible to pattern an array of cells, and then introduce plasmids at different locations to obtain spatially-differentiable cell transformation for cell-communication studies or biosensing applications.

Cellular behaviors can also be enhanced through the introduction of nanoparticles at the nano–bio interface. Nanoparticles with multiple functions can be engineered for specific biomedical applications and may include a matrix with a magnetic domain, fluorescent tags and antibodies, or small molecule incarceration for later targeted release [14,49] The particle shape can affect its survival time, flow characteristics and drug delivery efficiency. Toxicity of particles that are either soluble only in organic solvents, or are stabilized by surfactants that disrupt cell membrane integrity can be avoided by encapsulating the particles in a phospholipid shell to ensure biocompatibility [50]. When added to cells during CDA, coated gold nanoparticles, and magnetic iron oxide particles of interest for targeted delivery of materials and cell sorting, are organized at the cell surface and enter the cell efficiently through mild membrane permeabilization, as seen for plasmids.

6. Conclusion

In clinical medicine, the development of a complex disease such as diabetes or coronary artery disease is rarely the result of a single factor, but depends on multiple health and environmental influences, such as diet, genetics, sleep disorders or immune system. Physicians are changing their focus from the disease to the person, recognizing that system-level understanding of human health and disease is

needed. A reductionist approach to complex systems relies on understanding and identifying the individual components, but is deficient in describing emergent behaviors that result from the interplay between system parts. Systems biology, however, focuses on integration, rather than reduction, and looks at the diversity of cause and effect in system level networks. It recognizes that behavior of the whole system depends on interaction of the components, including the temporal characteristics of each component and its local environment, and the topographic relationships between components. However, trying to integrate in-depth knowledge of numerous parameters can be overly cumbersome when looking at living systems and a combination of reductionist and system biology approaches may be necessary to understand complex biological problems. The reduced, integrated platform of a cell confined in a robust porous matrix with an adaptable biomimetic interface that allows placement of additional system components, is an important step in understanding inherent biological complexity. It allows investigation of composition, space and time, recognizing the importance of interactions between parts of a system, but focusing on a controlled number of components, and should prove fundamentally important in studying cell biology, health and disease.

Acknowledgments

This work is supported by the Air Force Office of Scientific Research (FA 9550-07-1-0054); the NIH/Roadmap for Medical Research (PHS 2 PN2 EY016570B); the Defense Threat Reduction Agency (B0844671), the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering; the NSF Research Experiences for Undergraduates (DMR-0649132); and Sandia National Laboratories' LDRD program. ECC was supported by an NSF IGERT Fellowship (DGE-0549500). CEA was also supported by an NSF IGERT Fellowship (DGE-0504276). Fluorescence images were obtained at the UNM Cancer Center Fluorescence Microscopy Facility. SEM images were obtained at the UNM Center for Micro-Engineered Materials. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

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