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Biomimetic Coatings to Control Cellular Function through Cell Surface Engineering

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Biologically inspired materials and structures with tailored biological, physical, and chemical properties provide an indispensable platform to actively modulate and protect cell function in hostile and synthetic environments (e.g., devices or matrices). Herein, recent innovations are discussed in cell surface engineering methods based on bulk hydrogels, microgels, and ultrathin capsules derived from inorganic, polymeric, biomolecular, or nanoparticle materials applicable to various protective and interaction mediating applications. These biomimetic cell coatings can dramatically increase cell viability and stability in a hostile environment and expand their applicability for demanding biomedical, biotechnology, and bioelectronics applications. Some of the most recent studies of traditional inorganic and organic gels, polymeric and biomolecular microgels, and ultrathin conformal soft shells from polymers and proteins are also discussed. Proper selection of chemical composition and assembly conditions has potential to dramatically enhance viability of encapsulated cells by increasing their mechanical stability, masking the cell surface from immunological agents, increasing mechanical stability, and providing chemical resistance to aggressive environments. Some recent examples of such robust and viable protected cells for biotechnology and bioelectronics are presented.

1. Introduction

Biologically inspired materials are designed in an effort to mimic essential elements, properties, and processes naturally occurring in biological organisms.[1–4] Recent successes in engineering design inspired by organisms include snake-like reptation motion,[5,6] the formation of sponges,[7,8] walking on vertical surfaces by geckos,[9,10], micro-lenses by animal eyes and snake thermal receptors,[11,12] and adhesive behavior of marine mussels.[13] Other popular biomedical examples include surface modification to overcome nonspecific protein adsorption, precision immobilization of signaling groups, built-in programmable biodegradation profiles, or sophisticated three-dimensional (3D) supramolecular architectures for diagnostics, healing, immobilization, implantation, and tissue regeneration.[14,15] A number of biomaterials have traditionally been used for biomedical applications, where the need for enhanced in vivo survival rate of implanted materials must be combined with mimicry of biological recognition processes to achieve immunosuppression.

Recently, however, developments in micro- and bioelectronics such as cell-based biosensors, thermal and chemical sensors, bio-mimicking devices, and biochip technologies have emerged.[16–23] Novel demands for long-term cell viability, functionality, and sustainability in artificial environment (unfriendly or hostile nature) such as inorganic electrodes, dry environment under direct light illumination, or synthetic cytotoxic matrices are important for many of these developments. An important difference is that the biomedical applications typically rely on using much more sensitive mammalian cells, which often require only temporary immunosuppressive protection and selective affinity. In contrast, many bioelectronic applications utilize much more robust microbial cells with proper engineered functions (fungi, bacteria, or algae) but require long-term viability under hostile physical and chemical conditions.

A number of comprehensive reviews on biomaterials and their biomedical applications which rely on cell surface engineering have been published including those on naturally derived biomaterials or hybrid self-assembled materials that mimic the phospholipid structure of cell membranes.[24–28] A wide range of prospective applications beyond the more traditional biomedical efforts of cells encapsulated into various media (cell delivery and immobilization, device implantation and wound healing, tissue engineering) are widely considered.[29,30] However, very little has been summarized on recent biomimetic strategies in the design of robustly protected cells to be placed in artificial media for demanding bioelectronic devices. In rare examples, Fakhrullin, Paunov, Lvov, Choi, and their collaborators have recently discussed current biomedical and bioelectronics applications of cell surface engineering principles in excellent reviews with most attention paid to direct layer-by-layer (LbL) assembly of various components on cell surfaces.[31–33]

In this Feature Article we focus on several biomimetic engineering strategies for the fabrication of robust protected cells by mimicking function, morphology, and composition of various natural cell protective means in order to retain essential functions in an artificial environment. We consider very recent (mostly within the past five years) developments in assembling

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traditional and novel synthetic inorganic and polymeric gels, protein and peptide microgels, as well as ultrathin conformal soft shells from synthetic polymers, crosslinked gels, nanoparticles, and natural proteins.

2. Biological and Biomimetic Means for Cell Protection

All biological organisms ranging from the simplest unicellular to the most complex multicellular have essential means to isolate the internal cellular components from the external chemical and physical environment. This cellular protection allows a cell to maintain a distinct internal chemical environment, provides mechanical support, maintains osmotic pressure, regulates transport of biomolecules and ions in and out of the cell, and provides a means for assembly and communication with other cells. The basic component of cell protection in all organisms is a cell membrane that is comprised of a bilayer of amphiphilic molecules. It is well known that cell membranes are indispensable barriers which provide protection and support to sustain various essential cellular and physiological activities (**Figure 1**).[34] Beyond the cell membrane, many types of cells including both eukaryotes and prokaryotes (respectively defined as cells with and without a membrane enclosed nucleus) have additional means of protection, namely a cell wall. In this section, we provide a basic introduction on the composition and structure of cell membranes and cell walls, and discussion of design criteria for biomimetic strategies.

2.1. Cell Membrane Structure and Composition

The cell membrane serves as a selective barrier sensitive to the chemical/biological environment and mechanical stresses. Because of their construction and composition, cell lipid membranes provide the cell with an extensive framework within which components (e.g., membrane proteins) can control intercellular interactions, selective adhesion, and mass transport. For example, cell membranes control signal transduction through receptor-mediated signaling pathways in response to external stimuli.[34] In this way, highly dynamic biophysical and biochemical intracellular signaling may trigger a cascade that stimulates or inhibits internal activities, such as regulating gene expression and establishing cell fate. However, supported by the intracellular skeleton, these very thin (around 5 nm) lipid membranes play a role in the protection of the cell interior from only very modest disturbances because of their extremely low mechanical strength and shear resistance.

Mammalian cells are separated from the external matrix by a thin, fragile, yet flexible plasma membrane (PM) that is only 5 to 10 nm thick. The membrane is held together as a cohesive sheet by lipid-protein assemblies consisting of a bimolecular layer of amphipathic lipids (phosphoglycerines, sphingosine-based lipids, glycolipids, cholesterol), serving primary as a structural backbone and the barrier to prevent random movements of water-soluble materials into and out of the cell.[34] The two leaflets of the bilayer are composed of different types of lipids and different ratio of lipid/protein composition in respect to the type of the cell (cartilage, muscle, liver, etc.) and the state of the cell (malignant vs. healthy). Many cellular activities, including signal transduction, cell division, energy

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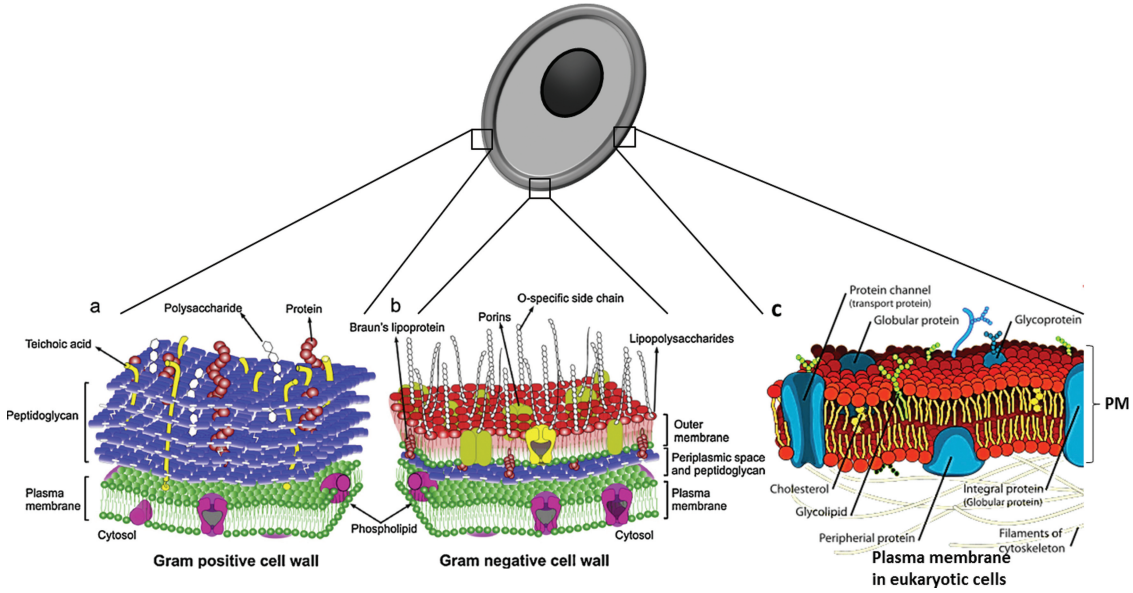


Figure 1. Structure and composition of the cell wall, plasma membrane (PM) and outer membrane in a) gram positive bacterial cells, b) gram negative bacterial cells and c) eukaryotic cells. a,b) Reproduced with permission.[34b] Copyright 2012, Elsevier. c) Reproduced with permission.[35]

transduction, and other various intracellular interactions rely on maintaining a dynamic fluid membrane in order to keep components mobile and capable of coming together to engage in many types of transient interactions.

In contrast, the microbial single-cell organisms utilized in many biotechnology applications including both prokaryotes (bacteria) and eukaryotes (fungi, algae) differ considerably from mammalian cells in their structure and composition. These cells show much higher resilience and hence can tolerate harsh artificial environments. Generally microbial cells are much smaller (usually 1–2 μm for bacterial and 4–8 μm for yeast cells) as compared to mammalian cells (usually >10 μm), shape persistent, and overall are less susceptible to mechanical stresses. A critical component of these cells is the presence of relatively thick and robust protective walls composed of different biomolecular elements (Figure 1). Considering that most of current non-biomedical applications utilize more robust microbial cells, we will focus this review mostly on this type of cells with proper surface engineered functions with, however, few examples mentioning mammalian cells as well.

2.2. Cell Wall Composition of Bacterial Cells

For bacterial cell survivability the presence of the robust but selectively permeable walls in additional to lipid membranes is critically important. Grown microbial cells possess cell walls with fungi, algae and gram-positive bacteria having thicker walls and gram-negative bacteria having thinner cell wall surrounded by an outer membrane (Figure 1).[34,35] There are significant differences in the cell wall structure and composition among the microbial cells themselves, which varies additionally based on the cell's growing stage (germinating, outgrowing and vegetative forms). As the cell matures over the time there is a higher complexity and increasing rigidity of the cell membrane and the wall that is built up, which prepares the cell to be more resilient to the external stresses. These robust cell walls mitigate further processing steps under unfriendly conditions such as cell surface functionalization, cell immobilization, and engineering into artificial media.

The cell wall is the exterior layer, fairly rigid and much stronger than a lipid membrane that usually lies outside of the lipid membrane (Figure 1). It is one of the most important prokaryotic structures because it supports the shape of the cell, helps to protect the cell from osmotic lysis and toxic substances, and it can contribute to pathogenicity. Gram-positive bacteria, named due to the ability to retain the crystal violet stain, have a relatively thick (20–80 nm) homogeneous layer of peptidoglycan (murein) and large amounts of teichoic acids that tether underlying cell membrane with the cell wall. In contrast, gram-negative bacteria, which cannot retain the crystal violet stain, lack teichoic acids and contain only one or two sheets of 2–7 nm peptidoglycan layer covered by a 7 to 8 nm thick outer membrane containing lipopolysaccharides and lipoproteins that are covalently joined to the underlying peptidoglycan layer (Figure 1).[36] These layers enhance the mechanical robustness of cell shape, control intracellular transport, support the shape and membrane integrity, promote the biofilm formation, and control the surface attachment. The geometry, structure and specific interactions among glycoprotein, lipoprotein and lipopolysaccharide molecules create a unique selectively permeable barrier restricting the entry of antibiotics and toxic substances, protecting pathogenic bacteria from hostile environment, or eliciting an immune response from the host.[36]

2.3. Cell Wall Composition of Yeast Cells

The yeast cell walls are chemically simpler than bacterial peptidoglycan. In *Saccharomyces cerevisiae* yeast cells, the cell wall

contains $\beta(1 \rightarrow 3)$-D-glucan, $\beta(1 \rightarrow 6)$-D-glucan, chitin, and mannoproteins.[37] The polysaccharides appear to have a structural function, whereas the mannoproteins may act as "fillers" and are important for controlling the permeability of the cell wall.[38] All components of the wall are integrated by covalent cross-linking, which provide essential rigidity and a continuous phase to the cell wall material. It has been suggested that only compounds of molecular weight smaller than 700 Da are capable of diffusing freely through the rigid walls of *S. cerevisiae* yeast cells after their maturity.[39] Because of its rigidity, the cell wall determines the persistence of the cell shape, protect cells from excessive mechanical stresses, and helps to maintain the cell morphogenesis.

Overall, the presence of elaborate and mechanically robust cell walls enhances the survival ability of microbial cells in hostile environments. Robust protective barriers help to withstand the osmotic stresses caused by concentration gradient of solutes and ions, as well as ionic and pH fluctuations in synthetic environments.[39] Fabrication of biomimetic synthetic walls and protective media for cell entrapment holds numerous advantages to develop semipermeable and robust artificial constructs capable for protecting cells in artificial environment while allowing passage of essential nutrients and signaling cues, all critical for cell integration and functioning in a device-based environment. Even if in most of these approaches chemical composition and organization of natural protective cell walls are not directly matched, their major properties and functions are mimicked in different manners.

2.4. Design Criteria for Cell Surface Modification

It is critically important to mimic essential properties of the extracellular matrix (ECM) with ability to create soft but mechanically-robust, porous but semipermeable with ligand-gated selectively permissive artificial membrane/scaffold that would not interfere with biological functions of the cell. Additionally, to mimic sophisticated intelligence of a plasma membrane (PM) in order to engage specific cellular interactions (cell adhesion, cell migration, cellular differentiation, cell growth, morphogenesis and overall survivability), biochemical signals in the form of certain peptide sequences need to be dynamically present either at the cell surfaces or extracellularly in ECM to provide healthy microenvironment for proper maintenance of a cell biological function. For example, a cell-binding site that contains the sequence arginine-glycine-aspartic acid (RGD) plays a major role in cell attachment and signal transduction at the early stages of cell differentiation in human mesenchymal stem cells, which however has to be down-regulated in order to support correct pathway for chondrogenesis.[40] In other cases, incompatibility of cells with host immune system during transplantation/injection, occurs specifically due to the presence of cell-bound compliment regulator biomolecules. Hence, conjugation of biomolecules to biomaterials, specifically those with low-molecular weight and immunogenicity, and cells can be used to avoid the potential cell-damaging and pro-inflammatory effects.[41]

3. Cell Protection via Media Immobilization

Many traditional methods for cell encapsulation, coating, or entrapment within synthetic polymer or silica hydrogels have been investigated as a means to create protective local environments for cells and cell colonies (**Figure 2**). Microencapsulation in macro scale gel beads,[42–44] emulsification,[45] micro-cell encapsulation using microfluidic devices,[46] discontinuous gradient density centrifugation,[47] formation of injectable bulk gels or sol–gel matrices and templates[48] have all been utilized and studied as methods to entrap and protect cells for different

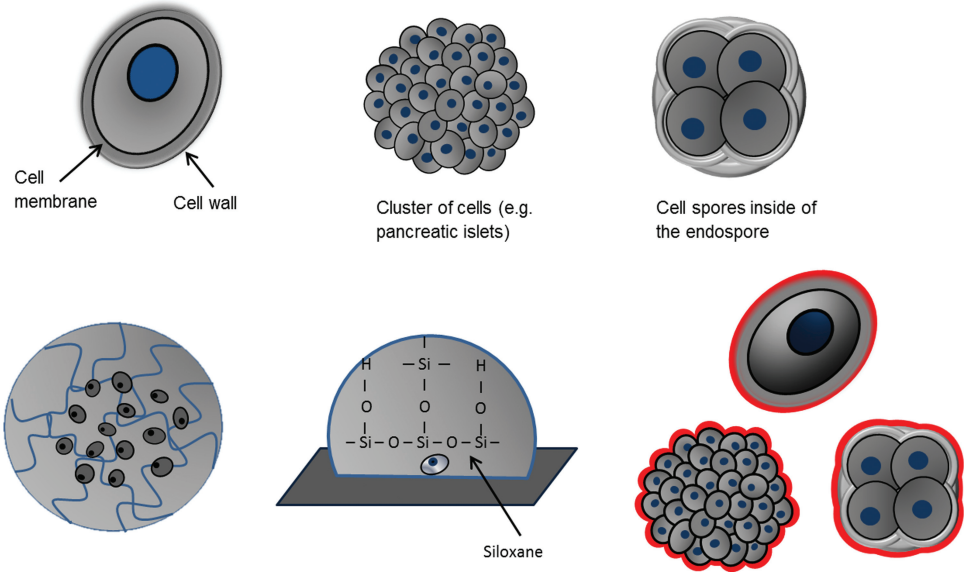


Figure 2. Cell protective barriers that occur in nature (top) and synthetic forms of cell protection (bottom): a) microencapsulation in bulk gel beads, b) sol–gel immobilization on the top of the substrate; c) engineered cell surfaces with thin conformal shells.

applications. Here, we discuss some representative results from recent studies (Figure 2).

3.1. Sol–Gel Silica Immobilization

Alkoxide-based sol–gel silica offers a number of advantages as an immobilization platform for biosensors owing to superior mechanical strength, chemical inertness, hydrophilic nature, and optical transparency (Figure 2).[49–51] However, the hydrolysis and condensation reactions during the encapsulation process usually require acidic or basic catalysts, with the former favoring the formation of a silica network characterized by high porosity with small pores. Since the process requires the use of harsh acidic or basic conditions, the sol–gel entrapment of biosystems is rather challenging. Enzymes may denature during formation of by-products (alcohol) in the gelling solutions, so that most of their activity can be lost upon confinement in sol–gel silica matrices.[52] Additionally, the masking of critical protein active sites might occur.[53]

Despite these challenges successful entrapment of microbial cells (*S. cerevisiae*, *S. aureus* and *E.coli*) has been demonstrated by dip-coating in cross-linkable silica solution.[54] Examples of immobilization of yeast cells and bacteria are not surprising due to the high tolerance of the cells to acidic media and the water-alcohol solvent, with former being significantly more sustained to the conditions necessary for sol–gel process. An aqueous route for synthesis of silica monoliths that uses sodium silicate as a precursor has been developed for more friendly immobilization of biological entities.[51] The approach avoids generation of alcohol and, at the same time, allows encapsulation to be carried out at appropriate neutral pH to preserve biological activity. However, even in this two-step method that makes the process more compatible with enzymes, the enzymatic activity may be only partially retained. The overall shape and appearance of various cells and their clusters can be preserved after encapsulation by the siliceous membranes as has been demonstrated for human fibroblasts by Carturan et al.[49]

Using an alternative approach of combining silica and organic materials, immobilization of several bacteria cells has been successfully demonstrated.[55] Entrapment of bacterial and yeast cells in hybrid sol–gel matrices which combines synthetic and natural polymers such as alginate, peptides, phospholipids, and polysaccharides is widely utilized.[56–58] In the case of cell immobilization with phospholipids, as structure-directing agents, the cell surfaces organize multilayered phospholipid vesicles that help to relieve drying stresses (**Figure 3**a,b).[58] These inorganic-organic matrices maintain cell viability, addressability and accessibility under dry conditions and enable stand-alone cell-based sensing. Complex cellular shapes can be also captured by silica biocomposites and fine details can be further preserved by calcination which, however, results in cell disintegration.[59]

It is important to note that during sol–gel entrapment of cells the lysis of cell membranes may be induced due to possible confinement within the silica matrix and the toxicity of chemical reagents.[55] Additionally, since bacteria in silica gels are entrapped in a confined space, their collective behavior (known as "quorum sensing", a phenomenon that is necessary for bacteria to communicate via small signaling molecules in order to regulate behavior such as luminescence, biofilm formation, and pathogenicity) might be disrupted.[60] Overall, sol–gel encapsulation provides the opportunity to control the density of homogeneously dispersed immobilized bacteria cells. However retaining the long-term viability and proper functioning of encapsulated cells remains a great challenge.

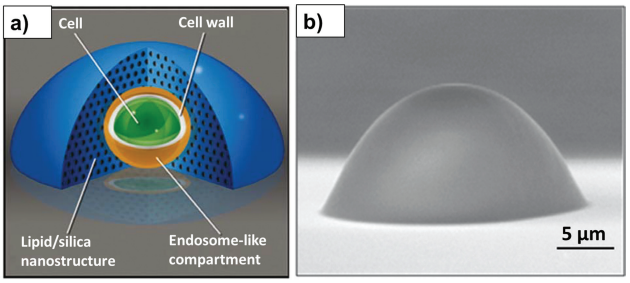


Figure 3. a) Schematic representation of a cell incorporated in an endosome-like lipid vesicle within a surrounding nanostructured lipid/silica droplet deposited on glass substrate. b) SEM image of the lipid-silica hemisphere confining *S. aureus* cell. Reproduced with permission.[58] Copyright 2009, Nature Publishing Group.

3.2. Synthetic Polymer Hydrogels

Polymer hydrogels are widely utilized for cell surface engineering owing to a faster processing time, better control over hydrogel chemistry and properties, cell-friendly functionalities and potential for controlled biodegradation.[61] Usually these hydrogels are loosely cross-linked polymer networks that are insoluble but highly swellable in an aqueous medium and if made from stimuli-responsive materials, can rapidly respond to changes in the external environment.[62,63] Hydrogels based on poly(lactic acid), poly(ethylene)glycol (PEG), poly(vinyl alcohol), meth(acrylates), poly(N-vinylpyrrolidone), or their co-modified macromers offer spatial and chemical control for cell encapsulation.[64–68] By creating complex heterogeneous hydrogels with segments of hydrophilic and hydrolytically degradable groups, with adjustable degree of crosslinking, the relevant macroscopic properties such as swelling rate, mechanical properties, diffusion and degradation profile can be controlled over a wide range to mimic the native extracellular matrix. When applied to cells, PEGylation procedure (formation of PEG coatings, or other biocompatible polymers) has the additional benefit of concealing surface structures that would otherwise trigger the activation of cascade reactions from the immune system and therefore protect encapsulated cells from both humoral and cellular host immunity.[69]

Typically, (meth)acrylate- functionalized biological monomers will react to form gels that cells can degrade through secretion of enzymes, and often the released products have a potent effect on the activity and function of the cells.[70] This ability is important for controlled protection of encapsulated cells during implantation followed by programmed hydrogel biodegradation to complete integration in a biological environment. It has been demonstrated that high molecular weight

hyaluronans can provide initial structural support but are degraded by migrating cells during wound healing events.[71] The low molecular weight fragments can be internalized to influence cell proliferation, matrix secretion, and gene expression. For example, by tuning the chemistry of dimethacrylated tri-block copolymer (polycaprolactone-b-poly(ethylene glycol)-b-polycaprolactone (PEG-CAP-DM), the degradation of PEG-based hydrogels can be controlled to accommodate growth of the extracellular matrix.[70]

Regular synthetic polymers lack any selected recognized functionality, and at best, facilitate non-specific interactions (e.g., charged-driven protein adsorption) that can be exploited with limited control of specificity.[70] Hence, the interest in development of hybrid gels with multifunctional monomers where one group is capable of polymerizing and the other group is capable of selective conjugation to drug, peptides, or protein is essential for creating matrices to promote efficient cell survival, proper morphogenesis and immobilization.[61,72] The bioactive molecules of interest can be either permanently linked or released over time through degradable linkers, depending on the requirements of the application. An example of this type of approach includes the tethering of transforming growth factor beta (TGFβ) to hydrogels through polymerization of monoacrylated PEG conjugates, which were found to increase production of extracellular matrix by smooth muscle cells.[73] Other examples of inserting biofunctionality in naturally antifouling PEG hydrogels include conjugation of acrylate with peptide sequences, specifically those that promote cell adhesion such as the tripeptide RGD sequence, and other integrin-binding chemokines to support attachment, migration and cell differentiation in three-dimensional structure.[74]

Despite the success in cell protection, growth and directed cell morphogenesis with hybrid hydrogel matrices, the processing conditions are frequently far from physiological conditions and detrimental to the encapsulated cells and accompanying proteins. The gelation process, which is usually initiated by harsh external stimuli (low/high pH, salt, temperature, or light) during chemical of physical polymerization, may be toxic to cells.[61] Other disadvantages of synthetic hydrogels may include low mechanical strength that pose significant difficulties in handling and long-term stability,[75] and uncontrollable and/or slow degradation that ultimately leads to mass loss under harsh conditions.[76]

3.3. Natural Hydrogels and Microgels

Naturally forming hydrogels prepared from extracellular matrix proteins such as collagen, fibrin, laminin, gelatin, or polysaccharides such as alginate[77–81] or hyaluronic acid[82] have become alternative choice of hydrogel matrices for less intrusive cell encapsulation.[83,84] Naturally derived materials offer a versatile alternative to synthetic silica and synthetic polymer hydrogels due to a chemical nature more akin to the extracellular matrix structure, which enables the encapsulated cell population to grow, proliferate and exhibit phenotypes more similar to those under in vivo conditions.[85]

Significant efforts have been devoted to demonstrate successful encapsulation of not only bacterial but also more

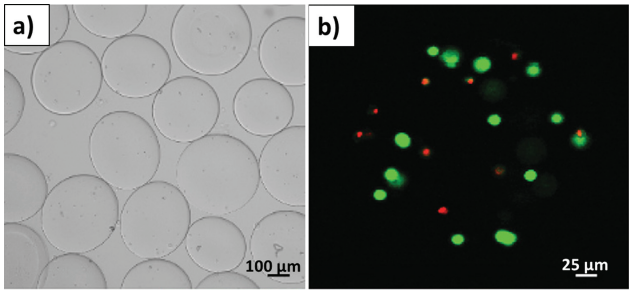


Figure 4. a) Alginate microbeads with human adipose-derived stem cells incorporated; b) Live/dead staining of cells within a single microbead (red = dead cells, green = live cells). Reproduced with permission.[78a] Copyright 2010, Elsevier.

environment-prone mammalian cells in alginate-derived microbeads (**Figure 4**). The successful encapsulation of probiotic microorganisms (such as bifidobacteria),[86] red blood cells with hemoglobin release,[87] pancreatic islets for post-transplantation studies,[88] adipose stem cells,[78] hepatocytes with assessment of proliferation and protein secretion[89] have been reported to date. However, optimization of the encapsulation procedure for long-term cell viability and mechanical robustness as well as controlled cell release still remains challenging.[90] Since a majority of chemical crosslinks and reactive functional groups are cytotoxic and result in non-injectable coagulated gels, physical hydrogels are preferred for many biomedical applications in order to temporary encapsulate multiple cell lines. These hydrogels are also readily injectable for noninvasive cell implantation applications.[91]

Encapsulation of cells in microbeads composed of crosslinked natural gels with relatively small dimensions is considered to be critical for controlling cell release as a result of enzymatic activities.[92,93] It is suggested that a well-defined number of cells and their colonies can be encapsulated in microscopic beads with dimensions of several hundred microns which are appropriate for the synthesis of injectable materials. Special attention must be paid to controlled release of cells from such alginate microbeads by designing more efficient biodegradable pathways with faster release of cells without compromising their ultimate function.[94] In a recent study, Leslie et al. have suggested smaller crosslinked alginate microbeads with diameter below 200 µm for the implantation of viable stem cells with controlled number of cells and pre-programmed cell release.[95] These engineered microbeads were explored to encapsulate rat adipose stem cells. Gradual cell release over many days with retained ability for differentiation after release was successfully demonstrated.

3.4. Peptide and Protein Matrices for Cell Encapsulation

Peptide-based materials for cell encapsulation represent another type of biomimetic matrices. These matrices were designed with several classes of self-assembling peptide materials, including peptide amphiphiles, Fmoc-peptides, self-complementary ionic peptides, and hairpin peptides to name a few.[96] Due to their amphiphilic nature, the peptides assemble into a

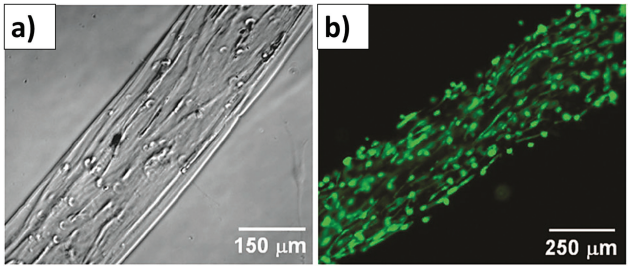


Figure 5. Aligned monodomain peptide amphiphile gels. a) Phase image of human mesenchymal stem cells (hMSCs) preferentially aligned along the string axis. b) Fluorescence image of calcein-stained hMSCs cultured in the aligned PA gel. Reproduced with permission.[96] Copyright 2010, Nature Publishing Group.

variety of supramolecular nanostructures such as spheres, cylinders, and tubes which resemble fiber-like natural materials. The advantage of using such small molecules is their potential for full and fast degradability and formation of shear-thinning gels that can be effectively injected as low-viscosity liquids. Additionally, cell-binding epitopes as bioactive sequences can be incorporated into peptide matrices to control adhesive and cell-differentiation properties. Cellular chambers, pores, channels, and microcapsules of different shapes can be also fabricated from protein hydrogels by using various microprinting and lithography approaches and these designed structures have been exploited for their loading capacity.[97–99]

A more complex and efficient architecture that has recently been reported is the development of lyotropic liquid crystalline peptide amphiphile that assembly as a nanofiber network forming aligned gels.[96] The monodomain gels were formed by inducing a thermally triggered dehydration of peptide amphiphiles into two-dimensional sheets followed by cooling when molecular sheets break into large bundles of aligned nanofibers, which can be formed into long, highly-aligned constructs (**Figure 5**). The resulting structured gels were capable of encapsulating and aligning cells with potential for directional guidance to facilitate axon regeneration across the damaged nerve.

Recently, it has been demonstrated that by mixing of aqueous solutions of oppositely charged peptide amphiphiles and polyelectrolytes, a dynamic assembly of nanofiber bundles can lead to a peptide-biopolymer hybrid membranes or enclosed sacs.[100] These membranes are 2–20 μm in thickness and have two chemically and morphologically distinct faces—one containing amorphous polyelectrolyte and the other displaying highly aligned peptide nanofiber bundles. These structures were shown to release proteins demonstrating a potential to be used as protein reservoirs or cell-isolating containers.

Another example of two-component protein hydrogels based on proline and tryptophan-rich residues was shown to support differentiation and morphogenesis of several cell lines due to injectable and self-healing properties of fast-gelling heterodomains.[91] These macroscopic heterogels may be particularly beneficial because the engineered peptides are recognized in the in vivo environment by additionally incorporating cell-binding domains more than a conventionally synthesized material. Furthermore, the enzymatic breakdown of protein heterogels into amino acids provides the ease for body to be able

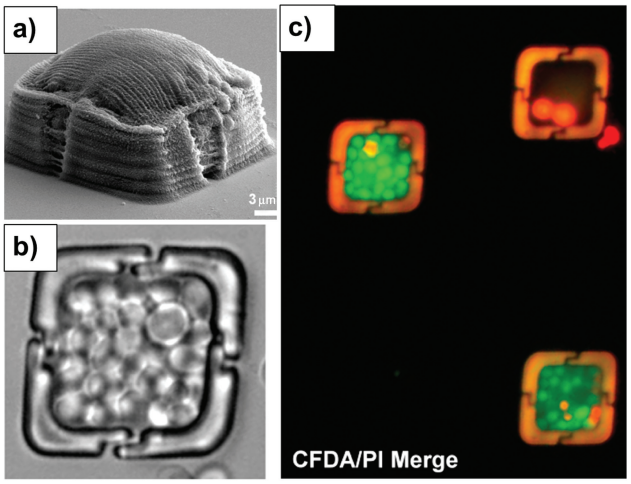


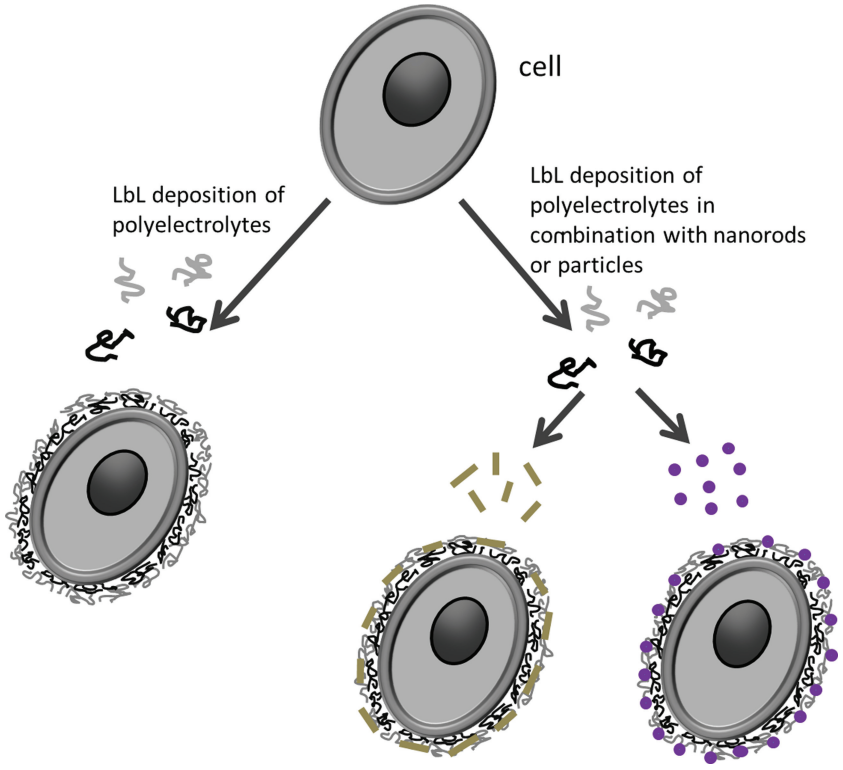
Figure 6. a) SEM image of a protein structure containing a high-density *S. cerevisiae* cell population. b) SEM image of confined colony in microfluidic droplet. c) Viability dye assays of *S. cerevisiae* cells displayed that the majority of cells that divide into dense populations remained viable (green = viable, red = apoptotic cells). Reproduced with permission.[103] Copyright 2012, American Chemical Society.

to use them as building blocks in cellular metabolism. Alternatively, protection of probiotic microorganisms with protein encapsulation has been demonstrated for oral delivery.[101] Picot et al. have developed methods for encapsulation of bifidobacteria in insoluble whey protein capsules. Microcapsules were formed by spray-drying and emulsion processes. Encapsulation of bacteria resulted in increased survivability of bacteria under extreme conditions such as digestive environments.[102]

An interesting example of biocompatible 3D isolation chambers from protein hydrogels for confinement of microbial cells and their colonies has been demonstrated by Harper et al. (**Figure 6**).[103] These microchambers can be fabricated from biocompatible precursors and under biocompatible conditions thus facilitating functioning of cells inside of these enclosures. These closed hydrogel structures can be used for physical protection of cells from hostile environment allowing cells to grow and function inside of closed microscopic space. These chambers from protein hydrogels are shape-persistent and biocompatible and, furthermore, capable of preserving their shape even during cell growth and external deformations.

4. Cell Protection with Ultrathin LbL Shells

Cell surface engineering using bottom-up assembly of ultrathin conformal soft shells has been introduced to overcome some of the issues prominent in bulk cell encapsulation with hydrogel media. Loss of cellular activity after bulk and microgel encapsulation, inflammatory host immune response, transport limitations of nutrients and signaling molecules, and void volume restrictions remain significant challenges for these traditional approaches.[104] In order to minimize these challenges, conformal ultrathin (2–100 nm) protective soft shells with different morphologies, various compositions, and intermolecular



Scheme 1. LbL assembly of oppositely charged polycations (black) and polyanions (gray) components with added nanostructures (nanotubes and nanoparticles) onto living cells.

interactions that facilitate fast transport and provide robust mechanical properties have been introduced and elaborated (**Scheme 1**).[31]

4.1. Synthetic Polyelectrolyte Shells

Over the past two decades LbL assembly has emerged as a powerful and versatile bottom-up approach for engineering diverse organized films with microstructure, morphology, composition, biological, mechanical, and chemical properties readily tailored through cell-friendly assembling routines.[105–107] Well established LbL assembly procedures for cell encapsulation include multiple steps of sequential deposition of "monolayers" of oppositely charged components onto the negatively charged cell surface. This sequential addition of material provides the ability to fabricate multilayered ultrathin soft shells with predefined number of layers (hence thickness) from a wide range of different components (including polyelectrolytes, proteins, and nanoparticles) (Scheme 1).[31]

Assembled through traditional alternating LbL assembly, ultrathin multilayered films are clearly different from naturally occurring cell membranes yet may be designed to confer similar protective and transport functions.[108] Through incorporation of naturally occurring polymers,[109] proteins,[110] nucleic acids,[111] liposomes,[112] biologically active nanoparticles,[113] or polymers with bioactive motifs[114] LbL shells provide wide opportunities for cell surface engineering.[108] The advantages of this approach include conformal coating of complex shapes, a precise control of the shell thickness, variable porosity and mass transport, enhanced mechanical resistance, and wide tunability of membrane functionalities and properties, as well as masking the cells immunologically invisible.[105,114] The ability to tailor transport and mechanical properties of these synthetic walls is of particular importance for encapsulation of living cells as cell viability and long-term shelf life critically depends on the diffusion of nutrients and waste release through the artificial polymer membrane.

The poly(allylamine hydrochloride)/poly(styrene sulfonate) (PAH/PSS) LbL films are the mostly explored polyelectrolyte pair were initially exploited to encapsulate cells.[115] PSS/PAH microcapsules were shown to exhibit pH-controlled responsive changes in size and porosity at extreme acidic and alkaline conditions.[116,117] Despite successful examples of the LbL-based cell encapsulation with polyelectrolytes, cytotoxicity from their polycation components poses severe limitations for this approach as a routine tool in cell surface engineering.[104,118] Moreover, the sensitivity of mammalian cells to polycations is more prominent compared to microbial cells since they lack effective cell wall machinery that helps cells to withstand membrane disruption.

Indeed, the cationic materials adsorbed on cell membranes during LbL assembly of PSS-PAH (and similar) polyelectrolyte shells can cause pore formation followed by cell damage as has been demonstrated by leaking of lactose dehydrogenase.[104] On the other hand, decreasing molecular weight of polycationic chains mitigates cell membrane disruption and facilitates the LbL shell formation without adverse effect on cell functioning. Furthermore, the cytotoxicity of these LbL shells can be significantly reduced by reducing cationic content or by using (if possible) cells with developed walls. For instance, the dramatic reduction in the cytoxicity can be achieved by decreasing the polycation charge density through proper grafting of PEG chains (**Figure 7**).[104,108] LbL shells from certain PEG-containing components have been shown not to interfere significantly with the viability of pancreatic islets. Composition, reactivity, thickness, and mechanical properties of such nanocomposite shells can be readily tailored by changing composition.

On the other hand, it seems that the viability of cells encapsulated in traditional LbL shells with significant cationic content is significantly higher if shells are assembled on more mature, stationary-phase cells, when they are functionally equivalent to spores. At this stage, matured cells drastically reduce their metabolic activity and hence increase their resistance to many environmental stresses.[31,119] However, the functionality of cells at this stage becomes very limited and thus the applicability of these protected cells in biosensing can be limited.

Introduction of metallic nanostructures into LbL shells is a well-known reinforcing approach in LbL technology.[105,120–123] Such an approach has been reported for LbL-modified cells as

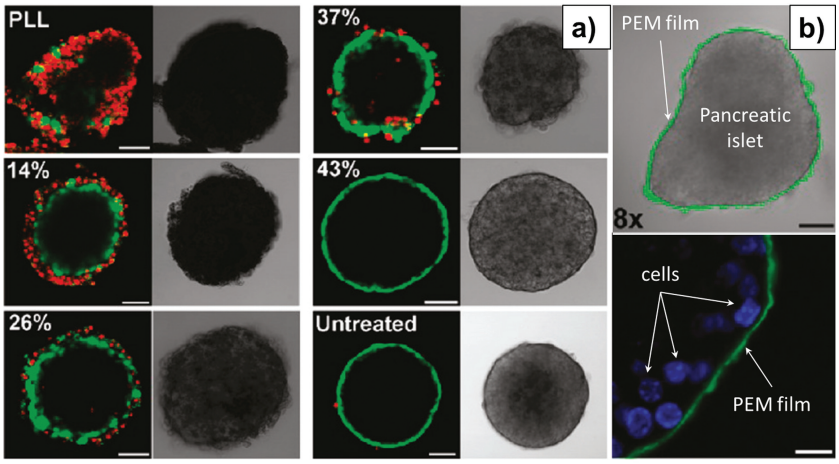


Figure 7. a) Polycations with enhanced cytocompatibility can be designed by tailoring the structure of PLL-g-PEG copolymers. Confocal and bright field micrographs of pancreatic islets stained with calcein AM (light grey, viable) and ethidium homodimer (dark grey, nonviable) after incubation with poly(L-lysine) copolymer with variable degrees of PEG grafting (scale bar: 50 µm). b) Confocal micrographs overlaid on bright-field images of islets coated using PLL copolymer and flourescein-labeled alginate (F-Alg) with eight bilayers. F-Alg is localized on the extracellular surface of cells, confirming the cell-surface-supported nature of films. Reproduced with permission.[108] Copyright 2011, American Chemical Society.

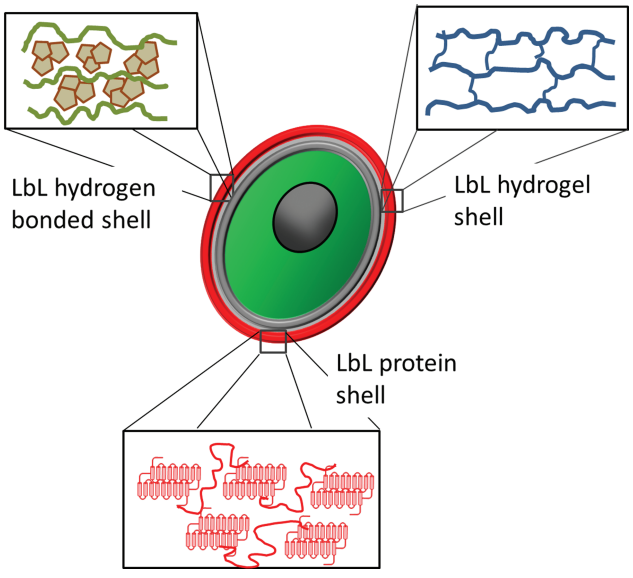
an effective mean to increase strength, enable cell conductivity, make cell magnetic, and control shell permeability. These nanostructures usually form dense and robust monolayers on bacterial cells with grafting density controlled by the LbL assembly procedure.[124,125] Proper placement of usually toxic nanoparticles on the outside of functionalized shells or inside of LbL shells prevents or limits their adverse effect on cell functions.[31] Silica nanoparticles inside LbL shells were shown to very modestly affect cell growth.[126,127] Carbon nanotubes and graphene flakes have been incorporated in LbL shells to improve robustness and induce conductivity of modified cells without compromising cell viability.[128,129] Finally, magnetic nanostructures have been utilized in LbL shells to enable the placement and controlled flow of magnetically modified cells with external magnetic field without significant effects on mature cell with developed walls.[130,131]

4.2. Synthetic Hydrogen-Bonded LbL Shells

Hydrogen-bonded LbL structures represent new opportunities for cell-friendly LbL shells of "soft" type with more cell-compatible components and assembly procedure (**Scheme 2**). The advantage of having such LbL shells assembled via non-covalent hydrogen-bonding interactions instead of traditional polyelectrolyte shells is that their micromechanical properties can be well controlled by changing pH, ionic strength, light conditions, salt concentration, or temperature.[132,133] However, many of the hydrogen-bonded LbL shells studied to date have been demonstrated to be unstable under certain conditions during cell processing that compromise mid-term stability of these shells and hence must be specifically addressed.[133] On the other hand, by adjusting conditions these shells can be controllably disassembled thus facilitating pre-programmed degradability.

The hydrogen-bonded LbL shells from biocompatible functional polymers such as PEG can be appropriate for cell surface engineering.[14,104] Another example of a non-toxic and biocompatible component is poly(N-vinylpyrrolidone) (PVPON) for these shells that can facilitate the conversion of hydrogen-bonded LbL shells to ultrathin hydrogel shells with responsive, low-fouling or/and biodegradable properties.[134] Tannic acid has been used to assemble hydrogen bonded shells with polymer counterparts during LbL assembly due to the presence of numerous terminal hydroxyl groups.[134] TEM analysis of freeze-dried cells further established the integrity of the cell membranes and hydrogen-bonded LbL shells (**Figure 8**). Binding tannic acid with several synthetic polymers has been shown to be pH-stable within a wide range from pH 2 to pH 10 with permeability properties inversely dependent on molecular weight in contrast to vast majority of hydrogen-bonded LbL shells based on poly(carboxylic acids).[134] High biological activity of tannic acid including antioxidant, antimicrobial, and anticarcinogenic properties can be screened by other shell components protecting the cells from oxidative damage.[135] Successful formation of hydrogen bonded LbL shells was also facilitated by primary poly(ethylene imine) (PEI) pre-layer which increases stability of the shells.

Cell surface engineering with hydrogen-bonded LbL shells with inclusion of tannic acid as a critical component preserved long-term survivability of the encapsulated cells with cell viability reaching 79% in contrast to ~20% viability for conventional PSS/PAH shells assembled on younger cells.[136] This



Scheme 2. Different compositions and morphologies of novel LbL shells for cell surface engineering.

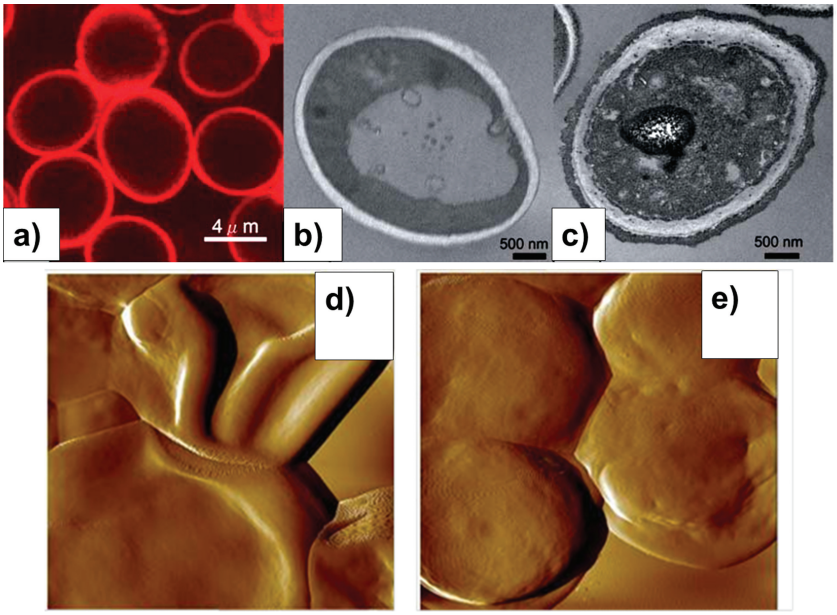


Figure 8. a) Confocal images of YPH501 *S. cerevisiae* yeast cells encapsulated with four bilayers of PEI(TA/PVPON)$_4$ before yEGFP expression with fluorescently labeled PVPON-co-Alexa Fluor 532 (Ex/Em = 543/560) as the top layer. TEM images of b) freeze-dried bare and c) PEI(TA/PVPON)$_6$-coated YPH501 yeast cells. Reproduced with permission.[136a] Copyright 2011, The Royal Society of Chemistry. AFM images of d) bare and e) LbL coated YPH501 yeast cells. Reproduced with permission.[118]

high cytocompatibility originates not only from the minimal exposure of the cells to toxic polycations, but also from highly permeable and more porous LbL shells favoring an easy access of nutrients to cell interior unachievable with traditional polyelectrolyte LbL shells. The pore sizes can reach several tens of a nanometer as has been obtained from fluorescent recovery after photobleaching experiments.[118,137,138]

Beyond cell viability, the function activity of cells in response to inducer molecules that simulated biosensing routine was shown to be higher for cells encapsulated in hydrogen-bonded LbL shells compared to electrostatically-assembled LbL shells. As a result of induction of the reporter protein, a significant increase in fluorescence intensity was observed from green fluorescent protein (GFP) for encapsulate yeast cells proving that these shells do not interfere with metabolic activity.[136] Depending on the thickness of the shell, their chemical composition, and the nature of interactions within the shell, the shell can delay cell growth to varying degrees but does not suppress the cell division. In fact, the cell proliferation eventually results in the budding cells rapturing the surrounding polymer shell (**Figure 9**). Furthermore, eliminating cationic PEI pre-layer resulted in truly non-ionic hydrogen-bonded LbL shells with high stability.[118] The absence of the cationic PEI pre-layer also caused the further increased viability of encapsulated cells.

4.3. Crosslinked Hydrogel Shells

The advantage of ultrathin hydrogel shells is their highly permeable network state, which is controlled by the crosslinking density and pH-responsive behavior.[137] The responsive nature of the hydrogel allows active control of cell function and growth in response to the changes in external pH environment. The formation of chemically-crosslinked permeable LbL hydrogel shells from amine-functionalized poly(methylacrylic acid) (PMAA-*co*-NH_2) has been reported.[138] Shells fabricated in this study contained a very low fraction of crosslinkable amine groups which facilitated a highly swollen state in aqueous environment. *S. cerevisiae* yeast cells encapsulated in such pH-responsive synthetic nanoshells demonstrated high viability rate of around 90% indicating high bio-compatibility of synthetic shells capable of permeating nutrients and waste into and out of cells through the porous shells. Such high survivability level of encapsulated cells was attributed to the high compliance of the porous hydrogel shells and very minor content of a cationic component.

Moreover, cell function can be mediated by the shell pH responsive physical properties. By keeping encapsulated cells above the pKa of the hydrogel system, the budding and replication cycle were effectively

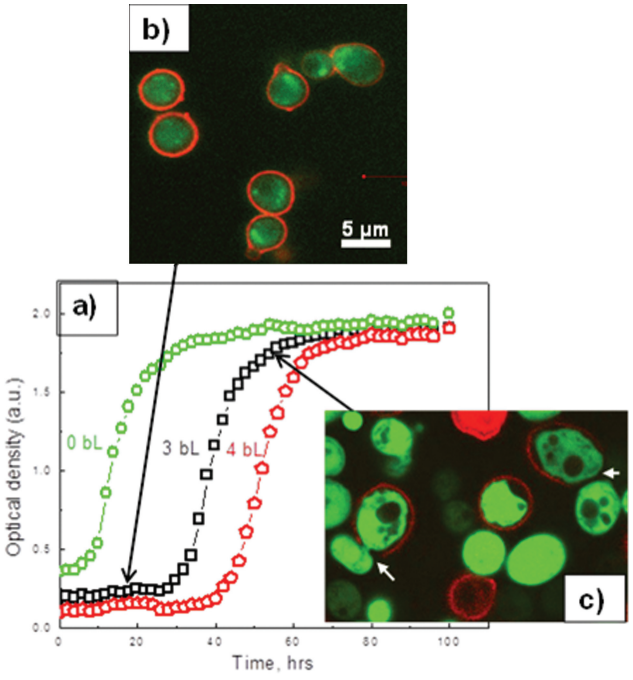


Figure 9. a) Growth of PEI(TA/PVPON)$_0$,$_{(3)}$,$_{(4)}$-coated YPH501 cells after yEGFP expression was induced. b,c) Confocal microscopy images of PEI(TA/PVPON)$_3$-coated cells after b) 10 h and c) 46 h of the yEGFP expression. White arrows represent breakage of the polymer shell due to formation of budding daughter cell. Reproduced with permission.[136a] Copyright 2011, The Royal Society of Chemistry.

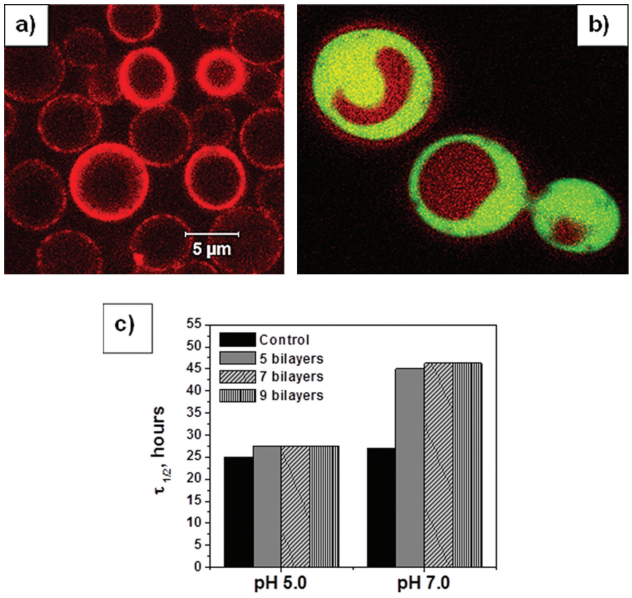


Figure 10. (PMAA-co-NH_2)$_9$ coated *S. cerevisiae* yeast cells kept in media adjusted to pH 7 a) before and b) after induction of GFP. c) 50% growth time of (PMAA-co-NH_2) coated yeast cells with different shell thicknesses as a function of pH. Reproduced with permission.[138] Copyright 2012, American Chemical Society.

delayed by about 24 hours as compared to encapsulated cells kept below pKa (**Figure 10**).[138] Such a strong effect of the presence of (PMAA-co-NH_2) shells on the functionality of encapsulated cells at higher pH can be associated with pH-triggered changes in the state of free carboxylate groups and ultimately increased shell thickness. Progressive deprotonation of carboxylic groups above pH 5 increases the degree of ionization and drives the surface potential to significantly higher net negative charge that resulted in almost doubled thickness of the shell wall. As a result, increased surface charge and thickness efficiently reduced transport of nutrients through the shell thus damping the replication process without inhibition of biosensing activity of the encapsulated cells. In this sense, pH-responsive LbL shells behave as an active ultrathin barrier that controls the growth behavior and metabolic function of cells. The ability to manipulate perceptible response from the cells by keeping them in "dormant" conditions (constrained replication) for extended time can be rewarding for biosensing applications with consistentlong-term performance.

4.4. Natural Proteins For Cell Shells

While encapsulation of a wide variety of cell types in a range of natural and synthetic polymers has been demonstrated, the successful encapsulation of living cells in protein shells represents a particularly unique challenge for various biotechnology applications. The motivating factors for this effort include the ability to reengineer the surface of the cell with proteins allowing for direct control of interactions with the extracellular environment and to create more biocompatible encapsulants for applications involving long-term in vivo implantation.

LbL films and capsules from proteins are well known for many years.[139–141] However, LbL approaches for the formation of protein shells around cells prove to be more challenging and only few examples have been demonstrated to date. Microcapsules have been fabricated from a range of proteins including hemoglobin, bovine serum albumin, and human serum albumin (HSA).[142] HSA capsules were formed by covalent crosslinking with L-a-dimyristoylphosphatitic acid. Hemoglobin capsules which retain activity were formed by crosslinking with glutaraldehyde. Cell shells from various natural materials have been assembled as well as show reasonable stability and high biocompatibility. Natural polyelectrolytes (e.g., polylysines), polysaccharides, DNA, and oligopeptides have been assembled on various cells, mostly for biomedical applications.[109] Encapsulation of living cells in such shells has proven more difficult due to the harsh crosslinking processing used which affect cell functions. Recently developed protein LbL shells from silk proteins that are suitable for cell encapsulation are discussed below.

4.4.1. Silk Fibroins for Shells

The excellent mechanical properties, availability, and biocompatibility of silk proteins have made them attractive candidates for biomedical and biotechnology applications.[143,144] Silk fibroin, isolated from the cocoon fiber of the *Bombyx mori* silkworm, consists of a heavy and light chains covalently linked through a disulfide bond. The heavy chain portion of the protein is divided into twelve crystalline regions separated by random coil regions of 30–40 amino acids in length. Advances in the processing, purification, and fabrication of silk materials have led to its use as a scaffold to support cell growth in tissue engineering studies and as a drug delivery material.[145] The recent ability to fabricate silk materials of various geometries including films, sponges, mats, and fibers has resulted in the reemergence of silk as an advanced biomaterial because of its slow degradation, excellent mechanical, micrfabricaiton, and optical properties,[146,149] and biocompatibility.[147,148] Numerous studies have proven the biocompatibility of silk substrates in supporting the adhesion and proliferation of mammalian cells.[149–151]

Silk microcapsules and microparticles are excellent candidates for delivery platforms, composite materials, and cell encapsulation.[152] Silk-based materials have also been demonstrated to stabilize the activity of enzymes and biomolecules in harsh environments, allowing for the potential to deliver treatments that may otherwise quickly lose efficacy.[153] However, to date, only a few examples of utilizing silk for the fabrication of microgels and cell shells have been reported. Hermanson et al. have reported fabrication of capsules by interfacial adsorption of ADF-4 synthetic spider silk proteins.[154,155] The Kaplan group has also demonstrated formation of silk nano- and microspheres using lipid vesicles[156] controlled gelation of silk solutions using sonication and vortexing.[157,158] The introduction of shear forces in solution helps promote the formation of β-sheet crystallites resulting in gelation. Cells can be introduced in the solution to yield encapsulated cells.

4.4.2. Silk LbL Microcapsules

More recently, biocompatible, ultrathin, highly porous silk microcapsules with ultrathin and tunable shell thickness and controlled permeability have been reported.[152] "Silk-on-silk" LbL assembly included silk adsorption from aqueous solution onto silica cores, followed by washing in water and redispersion in methanol to induce formation of a β-sheet-rich structure and stabilize the adsorbed silk layer, thus resulting in robust LbL shells stabilized by physical crosslinks (β-sheets) and hydrophobic–hydrophobic interactions. The next silk layer was further deposited in the same way and the procedure was repeated multiple times. The silk microcapsules show good stability and high permeability which is readily controlled by the thickness of the silk shells.

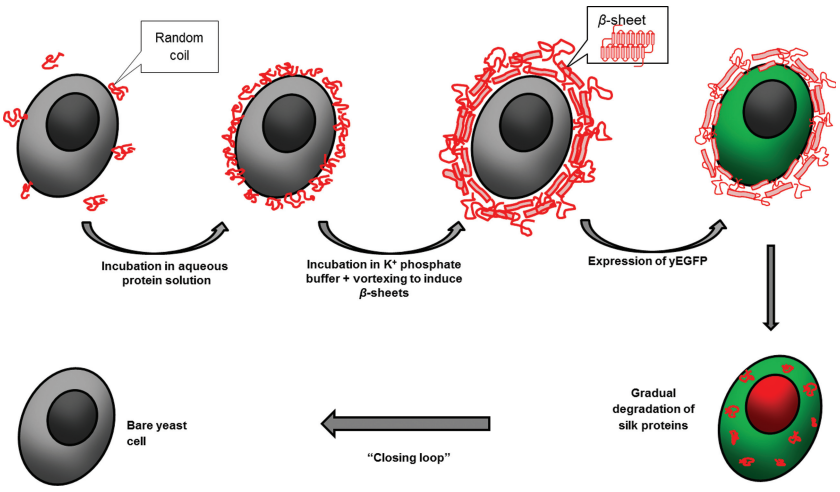


Figure 11. Silk-on-silk LbL deposition to form silk II rich shells on the surface of the yeast cells, followed by expression of yEGFP after formation of silk nanoshells; and gradual biodegradation with internalization of silk proteins and returning to its original bare state. Reproduced with permission.[161]

This method was later extended to functionalized silks and to promote the assembly of extremely robust and pH-responsive LbL microcapsules from silk fibroin counterparts modified with poly(lysine) and poly(glutamic) acid, which are based on biocompatible silk ionomer materials.[159,160] The microcapsules are extremely stable in a wide pH range from 1.5 to 12.0 and show a significant degree of reversible swelling/deswelling response in dimensions, as exposed to extreme acidic and basic conditions. These changes are accompanied by reversible variations in shell permeability that can be utilized for pH-controlled loading and unloading of large macromolecules. Finally, it was confirmed that these shells can be utilized to encapsulate yeast cells with a viability rate much higher than that for traditional synthetic polyelectrolyte LbL shells.[159]

4.4.3. Protection of Cells with Silk Shells

Another study demonstrated that stable silk protein shells can be locked in at the cell surfaces by inducing secondary transition from water-soluble Silk I to non-soluble β-sheet-rich Silk II by salting-out in a proper ionic mixture complemented with shear-thinning effect (**Figure 11**).[161] Gentle ionic treatment allowed stabilizing the shell protein structure without compromising the cell function/activity, as indicated by fast response of the encapsulated cells, with an immediate activation and synthesis of GFP protein by the inducer molecules. Very high viability rates (up to 97%) along with preserved metabolic activity of "silked" cells were achieved due to a greater cytocompatibility of the processing method along with highly porous and ultrathin silk nanoshells, which are rarely achieved with synthetic polymer shells. The ability of cells to bud (produce daughter cells) after the process of encapsulation was not affected and showed no changes in the ability of cells to replicate, with a small delay (by two hours or about 10% change) in reaching the maximum rate of log phase. Additionally, it has also been demonstrated the non-cytotoxic nature of silk nanoshells compared synthetic composites. Accumulation of yeast enhanced green fluorescent protein (yEGFP) production in response to the inducer molecule further confirmed weakly affected cell function with insignificant (only a few hours) lag (**Figure 12**).

It has been suggested that in contrast to synthetic polymer shells that shown to be slowly degraded extracellularly,

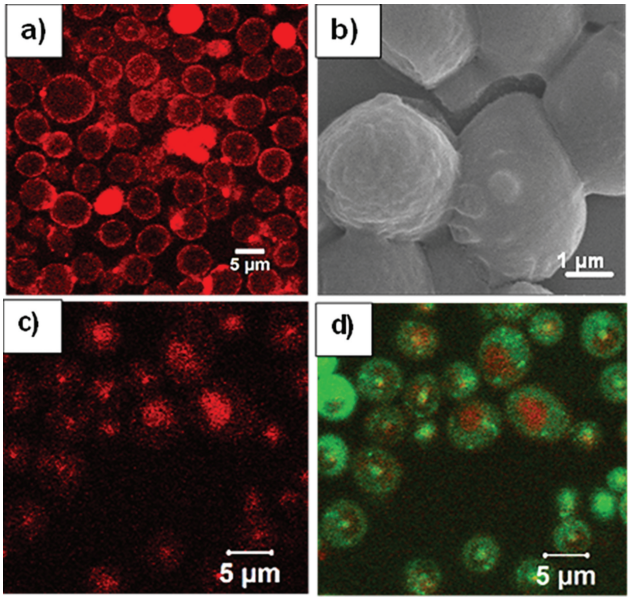


Figure 12. a) Confocal images of yeast cells encapsulated in silk shells as visualized by fluorescently labeled silk (silk-*co*-Alexa532) deposited as the top layer; b) SEM images of yeast cells encapsulated in $(silk)_8$ shells and treated with K^+ phosphate buffer; c) represents internalization of fluorescently labeled silk (red) inside of the cells after 25 h of GFP induction; d) represents colocalization of green and red filters, with yEGFP expression (green) within the interior of the cell and fluorescently labeled silk (red). Reproduced with permission.[161]

these protein coatings go through fast biodegradation intracellularly. The process of fast degradation begins not until the cells reach mid of the log phase in their growth behavior at the expense of partial degradation of silk during exocytosis of waste products (mainly CO_2, and ethyl alcohol). Under these conditions silk protein undergoes secondary structure transition to β-sheet secondary structure. Along with active extracellular enzymatic activity, cells might further promote digestion of silk protein, and endocytosis of degraded protein fragments (Figure 12). Thus, after initially serving as protective nanoshell, silk coating undergoes fast biodegradation and digestion, leaving behind mature cells with full functionality and no trace of the initial shell. Hence, the biodegradable silk nanoshells can be considered as an efficient way to provide short-term protection for the cells during implantation without affecting their metabolic activity.

5. Examples of Applications of Protected Cells

The demand for design of protective cell coatings that are robust, semipermeable, tunable to the external stimuli and effective for transport finds a number of novel applications. Robust living cell-based biosensors with long-term shelf life gain significantly in high real-time sensitivity detection in response to a target analyte,[162] protection from ex vivo environment,[163] and high viability/activity.[118,137,140]

Cells encapsulated in microbeads, microgels, or shells have been considered for shelf storage, selective adhesion to various substrates, selective transport of toxic substances, sustainability under excessive mechanical stresses and osmotic pressure, external field control of location and growth of cells, and enhancement of intercellular communication. Prospective nano- and biosensing applications of regular and genetically modified bacterial cells include detection of various toxic, explosive, and hazardous substances reported through corresponding changes in their optical appearance (e.g., fluorescence) or electrical (e.g., conductivity) properties in linear or logic circuitry manner.[162,164,165] These demanding applications usually require programmable and living arrays of multiple cell lines arranged in an organized manner on electrodes.[165,166] Interactions of cells with various substrates with different surface functionalities well beyond traditional electrodes (such as various flexible synthetic polymer and biopolymer substrates) must be further controlled by modification of their composition and affinity.[167]

The formation of cell colonies covered by and interconnected with LbL membranes can be considered as a means for controlling intercellular communication, potential conductivity of cell colonies, and cell aggregation.[168] Conductive cells with metal nanoparticle embedded in outer LbL shell can be a basis for thin film transistor biosensors with conductivity of the cell layer in this microelectronic device mediated by the environment conditions such as humidity or analyte presence.[169,170] Novel bio-adsorbents and biocatalytic materials have been suggested from cells modified with magnetic nanoparticles which can be separated from complex mixture by external magnetic field.[171]

6. Conclusions

Biologically inspired materials with tunable bio- and physicochemical properties provide an indispensable platform to actively control and support cell functionality in vitro and in vivo. Cell surface engineering with application of various cytocompatible protective, mediating, functionalized media comprised of inorganic, polymeric, biological, and nanoparticlulated microgels, microbeads, and shells dramatically increase cell viability and stability in synthetic environment and expand their applicability in biomedical, biotechnology, and bioelectronics fields. However, traditional inorganic and organic gel media do not readily support a number of modern applications because of very slow diffusion of nutrients or analytes into bulky and low porosity matrices, slow and no biodegradation of these materials, high enzyme resistivity, modest long term viability, slow response time, and questionable compatibility with microelectronic devices. Polymer and protein microgels and microbeads as media for cell encapsulation are appropriate for many applications because of several factors related to downscaling of their dimensions. High injectability of low viscosity dispersions, faster diffusion of analytes and nutritions, higher rate of biodegrabaility and cell release, and dimensional compatibility with microdevices all are critically important for future developments.

Finally, ultrathin protective LbL shells and microfabricated chambers from compatible synthetic polymeric or natural materials can be considered as the best choice for a wide range of applications. Proper selection of chemical composition and assembling conditions often dramatically increases the viability of encapsulated cells and increase their stability, robustness, and resistance to the external disturbances and aggressive environment. On the other hand, the minute thickness of these shells (well below 100 nm) and highly controlled permeability allow for fast diffusion of external substances, biodegradability, conformal behavior, cell release from shells at well-defined conditions, and abrupt changes in physical properties.

Some limitations of current cell surface engineering approaches to be considered for demanding applications and to be further addressed in future studies include passive/non-selective mass transport across shells, passive/non-selective adsorption and adhesion of modified cells, limited tunability of shell properties, and cumbersome and costly processing conditions. Inserting active biological and synthetic elements into cell shells such as active channels, tightly controlled pores, adding surface bioactive groups, shape supporting/forming shells, actuating and reconfigurable shells, compartmentalization of encapsulated media, or adding proper intracellular elements, all should be considered for further exploration. Eventually, a path to assembling of robust, sustainable, long-living, and functioning artificial cell-like, cell-mimicking, and cell-replacing mesoscale structures can be thought and might be reported in distant future.

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