



Surface engineering of microbial cells: Strategies and applications

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Microbial cells (bacteria, fungi, and algae) and viruses are important part of life; which besides their harmful effects, perform several useful functions owing to their unique cell surface properties. The unique structures present on their surfaces serve as barriers between the cell and its environment and bestow them with unique functional properties. The current review describes strategies to decorate microbial cells by using different materials. It details various strategies such as layer by layer (LbL) decoration, mineralization, encapsulation, and genetic engineering among others to modify the surfaces of different microbial cells for potential applications such as environmental biotechnology, toxicology, medical microbiology, and nano-biotechnology, etc. Besides, it discusses the effects of various materials on cell viability, physiology, and functionality used for surface engineering. This review provides fundamentals to the novice readers and insights to the seasoned researchers to pave way for their future research in the area.

Keywords: Microbial cell; Surface properties; Cell surface modification; Applications

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1. Introduction

Biom mineralization is a biological process used for the formation of protective structures around different microscopic single-cell organisms like diatoms algae and foraminifers. These microorganisms possess inorganic shells on their surfaces which are made of calcium carbonate and silica and potentially serve as a boundary between the cell and its environment. However, most of the microorganisms lack such structures that necessitates the introduction of artificial biomimetic shells on their surfaces.¹ The process of introducing minerals or macromolecules helps in the modification of microbial cell surface thereby imparting specialized functions to them. This is achieved by using two main biological strategies: functional integration and biomimetic approach for modification of living cells.² Entrapping live cells inside a polymer layer at a micrometer scale where the polymer coating restricts the cell movement within the microsphere and offer protection against the varying microenvironment (pH, temperature, ionic strength, etc.) is a strategy that has been broadly applied in recent years.^{3,4} The pores, present in most cases in the encapsulating layer, allow the diffusion of nutrients, oxygen, wastes, and electrolytes to move across the barrier, thus, maintaining cell growth.⁵ Cells can also be coated with magnetic nanoparticles that allow effective

spatial manipulation by application of magnetic fields. This property helps to improve control over size distribution, cell distribution and geometry within multicellular constructs, thus, giving way in tissue engineering which is a potential application in advanced regenerative medicine and many other fields.⁶ Polymer and nanoparticle coating of cells have been done on cells from different origin.¹ The mostly studied eukaryotic organism is the yeast cell because of its cell wall characteristics that provide cell resistance.⁷ Bacterial cells have also been decorated with polymers and magnetic nanoparticles to obtain functionalized cells.⁸⁻¹⁰ Viruses on their surface lack the negative charge so they have been engineered with different minerals and nanoparticles.¹¹

To date, a variety of strategies have been developed for the surface modification of microbial cells such as layer by layer (LbL) decoration,¹² mineralization,¹³ encapsulation¹⁴ and genetic engineering¹⁵ among others. For example, the LbL strategy is used to achieve magnetized functionalized cells by depositing magnetic (Fe₃O₄) nanoparticles onto the cell surface using different polymers as mediators for the immobilization of colloidal nanoparticles. The simplicity and versatility of the LbL assembly technique paves the way for extensive applications due to the production of hybrid nanostructures with promising collective and improved functional properties.¹⁶ However, the different techniques used for surface modification of microbial cells differ in their degree of biocompatibility, sensitivity, types of materials, and effect on the viability of target microorganisms. Thus, there is an extensive need for developing more compatible strategies to deposit a variety of materials for the fabrication of broad-spectrum functionalized microbial cells.¹⁷ To date, materials of different nature such as natural and synthetic polymers, organic, inorganic, and magnetic nanoparticles, polyelectrolytes, gene and DNA, etc. have been

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used for the surface modification of microbial cells. The polymer and nanoparticles-based fabrication of microbial cells has been achieved for a variety of microbial cells owing to their potential applications in different fields such as biotechnology and biomedicine.¹

Despite the greater potential of microbial cells to be surface-modified and availability of various materials used for their modification, the coating of living cells with certain types of nanoparticles, polymeric- and non-polymeric, and polyelectrolytes tend to have toxic effects towards their viability. Therefore, any microencapsulation strategy used for surface modification should ensure the viability of coated cells against any harmful effects of the materials used as well as environmental factors such as varying pH, ionic strength, temperature, metabolites, and osmotic stress, etc. Further, it should enhance the storage stability of the encapsulated cells. In line with cell viability, important considerations include the integrity of cell membrane, cell division, and intracellular enzymes of the functionalized cell.¹⁸ Recent interests of cell-surface modification by using various polymer nanofilms, hydrogels, minerals, and sol-gel shells, etc. have resulted in developments in several fields such as their applications in whole-cell biosensors^{19,20} toxicity microscreening devices¹⁷ and catalysts,²¹ tissue engineering,²² and bioanalytical chemistry.²³

The use of inorganic micro-shells of different varieties for biomimetic encapsulation of microbial cells has been the recent area of research whose target is mainly yeast, human normal and cancerous cell lines, and bacteria, etc. for diverse applications.¹⁸ Biofabrication of microbes has provided an insight for wide range of applications such as micro devices, bio-nanomaterials and micro/nanorobots due to their different shapes and sizes.²⁴ Therefore, this review is aimed to overview the current progress of surface engineering of a variety

of microbial cells through different strategies for various applications. Emphasis has been laid on several microbes that can be potentially modified by using compatible materials. Further, various strategies employed to encapsulate different types of live microbial cells by creating an artificial shell around them have been described along with their potential merits and limitations. In addition, it addresses the effect of microbial encapsulation towards the viability of target cells to pave the way to future developments of the cell surface engineering strategies. Several important applications of surface modified microbial cells in different fields such as biomedical, pharmaceuticals, environment, and industry, etc have been enumerated in detailed. Besides, this review provides a base for the development of new modification strategies, selection of appropriate materials and microbial cell types, and development of novel materials which can find potential applications in different fields.

2. Surface modification strategies

2.1. Layer by layer technique

Layer by layer (LbL) is the most commonly used technique for encapsulation of microbial cells and is illustrated in Fig. 1. It involves multilayer coatings formation by exposing the cells to polyelectrolytes by alternating the charges existing of an acidic and basic component. The living cells are mainly used as functional elements of polyelectrolyte such as attachment of multilayers to the surface of the cells and the incorporation of polyelectrolyte into multilayers.¹² This strategy involves formation of thin films and has received immense consideration owing to its wide choice of materials that can be used for coating particulate substrates and due to its ability to

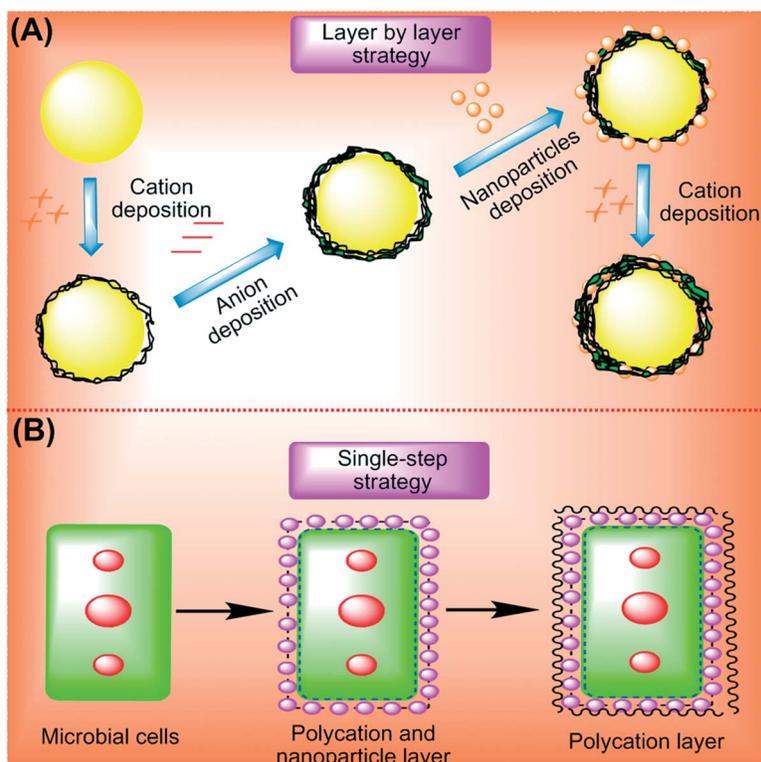


Fig. 1 Illustration of (A) layer by layer strategy and (B) single layer step of polycation stabilized with nanoparticles on the surface bacterial cell.

modulate nanometer control over film thickness. This fabrication technique has led to rise of functional and responsive thin films which have found potential applications in a various fields such as but not limited to bioelectronics, energy storage and conversion, drug delivery, and catalysis, etc.¹⁶

The LbL technique is a low-cost, simple, and possesses wall properties, such as texture or thickness and permeability. These properties can be controlled to a nanometer scale during the layer by modulating the ionic strength, pH or counteracting ions.²⁵ Briefly, the first layer deposited onto the cell is composed of a polycation (cells mainly possess the negative charge in water), the second layer deposited is comprised of a polyanion. This layer is repeated until the required bilayers are obtained. Washing is done after every layer has been deposited so as to remove the traces of polymers used and finally centrifugation is carried out.¹⁸ Several studies have reported about the application of LbL strategy to encapsulate microbial cells. For instance, Fakhrullin and Minullina demonstrated the LbL to encapsulate yeast cells into artificial inorganic shells of calcium carbonate (CaCO₃). Capsules of CaCO₃ were formed because of precipitation of Ca²⁺ and CO₃²⁻ ions onto the cell surfaces in aqueous solutions for several minutes. The resulting two component hybrid structures of cells and inorganic shells are formed, referred to as “core shell particles,” where the inorganic layer is 1–2 μm thick.²⁶

2.2. Genetic engineering

As stated earlier, viral engineering methods like genetic recombination, PEGylation, and covalent modulations have become disadvantageous owing to their irreversibility that can easily affect several processes like viral production, infection, and the transduction processes.^{27,28} Fabrication strategies such as genetic engineering are more advantageous compared to the previously reported strategies. Genetic engineering involves the transformation of coat proteins by inserting amino acids which act as ligation handles for introducing peptide-based affinity tags, bio-conjugation, and to insert peptides as epitopes or targeting ligands in order to provoke the immune response.²⁹ The changes lead to the insertion or exchange of individual amino acids to introduce side chains that allow functionalization, terminal extensions (adding sequences to C-terminus or N-terminus of each coat protein), or insertion of sequences that form surface loops^{30,31} or to alter the overall physicochemical properties of VNP.³² Examples of modifications include the introduction of targeting sequences that allow VNP to target-specific receptors, introduction of immunodetection tags/purification, and introduction of epitope sequences for functioning of VNP as a vaccine.^{33,34} The genetic material is located in the single-stranded or double-stranded fragments or in the interior of the capsid as circular. Enveloped viruses consists of a bi-lipid layer on the exterior which provides targeting specificity to the virus.³⁵ The addition of unnatural amino acids as unique handles for subsequent chemical reactions is also possible using similar recombinant expression techniques.³⁶

2.3. Encapsulation

Virus coat proteins self-assemble around the nucleic acids under physiological conditions, and this property, shared by the viral nanoparticles (VNPs), can be exploited to reassemble and disassem-

ble them into more desirable structures around other cargo molecules.¹⁴ At present, two different strategies are used to trigger the cargo encapsulation; (a) unique binding interactions that occur during self-assembly, and (b) electrostatic interactions and surface charge. For efficient encapsulation process of the foreign cargo, self-assembly of viral coat proteins around a negatively charged nucleic acid is warrant.¹⁴ In viral encapsulation, the size of the cargo is the main key factor due to different sizes and its radius of curvature, which could lead to the morphological and physical characteristics of the capsid to be altered.³⁷

2.4. Biomineralization

The deposition process of minerals around and in the cells and tissues of living organisms to accumulate and assemble is known as biomineralization. In viral nanoparticles (VNPs), this process involves the capability of virus coat proteins to nucleate mineralization or assemble around a mineral core.¹³ The biotemplate, that is a VNP, is exposed to other inorganic precursors or metallic, resulting in the nucleation of material on the internal or external surface due to the capsid amino acids interactions.¹³

A study by Pouget and Grelet described a novel mineralization process of a filamentous virus by stabilizing the virus surface with polyethylene glycol (PEG) covalently, followed by mineralization on the surface by use of silica and with titanium dioxide (TiO₂) to achieve high quantity of the mineralized rods. The results showed aggregation of 1-2 nm nanoparticles on the virus surface forming an incomplete non-homogeneous mineral layer. However, the mean thickness of coated mineral layer was constant on the whole length surface of the virus.¹¹ These three strategies have been summarized in Fig. 2.

3. Surface modification of microbial cells

The variability of live microbial cells in their sizes, morphologies, physiological properties, and biochemical activities give the possible ways to use them as objects to deposit different functional nanomaterials onto the their surface.¹ Several living microorganisms of different kinds, including magnetotactic bacteria, yeast, and viruses hardly makes their own shells, and hence been widely utilized by various researchers in nano modification by biomineralization which demonstrate their suitability by retaining their viability.² The following sections describe the surface modification of various types of microbial cells by different strategies (Table 1).

3.1. Yeast

Yeast, *Saccharomyces cerevisiae*, is an important microorganism for understanding eukaryotic biology at the cellular and molecular levels. Its cell wall is composed of chemical compounds, including weak negatively charged polysaccharides, N-acetyl glucosamine, and mannose and rarely contains any minerals on its surface which limits its surface modification.⁷ Therefore, the deposition of positively charged polyelectrolytes on its surface will enable its biomineralization (Fig. 3). This deposition of positively charged groups on surface of yeast cell wall serves as a link between the cell and deposited polyelectrolytes. For example, Yang et al. encapsulated the

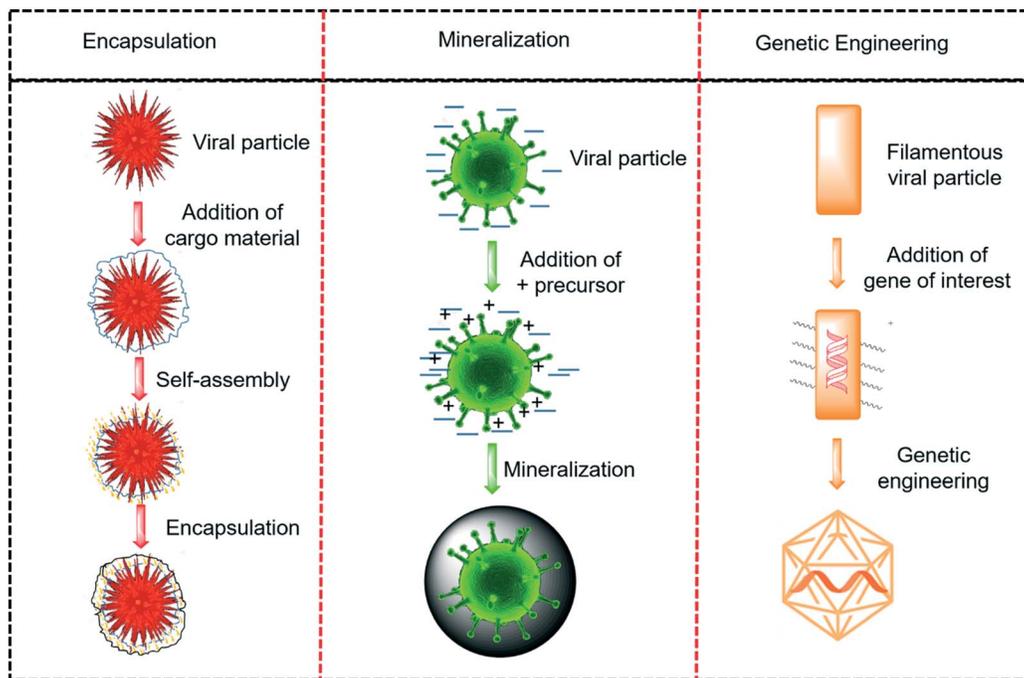


Fig. 2 Illustration of techniques used for surface modification of different types of viruses.

living yeast cells by forming silica shells in the presence of poly (diallyldimethylammonium chloride) (PDADMAC) and poly (styrene sulfonate) (PSS). This strategy was based on the preliminary modification of the cell surface by use of the LbL technique to form a multilayered film of PDADMAC/PSS and make the surface of the cell to act as a positive potential. The surface-modified cells were then placed in silicic acid which triggered the formation of a 50 nm thick layer of silica shell on the cell surface.³⁸ A similar technique was used by Wang et al. to form calcium phosphate micro shells by depositing PDADMAC/PSS/CaCl₂/Na₂HPO₄ on the surface of yeast cell wall.³⁹

3.2. Bacteria and algae

Bacterial and algal cells can be modified with different polyelectrolytes and nanoparticles layers through the LbL technique to form a functional artificial shell. Having a wide variety of applications, direct usage of bacterial and algal cells is challenging owing to the fact that their activity is highly dependent on several environmental factors. For example, the delivery of probiotic bacteria to the Gastro Intestinal Tract (GIT) has always been challenged by the specific pH of the target site. Therefore, in order to overwhelm such conditions, several techniques like microencapsulation of cells have been employed to protect the cells, enhance their viability, and improve their delivery to the target site by inducing a protective layer around them.⁵⁰ For applications such as bioremediation and agriculture, the encapsulated cells have demonstrated extended shelf-life and controlled microbial release.^{51,52} Fig. 4 summarizes LbL technique for coating the cell and also doping it with magnetic nanoparticles.

To date, different materials have been reported for their use in the encapsulation of bacterial and algal cells. Zhang *et al.*, encapsulated algae *Chlorella pyrenoidosa* by using poly (allylamine hydrochloride) (PAH)-stabilized magnetic nanoparticles (MNPs) through

a single-step technique of functionalization. The energy dispersive X-ray (EDX) spectroscopy associated with scanning electron microscope (SEM) confirmed the successful deposition of PAH-stabilized MNPs onto the algae cell surface forming a 90 nm thick nano-layer. The encapsulated cells retained their viability and were able to autofluorescence indicating the non-toxicity of PAH-MNPs towards the algal cell even when during their exposure to magnetic fields.⁴⁵ Similarly, *E. coli* cells have been surface modified by application of the LbL method by depositing different polyelectrolytes (CaCl₂, Na₂CO₃, PAH, PSS) and proteins (protamine). The surface-modified cells demonstrated up to 40% cell viability that could have been accounted by the capsules breaking causing damage to the cells. However, the encapsulated cells showed an enhanced lag phase in comparison to the non-encapsulated cells.⁴² In another study, the *Alcanivorax borkumensis* marine bacteria were encapsulated using PAH-stabilized MNPs through LbL method. The cells were successfully encapsulated and retained their viability.⁸

3.3. Virus

Unlike bacteria and yeast cells, most of the viruses do not have a high negative-charged surface for mineralization. Therefore, it is hard to induce mineral shell formation spontaneously. A biological or chemical modification is needed to boost the biomineralization process by introducing some nucleation-related functional groups.² Viruses have also been studied as human, animal, and plant pathogens and as subjects for understanding the molecular and cell biology. The structure of viruses is composed of multiple copies (up to thousands) of one or a few capsid protein subunits that are arranged either in helical (rod-shaped viruses) or in icosahedral (spherical viruses) symmetry. These proteins present on the capsid of viruses are very crucial in that they provide a wall for the attachment or

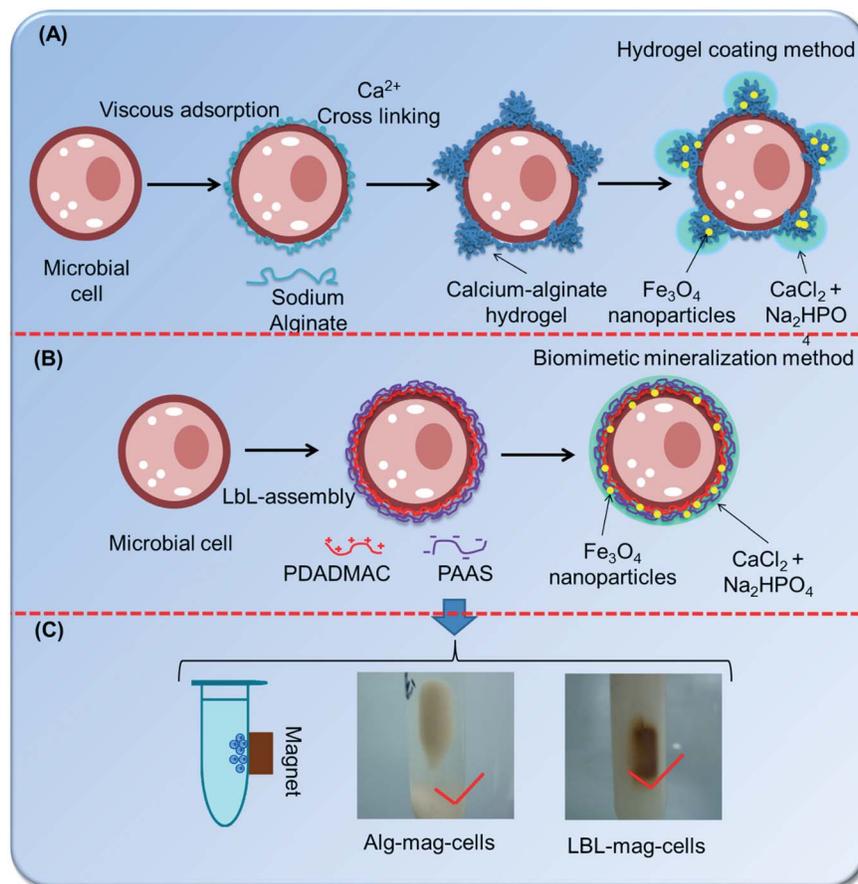


Fig. 3 Schematic representation of (A) the process of coating the microbial cell surface with the hydrogel, (B) biomimetic mineralization technique, and (C) testing of magnetic properties of the modified microbial cells. The figure has been modified from.²¹ Copyright@2016, John Wiley and Sons.

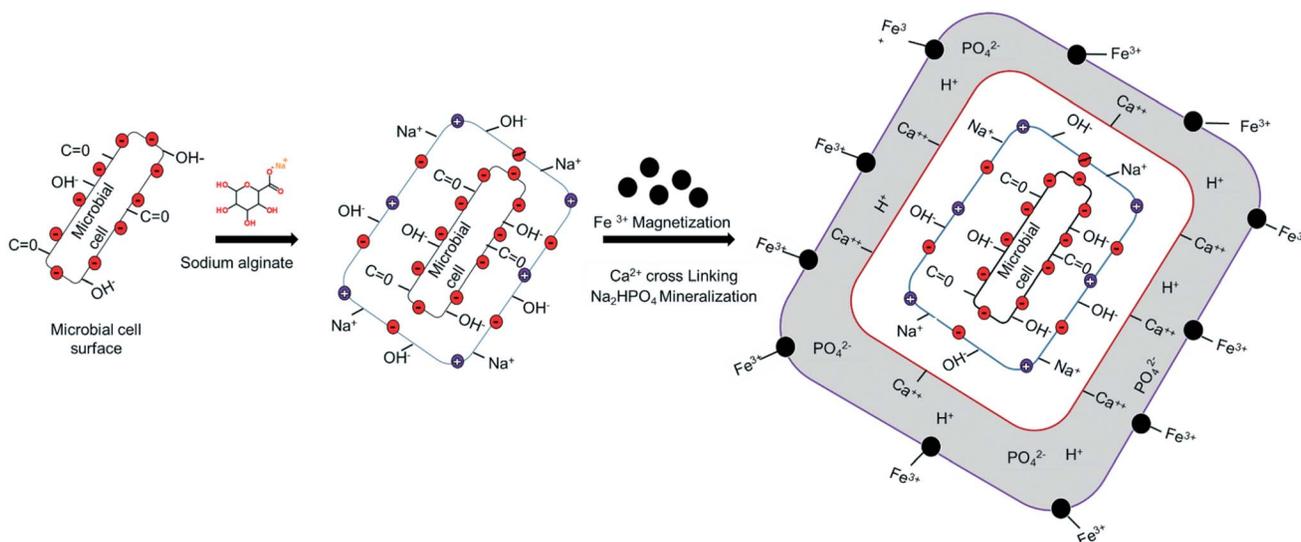


Fig. 4 Illustration of mineralization and magnetic modification of *E. coli* cells via single LbL method. The cells were surface-modified by forming alginate layer. Thereafter, the cells were cross-linked with calcium chloride and modified with polyelectrolytes Na_2HPO_4 (disodium hydrogen phosphate) and magnetic nanoparticles (Fe_3O_4).

incorporation of several functional groups to the cell, thus becoming a good choice in fabrication of new nanomaterials. Researchers have studied the viral capsids and found that these can be modified

into nanosized templates for incorporation or deposition of functional group like metals.^{53–55} In other studies, the viral capsids have been fabricated or engineered to nanosized carriers for drug delivery

and other therapeutic applications.^{56,57} However, viruses that infect plants have been exploited due to their advantageous properties of being nonpathogenic to animals and their empty virus-like particle noninfectious capsid can easily produce high yields.⁵⁸ Engineering or fabrication of viruses is viewed as a safer, less time consuming, and cost effective technique compared to the other living cells like yeast and bacteria.^{37,59} Engineering of viral surface is a useful strategy to tailor the viruses possessing the desired functions, besides; it tends to preserve the natural properties of the cell without alteration. The currently used viral engineering techniques such as genetic recombination, PEGylation, and covalent modulations, etc. have become irreversible which interfere with viral production, infection, and transduction process. Therefore, there is an extensive need to develop more advanced and safe strategies to solve the above challenges.^{28,60} Several advanced approaches have been developed for the modification of virus-based materials such as encapsulation, biomineralization, and genetic engineering, etc. which are discussed in section 3 and summarized in Table 2. Many cargos including synthetic nanoparticles, polymers, enzymes, and drugs, among others

have been successfully incorporated into the viral-like particles by employing these techniques.⁶¹

4. Effects of microbial surface modification

During cell encapsulation, the semi-permeable porous mineral shells formed around the cells must allow the transport of nutrients and excretion of metabolic byproducts to and outside the cell. Further, the hard mineral shells must safeguard the encapsulated cells by mimicking the function of uncoated cells, thus enabling the cells to function normally.⁴⁸ Fig. 5 summarizes the effects of surface modification on cells in terms of cell physiology, cell viability and cell toxicity.

4.1. Cell physiology

Allochromatium vinosum was encapsulated through LbL technique by using different polyelectrolyte ensured that the cell did not lose

Table 1 Microbial cell encapsulation and overview of the technique, coating substances used and general description of characteristics information obtained.

Microorganisms	Minerals used	Technique	Description	Ref.
<i>S. cerevisiae</i>	β -lactoglobulin (Blg) and alginate	Adsorption/ LbL	Microbial cell growth and membrane integrity, protection against environmental stresses	40
<i>S. cerevisiae</i>	Magnetically labeled Halloysite clay nanotubes (Mag-HNTs)	LbL	Direct and rapid cell surface engineering, cell viability and proliferation	41
<i>S. cerevisiae</i>	Sodium alginate/ CaCl ₂ / Fe ₃ O ₄ /Na ₂ HPO ₄	LbL	Viability, metabolism, cell morphology, successful magnetic modification	21
<i>E. coli</i>	CaCl ₂ , Na ₂ CO ₃ (Sodium carbonate), poly (allylaminehydrochloride) (PAH),PSS/protamine	LbL	Successful encapsulation with a narrow size distribution, enhanced lag phase in treated cells, cell viability (40%)	42
<i>S. cerevisiae</i>	CaCO ₃ /PAH/PSS/Ag nanoparticles	LbL	Cell viability, synthetic biofilm formation, development of polyelectrolyte multi-layers	43
<i>S. cerevisiae</i>	GO-NH ³⁺ , GO-COO ⁻ / PDDA/PSS/ Fe ₃ O ₄	LbL	Biocompatibility of GO, magnetic manipulation, GO multi-layers formation to serve as scaffold	44
<i>Alcanivoraxborkumensis</i>	Poly(allylamine hydrochloride) (PAH)-stabilized Fe ₃ O ₄	Deposition and adsorption-LbL	Magnetic manipulation of cells, cell proliferate and normal physiological activity	8
<i>Acinetobacter baylyi</i>	Poly(allylamine hydrochloride) (PAAH)-stabilized Fe ₃ O ₄	Deposition	High efficiency of Fe ₃ O ₄ (99.96%) attachment to the cells, spatial control of cells in external magnetic field, cell viability and normal function, unaltered catalytic activity	9
<i>S. cerevisiae</i>	Poly(allylamine hydrochloride)(PAH)-stabilized Fe ₃ O ₄	Direct single magnetization step	Integrity of intracellular enzymes and cell membrane	17
<i>Chlorella pyrenoidosa</i>	Poly(allylamine hydrochloride)(PAH)-stabilized Fe ₃ O ₄	Direct rapid single-step magnetization	Formation of a thick nano-layer on surfaces of the cell wall, magnetic manipulation of cells using a permanent magnet, cell viability	45
<i>Lactobacillus plantarum</i> CRL 1815 and <i>Lactobacillus rhamnosus</i> ATCC 53103	Gellan gum, xanthan gum, pullulan gum, jamilan	Extrusion	Microbial cell protection, recovery of viable cell number, metabolic response against bile exposure, resistance of polymers against extreme simulated bile conditions	46
<i>S. cerevisiae</i>	Alginate-poly-L-lysine-alginate (APA)	Extrusion	No perceivable adverse effects of oral administration of the capsules on the microbial flora of the human gastrointestinal tract (GIT)	47
<i>S. cerevisiae</i>	PDADMAC/PAA/CaCl ₂ / Fe ₃ O ₄ / Na ₂ HPO ₄	LbL	Cell viability and division, high cell survival rate protection	39
<i>S. cerevisiae</i>	PDADMAC /PSS/Silica	LbL	Maintained cell growth, cell viability, formation of thick shell around cell	38
<i>S. cerevisiae</i>	CaCl ₂ /Na ₂ CO ₃		Successful cell encapsulation, cell viability, high enzymatic activity	26
<i>S. cerevisiae</i>	Dopamine	Polymerization	Growth and cell viability, protection against foreign aggression, functionalization	48
<i>E. coli</i>	Sodium alginate, CaCl ₂ , Fe ₃ O ₄ / Na ₂ HPO ₄	Single LbL biomineralization and magnetization	Magnetization of the cells, Formation of artificial cell capsule on biomineralized cells, growth and cell viability maintained.	49

Table 2 Fabricated viruses with different techniques and polymers to obtain a functionalized Virus with different characteristic.

Type of virus	Technique	Polymer	Description	Ref.
Filamentous virus (<i>fd virus</i>)	PEGylation and Mineralization	Polyethylene glycol (PEG), TiO ₂ and SiO ₂	Well-dispersed hybrid rod-like particles obtained, highly monodisperse, and large aspect ratio	11
Filamentous <i>fd virus</i>	Mineralization	Silica	Narrow diameter distribution (0.5 to 2 μm), well-dispersed hybrid fiber and uniform diameter, synergistic assembly of positively charged <i>fd virus</i> and negatively charged silica particles	62
Adenovirus serotype 5 (Ad5)	Biomimetic Mineralization	Calcium phosphate (CaPi) and dibasic sodium phosphate	The resulting core shell like Ad5-CaPi possessed unique physical and chemical properties as compared with the native Ad5	63
Human enterovirus type 71 (EV71)	Genetic engineering and biomimetalization	A phosphate chelating agent calcium or (N6p) chelating agents (W6p and NWp)	The biomimetalized engineered vaccine exhibited overall improved thermostability and immunogenicity	64
Cowpea mosaic virus (CPMV)	Poly (diallyldimethylammonium chloride) (PDDA)	Layer by layer	Biologically active virus-based thin films obtained which can be a potential scaffold that can be used in cell adhesion studies	65
Tobacco mosaic virus	Self-assembly	PEG	High thermal stability against in organic solvents by TMV-PEG scaffold.	66
Hemagglutinating Japan enveloped virus (HVJ-E).	Layer by layer	Chitosan (CH), glycol chitosan (GC) and PolyL-lysine (PLL).	HVJ-E coated with GC showed great stability in PBS. Six layers of GC/ hyaluronic acid (HA) on HVJ-E formed. Degradation ability of hyaluronic acid (HA) layer by hyaluronidase.	67

its metabolic activity. Furthermore, the change in the surface charge of the *A. vinosum* did not affect the transport of insoluble elemental sulfur or the soluble sulfide substrate. In many cases, a lot of polymeric layers in the cell build up a physical barrier between the cell and its environment, thus affecting the cell permeability depending on the choice of polyelectrolyte. It is very essential for one to choose the polyelectrolytes carefully in order to avoid interference with the cell functionalization.⁶⁸

4.2. Cell viability

The toxic effect of the polyelectrolyte layer may be caused by direct penetration of polyelectrolytes into cellular membranes causing blockage of nutrients and ions uptake, destruction of cellular membranes, or retarding the cell division. This sometimes leads to the hibernation of the cells thus form shells under unfavorable conditions and cannot grow and divide. Microbial cell wall protects the cells from osmotic pressure fluctuation during the LbL modification of cell which involves the deposition of polyelectrolyte on their surface. Studies have suggested that the synthetic polyelectrolyte coatings cause cell death and suppression of green fluorescent protein (GFP) synthesis. However, other results have demonstrated very low toxicity for LbL coated microorganisms. Use of natural polysaccharides for coating microbial cells have also been identified to improve the cell viability of encapsulated cells.⁶⁹

Techniques like plate count or optical density analyses of encapsulated cells have confirmed the efficient cell growth and division, thus confirming the viability of the surface modified cells. Besides these techniques, the cell viability of surface modified cells is also investigated by using various dyes. Such dyes have their unique working principles and the methods used are mainly based on the permeability of selected dyes and allow the differentiation of living and dead cells by unique staining. Most of the cell membranes of viable cells are intact and mainly act as a boundary that separates the cell cytoplasm from media. These membranes do not allow several inorganic substances to pass through it. However, if the cell vi-

ability is interfered, the membrane integrity is disturbed too, thus allowing the dyes to enter into the cell.¹⁸ For instance, the encapsulations of yeast cells into inorganic and organic shells caused no significant effect on their viability, which was confirmed by vital dyes and direct microscopic observation over the cell germination period, as well as microbiological methods for controlling the microbial growth.^{38,39,48} The authors also concluded that porous mineral shells act as semipermeable barriers for nutrients and metabolic byproducts. The division and growth of cells start at the moment when the integrity of the external synthetic shell is broken, for instance, under the action of extracellular secretion of cells.

A study by Yang et al., showed an unusual long lag phase of the yeast cellular growth which implies that the encapsulation method is a long time storage of microorganism collections without regular reinoculations. Moreover, the synthetic inorganic shells protected the encapsulated shells from external stressors, which mimicked the functions of native shells.⁴⁸ The coated cells show enzymatic lysis resistance compared to the uncoated cells. The mineral shell prevents a direct enzyme contact with the cell wall surface. Moreover, the deposition of electrolyte nanoparticles on cell surface prevent the germination upon the cultivation in a nutrient medium which indicates that the shell enhances the resistance of encapsulated cells to a long lasting action.

4.3. Cell Toxicity

The use of organic and inorganic substances to form artificial shells in microbes by deposition has no effect on cell viability. The hard artificial shell formed around the living cells helps the cells to resist several environmental stresses, thus, serves as a promising application in the storage of cells for a long period of time.⁷⁰ The toxic effects of coated polymers are due to the hindrance of ions or nutrients passages due to the formation of layers; however, causing no harm to the cellular enzymes (enzyme activity inhibition), membrane (poly-ion-mediated pores formation in membranes), and cell division where the cells are unable to grow nor divide inside the shell. Repeated

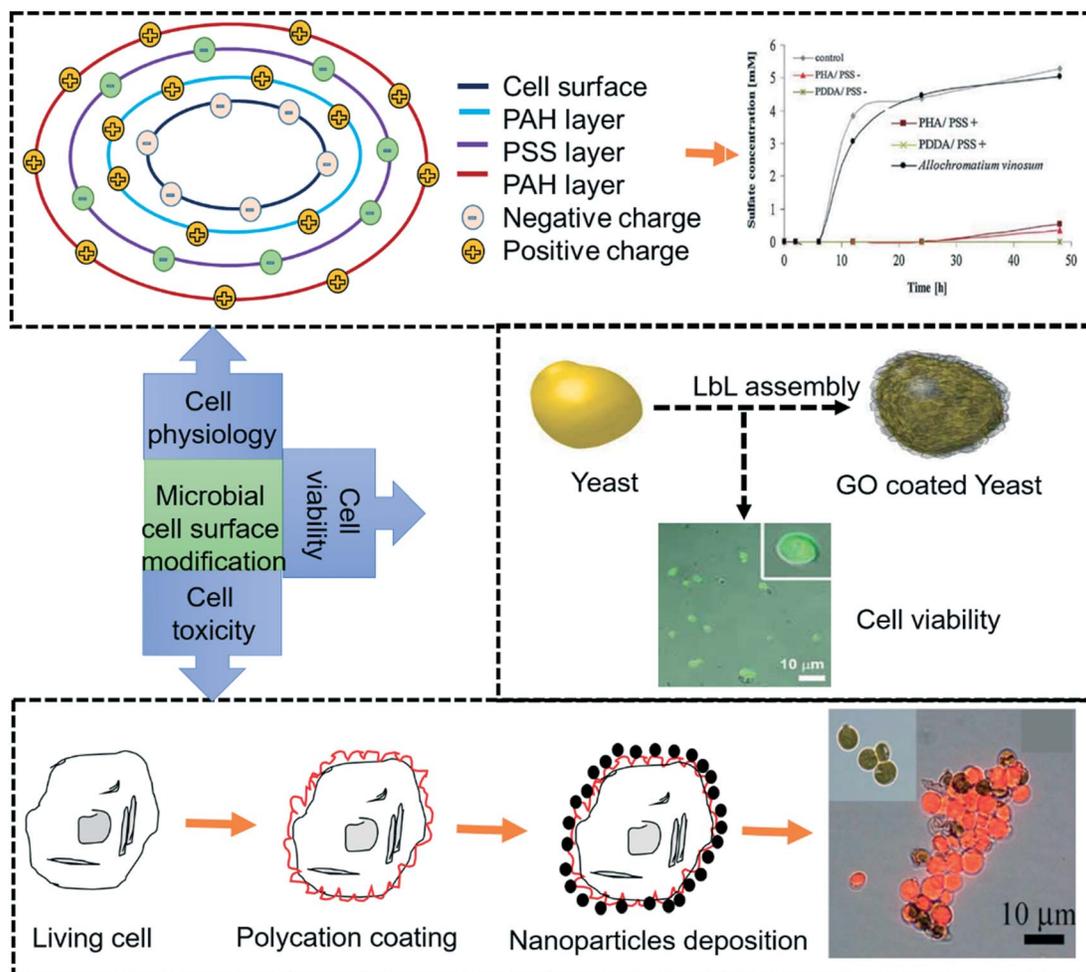


Fig. 5 Illustrations of effects of cell surface modification using several polymers on cell viability, toxicity, and physiology. The figure has been modified from.^{19,44,68} Copyright©2011, Royal Society Chemistry; 2011, John Wiley and Sons; 2010, John Wiley and Sons.

strategy of deposition of layers, incubation, and centrifugation may also affect the cell viability, hence needs a lot of care. The toxicity level of polyelectrolytes deposited on bacterial or yeast cell is different from when used in human cells. In most cases, the microbial cells (fungi, algae, and bacteria, etc.) are more likely to remain viable when modified with polymers in the functionalized shells as compared to mammal cells. Most microorganisms possess additional layer in the form of cell wall that protects them from environmental stresses such as osmotic pressure. In contrast, the human cells lack a cell wall, thus are more delicate and vulnerable to damage by external factors.¹⁸

Most studies have shown that the commonly used polyelectrolytes do not affect microbial cell viability. For example, the yeast cells and *E. coli* cells encapsulated within multilayers of sodium alginate/ CaCl_2 / Fe_3O_4 / Na_2HPO_4 respectively were observed to be fully viable and functional.^{21,87} Another case also proved that bacterial cells were able to retain viability when coated with Poly (diallyldimethylammonium chloride) (PDDA), poly (acrylic acid) (PAA), Poly (styrene sulfonate) (PSS) and Poly (glutamic acid) (PGA).⁶⁸ Permeability of LbL shells was demonstrated by the passage of the dyes and nutrients to the encapsulated microbial cells.⁶⁸ A low percentage of toxicity was

observed when yeast cells were coated with poly (allylamine hydrochloride) (PAH) and doped with magnetic nanoparticles. The cells were able to produce Green fluorescence protein(GFP), however, when coated with PAH, PSS, the GFP production was suppressed, and a cell death up to 89% was observed.⁶⁹ This high cell death might have been caused by the fluctuation of osmotic pressure especially during the centrifugation process, coating, and washing.⁷¹

5. Applications of surface engineered microbial cells

Microencapsulation has recently gained its popularity in industry and biomedical fields owing to their potential advantages related to their simple culturing, processing, and modification, which make them more affordable and accessible to many applications. Microencapsulation has been used widely for the encapsulation and immobilization of microorganisms.⁷² Significantly, bacterial cell encapsulation occurs naturally when bacterial cell proliferate and produce some polymers (mainly comprised of sugar residues) which have high molecular weight and act as exopolysaccharides.⁷³ The following

Table 3 Summary of applications of surface engineered microbes: Medical application (Cell delivery), pharmaceutical industry (toxicological screening), environmental microbiology (biodegradation), and biosorbents and catalysts.

Applications	Example	Microbial system	Description	Ref.
Medical	Hypocholesterolaemic effect	<i>Lactobacillus plantarum</i> , Lp91 and Lp21	Reduced plasma total cholesterol and LDL-cholesterol	74
	Uremic therapy	<i>Escherichia coli</i> DH5 α	Stable alginate-chitosan-alginate (ACA) microcapsules as a potential functionalized cell for oral therapy of uremia Significant <i>Reduction of</i> the urea concentration in the simulated culture medium by <i>Encapsulated E. coli</i> ,	75
	Renal failure treatment	<i>Saccharomyces cerevisiae</i>	Retention of yeast cells in the microcapsules through Gastro intestinal tract (GIT) transit Decrease urea levels	47
	Colon Diseases treatment	<i>Lactobacillus brevis</i> <i>L. acidophilus</i>	Changed ERIC-PCR profiles of fecal samples from diarrhea calves to healthy calves Increased colonic epithelial cell survival	76 77
Pharmaceutical industry	Toxicology screening	Magnetically modified yeast cells, GFP reporter yeast	Retention of magnetic modified yeast cells within microfluidic device, rapid screening toxicity, magnetization of cells with PAH-stabilized magnetic nanoparticles and release of cells upon removal of magnetic field	17,71
Industry	Food industry	<i>Brewing S. cerevisiae</i>	High rate of ethanol production and yeast growth	78
		<i>S. cerevisiae</i> AXAZ-1	High stability rate of immobilized cells at wide range of temperatures and high ethanol production	79
Environment	Biodegradation	<i>P. putida</i>	Low phenol concentration resulted in higher biodegradation by both immobilized and suspended cells with higher efficiency of magnetic nanoparticles	80
	Bioremediation	<i>Acinetobacter baylyi</i> ADP1	Controlled magnetic modified cells by external magnetic fields and detection of toxic compounds from sediments and soil	9
	Biosorbents and catalysts	<i>S. cerevisiae</i>	A higher adsorption rate of Cd ²⁺ and Pb ²⁺	81
Biomass yeast cell		Higher adsorption rate of lead ions at higher pH	82	

sections overview various potential applications of surface engineered microbial cells (Table 3).

5.1. Cell delivery

5.1.1. Hypocholesterolaemic effect. Two probiotic strains of *Lactobacillus plantarum*, Lp91 and Lp21, which produce bile salt hydrolase (Bsh), were evaluated on in Sprague–Dawley rats for high plasma cholesterol level that is the real cause of hypercholesterolaemia in humans. The probiotic bacterial cells were microencapsulated in sodium alginate matrix. Hypercholesterolaemic diet (HD) with *L. plantarum*Lp91 (HD91), a HD with microencapsulated *L. plantarum* Lp91 (HDCap91) and a HD with *L. plantarum* Lp21 (HD21) were tested for cholesterol reduction effect. The total cholesterol reduction after 21 days was 23.26, 15.71, and 15.01%, and taurodeoxycholic acid (TGA) reduction was 21.09, 18.77, and 18.17 % and finally 38.13, 23.22, and 21.42 % reduction in LDL-cholesterol. The study showed that Bsh active *L. plantarum* strains have the potential to be used in treatment of hypercholesterolaemia in patients since it was able to demonstrate reduction in plasma total cholesterol and LDL-cholesterol in rats fed with a diet high in cholesterol.⁷⁴

5.1.2. Uremic therapy. A study by Lin et al., 2008 used *Escherichia coli* DH5 α , a genetically modified strain encoded with urease gene, as a model for *in vivo* and *in vitro* studies to assess the alginate-chitosan-alginate (ACA) microcapsules as a potential functionalized cell for oral therapy of uremia. In the ACA microcapsules containing *E. coli*, the urea concentration in the simulated culture medium was significantly reduced from 429.20 mg/L to 37.06 mg/L in 2 h, and was undetectable after 4 h. The urea removal was accomplished better by free cells compared to bacteria encapsulated within the ACA or alginate-polylysine-alginate (APA) shells, which

may be attributed to the easy diffusion of the urea molecule through the cell compared to the immobilized cells. It was concluded that the ACA microcapsule membrane possesses superior mechanical and chemical stability in the simulated gastrointestinal conditions. *In vivo* experiments demonstrated that the ACA microcapsule is more stable than the APA microcapsule, because of increased resistance to gastrointestinal (GIT) enzymatic degradation. Therefore, it is anticipated that ACA microcapsules could allow safer and more effective oral delivery of live bacterial cell for various clinical applications.⁷⁵

5.1.3. Renal failure treatment. Prakash et al. were the first to study the microencapsulated yeast cells, *Saccharomyces cerevisiae* in renal failure. Live yeast cells were encapsulated in alginate-polylysine-alginate (APA) microcapsules and orally administered to uremic rat model. It was found that microencapsulated yeast cells were retained in the microcapsules through gastro-intestinal tract transit, however, it allowed urea to diffuse through the semi-permeable membrane of the microcapsule and were acted upon by yeast urease. There was 18% decrease of the urea levels during 8 week treatment period, thus, demonstrated to be a therapeutic method for eliminating the elevated levels of metabolites in renal failure. Plasma urea level rapidly returned to uremic level when administration of APA encapsulated yeast was terminated. They, therefore, concluded that the encapsulated yeast cells did not remain in the intestinal tract rather it was removed in the stool.⁴⁷

5.1.4. Colon Diseases treatment. Recently, microencapsulation of probiotic cells has been vastly studied and being identified for the treatment of various gastrointestinal and other health condition. Their delivery has been enhanced by microencapsulation which offers protection against harsh conditions of the upper gastro intestinal

tract (GIT).⁷³ The effect of the probiotic microencapsulation on therapy of neonatal calf-under field conditions was investigated using Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) methods. The analysis of ERIC-PCR fingerprints showed that the administration of microencapsulated *Lactobacillus brevis* had a strong beneficial effect on the replacement of the intestinal microflora of diarrhea calves. ERIC-PCR profiles of fecal samples from the diarrhea calves were different from that of control health calves. Diarrhea calves who were administered with probiotic capsules showed ERIC-PCR profiles similar to that of healthy calves. These findings demonstrated a positive signal for using probiotics capsules to treat neonatal calf diarrhea.⁷⁶ Several other studies have investigated the ability of APA microencapsulated *L. acidophilus* to suppress intestinal inflammation in mice, hence becoming one of the potential application in chronic inflammatory gut diseases such as inflammatory bowel syndrome and inflammatory bowel disease. A study showed that the cytokine level were lowered when microencapsulated cells were administered which enhanced the markers linked to colonic epithelial cell survival.⁷⁷

5.1.5. Drug delivery vehicles (Viral Nanoparticles). The invention of Viral Nanoparticles (VPNs) targeting specific cell types by loading toxic substances through encapsulation, infusion, and/or conjugation to eliminate them enhanced the removal of diseased cells or cancer cells without any effect on the non-targeted cell. The toxic cargos are always loaded into the VNP cavity to preserve them from chemical and enzymatic degradation and to prevent them from interacting with the healthy cells.⁸³ It is presumed that up to 300 doxorubicin molecules can be conjugated to the capsid surface of cowpea mosaic virus (CPMV).⁵⁷ Some studies have reported the designing of VNP for *in vitro* toxicity for drug delivery and the clinical trials demonstrated the *in vivo* efficacy reduced cardio toxicity of a doxorubicin-loaded VNP; specifically the cucumber mosaic virus (CMV) modified with folic acid to target the ovarian cancer.⁵⁶

5.2. Pharmaceutical industry

5.2.1. Toxicology screening. The use of magnetically modified yeast cells in microfluidic biosensor systems have been studied as the most cost effective method for industrial scale application for screening toxicity of various substances. A short communication study by Alonso et al. exposed the magnetically-PAH stabilized GFP reporter yeast to a genotoxic chemical (methyl methane sulfonate) and monitor the genotoxicity of the chemical to the cells within a microfluidic device. Gradient mixing was done to ensure simultaneous exposure of functionalized yeast to a various concentrations of toxins in order to measure effectively the emitted fluorescence from GFP. The magnetic modification on the cells ensured that the yeast cells were retained within the device. The rapid toxicity screening of a various range of chemicals and convenience was enhanced by their facile subsequent reloading and removal.^{17,71}

5.3. Industry Applications

5.3.1. Food industry. In food industry, microbial cell or enzyme immobilization is mostly carried out through entrapment or encapsulation.^{3,4} The process entails entrapping or coating the living cells inside a polymeric substance in order to obtain beads which are able to permit gases, metabolites, and nutrients within the cell to main-

tain cell viability.^{52,84} This encapsulation strategy leads to the entrapment of the cells within a micro (within size range of 1–1000 μm) and macro (within size range of a few millimeters to a few centimeters) polymeric beads.^{52,85} High rate of fermentation for beer production was observed when brewing yeast was encapsulated in alginate/chitosan polymers in matrix with a liquid core as compared to when it was done in free cell system. The high rate of ethanol production and yeast growth was attributed to the encapsulation technique protecting the cells from product and substrate inhibition.⁷⁸ Use of encapsulated *S. cerevisiae* AXAZ-1 cells in a multi-stage fixed bed tower bioreactor that has a capacity of 5000–10,000 L for wine production, lead to good operation stability even at a wide range of temperature. Time to complete fermentation with the immobilized yeast ranged from 290 h at 5 °C and 120 h at 40 °C to 25 h at 33 °C. The daily ethanol productivity reached maximum (88.6 g/l) and minimum (5.6 g/l) levels at 33 °C and 5 °C, respectively. Free cells were unable to ferment at temperatures greater than 35 °C, in contrast to immobilized yeast.⁷⁹

5.4. Environmental microbiology

5.4.1. Biodegradation. Phenol is one of the commonly used chemicals in various industries although it is hazardous to human health when released directly to the environment. Hence, there is a need to develop a method to reduce its concentration to safe levels and release the wastewater that has low phenol from industries to stream water. Several researchers have developed physical, chemical, and biological treatment methods to remove phenol from industrial water.^{86–89} Immobilization of cells and cell suspension are two commonly used strategies for biological treatment of water.²⁷

For instance, in a study, the bacterium *P. putida* was immobilized in sodium alginate beads in order to evaluate the degradation of phenol of different concentrations.⁸⁰ A low phenol concentration between 50–500 mgL^{-1} leads to higher biodegradation by both immobilized and suspended cells. However, an increased concentration above 500 mgL^{-1} leads to decreased biodegradation of phenol by the suspended cells as compared to immobilized cells which showed to smaller extent decrease in biodegradation rate. In conclusion, the TiO_2 immobilized cells showed a higher rate of biodegradation.

5.4.2. Bioremediation. Bioremediation uses biological organisms to assist in the removal of hazardous substances from polluted area. When compared to the planktonic bacteria, the immobilized bacteria also shield perturbations of environmental conditions, like the toxic compounds.⁹⁰ Zhang et al. used three strains chromosomally encoded bioreporters of *Acinetobacter baylyi* ADP1 to obtain magnetic function by stabilizing the cells with poly (allylamine hydrochloride) and magnetic nanoparticles (PAAH-MNPs). Genetic engineering was done to the cells in order to produce bioluminescence in the presence of toluene/xylene, alkanes and salicylate. The *Acinetobacter* bioreporters cells were reported to have higher efficiency of magnetic nanoparticles functionalization of about $99.96 \pm 0.01\%$. Moreover, the magnetic modified bioreporters were able to detect salicylate when applied to garden soils and sediments which were detected by measuring the bioluminescence and they were able to be recovered by use of a permanent magnet, thus, serving as a promising tool for cleaning of contaminated soil.⁹

5.4.3. Biosorbents and catalysts. Microorganisms, either unicellular or multicellular, can interact with a variety of nanoparticles

without interference of their viability to provide several potential applications.^{91,92} For example, the magnetic modified microbial cells can find potential applications as cell biocatalysts and adsorbents of several types of organic and inorganic xenobiotics.^{93,94} Ethylenediaminetetraacetic dianhydride (EDTAD) with magnetic nanoparticles (Fe_3O_4) was used to modify baker's yeast biomass to form a functionalized *S. cerevisiae*. The functionalized yeast cell obtained acted as a biosorbent for removal of heavy metals such as Cadmium (Cd^{2+}) and Lead (Pb^{2+}). A higher adsorption rate of 40.72 mg/g for Cd^{2+} and 88.16 mg/g Pb^{2+} was observed at pH 6.0 and 5.5, respectively.⁸¹ Similarly, biomass yeast cell was modified with ethylenediamine and doped with magnetic chitosan microparticles for the adsorption application of lead metal ions. Increase in pH leads to higher adsorption of lead ions and the highest adsorption rate was observed at pH 4.0-6.0.⁸²

6. Conclusion and future prospects

Surface engineering of microbial cells is a promising fabrication technique in industry, pharmaceutical, biomedical, and environmental sectors with promising applications. It is a simple, efficient, and cost-effective process that offers modification of a wide range of microbes for a wide range of applications. Recently, the surface modification processes have resulted in increased viability of microbes by the use of nontoxic polymers to encapsulate the cells, thus the development of different varieties of surface engineered organisms for different purposes. However, the selection of appropriate technique, polymers, and human beneficial microbial cells could help to extend the applications of this engineering process to other fields like advanced delivery of beneficial components to the human body. It is conceivable that this field with further advancements, will find major breakthroughs in the near future.

Conflict of interest

The authors declare that they have no conflict of interest.

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