

# Organism–Materials Integration: A Promising Strategy for Biomedical Applications

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In nature, organisms play an essential role in harnessing elements to produce materials. Being precisely integrated with the biological structures, the materials confer organisms with various unique functions such as protection, recognition guiding, biocatalysis, etc. Inspired by this phenomenon, elaborately designed materials can be grafted to different organisms such as cells, eukaryotes, and viruses via artificial incorporation strategies. Herein, progresses upon the methods and techniques of organism–materials integration are discussed, including spontaneous formation, artificial enhancement, and genetic engineering. The integration of organism and materials can alter the biological behavior and even offer the organism rationally designed functions, facilitating the biological applications of organisms in the field such as vaccine improvement, biomedical therapy, and biomedical imaging. These unique effects achieved by the combination of organisms and materials propose a new strategy for providing precise control over organisms. These promising strategies also offer new perspectives of biology and chemistry development, and show great potential in future biomedical therapy.

synthesized by small proportion of proteins and calcium carbonate, the shell of shellfish feature with a hierarchical and stratified structure, which denoted 3000 times harder than synthesized one. Inspired by such phenomenon, materials can be grafted to other organisms by artificial incorporation, which can provide organisms, such as cells, eukaryotes, and viruses, with optimized biological behavior or even rationally designed functions.<sup>[1]</sup> In general, co-culture or mixture of materials and organisms can result in the biological changes.<sup>[2]</sup> Nevertheless, the integration of materials and organisms represents an accurate process control, by which materials should be first synthesized according to the organism, either biologically or biomimetically, and further precisely integrated with the organism for regulation in return. For example, even though nanoparticles that are widely investigated for biomedical

## 1. Introduction


In nature, organisms can harness elements to synthesize hybrid materials for their own use, which improve their original metabolism or provide nonoriginal functions to the organisms. For instance, shellfish can produce a shell mainly consisting of calcium carbonate to protect its soft body from predators. Compared with normal monolithic calcium carbonate that is

applications are capable of delivering and controlled release of bioactive entities,<sup>[3]</sup> their influence are still limited to local interaction as compared with strategy of organism–materials integration, as they do work on their own rather than a combination with the organisms. We suggest that the integration is based upon three aspects: chemical or physical interactions, structure integration, and functional coordination.

In the past decades, advances have been made upon organism–materials integration, in the hope of endowing organisms with artificial functions. The integration of materials and organisms is mainly based upon microscale interactions, for instance, chemical bonds or noncovalent interactions.<sup>[4]</sup> Small molecules of chemical entities with specific function and target are widely used in modulation of organisms, however, to date, they are not capable of linking with organisms in microscale, which results in limited modulation of function due to the relative instability, poor interfacial interaction with organisms, and lack of interfacial features. In contrast, materials especially nanomaterials with high surface energy and unique structures could be facily deposited upon or swallowed by organisms. Moreover, compared with small molecules, materials are larger bulks, thereby the robustness and stability of the modification are basically guaranteed. In this case, the performance of materials may outstand simple small molecule with respect to long-term modification. Nonetheless, although numerous materials are considered to be biocompatible, the integration capability of the materials is still challenging and more attempts are needed. Materials might have toxic effects and nonspecific binding,

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and when this happens, a combination of small molecules and materials are suggested.

With respect to organisms, they are promising in environment remediation,<sup>[5]</sup> energy harvesting,<sup>[6]</sup> and most importantly, they show great advantage in biomedical field,<sup>[7]</sup> as they have the characteristics of versatile biological functions, large-scale preparation, and homogeneity. However, the extensive application of organisms alone still faces the obstacle of instability, hard collection, and tedious management process, due to the lack of efficient engineering strategy. With the aim of conferring organisms with on-demand functions, the biomimetic strategy is suggested through integration of materials that has specific chemical or physical characteristics such as reductivity, photoresposibility, etc. Thus, the characteristic of materials could furnish functionalities both in vitro and in vivo without doing harm to the original metabolism of the organism.<sup>[8]</sup> For example, a rebuilt red blood cell (RBC) loaded with different functional material cargos, such as doxorubicin (DOX), Fe<sub>3</sub>O<sub>4</sub> nanoparticles and ATP biosensors, could perform various functions such as oxygen delivery, drug delivery, magnetic manipulation, and toxin biosensing.<sup>[9]</sup> The transportation of the engineered cells was boosted in a controlled manner using these materials. Except for modifying the instinct functions of organisms, material–organisms integration might to some extent produce “new species”, which present noninstinct properties. For instance, a material-coated alga can produce hydrogen rather than oxygen through photosynthesis; and an implanted particle inside cell can act as organelles. In this case, we believe these results could shed light on deeper insights of organisms and their evolution process. According to nature evolution process that materials in environment may lead to natural selection, the artificial integration of materials with organisms might lead us to new aspects of co-evolution of material and creatures.

To date, the organism–materials hybrid is composited using three types of materials including inorganic minerals, metal complex, and polymer. In general, the integration process undergoes spontaneous incorporation, but sometimes when the interface properties are not suitable, artificial assistance could be applied. For example, a natural diatom’s cell wall is capable of direct silicification; however, in case of yeast cells, it should be coated with polymers to enhance the surface affinity to silicon precursors for shell formation.<sup>[10]</sup>

Together, in this review, we first discuss the recent advances regarding the integration of materials and organisms including cells, viruses, and eukaryotes. We principally focus upon the mutual interactions and the functions thereby produced by materials-integrated organisms. In the second part, we summarize several useful techniques for fabrication of organism–materials hybrid, including spontaneous formation, artificial enhanced integration, and genetic engineering. Then, we use specific examples to further clarify the strategy and the effect caused by the materials in the form of shells, bulk hydrogel, and intracellular scaffolds. The applications of organism–materials hybrids in biomedical fields including vaccine improvement, biomedical therapy, and biomedical imaging are emphasized, which will provide a better understanding for control over the organisms by materials. Finally, we present a brief summary and outlook of such research field. We believe organisms engineering by materials integration as a newly developed area and will provide a promising strategy for

biomedical use of organisms involving storage of biological products, therapy, and vaccination.

## 2. Basic Methods for Organism–Materials Integration

Due to their fragility, materials integration toward organisms should be operated carefully. To retain their bioactivity, the reaction conditions involve temperature, pressure, reagent toxicity, ion concentration, buffer environment, sustaining time, and biodegradability should be concerned.<sup>[11]</sup> The synthesizing conditions related to high temperature and high pressure should be avoided. In addition, the efficient coordination forces between organisms and materials must also be considered. In the recent decades, chemists and biological scientists have developed various strategies for achieving the materials incorporation, which show no clear harm to the original organisms. As far as now, the spontaneous integration method is mainly used for simpler systems that requires less treatment. The materials integration strategy could also be expanded to organisms that are not suitable for spontaneous integration using more complex components. With the help of artificial assistance, materials that are not suitable for the organisms can be introduced, and even multiple material components can be grafted to the organisms. Furthermore, genetic engineering is also used to provide organisms with variety of materials binding sites. These three commonly used methods involving material–organism interaction are introduced as follows.

### 2.1. Spontaneous Integration

In some cases, organisms, in particular for whose interfaces contain abundant active biomolecules, can offer appropriate binding sites for materials or precursors integration. Simply adding these materials or precursors will result in the interaction between materials and organism. The materials or precursors including polymer materials, mineral ions, and nanoscale particles could interact with the organisms via different binding forces, for instance, electrostatic forces, hydrogen bond, hydrophobic force, covalent bonds, etc.<sup>[12]</sup> The interacting process in which materials directly assemble to the organisms without additional modification is called a spontaneous integration process. Based on the types of precursors that interact with organisms by different interaction forces, spontaneous methods are divided into ionic binding, self-assembly and formation, as well as layer-by-layer (LBL) methods.

In general, organisms can produce inorganic components with ordered and functional structures, for example, bones, nacre, teeth, egg shells, etc.<sup>[13]</sup> Under the regulation of biomolecules, mineral materials can obtain structures with unique morphology and function, which are far distinct from chemically synthesized materials.<sup>[14]</sup> This process is usually called biomineralization process. Briefly, the organisms that have abundant biomacromolecules act as a template for mineral deposition. In a general understanding of biomineralization, both ionic binding and self-assembly strategy follow this principle. Metal ions, with positive charge, bind to negatively charged biomacromolecules, such as peptides, polysaccharides, proteins, and nucleic acids, especially those rich in carboxyl group.<sup>[15,16]</sup> The

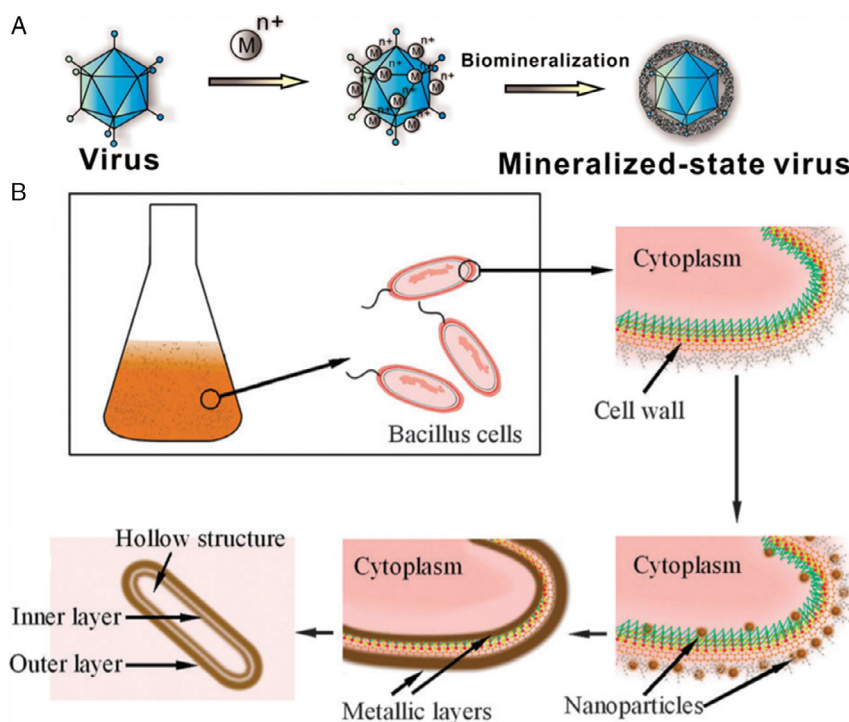
coordination can enrich inorganic ions, and when the ions are in the saturated state, they lead to the spontaneous formation of minerals upon the biomacromolecule's surface. In addition, to well control the nucleation, crystal growth, phase transformation, orientation, and particle assembly procedure, the introduction of molecular additive or stabilizer is suggested.<sup>[17]</sup> For example, triethylamine (TEA)-stabilized calcium phosphate (CaP) cluster has been synthesized, which promoted epitaxial growth of CaP crystals that are close to enamel.<sup>[18]</sup>

To date, only a few species of viruses and cells have been verified to exhibit the ability to form mineral shells spontaneously via direct ions absorption. Calcium ions are commonly discovered in nature and living creatures. Moreover, calcium ions have strong interactions with carboxyl groups, making them ideal candidate for mineral deposition. Thus, the organism–minerals integrates can be prepared via ion absorption and in situ mineralization. Japanese encephalitis vaccine (JEV) has been modified to form a CaP shell in this way. Because the viral surface displays lattice of negatively charged carboxyl-rich proteins, which lead to the aggregation of calcium ions onto the viral surface and promote the in situ nucleation of minerals upon the virus (Figure 1A). This shell presents heat-resistance property and thus preserves the stability of virus under room temperature.<sup>[19]</sup> Cell membranes and cell walls can also serve as nucleation sites for mineral deposition. In a previous research, Pd ions was introduced both in and out the cell wall of a bacteria, *Bacillus*. Attracted by the negatively charged carboxyl and phosphate groups on the cell wall, Pd ions were enriched and reduced by adding a mild reducing agent, therefore forming Pd nanoparticles that were detected at the inner and outer surface of the cell wall (Figure 1B).<sup>[20]</sup>

In summary, through the enrichment of metallic ions onto organism interfaces and the following spontaneous mineralization, a simple process of organism–mineral integrate could be facily constructed.

Self-assembly and self-formation emphasize the spontaneous integration of material units with the organism. Unlike ionic binding strategy, material monomers or nanoparticles that consist of multiple ions or functional residues can recognize the organism and form ordered structures as a consequence of specific interactions among the components themselves.<sup>[21]</sup> For example, researchers use Ag nanoparticles stabilized by positively charged polymer as the cell-engineering reagent. By simply shaking the mixture of materials and *Escherichia coli* for 15 min, the materials can assemble onto the bacteria's surface with nearly no side effects.<sup>[22]</sup> Polydopamine (PDA) is a wide-spread coating material for nanoparticles and shows good biocompatibility and modifiability. Choi and co-workers have raised a strategy to use dopamine monomers to directly synthesize a PDA shell on cell surface, because dopamine tends to be oxidized in mild basic solutions that dissolved O<sub>2</sub> from the air.<sup>[23]</sup> It is suggested that the monomers and oligomers of dopamine interact with the yeast cell surface by catechol and amine groups, resulting in the formation of a uniform polymer shell upon the cell.

Recent years metal–organic framework (MOF) materials, which belong to metal coordination complexes, have received more and more attention, and this type of materials is also applied to improve organisms.<sup>[24]</sup> Metal complexes are generally “minerals”, whose anions are mainly organic molecules containing coordinating groups such as carboxyl, hydroxyl, etc. The MOF materials generally displays porous network structures,



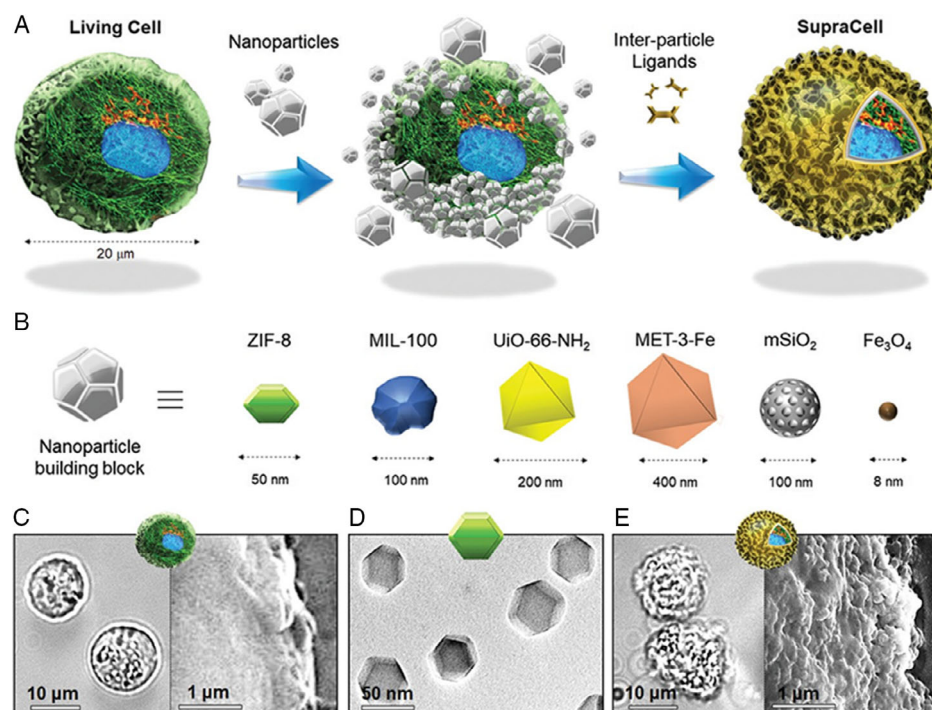
**Figure 1.** A) Schematic of virus biomaterialization via an ionic binding strategy. Reproduced with permission.<sup>[1a]</sup> Copyright 2012, Wiley-VCH. B) Schematic of the introduction of Pd nanoparticles and double metallic layers to individual *Bacillus* cells. Reproduced with permission.<sup>[20]</sup> Copyright 2012, Royal Society of Chemistry.

compared with dense and solid mineral materials, and might provide better permeability. However, unlike mineral salts, MOFs are constructed by coordination bonds, and have multiple metal ion centers. In this case, to form its ordered structure, most MOF materials tend to derive from nanoparticles rather than ions as building units for the integration, namely, they undergo a self-assembly process to link with organisms. For example, ZIF-8 is a star candidate for integration with organisms, due to its precursors' water solubility and low biological toxicity. The tobacco mosaic virus (TMV) has been encapsulated using ZIF-8. Similar to polymer materials that self-formed on the surface,<sup>[25]</sup> ZIF-8 nanoparticles absorb to the virion in the early stage and then link with each other by the growth of imidazole network, leading to the formation of a universal shell upon its surface. Falcaro's group has also applied ZIF-8 for cell improvements.<sup>[26]</sup> A galactosidase inner layer was first constructed upon the yeast cell surface, which have enzymatic effect to support the cell in environment containing low nutrients, while the outer shell is composed by self-assembling of ZIF-8 on the enzyme layer, which provides an extra shielding effect. Except for MOFs, other nanoparticles such as silica and  $\text{Fe}_3\text{O}_4$  can similarly self-assemble to integrate with cells (Figure 2).<sup>[27]</sup>

Polymer materials can be spontaneously deposited onto organism surfaces. Integration of organism with polymers and biomacromolecules can confer the organism with stabilization coatings or targeting guides.<sup>[28]</sup> In the past decades, polymer materials, such as polyaminoacids, polylactic acid (PLA), polyethylene glycol (PEG), etc., have been improved with stimuli-responsive features, recyclability, and biocompatibility,<sup>[29]</sup> therefore provided vast numbers of candidates for organism modification. The

designed polymer materials can interact with organisms by non-covalent forces, such as electrostatic force, hydrogen bond, and hydrophobic force.<sup>[30]</sup> The most significant advantage of polymer materials is its designable nature. Properties such as molecular weight, charge, geometry, and cross-linking degree can be adjusted through synthesis, thus a designable and more compatible organism–materials hybrid could be produced. Alternately, deposition of polymeric materials onto organism surface can produce a shell structure with controllable thickness and outermost charge. The process of repeated stacking of multiple polyelectrolytes layers is generally called LBL strategy, which has been widely used in construction of nanocapsule due to its biocompatibility and flexibility.<sup>[31]</sup>

In general, the LBL technique use polymers with opposite charges, which interact with each other mainly by electrostatic forces. Meanwhile, other forces including van der Waals forces, hydrophobic/hydrophilic forces, and hydrogen bonds may also contribute to the formation of LBL layers.<sup>[32]</sup> In some cases, the innermost material layer will have toxic effects upon the organism. However, with the development of LBL strategy, the biocompatible polymer layers can be prepared for organism coating. For instance, natural polymers like alginate, gelatin, hyaluronic acid, and synthesized polymers such as poly(diallyldimethylammonium chloride) (PDADMAC), poly(allylamine hydrochloride) (PAH), poly(sodium 4-styrenesulfonate) (PSS), and polyaminoacids have been widely applied in recent studies because of their biocompatible and easy-acquiring features.<sup>[33]</sup> With respect to the spontaneous integration of materials to organisms, LBL method generally produce a polymer shell with alternate polymer layers with opposite charge, and accordingly



**Figure 2.** A) Schematic of the formation of materials shell upon living cell using self-assembly of nanoparticles; B) Various nanoparticles as building blocks. C–E) Characterization of native cells, material nanoparticles, and material-coated cells, respectively, using electron microscopy. Reproduced with permission.<sup>[27]</sup> Copyright 2019, Wiley-VCH.



the hybrid of organism and material can acquire robustness improvement. For example, gelatin and alginate were chosen as the composition of LBL layer, because under neutral pH, gelatin with isoelectric point of 7.0–9.0 and alginate with isoelectric point of 5.4, display opposite charge.<sup>[34]</sup> Therefore, the negatively charged neural stem cells can be generally encapsulated by alternatively incubating with gelatin and alginate with opposite charge after mild washing process (Figure 3A). The obtained shell shows protecting functions against physical forces. In addition to LBL layer, hydrogel is also a choice for organism encapsulation when using polymer materials. The crosslinking net structure of gel can trap solvent in the net.<sup>[35]</sup> Gels usually have stronger mechanical properties, better biocompatibility, but larger size, which can even reach to the size of bulk materials. In other case, nanoscale hydrogel can be obtained. The macroscale hydrogel can be readily constructed to form large bulk materials, benefiting for loading with organisms via aforementioned forces. The amplified scale offers new applications of loading cells such as tissue regeneration, cell manufacture, and immunotherapy.<sup>[36]</sup> These spontaneous incorporation strategies are simple and efficient for organisms engineering.

## 2.2. Indirect Integration

Afterall, organisms in nature only display certain degree of affinity to material precursors, and most of them prefer only a few kinds of materials. For instance, a diatom cell shows perfect affinity with Si, but is not likely to interact with most of metal precursors. To better enhance the integration, the materials or molecule can be applied as “bridges” to enhance the interaction between designated materials and organisms in achieving the design. In this case, the introduction of transition layers in-between via LBL method, single-layer adsorption, covalent binding of molecules that can enhance charge density or enrich functional groups on the outer surface are suggested for the modification of organisms.<sup>[37]</sup> For example, because of Si-rich living environment, natural diatoms evolve to produce SiO<sub>2</sub> shell on their surface to retain more light from outside. However, without certain surface proteins, the cyanobacteria cannot synthesize their own SiO<sub>2</sub> shell under the same conditions. Tang and co-workers used LBL method to introduce bridge layers onto the cyanobacteria.<sup>[38]</sup> PDADMAC and PSS were chosen as the polycation and the polyanion, respectively. By assembling these bilayers with sixfolds, the outermost surface of the cells presents stronger affinity with the Si precursors via electrostatic forces, and SiO<sub>2</sub> shell was facily obtained under room temperature (Figure 3B). The coated cyanobacteria retain photosynthetic activity, and because of the SiO<sub>2</sub> shell, their photoinhibitory effect have been largely reduced.

LBL method offers an efficient method to enhance binding forces step by step, but the process is proved to be time-consuming. When the binding forces are sufficient for materials integration, single transition layer can also realize the modification as the LBL methods. Yeast cells cannot spontaneously silicify in the solution of tetraethyl orthosilicate (TEOS), which provides SiO<sub>2</sub> precursors. In this case, a “bridge” layer containing an elaborately designed peptide was used.<sup>[10]</sup> The peptide, namely R<sub>4</sub>C<sub>12</sub>R<sub>4</sub> (R: arginine; C: cysteine), in which arginine residue

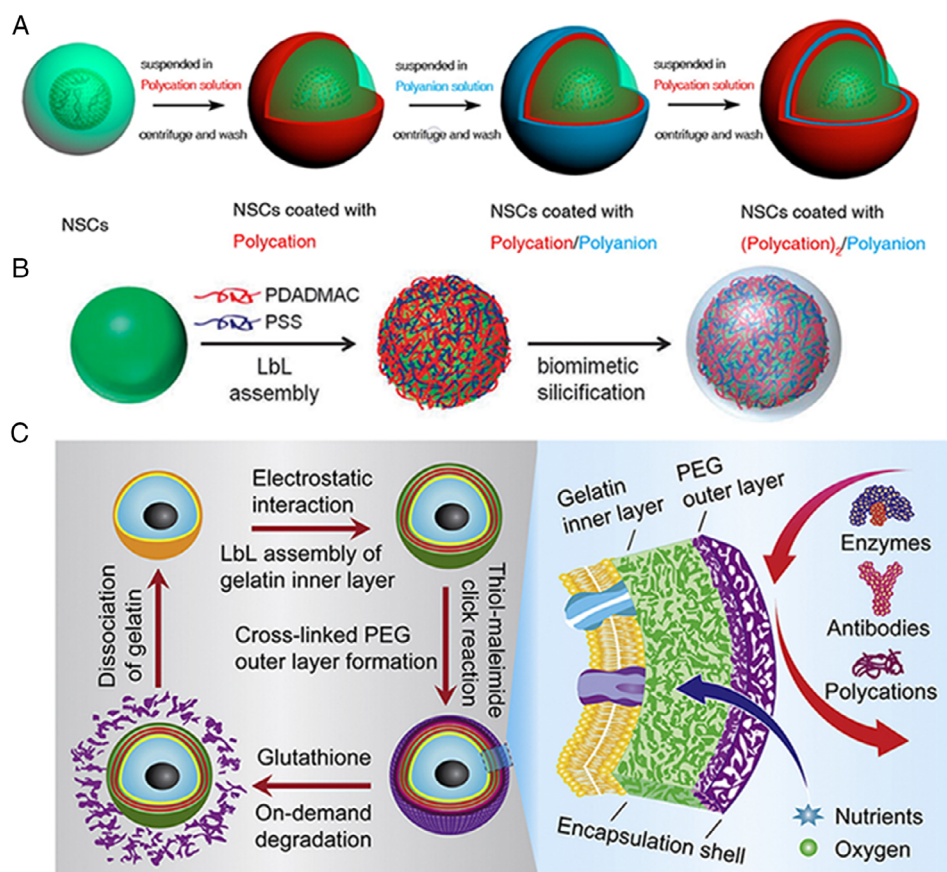
displays positive charge to link with the cell surface and the thiol-rich cysteine sequence mimics the TEOS hydrolysis site that was derived from natural silicatein- $\alpha$  protein, managing to integrate the outer SiO<sub>2</sub> layer and inner yeast cell together.

Unlike the universally used intermolecular forces, covalent bonds are much stronger and more stable, thus are more preferred when long integration sustaining period is required.<sup>[39]</sup> However, due to direct chemical binding to the organism, the instinct biological properties of the organism should be carefully examined and maintained. Chen and co-workers encapsulated single mammalian cells by in situ polymerization.<sup>[40]</sup> N-acryloxysuccinimide (NAS) dissolved in dimethyl sulfoxide (DMSO) and water was used to modify the cell surface, which results in acryloylation of cell membrane. Then monomer acrylamide (AAM) and glycerol dimethacrylate (GDMA) as a cross-linker were added and the in situ polymerization reaction was initiated to produce a shell-comprised polymer network. The cell's viability was about 75% due to the toxic effects of DMSO and initiators. This strategy is to some extent toxic, but the in situ chemical reactions still show the potential for cell modification due to its site-specific interaction and high yields.

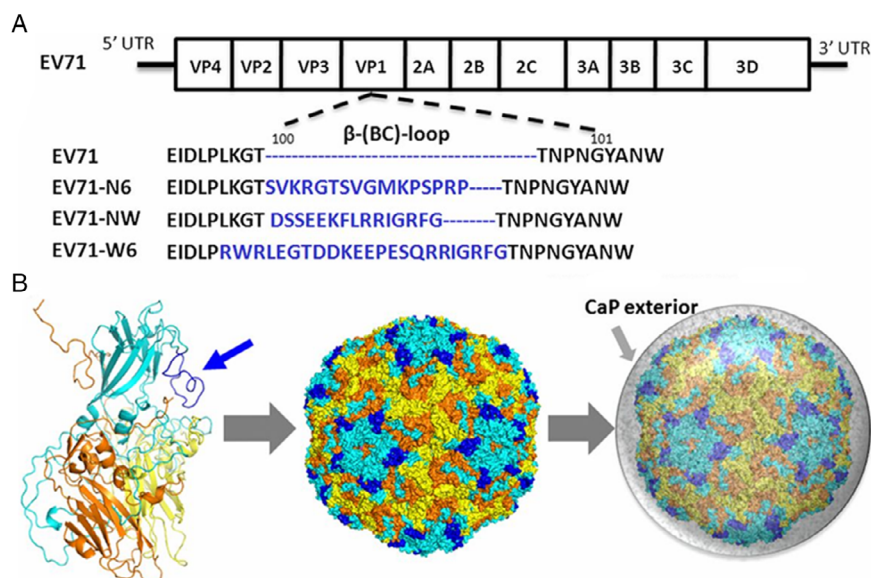
For now, a combination of different interaction forces has been gradually increased. For example, Huang and co-workers fabricated a sandwich-like structure on the surface of *Chlorella pyrenoidosa* cells.<sup>[41]</sup> First, a PDA layer was coated onto the cells through oxidative polymerization. This layer acting as a “bridge” can connect the cells and promote the formation of hydrogen bonds by interacting with thiol or amine moieties of laccase. Without the PDA layer, laccase would not have enough interaction force to bind to the cell wall. At last, tannic acid (TA) was introduced as the outmost layer through quick covalent reaction with the laccase to form a sandwich-like three-layer shell outside the cells. In another example, researchers developed a mild method for encapsulating a collection of mammalian cells.<sup>[42]</sup> The inner layer was formed by LBL technique that was composed with opposite-charged gelatin, whereas the outer layer contained cross-linked PEG that was covalently introduced through thiol-click chemistry, which provided a simple shield without doing harm to the cells (Figure 3C). The combination of chemical modification and biological interaction will provoke the artificial regulation of organism via materials hybrids.

## 2.3. Genetic Engineering for Integration

According to biological understandings, the instinct materials hybrid structures produced by organisms are ultimately controlled by genes. The example of diatoms that can produce SiO<sub>2</sub> shells are related to the transcription and translation of their genome with no exception. Scientists have been looking for strategies to regulate organisms, artificially evolve them or even create new species, which is beyond simple modifications. The combination of engineering and materials integration may help to realize such goals. A few efforts have been made to control the organisms' affinity with materials using gene engineering. Enterovirus 71 (EV71) virus has been genetically modified to self-mineralize CaP shell upon its surface, thus virus can acquire a protection against high temperature (Figure 4).<sup>[43]</sup> Two types of protein residue including a phosphate chelating N6p and



**Figure 3.** A) Self-assembled LBL method. Reproduced with permission.<sup>[34]</sup> Copyright 2015, American Chemical Society. B) LBL-mediated silicification of cyanobacteria. Reproduced with permission.<sup>[38]</sup> Copyright 2013, Royal Society of Chemistry. C) Schematic of mammalian cell encapsulated within multilayer shell. Reproduced with permission.<sup>[42]</sup> Copyright 2017, Elsevier.



**Figure 4.** Design and engineering of EV71. A) EV71 genome and the insertion site of the  $\beta$ -(BC)-loop of VP1. B) Homology model of the mutant viral protein, the engineered virus, and the biomimetalization of EV71 with CaP shell. Reproduced with permission.<sup>[43]</sup> Copyright 2013, National Academy of Science.

calcium chelating NWp and W6p were first chosen. The related nucleotide segments were cloned into genome of the virion, and the recombined virus retained its original infectious property. Apart from forming common shells upon organisms, intracellular materials with organelle-like properties could be fabricated in this way as well. Cui and co-workers have improved protein's magnetic response using genetic engineering method.<sup>[44]</sup> The modification was done by inserting a ferritin encoding segment to HEK 293T cells. The product is an improved protein added with more ferritin residues, which are peptide segments containing structured Fe binding sites based upon N-coordination and hydrogen bonds.<sup>[45]</sup> In nature, most magnetogenetic proteins do not contain enough iron, thus they are not sensitive enough to respond to weak magnetic fields. In contrast, these protein productions perform nine orders higher affinity to magnetic field when they were self-mineralized in the presence of iron, and this property showed no clear decline in the cells, making it a potential strategy for the remote control of cellular functions. Though attempts have been made, genetic engineering is in no way a simple strategy. It still faces problems such as gene mutations, side effects such as unpredictable change in organism character, complex characterization methods, etc. With deeper investigation of genomics and proteomics, genetic engineering might become a more practicable strategy that will promote the development of organism–materials integration.

### 3. Integration Strategies

To endow organisms with functions they do not possess in natural state, outer modifications such as material shells and inner materials integrations such as artificial organelles are commonly used strategies to achieve the goal.

#### 3.1. Interfacial Integration

Cell surfaces are rich in proteins and polysaccharides, which can serve as nucleation sites and specific binding sites for materials modification. Therefore, materials are generally integrated in the form of shells for improvements in cells. In addition, due to size and surface proportion of virus, virus surface proteins are more active than cells', which means they are even more suitable binding sites to metal ions, and similar shell could be fabricated upon virion as well. According to their degradability and repairability, material shells could be classified as static shells and dynamic shells.<sup>[46]</sup> Meanwhile, recent approaches of loading cells inside gel-based three-dimensional (3D) bulk matrices also represent a promising strategy for organism–materials integration. The aforementioned three aspects would be discussed briefly in the following parts.

##### 3.1.1. Static Shells

Static shells are condensed layer structures upon organisms. As regulators to the organisms, this type of shell only provides spatial functions, neither strongly interfere with the organism metabolism nor the environment. So static shells are generally robust protecting materials against outer stimulus.

Inorganic materials and metal complexes are commonly used as components for static shells, while a few polymers could have similar properties. Static shells have been first fabricated about a decade ago, and during the following years, quite a lot of attempts have been made by scientists. Materials, such as SiO<sub>2</sub>, silica–titania,<sup>[47]</sup> CaP, MOFs, graphene,<sup>[48]</sup> and polymers including natural biomacromolecules and artificially synthesized polymers have been incorporated to the cell surface, which mainly result in protecting effects against outer harsh environments, but can also have other functions such as conductivity, recognition, growth-promoting, etc. In the following paragraphs, we list several static shells upon cells and discuss about their functions.

Eukaryote cells are sturdier to harsh conditions and are widely chosen as templates for static shell synthesis. Protection robustness and duration periods have long been a topic for cell encapsulation.<sup>[49]</sup> Back in 2008, Tang and co-workers have deposited CaP upon yeast shells by introducing LBL bridge, which displayed protection properties from zymolyase.<sup>[50]</sup> This work shows the great potential of functional material shells upon organisms. ZIF-8 has also been applied to construct the protection shell for yeast cells, the porous structures of which can defend harmful reagent outside.<sup>[26]</sup> Recent studies using shells based upon microdroplet also gave strong protection to yeast cells from the entry of Fe<sub>3</sub>O<sub>4</sub> nanoparticles and the digestion of enzymes.<sup>[51]</sup>

Recently, yeast cells were coated with two layers of materials—the inner layer consists of cysteine-coated Au nanoparticles and the exterior layer constitutes porous SiO<sub>2</sub>. Such materials layer can protect the yeast cell from hostile environments including UV light, high temperature (≈40 °C), and lyticase.<sup>[52]</sup> Cystine residues undergo stronger interaction with the cell wall than those of polymers used in LBL methods, avoiding the chance of being damaged or losing interaction under harsh environments, thus the shell performs robustness and long durability. In addition to protecting the cells from outer harmful conditions, such static shells can offer additional functions to the cells.<sup>[53]</sup> Porous silica shell in outside layer of cells can selectively permit the passage of small-molecule nutrients to support cell survival, meanwhile exclude cell-damaging enzyme with relatively bigger size. Due to the multiple cycles of treatment, the formed shell is more condensed, which shows clear advantage for molecule selectivity. Moreover, as Au nanoparticles only display a weak response to electric field, graphene layer can be deposited onto yeast cell to boost its conductivity, which can produce a robust and electro-controllable cell.

Other eukaryotes such as alga are also modified to change its instinct response to photosynthesis. For example, CeO<sub>2</sub> shells upon algae *C. pyrenoidosa* was fabricated by a one-step self-assembly method to protect it from increasing ultraviolet (UV) irradiations.<sup>[54]</sup> Under strong UV light, the photosynthesis of algae could be suppressed, and the concurrent of reactive oxygen species (ROS) may together be fatal to the organism. In such alga–CeO<sub>2</sub> hybrid, most UV light can be filtered by the CeO<sub>2</sub> shell, and a small amount of ROS was also eliminated through redox reaction with the shell, leading to the protection of organism from UV light damage.

In another study, shells made up of mesoporous SiO<sub>2</sub> was facilely formed upon *E. coli* DH5α bioreporter strain 1598 to construct an efficient in vivo As sensor.<sup>[55]</sup> Such enhanced green fluorescent protein (EGFP) expressing bacteria can detect As



species in vitro; however, with respect to in vivo condition, the responses to As are eliminated by the immune system. In contrast, the SiO<sub>2</sub> encapsulated *E. coli* can efficiently trace As species in vivo because the materials layer blocks the recognition by phagocytes, the RAW264.7 cells, via antigen–antibody combination, therefore the shield effect of materials layer denotes the potential for in vivo application.

Efforts have also been done to take advantage of harmful effect of the materials shells. In nature, the periodic bloom of cyanobacteria poses threats to water resources and ecosystems of bloom area. Except for the vast volume of cyanobacteria, buoyancy is also a critical factor as dense cover upon the water surface causes oxygen deficit in deeper water. To solve this, an environmental-friendly organism–materials integration method is proposed (Figure 5C,D). In the presence of PDADMAC polymer, silica nanoparticles can directly assemble upon the cell wall of cyanobacteria to deposit a SiO<sub>2</sub> shell, which leads to the precipitation of the algae and reinforcement of further aggregation.<sup>[56]</sup> Field testing proved it as an efficient strategy for environment control.

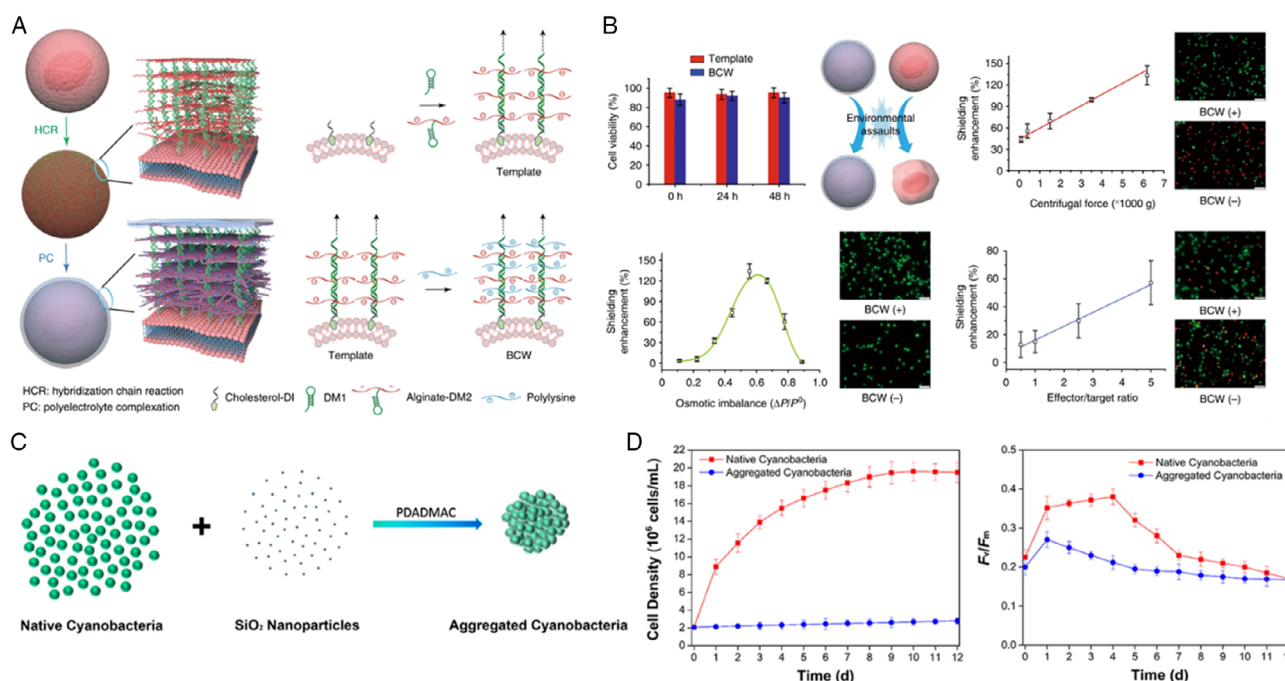
Protection of mammalian cells has received more and more attention, as it has been considered as one of the most challenging and applicable system. However, lacking the cell walls, an extra shielding layer, the mammalian cells are fragile to chemical modifications. Hence, materials selection and synthesizing route are limited as compared with eukaryotes. Similar to the CeO<sub>2</sub> shell mentioned earlier, when applied to mammalian cells, the self-assembly method might be harmful to the cell membrane. In this case, an alternative route such as LBL assembly of CeO<sub>2</sub> nanoparticle and alginate upon insulin-producing beta cells was developed. The biocompatible alginate as a bridge layer

enables immobilization of the CeO<sub>2</sub> nanoparticles and reduction in cytotoxicity. This hybrid performs anti-ROS property, providing an approach for protection of implants.<sup>[58]</sup>

Mammalian cells coated with solid and robust materials shells usually face the challenge of limited molecular transport, which limits their further applications. It has been pointed out that a cell wall is not a simple polymer layer but a structured matrix.<sup>[59]</sup> To mimic this matrix structure to the largest extent, researchers have designed a DNA framework with ordered steric structures rather than linear structures, which has been used as a template for further assembling of polymers. Alginate and polylysine were then filled in the matrix to form a biomimetic cell wall (BCW), which could provide physical robustness and long-period protection (Figure 5A,B). Notably, the strategy used the two selected polymers as examples; in principle, other biocompatible polymers that can undergo crosslinking reactions can also be applied.<sup>[57]</sup>

Zhu et al. recently developed a versatile method for integration of several kinds of materials to HeLa cell surface. In the experiment, MOF, Fe<sub>3</sub>O<sub>4</sub>, and mesoporous SiO<sub>2</sub> were self-assembled upon cell membrane, rendering a quick and simple procedure for modifying the cell surface. To avoid nanoparticle endocytosis, researchers first incubated the nanoparticles with cells in a short period of time ( $\approx 30$  s), then quickly added complexation ligands to crosslink the nanoparticles that absorbed upon the cell surface. Such operations resulted in the formation of an “armored supercell”. The materials–organisms integrates display different nonoriginal properties including intracellular sensing, multifluorescence, magnetism, and electrical conductivity.<sup>[27]</sup>

Viruses are organisms much smaller than cells and eukaryotes. Being simply composed of protein shell and nucleic acid



**Figure 5.** A) Schematic shows the synthesis of BCW including sequential hybridization chain reaction and polyelectrolyte complexation on the live cell. B) Evaluation of cell viability under different stimulus. Reproduced with permission.<sup>[57]</sup> Copyright 2019, Springer Nature. C) Schematic of the incorporation of silica nanoparticles on the cyanobacteria using PDADMAC. D) Cell densities (left) and photosynthetic activity (right) of native and aggregated cyanobacteria. Reproduced with permission.<sup>[56]</sup> Copyright 2017, American Chemical Society.



core, the virus can be somewhat hardly be called “alive” rather than a complex assembly of biomacromolecules. On the one hand, material integration with virus will be simpler, for there are not so many restrictions compared with the cells; on the other hand, however, it is difficult to construct a complex structure (e.g., multilayer shell) upon its surface, due to the tiny size and lack of long-range binding sites.

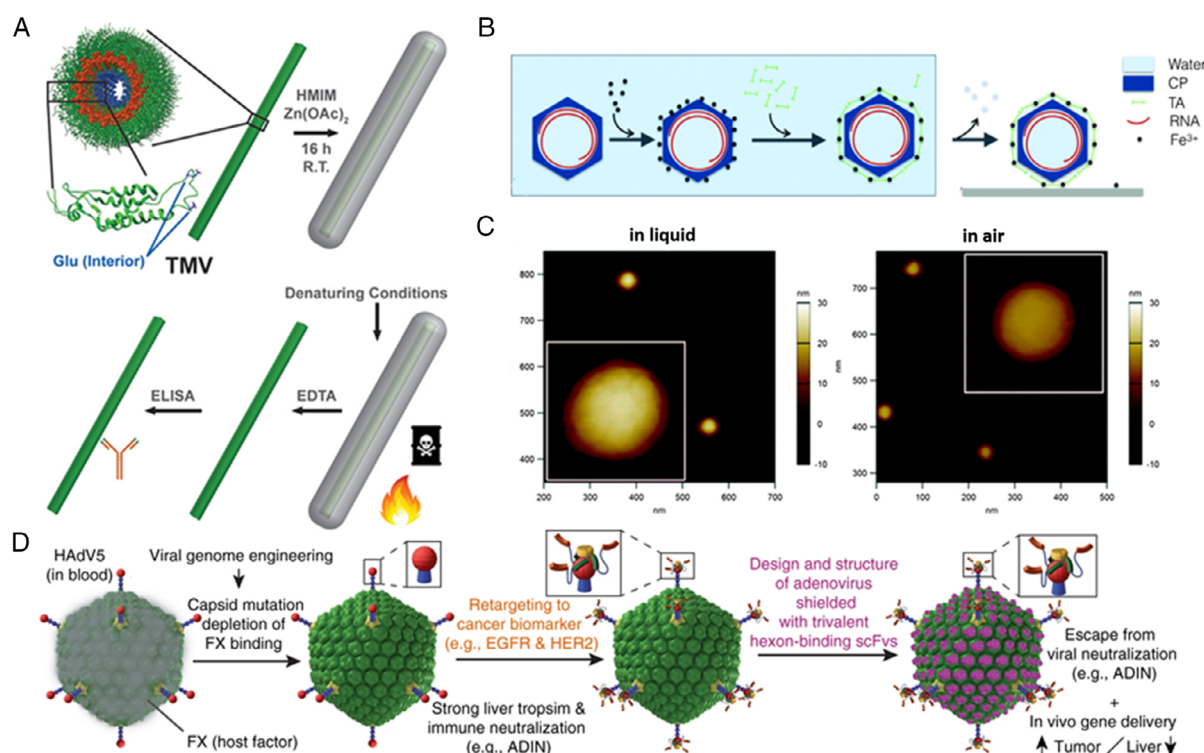
Virus surface proteins are more active than cells and might provide appropriate binding sites to metal ions. In the presence of  $\text{Ca}^{2+}$ , Adenovirus serotype 5 (Ad5) and JEV absorb these ions, and produce CaP shells after supplement of phosphate from the dulbecco's modified eagle medium culture medium within minutes.<sup>[19,60]</sup> This simple approach resulted in robust viruses that have boosted stabilization under room temperature. Under temperature of 37 °C, normal virus has few chances to survive in one day; however, the coated vaccines still perform infectivity in several days' time. Furthermore, the shell can resist neutralization effects, which benefits to improve the vaccines effectiveness.

MOF has been recently applied to coat biomacromolecule and virion due to its outstanding stability.<sup>[61]</sup> For example, ZIF-8 deposited upon oligo nucleic acids and proteins endows protection from high temperature above 100 °C. Recently, the usage of crystallized ZIF-8 on TMV has been achieved.<sup>[62]</sup> In the study, TMV–Zn interaction was found to play a vital role in morphology control, which can be composited as a core-shell nanoparticle with virus encapsulated within or as a large ZIF crystal with virus located on its outer surface (Figure 6A). Concerning the shielding function,

the former type was investigated to gain deeper sights, which showed protection against biological or environmental denaturing factors, such as heat and proteolytic agents.

Virus dehydration can result in the undesired aggregation and collapse of virion structure, which restricts the characterization of its physicochemical properties. A shell consisting of Fe(III) and TA was synthesized upon brome mosaic virus (BMV), a well-studied icosahedral plant virus, to preserve its structure.<sup>[63]</sup> First,  $\text{Fe}^{3+}$  spontaneously absorbed onto the virus surface according to electrostatic force, and TA was then introduced to link to the ions, encapsulating the virion with molecular network. The results of atomic force microscopy (AFM) tests demonstrated the virus particles maintained structural integrity upon exposure to air in long storage period (Figure 6). Remarkably, mass spectrometry showed an extra molecular weight gained after wrapping, which indicated the retention of water and other possible salts.

Apart from protecting effects, functional materials can change the original biorecognition. Adenoviruses (AdVs) are the most widely used vectors in clinical trials, however, its liver tropism and fragility to antibodies still limit their applications.<sup>[65]</sup> Genetic engineering has been used to construct a protein coat to shield the virus vector from antibodies (Figure 6D). When the shielded virion was equipped with adaptor proteins, the transfection of viral gene in xenografted tumors in vivo has increased, whereas liver accumulation and undesired immune neutralization were reduced.<sup>[64]</sup>



**Figure 6.** A) Schematic of encapsulation of TMV with ZIF-8 and shell remove. Reproduced with permission.<sup>[62]</sup> Copyright 2018, American Chemical Society. B) Schematic depicts the approach for wrapping a virus in a metal–organic network, and after evaporation of water the virus remains partially hydrate. CP indicated coat protein; TA indicated tannic acid. C) AFM observation of  $\text{Fe}(\text{III})$ –TA wrapped BMV stored at 4 °C for 1 year. Reproduced with permission.<sup>[63]</sup> Copyright 2016, Royal Society of Chemistry. D) Schematic of the generation of a new AdV vector via multiple engineering steps and the resulted properties. Reproduced with permission.<sup>[64]</sup> Copyright 2018, Springer Nature.

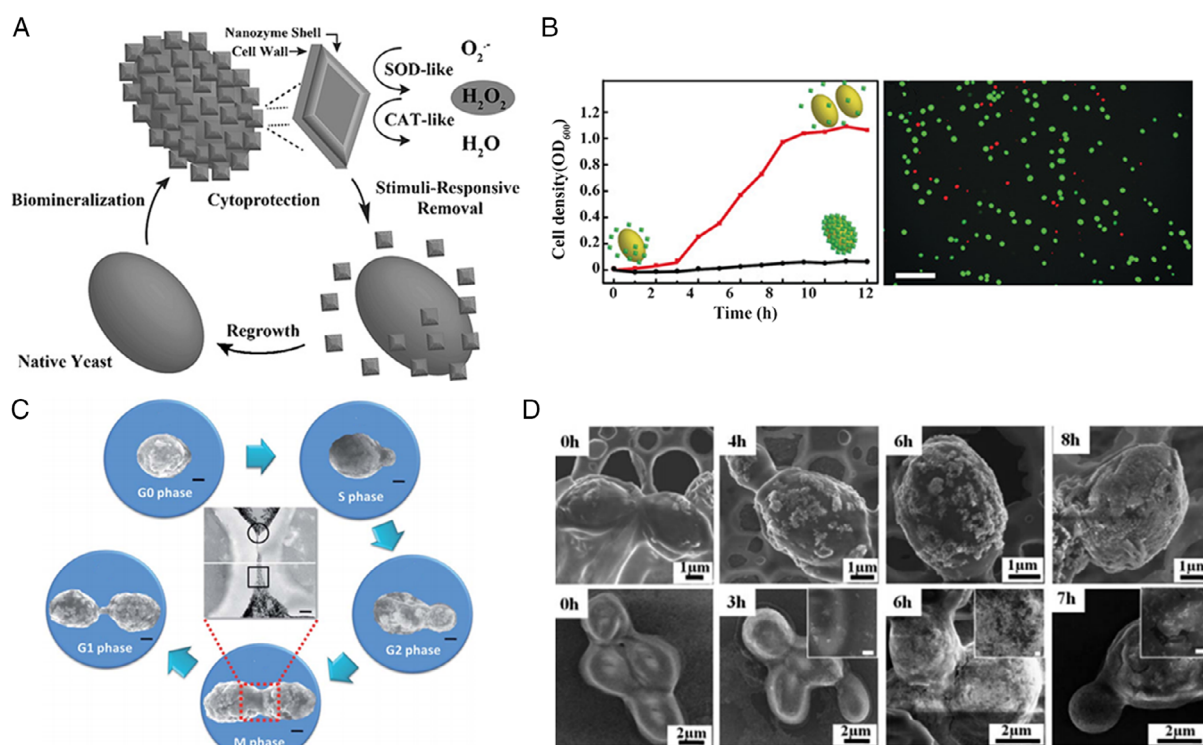
### 3.1.2. Dynamic Shells

Static shells upon cells usually limit the proliferation of the cells; however, in some cases, cell multiplication is required. Thus, a controlled release of the encapsulated cells should be realized, demanding a designable and degradable shell rather than a solid, lifeless shell. Compared with static shells, dynamic shell could offer functions of both space and time. They usually coordinate with the metabolism or a series of change in living environments of the organism, responding to these processes and do their work. To date, pH- or redox-sensitive minerals and versatile polymers are qualified candidates for dynamic shells.

Degradability of shells at required conditions is a crucial factor for producing an “intelligent” organism–materials hybrid.<sup>[66]</sup> For example, MnO<sub>2</sub> shell has been facilely fabricated upon yeast cells and *E. coli* cells via mixing of MnCl<sub>2</sub> and NaOH solutions. MnO<sub>2</sub> materials have superoxide dismutase (SOD) enzyme-like catalytic function, which is superior to CaP and SiO<sub>2</sub> shells. When integrated with the cells, the modified shells showed defensive function against ROS.<sup>[67]</sup> Most importantly, the mineral shell could be degraded under a controlled biocompatible environment using glutathione (GSH), a common biological product of organisms. GSH reacted with MnO<sub>2</sub> according to a redox procedure, and the cells released immediately to regain their full growth vitality (Figure 7A,B).

Protein shells generally have good degradability. Silk fibroin (SF) is a widely used natural protein in biotechnology and biomedical research due to its easy processability into fibers and films, etc.<sup>[69]</sup> Yeast cells have been encapsulated in SF via a salt-ink-out LBL process within phosphate buffer. The SF formed a  $\beta$ -sheets format, a strong crystallized state of SF, and provided a robust supporting effect on the structure. The biowaste, such as ethanol and CO<sub>2</sub> that is produced by cells metabolism, can gradually turn SF shell into structures that can be easily cleared by endocytosis due to the changes in its secondary structure. The shells could undergo a spontaneous degrading process, releasing the original cells to original states.<sup>[70]</sup> Similarly, researchers have used aminated and carboxylated SF as alternative layers of LBL method to coat mammalian cells, and the shell exhibited degradability within hours, suggesting potential applications in cell therapies and 3D printing of biomaterials.<sup>[71]</sup>

When long-term shell modification is desired, dynamic shells can preserve both the proliferation of the encapsulated cells and the function of materials during the cell division period. In recent works, shells with self-repairing properties have been achieved, which could redisperse to the offspring without internal destruction. The shell was synthesized using L-cystine and gold nanoparticles as self-assembly blocks, which could support the cell structure and defend against UV, enzyme, etc. During the division period, the void area between mother cells and

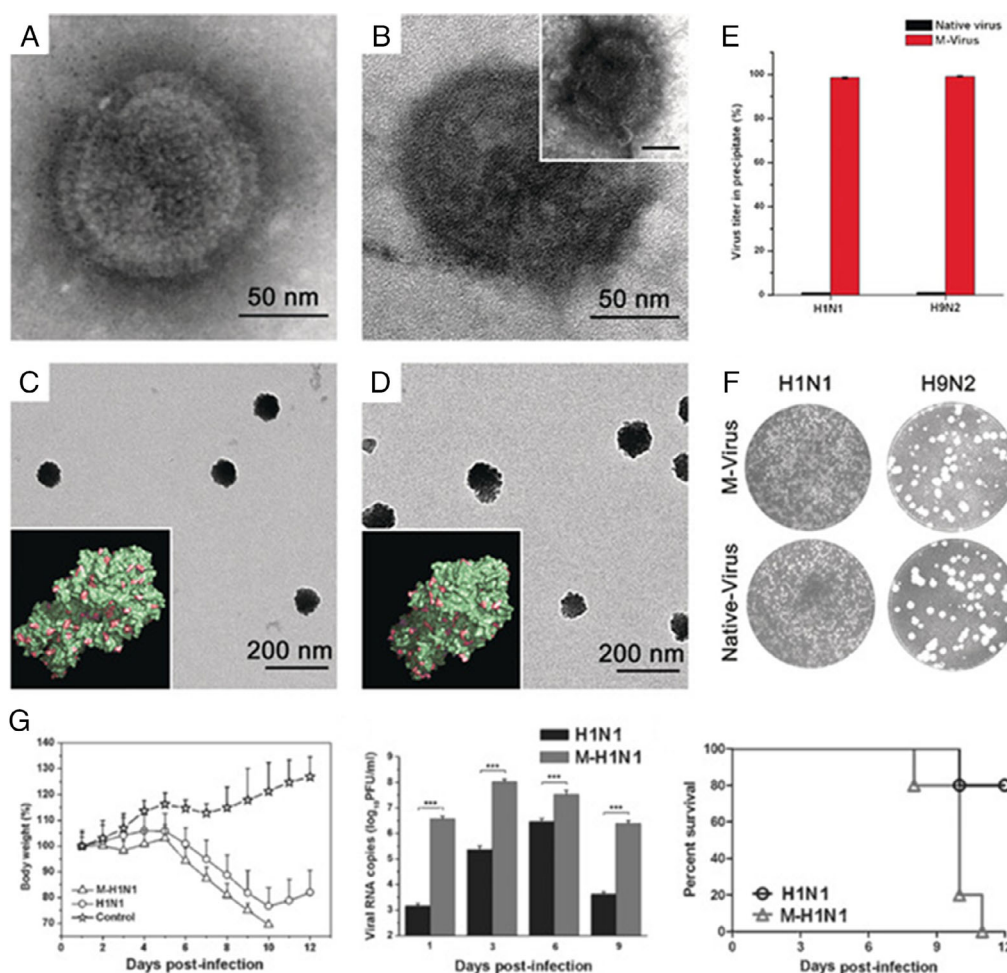


**Figure 7.** A) The schematic shows the formation and degradation of MnO<sub>2</sub> nanosheet shells on yeast cells. B) Growth curve of cells with (red) and without (black) adding GSH (left), and the live/dead stained cells after removal of shells (right). Scale bar: 50  $\mu$ m. Reproduced with permission.<sup>[67]</sup> Copyright 2017, Wiley-VCH. C) Self-repairing process of nanoshells during division of yeast cell. Scale bar: 250 nm. D) SEM images of yeast cells in biohybrid solution (upper row) and on silicon-modified surfaces (lower row) at various times. Insets are magnified images, scale bar: 200 nm. Reproduced with permission.<sup>[68]</sup> Copyright 2015, Royal Society of Chemistry.

daughter cells were still filled with material aggregates, coating the two cells without any further addition of raw materials (Figure 7C,D). The resulted shells were thinner, which suggested the fluidity of the shells and a redispersion process might have occurred.<sup>[68]</sup> Although this repairable property may exist in several generations, as the shells grow thinner, the functions could disappear within certain times. In another study, microdroplet shells fabricated by peptides also showed long-term protection as far as the third generation of daughter cells. Due to flexibility of the shells, they could be allocated to daughter cells during cell division.<sup>[51]</sup> To date, the constant self-repairing shell has not been successfully realized under in vitro culturing conditions or in vivo environment due to the incapability of cells to use precursors themselves.

Shells upon viruses can also display a dynamic feature. In some cases, the tunable shell may even be obtainable for virus in natural process. It has been reported that avian influenza virus, or simply called bird flu virus, is strictly limited when infecting humanity due to lack of related surface-binding sites with human cells. However, in recent years, bird flu has caused several influenza pandemics around the world with severe

mortalities, which suggests these viruses can transmit from Aves to humans.<sup>[72]</sup> Zhou et al. put forward a material-based virus mutant theory to explain these cases, as shown in **Figure 8**. Inspired by mineralization process of eggs, researchers tested the mineralization state of H9N2 bird flu virus in a simulated avian intestinal fluid (SIF). In the procedure of bird egg formation, high intestinal  $\text{Ca}^{2+}$  concentration (8.0–15.0 mM) may cause the deposition of calcium carbonate upon the surface of virus by ion binding. Interestingly, surface proteins of H9N2 exhibit rich aspartic and glutamic residues, which contain large amount of carboxyl groups to enrich  $\text{Ca}^{2+}$  and form minerals. With no doubt, mineralized state H9N2 virus (M-H9N2) was observed after SIF treatment.<sup>[73]</sup> The influenza virus H1N1 was found to have similar mineral shells, suggesting a mineralized state may be widespread in nature. Remarkably, though performing semblable plaque-forming properties, H9N2 and M-H9N2 showed largely different infectivity. The infectivity of mineralized state virus was enhanced because cells tend to invoke a nonspecific endocytosis procedure to absorb the virion, rather than surface protein recognitions. Therefore, the infectivity of M-H9N2 is boosted, and meanwhile, it can cross the barrier



**Figure 8.** A,B) Negatively stained TEM image of H9N2 and mineralized H9N2 (M-H9N2). C,D) TEM image of M-H1N1 and M-H9N2. E) Mineralization efficiency of H1N1 and H9N2. F) Plaque morphologies of different influenza virus. G) In vivo statistics of virus-infected mice. Reproduced with permission.<sup>[73]</sup> Copyright 2017, Wiley-VCH.



of cell-membrane receptor. This study explains the fact of how avian influenza viruses transmit from Aves to human.

### 3.1.3. 3D Bulk Hydrogel Matrices

It is not until recently that we recognize the importance of 3D microenvironment, which may now be called, biophysical fields. These 3D matrices offer biophysical cues including topological features, mechanical resistance, and chemical stabilization, which regulate cell behaviors such as spreading, proliferation, migration, differentiation, and apoptosis.<sup>[74]</sup> The 3D hydrogel matrices are large-scale and mainly serve as a culture environment or reservoir for the cells. Hydrogel matrix is an area that received vast attention and should not be limited to the topic of this review.

Simply using the plasticity of hydrogel materials to fabricate supportive constructs is one applicable strategy for cell-hydrogel integration. For instance, technology called “cell printing” can produce cell-hydrogel scaffolds to repair or create soft tissues based upon the integration of cells to gel compositions. Xu et al. developed a cross-linked, biodegradable polycaprolactone-poly (ethylene glycol)-polycaprolactone (PCL-PEG-PCL) triblock polymer hydrogel with visible light, high elasticity and flexibility for cell printing.<sup>[75]</sup> The cell-hydrogel hybrid can undergo stretching, compression, and twisting process without any clear breakage. Also, the cells can survive in the materials for up to 7 days of culture.

Adhesion is another property required for cell-hydrogel integration, especially for tissue repairing. For example, a recent study constructs a hydrogel to encapsulate fibroblasts.<sup>[76]</sup> The researchers indicated the catechol groups of hydrogels and the skin tissue could form strong covalent bonds in between. The complex performs biocompatibility and photodegradability,

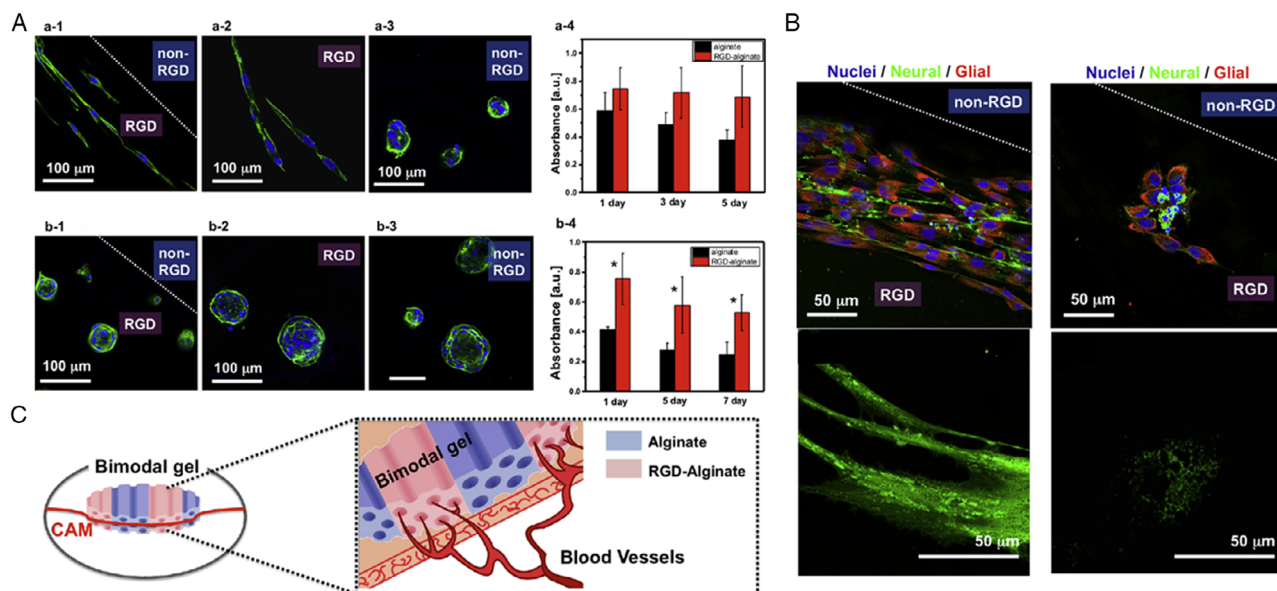
which guaranteed the survival and controlled release of fibroblast, exhibiting good potential in wound management.

Apart from providing mechanical properties, the regulation of cell growth and differentiation by combining bioactive factors to the hydrogels can provide complex effects upon cells. For example, residues of cell adhesion peptides (RGD peptides, containing Arg-Gly-Asp sequence) are generally used to modify alginate-based gel blocks. To control the spatial location of cell, the hydrogel constituted with the bimodal gel blocks was uniaxially lyophilized, which introduced anisotropically aligned microchannels, so that the RGD peptides can be presented on the microchannel as linking sites for cell binding (**Figure 9**). This material could readily control the spatial distribution of cells and guide the formation of ordered structures. Taking human bone marrow stromal cells (hBMSCs) as an example, once implanted into this hydrogel it can be immobilized and further differentiated into nerve-like tissue.<sup>[77]</sup>

Similar to the aforementioned strategy, cell growth factors incorporated in the hydrogels can regulate the cells. Heparin, which has high affinity with various types of growth factors, was immobilized to the hydrogel, where they interacted with cells and regulated the behavior of cells. For example, FGF-2 and VEGFs loaded hydrogel benefits to boost angiogenesis of cells.<sup>[78]</sup> In addition, functional groups of the hydrogels can influence the differentiation of cells. The PEG hydrogel functionalized with phosphate- and *t*-butyl-group promoted the differentiation of human mesenchymal stem cells (hMSCs) into osteogenic and adipogenic without any additives.<sup>[79]</sup>

### 3.2. Intra-Organism Integration

Though influence on the inner parts of cells or viruses are commonly avoided, some researches have succeeded in integrating functional entities of material into these organisms. In the



**Figure 9.** A) Confocal images of cell adhesion and cell viability in the bimodal gel. Upper row: cells trapped in microchannels; lower row: cells trapped in micropores. B) Confocal images of nerve-like tissue formed in hydrogel (upper row) and neural cells on microstructure walls (lower row). C) Schematic of hydrogel embedded in chick chorioallantoic membranes. Reproduced with permission.<sup>[77]</sup> Copyright 2015, Elsevier.

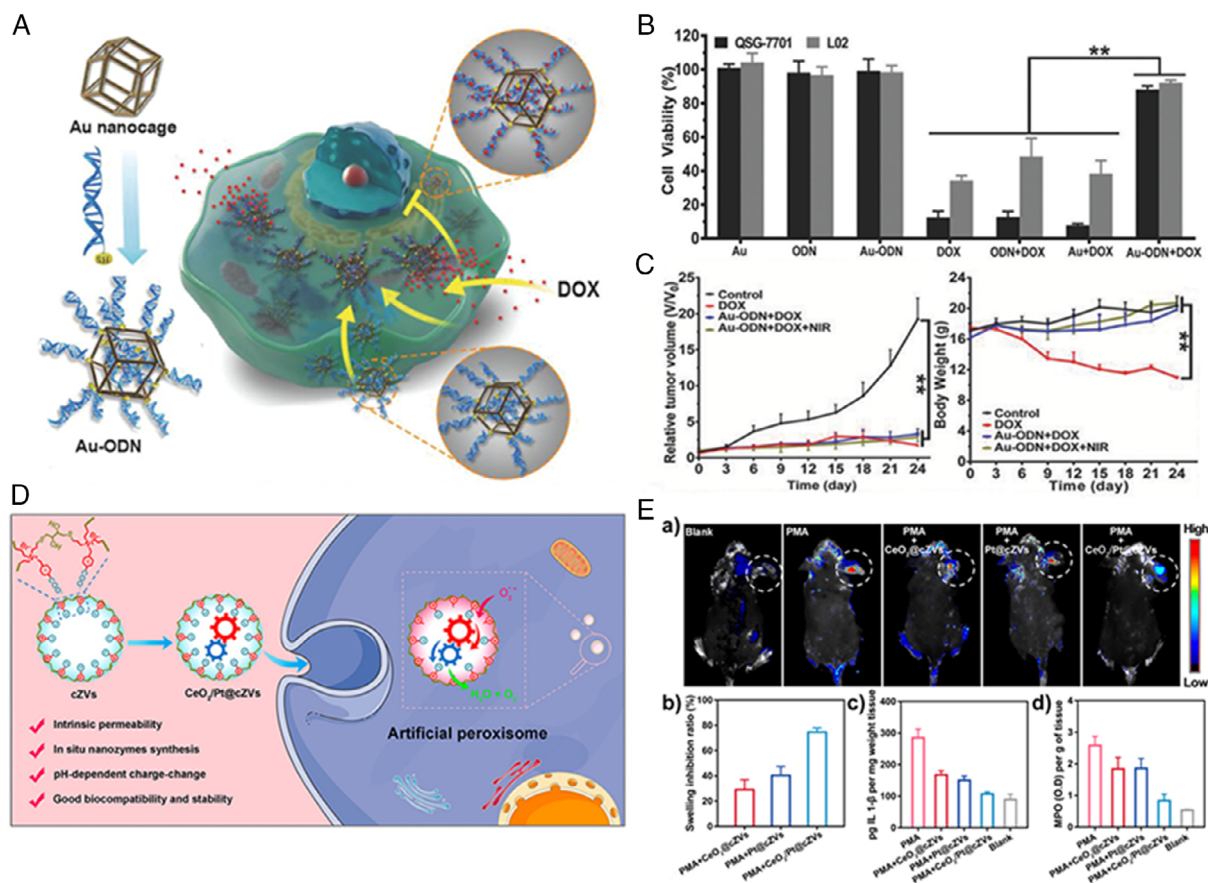


improvement of organisms, shell-forming is more straightforward and controllable, but materials generated in or implanted into the organism have also shown remarkable property. At cellular level, the intracellular materials are usually called artificial organelles or intracellular scaffolds, which have shown great potential in regulating the cell metabolism, such as drug resistance, drug delivery, and apoptosis induction.<sup>[80]</sup> In the following discussion, we introduce material organelles based upon metal, mineral, and polymer vesicles, and finally discuss modification of single virion with inner labeling.

Chemotherapy has long been used in cancer treatments. However, its side effects have also done great harms, leading to hepatic dysfunction, circulatory disorder, endocrine dyscrasia, etc.<sup>[81]</sup> As toxic chemicals, the drugs tend to concentrate in normal tissues, especially liver. To conquer this problem, a cell modification strategy to enhance drug resistance in normal cells was suggested by Tang and co-workers. It has been reported that oligonucleotides (ODNs) could bind with DOX, a common drug applied in chemotherapy. Thus, ODNs acting as a trap can capture DOX and protect the cell nucleus from its harmful effects. ODNs are yet unstable in cellular environment due to the existence of nucleases. Therefore, Au nanocage material was introduced to form a Au-ODN hybrid system, in which Au nanocage plays a

vital role in protecting the nucleotide from cytoplasmic nucleases.<sup>[82]</sup> This hybrid system was absorbed by cells to form an artificial organelle against DOX by simply incubating cells with hybrid materials. Meanwhile, the researchers found organelles in low concentration perform ignorable protecting function, whereas in high concentration defended the cells from toxic effects of DOX with viability up to 80% (**Figure 10A–C**). In vivo experiments showed normal cells, particularly liver cells, absorbed most of the organelles to realize antidrug function, while the organelles in cancer cells tended to remain at low concentration. Moreover, Au could be stimulated by near-infrared (NIR) light to release the trapped DOX absorbed in tumor, and a controlled photothermal therapy ensured the antitumor efficacy.

Materials composited artificial organelles are also applied in drug targeting and delivery process, during which the materials are encapsulated by cells and can be regarded as the organelles. By adding saturated  $\text{Ca}(\text{OH})_2$  solution in yeast culture, calcium carbonate crystals were in situ formed in *Saccharomyces cerevisiae* due to the reaction between calcium ions and  $\text{CO}_3^{2-}$  from water dissolved  $\text{CO}_2$ , byproducts of cell respiration.<sup>[84]</sup> The added calcium ions diffused into the cell, bound with biomacromolecule inside the yeast cell, and produced  $\text{CaCO}_3$  nanoparticles according to an ion adsorption method. The nanoparticles loading



**Figure 10.** A) Schematic illustrates the construction of Au-ODN and its working principle. B) Cell viability with and without OND organelle. C) Normalized tumor growth curves (left) and body weights curves (right) of mice. Reproduced with permission.<sup>[82]</sup> Copyright 2018, Wiley-VCH. D) Schematic indicates the fabrication of artificial peroxisome. E) In vivo imaging of mice with induced ear inflammation after treatment with the nanoparticles (upper row) and the effects of ROS elimination (lower row). Reproduced with permission.<sup>[83]</sup> Copyright 2020, American Chemical Society.

reached to 15% (w/w) without significant toxicity to the yeast cells. To make the simple calcite functional, DOX as a model drug was introduced by the interactions between the  $\text{Ca}^{2+}$  and carbonyl groups in DOX. The pH-sensitive property of the calcite immobilized DOX under physiological conditions (7.0–7.5), which could be released under lower pH (6.0) at tumor tissues due to the pH-responsive features of calcite.<sup>[85,86]</sup>

As water-in-oil (w/o) droplet vesicles can localize in distinct area of cells, it reveals the features of organelles.<sup>[87]</sup> Inspired by this, polymer vesicles-based artificial organelles have attracted much attention from researchers. Different from organelle based on material nanoparticles with reactive interface, polymer vesicles should be carefully sealed, and their inner components are biologically compartmentalized.<sup>[88]</sup> For example, zwitterionic cross-linked vesicles that mimicked peroxisome have been fabricated through in situ incorporation of nanoenzymes to cavities of vesicles. The artificial enzyme, contains  $\text{CeO}_2$  and Pt nanoparticles, mimics the natural SOD and catalase (CAT), which detoxicated ROS in cells (Figure 10).<sup>[83]</sup> In another study, artificial organelles were synthesized by microfluidics technology. An intracellular light-responsive calcium-loading vesicle, named as “calcium store” was fabricated using chemical chelator nitrophenyl egtazic acid. This artificial organelle mimics the mitochondria’s calcium binding but performs via a totally different mechanism. Moreover, a synthetic magnetosome which is inspired by other organisms has also been successfully fabricated using the same vesicle.<sup>[89]</sup>

Materials can also be integrated inside virus particles. Different parts of virus can be labeled using material particles. Researchers chose the widely used quantum dots (QDs) as the labeling material. By binding them to the nucleic acids of human immunodeficiency virus (HIV-1), a QD-integrated virus was formed. Notably, the integration of QD that is larger than the targeting RNA did not significantly affect the multiplication pathway of the virus, meanwhile enable the observation of infection procedure via fluorescence imaging of live virus in macrophages, including endocytosis, translocation, core releasing process. Using this method, the researchers discovered that a dynamic actin cytoskeleton is critical for HIV-1 entry and intracellular migration.<sup>[90]</sup> Another related work by labeling of single virus’ genome RNA, capsid, and matrix protein produced a “multicolor” virus, which benefits to trace live virus during infection process.<sup>[91]</sup>

In summary, intra-organism materials fabrication exhibits a new strategy in organism–materials integration with advantages and disadvantages appearing at the same time. Compared with material shells, intra-organism modification has stronger influence upon the organism’s physical activities, but it is still facing challenges such as short existing period (especially polymer-based ones), complex synthesizing route, and potential toxicity. With more research being done, this strategy will play a more important role in biomedical applications.

## 4. Biomedical Applications of Materials Integrated Organisms

In addition to applications in environment control,<sup>[92]</sup> biocatalysis,<sup>[93]</sup> energy production,<sup>[94]</sup> etc., the organism–material integration plays a vital role in biomedical applications. In this part,

we discuss the specific application of organism–materials hybrid in vaccine improvement, biomedical therapy, and bioimaging.

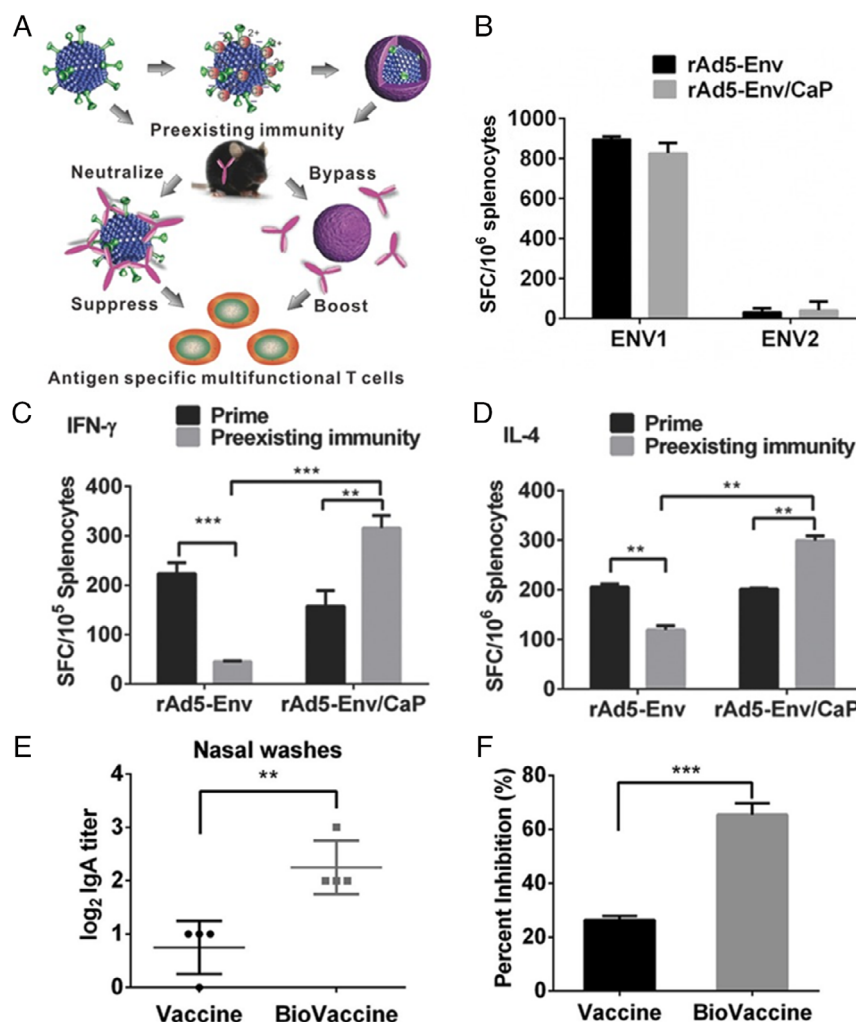
### 4.1. Vaccine Improvement

Vaccines as most important invention for public health, have saved millions of lives due to the wide application. To date, however, the production, preservation, and inoculation cost of vaccine remains at a high level, which especially increases the burden of poorer countries and regions. Improvement in vaccines mainly aim at reducing the cost and boosting its efficacy.

Preservation of vaccine have long been a topic for its improvement.<sup>[95]</sup> In general, vaccines should be stored under the temperature of  $-20^\circ\text{C}$  to avoid inactivation. In a most recent work, viruses have been protected from very harsh environment, which suggests a grander prospect for vaccine storage. TMV was facily encapsulated within ZIF-8 networks with mesoporous structures. After stored in organic toxic solvents, e.g., methanol, ethyl acetate, and protein denaturant guanidinium chloride, the structure of the virion hardly changed. Remarkably, heating the hybrid up to  $100^\circ\text{C}$  for 20 min have done little damage to the virus due to the protection of the ZIF shell. Further infection test in plants also showed the virus retained bioactivities after these stressing.<sup>[62,96]</sup> This research shows the possibility of a super-virus-based vaccines that do not require cold-chain transportation.

Except for the inactivation by external environment, the preexisting antiviral immunity can also affect the effectiveness of virus vaccination.<sup>[97]</sup> Taking Ad5-based vaccine as an example, previous study and clinical data have indicated widespread of anti-Ad5 immunity across the world, which could neutralize the virus before it enters the target cells, therefore suppress the effectiveness of the vaccine.<sup>[98]</sup> Biomineralization of Ad5 with amorphous CaP suggested an idea for solving the problem. By mixing the virion with a calcium-rich medium, CaP was produced by the ionic interactions between  $\text{Ca}^{2+}$  and surface protein of Ad5, which leads to the formation of a core-shell structure. Compared with native Ad5, the mineralized virus displayed a nonspecific endocytosis route rather than a receptor-dependent recognition. Furthermore, due to the shielding effect of CaP shell, the antibody binding sites exposed on the surface were blocked and thereby prohibited the virus from being recognized and neutralized by immune system. The biomineralization-based surface shielding is called “Trojan Horse” effect (Figure 11A–D). After entering cells, the CaP shell dissolved under mild acid environment of lysosome ( $\text{pH} < 6$ ) to release the encapsulated virion. Experiments indicated no significant difference in virus activity between the native Ad5 and those released from mineral shells. However, the mineralized Ad5 showed distinct enhancement of T cell immune response in mice with preexisting immunity, suggesting the “Trojan Horse” not only bypassed the anti-Ad5 immunity system but also generated robust cellular immune responses even under the condition of preexisting immunity.<sup>[99]</sup>

The material shells can also offer convenience in the operation of vaccination. In research followed in the aforementioned study, it was found that the CaP encapsulation strategy could have other advantages. Most of the currently available vaccines are facing the drawback of using needle sticks, which have the risk of spreading the infection and require skillful medical practitioners to operate.



**Figure 11.** A) Schematic shows the CaP-coated virus as Trojan Horse can bypass the preexisting immunity. B) IFN- $\gamma$  ELISPOT response of mice splenocytes after immunization. C) SIV-ENV specific IFN- $\gamma$  ELISPOT responses. D) IL-4 ELISPOT responses after administration of virus in the absence or presence of anti-Ad5 immunity. Reproduced with permission.<sup>[99]</sup> Copyright 2016, Wiley-VCH. E) DENV2-specific IgA antibody responses in nasal washes from intranasally immunized mice. F) The inhibition by receptor-independent clathrin-mediated endocytosis of different vaccines. Reproduced with permission.<sup>[100]</sup> Copyright 2016, Elsevier.

Intranasal mucosal vaccination is a promising candidate for future vaccines because of its high efficiency and safety. Previous works suggested that amorphous CaP has strong capability to adhere to tissues and cells, and in this case, ChinDENV2 was biomaterialized with CaP nanoshell.<sup>[100]</sup> Through simple nasal administration of vaccine, the antigens adhered to nasal mucosa initiated an enhanced local and systemic immune responses (Figure 11E,F).

#### 4.2. Biomedical Therapy

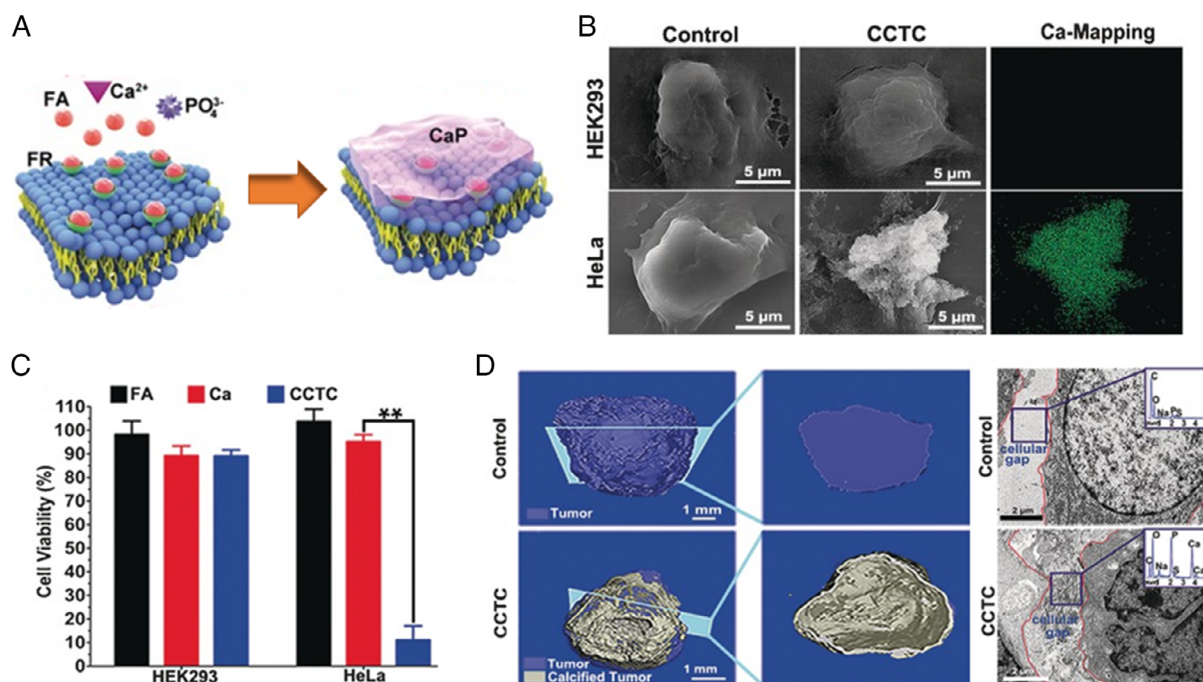
To date, the organism–materials hybrid has found many potential applications in biomedical therapies such as strategy in cancer treatment, blood transfusion, and cell transplantation.

Cancer has always been among the deadliest diseases. Though effective, the widely used chemotherapy is limited by its disadvantages such as adverse drug reactions, low therapeutic index,

drug tolerance, and poor targeting effect.<sup>[101]</sup> In addition to drug-targeting strategies,<sup>[102]</sup> the application of organism–materials integration can also be beneficial for tumor control.<sup>[103]</sup>

Integrating with materials can also result in abnormal death of cells. Zhao et al. developed a drug-free tumor therapy called cancer cell targeting calcification (CCTC) to minimize the injuries caused by chemotherapy treatment.<sup>[104]</sup> Cancer cells display abnormal properties compared to normal cells; one of these characteristics is the up-regulated folate receptors (FRs). FRs can specifically enrich folate acid, therefore folate residue has become a popular targeting functional group for drug delivery. In this work, simple folate acid molecule was used, for it also has two carboxyl groups, which are likely to bind with Ca<sup>2+</sup>. In vitro culture of HeLa cells in folate- (500  $\mu\text{g mL}^{-1}$ ) and Ca<sup>2+</sup> (10 mM) rich medium produced amorphous CaP biomaterials covered on HeLa cells in situ, whereas HEK293 cells have hardly been affected under the same conditions. The mineral shell inhibited





**Figure 12.** A) Schematic of CCTC. B) Micrograph of selective calcification. C) Cell viability after selective calcification. D)  $\mu\text{CT}$  detection of the tumors (left two rows) and TEM observation of calcified area in tumor tissues (right row). Reproduced with permission.<sup>[104]</sup> Copyright 2016, Wiley-VCH.

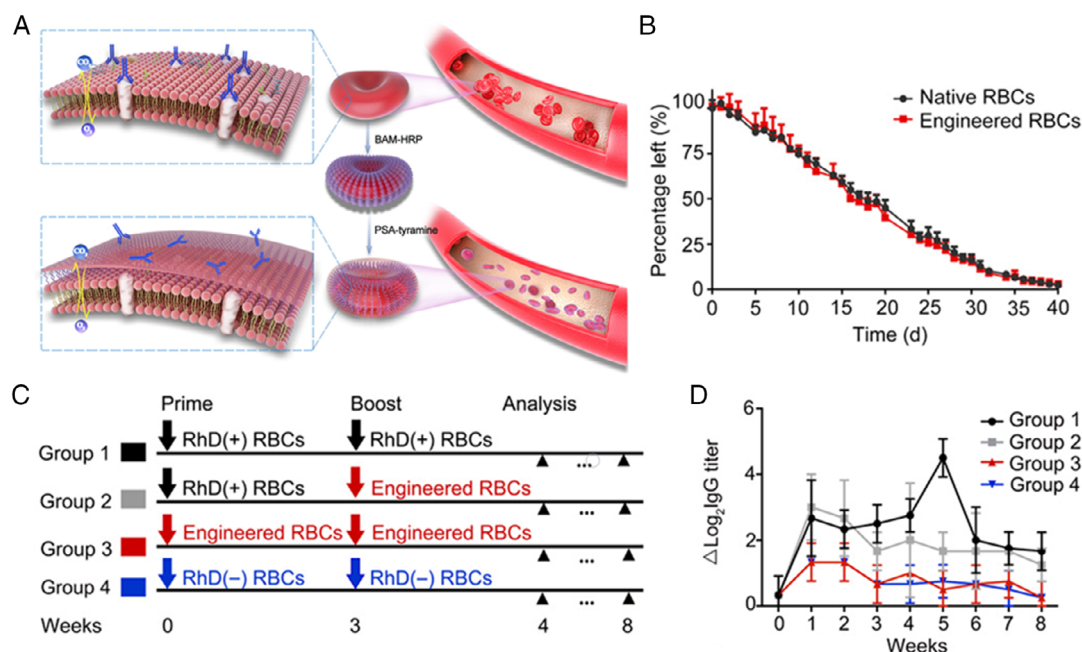
the entry of outer nutrients to the cancer cells, and served as foreign solid matter, continuously interacting with the membrane. Under these two harmful effects, the cancer cells were finally “oppressed to death” (Figure 12). After 24 h,  $\approx 90\%$  of cancer cells were clearly damaged. In vivo experiment indicated the CCTC strategy has comparable tumor inhibitory effect with DOX, and the mice showed low mortality (viability 90%) under this condition. This strategy raises an applicable method in cancer controlling without toxic side effects.

Cancer treatment can also be improved by the aid of a modified virus. Oncolytic virus is a type of virus that can specifically replicate in tumor cells and kill cancer cells, which can be modified by materials to improve its anticancer effect. To date, due to hepatic sequestration and preexisting antibodies, the curative efficacy of oncolytic virus is largely limited.<sup>[105]</sup> Recently, a polyethylenimine-induced silicification method was applied to oncolytic adenovirus (OA) by forming  $\text{SiO}_2$  protecting shell, which could effectively shield off antibodies and avoid aggregating in liver. The biomineralized virus showed increased uptake by cells in 24 h, and denoted toxicity effects toward cancer cells in 72 h.<sup>[106]</sup> The report presents an alternative strategy for virus-based cancer therapy.

The organism–materials integration strategy is also feasible to achieve blood transfusion applications. Blood transfusion is used world-wide in surgeries; however, blood-type matching is always a problem, especially when rare blood type is required.<sup>[107]</sup> According to different alloantigen, human blood group is composed of more than 20 blood group systems, in which ABO and Rhesus (Rh) blood group are the most related system. Unlike simple ABO classification, Rh blood group system includes more than 50 different serologic specificities, which greatly increases the difficulty in cell engineering or specific epitope deactivation

methods.<sup>[108]</sup> In this case, materials shells, which can nonspecifically cover most or all the surface epitopes of RBCs, show particular advantage. Previous research used LBL method to construct a camouflage shell with complex polymer components. The outermost PEG helped in the repulsion of normal RBCs, thus reduced aggregation. In vitro experiment showed the capability of cell survival and oxygen transportation under Rh antigen still existence.<sup>[109]</sup> However, the strategy can only attenuate the hemolysis and rejection reaction rather than completely avoiding it. Self-assembled PDA shell has also been attempted to add stealth effect to RBCs and eliminate the recognition by ABO system. In vivo experiment proved that the lifespan of coated RBCs remained for more than 40 days after primary infusion, which is very similar to the clearing rates of native RBCs.<sup>[110]</sup> Nonetheless, this hybrid shell not only covered the epitopes but also limited the membrane fluidity as well, causing fragility of the RBCs. According to studies, an ordered structure of hydrogel network that mimic cell-wall structure can efficiently reduce the side effect and could realize long-term survival. Very recently, a nanogel shell prepared by natural polymer has been suggested to encapsulate RBCs via milder interactions. Biocompatible anchoring molecule and horseradish peroxidase residue were primarily introduced, and further cross-linked with polysialic acid (PSA) and tyramine to form gel network (Figure 13). A flexible gel shell about 250 nm thick was finally obtained, which hardly influence the native fluidity of cell membrane. The coated RBCs showed no immune response to Rh antigens, retained oxygen dissociation curve (ODC) as native RBCs, and preserved in serum for 8 days, which is much longer than previous results. In vivo examinations indicated a complete stealth and indistinguishable difference compared with native RBCs. In summary, the newly





**Figure 13.** A) Schematic of RBC surface engineering and the blood transfusions of the obtained cell-material hybrids. B) Survival profiles of RBCs after blood transfusion in vivo. C) Scheme shows the immunization plan of native or engineered RBC. D) Antibody titers in rabbits after receiving immunostimulation. Reproduced with permission.<sup>[111]</sup> Copyright 2020, American Association for the Advancement of Science.

developed method provides potential applications in blood transfusion.<sup>[111]</sup>

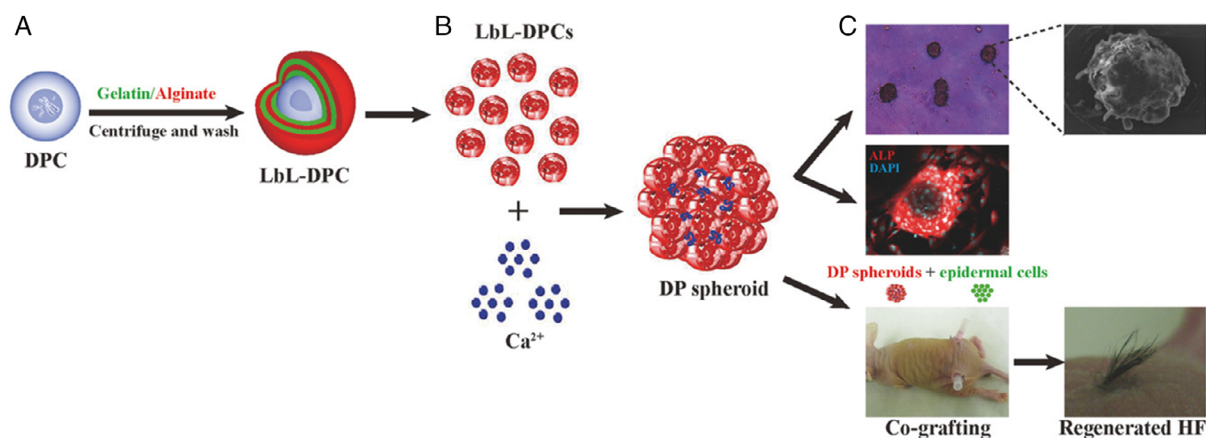
Materials could change the interaction between organisms and their environment, as mentioned earlier, they can also serve as a new microenvironment for the culturing organisms. Cell transplantation technology has been rapidly developed, while still facing challenges not only from immune rejection response but also from function loss during the procedure as well. Loading cells by materials could provide not only a camouflage but also an enclosure for cell growth and differentiation, which could help realize successful transplantation. For instance, dermal papilla cells (DPCs) is a kind of mesenchymal cell in the lower part of a hair follicle (HF), which plays a vital role in the regeneration of hair. However, when cultured in plane environment, DPCs are likely to lose their inductive functions. To solve the problem, Xing and co-workers used LBL method to encapsulate single DPC by nanogel. These cells were first encapsulated within four double layers using gelatin as the polycation and alginate as the polyanion, which were then artificially aggregated by adding calcium ions through reacting with the outermost alginate layer to produce DPC spheroids (Figure 14). The spheroids mimicking the 3D growing environment of DPC in living human body, thus retained their original inductive functions. In vivo experiment by nude mice showed that this nanogel–cell complex exhibited no toxicity, and hair shafts were induced in 4 weeks.<sup>[112]</sup> Problems of immune response has also been solved using material integration strategy. Pluripotent stem cells (PSCs) are capable for treating myocardial infarction (MI); however, with low concentration and short retention time, the effects cannot be guaranteed. To solve this problem, the cells were incubated in microcapsules containing alginate and chitosan hydrogel shell.<sup>[113]</sup> The cells

were let to grow and predifferentiate for 7 days inside the capsules to form vigorous aggregates, which performed much stronger defensive capacity against clearance in vivo, and the 3 day-degrading period decreased the fierce immune reactions.

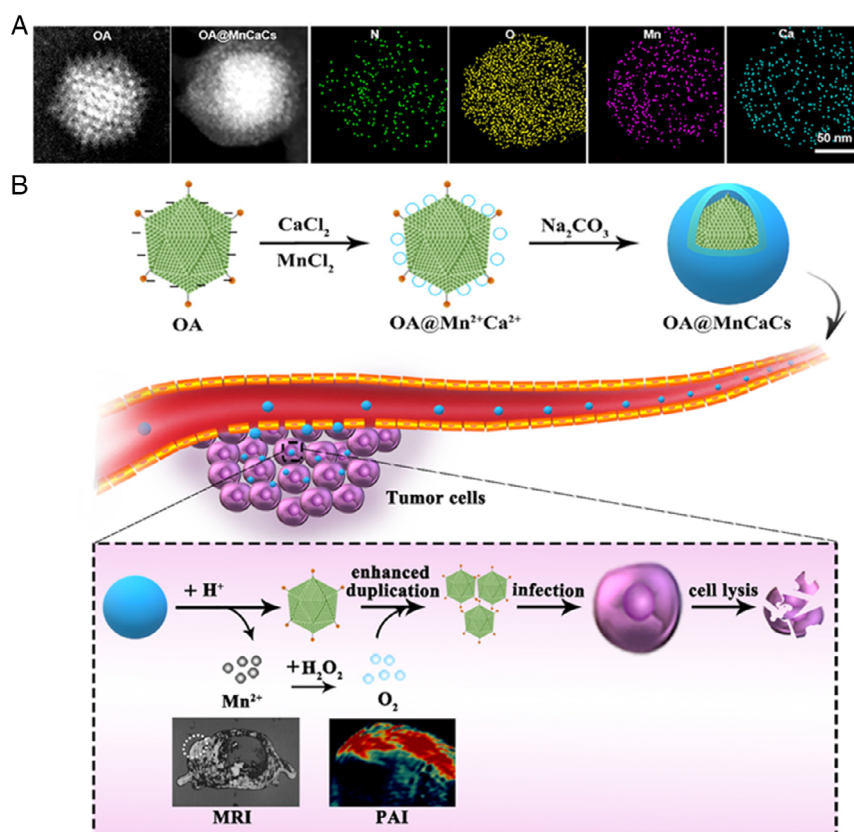
### 4.3. Bioimaging

Disease surveillance involves biosensing and bioimaging technology. More and more attention has been paid to the application of these technologies in clinical medical diagnosis. The development of noninvasive surveillance method is an important prerequisite for disease treatment. To date, numerous sensors and contrast agents with bioluminescence, fluorescence, photoacoustic effect, photothermal effect, etc., have been developed. Among these strategies, labeling targeted biomacromolecule is achievable using materials. For instance, carbon nanotubes, hydrogel fibers, metal and metal oxide nanoparticles can be linked to the targeted binding sites.<sup>[114,115]</sup>

Though performing similar function as biomacromolecules, and even better biocompatibility and more precise targeting effects, organism–materials hybrids are rare in such researches. In an innovating work, an OA-material construct was prepared by encapsulating OA with calcium and manganese carbonates (MnCaCs) mineral shells (Figure 15).<sup>[116]</sup> The shell offered immune stealth and prolonged in vivo circulation. Moreover, the mineral shell quickly dissolved under the acidic microenvironment of tumor cells, and released oxidizing  $Mn^{2+}$ , thus increasing the endogenous transformation of  $H_2O_2$  into  $O_2$ . The coexistence of  $Mn^{2+}$  and  $O_2$  enhanced the signal displayed in magnetic resonance imaging (MRI) and photoacoustic imaging (PAI), realizing dual-modality imaging combined with cancer therapy.



**Figure 14.** Establishment of DP spheroids for HF regeneration. A) LBL coating of DPCs. B) Ionic cross-linking through exposure to  $\text{Ca}^{2+}$ . C) In vitro (upper) and in vivo HF regeneration (lower) assay. Reproduced with permission.<sup>[112]</sup> Copyright 2018, Wiley-VCH.



**Figure 15.** A) TEM images of OA and OA@MnCaC and element distribution analysis of OA@MnCaC. B) Schematic of the preparation of OA@MnCaC nanoparticles and their application in dual-modality imaging-guided and synergistically enhanced anticancer therapy. Reproduced with permission.<sup>[116]</sup> Copyright 2019, American Chemical Society.

Without invading the cells with virus, on the other hand, in situ formation of materials can also serve as imaging reagent.<sup>[117]</sup> There is significant interest in the effective imaging in early cancer diagnosis, and an in situ strategy through biomineralization of metal oxide has been reported.<sup>[118]</sup> In a typical work, HeLa, U87, and HepG2 cancer cells were used as models. By introducing

aqueous  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  ions into the cancer cells, they formed magnetic  $\text{Fe}_3\text{O}_4$  nanoclusters and fluorescent  $\text{ZnO}$  nanoclusters inside cells, respectively. Thus, a multimodal cancer imaging system combining fluorescence imaging with MRI could be readily achieved. Normal cells, in the meantime, did not show nanoparticle formation due to the low ROS environment.

## 5. Outlook

In this review, we discussed the definition, fabricating methods, specific examples, and possible applications of organism–materials integration systems. To date, this field has attracted more attention from researchers. On the one hand, with the development of materials science, diverse kinds of materials have been applied to biological systems. On the other hand, materials with complicated forming process and properties have also long intrigued us to unravel the new functions. These two together push the development of organism–materials integration. Research in recent years have produced robust and functional cells, viruses, eukaryotes, presenting artificial improvement strategy of organisms. Remarkable progress in biological, biomedical, energy, environmental fields, indicates the integration of material and organisms is a promising strategy in the present and future applications.<sup>[119]</sup> Although great achievements have been made, challenges still remain. First, an attempt to integrate new materials with organisms is still very challenging and require lots of efforts. A general theory or guidance remains to be found for new materials. Second, due to simplicity, most studies are aiming at protection functions upon organisms. Although this is meaningful, organisms with other functions and other integrating formats should be paid more attention. Moreover, multifunction is demanded. To date, the multifunctional fabrication is limited by simply adding the number of components. However, as material science develops, different forms, crystallization states, and the location of the materials in the organism could also influence functions.

A successful integration process involves the innovation of materials and the expansion of targeting organisms. Many researchers choose yeast and bacteria, which are already robust to chemical stimulus and natural environment; however, other organisms such as fungi and plant cells are rarely seen. Moreover, in future, the integrations are not likely to be limited at cell and virus level. For instance, tissue modification with a combination of different types of cells should be proposed, and thus request studies related to their synergistic effects.

The integration of organisms has become an emerging interdisciplinary field. Studies have suggested the materials integration can be achieved either spontaneously or artificially, and based on these tactics, eukaryote cells, mammalian cells, and viruses have been modified to perform nonoriginal functions. Some organism–materials hybrids have found unique advantage in vaccine improvement, biomedical therapy, and bioimaging. In summary, great progress has been made, and in the meantime, more efforts are needed to make such strategy more applicable in the future.

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## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

artificial shells, biomedical applications, biomineralization, organism–materials integration, virus modifications

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